Syntheses and Spectrophotometric Studies of Some New Aralkyl Pyridinium Oximes

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Syntheses and some physical properties of seven aralkyl pyridinium oximes, potential antidotes against organophosphorus poisons and potential reagents for pentacyano compounds of iron, are described and discussed. Their UV absorption spectra in water and in water-dioxane solutions 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, and in water-dioxane mixtures they are attributed to the polarities of the solvents. One equilibrium is established in most of the compounds, however, in PhPA-4 and PhOPA-4 two equilibria exist. The dissociation constants of all compounds have been determined spectrophotometrically. The pK's of the compounds are discussed in terms of the structure of the compounds. A spectrophotometric method for the determination of the examined compounds is proposed.

INTRODUCTION

Oximes having different chemical structures are known to form complexes with a number of metal ions,[1] to act as strong inhibitors on the luminol reaction catalysed by organophosphorus compounds or iron complexes,[2] and to form water-soluble coloured complexes[3] with aquopentacyanoferrate(II) ions. Besides, a great number of oximes and particularly mono- and bis--quaternary pyridinium oximes are capable, *in vitro* and *in vivo,* of reactivating cholinesterases inhibited by organophosphorus compounds.[4] Some of them are used today in human therapy as antidotes in organophosphorus poisoning.

In the present work the syntheses of some new aralkyl pyridinium oximes are described and their absorption spectra at different pH's of the media and with regard to the polarities of the solvents are examined. The complexing and antidotal properties of these oximes will be reported on elsewhere. From the spectrophotometric data the pK's of the individual functional groups of the compounds have been determined, and a method for the determination of the compounds is proposed.

EXPERIMENTAL

Syntheses of the Compounds

The seven oximes presented in this work are listed in Table I. 1-Benzyl-4-pyridiniumaldoxime chloride (BPA-4) and 1-phenacyl-4-pyridiniumaldoxime chloride (PhPA-4) were prepared by known methods[5].

TABLE I

Aralkyl Pyridinium Oximes
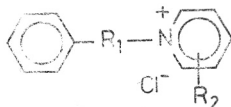
Oxime		R_1	R_2
Name	Abbr.		
1-Phenacyloxime-pyridinium chloride	PhOP	—C(NOH)—CH₂—	—H
1-Phenacyloxime-2-methyl- -pyridinium chloride	PhOPM-2	—C(NOH)—CH₂—	—CH₃
1-Phenacyloxime-4-methyl- -pyridinium chloride	PhOPM-4	—C(NOH)—CH₂—	—CH₃
1-Benzyl-2-pyridinium- aldoxime chloride	BPA-2	—CH₂—	—CH=NOH
1-Benzyl-4-pyridinium- aldoxime chloride	BPA-4	—CH₂—	—CH=NOH
1-Phenacyl-4-pyridinium- aldoxime chloride	PhPA-4	—CO—CH₂—	—CH=NOH
1-Phenacyloxime-4-pyridinium- aldoxime chloride	PhOPA-4	—C(NOH)—CH₂—	—CH=NOH

1-Benzyl-2-pyridiniumaldoxime chloride (BPA-2)

Into a three-necked flask fitted with a mechanical stirrer, dropping funnel and reflux condenser a solution of 0.025 mole of 2-pyridinealdoxime in 50 ml. of nitro-benzene was placed. The solution was heated to 110° and from the dropping funnel 0.03 mole of benzyl chloride was added during one hour under stirring. The heating and stirring were continued for seven hours. After standing at room temperature for five days, nitrobenzene was evaporated *in vacuo,* and green residue was dissolved in methanol. By addition of acetone a light green solid precipitated which was subsequently dissolved in ethanol and precipitated with ether. A light green sub-stance m. p. 214—215° was obtained; yield 50%.

Anal. C₁₃H₁₃ClN₂O (248.7) calc'd.: C 62.8; H 5.2; N 11.3; Cl 14.3%
found: C 62.6; H 4.9; N 11.0; Cl 14.5%

Phenacyloxime pyridinium Compounds — General Procedure

To a solution of phenacyloxime chloride, prepared according to Malatesta *et al.*[6], (0.007 mole) in ether (7 ml.) the corresponding pyridine compound (0.01 mole) in ether (2 ml.) [4-pyridinealdoxime was dissolved in ethanol and ether added (1 : 1)] was added and allowed to stand at room temperature for 7 days. The solution was then evaporated to a small volume and the remainder worked up in a way specific for each compound.

