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DEPENDENCE OF THE INHIBITOR LEAVING GROUP EFFECT ON THE STRUCTURE OF ITS PHOSPHORYL PART IN THE REACTION OF CHYMOTRYPSIN WITH O-*n*-ALKYL METHYLPHOSPHONIC ACID THIOESTERS

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It is shown that the influence of the structure of the aliphatic leaving group -SX in thiophosphonates $(C_2H_5O)(CH_3)P(O)SX$ (I) and $(n\text{-}C_6H_{13}O)(CH_3)P(O)SX$ (II), where X = $-C_nH_{2n+1}$ and $-(CH_2)_mSC_nH_{2n+1}$, upon the second-order rate constants of the inhibition of chymotrypsin activity can be described by the equation $\log k_i = \log k_{i,o} + \rho^* \sigma^* + \varphi\Pi$, where both the inductive effect and the hydrophobicity of X are considered. The substitution of the ethyl group in (I) for *n*-hexyl in (II) resulted in a change in the form of the dependence of the reaction rate upon the leaving group hydrophobicity, and a decrease in ρ^*. The data point to electrophilic solvation of the reaction center of the inhibitor in the "oxyanion hole" of the enzyme as a result of the interaction of the *n*-hexyl chain of (II) with the hydrophobic binding pocket in the chymotrypsin active site.

The Hammett ρ^- value for the leaving group effect in α-chymotrypsin-catalyzed hydrolysis of aromatic ester substrates has been shown to depend on the structure of the acyl portion of the compounds. Thus, the values of $\rho^- = 0.45$ and 0.63 have been obtained for N-acylamino acid derivatives[1,2] whereas the higher value $\rho^- = \sim 2$ has been determined in a series of phenyl acetates[3,4].

It has been suggested by Williams[1] that the low ρ^- values in the acylation of chymotrypsin (CT) by amino acid substrates are determined by electrophilic assistance of the formation of tetrahedral transition state by hydrogen bonding between the oxyanion, developed from the carbonyl group of the substrate, and the backbone NH groups of Gly 193 and Ser 195 of the enzyme, i.e. the oxyanion hole[5,6]. Alternatively, hydrogen bonding between the enzyme NH groups and the substrate leaving group oxygen has been proposed[2].

By analogy with non-enzymic nucleophilic displacement at the tetrahedral phosphorus in O,O-dialkyl phosphates and O-alkyl alkylphosphonates[7,8] it is reasonable to assume that the reaction of organophosphorus inhibitors $(RO)(CH_3)P(O)SX$ with CT proceeds *via* the direct displacement as well, and the P=O double bond is not affected by the process. The ρ^* value of the reaction should then be primarily affected by the solvation of the inhibitor leaving group rather than the P=O bond. Hence it is of interest to study whether the leaving group inductive effect in the reaction of CT with these compounds depends on the structure of their phosphoryl part. To clarify this question we have studied the kinetics of the reaction of CT with two series of the inhibitors $(RO)(CH_3)P(O)SX$. In the series (I), the substituent $R = -C_2H_5$ is too short for binding in the hydrophobic pocket[9] in the active site of CT whereas the n-hexyl group as R in the series (II) is of optimal length for binding in this pocket[10].

The second-order rate constants of the phosphorylation of the active site of CT by the above compounds are listed

in Table 1. Figure 1 shows the dependence of log k_i upon the substituent hydrophobicity constant π_X for the inhibitors of the series (I) with *n*-alkyl chain in the leaving group. It can be seen that, up to the length of normal hydrocarbon chain with n = 9, a linear relationship

$$\log k_i = C + \varphi\pi_X \qquad (1)$$

holds for the series which points to hydrophobic interaction between the leaving group of the inhibitors and the enzyme surface. The hydrophobicity constants π_X were calculated by additive scheme[11] using the value 0.50 for methyl and methylene groups.

In (II), log k_i for the compounds with *n*-alkyl groups in their leaving part increase in accordance with Eq. (1) only up to X = $-C_4H_9$-*n*. Further increase in the chain length (from n = 4 to 7) does not result in any considerable change in log k_i values (see Fig. 2). Apparently, in the series of inhibitors in which the length of the substituent in their acid moiety is optimal for the interaction with the hydrophobic pocket in the active site of CT, the interaction of their leaving group with the enzyme active surface appears only if its length does not exceed that of *n*-butyl radical.

In calculation of φ-coefficients for the series (I) and (II) the following equation

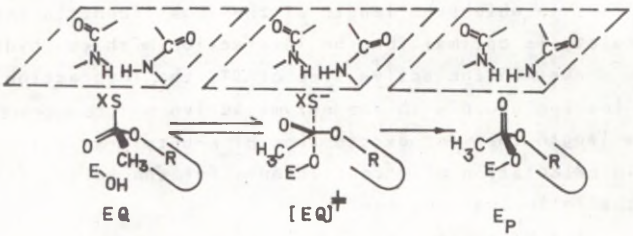
$$\log k_i^X = C + \rho^* \sigma_X^* + \varphi\pi_X \quad , \qquad (2)$$

where the term $\rho^* \sigma_X^*$ accounts for the leaving group inductive effect, was used. In taking into account the inductive effect of substituent X we have applied the scale of σ^* constants in which an infinitively long polymethylene chain is considered as the reference substituent with $\sigma^* = 0$[12,13]. This enables one to treat the alkyl and electronegative substituents in a common series. The σ^* values for the substituents $-(CH_2)_mSC_nH_{2n+1}$ were equalized with σ^* for the substituents $-(CH_2)_mSC_2H_5$[14]. Considering that in the series (II) only the fragment of the leaving group with the length not exceeding the length of thiobutyl chain can interact with the enzyme active surface, the "effective" π values for X

have been used for these compounds (see Table 1). The Π constants for the substituents $-(CH_2)_mSC_nH_{2n+1}$ were calculated by additive scheme making use of the partition coefficient of diethyl sulfide in the octanol-water system[11].

The parameters of the correlation of log k_i with σ^* and Π according to Eq. (2) are given in Table 2. It is evident from the Table that the values of φ are close in two series of the inhibitors whereas the ρ^* value in the series (II) with R = $-C_6H_{13}$-*n* is 1.5 times less than in the series (I) with R = $-C_2H_5$, and coincides with ρ^* for alkaline hydrolysis of the compounds $(RO)(CH_3)P(O)SX$ in water[14].

The observed decrease in ρ^* for the reaction of CT with the inhibitors $(n\text{-}C_6H_{13}O)(CH_3)P(O)SX$ could be explained assuming that the conversion of the initial complex of the enzyme with these inhibitors proceeds in accordance with the following scheme:



In the proposed scheme, the orientation of the alkoxy chain RO- is assumed to remain unchanged in the initial complex EQ, transition state $[EQ]^‡$ and phosphorylated enzyme EQ'. The leaving group of the inhibitor is directed towards the positive ends of the peptide bond dipoles Met 192-Gly 193 and Ser 195-Gly 196, i.e. towards the oxyanion hole. The interaction of the dipoles with the leaving group in the bipyramidal transition state $[EQ]^‡$, in which the P=O, P-O and P-C bonds are located on the same plane, provides the solvation of the negative charge on the leaving group sulfur atom. In the phosphorylated enzyme EQ', the P=O group is oriented as the leaving group in the initial noncovalent complex. The binding of alkyl chain in the hydrophobic

Table 1

The second-order rate constants of the reaction of α-chymotrypsin with O-*n*-alkyl-S-alkyl methylthiophosphonates, (RO)(CH_3)P(O)SX. Temperature 25.0 ± 0.1°C, pH 7.60 ± 0.02, phosphate or Na-veronal-HCl buffer solutions, μ = 0.05, 0-0.8 % (v/v) acetonitrile

No	X	k_i, $M^{-1}\,s^{-1}$		σ^*_X	π_X	π_{eff} c)
		R=-C_2H_5 a)	R=-C_6H_{13}-*n* b)			
1	-C_2H_5	$4.0\cdot10^{-4}$	0.77	0.067	1.0	1.0
2	-C_3H_7-*n*	$4.7\cdot10^{-4}$	0.86	0	1.5	1.5
3	-C_4H_9-*n*	$7.3\cdot10^{-4}$	1.66	0	2.0	2.0
4	-C_5H_{11}-*n*	$1.4\cdot10^{-3}$	1.55	0	2.5	2.0
5	-C_6H_{13}-*n*	$2.3\cdot10^{-3}$	-	0	3.0	-
6	-C_7H_{15}-*n*	$3.4\cdot10^{-3}$	1.72	0	3.5	2.0
7	-C_9H_{19}-*n*	$1.0\cdot10^{-2}$	-	0	4.5	-
8	-$CH_2SC_2H_5$	$3.6\cdot10^{-2}$	14.9	0.56	1.45	1.45
9	-$(CH_2)_2SC_2H_5$	$6.1\cdot10^{-3}$	3.76	0.28	1.95	1.45
10	-$(CH_2)_2SC_3H_7$-*n*	$1.2\cdot10^{-2}$	-	0.28	2.45	-
11	-$(CH_2)_2SC_4H_9$-*n*	$1.4\cdot10^{-2}$	-	0.28	2.95	-
12	-$(CH_2)_3SC_2H_5$	$3.8\cdot10^{-3}$	-	0.14	2.45	-
13	-$(CH_2)_4SC_2H_5$	$3.2\cdot10^{-3}$	4.16	0.07	2.95	2.0
14	-$(CH_2)_5SC_2H_5$	$5.8\cdot10^{-3}$	-	0.035	3.45	-
15	-$(CH_2)_6SC_2H_5$	-	2.68	0.018	3.95	2.0

a) Mean experimental error in k_i - 10%.

b) Mean experimental error in k_i - 4%.

c) Effective Π-constants for the series (II) with R = -C_6H_{13}-*n*, see text.

pocket ensures the proper orientation of the leaving group towards the two peptide bond dipoles thus creating a polar (electrophilic) microenvironment in the vicinity of the scissible bond, which is comparable with that of water medium. By the intensity of the leaving group inductive effect,

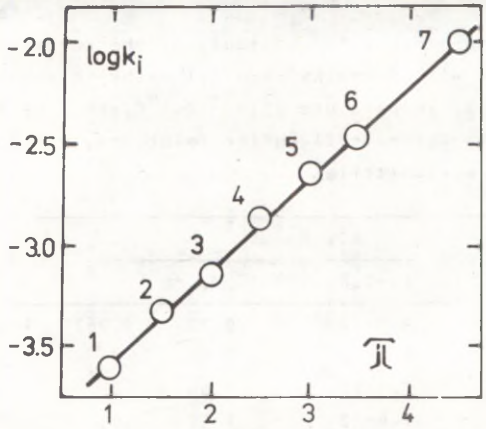


Fig. 1. Dependence of the logarithms of the second-order rate constants of the reaction of α-chymotrypsin with O-ethyl-S-alkyl methylthiophosphonates $(C_2H_5O)(CH_3)P(O)SX$, X = $-C_nH_{2n+1}$, upon Π-parameters of X. For the compound with X = $-C_2H_5$, the contribution of the alkyl group inductive effect, $\rho^{*}\sigma^{*}$, in accordance with Eq. (2), has been subtracted from the experimental value of log k_i. The numbers at the points refer to the inhibitors listed in Table 1.

