Bulletin of the Chemists and Technologists of Macedonia, Vol. **25**, No. 1, pp. 29–37 (2006)
GHTMDD – 480
ISSN 0350 – 0136
Received: February 21, 2006
UDC: 547.79 : 543.42
Accepted: June 12, 2006
Original scientific paper

BEHAVIOUR OF SOME NEWLY SYNTHESIZED SUBSTITUTED 1,2,4-TRIAZOLINE-3-THIONES IN SULFURIC ACID MEDIA

Mirjana Jankulovska[1*], Ilinka Spirevska[1], Katica Čolančeska-Rag̗enovik̗[2]

[1]*Institute of Chemistry, Faculty of Natural Sciences and Mathematics, Ss Cyril and Methodius University,*
Arhimedova 5, MK-1001 Skopje, Republic of Macedonia,
[2]*Faculty of Technology and Metallurgy, Ss Cyril and Methodius University,*
P. O. Box 580, MK-1001 Skopje, Republic of Macedonia
*jankulovska_m@yahoo.com

The protonation process of some newly synthesized substituted 1,2,4-triazoline-3-thiones (4-butyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione, 4-allyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione, 4-phenyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione) was investigated in aqueous sulfuric acid solutions, using the ultraviolet spectrophotometric method, at a room temperature. The acid-base equillibria were characterized qualitatively and quantitatively. The method of characteristic vector analysis (CVA) was used to reconstruct the experimental spectra. The protonation process, most likely occurs on the thiourea fragment of the 1,2,4-triazoline-3-thione ring. The pK_{BH^+} values were calculated using several methods: the Hammett acidity function method, Bunnett and Olsen, Tissier and Tissier (Bunnett and Olsen, Yates), Marziano, Cimino and Passerini, and the "Excess acidity" function method (Cox and Yates). There is a good agreement between the pK_{BH^+} values determined with these different methods.

Key words: 4-butyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione; 4-allyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione; 4-phenyl-5-octyl2,4-dihydro3H-1,2,4-triazoline-3-thione; UV spectrophotometry; protonation constants

ОДНЕСУВАЊЕ НА НЕКОИ НОВОСИНТЕТИЗИРАНИ СУПСТИТУИРАНИ 1,2,4-ТРИАЗОЛИН-3-ТИОНИ ВО СРЕДИНА НА СУЛФУРНА КИСЕЛИНА

Процесот на протонирање на некои новосинтетизирани супституирани деривати на 1,2,4-триазолин-3-тионот (4-бутил-5-октил-2,4-дихидро-3H-1,2,4-триазолин-3-тион, 4-алил-5-октил-2,4-дихидро-3H-1,2,4-триазолин-3-тион, 4-фенил-5-октил-2,4-дихидро-3H-1,2,4-триазолин-3-тион) беше испитуван во водни раствори на сулфурна киселина, со помош на методите на ултравиолетовата спектроскопија, на собна температура. Кисело-базната рамнотежа беше окарактеризирана квантитативно и квалитативно. Методот на карактеристична векторска анализа (CVA) беше користен за реконструкција на експерименталните спектри. Процесот на протонирање најверојатно се врши на сулфурот во прстенот на 1,2,4-триазолин-3-тионите. Вредностите на pK_{BH^+} беа пресметани со примена на неколку методи: Hammett acidity function method, на Bunnett и Olsen; на Tissier и Tissier (Bunnett и Olsen, Yates); на Marziano, Cimino и Passerini; на Cox-Yates (метод на „вишок на киселост").

Клучни зборови: 4-бутил-5-октил-2,4-дихидро-3H-1,2,4-триазолин-3-тион; 4-алил-5-октил-2,4-дихидро-3H-1,2,4-триазолин-3-тион; 4-фенил-5-октил-2,4-дихидро-3H-1,2,4-триазолин-3-тион; UV спектрофотометрија; константи на протонирање

INTRODUCTION

1,2,4-Triazole belongs to the heterocyclic class of compounds which possess a wide spectrum of biological activity that has been demonstrated in many studies [1, 2]. Because of the pharmaceutical characteristics of the compounds with 1,2,4-triazole nucleus, they can be used as antibacterial, antiviral and anticancer drugs [3]. In addition, 1,2,4-triazole and its derivatives have found wide use in the chemical industry and agriculture, because of their fungicidal, insecticidal and herbicidal characteristics [3].

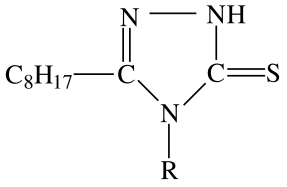
The characteristics of these compounds depend on the acid–base processes in defined media. For this reason, the acid–base equilibrium of 1,2,4-triazole and its derivatives in different media has been studied by many authors [3, 4]. Katritzky and Rees [3] determined the values of the ionization constants of 1,2,4-triazole as a base ($pK_B = 2.19$) and as an acid ($pK_{BH+} = 10.26$). In the literature, there is data about the pK values of some 1,2,4-triazoline-3-thiones in sulfuric acid and sodium hydroxide media determined using the ultraviolet spectroscopy [4].

The determination of the ionization constants of 1,2,4-triazole and its derivatives is important for studying the reaction mechanisms which take place in acidic media.

The subjects of our investigation were the following compounds: 4-butyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione, 4-allyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione, 4-phenyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione which were synthesized for the first time by Raǵenoviḱ et al. [5]. Their structure was determined by several methods: UV, IR, 1H NMR and 13C NMR spectroscopy.

It was found that the investigated compounds are biologically active [3], and it was deemed very important to investigate their acid–base properties.