1-Phenacyloxime-4-methyl-pyridinium chloride (PhOPM-4). — The obtained precipi-tate was sucked off and recrystallized from absolute ethanol. White crystals m. p. 233—235° (lit.[8] 235°) were obtained in 94% yield.

1-Phenacyloxime-4-pyridiniumaldoxime chloride (PhOPA-4). — The remainder was poured into 50 ml. of ether a green solid precipitated. After two recrystallizations from boiling ethanol with charcoal added, yellowgreen crystals m. p. 181[0] (lit.[7] 181——183[0]) were obtained. Yield: 67[0]/o.

1-Phenacyloxime-pyridinium chloride (PhOP). — The residual crystalline precipitate was sucked off, dissolved in ethanol and precipitated with ether. This was repeated twice. White crystals m. p. 210[0] (lit.[7] 208—210[0]) were obtained. Yield: 84[0]/o.

1-Phenacyloxime-2-methyl-pyridinium chloride (PhOPM-2). — The solution was evaporated to dryness and the sticky crystalline residue was recrystallized from an absolute ethanol—absolute ether (1 : 1) mixture to give white crystals m. p. 200[0]. Yield: 90[0]/o.

$$Anal.\ C_{14}H_{15}ClN_2O\ (262.7)\ \ calc'd.:\ C\ 64.0;\ H\ 5.7;\ N\ 10.7;\ Cl\ 13.5^0/o$$
$$found:\ C\ 63.6;\ H\ 5.4;\ N\ 11.0;\ Cl\ 13.2^0/o$$

Apparatus

The absorption spectra and all absorbances were measured with a Unicam SP 500 spectrophotometer and 1 cm silica cells were used. A pH-meter with a saturated calomel-glass electrode system was used for the pH measurements, accurate to $\pm$ 0.05 pH units.

The Samples for Measurements

The stock solutions of the compounds were prepared by dissolving the weighed amount in redistilled water. The solutions were stable for a few days.

The UV absorption spectra of the aqueous solutions of the examined oximes were measured at a fixed concentration of oximes and at various pH values in the spectrum range between 200 and 450 nm. The pH was varied from 1 to 12.2 by using appropriate amounts of the solutions of hydrochloric acid, sodium hydroxide and Britton-Robinson's buffer solutions. The absorption spectra of the oximes were also measured in acid and basic 10, 20 and 40[0]/o v/v dioxane-water mixtures. In the solutions the concentration of HCl or NaOH was 0.1 N. The dioxane used was of G. R. Merck. All measurements were made against water at room temperature (25[0] C $\pm$ 0.5) and with the same ionic strength (0.1) of the solutions and this was kept constant by means of sodium chloride. Because of the instability of the oximes in basic solutions the measurements were performed immediately after preparing the mixtures.

RESULTS AND DISCUSSION

The UV Absorption Spectra

The absorption spectra of aqueous solutions of the examined compounds in the UV region change with the pH of the media. The changes are essentially due to variations in the nature of the absorbing species in the solutions. The spectral data of the compounds are shown in Table II and the influence of the pH's on the absorption spectra in Figs. 1—4.

In an acid medium the spectra of the solutions of PhOP, PhOPM-2 and PhOPM-4 possess only one weakly pronounced absorption band or shoulder at about 240 nm, which with the increase of the pH of the medium gradually shifts bathochromically and gains in intensity (Fig. 1). This behaviour indicates an easy transition to the ionized species as a result of the dissociation of the ketoxime group. In a strongly alkaline medium, the absorption band at about 260 nm can be attributed[9] to the chromophore C_6H_5—CH$=$NO$^-$.

