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STABILITY OF NEW ANALGESIC ACTIVE COMPOUND, PYRROLO-[3,4-c]PYRIDINE DERIVATIVE, IN AQUEOUS SOLUTIONS

IZABELA MUSZALSKA and ELIZA WOJTYNIAK

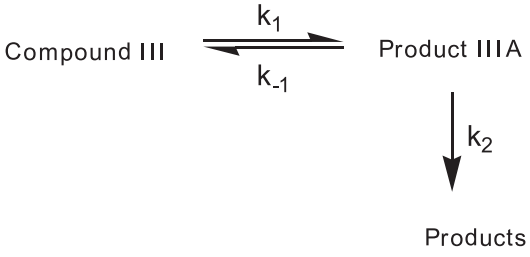
Department of Pharmaceutical Chemistry, Poznań University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland

Abstract: The kinetics of hydrolysis of 4-ethoxy-2-[2-hydroxy-3-(4-phenyl-1-piperazinyl)]propyl-2,3-dihydro-6-methyl-1,3-dioxo-1*H*-pyrrolo[3,4-c]pyridine (**III**) in aqueous solutions at 333, 343, 353 and 363 K over the pH range 0.4 – 5.0 was investigated. The decomposition was observed by means of the HPLC method. A sample solution was chromatographed on octadecyl-packed column (LiChrosorb® 100 RP-18 column 250 × 4.0 mm I.D., dp 5 μm) using a mixture of acetonitrile and phosphate buffer (pH 2, 0.01 mol/L) as an eluent (45:55, v/v – phase A or 30:70, v/v – phase B). The UV detection was performed at a wavelength of 239 nm. The stability of compound **III** was found to be dependent on pH. The pH-rate profile indicated specific acid- and base-catalyzed reactions as well as spontaneous water-catalyzed degradation. The ionic strength effect, the pK_a value (2.9; 6.0), the pH_{min} value (2.1) and thermodynamic parameters of the reaction (energy of activation (E_a), enthalpy ($\Delta H^{\neq}$) and entropy ($\Delta S^{\neq}$)) were determined.

Keywords: 4-alkoxy-2,3-dihydro-6-methyl-1,3-dioxo-1H-pyrrolo[3,4-c]pyridine derivatives, hydrolysis, stability, thermodynamics

The N-[2-hydroxy-3-(4-phenyl-1-piperazinyl)] propyl derivatives of 2-methoxy(ethoxy)-6-methyl-3,4-pyridinedicarboxyimides and 2,3-dihydro-1,3-dioxo-1*H*-pyrrolo[3,4-c]pyridine (3,4-pyridinedicarboximide) (1-3) have been shown to cause significant analgesic activity and do not display any noticeable toxic effects ($LD_{50} > 2000$ mg/kg). Most of the tested compounds showed interesting analgesic properties and in order to obtain further information concerning the structure/activity relationship, a modification of their chemical structure was made. In pharmacological tests (*'hot plate', 'writhing syndrome'*), compound **I** (Figure 1) containing the methoxy group in position 4 was more active than its ethoxy homologue **III** (Figure 1). The replacement of the methoxy group (**I**) by the ethoxy one (**III** – Figure 1) caused an almost fourfold decrease of the analgesic activity. The introduction of trifluoromethyl or methoxy groups (**IV** – Figure 1) into the phenyl substituent at N-4 of piperazine in compound **I** also weakens the analgesic properties. The analgesic activity of these compounds was superior to that of acetylsalicylic acid in two different tests. The prolongation of the side-alkyl chain at the nitrogen atom to C_4 and the elimination of hydroxy group decreased the analgesic activity. The results of the preliminary radioligand binding assay suggest that these compounds display a weak affinity (at micromolar concentration) to μ-opiod receptors. This probably plays a role in the mechanism of action of these compounds (4-6).

