Acta Poloniae Pharmaceutica – Drug Research, Vol. 60 No. 4 pp. 247–252, 2003

ISSN 0001-6837
Polish Pharmaceutical Society

HYDROLYSIS OF N– AND S–MONOMETHYL DERIVATIVES OF 2–THIOPHENOBARBITAL

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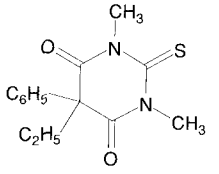
Abstract: Kinetics of hydrolysis of S–methyl–2–thiophenobarbital in aqueous solutions was investigated using the UV spectroscopic method within the pH range 1.5–12.9 at 60°C. Chromatography was used to separate and isolate the products of hydrolysis of this compound and its N–methyl isomer. The products were identified by spectroscopic methods and the course of hydrolysis of both isomers were compared.

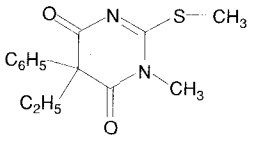
Keywords: N– and S–methyl–2–thiophenobarbital hydrolysis, kinetic parameters, hydrolysis products, stability

Kinetic investigations on the hydrolysis of 2–thiobarbituric acid derivatives are less frequent than for their 2–oxo analogs although some drugs of this class, such as thiopental (5–ethyl–5–(1–methylbutyl)–2–thiobarbituric acid) and thiamylal (5–allyl–5–(1–methylbutyl)–2–thiobarbituric acid), are still in use as intravenous anesthetics. The results of investigations of kinetics of hydrolysis of both these compounds (1, 2), as well as 5,5–diethyl–2–thiobarbituric acid (3) suggested the similarity of the process with that for 2–oxo analogs with the difference of the magnitude of kinetic parameters. Thus, the log k/pH profiles indicated hydroxyl ion–catalyzed degradation of the non–ionized and mono–ionized species (or kinetically equivalent water attack on the mono– and di–ionized species), whereas the apparent first–order rate constants were higher for the 2–thiobarbiturates.

Since the hydrolysis of three–substituted 2–thiobarbiturates was not investigated in detail we were interested in the effect of N–methyl and S–methyl substitution on the hydrolysis of 5–ethyl–5–phenyl–2–thiophenobarbital. The kinetics of hydrolysis of N–methyl derivative (**I**) was already reported (4) and was found to represent the same kinetic model as mentioned above. Here, we present investigations on kinetics of isomeric S–methyl derivative (**II**) and isolation and identification of several products of hydrolysis of both isomers.



I



II

EXPERIMENTAL

Monomethyl derivatives of 2–thiophenobarbital were obtained according to (5). Kinetics of hydrolysis of **II** was investigated in buffer solutions (Table 1) within the pH range 1.5–12.9 at a concentration of 4×10^{-5} mole/dm^3. UV spectrophotometric measurements were made with a Gilford–Response II (England) apparatus in thermostated stoppered quarz cuvettes and pH was measured on a microcomputer pH–meter CP–315M (Elmetron, Zabrze) with a combined electrode ESAgP–301W (Eurosensor, Gliwice) calibrated against standard buffers (pH=4.0, 7.0 and 10.0 – POCh, Gliwice).

The course of hydrolysis of 1% ethanol–buffer solutions of **I** (at pH = 10, at 30°C) and **II** (at pH 12.9 and 3.25, at 60°C) was followed by TLC using aluminium sheets 20 × 20 cm with silica gel 60 F_{254} (Merck) and the following mobile phases: a) n–hexane : ethanol : triethylamine (7:1:, v/v/v), b) chloroform: 25% ammonia (100:0,5, v/v), and c) chloroform : isopropanol (2:1, v/v) for **I** and d), chloroform : acetone (4:0.5, v/v) and e), chloroform : toluene : acetone (4.5:3:1, v/v/v) for **II**. Visualization – UV light 254 nm (HA–05 lamp – Haland, Warszawa), iodine vapors and 0.04% ethanol solution of bromocresol green. For identification of hydrolysis products, after 24 h the solutions degraded in flasks heated in a thermostated oil bath were evaporated to dryness, the residues were dissolved in methylene chloride and the products of hydrolysis were separated by column chromatography (silica gel 40, Merck, chloroform), circular chromatography (Model 8924 Chromatotron, Harrison Research, USA, silica gel 60 PF_{254}, Merck, mobile phases as for