The structure formulas of compounds are:



R

(I) —C_4H_9

(II) —CH_2—CH═CH_2

(III) —C_6H_5

(I) 4–butyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione

(II) 4-allyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione

(III) 4-phenyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione

It is expected that 1,2,4-triazoline-3-thiones in strong mineral acid media behave as weak bases and the protonation process should take place. The aim of our work was to analyze the behavior of the investigated compounds in strong mineral acid media and to determine the ionization constants, data which is not avaliable in the literature.

For the calculation of $pK_{BH}{}^+$ values several methods known from the literature were applied.

For the reaction of protonation of a weak base in strong mineral acid the following equation can be written:

$$B + H^+ \rightleftarrows BH^+. \tag{1}$$

The equilibrium constant according to Hammett [6] can be defined with the following equation:

$$pK_{BH}{}^+ = H_0 + \log\frac{c(BH^+)}{c(B)}. \tag{2}$$

H_0 is Hammett acidity function which represents acidity scale of the highly acidic media, and is used for the determination of a weak base strength [7].

$c(BH^+)$ is the concentration of the protonated form of the compound;

$c(B)$ is the concentration of the unprotonated form of the compound;

The original Hammett acidity function was upgraded by some authors. Their purpose was to reach more reliable $pK_{BH}{}^+$ values of the investigated compounds. Above all, it is found that H_0 acidity function depends on the class of the investigated compounds. Sometimes, there are differences for closely related compounds even with the same protonation center. Different values of H_0 are defined as H_X which describe most accurately the protonation process of different group of compounds.

Accordingly, $pK_{BH}{}^+$ values can be defined by the equation:

$$pK_{BH}{}^+ = \log I + mH_X. \tag{3}$$

A plot of $\log I$ vs. $-H_x$ gives a straight line with slope m, whose value is about 1.

I is a ratio between the concentration of the protonated and unprotonated form of the base, $c(BH^+)/c(B)$, known also as a ionization ratio.

For thiocarbonyl compounds H_x is noticed as H_T and its values are established for 10–90% sulfuric acid aqueous solutions [8].

For the determination of $\mathrm{p}K_{\mathrm{BH}}{}^{+}$ values Bunnett and Olsen [9] suggested the equation based on the linear free energy relationship:

$$\mathrm{p}K_{\mathrm{BH}}{}^{+} = H_0 - \phi[H_0 + \log c(\mathrm{H}^+)] + \log I. \qquad (4)$$

The value of H_{T} acidity function can be defined by the equation:

$$H_{\mathrm{T}} = H_0 - \phi[H_0 + \log c(\mathrm{H}^+)]. \qquad (5)$$

The final equation for determination of $\mathrm{p}K_{\mathrm{BH}}{}^{+}$ values, obtained from equations (4) and (5) is:

$$\mathrm{p}K_{\mathrm{BH}}{}^{+} = H_{\mathrm{T}} + \log I. \qquad (6)$$

In equation (4) ϕ is a parameter which characterizes the changing activity coefficient behavior of bases with changing acidity. According to this method it is found out that the parameter ϕ has negative value for the large polarizable molecules. These molecules have smaller extent of solvation than the protonated indicators. The value of ϕ for thiocarbonyl compounds is –0.4.

Bunnett and Olsen [9] calculated the values of $\mathrm{p}K_{\mathrm{BH}}{}^{+}$ using the H_0 values for lower concentration of sulfuric acid from Poul and Long [10], and for higher concentration of sulfuric acid from Jorgenson and Harter [11].

Values of $\log c(\mathrm{H}^+)$ are taken from literature [12] where they are determined for dibasic acids.

For determination of $\mathrm{p}K_{\mathrm{BH}}{}^{+}$ values, the authors Tissier and Tissier [13] utilized two approaches, the equation of Bunnett–Olsen [9], and the equation of Yates–McClelland [14]. Using the equation of Bunnett and Olsen they obtained the following equation for H_{T}:

$$H_{\mathrm{T}} = H_0' + \phi[H_0' + \log c(\mathrm{H}^+)] \qquad (7)$$

H_0' is the acidity function determined by Yates and McClelland [14].

The authors [13] examined 11 indicators and they found that the average value of the slope parameter ϕ is –0.36.

Using the equation of Yates and McClelland [14], Tissier and Tissier [13] found that $\mathrm{p}K_{\mathrm{BH}}{}^{+}$ values can be calculated using the equation:

$$\mathrm{p}K_{\mathrm{BH}}{}^{+} = -mH_0' + \log I,\ H_{\mathrm{T}} = -mH_0' \qquad (8)$$

where m is a slope parameter whose average value for thiocarbonyl compounds is 1.31, while the values of H_0' are taken from Yates and McClelland [14].

Marziano, Cimino and Passerini [15] established different access for calculation of $\mathrm{p}K_{\mathrm{BH}}{}^{+}$ values (M.C.P. method). They used the equation:

$$\mathrm{p}K_{\mathrm{BH}}{}^{+} = Mc \cdot n^{*} - \log c(\mathrm{H}^+) + \log \frac{c(\mathrm{BH}^+)}{c(\mathrm{B})}. \qquad (9)$$

The expression $Mc \cdot n^{*} - \log c(\mathrm{H}^+)$ represents the H_{T} acidity function, and n^{*} is the slope parameter whose value is in the range of 1.0 ± 0.1. This value is the average one, obtained from the values of 16 used thiocarbonyl indicators.

$Mc = \log(f_{\mathrm{B}} \cdot f_{\mathrm{H}}{}^{+}/f_{\mathrm{BH}}{}^{+})$, is the activity coefficient function based on a single investigated compound (hypothetical standard base). Its value can be determined by measurement of the ionization ratio at higher mineral acid concentration, using the n^{*} and $\mathrm{p}K_{\mathrm{BH}}{}^{+}$ values determined for the used thiocarbonyl compounds.

