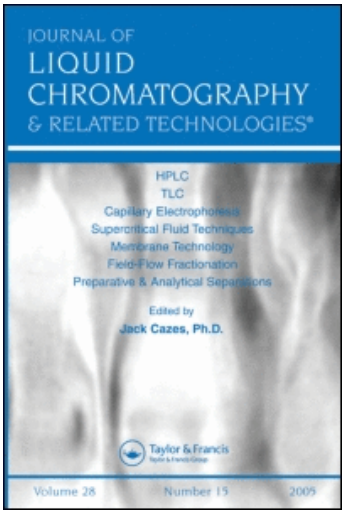

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Izabela Muszalska[a]; Szymon Bereda[a]
[a] Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Poznań, Poland

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Stability Study of New Analgesic Active Compound, 4-Methoxy-2-[3-[4-(2-methoxyphenyl)-1-piperazinyl)]-propyl] Derivative of Pyrrolo[3,4-c]pyridine, in Aqueous Solutions Using the HPLC Method

Izabela Muszalska and Szymon Bereda

Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Poznań, Poland

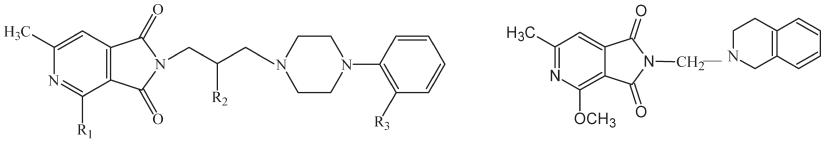
Abstract: A high performance liquid chromatographic method which has been applied for the determination of a 4-methoxy-2-[3-[4-(2-methoxyphenyl)-1-piperazinyl)]-propyl]-6-methyl-1*H*-pyrrolo[3,4-c]pyridine-1,3(2*H*)-dione (IV) (a new derivative of 3,4-pyridinedicarboximide with an analgesic activity), with 4-hydroxybenzoic acid ethyl ester as an internal standard (i.s.), is described. The samples of solution were chromatographed on LiChrosorb® RP-8 column (250 × 4.0 mm I.D., dp = 5 μm), using an eluent composed of: acetonitrile–propan-2-ol–phosphate buffer pH 2 (0.01 mol/L) (30:30:40). Ultraviolet detection was performed at the wavelength of 240 nm. The method was validated with respect to selectivity, linearity, accuracy, and precision. The method was found appropriate for kinetic studies with compound IV. The kinetics of hydrolysis of compound IV in aqueous solutions at 60, 70, 80, and 90°C over the pH range 0.4–7.0 was investigated. The stability of compound IV was found to be dependent on pH. The pH-rate profile indicated specific acid catalyzed and spontaneous water catalyzed degradation. The ionic strength, effect, and thermodynamic parameters of the reaction were determined.

Keywords: HPLC, Ionic strength effects, Log k–pH profiles

Correspondence: Izabela Muszalska, Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań Poland. E-mail: imuszals@ump.edu.pl

INTRODUCTION

N-substituted derivatives of 4-alkoxy-6-methyl-1*H*-pyrrolo[3,4-c]pyridine-1,3(2*H*)-dione (Figure 1) exhibited potent analgesic activity which was superior to that of acetylsalicylic acid in the "*writhing syndrome*" and "*hot plate*" tests. What's more, the results of the "*writhing syndrome*" test indicate that the compounds I–IV were more potent than ASA ($ED_{50} = 39.15$ mg/kg) and morphine ($ED_{50} = 2.44$ mg/kg). Furthermore, most of the investigated imides suppressed significantly spontaneous locomotor activity in mice and prolonged barbiturate sleep of these animals. All these imides were not toxic ($LD_{50} > 2000$ mg/kg) and the results of the preliminary radioligand binding assay suggest that these compounds display a weak affinity (in micromolar concentration) to μ-opioid receptors. They probably play a role in the mechanism of action of these imides.[1,2] Introduction of trifluorophenyl or methoxy groups into the phenyl at N-4 of piperazine in the imide I weakened the analgesic properties both in the "*writhing syndrome*" and "*hot plate*" tests. A similar relationship was observed in the case of the compound III. The prolongation of the side-alkyl chain at the nitrogen atom to C_4 and elimination of the hydroxyl group in compound I decreased the analgesic activity. Imides I and III displayed analgesic properties in the "*writhing syndrome*" test up to the dose of 0.39 mg/kg ($ED_{50} = 0.4$ mg/kg) (I), 1.56 mg/kg ($ED_{50} = 1.4$ mg/kg) (III).[1] In this test, compounds II and IV showed weaker analgesic agents than imide I and they showed stronger analgesic activity than imide III ($ED_{50} = 1.03$ mg/kg (II); $ED_{50} = 0.67$ mg/kg (IV)).[2] In the "*hot plate*" test, I and III displayed analgesic activity up to the dose of 12.5 mg ($ED_{50} = 11.9$ mg/kg) (I), 25 mg/kg ($ED_{50} = 17.6$ mg/kg) (III).[1] Compounds II and IV were less active in this test than imide I, but the analgesic action of IV was almost comparable with that of substance III.[2] Among the Mannich bases, the compound containing 4-phenyl-1-piperazinylmethyl substituent at the nitrogen atom showed the strongest analgesic activity in both tests. Simultaneously, it was the most toxic compound ($LD_{50} = 180.1$ mg/kg). Introduction of the methoxy group in position ortho of phenyl gave non-toxic imides endowed with weaker analgesic properties in comparison with those of the parent