Table 1. Values of experimental rate constants (k) for hydrolysis of **II** at different pH values

Buffer [9]	pH*	k × 10^{-5} [s^{-1}]	log k
Chloride acc. to	1.50	800	–2.097
Clark and Lubs	2.00	300	–2.523
Universal acc. to	2.52	86.940	–3.061
Britton and Robinson	3.15	26.120	–3.583
	3.41	8.217	–4.085
	3.71	4.630	–4.334
	4.00	3.655	–4.437
	4.50	1.834	–4.737
	4.95	1.370	–4.863
Phosphate acc. to			
Michaelis	5.20	0.854	–5.069
Phosphate acc. to	6.00	0.715	–5.145
Clark and Lubs	7.00	0.857	–5.067
Borate acc. to	8.00	0.795	–5.100
Bates and Bower	8.65	0.827	–5.082
	9.00	0.727	–5.138
	10.00	0.723	–5.141
Phosphate acc. to	11.00	0.897	–5.047
Bates and Bower	11.16	0.841	–5.075
	11.78	1.568	–4.805
	12.00	2.311	–4.636
Hydroxide acc. to	12.58	3.083	–4.511
Bates and Bower	12.70	3.987	–4.399
	12.80	4.370	–4.360
	12.90	6.281	–4.202

* experimental pH measured at 60°C

TLC) and HPLC/MS [LCQ/MS Finnigan MAT with Ion Trap, USA, column: Purospher RP18, 125 mm, 3 mm ID, 5 μm, precolumn: LichroCART 4 × 4 mm, with Lichrospher 60, 5 μm (Merck), gradient elution with A: 0.1% aqueous trifluoroacetic acid and B: 10% of A + 90% acetonitrile (programmed flow: 5% A + 95% B for 2 min., with 30 min increase to 70% A + 30 B and after 2 min decrease to 55% A + 95% B for 8 min, flow rate 0.4 ml/min, UV detection at 280 nm and MS detection with chemical ionization at atmospheric pressure]. The identification of the main products of hydrolysis was based on their IR (IFS 48 Bruker apparatus, Germany, in KBr discs), ^{1}H–NMR (Varian Mercury VX 300 MHz apparatus, USA, $CDCl_3$) and MS (Finnigan MAT 95S, USA, ionization energy 70 eV) spectra. m.p.'s were uncorrected and determined in glass capillaries on a Mel–Temp apparatus (Laboratory Devices Inc., USA). Calculations were performed using the program Quick Statistica PL. (Statsoft, Poland).

RESULTS

Linear absorbance–concentration relationship was checked in the $1 \times 10^{-5} - 5 \times 10^{-5}$ M range for buffer (pH = 10.0) and ethanol solutions of **II** (Figure 1), yielding linear correlation coefficients of 0.998 and 1.000, respectively.

Absorbance changes of **II** in time (Figure 2) at different pH values were linear (Figure 3) indicating pseudo–first order reaction. The rate constants (k) were calculated from the equation:

$$\log (A - A\infty) = \log (A_o - A\infty) - kt/2.303$$

where A_o is the absorbance at time t = 0; $A\infty$ is the final (residual) absorbance and A is the absorbance at time = t.