The final equation for calculation of $\mathrm{p}K_{\mathrm{BH}}{}^{+}$ values is similar to the equation (6).

The acidity function H_{T} and Mc, whose values depend on sulfuric acid concentration, can be found in the literature [15].

Further, the method of Cox and Yates [16], also known as the "excess acidity" function method, is often used in the literature for calculation of the $\mathrm{p}K_{\mathrm{BH}}{}^{+}$ values. According to this method, $\mathrm{p}K_{\mathrm{BH}}{}^{+}$ values can be calculated using the equation:

$$\mathrm{p}K_{\mathrm{BH}}{}^{+} = [\log I - \log c(\mathrm{H}^+)] - m^{*}X \qquad (11)$$

where m^{*} expresses the hydrogen-bonding solvation of the protonated base. Its value determined for thiocarbonyl compounds is in the range of 1.39 ± 0.14. X is a function which represents the difference between the observed acidity and that which the system would have if it was ideal („excess acidity"). The values of this function were determined depending on sulfuric acid concentration and they can be found in the literature [16].

EXPERIMENTAL

The investigated compounds, 4-butyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione (compound I), 4-allyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione (compound II), 4-phenyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione (compound III) were dissolved in absolute ethanol, at room temperature. There were no changes in UV spectra recorded for two months time, which meant that the ethanol solutions were stable for a long time. The stock solutions were prepared as primary standards, with concentration $1.000 \cdot 10^{-3}$ mol/dm^3 for compound I, $1.013 \cdot 10^{-3}$ mol/dm^3 for compound II and $1.036 \cdot 10^{-3}$ mol/dm^3 for compound III. Then, three series of the solutions of investigated compounds were prepared in sulfuric acid, with concentration from 1.000 mol/dm^3 to 12.000 mol/dm^3 and the constant concentration of the investigated compounds ($2.000 \cdot 10^{-5}$ mol/dm^3 for I; $2.020 \cdot 10^{-5}$ mol/dm^3 for compound II and $2.070 \cdot 10^{-5}$ mol/dm^3 for compound III).

The concentration of sulfuric acid was determined using volumetric titration method with standard solution of sodium hydroxide.

Simultaneously, the blanks were prepared with the same composition as the test solutions, except that they did not contain the investigated compound. The ethanol content in the test solutions and in the blanks was 1% (w/v). All measurements were carried out immediately after preparation of the solutions, because of their instability over the time.

The purity of the investigated compounds was confirmed by quantitative elemental analysis and the recorded IR spectra [5]. The sulfuric acid was of analytical grade p.a. (Alkaloid).

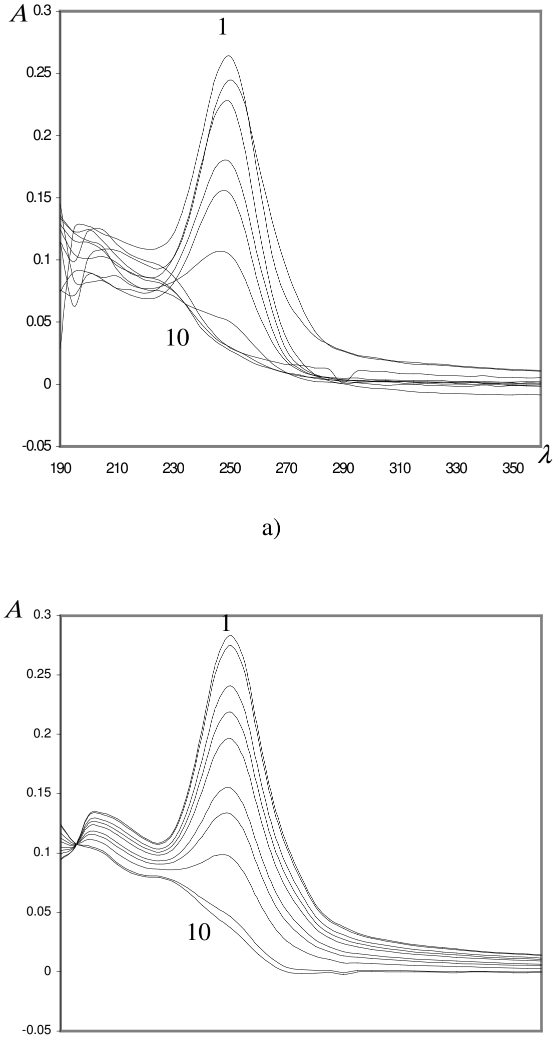
The UV spectra were recorded on a Varian Cary 50 spectrophotometer in the range from 190 nm to 360 nm wavelength at room temperature. The length of the quartz cell was 1 cm.

RESULTS AND DISCUSSION

The UV spectra of the aqueous sulfuric acid solution of the compounds: 4-butyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione (compound I), 4-allyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione (compound II) and 4-phenyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione (compound III), recorded in the wavelengths region between 190 nm and 360 nm, are shown in Figures 1a, 2a and 3a, respectively. Two bands can be noticed in the UV spectra of the investigated compounds. The first band, in the region from 190 to 210 nm was less intensive than the other one. The second band, with maximum about 250 nm for compound I and II about 255 nm for compound III, is probably the result of $\pi \rightarrow \pi^*$ electron transitions in the 1,2,4-triazoline-3-thione ring [15]. We considered that the second band is interesting for our further investigations.

When the sulfuric acid concentration increased from 1 mol/dm^3 to 12 mol/dm^3, the intensity of the second band decreased at constant concentration of the investigated compound, but its position did not change (see Figs. 1a, 2a and 3a).

