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Spectrophotometric Studies of Some Novel Derivatives of Pyridinium Chloride

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The UV-Vis and IR characteristics of five novel aralkyl derivatives of pyridinium chloride, found to be reversible inhibitors of acetylcholinesterase, are reported. The dissociation constants of the individual keto and oxime functional groups were determined spectrophotometrically and discussed in terms of the known p*K* values of compounds with similar structures. The three examined derivatives of the phenacylpyridinium type contain in solution a considerable proportion of the enol form while their next higher homologues are present predominately in their keto forms. AM1 molecular-orbital calculations show that the much higher acidity of α-CH_2 group of benzoylethylpyridinium type compounds is a consequence of an anomeric effect.

INTRODUCTION

Oximes having different chemical structures are known to form coloured complexes with a great number of metal ions[1] as well as with the aquapentacyanoferrate(II) ion.[2] Besides, many oximes, particularly those of the mono- and bis-pyridinium type, are capable of reactivating *in vitro* and *in vivo* the cholinesterase inhibited by organophosphorus compounds and seem

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to have otherwise multiple pharmacological actions.[3] Some of them are used in human therapy as antidotes against organophosphorus poisons.

The reactions of ketones with the aquapentacyanoferrate(II) were less investigated. Recently, an examination of the reactions of oxo-oximes (precisely quinone oximes) with the aquapentacyanoferrate(II) ion indicated that both the carbonyl and the oxime group participate in complex formation.[4]

Searching for compounds containing oxime and keto groups that could be interesting for further studies of the reactions with the aquapentacyanoferrate(II) ion, and at the same time chemically related to biologically and pharmacologically active molecules, led to the synthesis of five new derivatives of pyridinium chloride. Three of them (1-phenacyl-2-methylpyridinium chloride, 1-benzoylethylpyridinium chloride and 1-benzoylethylpyridinium-4-aldoxime chloride) were found to inhibit human blood acetylcholinesterase reversibly by binding to the catalytic or allosteric or to both sites of the enzyme.[5]

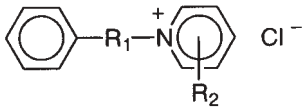
In the present work, UV-Vis and IR characteristics of the prepared compounds are presented. From spectrophotometric data, the dissociation constants of individual functional groups have been determined and the schemes of acido-basic equilibria prevailing in the examined solutions have been established. Complexing properties of these substances will be reported elsewhere.

EXPERIMENTAL

The five compounds examined in this work are listed in Table I. They were synthesized by the general procedure of mixing equimolar solutions of 2-chloroacetophe-

TABLE I

The examined derivatives of pyridinium chloride



Name	Abbr.	R_1	R_2
1-Phenacylpyridinium chloride	FP	$-COCH_2-$	–H
1-Phenacyl-2-methylpyridinium chloride	FPM-2	$-COCH_2-$	$-CH_3$
1-Phenacyl-4-methylpyridinium chloride	FPM-4	$-COCH_2-$	$-CH_3$
1-Benzoylethylpyridinium chloride	BEP	$-COCH_2CH_2-$	–H
1-Benzoylethylpyridinium-4-aldoxime chloride	BEPA-4	$-COCH_2CH_2-$	–CH=NOH

none or 3-chloropropiophenone (Aldrich®) and the corresponding pyridine compound (Fluka Chemie AG).[5] Their aqueous solutions were stable for several months. Britton and Robinson buffers were prepared by mixing 100 mL of a phosphoric, boric and acetic acid mixture (all 0.04 M) with different volumes of 0.2 M sodium hydroxide. The constant ionic strength of 0.1 M was maintained with sodium chloride. The pK values of particular functional groups were determined by spectrophotometric titration.[6] Absorption measurements were made at 25 °C against water on a Hewlett Packard 8451-A diode array spectrophotometer with 1 cm silica-glass cells. Spectra were run immediately and after the solutions were standing long enough to reach equilibrium (about one hour). The latter absorbance values were used for pK calculations. IR spectra were recorded in KBr pellets on a 580-B Perkin-Elmer apparatus.