The absorption spectra of aldoximes show two distinct pH-dependent absorption bands with one sharp isosbestic point. The absorption band in the acid medium at about 280 nm corresponds to the acid (molecular) form of the compounds; the absorption band in the alkaline medium at about 340 nm corresponds to the basic (ionized) form of the compounds. The gradual disap-

TABLE II

Spectral Data and pK Values of the Oximes. $\mu = 0.1$, $t = 25^0 C$

Oxime	λmax. nm	pH	ε	pK	Group
PhOP	246	1—7	6125		
	258	11—12	7500	9.78 ± 0.04	Ketoxime
PhOPM-2	238	1—8	5750		
	266	11—12	8713	9.99 ± 0.04	,,
PhOPM-4	246*	1—7			
	256	12	9725	9.72 ± 0.02	,,
BPA-2	296	1—6	9925		
	342	10—12	15950	8.01 ± 0.02	Aldoxime
	310**				
BPA-4	281	1—6	12825		
	343	11—12	19500	8.47 ± 0.02	,,
	305**				
PhPA-4	282	1—6	20750		
	260*	1—7			
	345	9.8	23450		
	248	9.8	16900	8.34 ± 0.01	,,
	335	12.2	21750	10.77 ± 0.08	Enol
	440	9.8—11			
	307**	1—9.8			
PhOPA-4	278	1—2.8	19125		
	243	9.4	11000	8.42 ± 0.04	Aldoxime
	340	11—12	21300	10.22 ± 0.06	Ketoxime
	282	11—12	9600		
	304**	1—8.4			

* shoulder; ** isosbestic point.

pearance of the first band and the gradual appearance of the second band with increase of the pH can be attributed to the appearance of acid-base equilibria in the systems. Similar phenomena have also been observed in the spectra of other *N*-substituted pyridinium aldoximes.[10]

The absorption spectra in acid and alkaline dioxane-water mixtures have shown that the ionized form of BPA-2, BPA-4, PhPA-4 and PhOPA-4 may exist as resonance hybrides of the zwitterionic and the quinoid forms of the compounds. The decrease of polarity of the solvent caused the bathochromic shift and diminishes the intensity of the band in the alkaline medium, which is characteristic of the zwitterionic form.[11] An example is given in Fig. 5.

While in solutions of BPA-2 and BPA-4, depending on the pH of the media, only two absorbing species exist, the PhPA-4 and PhOPA-4 solutions contain a larger number of such species. Consequently, the absorption spectra of these compounds, are much more complex. In the spectra of PhOPA-4 up to pH 7 there is one absorption band at 278 nm corresponding to nonionized form I (Scheme 1), while in a weakly alkaline medium where the aldoxime group becomes gradually dissociated, *i. e.* where form II appears, new bands appear at 340 and 243 nm. The latter is of highest intensity at pH 9.4. In the
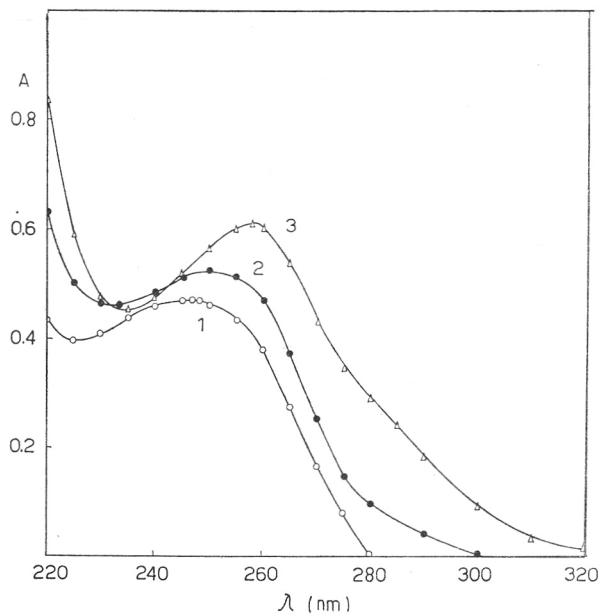
Fig. 1. Ultraviolet absorption spectra of 8×10^{-5} M PhOP solution at: 1 — pH = 1—7; 2 — pH = 9.7; 3 — pH = 12.2.
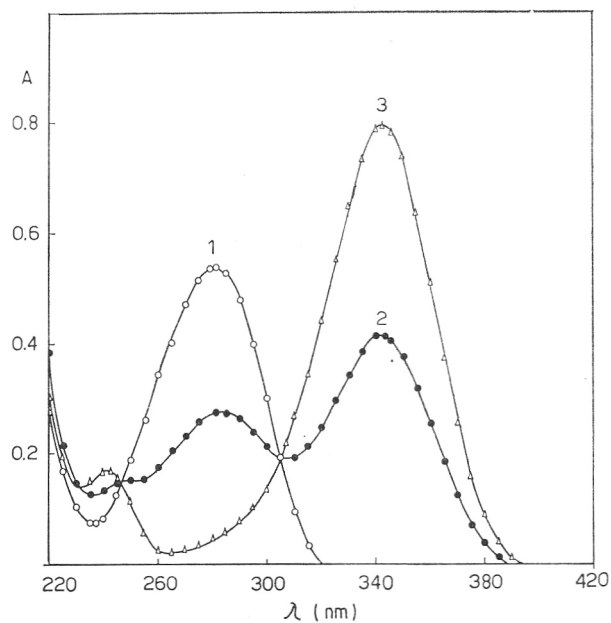
Fig. 2. Ultraviolet absorption spectra of 4×10^{-5} M BPA-4 solution at: 1 — pH = 2—6; 2 — pH = 8.5; 3 — pH = 12.2.