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

V

Figure 1. Chemical structures of 4-alkoxy-6-methyl-1*H*-pyrrolo[3,4-c]pyridine-1,3(2*H*)-dione compounds.

substance. Replacement of the N-substituted piperazinyl group by the 1,2,3,4-tetrahydroisoquinolinyl one (V) did not change the pharmacological profile ($ED_{50} = 12.70$ mg/kg in the "*writhing syndrome*" test and $ED_{50} = 15{,}4$ mg/kg in the "*hot plate*" test).[2]

These findings encouraged us to do a stability study in this group of compounds, in order to obtain further information concerning the structure/stability relationship. Our investigations were based on our previously kinetic studies on stability and degradation of compounds I–III[3–5] and the knowledge of other imides, such as barbituric acid derivatives[6–8] and thalidomide,[9–11] which showed that the hydrolysis of the imide bond by hydrogen and/or hydroxyl ions attack on the reactive forms is observed in aqueous solutions.

The literature indicates that many questions regarding the hydrolysis of derivatives of 4-alkoxy-6-methyl-1*H*-pyrrolo[3,4-c]pyridine-1,3(2*H*)-dione, such as mechanism, ionic strength effects, rate-pH profiles, have not been completely answered. This study develops an effective method (HPLC) for determination of compound IV and the influence study of the hydrogen ion and temperature on the stability, in order to establish the respective kinetic equations for the log k–pH profile.

EXPERIMENTAL

Materials

Compound IV, 4-methoxy-2-[3-[4-(2-methoxyphenyl)-1-piperazinyl)]-propyl]-6-methyl-1*H*-pyrrolo[3,4-c]pyridine-1,3(2*H*)-dione ($C_{23}H_{28}N_4O_4$; molecular mass 424.49; melting point 119°C–121°C) was synthesized and supplied by the Department of Chemistry of Drugs at Wrocław University of Medicine. Acetonitrile (Merck, Germany) was of an HPLC grade and other solvents or chemicals employed in this study were of an analytical reagent grade.

Apparatus

The high performance liquid chromatographic system (HPLC) was comprised of a Rheodyne 7120 20 μL fixed-loop injector, a LC3-UV detector (Pye Unicam, England), a L-6000 A pump (Merck-Hitachi, Germany), and an A/C transmitter. LiChrosorb® RP-8 packing was used as a stationary phase in the column of 250 × 4.0 mm I.D., 5 μm (Merck, Germany). The isocratic elution systems were developed utilizing a mobile phase consisting of acetonitrile–propan-2-ol–phosphate buffer pH 2 (0.01 mol/L) (30:30:40). The flow rate was 1.5 mL/min and the chromatograms were acquired at a wavelength of 240 nm. All analysis was carried out at laboratory temperature.