RESULTS

Electronic Spectra

The aqueous solutions of 1-phenacylpyridinium chloride (FP) and its 2- and 4-methyl derivatives (FPM-2 and FPM-4) exhibit analogous spectra with one maximum at 251–256 nm (ε = 16500–21500 M^{-1} cm^{-1}) in the pH range from 1 to 9, deriving from the unionized keto forms of the compounds. At higher pH values, this maximum loses in intensity along with the appearance of a new one of appreciably lower intensity (ε = 4000–6000 M^{-1} cm^{-1}) at 390–400 nm, which instantly begins to fall ($k_{\text{of the maximum fall}}$ = $2.58 \cdot 10^{-3}$ s^{-1}; $t_{1/2}$ = 268 s). It reaches maximal absorbance at pH about 11 and is attributed to the enolic form of the compounds. At pH about 12, a new maximum separates around 260 nm, probably belonging to the dissociated forms of the compounds (Figure 1 and Scheme 1). A plot of the absorbance measured at 251–256 nm *vs.* the solution pH (1–12.5) demonstrates the characteristic shape of a weak acid titration curve and gave the pK value of 10.95 ± 0.06 for FP and pK > 11 for its methyl derivatives. Exact pK values for the latter were not determined because it was impossible to reach higher pH values at the given ionic strength. Thus, the presence of methyl groups on the pyridine ring somewhat decreases the dissociation constant for the formation of the enolate ion.

The absorption spectra of 1-benzoylethylpyridinium chloride (BEP) show, in the pH range from 1 to 5, only one maximum at 252 nm (ε = 15000 M^{-1} cm^{-1}) characteristic of compounds of closed structures, as are the 1-phenacylpyridinium salt and its methyl derivatives. It is associated with the unionized keto form of the compound. With increasing pH, this maximum gradually weakens and shifts to 260 nm (ε = 12250 M^{-1} cm^{-1}), reaching maximal absorbance at pH about 8–11 where the compound is completely ionized and present in its basic form. At pH values over 11, this

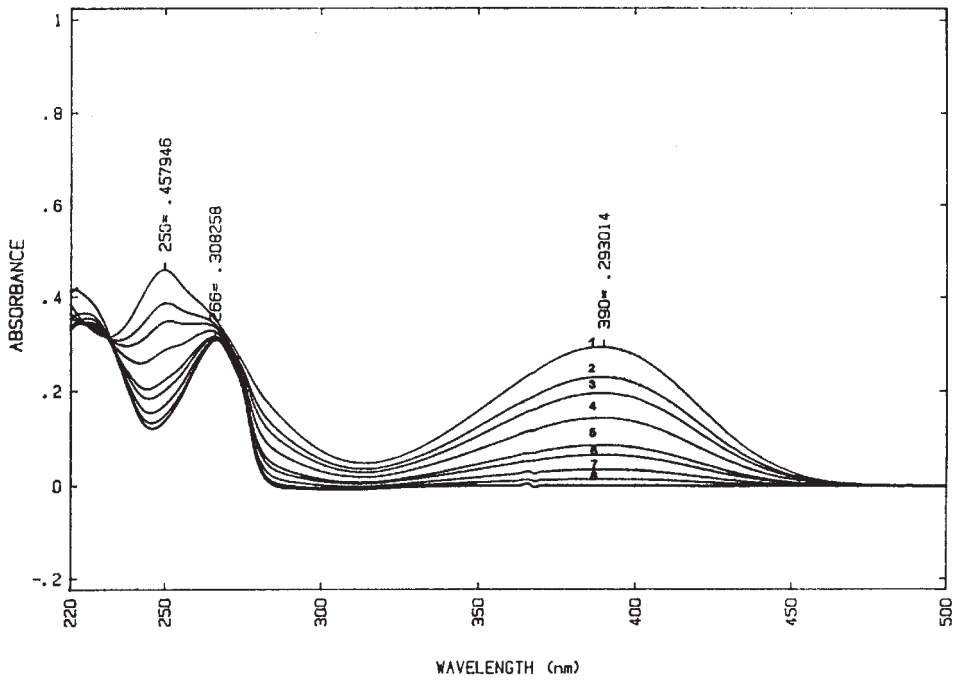
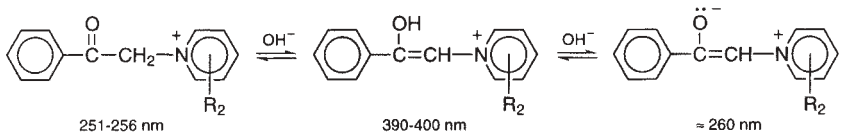