After the temperature, the second most important variable affecting drug degradation is pH. The effect of the pH degradation rate can be explained by the catalytic effects that hydrogen or hydroxide ions can have on various chemical reactions. Water itself is also a critical reactant. If the critical path in a reaction involves a proton transfer or abstraction step, other acids and bases present in the solution (usually buffer species) can affect the reaction rate. These reactions will also be pH-dependent because the fraction of any species present in its acid or base form will be dependent on its dissociation constant and the solution pH. Therefore, if the reactivity of



Scheme 1. The mechanism of degradation of **III** in the acetate buffer (pH 3.87 – 4.31) at 343, 353 and 363 K.

* Corresponding author: e-mail: imuszals@amp.edu.pl (I. Muszalska)

the drug depends on its form, this reactivity will be pH-dependent.

Kinetics studies of the degradation of compounds **I**, **II** and their degradation products have shown that in an acidic or basic medium at an increasing temperature, a hydrolysis of the imide bond is observed. Consequently, an indirect product with a carboxy group in either C_3 or C_4 position of the pyridine ring is formed. A positive staining with the ninhydrine reagent of a TLC chromatogram indicated that one of the subsequent degradation products is an amino derivative resulting from hydrolysis of an amide group which exists in this intermediate product (7-9). The pH-rate profile proved specific acid- and base-catalyzed reactions as well as spontaneous water-catalyzed decomposition of the molecules **I** and **II** (10, 11).

The purpose of this study involved investigating the stability of **III** as a function of the hydrogen ion concentration caused by buffer species and temperature, and establishing the respective kinetic equations for the log k – pH profile.

EXPERIMENTAL

Materials

The compound – 4-ethoxy-2-[2-hydroxy-3-(4-phenyl-1-piperazinyl)]propyl-2,3-dihydro-6-methyl-1,3-dioxo-1*H*-pyrrolo[3,4-c]pyridine (**III**) was obtained from the Department of Chemistry of Drugs at Wrocław University of Medicine. Oxazepam; $C_{15}H_{11}ClN_2O_2$ (serving as an internal standard – **i.s.**) was a product of Sigma Chemical Co. (St. Louis, USA). All chemicals and solvents were of analytical reagent grade.

Analytical method

The compounds studied were determined and their decomposition traced by means of high-performance liquid chromatography (HPLC) with a system consisting of a Rheodyne 7120, 20 μL fixed-loop injector, an LC 3 UV detector (Pye Unicam, England), an L-6000 A pump (Merck-Hitachi, Germany) and an A/C transmitter with Chromed software (Medson, Poland). The decomposition was monitored by the HPLC method with LiChrosorb® 100 RP-18 column (250 × 4.0 mm I.D., dp 5 μm). The mobile phase consisted of acetonitrile and phosphate buffer 0.01 mol/L pH 2 (45:55, v/v – phase A) or (30:70, v/v – phase B). The mobile phase B was only used for the determination of compound **III** and product **IIIA** observed in the acetate buffer (pH 4.98, μ 0.50 mol/L, 333 K). The flow rate used was 1.5 mL/min and UV detection was set at 239 nm (12). The analyzed solutions consisting of 1.0 mL of sample, 1.0 mL of **i.s.** (0.15 mg/mL) and 1.0 mL of water were injected directly onto the column for HPLC analysis. The retention times were ca. 4.0 min for **i.s.** and 6.2 min for **III** in mobile phase A or ca. 9.5 min and 17.3 min in mobile phase B, respectively.

Stability in aqueous medium

In order to shorten the time of analysis and determine thermodynamic parameters, the test of accelerated ageing was used at four different temperatures. All the studies in aqueous buffer solutions were performed at 333, 343, 353, 363 K. The ionic strength (μ 0.5 mol/L) was maintained constant for each solution by adjusting a calculated amount of sodium chloride. To obtain the desired pH, the following solutions were used: hydrochloric acid (pH

Table 1. The effect of ionic strength (μ) on pseudo-first-order rate constants for the degradation of **III** at pH 0.46 and 1.39.