The results are summarized in Table 1 and the log k/pH profile is shown in Figure 4. The rate

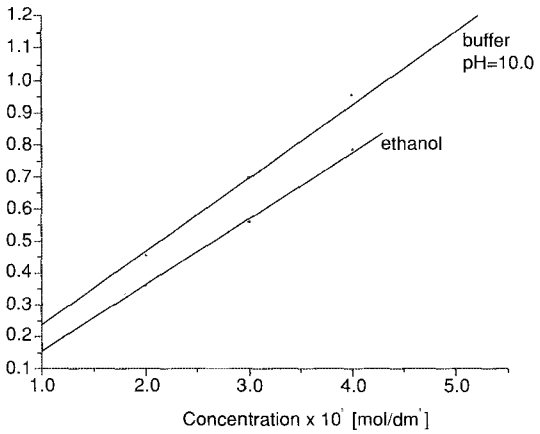


Figure 1. Absorbance–concentration relationship for compound **II**.

Table 2. Values of experimental rate constants (k) for hydrolysis of **II** at different temperatures

Temp. [K]	1/T × 10^{3} [1/K]	k × 10^{-6} [s^{-1}]	log k
318	3.145	2.291	–5.640
323	3.096	2.903	–5.537
328	3.049	4.445	–5.352
333	3.003	7.230	–5.141
338	2.959	1.134	–4.945

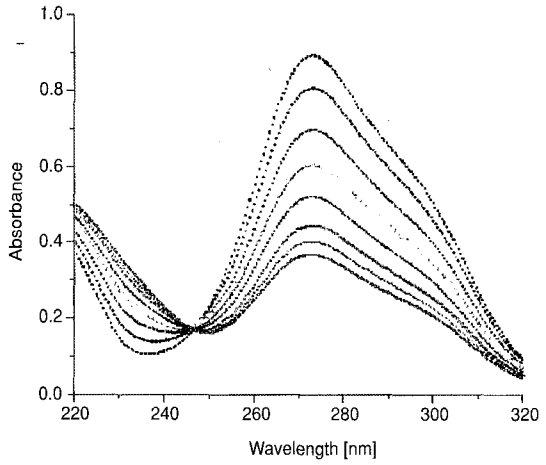


Figure 2. Spectral changes for hydrolysis of **II**.

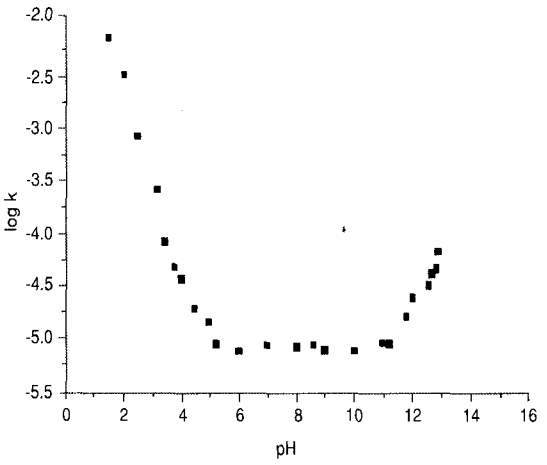


Figure 4. The log k/pH profile for hydrolytic degradation of **II** at 60°C.

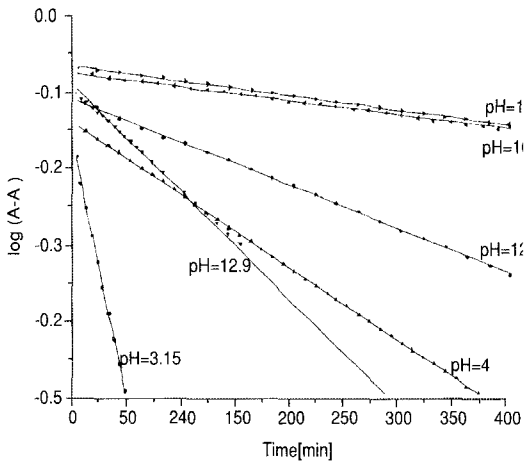


Figure 3. Apparent first-order plots for hydrolysis of **II** at different pH values at 60°C.