Figure 1. Variation of the spectrum of FPM-2 ($c = 5 \cdot 10^{-5}$ M) at pH = 10.8 as a function of time. Curves 1–9 were monitored from 0.5 to 35 min.



Scheme 1

maximum disappears and a new one forms at 248 nm ($\varepsilon \approx 14000$ M^{-1} cm^{-1}). From the dependence of the absorbances measured at 248, 252 and 260 nm on the pH of the solutions, a pK value of 6.67 ± 0.05 is obtained.

The absorption spectra of 1-benzoylethylpyridinium-4-aldoxime chloride (BEPA-4) are more complex. In very acidic medium, two maxima of similar absorption coefficients ($\varepsilon \approx 18000$–$19000$ M^{-1} cm^{-1}) appear at 256 and 280 nm, belonging to the keto and oxime group of the undissociated compound, respectively. With increasing pH, the second maximum drops in intensity while the first shifts to 252 nm and reaches maximal absorbance at pH about 8 when a new very unstable band at 340 nm ($k_{\text{of the band instability}} = 9.77 \cdot 10^{-4}$ s^{-1}, $t_{1/2} = 709$ s) begins to form (Figure 2). This reaches maximal absorbance at

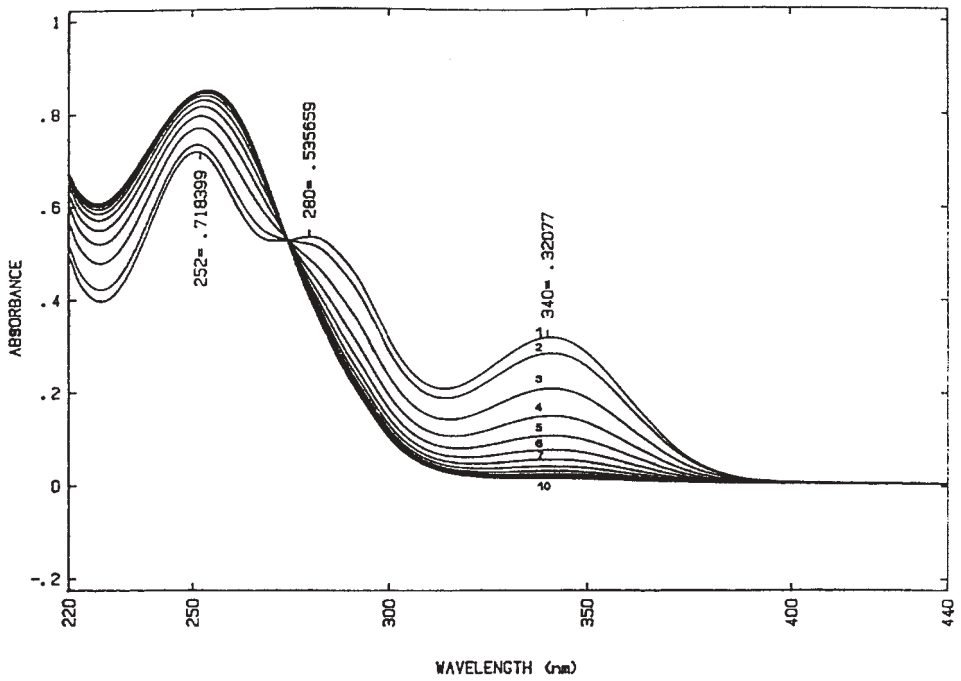
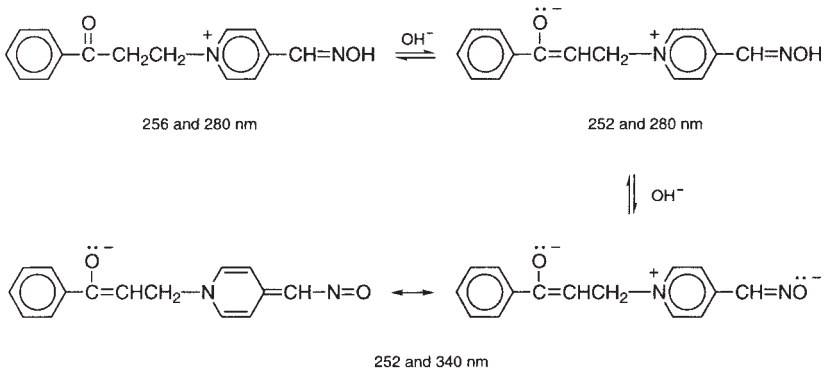


