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DETERMINATION OF THE DISSOCIATION CONSTANTS OF SOME NEWLY SYNTHESIZED DERIVATIVES OF 1,2,4-TRIAZOLINE-3-THIONE IN SODIUM HYDROXIDE MEDIA

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The behaviour of some newly synthesized derivatives of 1,2,4-triazoline-3-thione (4-buthyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione, 4-allyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione, 4-phenyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione) was studied in aqueous sodium hydroxide solutions at different pH values with UV spectroscopy. The measurements were carried out immediately after preparation of the solutions, in the wavelength range from 190 nm to 360 nm, on a Varian Cary UV spectrophotometer.

When the pH value of the solution increased from 5 to 9, the measured value of the absorbance decreased, while the absorption maximum shifted towards lower wavelengths.

The dissociation constants were determined using the equation $pK_{BH} = \log I + pH$ ($I = c(BH)/c(B^-)$). The ionic strength of the solutions of 0.10 mol/dm^3 was adjusted with addition of $NaClO_4$. The calculations were derived from the values of the absorbance from the spectra obtained experimentally and from the spectra reconstructed by using the method of characteristic vector analysis (CVA). The absorbance values were measured at four wavelengths, on and around the absorption maxima for each investigated compound. Also, the pK_{BH} values were determined graphically and they were in good agreement with those obtained numerically.

The obtained values of the pK_{BH} were in the range from 7.45 to 7.54 and they were in a good agreement with the expected ones for this kind of compounds.

Keywords: 4-buthyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione; 4-allyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione; 4-phenyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione; dissociation constant; UV spectroscopy; dissociation

ОПРЕДЕЛУВАЊЕ КОНСТАНТИ НА ДИСОЦИЈАЦИЈА НА НЕКОИ НОВОСИНТЕТИЗИРАНИ СУПСТИТУИРАНИ 1,2,4-ТРИАЗОЛИН-3-ТИОНИ ВО СРЕДИНА НА НАТРИУМ ХИДРОКСИД

Со UV спектроскопија беше испитано однесувањето на некои новосинтетизирани деривати на 1,2,4-триазолин-3-тионот (4-бутил-5-октил-2,4-дихидро-3*H*-1,2,4-триазолин-3-тион, 4-алил-5-октил-2,4-дихидро-3*H*-1,2,4-триазолин-3-тион и 4-фенил-5-октил-2,4-дихидро-3*H*-1,2,4-триазолин-3-тион) во водни раствори на натриум хидроксид со различни pH вредности. Мерењата беа вршени на UV спектрофотометар VARIAN CARY 50 во подрачјето на бранови должини од 190 nm до 360 nm, веднаш по подготвувањето на растворите.

Со зголемување на pH-вредноста на растворот од 5 до 9, вредноста на апсорбанцата се намалуваше, а апсорпциониот максимум се поместуваше кон пониски бранови должини. Определени беа константите на дисоцијација со помош на изразот $pK_{BH} = \log I + pH$ ($I = c(BH)/c(B^-)$). Јонската сила на растворите беше нагодена со додавање на $NaClO_4$ чија концентрација во конечниот раствор изнесуваше 0,1 mol/dm^3. Пресметките беа вршени од вредностите на апсорбанцата од експериментално добиените и од реконструираните спектри со примена на методот на карактеристичната векторанализа (CVA). Апсорбанцата потребна при овие пресметки беше мерена на четири бранови должини на и околу апсорпциониот максимум, за секое испитувано соединение соодветно. Вредностите на константите на дисоцијација беа определени и графички и беа во согласност со нумерички добиените вредности на pK_{BH}.

Добиените вредности за pK_{BH} се движеа во границите од 7,45 до 7,54 и беа во согласност со очекуваните за ваков вид соединенија.

Клучни зборови: 4-бутил-5-октил-2,4-дихидро-3*H*-1,2,4-триазолин-3-тион; 4-алил-5-октил-2,4-дихидро-3*H*-1,2,4-триазолин-3-тион; 4-фенил-5-октил-2,4-дихидро-3*H*-1,2,4-триазолин-3-тион; спектрофотометрија; константа на дисоцијација; дисоцијација

INTRODUCTION

1,2,4-triazoline and its derivatives belong to the heterocyclic class of compounds and possess interesting biological characteristics [1–5]. These compounds can be used in medicine as antibacterial, antiviral, anticancerous, antiasthmatic, analgesic and antiinflammatory drugs because of their pharmaceutical properties [1–5]. Hence, there is a considerable interest in synthesizing these compounds [6]. The derivatives of 1,2,4-triazole demonstrate less toxicity and more pharmacological activity compared to other drugs.