Fig. 3. Ultraviolet absorption spectra of 4×10^{-5} *M* PhPA-4 solution at: 1 — *p*H = 2—6; 2 — *p*H = 8.2; 3 — *p*H = 9.3 4 — *p*H = 11.7.
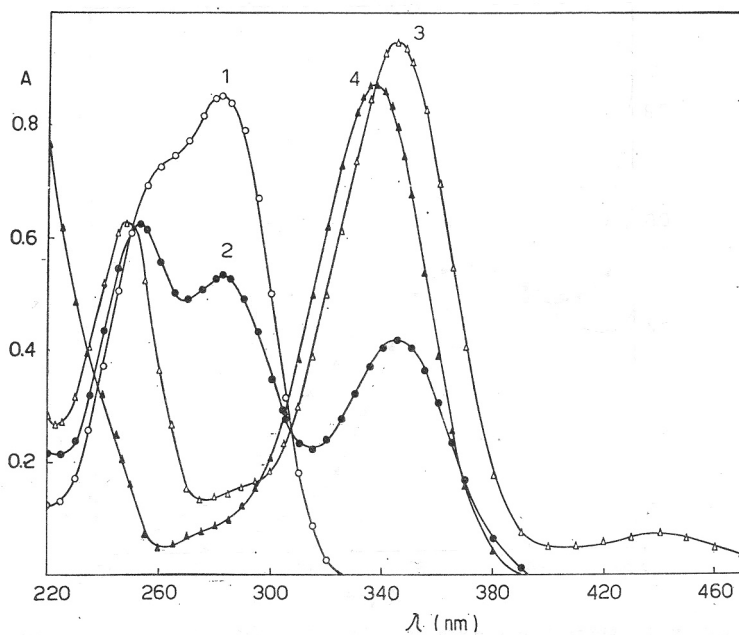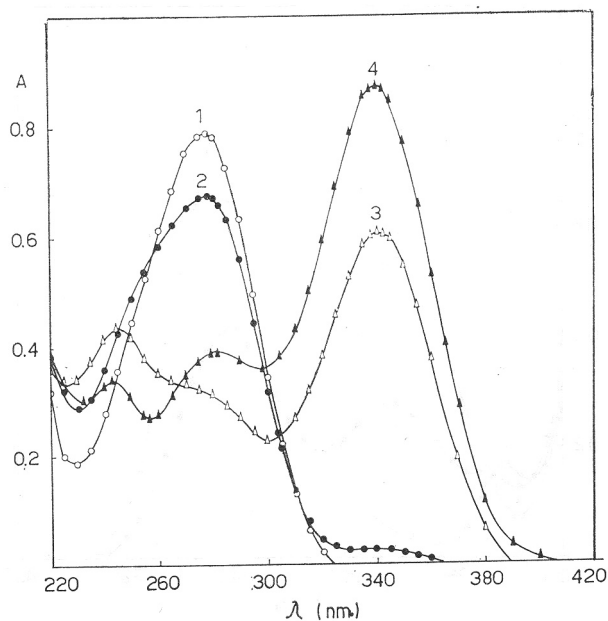

Fig. 4. Ultraviolet absorption spectra of 4×10^{-5} *M* PhOPA-4 solution at: 1 — *p*H = 1.5—2.8; 2 — *p*H = 7; 3 — *p*H = 3.9; 4 — *p*H = 11.5.

spectra an isosbestic point is present up to pH 8.4. The second dissociated form, form III, which is the result of the dissociation of the ketoxime group, is characterized by the appearance of a band at 282 nm and one existing at 340 nm. Both bands reach maximum intensity in a strongly alkaline medium.

