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Spectrophotometric and Potentiometric Determination of Acidic Constants of Oxo-Phenyl Pyridinium Monoxime and Dioxime

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The UV absorption spectra of 1-(1-hydroxyimino-2-oxo-2-phenyl) pyridinium chloride (compound I) and 1-(1-hydroxyimino-2-oxo-2-phenyl)-4-hydroxyiminomethyl pyridinium chloride (compound II) in water solution at different *pH* values have been measured. The spectral changes, with changing *pH*, in aqueous solutions are attributed to the dissociation of individual functional groups of the compounds. The mixed acidic constants (*pK'a*) of the investigated monoxime and dioxime, have been determined spectrophotometrically in the series of *Britton-Robinson*'s buffer solutions in the *pH* range 3.0–5.19 and 7.70–9.90 ($t = 25 \pm 0.5$ °C, $I = 0.2$). The following *pK'a* values have been obtained for monoxime $pK'a_1 = 4.30$ and for dioxime $pK'a_1 = 4.28$, $pK'a_2 = 8.36$.

Thermodynamic acidic constants (*pKa*) have been determined on the basis of potentiometric titrations and they have been found to be $pKa_1 = 4.32$ for compound I and $pKa_1 = 4.27$, $pKa_2 = 8.51$ for compound II. The values obtained by transferring *pK'a* into *pKa* are in good agreement with the values obtained potentiometrically.

(Keywords: Acidic constants; Potential antidotes; Pyridinium oximes)

Spektrophotometrische und potentiometrische Bestimmung der Aciditätskonstanten von Oxo-Phenyl-Pyridinium-Monooxim und -Dioxim

Die UV-Absorptionsspektren von 1-(1-Hydroxyimino-2-oxo-2-phenyl)-pyridiniumchlorid (Verbindung I) und 1-(1-Hydroxyimino-2-oxo-2-phenyl)-4-hydroxyiminomethylpyridiniumchlorid (Verbindung II) wurden in wäßrigen Lösungen bei verschiedenen *pH*-Werten aufgenommen. Die Änderungen in den Spektren, die in wäßrigen Lösungen mit der *pH*-Änderung entstehen, können der Dissoziation der einzelnen funktionellen Gruppen der untersuchten Verbindungen zugeschrieben werden. Die Mischaciditätskonstanten (*pK'a*) des untersuchten Monooxims und Dioxims wurden spektrophotometrisch in einer

Reihe von *Britton-Robinson*-Pufferlösungen in *pH*-Intervallen 3.0—5.19 und 7.70—9.90 ($t = 25 \pm 0.5\,^{\circ}C$; $I = 0.2$) bestimmt: für das Monooxim $pK'a_1 = 4.30$ und für Dioxim $pK'a_1 = 4.28$ und $pK'a_2 = 8.36$. Die thermodynamischen Aciditätskonstanten (*pKa*) wurden aufgrund der potentiometrischen Titration berechnet: $pKa_1 = 4.32$ für die Verbindung I und $pKa_1 = 4.27$ und $pKa_2 = 8.51$ für die Verbindung II. Die durch Übertragung $pK'a$ in *pKa* erhaltenen Werte sind mit den über die potentiometrische Methode erhaltenen Werten in guter Übereinstimmung.

Introduction

Despite of the fact that hundreds of pyridinium oximes were submitted to testing as antidotes in experimental organophosphate (pesticides, nerve gases) poisoning [1, 2], only four of them (PAM-2Cl, TMB-4, obidoxime chloride and HI-6) are used in clinical medicine [3–6].

Recently, the new group of oxo-phenyl-pyridinium monoximes and dioximes have been synthesized and will be tested as potential antidotes. The dioximes of these compounds are characterized by the hydroxyiminomethyl group in the position 4 of the pyridine ring.

The values of acidic constants (*pKa*) are of prime importance for the reactivating efficiency and according to some authors [7–9], it should be such, that sufficient amount of ionized form of oxime is present at physiological *pH*. The optimal *pKa* value of pyridinium oximes is evaluated to be about 8, though oxime HI-6 ($pKa = 7.2$) is an excellent reactivator [10].