Figure 2. Variation of the spectrum of BEPA-4 ($c = 4 \cdot 10^{-5}$ M) at pH = 8.31 as a function of time. Curves 1–10 were monitored from 0.25 to 60 min.



Scheme 2

pH about 11.5 (ε = 17500 M^{-1} cm^{-1}) and corresponds to the ionized, basic form of the oxime (Scheme 2). Two dissociation constants have been determined (Figure 3). The first, $pK_1 = 6.70 \pm 0.05$, probably refers to the α-methylene group while the second, $pK_2 = 9.97 \pm 0.04$, to the oxime group.

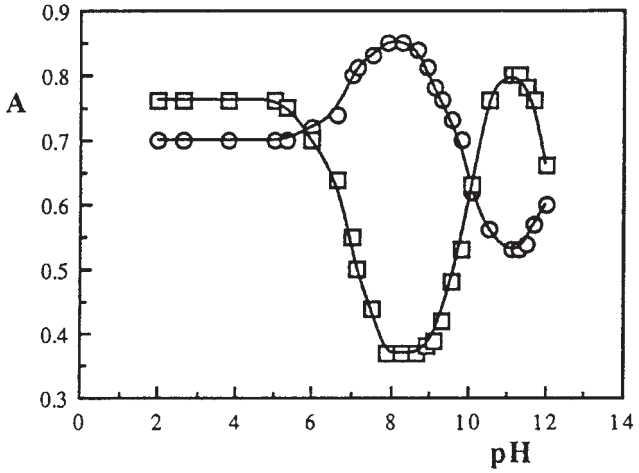


Figure 3. Absorbance dependence of BEPA-4 solutions ($c = 4 \cdot 10^{-5}$ M) on pH. □ – 252 nm; ○ – 280 nm.

The spectral data and pK values of the examined compounds are summarized in Table II.

TABLE II

Spectral data and pK values of the examined compounds

Abbr.	pH range	λ_{max}/nm	ε/M^{-1} cm^{-1}	pK
FP, FPM-2 FPM-4	1–9	251–256	16500–21500	10.95 ± 0.06 >11 >11
	≈11	251–256 390–400	≈9200 4000–6000	
	>11	≈270	≈6200	
BEP	1–5	252	15000	6.67 ± 0.05
	8–11	260	12250	
	>11	248	14000	
BEPA-4	1–5	256 280	18000 19000	6.70 ± 0.05 9.97 ± 0.04
	8	252 280 340	18000[a], 21250[b] 13400[a] 8000[a], 0[b]	
	11.5	340	17500	

IR Spectra

The wavenumbers of the characteristic IR absorption bands of three of the examined compounds and their tentative assignements are as follows:

1-Phenacyl-2-methylpyridinium chloride

1710 cm^{-1} ($\nu_{C=O}$), 698 cm^{-1} and 768 cm^{-1} (substitution of the two aromatic rings at position 1), 723 cm^{-1} (substitution of the pyridine ring at position 2).