Fig. 5. Ultraviolet absorption spectra of 4×10^{-5} M BPA-4 in acid and basic dioxane-water mixtures.
In 0.1 N HCl: 1 — water; 2 — 20 v/v % dioxane; 3 — 40 v/v % dioxane.
In 0.1 N NaOH: 4 — water; 5 — 20 v/v % dioxane; 6 — 60 v/v % dioxane.

SCHEME 1

λ = 278 nm

λ = 340 nm
and 243 nm

λ = 340 nm and 282 nm

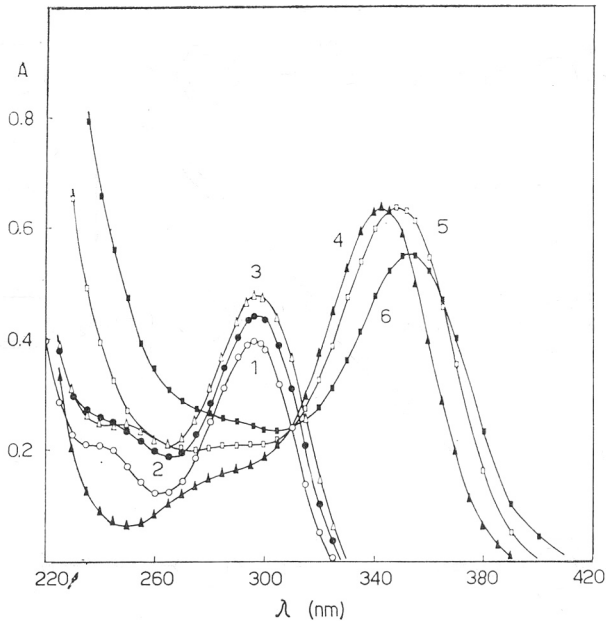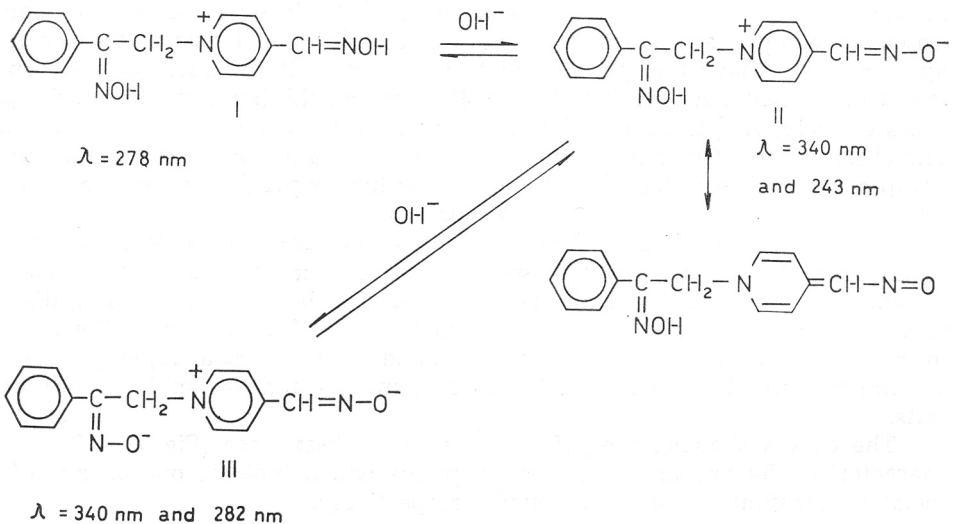Scheme 2 shows the dissociation equilibria and absorbing species in solutions of PhPA-4. The spectral data show that in the media of pH 1—6 only the nonionized form I (at 282 nm) is present in the solution. An increased pH leads to the appearance of form II, characterized by a band at 345 nm. If the

SCHEME 2

λ = 345 nm and 248 nm   λ = 440 nm and 335 nm

pH is above 6, a band appears at 248 nm and has its highest intensity at pH 9.8, which may be attributed to the forms III and IV. This band is also present in the spectra of acid solutions as a shoulder (it is overlaid by the high--intensity band of acid form I). A sharp isosbestic point exists in the spectra up to pH 9.8. Above this pH the band at 345 nm shifts hypsochromically and looses in intensity. At pH 12.2 it is at 335 nm and the band at 248 nm disappears entirely. At pH above 9.8 a new low-intensity band appears at 440 nm. The observed changes indicate that in a weakly alkaline medium the aldoxime group becomes dissociated at first and only at higher pH the enolic form of the compound is dissociated, *i. e.* form V appears.