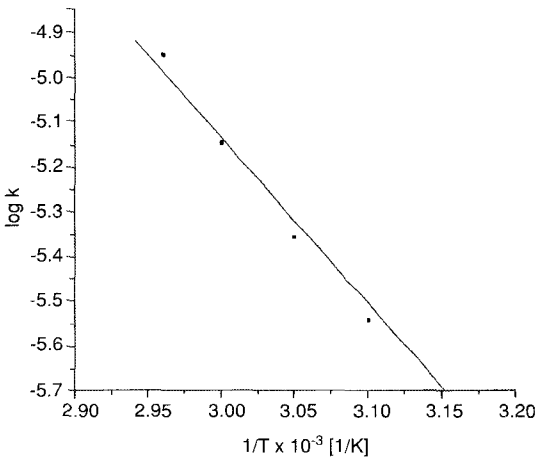


Figure 5. The Arrhenius plot for hydrolysis of **II** in borate buffer pH = 10.0.

constants at different temperatures (45–65°C) are presented in Table 2. The linear relationship log $k = f(1/T)$ is shown in Figure 5 and from Arrhenius equation the activation energy for hydrolysis of **II** at pH=10.0 was calculated yielding Ea = 73.35 kJ/mole.

The spectrophotometric pKa value for **II** calculated from the Henderson–Hasselbalch equation (10) was 5.85 ± 0.29 at 25°C.

The results of TLC monitoring of hydrolysis course of **I** and **II** are shown in Figures 6 and 7.

The spectroscopic data for identified main products of hydrolysis of **I** (compounds **III** and **IV** – Figure 8) and **II** (compounds **V** and **VI** – Figure 8) are presented in Table 3. Additionally, among the products of hydrolysis of **I** N-methylthiourea was separated and identified by m.p. 119–121°C, comparison of TLC results (Rf = 0.12 and 0.68 in the solvent systems b and c, respectively) and m.p. of the mixture (no depression) with an authentic specimen (Fluka).

DISCUSSION

From the results it follows that the kinetics of hydrolysis of **II** is a pseudo-first order reaction and in this respect is similar to the hydrolysis of **I** investigated earlier (4). However, there is a distinct difference in the rate of hydrolysis of both reactions in alkaline medium. For instance, the calculated, from the Arrhenius equation, pseudo-first order rate constant at pH 10.0 at 25°C for hydrolysis of **II** is 3.21×10^{-7} s^{-1} (4). meaning that under these conditions **I** is more susceptible to hydrolysis. The opposite is true for acidic region where the unionized form of **II** is hydrolyzed with evident H^+ ion catalysis – a feature rather uncom-

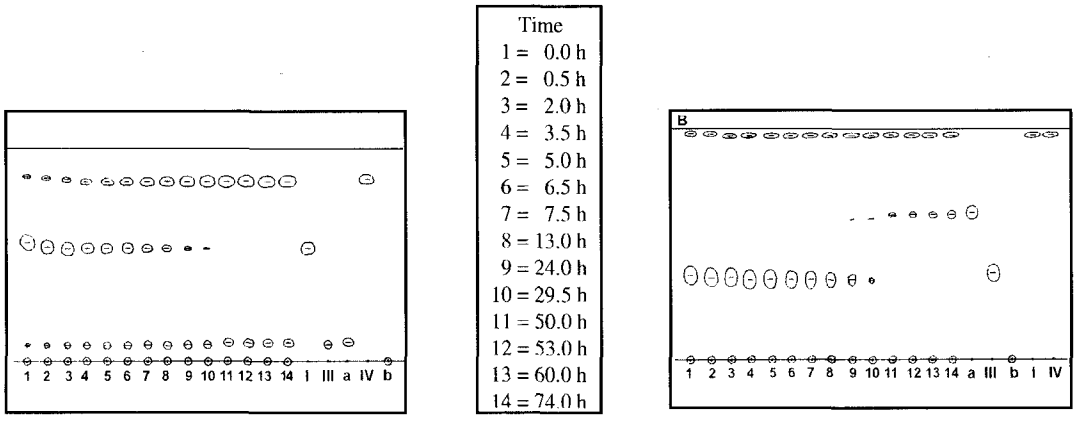


Figure 6. TLC monitoring of hydrolysis of **I** (pH = 10, 30°C). A – mobile phase: n–hexane : ethanol : triethylamine (7:1:1, v/v/v), B – mobile phase: chloroform : 25% ammonia (100:0.5, v/v), **I**, **II**, **IV** – standards of compounds designed as in text, a – N–methylthiourea, b – borate buffer pH 10,0.