Because of the wide use of the derivatives of 1,2,4-triazole, it is important to define their acid-base properties and to study the mechanisms of reaction taking place in acid or base media. The biological activity of weak base systems such as 1,2,4-triazoline-3-thione depends on their acid-base equilibrias.

1,2,4-triazoline in a base media behaves as a weak acid and the value of its dissociation constant is 10.26 [7]. The dissociation constants in sodium hydroxide media have been determined only for some substituted derivatives of 1,2,4-triazole [8].

The chemical formulae of the derivatives of 1,2,4-triazole are presented in the Fig. 1.

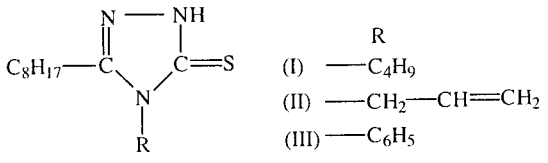


Fig. 1. Chemical formulae of the investigated compounds: (I) 4-buthyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione, (II) 4-allyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione, (III) 4-phenyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione

The investigated compounds were synthesized by Ragenovic and co-workers [9] and their structure was determined using the UV, IR, ^{1}H NMR and ^{13}C NMR spectrophotometric methods. However, there is no literature data for their acid-base properties. The aim of our work was to determine the values of dissociation constants of these compounds.

The dissociation of weak organic acids, such as derivatives of 1,2,4-triazole in base media, can be presented with the equation:

$$BH \rightleftarrows B^- + H^+. \qquad (1)$$

The equillibrium constant for the dissociation reaction presented with the equation (1) can be defined with the equation:

$$K^o_{BH} = \frac{a(B^-)\cdot a(H^+)}{a(BH)}, \qquad (2)$$

i.e. the stoichiometric dissociation constant is:

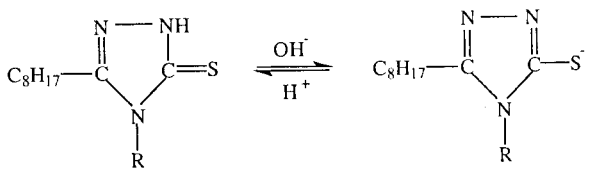
$$K_{BH} = \frac{c(B^-)\cdot c(H^+)}{c(BH)}\cdot\frac{1}{c^o}. \qquad (3)$$

The stoichiometric dissociation constant (pK_{BH}) can be calculated using equation (4):

$$K_{BH} = \log I + \mathrm{pH}, \qquad (4)$$

where $I = \frac{c(BH)}{c(B^-)}$ is the ratio between the concentration of the neutral form (BH) and the dissociated form of the investigated compounds (ionization ratio). The ionic strength of the solution was adjusted with addition of $NaClO_4$ and was 0.10 mol/dm^3.

The substituted 1,2,4-triazoline-3-thiones in aqueous solution of bases behave as weak acids and the values of theirs dissociation constants could be determined. The dissociation reaction of the neutral form of the investigated compounds in a base media can be presented with the following equation (5):



(5)

EXPERIMENTAL

Three series of solutions with different concentration of the investigated compounds: 4-buthyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione (compound I), 4-allyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione (compound II), and 4-phenyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione (compound III) were used for determination of the dissociation constants in sodium hydroxide media.

The investigated compounds were dissolved in 96 % ethanol, at room temperature. The stability of the stock solutions was confirmed by recording UV spectra over a two month period. The concentration of the investigated compounds in the stock solutions were: $1.0 \cdot 10^{-3}$ mol/dm^3, for compound I; $9.7 \cdot 10^{-4}$ mol/dm^3, $1.0 \cdot 10^{-3}$ mol/dm^3 and $9.5 \cdot 10^{-4}$ mol/dm^3 for compound II; and $1.0 \cdot 10^{-3}$ mol/dm^3 for compound III.

The concentration of the investigated compounds in the test solutions was about $2.0 \cdot 10^{-5}$ mol/dm^3. These solutions contained $NaClO_4$ with a concentration of 0.10 mol/dm^3 and sodium hydroxide in order to achive a desired pH value of the solution.