Conditions	μ, mol/L	$(\sqrt{\mu}/(1+\sqrt{\mu}))$	$k_{obs} \pm \Delta k_{obs}$, s^{-1}	Parameters of the equation: $\log k_{obs} = \log k_o + 2QZ_AZ_B(\sqrt{\mu}/(1+\sqrt{\mu}))$
pH 0.46	0.50	0.4142	$(1.33 \pm 0.10)\cdot10^{-4}$	$2QZ_AZ_B = 1.968 \pm 0.851$
(0.50 mol/L HCl,	0.60	0.4365	$(1.49 \pm 0.15)\cdot10^{-4}$	$k_o = 2.00 \times 10^{-5}$, s^{-1}
353 K)	0.70	0.4555	$(1.47 \pm 0.09)\cdot10^{-4}$	r = 0.9548
	0.80	0.4721	$(1.71 \pm 0.12)\cdot10^{-4}$	$t_a = 6.421 > t_{0,05}(5) = 2.571$
	0.90	0.4868	$(1.76 \pm 0.16)\cdot10^{-4}$	
	1.00	0.5000	$(2.03 \pm 0.09)\cdot10^{-4}$	
pH 1.39	0.05	0.183	$(1.81 \pm 0.15)\cdot10^{-5}$	$2QZ_AZ_B = 0.986 \pm 0.460$
(0.05 mol/L HCl,	0.10	0.240	$(1.78 \pm 0.20)\cdot10^{-5}$	$k_o = 1.11 \times 10^{-5}$, s^{-1}
353 K)	0.20	0.309	$(2.16 \pm 0.24)\cdot10^{-5}$	r = 0.9478
	0.30	0.354	$(2.42 \pm 0.32)\cdot10^{-5}$	$t_a = 5.944 < t_{0,05}(5) = 2.571$
	0.40	0.387	$(2.54 \pm 0.20)\cdot10^{-5}$	
	0.50	0.414	$(3.11 \pm 0.21)\cdot10^{-5}$	

t_a – t-Student's test for the value $a = 2QZ_AZ_B$

range 0.44 to 1.39, experimental concentration 0.05 – 0.50 mol/L), phosphate buffers (pH range 2.19 to 3.20, experimental concentration 0.1 – 0.4 mol/L), and acetate buffer (pH range 3.36 to 4.98, experimental concentration 0.1 – 0.4 mol/L). The pH values for HCl were calculated with the equation:

$$pH = - \log f_{HCl} \times [HCl] \quad (1)$$

The activity coefficients f_{HCl} were obtained from the literature (12). Other pH values were measured using a potentiometric pH-meter (CD-401, Elmetron, Zabrze, Poland) at the respective experimental temperatures. The reaction was initiated by adding 0,5 mL of hydrochloric acid (0.1 mol/L)-methanol stock solution of compound **III** to 14.5 mL of preheated solution to make a final concentration of 0.25 mg/mL. When necessary, 2 mL of the obtained solutions were transferred into 5 mL ampoules and sealed. The remaining ampoules were immediately heated to 333, 343, 353 or 363 K, and cooled to room temperature at experimentally determined time intervals to stop the reaction. The samples were collected at time intervals depending on the reaction rate at a given pH.

RESULTS

Ionic strength effect

The effect of ionic strength on the hydrolysis of **III** was studied at a constant pH (0.46 and 1.39) and temperature (353 K). The different ionic strengths of the solution were obtained by adding sodium chloride solution (4.0 mol/L). The results calculated for these pH values and in the ionic strength range 0.50 – 1.00 mol/L and 0.05 – 0.50 mol/L are given in Table 1. The dependences log k_{obs} as a function of $(\sqrt{\mu}/(1 + \sqrt{\mu}))$ were described by the equation:

$$\log k_{obs} = \log k_0 + 2QZ_AZ_B (\sqrt{\mu}/(1 + \sqrt{\mu})) \quad (2)$$

At pH 0.46 and pH 1.39 the slopes of the plots are positive and equal to $2QZ_AZ_B$, where Q is a constant for the solvent at a given temperature. Z_A and Z_B are the charges of the reaction species A and B, respectively. This is characteristic of the reaction of two positive-charged species: the dication of **III** and the proton.