Kinetic Procedures

The solutions of hydrochloric acid and buffer were equilibrated at the temperature of the study prior to initiation of the reaction. The reaction flasks were maintained at constant temperature controlled within 0.1°C, at selected temperatures 60, 70, 80, and 90°C. Acidic solutions were prepared by dilution of a standardized hydrochloric acid with deionized water. To obtain the desired pH, the following solutions were used: hydrochloric acid (pH range 0.44 to 1.39, experimental concentration 0.05–0.50 mol/L), phosphate buffers (pH range 2.25 to 3.37, 0.1–0.4 mol/L), acetate buffer (pH range 3.92 to 5.94, 0.1–0.4 mol/L), and phosphate buffer (pH range 6.15 to 7.04, 0.10–0.35 mol/L). The effect of ionic strength (μ) on the reaction rate was checked by adding varying amounts of sodium chloride solution (4.0 mol/L) to the reaction flasks. All reactions run in hydrochloric acid and buffer solutions were maintained at a constant ionic strength of 0.50 mol/L. The reaction was initiated by adding 0.5 mL hydrochloric acid (0.1 mol/L) methanol stock solution of compound IV to 14.5 mL preheated solution to a final concentration of 0.36 mg/mL. When necessary, 2 mL of the obtained solutions were transferred into 5 mL ampoules and sealed. Samples were withdrawn at suitable time intervals and cooled to room temperature. The analyzed solutions consisting of 1.0 mL sample, 1.0 mL of i.s. (0.30 mg/mL), and 1.0 mL of water were injected directly onto the column for HPLC analysis.

pH Measurements

The pH values of the hydrochloric acid at the temperature of the study were calculated from the activity coefficient data in literature.[12] The pH values of the buffer solutions were measured directly at the temperature of study on a CD-401 pH meter (Elmetron, Poland), which was standardized with standard buffer solutions.

RESULTS AND DISCUSSION

HPLC Determination

High performance chromatography, especially with reversed phase, is a form of chromatography used frequently in analytical chemistry. The separation was achieved using the stationary phase LiChrosorb® RP-8 and the mixture of acetonitrile–propan-2-ol–phosphate buffer pH 2 (0.01 mol/L) (30:30:40) as the mobile phase. Retention times were: ca. 2.3 min for i.s. (4-hydroxybenzoic acid ethyl ester (100 μg/mL)), ca 7.3 min for IV (120 μg/mL), and ca. 1.8 and 3.9 for the products of degradation (Figure 2). The repeatability of

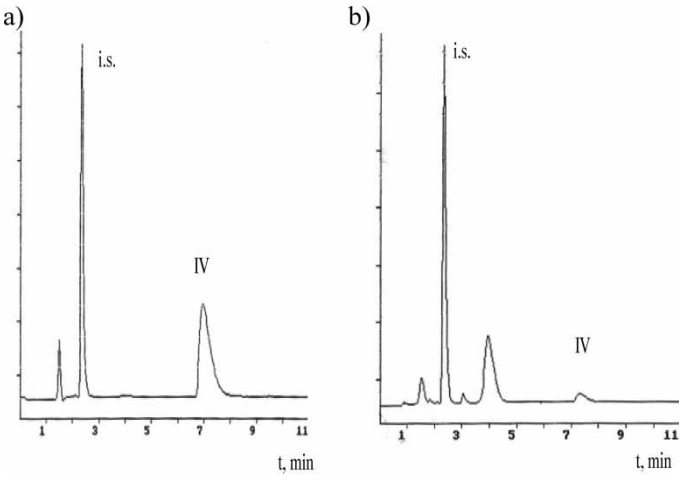


Figure 2. Typical chromatogram of compound IV, its degradation products and i.s. after hydrolysis in HCl (0.50 mol/L, 80°C); a) after 30 min, b) after 5 h.

retention times was determined from 8 injections of the standard solution (120 μg/mL), and it was under 1.1%. The calibration line was constructed by plotting the peak area as a function of standard concentration values in the range of 10.1 μg/mL to 204.0 μg/mL (10 concentrations, each were measured in triplicate) in the mobile phase. To the investigated solutions, the internal standard solution was added at the concentration 100 μg/mL. The linearity was calculated by the least squares method ($y = ax$, because values of intercept (b) were statistically insignificant), giving a correlation coefficient $r^2 > 0.99$. The obtained linear regression equation was: $y = (0.008928 \pm 0.000510)x$. Precision was determined from the analysis of 9 replicate injections of standard solutions containing compound IV dissolved in the mobile phase at the concentration: 64.0 μg/mL, 250 μg/mL, or 300 μg/mL in the mixture with the internal standard solution at the concentration 200 μg/mL (1:1). The results concerning precision and accuracy of the developed method are given in Table 1. RSDs were lower than 1.01%. The validation parameters were satisfactory, so the proposed HPLC method can be used for the quantitative or the stability study of compound IV.