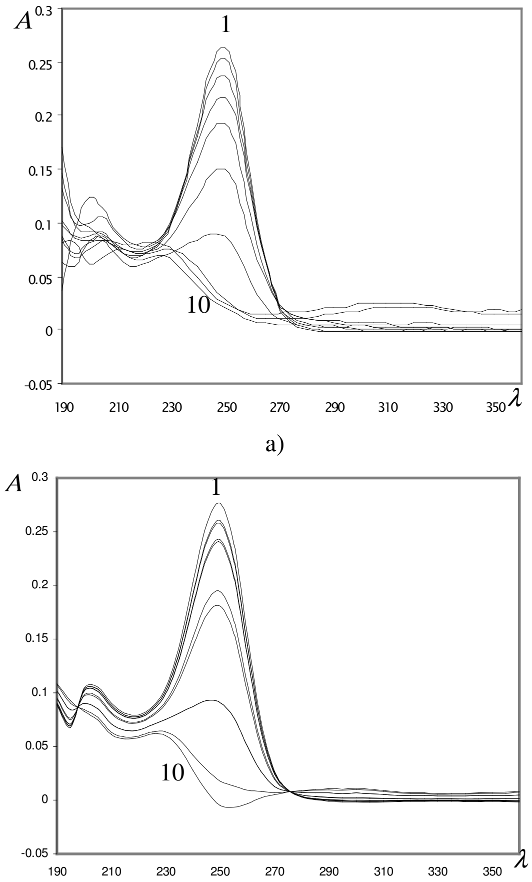
Reconstruction of the spectra is accomplished using the method of Characteristic Vector Analysis (CVA) [18]. The results are shown in the Figures 1b, 2b and 3b.



a)

b)

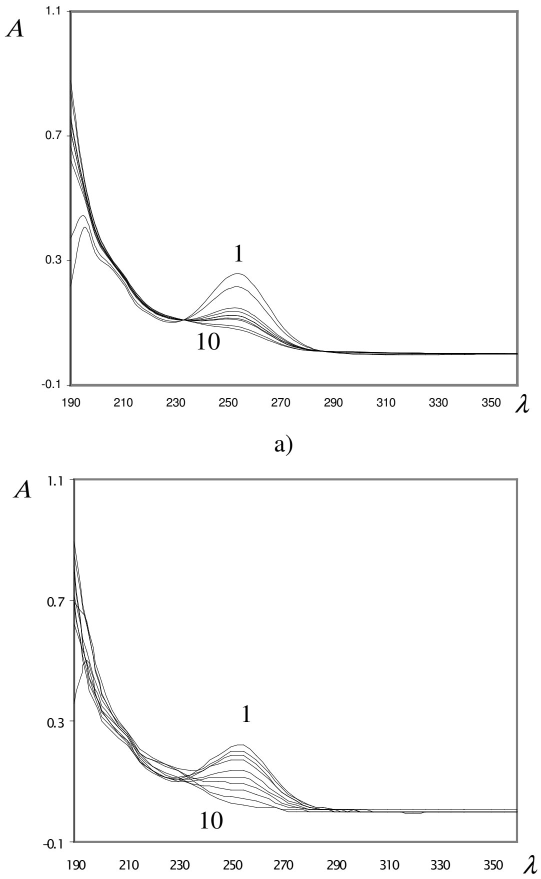
Fig. 1. UV spectra of 4-butyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione (compound I) in sulfuric acid media: (a) experimental spectra and (b) reconstructed spectra; $c(\mathrm{I}) = 2.000 \cdot 10^{-5}$ mol/dm^3, $c(H_2SO_4) = 1.000$ mol/dm^3 – 2.000 mol/dm^3



a)

b)

Fig. 2. UV spectra of 4-allyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione (compound II) in sulfuric acid media: (a) experimental spectra and (b) reconstructed spectra; $c(\mathrm{I}) = 2.020 \cdot 10^{-5}$ mol/dm^3, $c(H_2SO_4)$ = 1.000 mol/dm^3–12.000 mol/dm^3



a)

b)

Fig. 3. UV spectra of 4-phenyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione (compound III) in sulfuric acid media: (a) experimental spectra and (b) reconstructed spectra; $c(\mathrm{I}) = 2.070 \cdot 10^{-5}$ mol/dm^3, $c(H_2SO_4)$ = 1.000 mol/dm^3–12.000 mol/dm^3

In the reconstructed spectra for compounds I and III, the isosbestic points can be noticed at wavelengths of 200 nm and 235 nm, respectively. For compound II there are two isosbestic points at 200 nm and at 275 nm, indicating that there are probably two forms able to absorb UV radiation in the system.

As can be seen from the spectra, the value of the absorbance depends on the concentration of sulfuric acid, which can be better seen from the plot of absorbance at λ_{max} vs. sulfuric acid concentration. This dependence is presented in Fig. 4 and Fig. 5 for the compounds I and III, respectively, at wavelength of 250 nm, and in Fig. 6 for the compound III, at wavelength of 255 nm.

Figures 4–6 show that the dependence curve of the absorbance *vs.* sulfuric acid concentration represents a sigmoid curve („S" curve). It indicates that most likely a reaction of protonation of weak bases in sulfuric acid media takes place. The existence of only one plateau on the sigmoid curve of the investigated compounds suggests that the protonation reaction occurs in one step.