Having in mind the above finding, as well as the fact that we could not find any data on acidic constants of similar oximes to the group of oxo-phenyl pyridinium compounds in the available literature, it seemed to us of interest to determine their *pKa* values and thus to contribute to better understanding of their antidotal efficiency.

This report is a continuation of our systematic study on the spectrophotometric and potentiometric determination of acidic constants of phenyl-hydroxyiminoethyl-quinolinium compounds [11].

Experimental

UV spectra in the wave length range 196–450 nm were recorded on a spectrophotometer Varian Super Scan TM-3 in hydrochloric acid and sodium hydroxide solutions, but spectra in *Britton-Robinson*'s buffer solutions in the *pH* range 2.20–10.50 were obtained on Pye Unicam SP-6-550 in λ range 240–370 nm, with quartz cells of 10 mm. A PHM-62 Standard *pH*-meter (Radiometer, Copenhagen) equipped with a glass electrode (Radiometer G 202 B) was used for all *pH* determinations (accuracy $\pm$ 0.01 *pH* units). For potentiometric titrations a TTT 60 titrator with autoburrete ABU 12 (Radiometer, Copenhagen) was used (accuracy 0.001 ml). Ultra-Thermostat Medigen (Dresden) was used for maintaining a constant temperature ($25 \pm 0.2\,^{\circ}C$) during the titrations. The *pKa* values were calculated using a Texas Instruments TI 59.

The compounds I and II were synthesized in the Laboratory of Organic Chemistry, Bosnalijek Sarajevo and were > 99.5% pure. All other chemicals used were of analytical grade purity (Merck); the water was bidistilled. Boiled bidistilled water was used to prepare all the solutions for potentiometric *pKa* determinations.

For the spectrophotometric determination of *pK'a* values, freshly prepared standard water solutions of compounds I and II ($2.5 \cdot 10^{-3}\,M$) were used. The solutions were stable for only one day. The *pH* was adjusted by using 2 *M* solutions of hydrochloric acid and sodium hydroxide. The *Britton-Robinson*'s buffer solutions [12] were used for determinations in the *pH* range (2.20–10.50). The mixtures of phosphoric, boric and acetic acid (0.04 *M*) were stirred together with the corresponding volumes of sodium hydroxide solution (0.2 *M*). The ionic strength of 0.1 *M* was kept constant by addition of potassium chloride solution (2 *M*). The ionic strength of the solutions used for spectrophotometric determinations was 0.2 *M* and it was kept constant by the addition of 2 *M* potassium chloride solution.

For potentiometric determination of thermodynamic *pKa* values, for each probe, freshly prepared solutions were obtained by dissolving corresponding accurately weighted solid substances in boiled and cooled bidistilled water. Standard sodium hydroxide solution (0.1180 *M*) was used for potentiometric titrations, the concentration was determined by titrating standard potassium hydrogenphthalate solution, using phenolphthalein as indicator.

Spectrophotometric Determination of $pK'a_1$ and $pK'a_2$

Standard oxime solutions (1.00 ml of the compound I or 0.50 ml of the compound II) were transferred to a 50 ml volumetric flack, 25.00 ml of *Britton-Robinson* buffer solution (*pH* = 2.20–10.50) and 3.75 ml of potassium chloride solution were added, and the flask was filled up with bidistilled water to the mark.

By the same procedure, the oxime solutions of the same concentrations were prepared in 0.1 *M* solutions of hydrochloric acid and sodium hydroxide.

The measurements were performed immediately after the preparation of probes against reference solutions at 25 ± 0.5 °C and at constant ionic strength (0.2 *M*).

The $pK'a_1$ and $pK'a_2$ values were calculated according to *Albert* [13] using the following equation:

$$pK'a = pH + \log \frac{A_I - A}{A - A_M} \tag{1}$$

A_I and A_M represent the absorbance of the basic (ionized) form and the acid (molecular) form of the compound and A the absorbance obtained at given *pH* and wavelength.