Blanks were prepared with the same composition as the test solution, without the investigated compound. The ethanol content was 1 % in both the test solutions and the blanks.

The UV spectra were recorded on a Varian Cary 50 spectrophotometer, in the wavelenght range from 190 nm to 360 nm, at a room temperature. The pH values were measured on a MA 5705 pH meter. The measurements were carried out immediately after the preparation of the test solutions, because of their instability over the time.

The investigated compounds and all of the other substances used in the experiments were of analytical grade p.a.

RESULTS AND DISCUSSION

The UV spectra of the compounds 4-buthyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione, 4-allyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione, and 4-phenyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione, recorded at different acidity of the solution (from pH 5 to 10) are shown in Figs. 2a, 3a and 4a, respectively. In the wavelength range between 190 nm and 360 nm there is only one intensive band with absorption maximum at about 250 nm for compounds I and II, and at about 255 nm for compound III. This band is probably the result of electron transitions in the 1,2,4-triazoline-3-thione ring [10].

When the pH value of the solution increases from 5 to 9, the absorption maxima shifts towards lower wavelengths. The absorption maximum shifts as follows: for compound I from 250 nm at pH = 5.71 to 235 nm at pH = 9.27; for compound II from 250 nm at pH = 5.64 to 235 nm at pH = 9.58, and for compound III from 255 nm at pH = 5.81 to 240 nm at pH = 9.53. These changes indicate that most likely, a dissociation process took place in the system.

In Figs. 2a, 3a and 4a there is no clearly defined isobestic point around 235 nm. This absence of clearly defined isobestic point is probably the result of the influence of the solvent. The behaviour of these compounds in strong acid media [11] and the investigations of the acid-base reactions for similar compounds [8] showed the influences of the solvent on the spectra. In order to eliminate this influence the experimental spectra were reconstructed using the method of characteristic vector analysis (CVA) [12]. The obtained results are shown in Figs. 2b, 3b and 4b.

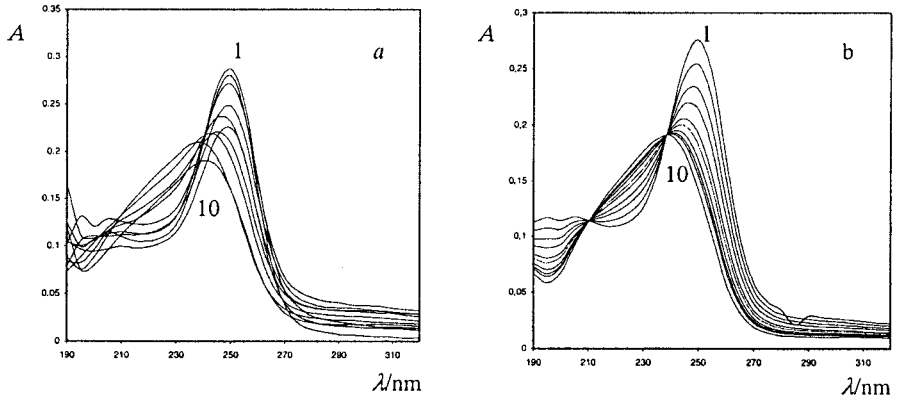


Fig. 2. UV spectra of 4-buthyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione in sodium hydroxide media (*a*) experimental spectra and (b) reconstructed spectra, $c(I) = 2.1 \cdot 10^{-5}$ mol/dm^3, pH from 5.71 (spectar 1) to 9.20 (spectar 10)

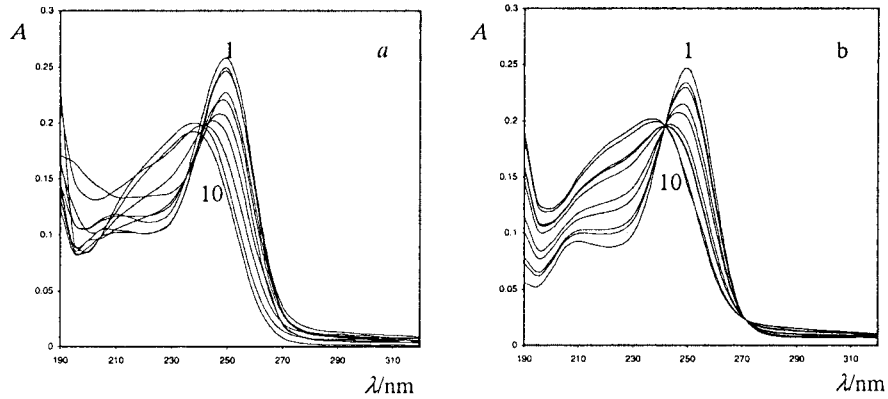


Fig. 3. UV spectra of 4-allyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione in sodium hydroxide media (*a*) experimental spectra and (b) reconstructed spectra, c(II) = $1.9 \cdot 10^{-5}$ mol/dm^3, pH from 5.83 (spectar 1) to 9.48 (spectar 10)

