Acta Poloniae Pharmaceutica – Drug Research, Vol. 66 No. 2 pp. 123–128, 2009 ISSN 0001-6837
Polish Pharmaceutical Society

HYDROLYSIS OF 2,4-DITHIOPHENOBARBITAL

MONIKA TARSA*, GRZEGORZ ŻUCHOWSKI, ANNA STASIEWICZ-URBAN and JACEK BOJARSKI

Department of Organic Chemistry, Jagiellonian University, Faculty of Pharmacy
9 Medyczna St., 30-688 Kraków, Poland

Abstract: Hydrolysis of 2,4-dithiophenobarbital in aqueous solutions of pH 2 – 12 was investigated at 40 and 60°C using UV spectrophotometry. The values of reaction order, rate constants, pKa1 and pKa2 and activation energy were determined. The preliminary estimation of degradation products was accomplished using thin layer chromatography. The major products were isolated by circular chromatography and identified by spectroscopic and classical methods.

Keywords: 2,4-dithiophenobarbital, hydrolysis, kinetic parameters, degradation products

This study is a continuation of our earlier work on hydrolysis of thiobarbituric acid derivatives (1-5), to investigate the products, course and kinetics of the reaction and to compare them with those of their oxo counterparts and among them the representatives of this group of drugs.

2,4-Dithiophenobarbital (DTPB) is a dithioderivative of phenobarbital, a well known drug with hypnotic and antiepileptic activity. The stability of the title compound in aqueous solutions within the pH range 2 – 12 was investigated following the UV spectral changes at analytical wavelengths at 40 and 60°C. The reaction order, rate constants, pKa and activation energy values were determined and compared with those of phenobarbital, 2-thiobarbital, and 2-thiophenobarbital. The products of degradation were analyzed by TLC and isolated by circular chromatography. Their structures were confirmed by spectral methods.

EXPERIMENTAL

2,4-Dithiophenobarbital was obtained by thionation of carbonyl groups of phenobarbital with Lawesson reagent (6).

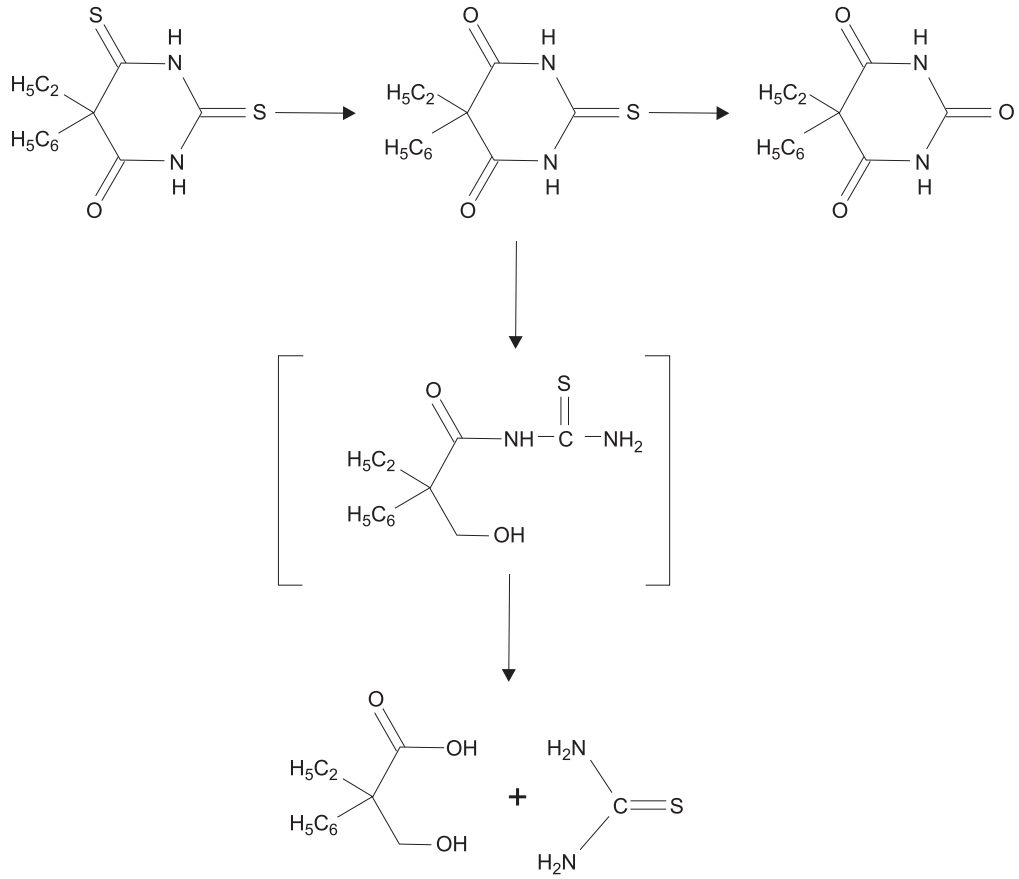
Kinetic measurements and UV spectra were obtained using an UV-VIS Cecil 7200 apparatus (UK). ^{1}H NMR spectra were recorded in the Laboratory of NMR Spectroscopy, Jagiellonian University, Medical College, on a Varian -Mercury – VX 300 MHz apparatus (USA) and standardized on the $CDCl_3$ signal.