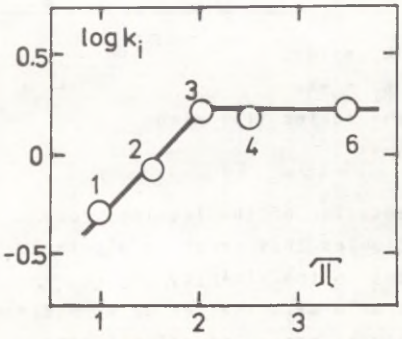


Fig. 2. Dependence of the logarithms of the second-order rate constants of the reaction of α-chymotrypsin with O-*n*-hexyl-S-alkyl methylthiophosphonates $(n\text{-}C_6H_{13}O)(CH_3)P(O)SX$. The meaning of the numbers and the procedure of the normalization of the log k_i value for the inhibitor with X = C_2H_5 as in Fig. 1.

Table 2

Dependence of the second-order rate constants of the reaction of α-chymotrypsin with organophosphorus inhibitors upon the structure of their leaving proup. The results of the correlation analysis of the data in Table 1 by Eq. (2).

Reaction series	Parameters of Eq. (2)	n	Correlation coeff., r	S.D.	Correlation coeff. between σ^* and Π, r_x
$C_2H_5O-\overset{\overset{O}{\|\|}}{\underset{\underset{CH_3}{\|}}{P}}-SX$	$\rho^* = 3.36 \pm 0.10$ $\varphi = 0.46 \pm 0.02$ $\rho^*_{OH} = 1.90 \pm 0.03$ [a]	14	0.995	0.059	0.366
$n\text{-}C_6H_{13}O-\overset{\overset{O}{\|\|}}{\underset{\underset{CH_3}{\|}}{P}}-SX$	$\rho^* = 2.21 \pm 0.21$ $\varphi = 0.60 \pm 0.11$	9	0.974	0.103	0.426

a) ρ^* for alkaline hydrolysis[14].

the reaction then resembles the alkaline hydrolysis of organophosphorus esters in water (see Table 2). If the alkoxy group in the phosphoryl part of inhibitors, as $-C_2H_5$ in (I), is too short for effective binding in the hydrophobic pocket, the localization of the leaving group relative to the described solvation cell may probably remain inexact. For the highly polar transition state it should mean a transfere of the reaction into a less polar medium with concomitant increase in ρ^* up to the values considerably higher than the ρ^* values of alkaline hydrolysis of the compounds in water.

The high value of ρ^- in the reaction of CT with phenyl acetates[3,4] could be explained similarly. It has been shown,

particularly, that ρ^{o} for alkaline hydrolysis of the aryl-substituted phenyl acetates increased from the values of $\rho^{o} = 0.947 \pm 0.037$ in water up to the values of 1.6-1.8 in water-dioxan, water-ethanol and water-acetone mixtures[15,16] thus reaching the value observed in their reaction with CT[3,4].

In conclusion, it may be stated that the dependence of the leaving group inductive effect upon the structure of acyl part in the reactions of CT with organophosphorus inhibitors or carboxylic ester substrates could be explained without introducing special requirements into detailed mechanism of the reaction with "good" (amino acid) substrates, e.g. the formation of additional hydrogen bonds at their conversion. It is sufficient to assume that the low values for the leaving group inductive effect in the reactions of CT with its specific substrates are brought about by electrophilic solvation of the substrate reaction center in the cell formed by NH groups of Gly 193 and Ser 195 in the enzyme.

Experimental section

Organophosphorus inhibitors were prepared by conventional methods[17,18] and purified on a silica gel column[19]. The structure and purity of the compounds were confirmed by their ^{13}C NMR spectra, elemental analysis data, infra-red spectra and by titration of the thiols liberating in the course of their alkaline hydrolysis[20]. The inhibition of CT activity in large excess of inhibitors was performed, as described elsewhere[21], by measuring the residual activity of the enzyme. The second-order rate constants of the inhibition reaction (Table 1) were calculated from the dependencies of pseudo monomolecular rate constants upon the inhibitor concentrations. Spontaneous inactivation of the enzyme in the course of the inhibition reaction has been taken into account.

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Organic Reactivity
Vol.23 3(83) 1986

HYDROPHOBIC INTERACTION IN THE ACTIVE SITES OF SERINE PROTEASES FROM THERMOACTINOMYCETS

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The second-order rate constants of the reactions of thermitase and a serine protease from *Thermoactinomyces vulgaris* strain RA 11-4a with the substrates $CH_3C(O)NHCH(R)C(O)OCH_3$ (I) and irreversible organophosphorus inhibitors $(RO)(CH_3)P(O)OC_6H_4NO_2$-*p* (II), where R stands for alkyl and/or aryl alkyl substituents, have been determined. The substrate specificity of both enzymes in these reactions has been shown to be the same. At he length of R not exceeding that of the *n*-butyl group, the dependence of the rate of enzymic hydrolysis of substrates (I) upon their structure is described by the equation $\log k_{II} = 3.54 + 1.19 \log P$, where P is the substrate partition coefficient in the octanol-water system. For (II) the linear relationship between $\log k_{II}$ and the compound hydrophobicity holds for the inhibitors with R from $-C_3H_7$-*n* to $-C_6H_{13}$-*n*. It is suggested that, as in α-chymotrypsin, the primary substrate specificity of the proteases from *Thermoactinomyces vulgaris* is determined by a special hydrophobic slit in their active sites, which principally differentiates them from bacterial subtilisins which do not show hydrophobic selectivity with respect to (I).

Recently a number of serine proteases which split peptide bonds after hydrophobic amino acids and contain a free thiol group, essential for their enzymic activity, have been purified from various strains of thermophilic actino-

mycets and some other microorganisms[1-8]. Thermitase, a thermostable serine protease from *Thermoactinomyces vulgaris*[1], has been most extensively studied in this subfamily[7,8] of serine proteases. On the basis of the comparison of the amino acid sequences of the microbial enzymes[9-12] as well as their specificities against some short polypeptide substrates[13,14], thermitase has been classified as a subtilisin-like enzyme.

However, a study of the interaction of another SH-containing serine protease, obtained from *Thermoactinomyces vulgaris* strain RA II-4a[3], with a series of N-acetyl-L-amino acid methyl esters, $CH_3C(O)NHCH(R)C(O)OCH_3$, has shown that the enzyme principally differs from subtilisins in primary substrate specificity. For the protease from *Thermoactinomyces vulgaris* strain RA II-4a, a linear relationship between $\log(k_{cat}/K_M^{app})$ and the hydrophobicity of the substituent R, covering the change of the rate constant within two orders of magnitude, has been found[3], while subtilisins do not display hydrophobic selectivity with respect to these substrates[15]. The interaction of R with a hydrophobic slit in the active site of the protease as well as the occurrence of the relationship "better binding:better reaction"[3] in this series of substrates make the enzyme look rather similar to α-chymotrypsin.

In the present work the study of the interaction of the series of N-acetyl-L-amino acid methyl esters with microbial proteases has been extended to thermitase, in order to correlate the primary substrate specificities of SH-containing serine proteases from different strains of *Thermoactinomyces vulgaris*. The second-order rate constants of the hydrolysis of the substrates, k_{II}, obtained under the conditions of $[S] \ll K_M^{app}$, have been used to quantify the specificities of the studied enzymes.

To account for the hydrophobicity of the substrates we have used the logarithms of their partition coefficients in the octanol-water model system; the hydrophobicity constants

Π for amino acid side chains as calculated from the partition coefficients of various amino acid derivatives, have been found to be at variance with each other[16].

In the study of the topographies of the active sites of the proteases from *Thermoactinomyces* we have also used a series of organophosphorus inhibitors, $(RO)(CH_3)P(O)OC_6H_4NO_2$-*p*, where R = $-C_nH_{2n+1}$-*n* (n=2-8). The series has been used earlier in the studies of subtilisins[17,18] and intracellular proteases[18] from *Bacillus amyloliquefaciens*.

Experimental section

The protease from *Thermoactinomyces vulgaris* RA 11-4a was purified on Sephadex G-50 and CM-cellulose columns as described elsewhere[3].

Thermitase from Serva (FRG) was used without further purification.

The enzyme stock solutions, pH 5.0, μ=0.05, were made in acetate buffer containing 10^{-2} M $CaCl_2$. The active sites of the enzymes were titrated by O-*n*-hexyl-*p*-nitrophenyl methylphosphonate; the release of *p*-nitrophenol was monitored at 25°C, pH 7.6, Na-veronal-HCl buffer, μ=0.05 (ε= =13700 at 400 nm) on a Varian Techtron 635 or Beckman 5260 spectrophotometer.

N-acetyl-L-amino acid methyl esters were prepared as described earlier[3]. Purity of the compounds was checked by titrating the amount of acid which liberated in the course of their complete thermitase-catalyzed hydrolysis. Substrate stock solutions were prepared in acetonitrile.

Kinetic measurements were carried out on a pH-stat (Radiometer, TTT 2/SBR 3/ABU 12) at 25°C and pH 7.6 in the solution containing 0.2 M KCl, 10^{-2} M $CaCl_2$ and 5% (v/v) acetonitrile.

In accordance with the reaction scheme

$$E + S \underset{}{\overset{K_M}{\rightleftharpoons}} ES \xrightarrow{k_2} EA \xrightarrow[H_2O]{k_3} E + P_2, \qquad (1)$$

$$ES \xrightarrow{} P_1$$

at $[S]_o \ll K_M^{app}$ the reaction follows the first-order kinetics, with $k = (k_2/K_M)\,[E]_o = k_{II}[E]_o$, which enables to calculate the second-order rate constant of the enzyme acylation as $k_{II} = k/[E]_o$; the k values were obtained by the differential method of Rudakov[19].

The synthesis and purification of O-*n*-alkyl-*p*-nitrophenyl methylphosphonates have been described earlier[20]. Stock solutions of the compounds were made in absolute ethanol.

Protease inhibition was carried out under the pseudomonomolecular reaction conditions ($[I]_o \gg [E]_o$) at 25°C and pH 7.6 in 0.04 M Na-veronal-HCl buffer containing 10^{-2} M $CaCl_2$ and 2% (v/v) of ethanol. At predetermined time intervals aliquots were withdrawn from the reaction vessels and assayed for the residual enzyme activity using $7 \cdot 10^{-3}$ M N-acetyl-L-phenylalanine methyl ester as substrate. The first-order rate constants $k = (k_2/K_M)[I]_o = k_{II}[I]_o$ were obtained from the plots of log(A%) against t in accordance with the equation

$$\log(A\%) = 2 - k_{II}[I]_o t/2.3033 \qquad (2)$$

which follows from scheme (1) with $k_3 = 0$. The second-order rate constants of the irreversible inhibition of protease activities were calculated from the slopes of the straight lines in the coordinates k *versus* $[I]_o$ (see[18]).

Partition coefficients of N-acetyl-L-amino acid methyl esters in the octanol-water system (0.1 M ammonium phosphate buffer, pH 2.2) were determined at room temperature. Initial $(0.1\text{-}1.0)10^{-3}$ M solutions of the compounds were prepared either in the buffer solution saturated with octanol or in octanol saturated with the buffer solution. Then 2.0 ml of each of the solutions in stoppered test tubes were mixed with the second phase by inversion of the tube for 200 times during 10 min. After the separation of the phases, the concentrations of the compounds in both phases were determined by high performance liquid chromatography (Du Pont 8800, Zorbax ODS column, 4.6 mm x 25 cm, 50:50 methanol and 0.1 M

ammonium phosphate buffer, pH 2.2, 35°C, detection by the absorbance at 205 nm). The partition coefficients were shown to be independent of the number of tube inversions and the concentrations of the compounds; the calculated values of the partition coefficients also remained unchanged when the concentrations were determined 10 hours after mixing the phases. The logarithms of the obtained partition coefficients, log P, for N-acetyl-L-amino acid methyl esters are given in Table 1.