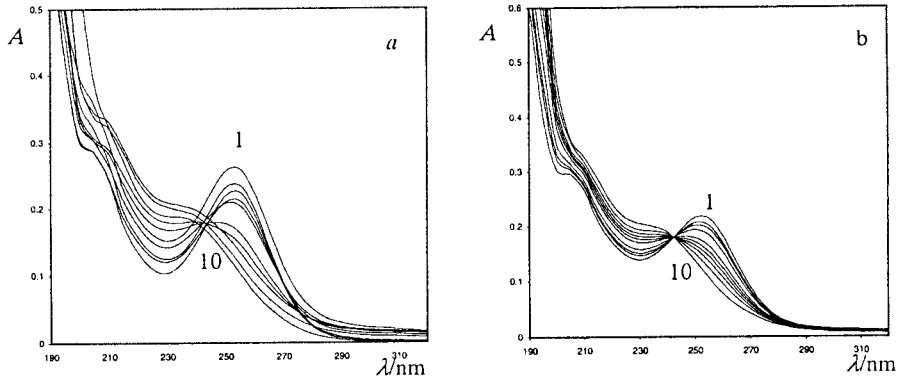


Fig. 4. UV spectra of 4-phenyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione in sodium hydroxide media (*a*) experimental spectra and (b) reconstructed spectra, c(III) = $2.0 \cdot 10^{-5}$ mol/dm^3, pH from 5.65 (spectar 1) to 10.23 (spectar 10)

In the reconstructed spectra of the compound I (Fig. 2b), two isobestic points at 210 nm and 235 nm are observable, while in the reconstructed spectra of the compounds II and III presented in the Figs. 3b and 4b, there is only one isobestic point at 240 nm.

The changes in the spectra could be better noticed from the plot of the absorbance, at given wavelenght, vs. the pH value of the solution (Fig. 5).

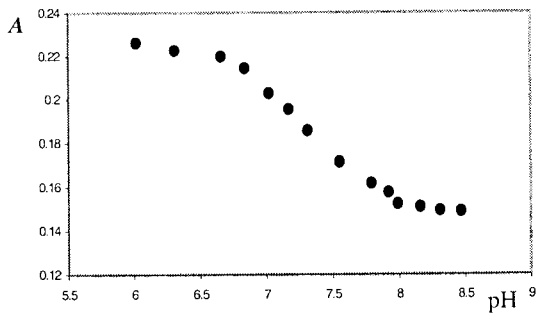


Fig. 5. Absorbance values (λ = 250 nm) as a function of pH of the solution for 4-buthyl-5-octyl-2,4-dihydro-3*H*-1,2,4-triazoline-3-thione with concentration $2.0 \cdot 10^{-5}$ mol/dm^3 in sodium hydroxide media

As it can be seen, this dependence is a sigmoidal curve („S" curve) and has one plateau, suggesting that the dissociation process occurs in one step. The „S" curves of compounds II and III were similar to that of the compound I (plots not shown).

When the pH value increases there is no significant changes in the absorbance value, as it can be noticed from the initial part of the „S" curve (Fig. 5, upper plateau of the „S" curve). In this range of pH values the neutral form of the compounds is most likely dominant.The similar plateau of the "S" curve could be noticed at higher pH values (lower plateau of the „S" curve), where only the dissociated form of the investigated compounds exist in the solution. The interval of pH when the dissociation process took place can be determined from the steep part of the „S" curve.

Namely, the dissociation process of the investigated compounds occurs in the pH values range: from 7.0 to 8.0. These results indicates that the derivatives of 1,2,4-triazole-3-thione are weak acids. The calculations of the pK_{BH} values were made in this pH range.

To determine the pK_{BH} values of the investigated compounds, the values of the absorbance, measured at four selected wavelenghts around the absorption maximum (for the compounds I and II at: 240 nm, 245 nm, 250 nm and 255 nm, while for the compound III at: 230 nm, 240 nm, 250 nm and 260 nm) were used.