Buffer solutions were prepared from stock solutions and distilled water acc. to (7). The pH determinations were done with microcomputer pH-meter CP-315m (Elmetron, Zabrze, Poland) using combined electrode ESAgP-301W (Eurosensor, Gliwice, Poland) calibrated with standard buffer solutions of pH 4, 7 and 10 (POCH, Gliwice,

Table 1. The rate constants, their logarithms and half-life times for hydrolytic degradation of 2,4-dithiophenobarbital in buffers of different pH at 40°C.

pH	k [s^{-1}]	log k	$t_{0.5}$ [h]
1.82	3.902×10^{-6}	-5.409	49.33
3.02	3.208×10^{-6}	-5.494	60.0
5.19	4.008×10^{-6}	-5.4397	48.0
5.90	7.174×10^{-6}	-5.155	26.83
8.14	10.60×10^{-6}	-4.973	18.16
9.43	22.21×10^{-6}	-4.657	8.67
10.41	38.12×10^{-6}	-4.42	5.05

* Corresponding author: mftarsa@cyf-kr.edu.pl



Scheme 1. The course of hydrolytic degradation of 2,4-dithiophenobarbital in alkaline medium.

Table 2. The rate constants, their logarithms and half-times for hydrolytic degradation of 2,4-dithiophenobarbital in buffers of different pH at 60°C.

pH	k [s^{-1}]	log k	$t_{0.5}$ [h]
1.82	$1.768 \cdot 10^{-5}$	-4.753	10.89
3.02	$1.574 \cdot 10^{-5}$	-4.803	12.23
5.19	$1.842 \cdot 10^{-5}$	-4.735	10.45
5.90	$3.32 \cdot 10^{-5}$	-4.489	5.8
6.96	$2.68 \cdot 10^{-5}$	-4.582	7.18
8.14	$8.2 \cdot 10^{-5}$	-4.086	2.35
9.43	$1.3 \cdot 10^{-4}$	-3.876	1.48
10.41	$2.75 \cdot 10^{-4}$	-3.56	0.7
11.91	$7.72 \cdot 10^{-4}$	-3.113	0.25

Poland). Temperature of DTPB degradation was maintained with the precision of ± 0.1°C using an ultrathermostat U-15 (VEB Prufgerate Werk, Medingen, Germany).

The preliminary estimation of degradation products was accomplished using thin layer chromatography. 1% solution of DTPH in the buffer (pH = 10) was degraded at 70°C. 5 mL samples were

Table 3. The rate constants, their logarithms and half-times for hydrolytic degradation of 2,4-dithiophenobarbital in buffer of pH 10 at different temperatures.