Rate Constants

The kinetic parameters describing the hydrolysis of the compound IV were determined by following the loss of a peak area (p) as a function of time.

Table 1. Accuracy and precision of the measurement of compound IV in the mobile phase (n = 9)

Day of analysis	Nominal concentration (μg/mL)	Measured concentration (μg/mL)	RSD (%)	Accuracy (%)
Day 1	64.0	63.8 ± 0.4	0.92	99.7
	250.0	249.9 ± 1.0	0.54	99.9
	300.0	300.4 ± 1.0	0.45	100.1
Day 2	64.0	64.5 ± 0.8	1.01	100.8
	250.0	248.2 ± 1.1	0.73	99.3
	300.0	298.7 ± 0.9	0.94	99.6

Plots of $\ln(p_t/p_i)$ *versus* time were reasonably linear in the pH region 0.44 to 4.53 and 6.15 to 7.04 (Figure 3) according to the first-order expression:

$$\ln\left(\frac{p_t}{p_i}\right) = \ln\left(\frac{p_0}{p_i}\right) - k \cdot t \tag{1}$$

where p_0, p_t are the peak area of IV at zero and time t, respectively; p_i is the peak area of i.s. and k is the apparent first order rate constant. 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

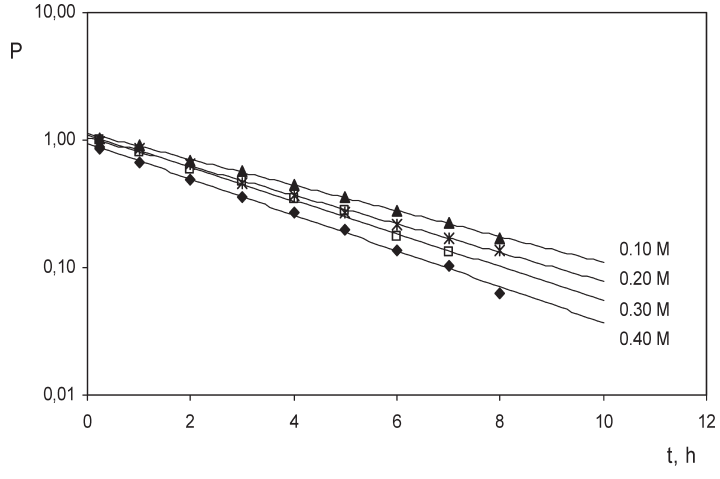


Figure 3. Apparent first-order plots for degradation of compound IV (phosphate buffer pH 3.37, 0.10–0.40 mol/L, μ 0.50 mol/L, 90°C).

acid base catalysis is represented by the equation:

$$k = k_{pH} + k_B[B]_T \quad (2)$$

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 (Table 2). The significant buffer catalysis in the hydrolysis of IV was observed in the phosphate buffers and was not observed in the acetate buffers, where the hydrolysis consisted of two stages (Figure 4), but not in the whole pH range. In this medium, the mechanism of degradation depended on the pH value and the temperature. That is, a complete degradation was observed as the first order reaction with a linear dependence log $(p_t/p_{i.s.})$ as a function of the time, in the solutions of IV at pH 3.9. Whereas, at pH 4.5–5.9 only the reversible reaction between IV and a

Table 2. Rate constants k_{pH} i k_B for hydrolysis of IV in hydrochloric acid and phoshate buffers (μ 0.50 mol/L)