The absorbance values at the selected wavelenghts, obtained from the experimental spectra, for different pH values, are shown in Tables 1–3. The pH values below 7 (Tables 1–3) were reached using diluted aqueous solution of sulfuric acid.

The molar absorption coefficient values necessary to calculate $c(BH)$ and $c(B^-)$ for the investigated compounds were determined (at the wavelengths meantioned above) using three solutions with different concentration of each investigated compound: $1.7 \cdot 10^{-5}$ mol/dm^3, $2.1 \cdot 10^{-5}$ mol/dm^3 and $2.5 \cdot 10^{-5}$ mol/dm^3 for compound I; $1.6 \cdot 10^{-5}$ mol/dm^3, $1.9 \cdot 10^{-5}$ mol/dm^3 and $2.3 \cdot 10^{-5}$ mol/dm^3 for compound II; and $1.6 \cdot 10^{-5}$ mol/dm^3, $2.0 \cdot 10^{-5}$ mol/dm^3 and $2.4 \cdot 10^{-5}$ mol/dm^3 for compound III.

Table 1

Absorbance values vs. pH for 4-buthyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione with concentration $2.1 \cdot 10^{-5}$ mol/dm^3

pH	*A* (240 nm)	*A* (245 nm)	*A* (250 nm)	*A* (255 nm)
5.71	0.2096	0.2609	0.2799	0.2402
6.09	0.2151	0.2679	0.2867	0.2501
6.63	0.2166	0.2622	0.2787	0.2433
6.82	0.2124	0.2563	0.2709	0.2375
7.07	0.1989	0.2366	0.2479	0.2162
7.16	0.1859	0.2260	0.2312	0.1993
7.25	0.1797	0.2160	0.2248	0.1886
7.37	0.1753	0.2103	0.2121	0.1824
7.48	0.1702	0.2107	0.2086	0.1693
7.59	0.1666	0.1865	0.1938	0.1508
7.72	0.1652	0.1895	0.1822	0.1458
7.81	0.1724	0.1704	0.1731	0.1437
7.93	0.1686	0.1789	0.1632	0.1391
8.10	0.1903	0.1846	0.1594	0.1198
8.28	0.1964	0.1866	0.1594	0.1167
8.43	0.2029	0.1891	0.1537	0.1058
8.51	0.1998	0.1863	0.1517	0.1050
8.73	0.1968	0.1812	0.1467	0.0987
8.88	0.2061	0.1882	0.1538	0.1072
9.27	0.2086	0.1938	0.1599	0.1151

Table 2

Absorbance values vs. pH for 4-allyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione with concentration $2.1 \cdot 10^{-5}$ mol/dm^3

pH	*A* (240 nm)	*A* (245 nm)	*A* (250 nm)	*A* (255 nm)
5.64	0.1885	0.2324	0.2477	0.2114
6.16	0.1869	0.2337	0.2481	0.2131
6.47	0.1874	0.2220	0.2377	0.2129
6.73	0.1754	0.2204	0.2365	0.2021
6.80	0.1797	0.2216	0.2339	0.1973
7.04	0.1831	0.2135	0.2275	0.1949
7.18	0.1845	0.2054	0.2200	0.1721
7.32	0.1858	0.2124	0.2135	0.1681
7.44	0.1832	0.1987	0.1993	0.1640
7.62	0.1844	0.1958	0.1837	0.1361
7.87	0.1883	0.1976	0.1761	0.1335
7.98	0.1885	0.1830	0.1677	0.1263
8.08	0.1942	0.1859	0.1584	0.1156
8.27	0.2014	0.1869	0.1530	0.1074
8.50	0.2007	0.1862	0.1526	0.1073
8.72	0.2072	0.1890	0.1517	0.1026
8.91	0.1966	0.1795	0.1433	0.0969
9.01	0.2000	0.1819	0.1443	0.0968
9.24	0.1977	0.1798	0.1426	0.0953
9.58	0.1977	0.1792	0.1402	0.0903