Results and discussion

As can be seen from Table 1, an increase in the substrate side chain hydrophobicity leads to the increase in the second-order rate constants of acylation of the active sites of the studied proteases. The dependence of log k_{II} upon log P for the compounds No 1-4 (Table 1) with normal hydrocarbon radicals as R, is given by a common equation for both proteases,

$$\log k_{II} = (3.55 \pm 0.03) + (1.19 \pm 0.07) \log P \quad (3)$$

where r = 0.991, s = 0.096 and n = 8. In Figure 1 these data are presented as a log k_{II} *versus* log P plot. As a characteristic fact, it is seen in Figure 1 that log k_{II} for N-acetyl-L-phenylalanine methyl ester substrate with aromatic amino acid side chain R = $-CH_2-C_6H_5$ quite satisfactorily falls on the extension of the straight line conducted through the points for log k_{II} of aliphatic amino acid substrates from alanine (R = $-CH_3$) to norleucine (R = $-C_4H_9$-*n*). Inclusion of the phenylalanine substrate as well as the derivative of leucine with a branched side chain R = $-CH_2CH(CH_3)_2$ into the correlation does not change the slope of the straight line but only lowers the quality of the correlation in some extent:

$$\log k_{II} = (3.54 \pm 0.06) + (1.19 \pm 0.08) \log P \quad (4)$$

where r = 0.976, s = 0.163 and n = 12. At the same time, the substrates with normal hydrocarbon radicals in their side chains longer than *n*-butyl group give systematic negative deviations of log k_{II} from the correlation line (see Fig.1).

Table 1

Hydrolysis of N-acetyl-L-amino acid methyl esters, $CH_3C(O)NHCH(R)C(O)OCH_3$, catalyzed by thermitase and the serine protease from *Thermoactinomyces vulgaris* RA II-4a. Temperature 25°C, pH 7.6, 0.2 M KCl, 5% (v/v) acetonitrile. The second-order rate constants are given with ±S.E.

No	R	log P	k_{II} 10^{-2}, M^{-1} s^{-1}	
			thermitase	protease RA II-4a
1	$-CH_3$	-0.57	9,9±0.2	5.3±0.1
2	$-C_2H_5$	-0.17	22±1	20±2
3	$-C_3H_7$-*n*	0.33	95±1	114±3
4	$-C_4H_9$-*n*	0.79	282±9	277±6
5	$-C_5H_{11}$-*n*	1.28	180±10	216±9
6	$-C_6H_{13}$-*n*	1.70	29±1	32,8±0,9
7	$-CH_2CH(CH_3)_2$	0.84	179±7	232±4
8	$-CH_2C_6H_5$	0.92	760±10	660±20
9	$-CH_2C_6H_4OH$-*p*	0.33	323±6	445±6

The observed dependence of log k_{II} upon the length and hydrophobicity of the hydrocarbon radicals in the acyl part of substrates is quite similar to the structure-activity relationship in chymotrypsin-catalyzed hydrolysis of these compounds[21-23] Since the primary substrate specificity of chymotrypsin has been shown to be determined by a specific hydrophobic slit in its active site[24] for the binding of the side chains of amino acids in the P_1-position of polypeptide substrates (notion of amino acid residues in substrates as in Schechter and Berger[25]) one can conclude that the primary specificity of SH-containing microbial serine proteases is determined by the binding of the amino acid substrate side chain in a special hydrophobic slit in the enzyme active site as well.

However, by the size and the intensity of hydrophobic interaction with the substrate side-chain, the hydrophobic slit in the active sites of the proteases from actinomycets

must be different from that in chymotrypsin. For chymotrypsin the linear relationship between log k_{II} and log P in this series of substrates holds up to α-aminoheptanoic acid methyl ester (R = $-C_5H_{11}$-*n*) and the slope of the observed straight line is about two:

$$\log k_{II} = (1.54 \pm 0.10) + (1.96 \pm 0.13) \log P , \quad (5)$$

where r = 0.993, s = 0.043 and n = 5 (the compounds No 1-5 in Table 1, the values of k_{II} by Jones et al.[21]). In addition, it is characteristic for chymotrypsin that the reactivities of the derivatives of aromatic amino acids Phe and Tyr do not obey Equation (5); $\log k_{II}^{exp} - \log k_{II}^{calc}$ is 1.3 for Phe and 2.8 for Tyr.

Inconsistency of the extraction model[26,27] to describe the hydrophobic selectivity of chymotrypsin in the reaction with aromatic amino acid substrates if the "initial" parameters log P for substrate hydrophobicity are used, needs special analysis elsewhere. According to the results of the present study, the SH-containing serine proteases from *Thermoactinomyces* do not prefer Phe over the aliphatic amino acids but log k_{II} for N-acetyl-L-tyrosine methyl ester still has the value of 0.6 log units higher than predicted by Equations (3) and (4).

It is interesting to note that for aliphatic amino acid substrates the log k_{II} values for proteases from *Thermoactinomyces* are considerably higher than log k_{II} for chymotrypsin. Vice versa, in the reaction of organophosphorus inhibitors O-*n*-alkyl-*p*-nitrophenyl methylphosphonates with the active sites of microbial proteases, the k_{II} values (in Table 2) are lower than for chymotrypsin[27]. The relationship between log k_{II} and the hydrophobicity parameter π [18,28] for substituent R in $(RO)(CH_3)P(O)OC_6H_4NO_2$-*p* is given in Fig. 2. The straight line covers the the log k_{II} values for the inhibitors with R from $-C_3H_7$-*n* to $-C_6H_{13}$-*n* and is characterized by a correlation equation

$$\log k_{II} = (-0.69 \pm 0.15) + (0.57 \pm 0.07) \pi , \quad (6)$$

with r = 0.962, s = 0.104 and n = 8. Further increase in the length of *n*-alkyl chain R leads to a decrease in log k_{II}

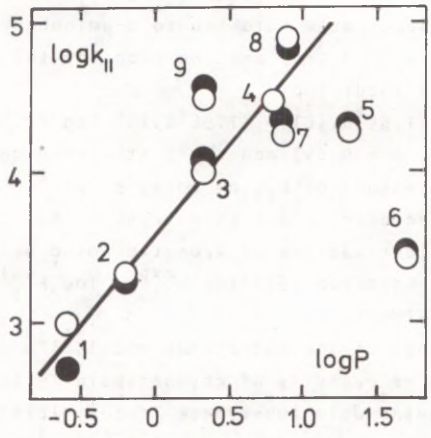

Fig. 1. Dependence of log k_{II} upon log P for the hydrolysis of the substrates $CH_3C(O)NHCH(R)C(O)OCH_3$ catalyzed by thermitase (o) and the serine protease from *Thermoactinomyces vulgaris* RA II-4a (•). The numbers refer to the substrates listed in Table 1.

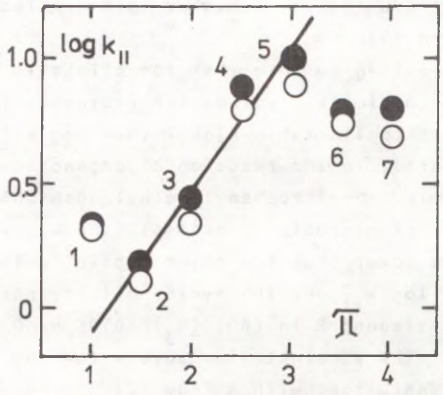

Fig. 2. Dependence of log k_{II} upon Π for the phosphorylation of the active sites of thermitase (o) and the serine protease from *Thermoactinomyces vulgaris* RA II-4a (•) by O-*n*-alkyl-*p*-nitrophenyl methylphosphonates, $(RO)(CH_3)P(O)OC_6H_4NO_2$-*p*. The numbers refer to the inhibitors listed in Table 2.

Table 2

The second-order rate constants of the inhibition of the activities of thermitase and the serine protease from *Thermoactinomyces vulgaris* RA II-4a by O-*n*-alkyl-*p*-nitrophenyl methylphosphonates, $(RO)(CH_3)P(O)OC_6H_4NO_2$-*p*. Temperature 25°C, pH 7.6, 0.04 M Na-veronal-HCl buffer with 10^{-3} M $CaCl_2$; 2% (v/v) ethanol. The constants are given with ±S.E.

No	R	π[a]	k_{II}, M^{-1} s^{-1}	
			thermitase	protease RA II-4a
1	$-C_2H_5$	1.0	2.1±0.5	2.2±0.5
2	$-C_3H_7$-*n*	1.5	1.3±0.1	1.6±0.1
3	$-C_4H_9$-*n*	2.0	2.2±0.3	2.9±0.5
4	$-C_5H_{11}$-*n*	2.5	6.5±0.9	8.0±0.6
5	$-C_6H_{13}$-*n*	3.0	8±1	10±1
6	$-C_7H_{15}$-*n*	3.5	5.5±0.4	6±2
7	$-C_8H_{17}$-*n*	4.0	5±1	6.3±0.3

a) Additive values[18].

which points to the binding of the inhibitor alkoxy group in a hydrophobic area of limited size in the active sites of the studied proteases. This hydrophobic area is apparently different from the hydrophobic slit for the binding of amino acid side chain R of the substrates $CH_3C(O)NHCH(R)C(O)OCH_3$ since the length of the seven-membered alkoxy group $-OC_6H_{13}$-*n* at which log k_{II} in the series of the inhibitors reaches its maximum value, differs from the length of the group $-\underset{|}{CH}-C_4H_9$-*n* in the substrate which has the highest rate constant of the acylation of the enzyme active site in the studied series.

In conclusion it may be said that despite rather extensive sequential homologies between the two groups of enzymes, the SH-containing serine proteases from *Thermoactinomyces* principally differ from bacterial subtilisins by their primary substrate specificity. The specificity of the enzymes

from actinomycets in the reaction with the substrates $CH_3C(O)NHCH(R)C(O)OCH_3$ can be explained by the presence of a hydrophobic slit in their active sites, which is similar to the hydrophobic pocket in the active site of α-chymotrypsin. Subtilisins do not show hydrophobic selectivity in the reaction with N-acetyl-amino acid methyl esters[15].

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A STUDY OF KINETICS OF AMINOLYSIS REACTIONS OF ACYLHALOGENIDES, CATALYZED BY 4-N,N-DIMETHYLAMINOPYRIDINE IN METHYLENE CHLORIDE

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Interaction kinetics of benzoyl-4-N,N-dimethylaminopyridine chloride with p-nitroaniline in methylene chloride has been studied. It is shown that the kinetics of the rate determining stage of aminolysis reaction of acylhalogenides, catalyzed by 4-N,N-dimethylaminopyridine (as it was also in case of N-alkylimidazoles, studied earlier) is a multichannel process, in the initial stage characterized by the formation of the H-complexes of nucleophiles with the intermediate and the initial tertiary amine. Reactivity of similar complexes with arylamines is close to those of phenols, which can be explained by the different level of proton transfer in the transition state.

The reaction of acylhalides with aromatic amines, catalyzed by the tertiary amines proceeds in aprotic media according to the nucleophilic mechanism (Scheme 1) via the intermediate (1) having the structure of acylammonium chlorides:

$$RCOX + N\lesssim \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \left[RCON^{+}\lesssim\right] X^{-} \xrightarrow{k_2, ArNH_2} ArNHCOR + \left[\overset{+}{H}N\lesssim\right] X^{-} \quad (1)$$

In the reaction of acetyl cloride with p-nitroaniline in CH_2Cl_2, catalyzed by N-methylimidazole, a thermodynamically favorable formation of the acetyl-3-methylimidazole intermediate product is observed, which gives its rate determining stage a multichannel character[1-4].