	Hydrochloric acid		Phosphate buffer		
Temperature (°C)	pH	k_{pH} (s^{-1})	pH	k_{pH} (s^{-1})	k_B ($mol^{-1} \cdot L \cdot s^{-1}$)
60	0.44	$1.46 \cdot 10^{-5}$	2.25	$2.57 \cdot 10^{-6}$	$8.91 \cdot 10^{-7}$
	0.54	$1.17 \cdot 10^{-5}$	2.73	$2.86 \cdot 10^{-6}$	$2.17 \cdot 10^{-6}$
	0.66	$1.04 \cdot 10^{-5}$	3.06	$3.71 \cdot 10^{-6}$	$2.72 \cdot 10^{-7}$
	0.83	$7.18 \cdot 10^{-6}$	3.39	$4.73 \cdot 10^{-6}$	$1.55 \cdot 10^{-6}$
	1.11	$4.92 \cdot 10^{-6}$	6.30	$5.84 \cdot 10^{-4}$	$1.68 \cdot 10^{-3}$
	1.39	$3.68 \cdot 10^{-6}$	6.99	$1.69 \cdot 10^{-3}$	$6.68 \cdot 10^{-3}$
70	0.45	$5.10 \cdot 10^{-5}$	2.26	$6.40 \cdot 10^{-6}$	$5.71 \cdot 10^{-6}$
	0.54	$3.66 \cdot 10^{-5}$	2.72	$7.85 \cdot 10^{-6}$	$5.55 \cdot 10^{-6}$
	0.66	$2.98 \cdot 10^{-5}$	3.06	$7.79 \cdot 10^{-6}$	$1.03 \cdot 10^{-5}$
	0.83	$2.13 \cdot 10^{-5}$	3.37	$8.89 \cdot 10^{-6}$	$4.10 \cdot 10^{-6}$
	1.11	$1.38 \cdot 10^{-5}$	6.25	$1.17 \cdot 10^{-3}$	$4.57 \cdot 10^{-3}$
	1.39	$1.04 \cdot 10^{-5}$	7.04	$4.34 \cdot 10^{-3}$	$1.27 \cdot 10^{-2}$
80	0.46	$1.33 \cdot 10^{-4}$	2.27	$1.66 \cdot 10^{-5}$	$3.40 \cdot 10^{-5}$
	0.55	$1.14 \cdot 10^{-4}$	2.70	$2.13 \cdot 10^{-5}$	$1.06 \cdot 10^{-5}$
	0.66	$8.97 \cdot 10^{-5}$	3.07	$2.58 \cdot 10^{-5}$	$1.35 \cdot 10^{-5}$
	0.84	$7.04 \cdot 10^{-5}$	3.36	$2.27 \cdot 10^{-5}$	$3.13 \cdot 10^{-5}$
	1.11	$4.06 \cdot 10^{-5}$	6.63	$7.02 \cdot 10^{-3}$	$2.51 \cdot 10^{-2}$
	1.39	$2.88 \cdot 10^{-5}$			
90	0.46	$3.49 \cdot 10^{-4}$	2.27	$4.36 \cdot 10^{-5}$	$3.33 \cdot 10^{-5}$
	0.56	$2.78 \cdot 10^{-4}$	2.71	$5.20 \cdot 10^{-5}$	$3.85 \cdot 10^{-5}$
	0.67	$2.16 \cdot 10^{-4}$	3.08	$5.97 \cdot 10^{-5}$	$5.66 \cdot 10^{-5}$
	0.84	$1.53 \cdot 10^{-4}$	3.37	$5.56 \cdot 10^{-5}$	$8.73 \cdot 10^{-5}$
	1.11	$1.07 \cdot 10^{-4}$	6.29	$5.81 \cdot 10^{-3}$	$2.06 \cdot 10^{-2}$
	1.40	$7.22 \cdot 10^{-5}$	6.92	$1.65 \cdot 10^{-2}$	$3.19 \cdot 10^{-2}$

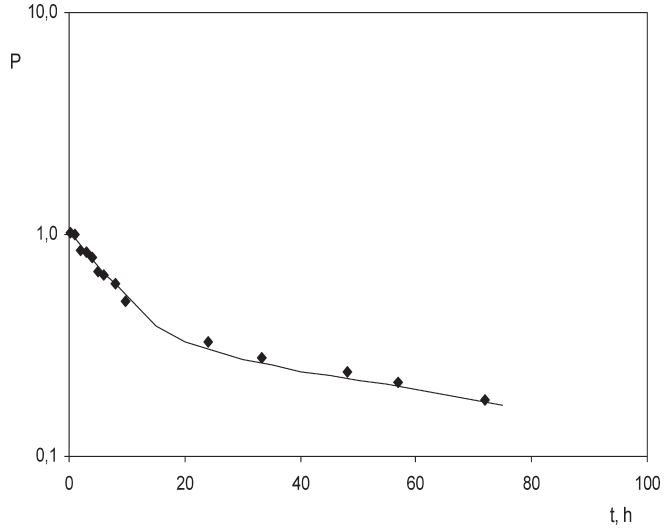


Figure 4. Apparent first-order plots for degradation of compound IV (acetate buffer pH 4.53, 0.40 mol/L, μ 0.50 mol/L, 60°C).