Table 3

Absorbance values vs. pH for 4-phenyl-5-octyl-2,4-dihydro-3H-1,2,4-triazoline-3-thione with concentration $2.1 \cdot 10^{-5}$ mol/dm^3

pH	*A* (230 nm)	*A* (240 nm)	*A* (250 nm)	*A* (260 nm)
5.65	0.1199	0.1579	0.2298	0.1996
6.12	0.1240	0.1612	0.2313	0.1999
6.62	0.1515	0.1853	0.2361	0.1839
7.12	0.1535	0.1684	0.2192	0.1765
7.34	0.1585	0.1694	0.2100	0.1487
7.39	0.1583	0.1689	0.2084	0.1442
7.43	0.1527	0.1724	0.1873	0.1429
7.55	0.1614	0.1897	0.1741	0.1453
7.72	0.1683	0.1861	0.1649	0.1376
7.93	0.1785	0.1859	0.1602	0.1208
8.23	0.1895	0.1876	0.1654	0.0987
8.53	0.2015	0.1882	0.1443	0.0872
8.71	0.2001	0.1879	0.1471	0.0916
9.13	0.2032	0.1889	0.1439	0.0877
9.31	0.1999	0.1857	0.1411	0.0857
9.57	0.2007	0.1567	0.1410	0.0828
9.71	0.1997	0.1871	0.1394	0.0827
9.92	0.2053	0.1822	0.1435	0.0837
10.15	0.1884	0.1795	0.1322	0.0738
10.23	0.1929	0.1817	0.1347	0.0757

The values of the absorbance were measured at pH values at which the neutral form of the investigated compounds exist (pH = 6.6 for compound I, pH = 6.7 for compound II, and pH = 6.6 for compound III), and at pH values at which the dissociated forms exist (pH = 8.3 for compound I, pH = 8.7 for compound II and pH = 8.2 for compound III). The results obtained are presented in Table 4.

Table 4

Molar absorption coefficient values of the neutral form and the dissociated form determined from the experimental and the reconstructed spectra for compounds I, II and III

Compound I		*ε (240 nm)	ε (245 nm)	ε (250 nm)	ε (255 nm)
Experimental spectra	ε(B⁻)	70.59	71.12	62.81	46.45
	ε(BH)	101.74	127.25	136.25	118.47
Reconstructed spectra	ε(B⁻)	72.38	73.12	64.55	47.81
	ε(BH)	101.78	127.36	136.36	118.54
Compound II		ε (240 nm)	ε (245 nm)	ε (250 nm)	ε (255 nm)
Experimental spectra	ε(B⁻)	95.84	89.01	73.41	51.66
	ε(BH)	86.95	106.66	113.71	97.82
Reconstructed spectra	ε(B⁻)	95.87	89.08	73.51	51.72
	ε(BH)	86.94	106.66	113.68	97.81
Compound III		ε (230 nm)	ε (240 nm)	ε (250 nm)	ε (260 nm)
Experimental spectra	ε(B⁻)	111.18	98.70	80.30	54.73
	ε(BH)	61.49	78.63	113.08	99.11
Reconstructed spectra	ε(B⁻)	111.18	98.72	80.27	54.80
	ε(BH)	61.46	78.66	113.14	99.15

* $\varepsilon - 10^3$ /mol^{-1}dm^{-2}

In accordance with the Beer's law, the concentrations of the neutral and the dissociated forms were calculated from the calculated values of the molar absorption coefficients and from the absorbances at the selected wavelenghts. An overdetermined system of four equations with two unknown parameters was used for these calculations. Then, the dissociation constants were calculated using the equation (4). The Excel computer program was used for all calculations.

Also, the pK_{BH} values of the investigated compound were determined using values of absorbances measured at one wavelength which corresponded to the absorption maximum (at 250 nm for the compounds I and II and at 255 nm for the compound III). In this case, the value of I can be calculated using the measured absorbance values of the neutral form, the dissociated form, and the absorbance values of the test solution at given pH, according to equation (6):

$$I = \frac{A - A_B}{A_{BH} - A}, \qquad (6)$$

where A_{BH} is the absorbance of the neutral form; A_B^- is the absorbance of the dissociated form; A is the absorbance of the test solution at given pH value.

A_{BH} and A_B^- were determined as average values from the measurement of the absorbances of three solutions with different concentrations of the investigated compounds, and at pH value about 6.6 and about 8.7.

The dissociation constants of the compounds in sodium hydroxide media were also determined graphically. In this case, the graphic value of the dissociation constant was determined as an intercept of the plot of log$I$ vs. pH values.

The average values of the pK_{BH} obtained from the three series of measurements are shown in the Tables 5–8. The values of the standard deviation and the coefficient of variance with 95 % confidence level were determined, too.