Observed rate constants (k_{obs})

The apparent first-order rate constants of hydrolysis of **III** were determined using the HPLC method, according to the equation:

$$\ln(p_t/p_i) = \ln(p_0/p_i) - k_{obs} \times t \quad (3)$$

where: p_0, p_t are the peak area of **III** at zero and time t respectively, and p_i is the peak area of **i.s.** In the solution of HCl, the observed rate constants (k_{obs}) are equal to rate constants describing the influence of pH on stability in aqueous media (k_{pH}) (Table 2). The degradation of **III** was found to be subjected to general acid-base catalysis. The catalytic effect was determined by measuring the rate of degradation at constant pH, ionic strength and temperature; only the buffer concentration at a specific pH was different. The observed rate constant, in the presence of general acid-base catalysis is represented by the equation:

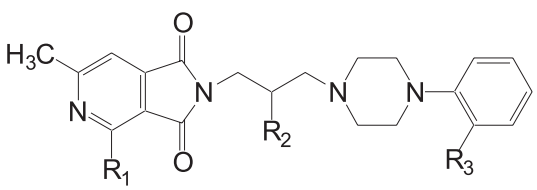
$$k_{obs} = k_{pH} + k_B[B]_T \quad (4)$$

where: $[B]_T$ represents the total buffer concentration, k_{pH} is the rate constant at zero buffer concentration, and k_B represents the catalytic effect of the buffers. The significant buffer catalysis in the hydrolysis of **III** was observed in the phosphate buffers (Table 2) and was not observed in the acetate buffers, where the hydrolysis consisted of two stages (Scheme 1 and Figure 2), but not in the whole pH range (Table 3). In this medium, the mechanism of degradation depended on the pH value and the temperature. That is, a complete degradation was observed as the pseudo-first-order reaction with a linear dependence log $(p_t/p_{i.s.})$ as a function of time, in the solutions of **III** at pH 3.4, whereas at pH 3.8 – 5.0 and temperature 333 K only the reversible reaction between **III** and **IIIA** occured and for that reason the k_{pH} value was equal to $k = k_1 + k_{-1}$.

The k_1, k_{-1}, k_2, k, k_{pH} and K (the equilibrium constant of the reaction) were calculated based on the following equations:

$$\ln(p_t/p_i) = \ln(p_0/p_i) - k_2 \times t$$
(for p_t/p_i observed in the second stage) (5)

$$\ln(P - P')_t = \ln(P - P')_0 - k \times t$$
(for p_t/p_i observed in the first stage) (6)



I; $R_1 = OCH_3$; $R_2 = OH$; $R_3 = H$

II; $R_1 = OCH_3$; $R_2 = H$; $R_3 = H$

III; $R_1 = OC_2H_5$; $R_2 = OH$; $R_3 = H$

IV; $R_1 = OCH_3$; $R_2 = H$; $R_3 = OCH_3$

Figure 1. Chemical structures of 3,4-pyridinedicarboximide compounds.

Table 2. Rate constants k_{pH} i k_B for hydrolysis of **III** in hydrochloric acid and phosphate buffers pH 2.0 – 3.0 (μ = 0.50 mol/L).

Temperature (K)	Hydrochloric acid		Phosphate buffer ($H_3PO_4 + H_2PO_4^-$)		
	pH	k_{pH} (s^{-1})	pH	k_{pH} (s^{-1})	k_B ($mol^{-1} \times L \times s^{-1}$)
333	0.44 0.54 0.66 0.83 1.11 1.39	1.42×10^{-5} 1.30×10^{-5} 1.10×10^{-5} 8.66×10^{-6} 5.82×10^{-6} 3.70×10^{-6}	2.27 2.65 2.92 3.20	2.30×10^{-6} 2.44×10^{-6} 2.45×10^{-6} 2.84×10^{-6}	4.45×10^{-6} 5.60×10^{-6} 4.09×10^{-6} 2.91×10^{-5}
343	0.45 0.54 0.66 0.83 1.11 1.39	4.95×10^{-5} 4.31×10^{-5} 3.65×10^{-5} 2.37×10^{-5} 1.54×10^{-5} 1.22×10^{-5}	2.19 2.93 3.20	8.03×10^{-6} 8.82×10^{-6} 9.80×10^{-5}	7.02×10^{-6} 7.43×10^{-5} 6.78×10^{-6}
353	0.46 0.55 0.66 0.84 1.11 1.39	1.20×10^{-4} 1.05×10^{-4} 8.57×10^{-5} 6.76×10^{-5} 4.27×10^{-5} 3.11×10^{-5}	2.20 2.63 2.88 3.15	1.93×10^{-5} 1.79×10^{-5} 2.04×10^{-5} 2.01×10^{-5}	1.81×10^{-5} 1.85×10^{-5} 1.88×10^{-5} 2.11×10^{-5}
363	0.46 0.56 0.67 0.84 1.11 1.40	3.13×10^{-4} 2.43×10^{-4} 1.90×10^{-4} 1.61×10^{-4} 7.78×10^{-5} 7.28×10^{-5}	2.21 2.64 2.89 3.18	3.71×10^{-5} 3.78×10^{-5} 4.01×10^{-5} 4.28×10^{-5}	4.86×10^{-5} 1.14×10^{-4} 2.70×10^{-5} 8.13×10^{-5}