1-Benzoylethylpyridinium chloride

1690 cm^{-1} ($\nu_{C=O}$), 690 cm^{-1} and 770 cm^{-1} (monosubstitutions at the two aromatic rings)

1-Benzoylethylpyridinium-4-aldoxime chloride

1700 cm^{-1} ($\nu_{C=O}$), 1620 cm^{-1} and 1660 cm^{-1} ($\nu_{C=N}$), 1010 cm^{-1} (ν_{NO}), about 3000 cm^{-1} and 1460 cm^{-1} (ν_{OH}), 703 cm^{-1} and 768 cm^{-1} (substitution of the two rings at position 1), 826 cm^{-1} (substitution at position 4)

DISCUSSION

The UV absorption bands of the examined compounds situated in the spectral region from 246 to 340 nm correspond to $\pi \rightarrow \pi^*$ transitions. Those located around 250 nm belong to transitions of aromatic systems while the two bands at 280 and 340 nm, which appear in the spectra of BEPA-4, are characteristic of pyridinium aldoximes and can be attributed to the conjugation of the oxime group with the aromatic nucleus.[7,8] The lower intensity unstable absorptions at 390–400 nm observed in the spectra of FP and its methyl derivatives (FPM-2 and FPM-4) are attributed to $n \rightarrow \pi^*$ transitions within their enolic forms, as it was done for other compounds of the phenacylpyridinium and acetonylpyridinium type.[9] They undergo a blue shift with increasing polarity of the solvent.

No such $n \rightarrow \pi^*$ transitions have been detected in the spectra of BEP and BEPA-4. One of the reasons can be found in the extreme instability of these absorptions, but the fact probably refers to the different keto-enol equilibrium existing in the solutions of the latter two compounds. The formation of the enol form of 1-phenacylpyridinium chloride and its methyl derivatives leads to the conjugation of the double bond with the pyridine and the benzene nucleus while such conjugation is not possible in the case of BEP and BEPA-4 having one methylene group more.

The hydrogen atoms of the α-methylene group in FP and its methyl derivatives are doubly activated by the carbonyl group and by the neighbouring positive charge of the pyridinium nitrogen. Thus, one might expect that the solutions of FP, FPM-2 and FPM-4 contain considerable proportions of the enol form, while the equivalent equilibrium in BEP and BEPA-4 is more displaced toward their keto forms. Contrary to that, the pK values of 6.67 and 6.70 obtained for the latter two compounds indicate their accentuated dissociation ability in comparison with FP, FPM-2 and FPM-4 (p$K \approx 11$), 1-phenacyl-4-pyridiniumaldoxime chloride (pK = 10.77)[9] and benzoylacetone (pK = 8.24).[10]