T [°C]	T [K]	$1/T \times 10^{-3}$	k [s^{-1}]	log k	$t_{0.5}$ [min]
40	313	3.195	2.29·10-5	-4.641	504.37
50	323	3.096	1.3·10-4	-3.886	88.85
60	333	3.003	2.54·10-4	-3.595	45.47
70	343	2.915	6.0·10-4	-3.222	19.25
80	353	2.833	7.91·10-4	-3.012	14.60

Table 4. Comparison of kinetic parameters for hydrolytic degradation of different barbituric and thiobarbituric acid derivatives.

Compound	Activation energy [kJ/mol]	Rate constant [s^{-1}] at t = 80°C
Phenobarbital	78.96 (at pH 10.12)	3.8×10^{-6} (at pH 9.89)
2-Thiobarbital	88.60 (at pH 10.32)	9.82×10^{-6} (at pH 10.32)
2-Thiophenobarbital	64.06 (at pH 10.84)	6.02×10^{-4} (at pH 9.82)
2,4-Dithiophenobarbital	78.80 (at pH 10.00)	7.91×10^{-4} (at pH 10.00)

withdrawn with Hamilton syringe and spotted on thin layer plates (silica gel 60 F_{254} plates, Merck, Darmstadt, Germany) at appropriate time intervals (0; 0,5; 1; 1,5; 2; 3; 4; 5; 6; 7; 8; 24 h). The standards of DTPH, 2-thiophenobarbital, phenobarbital, thiourea and ethylphenylmalonic acid were also spotted on the plates. The plates were developed in the systems: *n*-hexane : ethanol : triethylamine (7:1:1, v/v/v), cyclohexane : ethyl acetate (2:1, v/v), chloroform : *n*-hexane (2:1, v/v) and chloroform : 2-propanol (1:1, v/v). Visualization of spots was accomplished under UV light at 254 nm using HA-05 lamp (Haland, Warsaw, Poland).

Semipreparative hydrolysis was run with 0.1 g of DTPB in the buffer of pH 10 at 70˚C for 24 h. After evaporation under reduced pressure the residue was dissolved in a minimal amount of ethanol and the degradation products were separated by circular chromatography using Model 8924 chromatotron (Harrison Research, USA) and silica gel PF_{254} (Merck, Darmstadt, Germany) collecting fractions of ca. 20 mL volume and monitoring the separation with UV lamp at 254 nm. The following gradient solvent systems in volumetric ratios were used for elution of subsequent fractions (in parenthesis): cyclohexane : ethyl acetate 2 : 0.5 (5), cyclohexane : ethyl acetate 2 : 1 (10), chloroform : isopropanol 4 : 1 (2), chloroform : isopropanol 2 : 1 (2), chloroform : isopropanol 1 : 1 (1).. Eluates were analyzed by TLC and homogeneous fractions were combined and the solvents were distilled off on rotary evaporator (Laborota 4000 Heidolph, Germany).

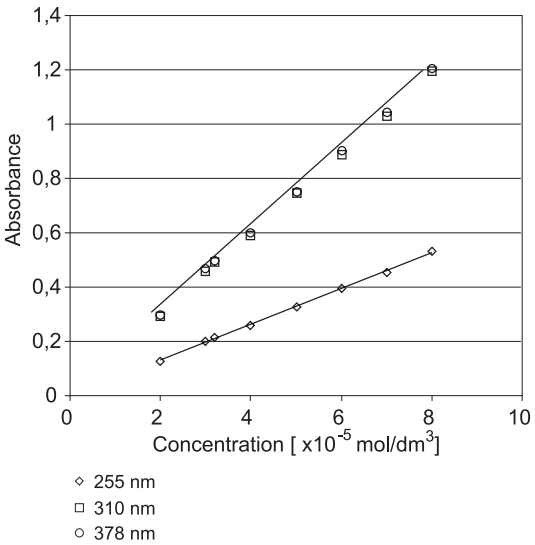


Figure 1. The absorbance – concentration relationship for DTPB in buffer of pH 8.14 at 25°C.

All reagents were of analytical reagent grade (POCH, Gliwice). Melting points were determined on Mel-Temp apparatus (Laboratory Devices Inc. USA) and are uncorrected. Calculations were done using Ouick Statistica PL program (Statsoft, Poland).