Table 3. Equilibrium constants of the reversible reaction (K) and rate constants: $k = k_1 + k_{-1}$, k_1, k_{-1}, k_2 and $k_{pH} = k_1 + k_{-1} + k_2$ for hydrolysis of **III** in acetate buffers pH 3.5 – 5.0 (μ = 0,50 mol/L).

Temp. (K)	pH	K	k (s^{-1})	k_1, (s^{-1})	k_{-1}, (s^{-1})	k_2, (s^{-1})	k_{pH}, (s^{-1})
333	3.36 3.77 4.28 4.98	– 0.272 0.128 0.088	6.74×10^{-6} 2.73×10^{-5} 6.93×10^{-5} 2.83×10^{-4}	– 2.15×10^{-5} 6.15×10^{-5} 2.60×10^{-4}	– 5.85×10^{-6} 7.81×10^{-6} 2.29×10^{-5}	– – – –	6.74×10^{-6} 2.73×10^{-5} 6.93×10^{-5} 2.83×10^{-4}
343	3.24 3.88 4.30	– 0.362 0.170	1.30×10^{-5} 5.47×10^{-5} 1.44×10^{-4}	– 4.01×10^{-5} 1.25×10^{-4}	– 1.45×10^{-5} 1.86×10^{-5}	– 3.76×10^{-6} 2.66×10^{-6}	1.30×10^{-5} 5.85×10^{-5} 1.46×10^{-4}
353	3.10 3.89 4.31	– 0.501 0.224	2.71×10^{-5} 1.53×10^{-4} 3.60×10^{-4}	– 1.02×10^{-4} 2.94×10^{-4}	– 5.11×10^{-5} 6.57×10^{-5}	– 1.32×10^{-5} 9.30×10^{-6}	2.71×10^{-5} 1.66×10^{-4} 3.69×10^{-4}
363	3.12 3.87 4.30	– 0.698 0.308	5.88×10^{-5} 5.35×10^{-4} 8.28×10^{-4}	– 3.15×10^{-4} 6.32×10^{-4}	– 2.66×10^{-4} 2.28×10^{-4}	– 4.61×10^{-5} 3.25×10^{-5}	5.88×10^{-5} 5.81×10^{-4} 8.60×10^{-4}

Table 4. Specific rate constans and thermodynamic parameters (293 K) of **III**.

Temp., K	$10^5(k_{H^+} \pm \Delta k_{H^+})$	$10^6(k_{H_2O} \pm \Delta k_{H_2O})$	$k_{OH^-} \pm \Delta k_{OH^-}$
333	4.02 ± 1.24	2.51 ± 1.72	33180 ± 3700
343	12.40 ± 1.77	7.01 ± 3.86	53770 ± 21997
353	29.04 ± 2.79	22.18 ± 5.99	82224 ± 30386
363	78.70 ± 14.93	33.80 ± 31.68	130170 ± 165960
E_a, kJ/mol	98.2 ± 12.8	90.3 ± 43.3	45.5 ± 3.4
$\Delta H^{\neq}$, kJ/mol	95.8 ± 12.8	87.9 ± 43.3	43.0 ± 3.4
$\Delta S^{\neq}$, J/K/mol	-33.8 ± 36.7	-80.2 ± 124.5	-21.7 ± 9.7