The absorption bands of the examined compounds, situated in the spectral region from 240 to 340 nm, correspond to the $\pi \rightarrow \pi^*$ transitions of aromatic systems. The low-intensity and unstable absorption band observed for PhPA-4 at 440 nm can, however, be attributed to the $n \rightarrow \pi^*$ transition of the enolic form of the phenacyl group. The $n \rightarrow \pi^*$ band in the spectral region at about 400 nm has also been observed in other phenacyl- and acetonyl- pyridinium salts.[12]

The curves showing the pH dependence of absorbance (Fig. 6 and 7) are characterized by one or two inflection points which indicate one or two dissociation constants of the compounds, respectively.
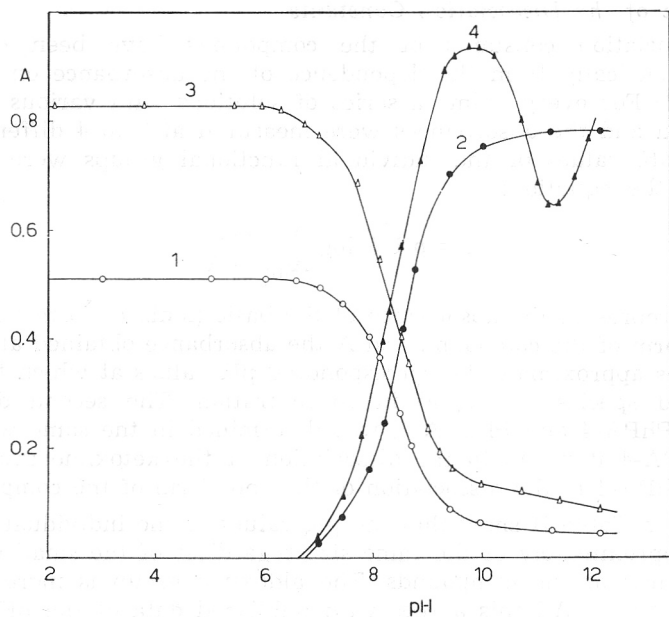
Fig. 6. Absorbance — pH curves. 1 — BPA-4 281 nm; 2 — BPA-4, 343 nm; 3 — PhPA-4, 282 nm; 4 — PhPA-4, 345 nm.
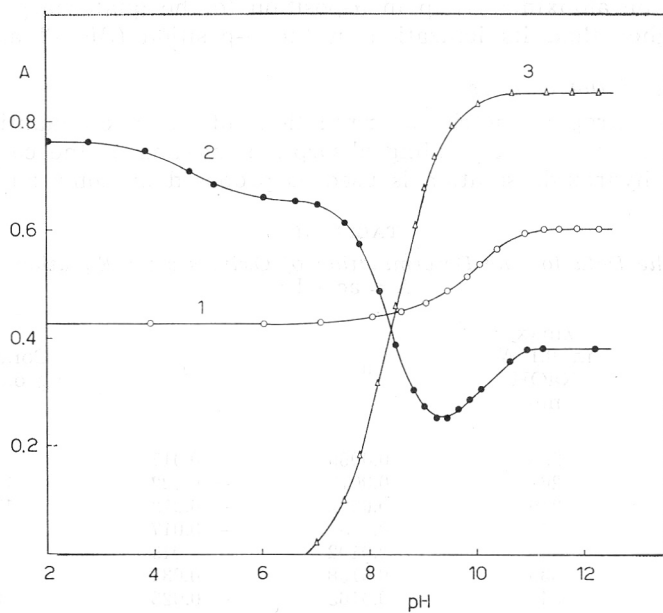

Fig. 7. Absorbance — pH curves. 1 — PhOP, 258 nm; 2 — PhOPA-4, 278 nm; 3 — PhOPA-4, 340 nm.