RESULTS AND DISCUSSION

To find analytical wavelengths, the UV spectra of DTPB at the concentration 6×10^{-5} mol/L were

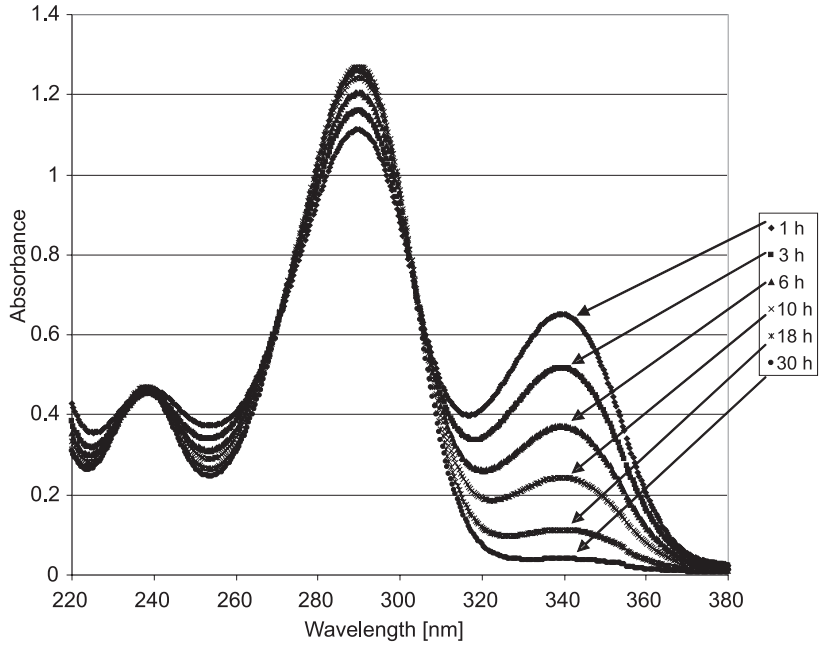


Figure 2. The absorbance changes for DTPB in buffer of pH 3.02 at 60°C as a function of time.

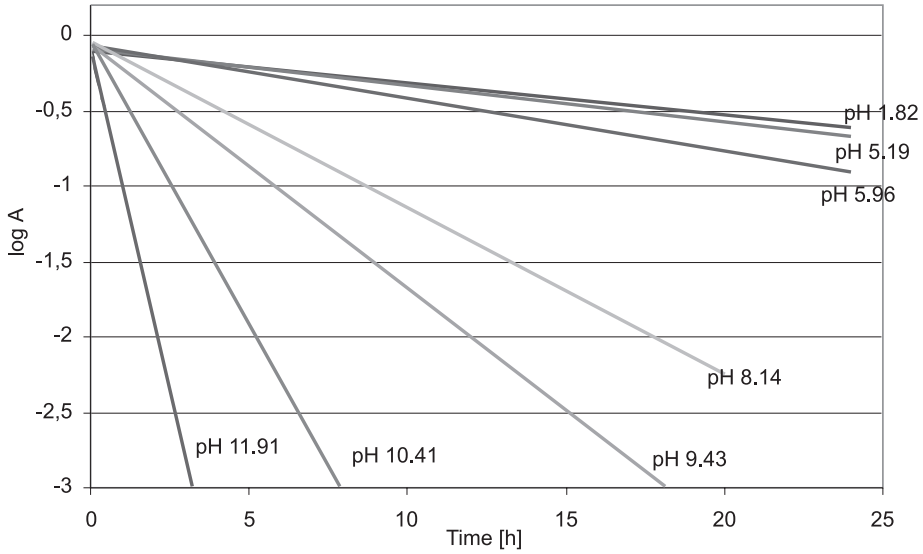


Figure 3. The relationship log A = f (t) for DTPB ($c = 6 \times 10^{-5}$ mol/L) at 60°C, at different pH values.

measured in buffer solutions of different pH values. To check the Lambert Beer law, the solutions of DTPB at the concentration $2 - 8 \times 10^{-5}$ mol/L in ethanol and in the buffer of pH 8.14 were prepared and their absorbance within the range 200 – 400 nm was measured (at 25°C). The absorbance value at the λ_{max} was determined and the molar absorption coeffficient was calculated. The relationship A = f (c) is linear (Figure 1) and allows to use UV spectroscopy to monitor the changes of absorbance in time.