The mixed acidic constants (*pK'a*) were transferred into thermodynamic *pKa* values using the equation:

$$pKa = pK'a + \frac{0.507\, I^{1/2}}{1 + 1.5\, I^{1/2}} \tag{2}$$

also according to *Albert* [13].

Graphical Determination of $pK'a_1$ and $pK'a_2$

A graph $\log\frac{A_I - A}{A - A_M}$ vs. *pH* gives a straight line, the intercept at the abscissa giving the *pK'a* value.

The *pK'a* values can also be determined by following the absorbance changes as a function of *pH* at wavelengths, where these changes are most pronounced.

Potentiometric Determination of pKa_1 and pKa_2

Quantities of 0.01314 g or 0.02627 g of the compound I, and 0.01223 g or 0.01528 of the compound II were transferred into a special potentiometric titration vessel with a double bottom (which could be thermostated) and bidistilled water added up to 20.00 ml. During the titrations the sodium hydroxide solution was added in portions of 0.01 ml until a constant *pH* value is obtained.

A neutralization region *a* (0.3–0.7) was used for the determination of the first acidic constant (pKa_1), for the both compounds (I and II), and *a* (1.3–1.7) for the second acidic constant of compound II, where *a* is the degree of titration which represents the ratio of the amount of added base and the amount of the base required for total neutralization.

Before beginning the titrations, a stream of nitrogen was passed through the solution for 5–10 min, and the inert atmosphere was maintained during the titrations. The solutions were stirred during the addition of sodium hydroxide solution, each *pH* value was read several times and the stirrer was stopped only during readings. The temperature during titrations was kept constant (25 $\pm$ 0.2 °C), using a thermostat.

Before each titration, the *pH*-meter was calibrated by using standard buffers (4.01 $\pm$ 0.01 and 9.18 $\pm$ 0.01).

Determination of pKa_1 and pKa_2 Values

For the calculations of thermodynamic acidic constants (pKa_1 and pKa_2) of compounds I and II the same equations were used as presented in our previous work [11] on the basis of data obtained by potentiometric titrations with sodium hydroxide solutions.

Results and Discussion

The absorption spectra of aqueous solutions of compounds I and II in the UV region are changing with the *pH* of the media. The nature of the absorbing species of the compounds I and II in the solution is the cause of the essential variations in the absorption spectra. The influence of the *pH*'s on the absorption spectra of the compounds are presented in Fig. 1. In acid medium, in the *pH* range 1.10–2.50, the spectrum of the aqueous solution of the compound I possess two absorption maxima at 203 nm and 263 nm, and one weakly pronounced absorption band or shoulder at about 240 nm. At *pH* higher than 2.50, the first absorption maximum slightly shifts bathochromically, while the second maximum and the shoulder gradually shift also bathochromically for about 30 nm and gain in intensity. This behaviour indicates a transition to the ionized species as a