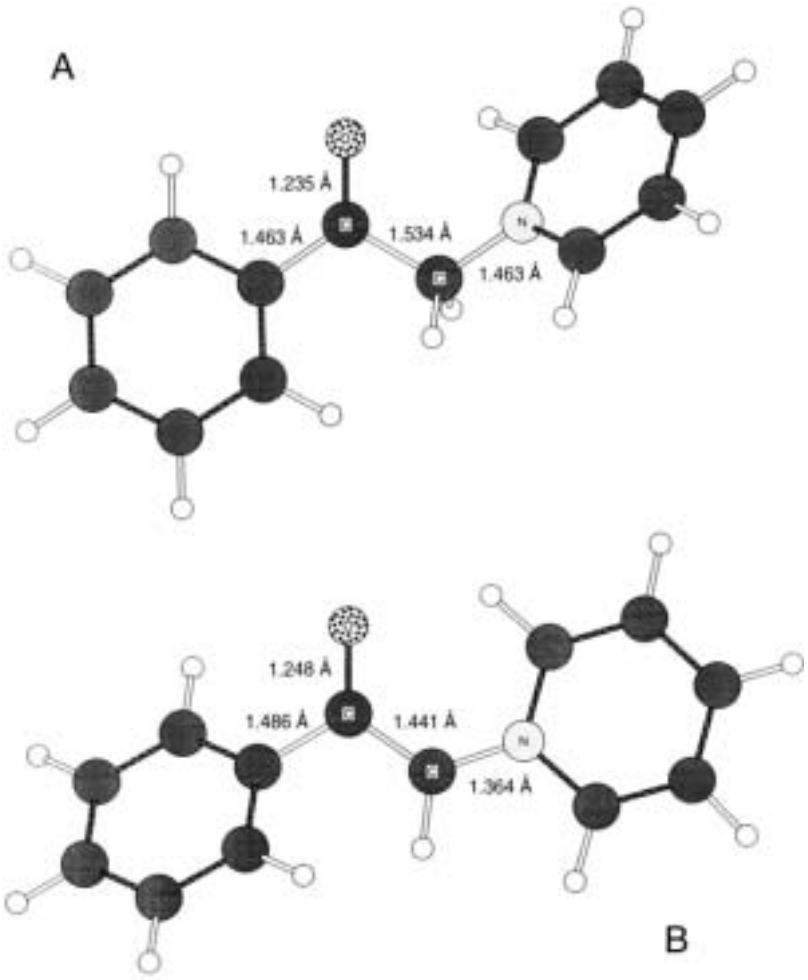


Figure 4. The most stable gas phase conformer of FP (A) and its conjugated base (B), calculated by AM1 method.

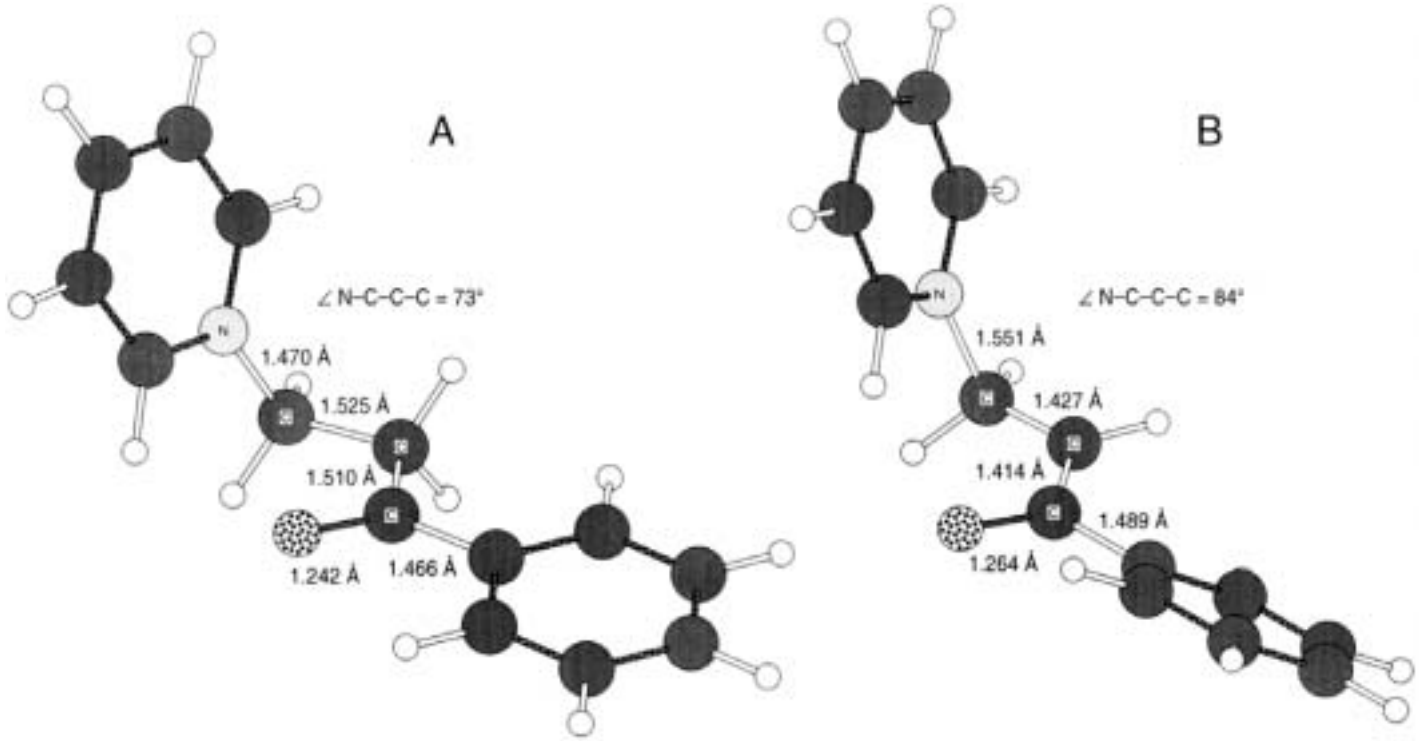


Figure 5. The most stable gas phase conformer of BEP (A) and its conjugated base (B), calculated by AM1 method.