Figure 2 presents the typical changes of absorbance of DTPB in time in the buffer of pH 3.02 at 60°C ($c = 6 \times 10^{-5}$ mol/L).

The following analytical wavelengths were chosen for monitoring the changes of UV chromophores in time:

310 nm for solutions of pH 8.14, 9.43, 10.41

316 nm for solutions of pH 11.91

340 nm for solutions of pH 1.82, 3.02, 5.19, 5.90, 6.96

The changes of absorbance at those vawelengths were measured at 40 and 60°C for 20 h. Typical relationships log A = f (t) are presented in Figure 3. The rate constants for DTPH hydrolytical degradation were calculated from the equation:

$\log (A - A_\infty) = \log (A_0 - A_\infty) - k\ t/2.303$

where A – absorbance of the solution at time t, A_0 – absorbance of the solution at time t = 0, A_∞ – final absorbance of the solution, t – time and k – rate constant.

The results are presented in Tables 1 and 2 and the relationship log k = f (pH) is presented in Figure 4.

Determinations of logarithms of dissociation constants for DTPB were based on Henderson-Hasselbalch equation:

$pKa = pH + \log [(A_n - A) / (A - A_d)$

where A_n is the absorbance of neutral form and A_d is the absorbance for monodissociated form for pKa1 and monodissociated form and double dissociated form for pKa2, respectively; A is the absorbance of the solution at the given pH value. The experimental pKa1 and pKa2 values were 6.73 and 10.79, respectively, at 25°C.

The value of activation energy for DTPB degradation in buffer pH 10.00 (for $c = 6 \times 10^{-5}$ mol/L) was determined from the Arrhenius equation and from absorbance readings at 310 nm for kinetic runs at at 40, 50, 60, 70 and 80°C (Table 3, Figure 5). The calculations yielded the value of 78.8 kJ/mol.

The products of degradation in alkaline medium were: 2-thiophenobarbital, phenobarbital, thiourea and ethylphenylmalonic acid.

The preparative separation of main degradation products after 24 h hydrolysis of DTPB in buffer of pH 10 at 70°C was accomplished by circular chromatography. The following products were identified: thiourea (**I**) and ethylphenylmalonic acid (**II**).

I: m.p. 172-175°C [lit. 174-6°C (8)], $R_f = 0.82$ (TLC, $CHCl_3$: isopropanol, 1:1, v/v) UV (etanol,

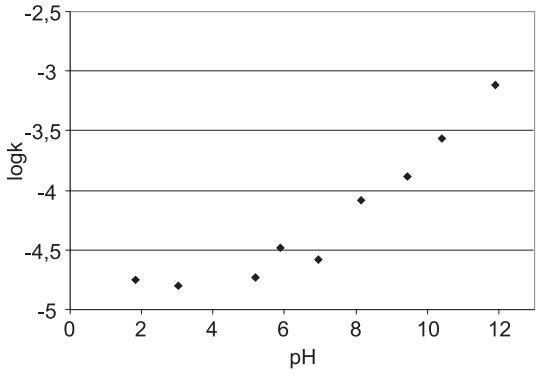


Figure 4. The log k – pH profile for hydrolytic degradation of DTPB at 60°C.