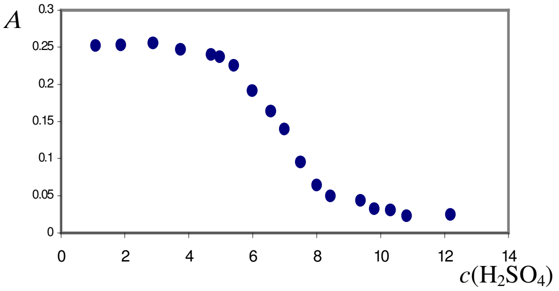


Fig. 4. The absorbance values (λ = 250 nm) as a function of sulfuric acid concentration for 4-butyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione with concentration $2.000 \cdot 10^{-5}$ mol/dm^3, $c(H_2SO_4)$ = 1.000 mol/dm^3 –12.000 mol/dm^3

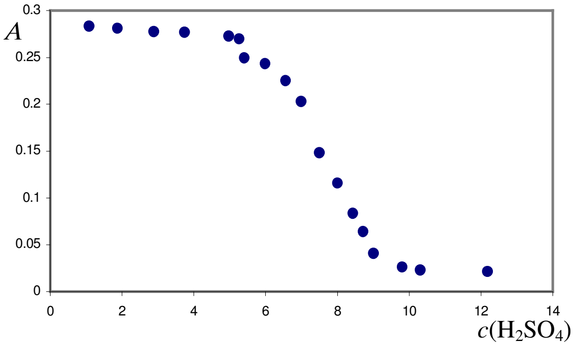


Fig. 5.The absorbance values (λ = 250 nm) as a function of sulfuric acid concentration for 4-allyl-5-octyl-2,4–dihydro-3H-1,2,4-triazoline-3-thione with concentration 2.020·10^{-5} mol/dm^3, $c(H_2SO_4)$ = 1.000 mol/dm^3 –12.000 mol/dm^3

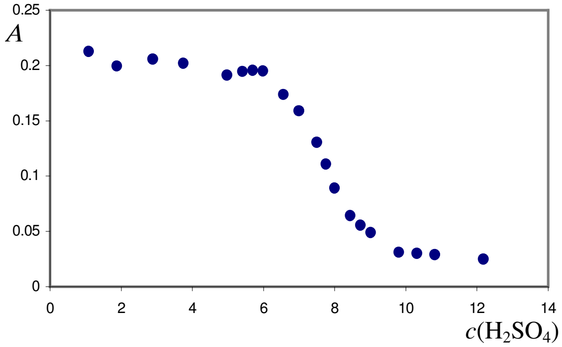


Fig. 6. The absorbance values (λ = 250 nm) as a function of sulfuric acid concentration for 4-phenyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione with concentration 2.070 ·10^{-5} mol/dm^3, $c(H_2SO_4)$ = 1.000 mol/dm^3 –12.000 mol/dm^3

The initial part of the „S" curve refers to the absorption of UV radiation of the unprotonated form (B), and the final one to the protonated form (BH^+) of the investigated compounds, respectively. From the linear step of the „S" curve, it could be seen that the protonation process of compound I is carried out at sulfuric acid concentration between 5.410 mol/dm^3 and 8.430 mol/dm^3, and from 5.980 mol/dm^3 to 9.010 mol/dm^3 for compounds II and III. Below sulfuric acid concentration of 5.410 mol/dm^3 only unprotonated forms of the compounds could be found in the media, while above sulfuric acid concentration of 9.010 mol/dm^3 there are only protonated forms of the investigated compounds.

The methods described in the introduction were used for the evaluation of p*K* values of the investigated compounds. The absorbance was measured at four selected wavelengths. The following wavelengths were used for the compounds I and II: 230 nm, 240 nm, 250 nm and 260 nm, while for the compound III, 240 nm, 250 nm, 260 nm and 270 nm were used.

The molar absorption coefficient values ($\varepsilon_{\lambda,i}$) were obtained by measuring the absorbance of investigated compounds at the concentrations 1.600·10^{-5} mol/dm^3, 2.000·10^{-5} mol/dm^3 and 2.400·10^{-5} mol/dm^3 at the same selected wavelength. The molar absorption coefficients of unprotonated form of the investigated compounds were determined in sulfuric acid concentration of 1.000 mol/dm^3, while that of the protonated form in sulfuric acid concentration of 10.310 mol/dm^3. The average values for the molar absorption coefficients are shown in Table 1.

Table 1

Molar absorption coefficient values of the investigated compounds: $c(H_2SO_4)$ *= 1.000 mol/dm^3 for unprotonated form,* $c(H_2SO_4)$ *= 10.310 mol/dm^3 for protonated form*

Compound I		*ε (230 nm)	ε (240 nm)	ε (250 nm)	ε (260 nm)
Experimental	ε(B)	55.18	94.38	131.94	85.90
	$\varepsilon(BH^+)$	43.66	25.58	13.72	9.241
Reconstructed	ε(B)	56.38	94.28	131.2	86.44
	$\varepsilon(BH^+)$	43.51	25.66	13.76	9.055
Compound II		ε 230 nm	ε 240 nm	ε 250 nm	ε 260 nm
Experimental	ε(B)	52.59	99.72	140.64	79.89
	$\varepsilon(BH^+)$	32.23	19.86	10.86	6.255
Reconstructed	ε(B)	54.63	100.2	139.9	79.49
	$\varepsilon(BH^+)$	28.51	18.21	12.83	7.198
Compound III		ε (240 nm)	ε (250 nm)	ε (260 nm)	ε (270 nm)
Experimental	ε(B)	73.27	102.4	90.61	43.70
	$\varepsilon(BH^+)$	44.23	21.29	13.68	6.865
Reconstructed	ε(B)	71.97	102.6	90.74	43.83
	$\varepsilon(BH^+)$	37.78	18.49	12.11	7.444

* $\varepsilon\cdot10^3/\mathrm{mol}^{-1}\mathrm{dm}^{-2}$

Furthermore, a system of four equations (four absorbance values) with two unknown parameters (concentration of protonated and unprotonated form) was used for determination of the concentration of unprotonated and protonated form (the ionization ratio) of compounds existing in the reaction mixture, for different sulfuric acid concentration. The pK_{BH^+} values were calculated using the values of log*I* and suitable values of the acidity functions. The calculations were carried out using the computer program Excel. These rethe computer program Excel. These results are shown in Table 2.