4-N,N-dialkylaminopyridines belong to another group of bases that are able to form stable acylammonium salts in aprotic media due to their higher basicity (pK_a= 9.36 - 9.6) and tendendency towards a more substantial resonance stabilization than N-alkylimidazoles. Moreover, 4-N,N-dimethylaminopyridine (DMAP) is of great interest from the point of view of its application as an efficient catalyst for preparatory acylation[5-9].

Taking into consideration the aforesaid, the present paper gives a direct kinetic investigation into the reactivity of the benzoyl-4-N,N-dimethylaminopyridine chloride (1) in the reactions of aminolysis in methylene chloride.

Experimental

Methylene chloride[10] and benzoylchlorides[11] were purified according to standard methods. 4-N,N-Dimethylaminopyridine was synthesized and purified as in paper[12] and then followed its vacuum sublimation. p-Nitroaniline was purified by a three-fold recrystallization from water and dried in vacuum, the melting point being 147°C[13].

Benzoyl-4-N,N-dimethylaminopyridine chloride was obtained by mixing equimolar quantities of benzoylchloride (BC) and DMAP in absolute diethyl ether at room temperature. The salt precipitate was several times washed on the Schott's filter with absolute ether, it was used without a further purification. Its melting point was 147-148°C. The % obtained : C 64.23; H 5.68; Cl 13.40; N 10.89. The % calculated:C 64.01; H 5.75; Cl 13.49; N 10.66.

The BC reaction with p-nitroaniline (PNA) in the presence of DMAP was carried out under the pseudofirst order conditions according to amine. The process was monitored spectrophotometrically measuring the PNA decrease at 380 nm.Kinetic

measurements were conducted in methylene chloride at 25°. Operations concerning the synthesis (1), preparation of solvents, filling of the quartz cells of the spectrophotometer as well as taking of samples were carried out in the box, carefully dried by means of phosphoric anhydride. The observed rate constants were calculated as follows:

$$k_H = \frac{I}{t} \ln \frac{D_o - D_\infty}{D_t - D_\infty} \qquad (2)$$

where D_∞ , D_o, D_t denote the solvent's optic density by the termination of the reaction, at the initial time moment and at time moment t. Linear dependences were treated according to the method of least squares[14].

Discussion of Results of Kinetic Measurements

The reaction of acylhalogenides RCOX X = (Cl, Br, I) with p-nitroaniline if RCOX ≫ PNA in methylene chloride proceeds according to scheme:

$$RCOX + H_2NC_6H_4NO_2 \longrightarrow RCONHC_6H_4NO_2 + HX \qquad (3)$$

Introduction of 4-piperidinopyridine (pK_a = 9.6) into the DMAP system leads to a rapid formation of (1). In case of 4-morpholinopyridine having a weaker basicity (pK_a = 8.6) such a situation can form only at a five-fold excess of acyl halide. In the IR spectrum of the mixture, the absorption bands of BC and pyridine base disappear and another absorption band crops up in the 300-320 nm range, The analogous phenomenon is observed also in the IR range. The IR and UV spectra obtained when mixing the equimolar BC and DMAP solutions, coincide with the spectrum of acylammonium salt, obtained preparatorily.

The character of kinetic interaction of I with aryl - amines as well as the nature of the reaction in general depend on the ratio of BC, DMAP and (I). It is useful to study three cases of I. $[BC]_o = [DMAP]_o$. It is shown in Fig. 1 that there exists a linear dependence between the k_H/I and $[DMAP]_o$ (consequently, $[I]$) values and that of the $k_3 \neq 0$

slope, corresponding to Eq. (4):

$$k_H = k_2[I] + k_3[I]^2 \qquad (4)$$

where k_2 characterizes the bimolecular interaction (I) with arylamines; k_3 denotes the interaction of (I) with arylamine in case of the contribution of the second salt molecule.

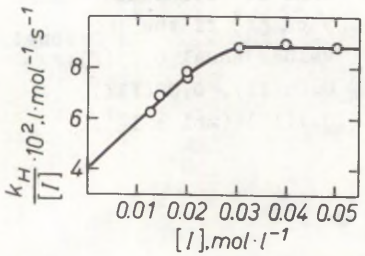


Fig. 1. Dependence of $k_H/[I]$ on the $[I]$ of benzoylchloride reaction with p-nitroanilino, catalyzed by DMAP in methylene chloride at 25°C. $[BH]_o = [DMAP]_o$ = I.

However, a wide variation range of the (I) concentration has shown that the $k_H/[I]$ linearly depends on (I) up to the salt concentration equalling ≈ 0.025 mole · l^{-1} (Fig. 1). In case of higher values, a negative deviation from the linearity connected with the formation of more complicated ionic aggregate (I) is observed. It will be studied in a separate publication.

2. If $[BC]_o > [DMAP]_o$, the reaction rate constant obeys the same equation as those catalyzed by N-alkylimidazoles[1]:

$$k_H = k_o[BC] + k_2[I] + k_3[I]^2 + k_4[BC][I] \qquad (5)$$

The reaction proceeds according to four parallel routes, including also k_2 and k_3, the non-catalytic reaction of k_o, as well as the k_4 route whose rate depends on the $[I]$ and BC concentration. The k_3 and K values were calculated from relationship (6), if [I] = const, eq. (5) transforms to eq. (6):

$$y = \frac{k_H - k_o[BC]}{[I]} = K + k_3[I] \qquad (6)$$

where $K = k_2 + k_4[BC]$ const (7)

The k_2 and k_4 values (figs. 2,3, Table 1) were found according to Eq. (7). A fairly good coincidence of the k_2 values found at various concentrations (Eqs. (4) and (7)), as well as the similarity of constants k_3 calculated according to Eqs.(4) and (6) confirm the validity of Eq. (5)*.

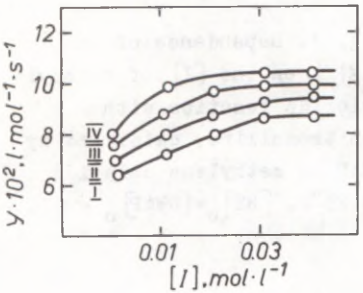


Fig. 2. Dependence of y on $[I]$ if the $[BC]_{const}$ values equal 0.03(I), 0.05(II), 0.08(III), 0.1(IV) (mol · l^{-1}).

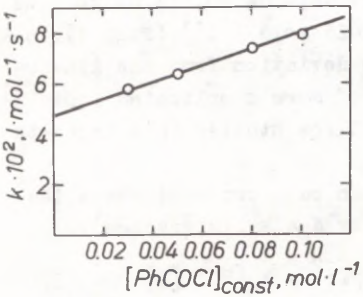


Fig. 3. Dependence (Eq.7) of the $K(l \cdot mol^{-1} s^{-1})$ values on the concentration of the $[BC]_{const}$ in case of the PNA acylation reaction in methylene chloride at 25°C.

3. $[BC]_o < [DMAP]_o$. Under these conditions, the observed rate constant depends linearly on the DMAP concentration, equalling $[DMAP]_o - [BH]_o$. This refers to the fact that in the studied system, also a reaction catalyzed by DMAP takes place in addition to the bimolecular route.

* At the concentration of (I) 0.025 mole · l^{-1} as well as in case of (4), a negative deviation from dependence (6) is observed.

Therefore

$$k_H = k_2 [I] + k_N [I] [DMAP] \quad (8)$$

From Eq. (8), the k_N value was found (Table 1).

Thus, the kinetic character of the DMAP effect on the benzoylchloride aminolysis reaction is similar to that of N-alkylimidazoles, the only difference being that in case of DMAP functions the route k_N.

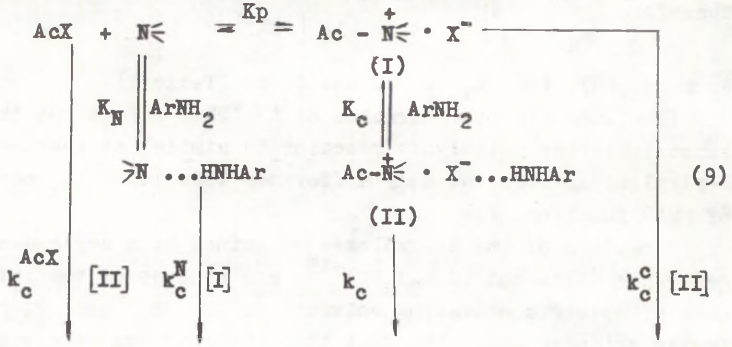
Formation of the H-complexes of amines with acylammonium reference salts and $(C_2H_5)_4\overset{+}{N}Cl^{-}$[15] and studies of the influence of specific solvating solvents on the k_2 and k_3[1,16] routes evidence about the fact that the reaction of acylammonium salts with amines proceed via the H-complexes only, and not via the direct bimolecular interaction.

Table 1

Results of Kinetic Studies of Reaction of Acylhalogenides with p-Nitroaniline, Catalyzed by N-Methylimidazole (I) and 4-N,N-Dimethylaminopyridine (2,3) in Methylene Chloride at 25°C

	RCOX	$k_0 \cdot 10^4$ $l \cdot mol.^{-1} \cdot s^{-1}$	k_2 $l \cdot mol.^{-1} \cdot s^{-1}$	k_3 $l^2 \cdot mol^{-2} \cdot s^{-1}$	k_4 $l^2 \cdot mol.^{-2} \cdot s^{-1}$	k_N $l^2 \cdot mol.^{-2} \cdot s^{-1}$
1.	C_6H_5COCl	1.0±0.2	0.11±0.02	17 ± 1	0	0
2.	C_6H_5COCl	1.0±0.2	0.042± ±0.003	1.79±0.06	0.22± ±0.04	2.52± ±0.12
3.	CH_3COCl	18 ± 2	0.02±0001	2.1± 0.2	-	-

Taking into account the formation of such complexes, the general scheme of aminolysis can be given as follows:



(9)

and described by Eq. (10).

$$k_H = \frac{k_c \cdot K_c \cdot [I]}{1 + K_c \cdot [I] + K_N \cdot [N\leq]} + \frac{k_c^c \cdot K_c [I]^2}{1 + K_c [I] + K_N [N\leq]} + \frac{k_c^N \cdot K_N [I] \cdot [N\leq]}{1 + K_c \cdot [I] + K_N [N\leq]} + \frac{k_c^{AcX} \cdot K_c [I]}{1 + K_c \cdot [I] + K_N \cdot [N\leq]} \qquad (10)$$

From the IR-spectroscopic data, in the range of the N-H-stretching frequencies, we determined the K_c of the formation of H-complexes of various arylamines with tetraalkylammonium chloride and bromide*. The obtained data are given in Table 2. It is evident that the $K_c[I] + K_N[N\leq] \ll 1$ and Eq. (10) transform into (11):

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* As it was shown in[17] on the example of the H-complexes of phenols with chloride ions, the K_c value does not practically depend on the salt cation nature.

$$k_H = k_c K_c[I] + k_c^c K_c[I]^2 + k_c^N K_N[I]\,[N\leqslant] + k_c^{AcX} \cdot K_c \cdot [I] \quad (11)$$

where $k_c K_c = k_2$; $k_c^c K_c = k_3$; $k_c^N K_N = k_N$; $k_c^{AcX} K_c = k_4$ (12)

The rate constants of the intramolecular k_c and of all intermolecular catalytic routes of k_c^c, k_c^N and k_c^{AcX} (Table 2) were calculated according to (12).