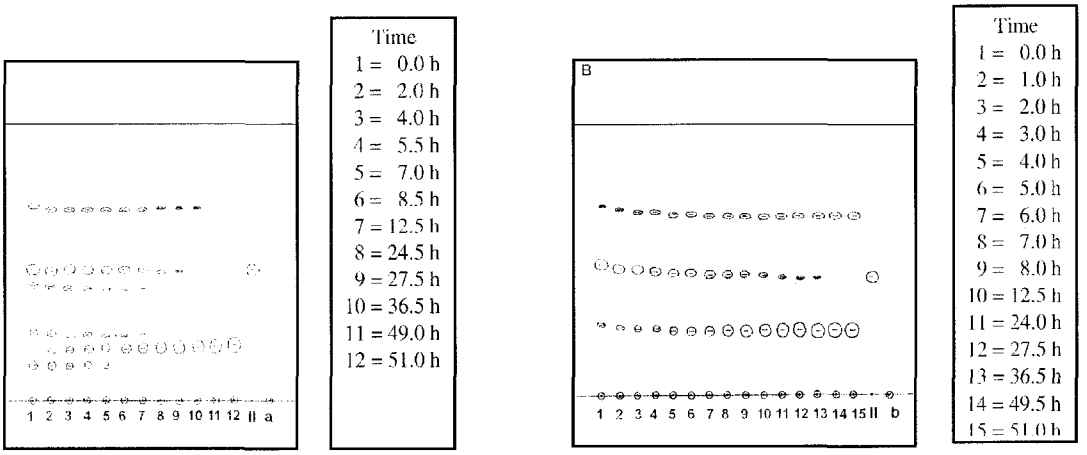


Figure 7. TLC monitoring hydrolysis of **II** in acid (A – universal buffer pH = 3.25) and alkaline (B – hydroxide buffer pH = 12.9) medium at 60°C. Mobile phase: chloroform : toluene : acetone (4.5:3:1, v/v/v). Standard of **II** and respective buffers (a, b) applied for comparisons.

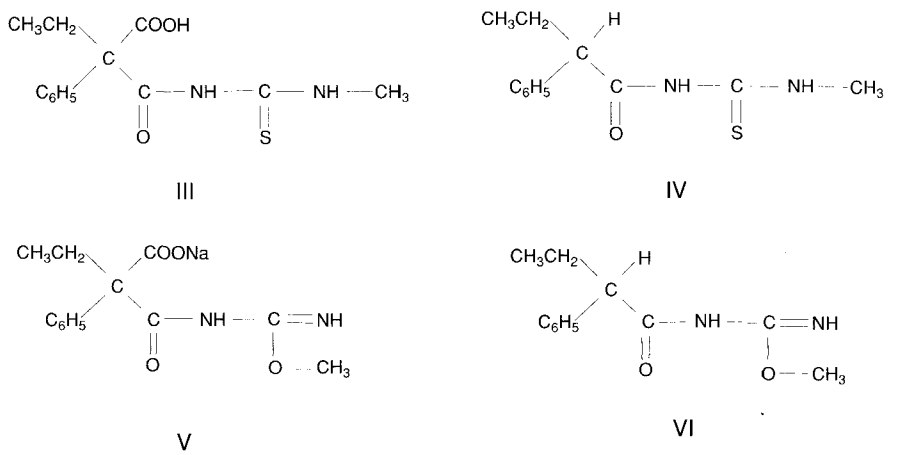


Figure 8. Structures of main products of hydrolysis of **I** (**III** and **IV**) and **II** (**V** and **VI**).