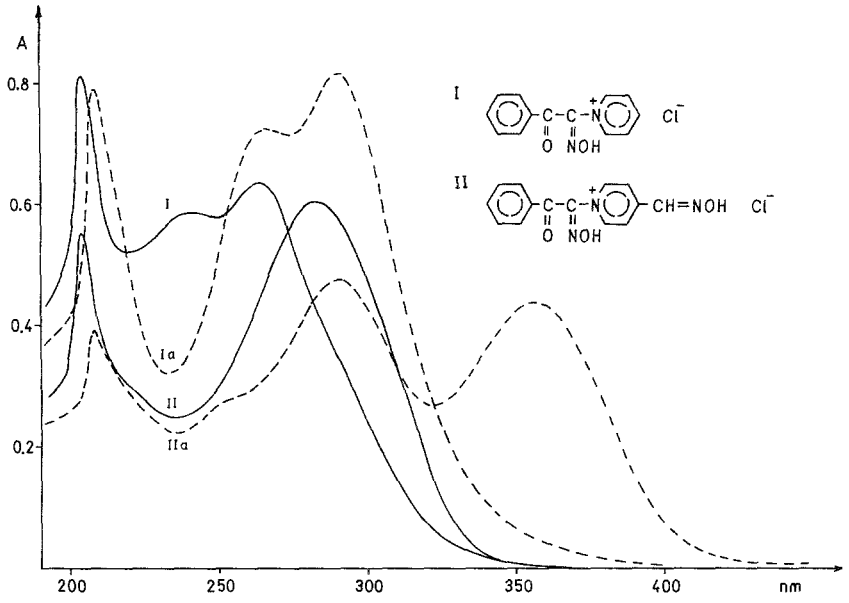


Fig. 1. Absorption spectra of compound I (curves I and Ia) and compound II (curves II and IIa) conc. $5 \cdot 10^{-5} M$ and $2.5 \cdot 10^{-5} M$, respectively; curves I and II were obtained in HCl (0.1 *M*) and curves Ia and IIa in NaOH (0.1 *M*)

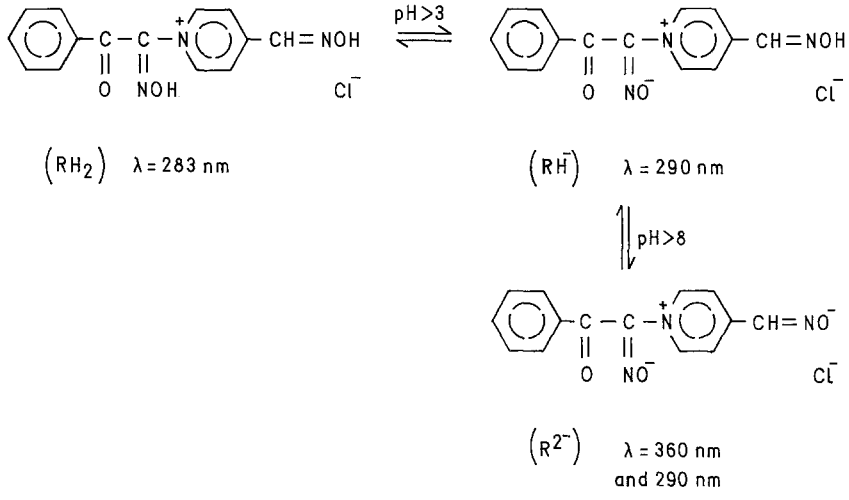
result of the dissociation of the ketoxime group. In the *pH* range 5.58–11.50 there are no significant changes in the spectra, and the absorption band at about 290 nm can be attributed to the chromophore $C_6H_5—\underset{\underset{O}{\|}}{C}—\underset{\underset{NO^-}{|}}{C}—$.

In the absorption spectra of compound II, two distinct *pH*-dependent absorption bands with one sharp isobestic point at about 310 nm were observed. In the *pH* range 1.10–2.40 two absorption maxima at 203 nm and 283 nm were obtained, and the second one corresponds to the acid (molecular) form of the compound II. The new absorption band at about 355–360 nm in the alkaline medium, at *pH* higher than 8.02, corresponds to the basic (ionized) form due to the dissociation of the aldoxime group (of the compound II). Similar phenomena have also been observed in the spectra of other N-substituted pyridinium aldoximes [14]. The first and the second maximum show negligible bathochromic shifts in basic media. The decrease of the second band and the gradual appearance of the third band with increasing *pH* can be attributed to the existence of acid-base

equilibria in the system. In the *pH* region 5.80–6.90 there are no significant changes in the absorption bands.

The dissociation of ketoxime and aldoxime groups of the compound II are presented in Scheme 1.

Scheme 1



On the basis of absorption spectra recorded in *Britton-Robinson*'s buffer solutions in the *pH* range 3.11–5.19 the mixed acidic constant $pK'a_1$ of the compound I was calculated according to Eq. (1) for the wavelengths 235 nm, 280 nm, and 290 nm. For the compound II the first acidic constant ($pK'a_1$) was calculated in the *pH* range 3.0–5.02 at 280 nm, and 290 nm, and for the second acidic constant the *pH* range 7.70–9.90 and wavelengths 270 nm, 350 nm, and 360 nm were used. The results are shown in Table 1. The thermodynamic *pKa* values obtained from mixed $pK'a$ constants according to Eq. (2) are in good agreement with those obtained from potentiometric titrations (Table 2).