Table 2

Equilibrium Constants K_c of Formation of Hydrogen-Bond and K_N (No 6) Complexes in Methylene Chloride at 25°C

No	Proton donor	pK_a	Proton acceptor	Equilibrium constant l/mole
1.	3,5-dinitro-aniline	0.22	tetraethylammonium chloride	38*
2.	p-nitroaniline	1.02	tetraethylammonium chloride	24 ± 3
3.			tetraethylammonium bromide	16 ± 1.5
4.	3-nitro-5-carbomethoxy aniline	1.54	tetraethylammonium chloride	14 ± 1.2
5.	m-nitroaniline	2.50	tetraethylammonium chloride	10 ± 1**
6.			4-N,N-dimethylaminopyridine	1.62
7.	4-aminoazobenzene	2.82	tetraethylammonium chloride	8.2± 0.7

* Calculated from the Brønsted equation.
** Measured by I.A. Belousova[18].

Thus, the function of amines' complex-formation does not differ much from that of phenols, though, owing to the weaker hydrogen-bond complexes, in the aminolysis, their formation has no kinetic character.

Some structural-chemical peculiarities of the routes will be discussed below.

The kinetic data, characterizing the dependence of constants k_c and k_c^c in the aminolysis reaction as well as k_c

and k_c^N in the phenolysis reaction on the pK_a values of the corresponding nucleophiles and leaving groups in the intermediate acylammonium halogenides, are given in Table 3. In Fig. 4 are given the logarithmic dependences of the k_c reactivity of H-complexes of anilines and phenols on the pK_a of these nucleophiles in water, whose slope $\beta = 0.45$ in case of substituted anilines and -0.45 in case of phenols[19]. In case of the alcoholysis reactions, the β value can be derived from the data of[20] for substituted benzylic alcohols. Regardless of the narrow range of structural variation, the aforesaid statement reveals that the electron-acceptor substituents tend to increase (contrary to the amines) the reactivity of the complex "intermediate product - alcohol".

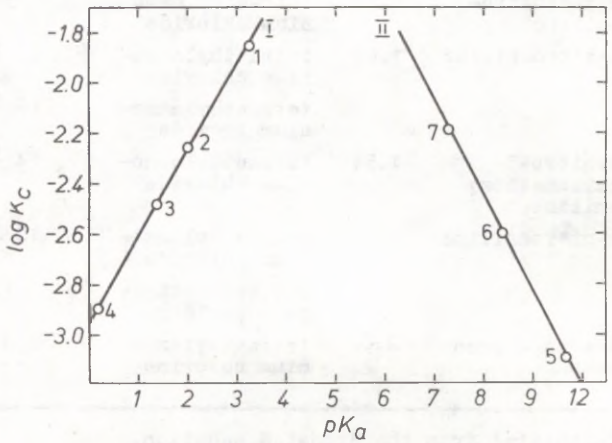


Fig. 4. Dependence of constants k_c of reactions of anilines (I) and phenols (II) with 1-acetyl-3-methylimidazole chloride on the pK_a of nucleophiles in water. Point numbers correspond to those of Table 3.

These data make it possible to find that $\beta \sim 0 \div -0.1$ for benzylic alcohols.

Thus, during the transition from amines to alcohols and phenols, i.e. from the compounds with basic properties to those having acidic properties, takes place the inversion

Table 3

Values of k_c, k_c^C and k_c^N for Reactions of 1-Acyl-3-Methylimidazole Chlorides with Anilines and Phenols in CH_2Cl_2 at 25°C

Nucleophile	1-acyl-3-methylimidazole chloride			1-(p-methoxybenzoyl)-3-methylimidazole chloride		pK of nucleophile
	$k_c \cdot 10^{-3}$ s^{-1}	k_c^C $l \cdot mol^{-1} \cdot s^{-1}$	k_c^N $l \cdot mol^{-1} \cdot s^{-1}$	$k_c \cdot 10^3$ s^{-1}	k_s^N $l \cdot mol^{-1} \cdot s^{-1}$	
1. 4-Aminoazobenzene	17±7	115±3	0	-	-	2.82
2. 3-nitro-5-carbomethoxyaniline	5.2±1.8	6.4±3	0	-	-	1.54
3. 4-nitroaniline	3±0.3	0.82±0.04	0	1.3±0.2	-	1.02
4. 3,5-dinitroaniline	1.2±0.1	0.13±0.1	0	-	-	0.22
5. 8-naphtene	0.89±0.15	0	11±1	0.93±0.2	13±1	9.63
6. 4-phenylazophenol	2.8±0.5	0	104±8	2.0 ±0.4	242±12	8.20
7. 4-nitrophenylazophenol	1.5±0.15	0	1400±70	7.0 ±0.15	3500±100	7.20

* The k_c values in case of reactions of benzyl, 4-methyl- and Br- benzyl alcohols, obtained via the recalculation of the data[20] for 4-N,N-dimethylaminobenzoyl-(4-N,N-dimethylamino) pyridine chloride in case of 4-methoxybenzoylic derivative, equal $5 \cdot 10^{-4}$s.

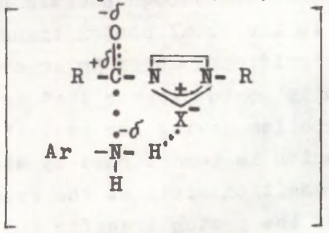
of the reaction's susceptibility sign towards the substituent effect. At the same time, such a varied character of the susceptibility of the reactions of acylammonium halogenides to the nucleophilic nature, the susceptibility to the leaving group basicity yields surprisingly close β_x values: -0.20 and -0.16 for aminolysis (1-acetyl- and 1-benzoyl-3-alkylimidazole chlorides, respectively), -0.2 for alcoholysis (calculated according to data[22] for the propanolysis of 1-cinnamoyl-3-methylimidazole and 1-cinnamoyl-4-N,N-dimethyl-aminopyridine chlorides in methylene chloride) and -0.26 for phenolysis. Such a small susceptibility to the leaving group nature in acylammonium halogenides is not connected with the effect of the anion and solvation nature, as the β values which are quite close to those given above were obtained for the aminolysis of the corresponding acylammonium cations in acetonitrile (β = -0.15)[23] and hydrolysis in water ($\sim$0)[24].

Consequently, the nature of interaction of acylammonium halogenides with the various nucleophiles having small basicities does not depend either on the acidity of their proton, or on their transition level in the transition state and is characterized by a practically similar and extremely small susceptibility to the leaving group nature.

The presented data evidence about the fact that the formation of the C-N bond with the H-complex is the predominating factor in the transition state of all the reactions studied, depending on the character of the stabilizing effect of the $N\bar{u}$-H...X^- type interaction, which is determined by the nature of the final nucleophile.

In the reactions of aminolysis, the amine structure of $\beta > 0$ in case of the hydrogen-bond "amine-anion" complex corresponds to the direct substituent effect on the nucleophile attack of the reaction center and on the C-N bond formation. The character of the susceptibility of the hydrogen-bond complex reaction of arylamines with an intermediate product to the structure of the attacking nucleophile and leaving group in the cation corresponds to the reagent-like transition state.

with a remarkable formation of reacting bonds having a low breaking level. High susceptibility to the substituent effect in the acylic region of the cation ($\beta = 2.1$)[25] refers to a highly changing character of the C=O bond in the transition state, thus indicating its similarity with the tetrahedral intermediate product (TIP) (13)



(13)

The situation is quite different in case of the reactions with alcohols and phenols. As to the nucleophile structure, the $\beta < 0$ value being in correspondence with the accelerating electron-acceptor substituent effect evidences about the appearance of the effective negative charge at its reaction center. In case of phenols, the absolute value of the charge is considerably higher than in case of alcohols. It means that the reactivity of these compounds is determined first of all by their acidity and not by their nucleophility Consequently, in case of such "acidic" nucleophiles, the proton shift the level of which is rising during the transition from alcohols to phenols, is the dominating factor in the formation of the transition state. Evidently, in the arylamines-alcohols-phenols series the anion of the intermediate product causes either the proton shift or transfer in the transition state at such a level which is necessary to achieve the effective NuH N-H nucleophility , thus ensuring the ejection of the leaving group. It agrees with the fact that the reactivity levels of H-complexes of arylamines, alcohols and phenols in such a broad variation range of their nucleophilic properties practically overlap (Fig. 4). It means that remarkable differences in the nucleophility of the observed series of acylated compounds are compensated by a more substantial proton shift to the anion of the intermediate

product. In the result of that the stabilization of the transition state varies insignificantly in all cases. It also seems to explain the practically similar bond-breaking level of the leaving group in the transition state.

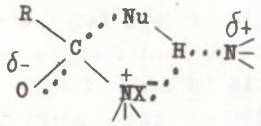
Still, a significant proton shift to the anion is not thermodynamically favorable and though phenols (unlike anions) can be subjected to the total proton transfer, the latter undergoes an insignificant shift in accordance with the β values. It permits us to presume that in case of the whole set of nucleophiles having low basicity, the catalytic effect of the anion is conditioned by stabilization of the corresponding transition state at the expense of the shift and not because of the proton transfer and the increasing firmness of hydrogen bond, in comparison with the initial complexes Nu - H...X^- $\geqslant N^+Ac$ (13). Therefore, the bond breaking of the leaving group in the intermediate product is not connected with the proton transfer but rather precedes it, since the positively charged (nitrogen atom) salt fragment lacks basicity. Evidently, the formation of a new bond and the breaking of the old one proceeds in accordance with the proton shift towards the anion, which guarantees the necessary nucleophility level for the reacting nucleophile. Thus, the transition states of the k_c route are similar, as regards the nature of their forming and breaking bonds, being conditioned by a different level of proton shift from the nucleophile to the anion of the intermediate product, that changes in the series phenols > alcohols > arylamines. Evidently, in the reactions with the strong enough nucleophiles (like amines e.g.) of the corresponding basicity, the participation of the nucleophilic proton is not necessary for the leaving group substitution in acylammonium salts. The course of the symmetric exchange reaction[25] confirms this statement. On the other hand, there are remarkable differences between the kinetic nature of the Nu-H...X^- $\geqslant$NAc complex in the phenolysis and alcolysis reactions and that of aminolysis. In the first two processes, the k_c route is the only possible reaction, where the participation of the complex is guaranteed (its contribution

to the total rate does not exceed 15-20 %). But in the aminolysis two additional, formally trimolecular routes can be traced: $k_3 = k_c^c \cdot K_c$ and $k_4 = k_c^{AcX} \cdot K_c$, whose mechanisms have been discussed earlier [1-4]. It should be stressed that not only the k_3 route but also k_2 gives the basic contribution to the rate of the slow stage of the catalytic reactions of the aminolysis, except for the acetylbromide reaction[1]. The absence of the routes corresponding to the k_3 and k_4 in the aminolysis in case of the phenolysis and alcoholysis reactions, also evidences about the fact that in case of such weak nucleophiles as alcohols and phenols, the proton shift otherwise guaranteed by the anion in the intermolecular process, is not great enough to substantially decrease the energy barrier of the reaction. A remarkable proton transfer is guaranteed by the tertiary amine only, taking into consideration the thermodynamic side. The effective catalysis of k_c^N by N-alkylimidazoles in the phenolysis and DMAP in the alcoholysis[20] can also be explained with this fact.