Table 2

pK_{BH}^{+} values for compounds I, II and III in sulfuric acid media (experimental spectra)

Compound	Hammett acidity function method		Bunnett and Olsen	
	Numerically	Graphically	Numerically	Graphically
I	–3.05±0.05 $^{*}s = 0.04$ $^{*}V = 1.42$ $^{*}R = 0.984$ $n^{*} = 9$	–3.01±0.03 $s = 0.03$ $V = 1.02$	–2.93±0.04 $s = 0.03$ $V = 1.11$ $R = 0.993$ $n = 9$	–2.93±0.08 $s = 0.07$ $V = 2.45$
II	–3.14±0.04 $s = 0.04$ $V = 1.16$ $R = 0.988$ $n = 8$	–3.14±0.04 $s = 0.03$ $V = 1.16$	–3.03±0.05 $s = 0.04$ $V = 1.35$ $R = 0.992$ $n = 8$	–3.09±0.07 $s = 0.06$ $V = 2.11$
III	–3.17±0.06 $s = 0.05$ $V = 1.72$ $R = 0.996$ $n = 9$	–3.18±0.07 $s = 0.06$ $V = 2.09$	–3.07±0.04 $s = 0.04$ $V = 1.18$ $R = 0.987$ $n = 9$	–3.06±0.07 $s = 0.06$ $V = 2.07$
	Tissier a nd Tissier (Bunnett and Olsen)[**]		Tissier and Tissier (Yates)	
I	–2.85±0.06 $s = 0.02$ $V = 0.82$ $R = 0.995$ $n = 9$	–2.88±0.05 $s = 0.04$ $V = 1.582$	–3.02±0.02 $s = 0.02$ $V = 0.64$ $R = 0.996$ $n = 9$	–3.07±0.03 $s = 0.03$ $V = 0.86$
II	–2.96±0.06 $s = 0.042$ $V = 1.42$ $R = 0.996$ $n = 8$	–3.07±0.06 $s = 0.05$ $V = 1.70$	–3.14±0.04 $s = 0.04$ $V = 1.15$ $R = 0.991$ $n = 8$	–3.13±0.03 $s = 0.02$ $V = 0.81$
III	–2.99±0.06 $s = 0.04$ $V = 1.32$ $R = 0.994$ $n = 9$	–3.05±0.07 $s = 0.06$ $V = 2.06$	–3.16±0.03 $s = 0.03$ $V = 0.95$ $R = 0.991$ $n = 9$	–3.13±0.03 $s = 0.03$ $V = 0.88$
	M. C. P.		„Excess acidity“ function method	
I	–3.07±0.04 $s = 0.04$ $V = 1.18$ $R = 0.987$ $n = 9$	–3.03±0.02 $s = 0.02$ $V = 0.66$	–2.97±0.05 $s = 0.05$ $V = 1.57$ $m^{*} = 1.43$ $R = 0.989$ $n = 9$	–2.98±0.04 $s = 0.04$ $V = 1.21$
II	–3.15±0.04 $s = 0.04$ $V = 1.25$ $R = 0.992$ $n = 8$	–3.15±0.04 $s = 0.04$ $V = 1.210$	–3.14±0.04 $s = 0.04$ $V = 1.15$ $m^{*} = 1.47$ $R = 0.995$ $n = 8$	–3.14±0.04 $s = 0.04$ $V = 1.15$
III	–3.17±0.05 $s = 0.04$ $V = 1.38$ $R = 0.994$ $n = 9$	–3.15±0.03 $s = 0.02$ $V = 0.82$	–3.18±0.05 $s = 0.05$ $V = 1.45$ $m^{*} = 1.46$ $R = 0.989$ $n = 9$	–3.18±0.05 $s = 0.05$ $V = 1.45$

[*] s – standard deviation, V – variance (%), R – correlation coefficient, n – number of points

[**] See introduction

Similarly, calculations were made using the data from reconstructed spectra where the solvent influence was eliminated. The obtained pK_{BH}^{+} values are shown in Table 3.

Table 3

pK_{BH}^{+} values for compounds I, II and III in sulfuric acid media (reconstructed spectra)