degradation product (A) was performed 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) (Table 3) were calculated according the following equations:

$$\ln(p_t/p_i) = \ln(p_0/p_i) - k_2 \cdot t$$
(for p_t/p_i observed in the second stage) (3)

$$\ln(P - P')_t = \ln(P - P')_0 - k \cdot t$$
(for p_t/p_i observed in the first stage) (4)

or

$$\ln(P_t - P_\infty) = \ln(P_0 - P_\infty) - k \cdot t \quad (5)$$

$$k = k_1 + k_{-1} \quad (6)$$

$$K = k_{-1}/k_1 = c_{IVe}/c_{IVA,e} \quad (7)$$

$$k_{pH} = k_1 + k_{-1} + k_2 \quad (8)$$

where $P = p_t/p_i$, P' values were carried out by using Eq. (3) for p_t/p_i observed in the first stage, $c_{IV,e}$ and $c_{IVA,e}$ are the concentration of IV and a product (A, t_R 3.9 min) 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 IV and its product (A) [for IV: $k = (5.10 \pm 0.75) \cdot 10^{-3}$, s^{-1}; $k_1 = 5.09 \cdot 10^{-3}$, s^{-1}; $k_{-1} = 8.65 \cdot 10^{-5}$,

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 IV in acetate buffers (μ 0,50 mol/L)

Temp. (°C)	pH	K	k (s^{-1})	$k_{1,\ pH}$ (s^{-1})	$k_{-1,\ pH}$ (s^{-1})	$k_{2,\ pH}$ (s^{-1})	$k_{,\ pH}$ (s^{-1})
60	4.93	0.3514	$6.67 \cdot 10^{-5}$	$4.93 \cdot 10^{-5}$	$1.73 \cdot 10^{-5}$	$2.77 \cdot 10^{-6}$	$6.94 \cdot 10^{-5}$
	5.41	0.08924	$2.10 \cdot 10^{-4}$	$1.93 \cdot 10^{-4}$	$1.72 \cdot 10^{-5}$	$3.93 \cdot 10^{-6}$	$2.14 \cdot 10^{-4}$
	5.94	0.03266	$4.15 \cdot 10^{-4}$	$4.05 \cdot 10^{-4}$	$9.73 \cdot 10^{-6}$		$4.15 \cdot 10^{-4}$
70	3.94	—	—	—	—	—	$2.31 \cdot 10^{-5}$
	4.52	1.6935	$1.10 \cdot 10^{-4}$	$4.08 \cdot 10^{-5}$	$6.92 \cdot 10^{-5}$	$2.55 \cdot 10^{-5}$	$1.35 \cdot 10^{-4}$
	4.92	0.4762	$1.64 \cdot 10^{-4}$	$1.11 \cdot 10^{-4}$	$5.29 \cdot 10^{-5}$	$2.18 \cdot 10^{-5}$	$1.86 \cdot 10^{-4}$
	5.40	0.1054	$4.73 \cdot 10^{-4}$	$4.28 \cdot 10^{-4}$	$4.51 \cdot 10^{-5}$	$3.91 \cdot 10^{-5}$	$5.12 \cdot 10^{-4}$
	5.93	0.02169	$8.73 \cdot 10^{-4}$	$8.54 \cdot 10^{-4}$	$1.85 \cdot 10^{-5}$	—	$8.73 \cdot 10^{-4}$
80	3.93	—	—	—	—	—	$8.25 \cdot 10^{-5}$
	4.51	1.3324	$2.40 \cdot 10^{-4}$	$1.03 \cdot 10^{-4}$	$1.37 \cdot 10^{-4}$	$5.62 \cdot 10^{-5}$	$2.96 \cdot 10^{-4}$
	4.91	0.4528	$4.02 \cdot 10^{-4}$	$2.77 \cdot 10^{-4}$	$1.25 \cdot 10^{-4}$	$4.96 \cdot 10^{-5}$	$4.52 \cdot 10^{-4}$
	5.39	0.1162	$1.26 \cdot 10^{-3}$	$1.13 \cdot 10^{-3}$	$1.31 \cdot 10^{-4}$	$7.48 \cdot 10^{-5}$	$1.33 \cdot 10^{-3}$
	5.92	0.02042	$2.18 \cdot 10^{-3}$	$2.14 \cdot 10^{-3}$	$4.36 \cdot 10^{-5}$	—	$2.18 \cdot 10^{-3}$
90	3.92	—	—	—	—	—	$1.06 \cdot 10^{-4}$
	4.90	0.4328	$8.86 \cdot 10^{-4}$	$6.18 \cdot 10^{-4}$	$2.68 \cdot 10^{-4}$	$7.26 \cdot 10^{-5}$	$9.59 \cdot 10^{-4}$
	5.38	0.1281	$2.36 \cdot 10^{-3}$	$2.09 \cdot 10^{-3}$	$2.68 \cdot 10^{-4}$	$1.15 \cdot 10^{-4}$	$2.47 \cdot 10^{-3}$
	5.91	0.01727	$5.10 \cdot 10^{-3}$	$5.09 \cdot 10^{-3}$	$8.65 \cdot 10^{-5}$	—	$5.10 \cdot 10^{-3}$