k_{H^+}, k_{OH^-}, $mol^{-1} \times L \times s^{-1}$; k_{H_2O}, s^{-1}

$$\text{or } \ln(P_t - P_\infty) = \ln(P_0 - P_\infty) - k \times t \quad (7)$$

$$k = k_1 + k_{-1} \quad (8)$$

$$K = k_{-1}/k_1 = c_{III,e}/c_{IIIA,e} \quad (9)$$

$$k_{pH} = k_1 + k_{-1} + k_2 \quad (10)$$

where: $P = p_t/p_i$, P' values are calculated from equation (5) for p_t/p_i observed in the first stage, $c_{III,e}$ and $c_{IIIA,e}$ are the concentration of **III** and **IIIA** in the state of equilibrium, respectively.

The dependences of the equilibrium constant of the reaction (K) as the function of the hydrogen ion activity (a_{H^+}) were linear. The observed rate constants ($k = k_1 + k_{-1}$) of the reversible reaction between **III** and its product **IIIA** [for **III**: $k = (2.83 \pm 0.30) \times 10^{-4}$, s^{-1}; $k_1 = 2.60 \times 10^{-4}$, s^{-1}; $k_{-1} = 2.29 \times 10^{-5}$, $s^{-1}$and for **IIIA**: $k = (2.82 \pm 0.66) \times 10^{-4}$, s^{-1}] did not show any significant statistical differences in the acetate buffer (pH 4.98, m 0.50 mol/L, 333 K).

The log k-pH profile

The semilogarithmic dependence $\log k_{pH} = f(pH)$ (Figure 3) indicates that specific acid-base hydrolysis of **III** is composed of hydrogen ion- and hydroxide ion-catalyzed reaction and water-catalyzed decomposition of two dissociated molecules of **III**.

The overall reaction of hydrolysis can be described by the following equation:

$$k_{pH} = k_{H^+} \times a_{H^+} \times f_1 + k_{H_2O} \times f_1 + k_{OH^-} \times a_{OH^-} \times f_2 \quad (11)$$

where: k_{H^+} represents the second-order rate constant for the specific hydrogen ion-catalyzed degradation of the dication form; k_{H_2O} is the first-order constant of the dicationic form of **III** for water-catalyzed degradation; k_{OH^-} is the rate constant for the hydroxide ion-catalyzed hydrolysis of the monocation form; a_{H^+}, a_{OH^-} refer to the hydrogen and hydroxide ion activity; f_1, f_2 are fractions of the compounds in the dicationic (f_1) and monocationic (f_2) forms of compound **III**.

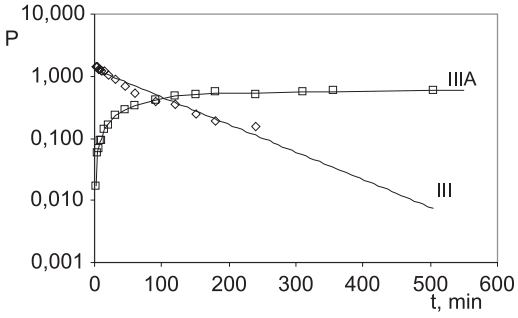


Figure 2. Plots of compound **III** degradation and the product **IIIA** formation in the acetate buffer at 333 K (μ = 0.50 mol/L; pH 4.98).

The pK_a values of **III** were determined by the potentiometric titration at room temperature (pK_{a_1} 2.9; pK_{a_2} 6.0).