Compound	Hammett acidity function method		Bunnett and Olsen	
	Numerically	Graphically	Numerically	Graphically
I	–2.99±0.05 $s = 0.04$ $V = 1.37$ $R = 0.993$ $n = 9$	–3.03±0.06 $s = 0.05$ $V = 1.771$	–2.93±0.06 $s = 0.06$ $V = 1.9$ $R = 0.993$ $n = 9$	–2.92±0.04 $s = 0.03$ $V = 1.18$
II	–3.13±0.07 $s = 0.07$ $V = 2.13$ $R = 0.998$ $n = 8$	–3.15±0.07 $s = 0.06$ $V = 1.98$	–3.01±0.02 $s = 0.02$ $V = 0.72$ $R = 0.995$ $n = 8$	–3.11±0.06 $s = 0.05$ $V = 1.75$
III	–3.17±0.06 $s = 0.05$ $V = 1.78$ $R = 0.998$ $n = 9$	–3.17±0.06 $s = 0.06$ $V = 1.80$	–3.07±0.04 $s = 0.04$ $V = 1.28$ $R = 0.993$ $n = 9$	–3.11±0.02 $s = 0.01$ $V = 0.43$
	Tissier and Tissier (Bunnett and Olsen)		Tissier and Tissier (Yates)	
I	–2.92±0.02 $s = 0.02$ $V = 0.69$ $R = 0.998$ $n = 9$	–2.92±0.02 $s = 0.01$ $V = 0.41$	–2.98±0.08 $s = 0.07$ $V = 2.59$ $R = 0.998$ $n = 9$	–2.97±0.04 $s = 0.03$ $V = 1.12$
II	–2.94±0.05 $s = 0.05$ $V = 1.58$ $R = 0.997$ $n = 8$	–3.07±0.03 $s = 0.02$ $V = 0.83$	–3.11±0.04 $s = 0.0$ $V = 1.15$ $R = 0.996$ $n = 8$	–3.09±0.01 $s = 0.01$ $V = 0.33$
III	–2.98±0.04 $s = 0.04$ $V = 1.33$ $R = 0.994$ $n = 9$	–3.08±0.02 $s = 0.01$ $V = 0.43$	–3.16±0.03 $s = 0.02$ $V = 0.78$ $R = 0.996$ $n = 9$	–3.15±0.02 $s = 0.02$ $V = 0.63$
	M.C.P.		„Excess acidity“ function method	
I	–2.95±0.08 $s = 0.07$ $V = 0.90$ $R = 0.996$ $n = 9$	–2.95±0.09 $s = 0.08$ $V = 2.97$	–3.00±0.06 $s = 0.05$ $V = 1.88$ $m^{*} = 1.47$ $R = 0.997$ $n = 9$	–3.00±0.04 $s = 0.04$ $V = 1.24$
II	–3.13±0.03 $s = 0.03$ $V = 0.90$ $R = 0.998$ $n = 8$	–3.12±0.04 $s = 0.03$ $V = 1.13$	–3.11±0.05 $s = 0.04$ $V = 1.42$ $m^{*} = 1.48$ $R = 0.996$ $n = 8$	–3.17±0.02 $s = 0.02$ $V = 0.665$
III	–3.16±0.05	–3.15±0.02	–3.18±0.04	–3.17±0.03

$s = 0.04$	$s = 0.02$	$s = 0.03$	$s = 0.03$
$V = 1.44$	$V = 0.56$	$V = 1.03$	$V = 1.03$
$R = 0.996$ $n = 9$		$m^* = 1.48$	
		$R = 0.995$ $n = 9$	

The values of the dissociation constants of protonated forms of the compounds were also determined graphically using the method of Davis and Geissman [19]. Namely, when the concentration of the protonated and unprotonated form is equal, log$I$ = 0, and $pK_{BH^+} = H_T$.

The dependence of log$I$ on $-H_T$ is linear with different slope, depending on the method of calculation, and with an intercept equal to the graphic value of the pK_{BH^+} for a given compound. In the „excess acidity" function method (Cox–Yates method), the dependence of log$I$–log$c$(H^+) *vs.* X is linear.

For illustration, the method used for determination of pK_{BH^+} values graphically in sulfuric acid media for 4-phenyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione, using the Hammett acidity function method, is presented in Fig. 7.

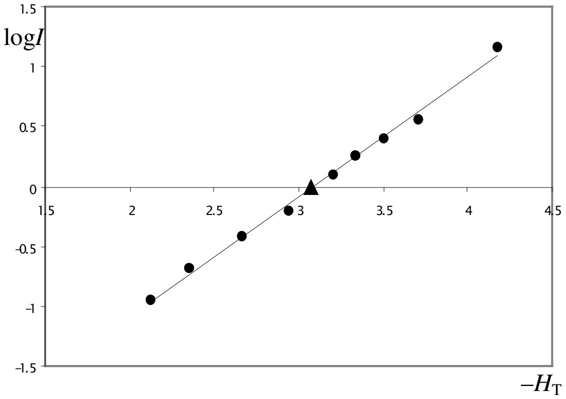


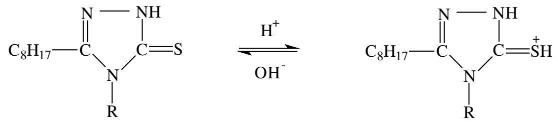
Fig. 7. Dependence of log$I$ on $-H_T$ for 4-phenyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione ($c = 2.070 \cdot 10^{-5}$ mol/dm^3) in sulfuric acid media according to Hammett method

From Fig. 7 it can be seen that the pK_{BH^+} value obtained graphically for 4-phenyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione according to Hammett is around –3.1.

The obtained results for the numerically and graphically calculated values of pK_{BH^+} using five methods from the absorbance data, at four selected wavelengths, are shown in Table 2 (for experimental spectra) and in Table 3 (for reconstructed spectra). Also, in Tables 2 and 3 are given the values of the standard deviations, correlation coefficients, and coefficients of variance, with 95% confidence level, for the performed measurement and the values of the slope (m^*) in according on the "excess acidity" function method.

The obtained results indicate that the thiones behave as bases in mineral acid media and could be protonated in high acid concentration.

The protonation process probably occurs on the thiourea fragment of the 1,2,4-triazoline-3-thione ring. This is confirmed by the value of the slope m^* which indicates that the protonation process is in agreement with the H_T acidity function determined for similar type of compounds. Namely, the value of the slope m^* for all investigated compounds is in the range 1.39 ± 0.14 (see Tables 2 and 3), which is characteristic for the protonation of sulfur [16]. The protonation process is illustrated by the equation:



The protonation process of compounds II and III was carried out at higher sulfuric acid concentration, compared to that of compound I. Compound III has the highest pK_{BH^+} values. It means that this compound is a weaker base, probably as a result of the influence of the phenyl group.

Different methods were used for determination of the pK_{BH^+} values of the investigated compounds. Also, the standard deviation and the variances were calculated from the obtained results for the dissociation constants of the protonated forms from three series of measurements. These calculations are made to choose the method that best describes the protonation reaction (Tables 2 and 3). It can be concluded that the standard deviation values are lower for the protonation constants calculated from the data of reconstructed spectra, but they are not much different from the values calculated from the data of the experimental spectra. It indicates that the pK_{BH^+} values obtained from reconstructed spectra are in good agreement with the pK_{BH^+} values obtained from experimental spectra, meaning that there is a little influence of the solvent on the appearance of the spectra.