Appearing of the catalysis by tertiary amines in the aminolysis reaction depends on the nature of the anion in I and that of the tertiary amine. In the reactions catalyzed by N-alkylimidazoles, it can be found only in case of 1-acetyl-3-alkylimidazole bromides, and not in case of chlorides[1]. The catalysis by the DMAP having a stronger basicity can also be detected in case of the corresponding chlorides (Table 3).

The mechanism of the catalytic effect of the tertiary amine in all these reactions is as follows: the tertiary amine functions like the general basic catalyst, as far as the nucleophilic attack at the corresponding acylammonium halogenide is actually a symmetrical substitution reaction, proceeding considerably faster than the attack by the nucleophile-tertiary amine complex. Evidently, these are the nature and level of the proton transfer that form the difference between the transition states of reactions of various nucleophiles.

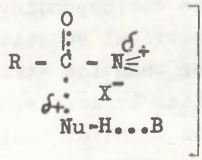
Proceeding from the fact that in the reaction of the NuH nucleophiles (here NuH = $ArNH_2$) having small basicites with (1) participate the hydrogen-bond complexes only, in general, the transition states of the reactions with participation of the anion I and tertiary amine can be described as follows:



(14)

where the interactions of $X^-...H-Nu$ and $Nu-H...N\leqslant$ compete with each other. The contribution of each of them is determined by the nature and level of proton transfer in the transition state. Their contributions are not in correlation with the basicity. This is quite natural since in the aminolysis the level of proton transfer cannot be very significant being more dependent on the proton-acceptor activity of K_c (Table 3).

Even in case of bromides, where the catalysis of N-methylimidazole is observed, the $K_c^{Br} > K_c^{Im}$*, though here the differences are much smaller than in case of the complex "chloride-ion-N-methylimidazole". It means that the proton transfer from the arylamine to the tertiary amine starts to prevail the transfer towards the halogenide ion in the transition state only. The level of the transfer, like in the course of the catalysis with the anion of the second salt molecule (k_c^c), corresponds only to the polarization of the N-H bond. Therefore the transition states of the aminolysis reactions with the participation of the $Nu-H...X^- \rightarrow N^+Ac$ (k_c^c) and $Nu-H...N\leqslant$ k_c^N have a somewhat similar structure (15):



(15)

* According to the data by Yu.S. Simanenko $K_c^{Im} \sim 1.0$ l·mol.$^{-1}$

where B is the tertiary amine or the second molecule of the intermediate product.

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THE SEPARATE DETERMINATION OF REACTIVITY OF IONS AND ION PAIRS OF ACETYLIMIDAZOLE SALTS IN AMINOLYSIS REACTIONS

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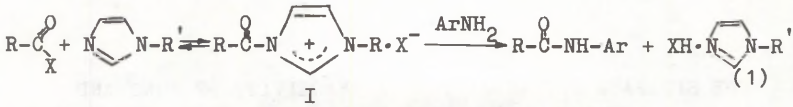
Reactivity of ionic forms of 1-acetyl-3-methylimidazole salts being the intermediate products of the acetylchloride aminolysis in acetonitrile catalyzed by N-methylimidazole, has been determined separately. It has been demonstrated that in the reactions of ion pairs, anions affect according to two mechanisms: stabilizing the transition state owing to the formation of a hydrogen bond with the chloride-ion arylamine and destabilizing it on the expense of steric hindrance to the nucleophile (ClO_4^-, $B(C_6H_5)_4^-$) attack. In the cation reactions, the solvent specific solvation of the attacking nucleophile is the factor of the general-basic character.

In the previous contributions[1-3] we have developed a new approach in order to study the mechanism of nucleophilic catalysis in the reactions of acylic transfer in the low polarity media. Making use of the ability of the N-substituted imidazoles to form the resonance stabilized acylammonium salts in the aminolysis reactions of acylhalogenides catalyzed by N-alkylimidazoles and establishing that they are stable enough even in the media having a relatively low polarity (CH_2Cl_2, $CHCl_3$, etc.), we studied the reactivity of these compounds as the intermediate products of the nucleophilic

catalysis by the N-substituted imidazoles in the aminolysis and phenolysis reactions of acylhalogenides.



I (1)

In the low-polarity media, the intermediate product exists mainly in the form of ion pairs and more complex ion associates. Its reactivity is determined by the cation structure as well as by the nature and character of solvation of the corresponding anion, i.e., two structural components of the system simultaneously control it. At the same time, it would be very interesting to get information about the reactivity of the ion pairs, as well as about the cation of the intermediate product, since the ion associate often markedly differs from the free ion, though the driving force is the same in both cases. In case of the reactions in the media of low polarity, the problem remains unsolved, since in these solvents the intermediate products are almost completely associated. Therefore we used a comparatively polar acetonitrile ($\mathcal{E}$ = 36.1) as a solvent, the substrate was 4-aminoazobenzene (AAB).

It was shown by some special experiments that in acetonitrile these salts are formed quantitatively in the studied concentration range of the components, as might be expected. It permits to carry out a direct investigation into the pecularities of the mechanism of the intermediate product transformation in acetonitrile in comparison with the low-polarity methylene chloride ($\mathcal{E}$ = 8.9).

A series of kinetic measurings at different initial concentrations of acetyl chloride [AcCl] and at varying concentrations of N-methylimidazole [N≤] were carried out if $[N\leq]_o > [AcCl]_o$, which demonstrated that the second order rate constant did not practically depend on the concentration [N≤] equalling $[N\leq]_o - [AcCl]_o$. Thus, there is no channel catalyzed by N≤ (k_N) in the studied system.

In case of $[N\leq]_o < [AcCl]_o$, the catalytic effect has a different character. In methylene chloride, the reaction

proceeds according to four parallel channels[2], including not only the catalytic one k_o but also the bimolecular interaction (1) with arylamine k_2, the k_3 channel interaction (I) with arylamine with participation of another salt molecule and channel k_4, representing the AcCl interaction with arylamine, catalyzed by the intermediate product[1]:

$$k_H = k_o \cdot [AcCl] + k_2 \cdot [I] + k_3 \cdot [I]^2 + k_4 \cdot [AcCl][I] \qquad (2)$$

or

$$y = \frac{k_H - k_o \cdot [AcCl]_{const}}{[I]} = k + k_3[I] \qquad (3)$$

from which can be derived the k and k_3 values

where $k = k_2 + k_4 [AcCl]_{const}$ (4)

Varying in the experiments the $[AcCl]_{const}$ value, from (3) was obtained a set of parallel straight lines (Fig.1), described by the slope $k_3 \approx 0$, evidencing about the absence of the indicated channels and by different values of the intercepted sections of k. The latter refers to the existence of the k_4 channel. As far as the k_4 is not directly connected with the nucleophilic mechanism of catalysis, we shall not dwell upon it.

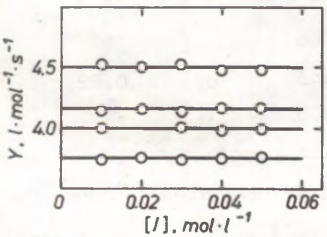


Fig. 1. Dependence (3) at $[AcCl]_{const}$ values, equalling respectively, 1(0.03); 2(0.05); 3(0.08); 4(0.1) $mol \cdot l^{-1}$ in acetonitrile at 25°.

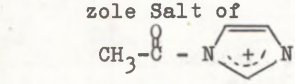
Thus, the analysis of the character of the k_H concentration dependence on $[N\leqslant]_o$ and $[AcCl]_o$ has shown that the I reaction with arylamine if $[N\leqslant]_o \geqslant [AcCl]_o$ proceeds according

to a single channel, i.e. the I bimolecular interaction with arylamine.

In acetonitrile, the acylammonium salts exist in the form of the equilibrium mixture of ions, ion pairs and other associates[4]. In order to find the ratio of the ionic forms of 1-acetyl-3-methylimidazole salts, the latter were studied conductometrically. The results are given in Table 1.

Table 1

Association Parameters and Rate Constants of Interaction of Ions and Ion Pairs of 1-Acetyl-3-Methylimidazole Salt of



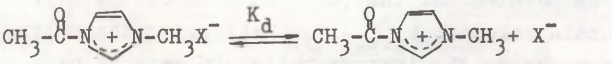
Type where X^- = Cl^-(I), Br^-(II); ClO_4^- (III); $B(C_6H_5)_4^-$ (IV), with 4-Aminoazobenzene in Acetonitrile at 25°

No	λ_o	λ^+_o	λ^-_o [5]	K_d mol·l^{-1}	k_i.e. l·mol^{-1} s^{-1}	k_i, l·mol^{-1} s^{-1}	k_i.e / k_i.
I.	164.28	72.68	91.6	0.0096± ±0.0004	3.4	0.81	4.2
II.	173.37	72.67	100.7	0.0153± ±0.0004	0.21	0.84	0.25
III.	175.44	72.44	103.0	0.0414± ±0.0006	0	0.79	-
IV.	132.88	74.68	58.2	0.0529± ±0.0008	0	0.82	-

Note: Dimension of λ (ohm^{-1}, gram-equivalent^{-1}, cm^{-2}).

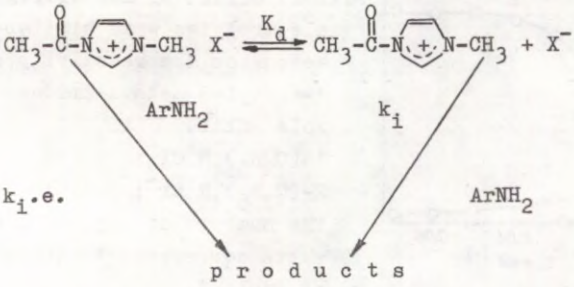
It follows from these data that the 1-acetyl-3-methylimidazole cation mobility (λ^+_o), calculated from the Kohlrausch additivity rule is the same in case of salts with different anions, evidencing about the correctness of the obtained results. The limiting equivalent conductivity of

the salts studied (λ_o) varies in the range of 140-180 ohm^{-1}. g-eq.$^{-1}$.cm^2, which is in keeping with the literature data for tetraalkylammonium salts[5-6] that are structurally analogous to the studied compounds. The dependence $\lambda = f(\sqrt{c})$ has linear character in all cases. From it follows that 1-acetyl-3-methylimidazole salts are in acetonitrile as an equilibrium mixture of ion pairs and ions (Scheme (5)). Evidently there are no bigger ionic associates there[7].



(5)

Taking into consideration the aforesaid, we can assume that the interaction of 1-acetyl-3-methylimidazole salts with AAB proceeds according to the following scheme:



(6)

the second order rate constant is determined according to the Acree equation

$$k_2 = k_{i.}\alpha + k_{i.e}(1-\alpha) \qquad (7)$$

where $k_{i.}$ and $k_{i.e}$ are the rate constants of acylammonium cation and the ionic pair of the salt, correspondingly. α denotes the dissociation level of the ion pair.

In order to check the validity of Eq.(7), the $k_{i.}$ and $k_{i.e}$ values were found also according to the kinetic data on the effect of the supporting electrolytes with the similar anions ($(C_2H_5)_4N^+Cl^-$, $(C_2H_5)_4N^+Br^-$, $ZiClO_4$). The initial concentration of acylammonium salts was approximately equal to $1 \cdot 10^{-3}$ mol$\cdot$l^{-1}, referring to their practically complete dissociation ($\alpha \geqslant 0.95$). It follows from the found depend-

ences (Fig.2) that if the concentration of a similar anion increases, the k_2 values will approach those equal to the reactivity of the corresponding ion pairs. The obtained $k_{i.e.}$ and $k_{i.}$ values are close to those found by Eq.(7) (Table 1). The given data show that the $k_{i.}$ values coincide in case of 1-acetyl-3-methylimidazole (In Fig. 2, the dependences for different salts intercept practically in the same point whose ordinate corresponds to the cation reactivity).