Table 5

pK_{BH} values (experimental UV spectra) for compounds I, II and III in sodium hydroxide media, determined at four wavelenghts, μ = 0.1 ($NaClO_4$), t = 20 °C

Compound	pK_{BH} (numerically)	pK_{BH} (graphically)
I	7.60 ± 0.07 *s = 0.06 V = 0.81	7.56 ± 0.05 s = 0.04 V = 0.59
II	7.56 ± 0.02 s = 0.02 V = 0.27	7.55 ± 0.02 s = 0.01 V = 0.21
III	7.46 ± 0.03 s = 0.03 V = 0.39	7.46 ± 0.03 s = 0.02 V = 0.36

* s – standard deviation; V – variance

Table 6

pK_{BH} values (reconstructed UV spectra) for compounds I, II and III in sodium hydroxide media, determined at four wavelenghts, μ = 0.1 ($NaClO_4$), t = 20 °C

Compound	pK_{BH} (numerically)	pK_{BH} (graphically)
I	7.46 ± 0.01 s = 0.007 V = 0.11	7.46 ± 0.01 s = 0.006 V = 0.08
II	7.54 ± 0.02 s = 0.01 V = 0.23	7.53 ± 0.01 s = 0.01 V = 0.19
III	7.45 ± 0.005 s = 0.004 V = 0.06	7.46 ± 0.005 s = 0.004 V = 0.06

Table 7

pK_{BH} values (experimental UV spectra) determined at 250 nm for compounds I and II, and at 255 nm for the compound III, in sodium hydroxide media, μ = 0.1 ($NaClO_4$), t = 20 °C

Compound	pK_{BH} (numerically)	pK_{BH} (graphically)
I	7.53 ± 0.04 s = 0.04 V = 0.58	7.53 ± 0.04 s = 0.03 V = 0.48
II	7.52 ± 0.006 s = 0.005 V = 0.07	7.53 ± 0.01 s = 0.009 V = 0.12
III	7.45 ± 0.02 s = 0.01 V = 0.22	7.45 ± 0.02 s = 0.016 V = 0.22

Table 8

pK_{BH} values (reconstructed UV spectra) determined at 250 nm for compounds I and II, and at 255 nm for the compound III, in sodium hydroxide media, μ = 0.1 ($NaClO_4$), t = 20 °C

Compound	pK_{BH} (numerically)	pK_{BH} (graphically)
I	7.44 ± 0.04 s = 0.04 V = 0.54	7.44 ± 0.04 s = 0.03 V = 0.04
II	7.50 ± 0.005 s = 0.01 V = 0.06	7.50 ± 0.008 s = 0.007 V = 0.09
III	7.45 ± 0.01 s = 0.01 V = 0.14	7.46 ± 0.01 s = 0.01 V = 0.18

Parallely, the values of the dissociation constants for the investigated compounds were calculated from the absorbances obtained after reconstruction of the experimental spectra. These results are shown in the Tables 5–8.

1,2,4-triazoline-3-thione ring can exist in the thiol-form, i.e. it can behave as an acid, as is the case with other heterocyclic thiones. The obtained pK_{BH} values for compounds I, II and III are in a good agreement with the literature data for the pK_{BH} values for the –SH group of the thiol form of pyrimidine-thione, which are in the range between 7.45 and 7.50 [13].

The pK_{BH} values determined using the data obtained from the experimental spectra are higher than those determined from the data of the reconstructed spectra. These differences in the pK_{BH} values appear to the result of the influence of the solvent [11].

The values of the standard deviation for pK_{BH} determined from the data of the reconstructed spectra are lower than the values of the standard deviation for pK_{BH} determined from the data of the experimental spectra. This indicates that the pK_{BH} values obtained from the data of reconstructed spectra are more precise, as expected [11]. The same conclusion could be drawn for the values of the coefficient of variances presented in Tables 5–8.

Compound III has lower values of the dissociation constant compared to the compounds I and II, which is probably the result of the influence of the phenyl group in position 4 of this investigated compound (compounds I and II have an alkyl group in position 4). Hence, compound III is stronger acid than compounds I and II.

The average pK_{BH} values, the value of standard deviation, and the values of the coefficient of variances calculated from the absorbance measured at four wavelenghts from the reconstructed spectra are similar to the pK_{BH} values calculated from the absorbance values measured at the wavelenght which correspond to the absorption maximum (Tables 5–8). Hence, the pK_{BH} values could be determined using the absorbance at one wavelenght, simplifying the calculations.

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