Determination of the Dissociation Constants

The dissociation constants of the compounds have been determined spectrophotometrically from the dependence of the absorbance on the pH of the solutions.[13] For every oxime a series of solutions with various pH values were prepared and the absorbances were measured at 3 to 4 different wavelength. The pK values of the individual functional groups were calculated according to the equation:

$$pK = pH + \log \frac{A - A_{B^-}}{A_{HB} - A}$$

A_{B^-} and A_{HB} represent the absorbance of the basic (ionized) form and the acid (molecular) form of the compound and A the absorbance obtained at given pH. The pH values approximate the corresponding pK values at which the ionized and unionized species are equal in concentration. The second dissociation constant for PhPA-4 and PhOPA-4, was determined in the same way. In the case of PhOPA-4 it refers to the dissociation of the ketoxime group and in the case of PhPA-4 to the dissociation of the enol form of the compounds.

The data in Table II show that the pK values of the individual functional groups are approximately of the same size regardless of the small differences in the structures of the compounds. The aldoxime group is more acid than the ketoxime group. All this agrees with published data of the pK values of these groups in other similar N-substituted pyridinium oximes.[14] The methyl group in α-position on the pyridine ring (PhOPM-2) somehow decreases the ionization of the ketoxime group, while the methyl group in γ-position (PhOPM-4) has no influence on the dissociation of the group. The position of the aldoxime group on the pyridine ring somewhat changes its ionization. The ionization of the aldoxime group in α-position to the quaternary nitrogen is somewhat higher than its ionization in the γ-position (BPA-2 and BPA-4).

Determination of the Oximes

For the spectrophotometric determinations of the microquantities of the examined oximes the corresponding absorption maxima of the compounds in 0.1 N sodium hydroxide solution is used as proposed by some authors.[15] The

TABLE III

The Data for the Determination of Oximes after Equation
A = ac + b*

Oxime	λmax in 0.1 N NaOH nm	a	b	Conc. range μg oxime/5 ml
PhOP	258	0.0065	— 0.017	10—120
PhOPM-2	266	0.0073	— 0.022	10—120
PhOPM-4	256	0.0082	— 0.012	10—120
BPA-2	342	0.0131	— 0.017	8—60
BPA-4	343	0.0192	— 0.019	8—60
PhPA-4	335	0.0158	— 0.031	8—60
PhOPA-4	340	0.0163	— 0.023	8—60

* A = absorbance measured at λ_{max}. in a 1 cm silica cell; c = μg of oxime in 5 ml 0.1 N NaOH.

standard curves were established by plotting the absorbances against the oxime concentrations. The solutions of oximes obey Beer's law in the concentration range between 10 to 120 µg/5 ml for PhOP, PhOPM-2 and PhOPM-4 and between 8 to 60 µg/5 ml for the other oximes. The data for the equations of the calibration curves calculated by the least square method are given in Table III. Probability errors of analyses for the individual oxime vary from 0.12 to 0.17 µg. The sensitivity of the method (expressed as the amount of oximes corresponding to an absorbance value of 0.05 in a 1 cm cell) is for PhOP, PhOPM-2 and PhOPM-4 from 6 to 11 µg and for the other oximes from 3 to 4 µg.

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IZVOD

Sinteze i spektrofotometrijske studije nekih novih aralkil piridinijumskih oksima

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Opisana je priprema i neke fizikalne karakteristike sedam aralkil piridinijum-skih oksima, spojeva koje možemo smatrati potencijalnim antidotima u trovanjima organofosfornim otrovima i potencijalnim reagensima na pentacijanidne spojeve željeza. Ispitivanim spojevima izmjereni su UV apsorpcijski spektri u vodenim i voda-dioksan otopinama pri različitim pH-vrijednostima. Rezultati su pokazali da postoji znatna ovisnost apsorpcije svijetla o pH vrijednosti otopina kao i o polaritetu otapala. Date su sheme acidobaznih ravnoteža u otopinama ispitivanih spojeva. Spektrofotometrijski su određene konstante disocijacije funkcionalnih grupa spojeva a dobivene pK vrijednosti diskutirane su u vezi sa strukturom spojeva. Na osnovu mjerenja ekstinkcija lužnatih otopina predložena je metoda za njihovo mikroodređivanje.

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