It should be pointed out that only in case of 1-acetyl--3-methylimidazole chloride $k_{i.e.} > k_{i.}$, while for the salts with other anions holds the inverse ratio. In case of perchlorate and tetraphenylborate $k_{i.e}=0$ and the reaction proceeds on the expense of the cation only.

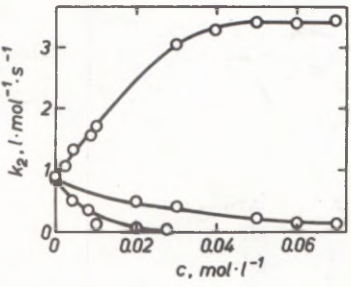
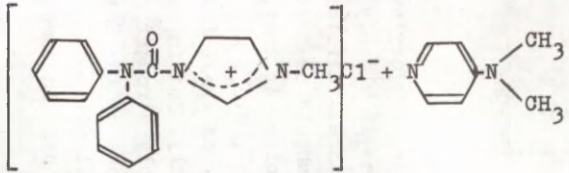
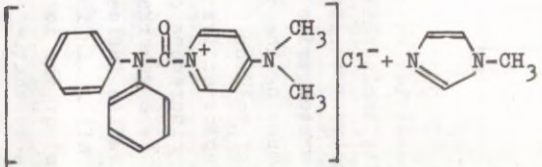


Fig. 2. Effect of supporting electrolytes with similar anions on the reactivity of 1-acetyl-3-methyl-imidazole salts. 1-$(C_2H_5)_4N^+Cl^-$; 2-$(C_2H_5)_4N^+Br^-$; 3-$ZiClO_4$. The numbers of acylammonium salts correspond to those of Table 1.

The data for chloride seem to contradict the idea that the ion pairs are not, in general, so reactive as the corresponding free ions. Therefore it is possible to suppose that in case of chloride ions appears a kind of additional factor stabilizing the transition state. As it has been shown[8], in the low-polarity media the chloride ion has a stabilizing effect because of the hydrogen bond with the attacking nucleophiles. There is ground to think that in acetonitrile, in the ion pair (I), it has the same function. It is also confirmed by the fact that in the reactions proceeding without the proton transfer, e.g. the reaction of diphenylcarbamoyl-N--imidazole chloride with 4-N,N-dimethylaminopyridine (Table 2), holds the ratio $k_{i.} > k_{i.p.}$. In this case, as in

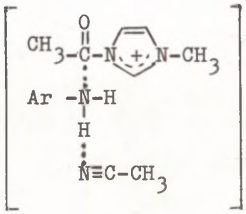
Table 2

Interaction of Diphenylcarbamoyl-N-Methyl-Imidazole Chloride with 4-N,N-Dimethyl Aminopyridine in Acetonitrile at 25°C.

	k_2 $l \cdot mol^{-1} \cdot s^{-1}$	α	$k_{i.}$ $l \cdot mol^{-1} \cdot s^{-1}$	$k_{i.e.}$ $l \cdot mol^{-1} \cdot s^{-1}$	$\frac{k_{i.}}{k_{i.e.}}$
	1.59	0.662			
	1.65	0.764			
	1.71	0.802	1.87	1.00	1.87
	1.73	0.814			
	1.77	0.834			

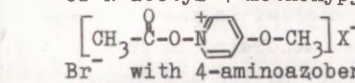
case of reactions of 1-acetyl-3-methylimidazole bromide, perchlorate and tetraphenylborate with arylamines, the anions restrain the reaction of ion pairs, if compared with the free cations.

As it has already been mentioned, in the low-polarity media, the reaction with arylamines proceeds at the general basic assistance of the weakly solvated anion of the ion pair. In case of the k_i route in acetonitrile, it seems to be the solvent itself that functions as such a factor, forming the hydrogen-bond complexes with arylamines thus favoring the increase of their reactivity. At the same time, by force of the proton-acceptor activity and high polarity, acetonitrile hinders the formation of such associates with the basis situated in the system[9]. Evidently, it is hardly possible to speak about the reactivity of a cation as such in acetonitrile and analogous solvents.



(8)

The reactivity of the free acylammonium cation with the low-basicity nucleophiles is closely linked with the general-basic solvent assistance. Therefore in the reactions of ion pairs acetonitrile should be treated as the proton-acceptor, competing the anion. At that, in case of the anions with greater volumes (Br^-, ClO_4^-, $B(C_6H_5)_4^-$) dominates the proton-acceptor activity of the solvents, the anion causing the steric hindrances only. But in case of the chloride ion, which is a remarkably stronger proton-acceptor, either predominates its influence or the both mechanisms are realized in parallel. An insignificant growth in the reactivity ($\approx$ 4 times) of the ion pair in comparison with the cation corresponds to that.

It can be mentioned for comparison that in the case of acyloxypyridine salts, where the reaction center is eliminated from the anion, the ion pairs with the chloride and bromide ions and the acyloxypyridine cation have practically similar reactivities. For instance, in the reactions of N-acetyl-4-methoxypyridines of the  halogenides, where $X^- = Cl^-$ and Br^- with 4-aminoazobenzene, $k_{i.} = k_{i.e} = 1.48\ l \cdot mol^{-1} \cdot s^{-1}$. A detailed comparison of the reactivity of acylammonium and acyloxyammonium intermediate products of the nucleophilic catalysis will be given in our following contributions.

Experimental

Acetylchloride, acetylbromide[10], diphenylcarbamoyl chloride[11] were synthesized and purified according to the standard methods. 3-methylimidazole[12] and 4-N,N-dimethylaminopyridine[13] were synthesized and purified as described in literature. Acylammonium salts were obtained applying the methods given in[11], 4-aminoazobenzene was purified by standard methods[14]. The UV spectra were taken on a spectrophotometer "Specord UV VIS" in the quartz cells (thickness 1 cm).

The reactions were conducted in the pseudofirst order of arylamine. The process was monitored spectrophotometrically according to the arylamine decrease at 390 nm. In case of the interaction of diphenylcarbamoylimidazole chloride and 4-N,N-dimethylaminopyridine, the process was checked according to the accumulation of diphenylcarbamoyl--4-N,N-dimethylaminopyridine chloride at 320 nm.

The rate constants k_2 were calculated according to equation

$$k_2 = \frac{1}{a \cdot t} \ln \frac{D_o - D_\infty}{D_t - D_\infty} \qquad (9)$$

where D_o, D_∞ and D_t denote the optical density of the solvents at the initial time moment, at the termination of the reaction and at time moment t; a is the concentration of acylammonium salt.

The linear dependences were calculated according to the

least squares' method.

The conductometric measurements were conducted by means of a differential-transformer bridge[15]. A constant-temperature cell with parallel-plate platinum electrodes, covered with platinum black were applied. The purity of the solvent was checked conductometrically , it was similar to the literature data[16]. The solutions were prepared according to the weight-space method with a following dilution. The electrical conductance was measured in the $5 \cdot 10^{-2}$ — $1 \cdot 10^{-4}$M concentration range of the electrolyte at 25°C. The thermostatic control was conducted in a water thermostate, the accuracy being 0.02°C. Because of a high hydroscopicity of the salts, the solutions were prepared, the electrical conductance was measured, the cells of the spectrophotometer were filled and the samples of the compounds were taken in a box which was carefully dried with phosphoric anhydride. Measurings were carried out during no more than 5 hours after the purification of the solvent.

The dissociation constants were calculated from the concentration dependence of electrical conductivity according to the methods of Shedlovskij[17] and Fuoss-Onsager-Scinner[18] on a computer EC-1022. Both methods yielded close results.

Proceeding from values of dissociation coefficients, the initial concentrations of acetylimidazole salts in kinetic studies were chosen so that the α values would make 50-80%, i.e. the concentration of ions and ion pairs would be commesurable.

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Organic Reactivity
Vol. 23 3(83) 1986

REGULARITIES OF STERIC EFFECT OF ALKYL GROUPS IN ALIPHATIC AMINES ON THEIR REACTIVITY

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The Menshutkin reaction has been used to prove the nonadditivity of the steric effect of alkyl substituents in amine. The contribution of the non-additive steric effect, depending on the other factors influencing the reaction rate has been found.

By the time being, a substantial number of examples demonstrating both the additive as well as the cumulative steric effect of certain substituents has been gathered. Thus, in the reactions of bromine addition to the substituted ethylenes, the additive steric effect is observed.[1] Similar action of methylic groups was found in the S_N2 reactions at the saturated carbon atom[2]. At the same time, in case of the alkaline hydrolysis of the $R^1C(O)OR^2$ and $R^1C(O)SR^2$ ethers, the non-additive steric effect of alkylic groups has been referred to[3]. In addition to that, out of the reactions, suggested for estimation of the steric constants $E_S(E_S^o)$ of the radical $R^1R^2R^3C$ (see, e.g.[4]), those taking into consideration the non-additive action of the R^1,R^2,R^3 substituents proved to be the most appropriate ones.

As it has been reported earlier[5], the nucleophility of aliphatic amines remarkably depends on their steric structure. Therefore it was interesting to calculate the actual differences between the steric action of alkyl radicals in amines

during their reactions with various substrates. With that aim in view were also determined (Table 1) the rate constants of the Menshutkin reaction (1)

$$R^1R^2NH + R^3R^4CHI \longrightarrow R^1R^2CHN^+HR^3R^4I^- , \qquad (1)$$

where R^1,R^2= Me, Et,i-Bu, i-Pr, R^3=H, R^4=H, Me; R^3,R^4=Me.

It can be considered when assessing the R^1 and R^2 influence that the change in the reaction rate caused by the difference in their inductive activity is negligible in comparison with that taking place because of the steric effect.

Table 1.

Rate Constants ($k \cdot 10^3$, $M^{-1} \cdot s^{-1}$) of Reactions of Amines R^1R^2NH with Alkyliodides in Acetonitrile at 25°C

R^1	R^2	MeI	EtI	i-PrI[a]
Me	Me	319±14	13.4±0.3	0.275±0.020
Me	Et	182±10	5.34±0.30	0.0733±0.0035
Me	i-Bu	107±7	2.38±0.20	0.0465±0.0025
Me	i-Pr	75.0±2.0	1.18±0.08	0.0135±0.0006
Et	Et	77.8±1.0	1.27±0.01	0.00914±0.00045
Et	i-Bu	36.0±0.5	0.694±0.040	0.00527±0.00042
Et	i-Pr	21.2±0.9	0.187±0.008	0.000744±0.000065
i-Bu	i-Bu	22.1±0.2	0.274±0.008	0.00231±0.00009
i-Bu	i-Pr	9.83±0.09	0.0862±0.0050	0.000498±0.000050
i-Pr	i-Pr	3.22±0.04	0.0187±0.0007	0.000020±0.000008

a) The presented constants of substitution rate were obtained substracting the elimination rate found independently[6] from the total reaction rate.