In Fig. 2 the dependence of the percentage of mole fraction of each molecular species on *pH* for compound II is presented. The *pH* values are calculated according to the equation:

$$pH = pKa + \log \frac{C_I}{100 - C_I} \tag{3}$$

on the basis of the obtained thermodynamic pKa_1 and pKa_2 constants, where C_I represents the concentration of the ionized species expressed as percentage.

Table 1. *Statistical data on spectrophotometric determinations of* $pK'a_1$ *and* $pK'a_2$; $I = 0.2$ (KCl); $t = 25 \pm 0.5\,^{\circ}C$

Compounds	$pK'a$	$\bar{x}$	n	SD	$S\bar{x}$	$CV(\%)$	pKa
I	$pK'a_1$	4.31	21	0.0740	0.0162	1.72	4.44
II	$pK'a_1$	4.28	14	0.0525	0.0140	1.23	4.41
	$pK'a_2$	8.37	21	0.0708	0.0155	0.85	8.51

$\bar{x}$ mean value; n the number of determinations; SD standard deviation; $S\bar{x}$ standard deviation of mean value; pKa thermodynamic acidic constants for $I = 0$ following Eq. (2)

Table 2. *Potentiometric determinations of* pKa $(t = 25 \pm 0.2\,^{\circ}C)$

Compound	c_{tot}	pKa	$\bar{x}$	n	SD	$S\bar{x}$	$CV(\%)$
I	$2.5 \cdot 10^{-3}$	pKa_1	4.35	18	0.0226	0.0053	0.52
	$5 \cdot 10^{-3}$	pKa_1	4.29	18	0.0303	0.0071	0.71
II	$2 \cdot 10^{-3}$	pKa_1	4.27	25	0.0542	0.0108	1.27
	$2.5 \cdot 10^{-3}$	pKa_1	4.27	16	0.0571	0.0143	1.34
	$2 \cdot 10^{-3}$	pKa_2	8.51	25	0.1480	0.0296	1.74
	$2.5 \cdot 10^{-3}$	pKa_2	8.52	18	0.1470	0.0347	1.73

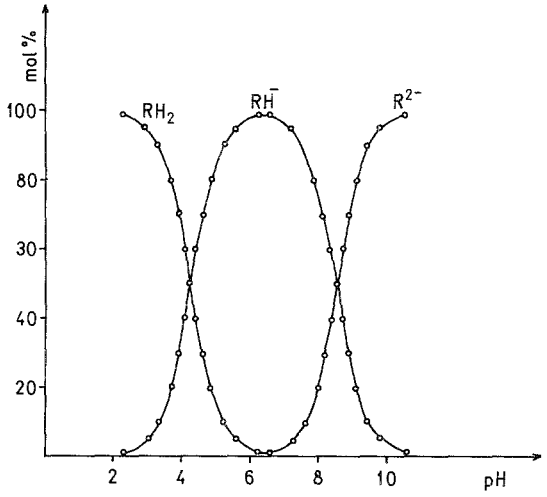


Fig. 2. The dependence of the percentage of mole fraction of each species on pH

In Fig. 3, the dependence of $\log \frac{A - A_I}{A - A_M}$ on pH at $\lambda = 290$ nm for $pK'a_1$ constants of compounds I and II, and at 360 nm for $pK'a_2$ of compound II, are presented. The value of $pK'a_1$ of compound I is 4.30 and of compound II $pK'a_1$ is 4.27 and $pK'a_2 = 8.35$.