Table 3. Spectroscopic data for degradation products of compound **I** and **II**

	H^1 NMR ($CDCl_3$) [ppm]	MS (%)	IR (KBr) [cm^{-1}]
Compound III		280 (2) M^+	
		235 (100) $[C_{12}H_{15}N_2OS]^+$	
		162 (20) $[C_{10}H_{12}NO]^+$	
		117 (20) $[C_8H_5O]^+$	
		91 (3) $[C_7H_7]^+$	
Compound IV	0.896 (t, 3H, J = 7.5, CH_3–CH_2–)	236 (61) M^+	3237, 3161 N–H
	1.774–1.889 (m, 1H, CH_3–CH_2–)	146 (100) $[C_{10}H_{10}O]^+$	
	2.068–2.211 (m, 1H, CH_3–CH_2–)	118 (13) $[C_9H_{10}]^+$	3049 C–H aromat.
	3.157 (s, 3H, CH_3–NH–)	91 (57) $[C_7H_7]^+$	2967 C–H aliph.
	3.302 (t, 1H, J = 7.5, C_2H_5–CH–C_6H_5)		1681 C=O
	7.240–7.384 (m, 5H, –C_6H_5)		1567, 1518 N–H
	8.545 (s, 1H, –NH–CH_3)		
	10.457 (s, 1H, –CO–NH–CS–)		1415 C–H aliph.
			1359, 1262 C–C
			1205 C–N
			1169 C=S
Compound V		286 (2.2) M^+	
		287 (3.2) $[C_{13}H_{16}N_2O_4Na]^+$	
		263 (2) $[C_{13}H_{15}N_2O_4]^+$	
		221 (5) $[C_{12}H_{17}N_2O_2S]^+$	
		220 (1.2) $[C_{12}H_{16}N_2O_2S]^+$	
		219 (3.4) $[C_{12}H_{15}N_2O_2S]^+$	
		164 (3.3) $[C_{10}H_{13}NO]^+$	
		162 (1.2) $[C_{10}H_{12}NO]^+$	
		119 (1.7) $[C_9H_{11}]^+$	
		91 (2.4) $[C_7H_7]^+$	
Compound VI	1.004 (t, 3H, J = 7.5, CH_3–CH_2–)	220 (12) M^+	
	2.077–2.188 (m, 2H, CH_3–CH_2–)	219 (5) M^+–H	
	3.485 (t, 1H, J = 7.5, C_2H_5–CH–C_6H_5)	164 (3.3) $[C_{10}H_{13}NO]^+$	
	5.4 (s, 3H, CH_3–O–)	162 (1.2) $[C_{10}H_{12}NO]^+$	
	7.27–7.457 (m, 5H, –C_6H_5)	119 (1.7) $[C_9H_{11}]^+$	
	8.362 (s, 1H, –CO–NH–C=)	91 (2.4) $[C_7H_7]^+$	
	10.349 (s, 1H, =C=NH)		

mon for other barbituric and thiobarbituric acid derivatives.

The identified products of hydrolysis of **I** indicate the hydrolytic pyrimidine ring opening at 1–6 position and are typical for other barbiturates (especially 2–oxo–analog (6) and thiobarbiturates (3, 7). On the other hand, the products of hydrolysis of **II** prove desulfuration of the parent compound and formation of derivatives of isourea. The exact mechanism of such hydrolysis course needs further elucidation.

Acknowledgment

The work was supported by the Grant No. 4P05F02915 from the Polish State Committee for Scientific Research (KBN), Warszawa, Poland. The authors are indebted to Dr. hab. M. Kłys, from the Department of Toxicology, Institute of Forensic Medicine, Medical College of the Jagiellonia University for enabling LC/MS measurements.

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Received: 10.02.2003