$s^{-1}$and for A: $k = (5.08 \pm 0.51) \cdot 10^{-3}, s^{-1}$] did not show any significant statistical differences in the acetate buffer (pH 5.94, μ 0.50 mol/L, 90°C).

Ionic Strength Effects

Variations in the apparent first order rate constants with ionic strength changes were observed for the hydrolysis of compound IV at pH 1.39 (HCl 0.05 mol/L, 80°C) where the protonated species of compound IV would exist. The dependences log k as a function of $(\sqrt{\mu}/(1+\sqrt{\mu}))$ were described by the Brönstedt-Bjerrum equation:

$$\log k = \log k_0 + 2QZ_AZ_B\left(\frac{\sqrt{\mu}}{(1+\sqrt{\mu})}\right) \tag{9}$$

At pH 1.39, the plot had significant positive slope of 0.995 ± 0.167 and equal to $2QZ_AZ_B$, where Q is a constant for the solvent at a given temperature. The value of 2Q at 80°C is 1.145.[13]

The Log k–pH Profiles

The rate pH profiles for the hydrolysis of compound IV were constructed from the logarithm of rate constants k_{pH} and the pH values at 60, 70, 80, and 90°C. The profiles were similar for all temperatures (Figure 5). The shape of these curves indicates hydrogen ion catalyzed degradation of the protonated species and water attack on both, protonated and neutral species of IV. Accordingly, the overall reaction of hydrolysis can be defined as:

$$k_{pH} = k_{H^+} \cdot a_{H^+} \cdot f_1 + k_{H_2O} \cdot f_1 + k'_{H_2O} \cdot f_2 \tag{10}$$

or, as its kinetic equivalent

$$k_{pH} = k_{H^+} \cdot a_{H^+} \cdot f_1 + k_{H_2O} \cdot f_1 + k_{OH^-} \cdot a_{OH^-} \cdot f_1 \tag{11}$$

where k_{H^+}, k_{OH^-} are the bimolecular rate constans for hydrogen or hydroxyl ion attack on the protonated (f_1) form of the compound IV and k_{H_2O}, k'_{H_2O} are the first order rate constants for water attack on the protonated (f_1) and neutral (f_2) forms, respectively, a_{H^+}, a_{OH^-} refer to the hydrogen and hydroxide ion activity. The pK_a value (7.1) of IV was determined based on the potentiometric titration at room temperature (pK'_a 6.8, 6.7, 6.5, and 6.4 at 60, 70, 80, and 90°C, respectively). At the pH range 2.70 to 7.04, the plots of the logarithm (p_t/p_i) as a function of pH had significant positive slopes of 0.64 at all temperatures. The specific rate constants determined are given in Table 4.