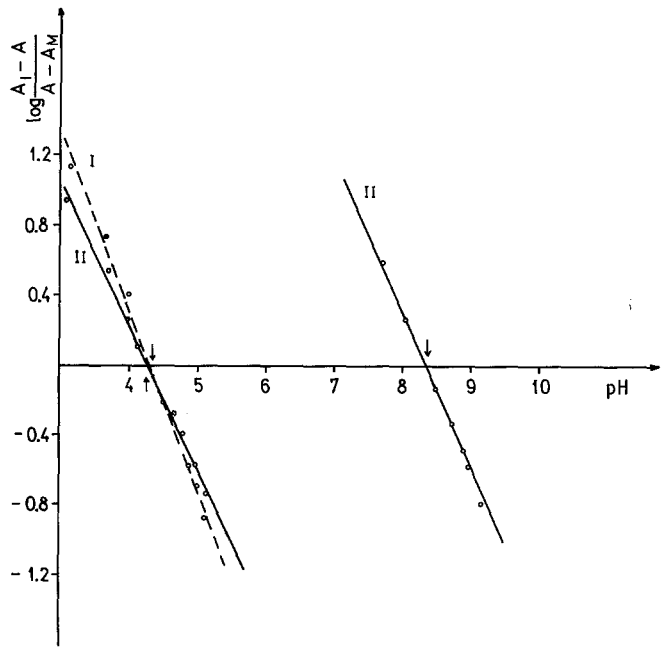


Fig. 3. Spectrophotometric determination of pKa_1 of compound I and II at $\lambda = 290$ nm; pKa_2 of compound II at $\lambda = 360$ nm; $I = 0.2$ (KCl); $t = 25 \pm 0.5$ °C

The $pK'a_1$ values obtained from the inflection points of the titration curves (Fig. 4) are 4.30 and 4.28 for compounds I and II, respectively, and 8.35 for $pK'a_2$ of the compound II.

The thermodynamic acidic constants (pKa) were determined on the basis of potentiometric titrations of compounds I and II with standard sodium hydroxide solution, using the same equations which were presented in our previous work [11]. Each compound was titrated twice with two different initial oxime concentrations. The pKa calculations were performed in the corresponding above mentioned neutralization regions, and the values are given in Table 2. The pKa values obtained from two different titrations are in good agreement. Since the statistical data on potentiometric determinations show that the results are reproducible, which is important for estimating the reactivating efficiency of oximes

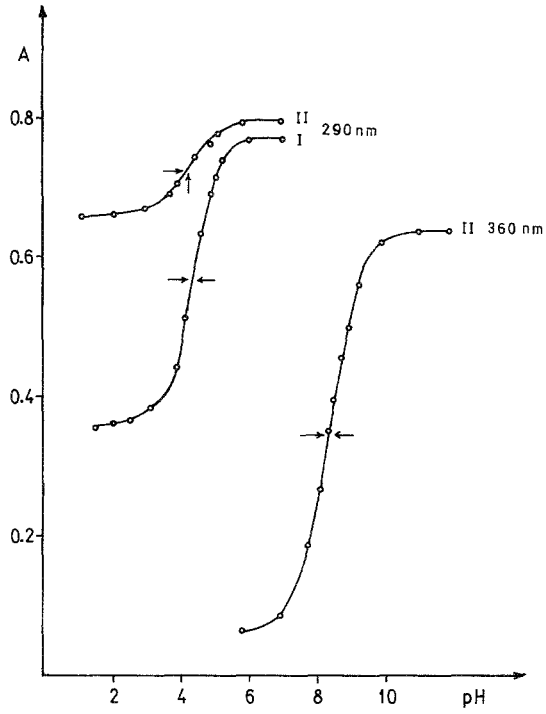


Fig. 4. Changes in the absorbance of oxime solutions as a function of pH; compound I: conc. $5 \cdot 10^{-5} M$; compound II: conc. $2.5 \cdot 10^{-5} M$; $I = 0.2$ (KCl); $t = 25 \pm 0.5\,^{\circ}C$

[15], this method can be successfully applied for the determination of pKa values of oximes.

We presume that the low values of the acidic constants (pKa_1) of the ketoxime group 4.33 and 4.27 of compounds I and II, respectively, are due to the influence of the CO group next to the hydroxiimino group, which is in accordance with the results of pKa of oximes with similar structure [16]. On the basis of this fact, we can say that compound I can't be an effective antidote, because for the reactiving efficiency the pKa value should be about 7–8.

On the basis of the value of the second acidic constants (pKa_2) of the compound II, we presume that this pyridinium dioxime could be used as antidote which will be confirmed by further investigations.

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