To explain this seemingly paradoxical observation, we conducted a series of semiempirical molecular-orbital quantum-mechanical calculations on both ionized and non ionized forms of FP and BEP. At AM1 level of theory,[11] geometries of all conformers of the four relevant species were fully optimized (MOPAC 97[12]), and structure changes and energy differences between ionized and non-ionized forms were compared. The most stable conformers in the gas phase are depicted in Figures. 4 and 5.

Calculated gas phase energy required to form enolate from the corresponding ketone is by 26 kcal/mol (109 kJ/mol) smaller for BEP than for FP, which is in qualitative agreement with experimental p*K* values.

Analysis of molecular orbitals of the most stable conformer of the ionized form of BEP (A, Figure 5) shows that its extra stabilization, and consequently enhanced acidity of BEP, comes from the interaction between enolate HOMO (ψ_2) and the neighbouring, conveniently positioned antibonding C_β–N orbital (σ_{CN}*) (Figure 6). The result of this interaction (anomeric effect[13]) is a significant C_β–N bond elongation (0.081 Å calculated by AM1 method), and C_α–C_β bond shortening (0.098 Å), which can also be seen as increased contribution of structure C in the enolate ion resonance (Scheme 3).

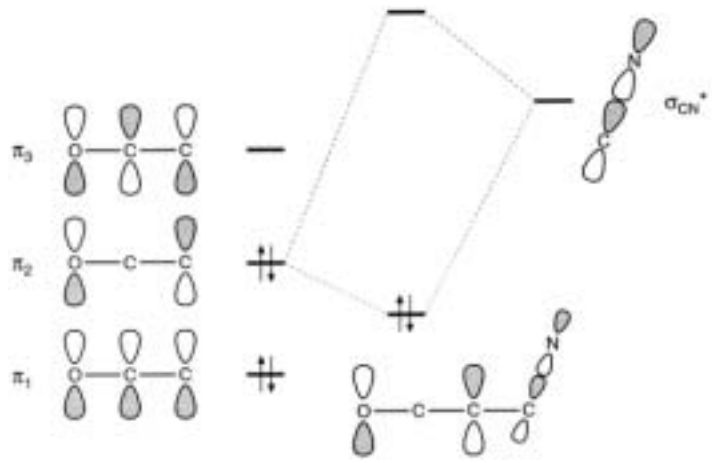
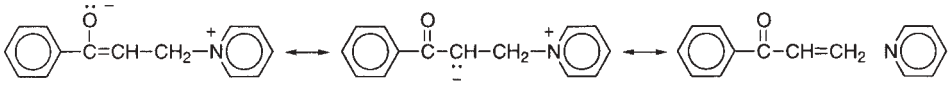


Figure 6. Stabilizing interaction between HOMO of enolate ion (ψ_2) and the antibonding sigma orbital of C–N bond (σ_{CN}*).



A B C

Scheme 3

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SAŽETAK

Spektrofotometrijske studije nekih novih derivata piridinijeva klorida

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Opisane su UV-Vis i IR spektralne karakteristike pet novih aralkil-derivata piridinijeva klorida za koje je poznato da su reverzibilni inhibitori acetilkolinesteraze. Spektrofotometrijski su određene konstante disocijacije pojedinačnih karbonilnih skupina te oksimske skupine, te su dobivene vrijednosti uspoređene s literaturnim vrijednostima p*K* tih skupina u spojevima sličnih struktura. Tri ispitivana spoja fenacilpiridinijeva tipa sadrže u otopini znatan udio enolnog oblika dok su njihovi neposredni viši homolozi prisutni pretežno u svojim keto-oblicima. Molekulsko-orbitalni računi AM1 pokazuju da je znatno veća kiselost α-CH_2 skupine kod spojeva benzoiletilpiridinijeva tipa posljedica anomernog efekta.