The microscopic rate constant (k_{H^+}) (Table 4) describing the catalytic effect of the hydrogen ion on the hydrolysis of **III** was calculated with the equation:

$$k_{pH}/f_1 = k_{H^+} \times a_{H^+} \times k_{H_2O} \quad (12)$$

using values of k_{pH} in the pH-region 0.4 – 1.4. In this pH-region the value $f_1 \rightarrow 1$. The plots $k_{pH}/f_1 = f(a_{H^+})$ were linear ($y = ax + b$) and the slopes (a) were positive, and equal to k_{H^+}. The value k_{H_2O} was calculated as the value b ($k_{H_2O} = b$). The microscopic rate constant (k_{OH^-}) (Table 4) which characterizes the catalytic effect of the hydroxide ion on the hydrolysis of **III** was calculated with the equation:

$$k_{pH}/f_2 = k_{OH^-} \times a_{OH^-} + k'_{H_2O} \quad (13)$$

using values of k_{pH} in the pH-region 3.2 – 5.0. The plots $k_{pH}/f_2 = f(a_{OH^-})$ were linear ($y = ax + b$) and the slopes (a) were positive, and equal to k_{OH^-}. The value k'_{H_2O} was calculated as the value b and it is not statistically significant ($k'_{H_2O} = b = 0$).

The pH_{min} values was calculated using the equation:

$$pH_{min} = 0.5\ pK_w + 0.5\ \lg(k_{H^+}/k_{OH^-}) \quad (14)$$

for all temperatures: 333 K, 2.05; 343 K, 2.09; 353 K, 2.09; and 353 K, 2.12.

Thermodynamic parameters

Using the catalytic rate constants derived from the Arrhenius equation ($\ln k_i = \ln A - a/T$), the slope (a) of the plots $\log k_i = f(1/T)$ (Figure 4) and the value of ln A (A – frequency coefficient) for particular reactions were calculated. These values were used to determine energy of activation (E_a), enthalpy ($DH^{\neq}$) and entropy ($DS^{\neq}$) at 293 K (Table 4) which was calculated with following equations:

$$DH^{\neq} = E_a - RT$$

$$DS^{\neq} = R(\ln A - \ln(\overline{k} \cdot T/h))$$

where: R is the gas constant (8,3144, J K mol^{-1}); $\overline{k}$ is the Boltzman constant (1,3805 × 10^{-23}, J K^{-1}); h is the Planck constant (6,6256 × 10^{-34}, J s^{-1}).

DISCUSSION

For most pharmacologically active compounds, hydrolysis is the main degradation pathway, especially when they have an ester, amide or imide functional group. The knowledge of the stability in the wide pH range is important in the design of potentially acid- or base-labile compounds, drugs and their dosage forms. Most drug substances are fairly stable at the neutral pH values found in the small intestine but can be unstable at pH values found in the stomach. The formulator must show that the drug is stable under the pH conditions found in the gastrointestinal tract, if the drug is intended for oral use. It has been noted earlier that the molecular structure of an active compound determines its degradation pathways and that substituents around the reaction center can strongly influence its reactivity.

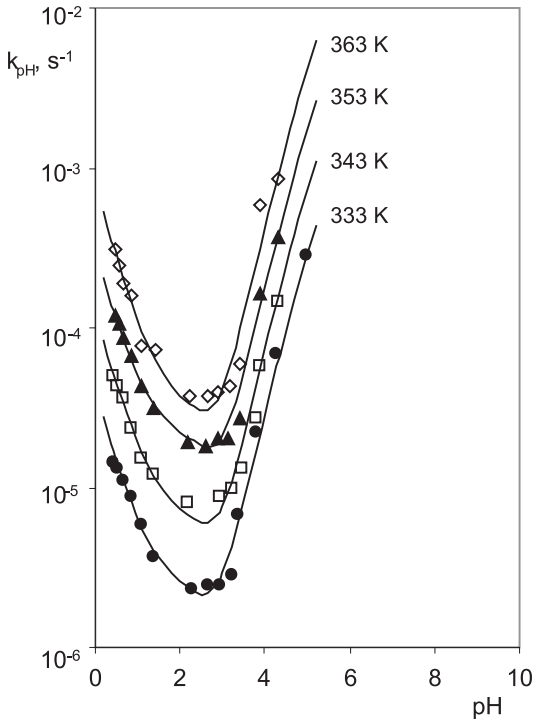


Figure 3. The log k-pH profile for the hydrolysis of **III** at 333, 343, 353 and 363 K. The points are the apparent first-order rate constants. The lines were derived from equation 11.