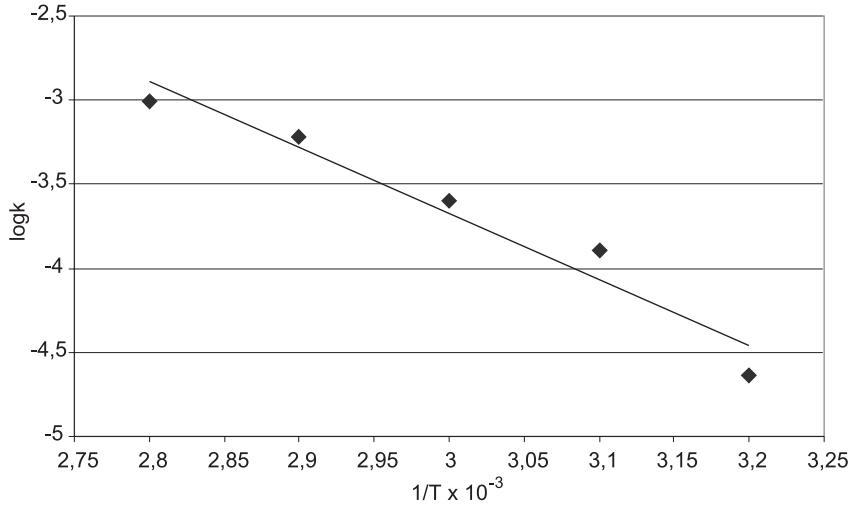


Figure 5. Arrhenius plot [log k = f (1/T)] for degradation of DTPB in the buffer of pH 10.

nm): 242,8 nm, ^{1}H NMR (DMSO) (δ, ppm): 7.037 (s, 4H, 2 × CH_2). Positive reaction with lead acetate after melting with sodium.

II: m.p. 150-152°C [lit. 153-155°C (9)], R_f = 0.46 (TLC, $CHCl_3$: isopropanol, 1:1, v/v), UV (etanol, nm): 253.2, 259.6, 265.2, ^{1}H NMR (DMSO) (δ, ppm): 0,825 (t, 3H, *J* = 7.5 Hz, $C\underline{H}_3$-CH_2-), 2.200 (q, 2H, *J* = 7.5, CH_3-$C\underline{H}_2$-), 7.20-7.40 (m, 5H, C_6H_5-), acidic properties – decoloration of basic phenolphtalein solution.

The results presented above allow to postulate that under the conditions employed the degradation of DTPB is a pseudo-first order reaction (Figure 3). The OH^- ions catalyze the degradation and in alkaline region the rate constants increase with an increase of pH. This feature is common also for other barbituric and thiobarbituric acid derivatives (9). The comparison of kinetic parameters for some of them (Table 4) indicates that phenobarbital (10) and 2-thiobarbital (1) are more stable under alkaline conditions than 2-thiophenobarbital (5) and DTPB. This means that the phenyl substituent at the C5 position of the pyrimidine ring acceletares the rate of degradation of respective derivatives.

The main reaction course of DTPB hydrolytic degradation is presented in Scheme 1. In the first step, there is the C = S → C = O transformation at the C4 position with formation of 2-thiophenobarbital that is degraded further on two pathways i.e. by another similar transformation at the C2 position (with formation of phenobarbital) and by the pyrimidine ring opening leading to the identified final products **I** and **II**.

REFERENCES

1. Bojarski J.: Rocz. Chem. 48, 619 (1974).
2. Tarsa M., Bojarski J.: Bull. Pol. Acad. Sci. Chemistry 45, 63 (1997).
3. Tarsa M., Żuchowski G., Bojarski J.: Acta Pol. Pharm. Drug Res. 60, 247 (2003).
4. Tarsa M., Żuchowski G., Bojarski J.: Acta Pol. Pharm. Drug Res. 60, 253 (2003).
5. Żuchowski G., Tarsa M., A. Stasiewicz-Urban A., Bojarski J.: Acta Pol. Pharm. Drug Res. 62**,** 335 (2005).
6. Stasiewicz-Urban A., Kubaszek M., Żylewski M. Cegła M., Bojarski J., Polish J. Chem. 78, 2105 (2004).
7. Physicochemical Manual WNT, Warsaw 1974 (in Polish).
8. Lessari A., Mata S., Blanco S., López J.C., Alonso J.L.: J. Chem. Phys. 120, 6191 (2004).
9. Norris J.F., Tucker H.F.: J. Am. Chem. Soc. 55, 4697 (1933).
10. Garrett E.R., Bojarski J., Yakatan G.Y.: J. Pharm. Sci. 60, 1145 (1971).

Received: 04. 07. 2008