The standard deviation values calculated for all methods are low, indicating good reliability for these calculations. The same conclusion can be derived from the obtained results for variances given in Tables 2 and 3.

To compare the numerical values of p$K_{BH}{}^+$ obtained from the data of the experimental and of the reconstructed spectra, a *t*-Test (Two-Sample Assuming Unequal Variances) is performed. For compound I, the obtained value of *t* was –0.399, for compound II it was 0.159, and for compound III the *t* value was –0.061. The absolute values, obtained from the *t*-Test are smaller than the critical value, which is 2.23. Hence, it can be concluded that the p$K_{BH}{}^+$ values numerically determined from the data of experimental spectra and those obtained from the reconstructed spectra for the investigated compounds are not significantly different. Also, there is a good agreement between the values obtained with the different methods.

The obtained results for the *t*-Test, the standard deviation and the variances, confirmed that all methods can be used successfully for determination of dissociation constants of protonated form of this kind of compounds.

Also, the p$K_{BH}{}^+$ values calculated using the absorbance values, measured at one wavelength, have similar values with those calculated from the data for absorbance measured at four wavelengths.

REFERENCES

[1] A. R. Jalilian, S. Sattari, M. Bineshmarvasti, A. Shafiee, M. Daneshtalab, Synthesis and in vitro antifungal and cytotoxicity evaluation of thiazolo-4H-1,2,4-triazoles and 1,2,3-thiadiazolo-4H-1,2,4-triazoles-1,2,4-4H-triazoles-thiazoles-1,2,3-thiadiazoles, *Arch. der Pharmazie*, **333**, 347–354 (2000).

[2] N. Guelerman, S. Rollas, M. Uelgen, Synthesis and *in vitro* microsomal metabolism of 4-ethyl-5-(4-fluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione and its potential metabolites, *Boll. Chim. Farm.*, **137** (5), 140–143 (1998).

[3] A. R. Katritzky, C. W. Rees, *Comprehensive Heterocyclic Chemistry*, Vol. **5**, Pergamon Press LTD, London, 1984.

[4] L. Arman-Zubiċ, N. Perišiċ-Janiċ, M. Lazareviċ, A study of the behaviour of some substituted 1,2,4-triazoline-3-thiones in different media, *J. Serb. Chem. Soc.*, **65** (9), 619–630 (2000).

[5] K. C. Ragenovic, V. Dimova, V. Kakurinov, D. M. Gabor, Synthesis of 1-nonanoyl/octadecanoyl 1-4-substituted thiosemicarbazides and substituted 1,2,4-triazoles as biological active compounds, *J. Hetrocyclic Chem.*, **40,** 905–908 (2003).

[6] L. P. Hammett, A. J. Deyrup, A series of simple basic indicators. I. The acidity functions of mixtures of sulfuric and perchloric acids with water, *J. Am. Chem. Soc.*, **54,** 2721–2739 (1932).

[7] H. C. Brown, P. W. R. Schleyer, *The Non-Classical Ion Problem*, Plenum Press, New York, 1977.

[8] J. T. Edward, I. Lantos, G. D. Derdall, S. C. Wong, An acidity function based on thiocarbonyl indicators, *Can. J. Chem.*, **55,** 812–821 (1977).

[9] J. F. Bunnett, F. P. Olsen, Linear free energy relationship concerning equilibria in moderately concentrated mineral acids, *Can. J. Chem.*, **44**, 1899–1916 (1966).

[10] M. A. Paul, F. A. Long, H_0 and related acidity functions, *Chem. Rev.*, **57**, 1–45 (1957).

[11] M. J. Jorgenson, D. R. Harrter, A critical Re-evaluation of the Hammett Acidity Function at Moderate and High Acid Concentrations of Sulfuric Acid. New H_0 Values Based Solely on a Set of Primary Aniline Indicators, *J. Am. Chem. Soc.*, **85**, 878–883 (1963).

[12] R. A Cox, K. Yates, Acidity functions: an update, *Can. J. Chem.*, **61,** 2225–2243 (1983).

[13] C. Tissier, M. Tissier, Propriétés acido-basiques des amides et thioamides, *Bull. Soc. Chim. Fr.*, 2109–2117 (1972).

[14] K. Yates, R. A. McClelland, Mechanisms of Ester Hydrolysis in Aqueous Sulfuric Acids, *J. Amer. Chem. Soc.*, **89**, 2686–2692 (1967).

[15] N. C. Marziano, G. M. Cimino, R. C. Passerini, The M_C Activity Coefficient Function for Acid-Base Equillibria. Part I. New Methods for Estimating pK_a Values for Weak Bases, *J. Chem. Soc. Perkin Trans.* II, 1915–1926 (1973).

[16] R. A Cox, K. Yates, Excess Acidities. A Generalized Method for the Determination of Basicities in Aqueous Acid Mixtures, *J. Am. Chem. Soc.*, **100,** 3861–3867 (1978).

[17] A. Albert, E. P. Serjeant, *The Determination of Ionization constants, Chapman*, London, 1971.

[18] T. E. Edward, S. C. Wong, Ionization of Carbonyl Compounds in Sulfuric Acid. Correction for Medium Effects by Characteristic Vector Analysis, *J. Am. Chem. Soc.*, **99**, 4229–4232 (1977).

[19] C. T. Davis, T. A. Geissman, Basic Dissociation Constants of Some Substituted Flavones, *J. Am. Chem. Soc.*, **76,** 3507–3511 (1954).