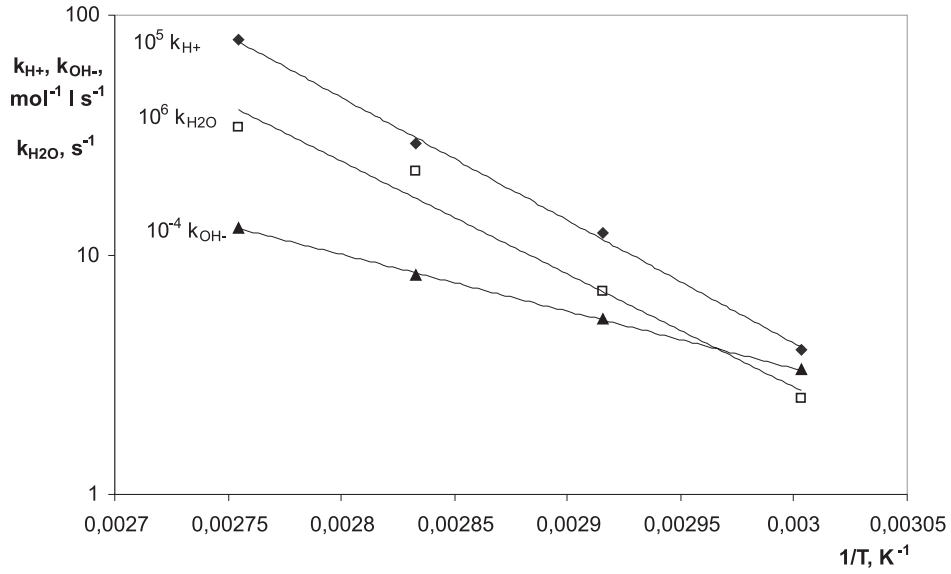


Figure 4. The linear Arrhenius plots for the hydrolysis of **III** at 333, 343, 353 and 363 K.

The degradation of 4-ethoxy-2-[2-hydroxy-3-(4-phenyl-1-piperazinyl)]propyl-2,3-dihydro-6-methyl-1,3-dioxo-1*H*-pyrrolo[3,4-c]pyridine (**III**) in aqueous solutions is independent of pH. The compound **III** was hydrolyzed according to the first-order kinetics. The influence of pH on the rate of hydrolysis of compound **III** is shown in Figure 3, Table 2 and 3. The influence of phosphate buffer species on the rate of hydrolysis of compound **III** by varying the buffer concentration was determined (Table 2). In the acetate buffer, the mechanism of degradation depended on the pH value and the temperature (Table 3) where the reversible and successive reactions were observed (Scheme 1).

The studies of the effect of ionic strength (Table 1) and the potentiometric titration suggest that compound **III** has two pK_a values equal to 2.9 and 6.0. In the pH region 0.4 – 5.0 it can exist primarily as a dicationic and monocationic moiety. The proposed kinetic equation describing the overall reaction of hydrolysis (11) as the correct interpretation of profiles k-pH, proved that the pK_a value is equal to 2.9.

Although the dependence log k_{pH} as a function of pH indicates that in the pH region 0.4 – 5.0 hydrogen ion- and hydroxide ion-catalyzed hydrolysis is predominant, water-catalyzed degradation of the dicationic form of **III** occurs in this pH region, too. The results obtained here show that the maximal stability of **III** is observed at pH ca. 2.1 and that the degradation is subject to specific and general acid-base catalysis. The linear Arrhenius plots (Figure 4) indicate that, once degradation rates are obtained at several different temperature levels, the degradation rate at some other specific temperatures can be estimated. Therefore, the degradation mechanism is not changed with temperature.

In summary, this study provides new fundamental data on the stability of a new analgesic active compound (**III** – pyrrolo[3,4-c]pyridine derivative) in aqueous solutions. The results show that the hydrogen and hydroxide ions have the main influence on its degradation and the decomposition product, formed by the ring-opening hydrolysis (an imide functional group), is susceptible to further decomposition reaction depending on pH.

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