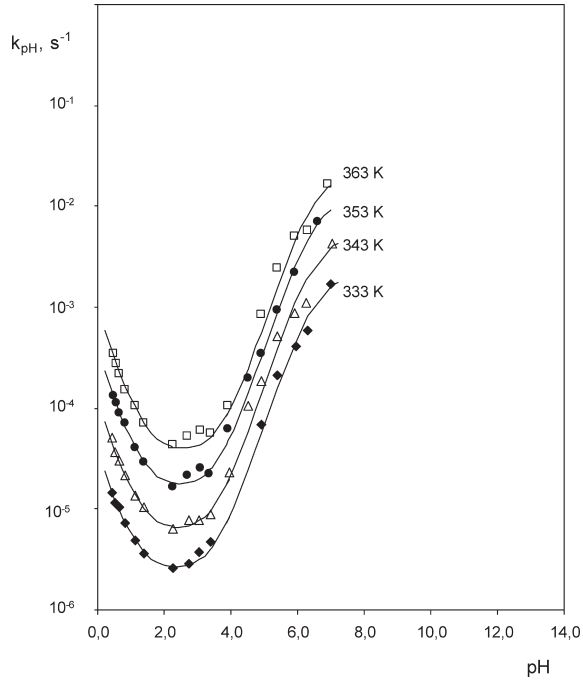


Figure 5. The log k–pH profiles for the hydrolysis of compound IV at 90, 80, 70, and 60°C. The circles represent the experimentally determined values. The lines represent the theoretical curves drawn from Eq. (10).

Dependence of Rate on Temperature

The Arrhenius parameters for the hydrolysis of compound IV were calculated from the slops and intercepts of plots of the logarithm of the specific rate

Table 4. Specific rate constants and thermodynamic parameters (20°C) from the hydrolysis of compound IV

Temp. °C	10^5 ($k_{H^+} \pm \Delta k_{H^+}$)	10^6 ($k_{H_2O} \pm \Delta k_{H_2O}$)	10^3 ($k'_{H_2O} \pm \Delta k'_{H_2O}$)
60	3.37 ± 0.28	2.36 ± 0.57	2.73 ± 0.21
70	10.75 ± 0.44	5.76 ± 0.71	6.14 ± 0.09
80	34.46 ± 2.24	15.52 ± 4.44	12.00 ± 0.96
90	88.32 ± 5.79	34.89 ± 11.37	21.31 ± 3.24
E_a, kJ/mol	110 ± 9	91.2 ± 8.7	68.9 ± 10.6
$\Delta H^{\neq}$, kJ/mol	108 ± 9	88.7 ± 8.7	66.4 ± 10.6
$\Delta S^{\neq}$, J/K/mol	0.581 ± 26.772	−78.9 ± 25.1	−86.7 ± 17.3

k_{H^+}, $mol^{-1} \cdot L \cdot s^{-1}$; $k_{H_2}O$, $k'_{H_2}O$, s^{-1}.

constants (k_{H^+}, k_{H_2O}, k'_{H_2O}) *versus* the reciprocal of the absolute temperature (T), in accordance with the expression:

$$\ln k_i = \ln A - \frac{E_a}{(R \cdot T)} \tag{12}$$

where A is the frequency coefficient and R is the gas constant (8.3144, $J \cdot K \cdot mol^{-1}$). These values were used to determine energy of activation (E_a), enthalpy ($\Delta H^{\neq}$), and entropy ($\Delta S^{\neq}$) at 20°C (Table 4), which was calculated with the following equations:

$$\Delta H^{\neq} = E_a - RT \tag{13}$$

$$\Delta S^{\neq} = R\left(\ln A - \ln\left(\bar{k} \cdot \frac{T}{h}\right)\right) \tag{14}$$

where $\bar{k}$ is the Boltzman constant ($1.3805 \cdot 10^{-23}$, $J \cdot K^{-1}$) and h is the Planck constant ($6.6256 \cdot 10^{-34}$, $J \cdot s^{-1}$).

CONCLUSION

The results obtained here show that the maximal stability of IV is observed at pH ca. 2.4 and that degradation is subject to specific and general acid base catalysis. In summary, this study provides new fundamental data on the stability of a new analgesic active compound (IV – pyrrolo[3,4-c]pyridine derivative) in aqueous solutions. The results show that at the pH region 0.44 to 7.04 the hydrogen ions and water have the main influence on its degradation by ring opening hydrolysis (an imide functional group). The ionic strength effect increases the rate of compound IV degradation by the attack of a hydrogen ion on the protonated species, in the acidic hydrolysis. Our previously kinetic studies, and the data obtained in this work, suggested that the lipophilicity coefficients (log P) of studied derivatives can have the main influence on their stability in solutions.

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