The fact that the reactivity of the amines with a similar number of alkyl radicals depends mainly on the steric constants E_N confirms the aforesaid statement. E.g., the data[7] on the reactivity of the secondary amines with EtI can be depicted by equation:

$$\log k = (-1.35\pm0.08) + (0.84\pm0.04)E_N; \qquad (2)$$

$$N = 7;\ s_o = 0.11;\ r = 0.995$$

To characterize the R^1 and R^2 substituents, we made use of the intrinsic operational scale[8], which enabled to find small cross terms.

$$\log k_{(R^1R^2)} = \log k_{(R_o^1,R_o^2)} + a_1E_R{}^1 + a_2E_R{}^2 + a_{12}E_R{}^1E_R{}^2 \qquad (3)$$

$E_R{}^1$ and $E_R{}^2$ are the constants characterizing the activity of the R^1 and R^2 substituents in amine, calculated according to equations (4) and (5) but $R_o^1 = R_o^2 = Me$.

$$E_R{}^1 = \log k_{(R^1,R_o^2)} - \log k_{(R_o^1,R_o^2)} \qquad (4)$$

$$E_R{}^2 = \log k_{(R_o^1,R^2)} - \log k_{(R_o^1,R_o^2)} \qquad (5)$$

Taking into account that $a_1 = a_2 = 1$ according to[8], equation (3) can be transformed into (6)

$$\log k_{(R^1R^2)} = \log k_{(R_o^1,R_o^2)} + a_1(E_R^1+E_R^2) + a_{12}E_R^1E_R^2 \qquad (6)$$

Treatment of the data of Table 1 according to this equation shows (Table 2) that, first, the $\log k_{(R_o^1,R_o^2)}$ values and the experimentally found values of the rate of Me_2NH interaction with the corresponding substrates coincide. Secondly, the $a_{1(nat.)}$ values remarkably differ from 1, as was expected mathematically. Finally, in case of all reaction series the cross term $a_{12}E_R{}^1E_R{}^2$ being the correction for the nonadditivity* of the action of the R^1 and R^2 substituents at the nitrogen atom in nucleophile, turns out to be significant .

A negative value of the a_{12} coefficient refers, as it has already been mentioned[3], to the fact that the steric interac-

* An analogous regularity was observed[3] at the treatment of the data on the hydrolysis of the ester of the $R^1C(O)OR^2$ and $R^1C(O)SR^2$ types.

Table 2

Correlation Parameters of Rate Constants of Reactions of Aliphatic Amines with Alkyliodides According to Eq.(6)

Substrate	$\log k_{(R_o^1, R_o^2)}$	a_1		a_{12}		s_o	R	N
		nat.	norm.	nat.	norm.			
MeI	-0.51 ± 0.07	0.99 ± 0.16	0.63 ± 0.10	-1.72 ± 0.45	-0.39 ± 0.10	0.08	0.993	10
EtI	-1.88 ± 0.08	1.01 ± 0.12	0.76 ± 0.09	-0.56 ± 0.20	-0.25 ± 0.09	0.10	0.994	10
i-PrI	-3.58 ± 0.07	0.98 ± 0.08	0.62 ± 0.05	-0.87 ± 0.11	-0.40 ± 0.05	0.08	0.998	10

tion of the R^1 and R^2 substituents tends to destabilize the transition state of the studied reaction.

Besides, the contribution of the nonadditivity factor of the steric substituent effect in amine, estimated according to the $a_{12(norm.)}$ [9] being~35%, does not particularly depend on the substrate type.

It was interesting to process the whole data file of Table 1 according to the single equation, simultaneously characterizing the structural effect of the substrate and nucleophile. For that purpose, Eq. (7) was used:

$$\log k = \log k_o + a_1 (E_R'^1 + E_R'^2) + \gamma' v' + a_{12} E_R'^1 E_R'^2 + a_{13}(E_R'^1 + E_R'^2) v' + a_{14} E_R'^1 E_R'^2 v' \quad (7)$$

where $E_R'^1$ and $E_R'^2$ are the constants* characterizing the substituents' activity in amine; v' denotes the steric con-

* Since $E_{R^1(R^2)}$ have certain values for each reaction series, we applied the mean values of these constants for the treatment according to (7), and denoted them by $E_{R'^1(R^2)}$. In this case, the $a_1 \neq 1$.

stants of Charton[2] for the S_N2 reactions characterizing the substituent effect in the substrate, $a_1, a_{12}, a_{13}, a_{14}$ and ψ'' are the coefficients of the susceptibility to the corresponding factors.

According to the analysis of the coefficients of Eq.(7) in the normed scale (Table 3), these are the volume of the substituents in the substrate ($|\psi'| = 0.69$) and their total in the nucleophile ($|a_1| = 0.22$) that mainly influence the process rate in this case. The $a_{13}(E_R'1 + E_R'2)v'$ term refers to the changing of the transition state on the reaction coordinate[6].

Cross term $a_{12}E_R'1\ E_R'2$($|a_{12}| = 0.09$), characterizing the substituents nonadditive activity in the amine, gives a remarkably smaller contribution than in case of the correlation treatment of the reaction series with a fixed substrate, though the ratio a_1 norm./a_{12} norm. = 2.45 is approximately equal to that (1.92) for the series where the amine structure is the only variable parameter. The contribution of the triple cross term $a_{14}E_R'1\ E_R'2v'$ is also negligible ($|a_{14}| = 0.10$).

Table 3.

Correlation Parameters of Rate Constants of Reactions of Aliphatic Amines with Alkyliadides According to Eq.(7)

$\log k_{(R_o^1R_o^2)}$	a_1		ψ'		a_{12}	
	nat.	norm.	nat.	norm.	nat.	norm.
-0.44	0.70±0.16	0.22±0.05	-4.43±0.24	-0.69±0.04	-0.53±0.29	-0.09±0.05

$\log k_{(R_o^1R_o^2)}$	a_{13}		a_{14}		s_o	R	N
	nat.	norm.	nat.	norm.			
-0.44	0.83±0.36	0.15±0.06	-1.12±0.64	-0.10±0.06	0.14	0.998	30

The a_1, ψ', a_{13} coefficients are significant on the 0.95 level according to the t-criterion; the a_{12}, a_{14} coefficients are significant on the 0.90 level according to the t- criterion.

Thus, in case of small R^1 and R^2 volumes as well as in case of a remarkable effect of the substrate's structure on the reaction rate (or in case of some other factors), the nonadditivity of the substituents' steric activity in the amine is suppressed by the functioning of these factors. Still, as to the sterically complicated amines, the nonadditivity may have a certain significance in their characterization.

Experimental

Dimethylamine, diethylamine, diisobutylamine, diisopropylamine and alkyliodides were purified according to the known methods. Methylethylamine and methylisobutylamine were obtained by means of the hydrolysis of the corresponding N,N'-dialkylsulfamides[10,11]. Methylisopropylamine, ethylisopropylamine, ethylisobutylamine and isobutylisopropylamine were obtained via the reduction of the corresponding azomethines by the lithium aluminium hydride[12]. The physico-chemical characteristics of the obtained compounds corresponded to those given in literature. Kinetic measurements were carried out as described earlier[6,7].

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Organic Reactivity
Vol. 23 3(83) 1986

FACTORS AFFECTING FORMATION RATE OF ALKENES IN CASE OF INTERACTION OF ALKYLIODIDES WITH ALIPHATIC AMINES

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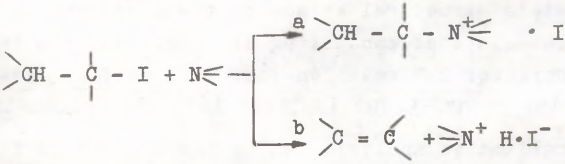
It has been demonstrated on the basis of the studies of the interaction kinetics of alkyliodides with aliphatic amines (in acetonitrile at 25°) that the reaction has two possible channels of proceeding: substitution and elimination. The dependence of the rate of elimination reaction and the ratio of the elimination and substitution rate on the reagent's structure have been studied. The elimination rate dependence on the nucleophile structure turned out to be much weaker than that of substitution rate.

It is known that the reactions of nucleophilic substitution and splitting can be parallel in several cases (see eg.[1]), the Menshutkin reaction included. Menshutkin[2] referred to the formation of isobutene from tertiary butyl iodide and perhaps also that of propene from isopropyliodide in the presence of amines. Therefore when studying the amines' interaction with alkyliodides (1), two goals have been determined in the present work:



(1)

studies of substitution in "pure" cases* and establishing

* The effect of the reagent's structure on the rate of the Menshutkin reaction has been reported in[3,4].

the factors affecting the relations between the substitution (1a) and elimination (1b).

The obtained data (Tables 1 and 2) indicate first of all a clear dependence of the elimination channel (%E) on the alkyliodide structure.

Table 1

Rate Constants ($k \cdot 10^6$ $M^{-1} \cdot s^{-1}$) of Interaction of Alkyliodides with Et_3N in Acetonitrile at 25° Concerning the Substitution (S) and Elimination (E).

	EtI	n-PrI	i-AmI	i-BuI	i-PrI	s-BuI
k_S	228± ±5[a]	38.2± ±0.6[a]	6.85± ±0.30	3.19± ±0.17[b]	0.26± ±0.03[b]	-
k_E	0.21± ±0.02	0.86± ±0.08	0.29± ±0.03	1.65± ±0.08	1.18± ±0.04	3.05± ±0.10
%E	0.09	2.2	4.0	34	82	100

[a] From paper[3]. [b] From paper[4].

Thus, this contribution increases almost 3 times in the case of transition from EtI to i-PrI. In case of S-BuI (Table 2) it was not possible to find the substitution channel of a number of amines. This is mainly caused by the decrease of the substitution rate in the series of the compared sub - strates if the screening level of their reaction center is growing.

The substrate's structural effect on the dehydroiodination rate* reveals that conducting of alkyl radicals into its molecule increases the reaction rate to a certain extent. Thus, if the methyl group is taken into the ß-position

* Only the qualitative analysis can be carried out as far as the rate variation is not great enough and the set of substrates is too small for a reliable quantitative analysis of the data concerning the given reaction series.

Table 2

Rate Constants ($k_E \cdot 10^6$ $M^{-1} \cdot s^{-1}$) and Contribution of Elimination Channel to the Total Rate of Alkyliodide Reaction with Amines

	i-PrI		i-BuI		s-BuI	
	k_E	%E	k_E	%E	k_E	%E
$MeNH_2$	0.567±0.040	0.93	2.13±0.20	4.7	4.86±0.16	11
i-$PrNH_2$	0.267±0.10	2.9	1.06±0.05	10	-	
t-$BuNH_2$	0.220±0.009	6.9	0.670±0.030	14	2.13±0.16	63
piperidine	3.26±0.06	1.4	17.6±0.9	7.7	28.2±1.5	24
Et_2NH	1.38±0.05	13	5.26±0.30	24	10.9±0.30	94
$(i\text{-}Bu)_2NH$	0.334±0.012	13	0.865±0.027	16	-	
$(i\text{-}Pr)_2NH$	0.217±0.008	92	0.160±0.008	40	-	
Me_3N	4.50±0.35	4.0	14.3±1.0	6.4	21.9±1.5	67
Me_2BuN	1.96±0.08	12	9.79±0.40	17	10.7±0.7	100
Me_2BzN	0.350±0.012	8.1	0.969±0.040	8.9	-	
$MeEt_2N$	1.90±0.11	41	5.55±0.40	25	-	
Et_3N	1.18±0.04	82	1.65±0.08	34	3.05±0.10	100

(cf. n-PrI and EtI, i-BuI and n-PrI, s-BuI and i-PrI in Table 1), the rate of the process increases 2-4 times, while in the α-position the rate increases 4-5 times (cf. i-PrI and EtI, s-BuI and i-PrI).

Acceleration of the donor substituents' action in the elimination reaction according to the Thornton-O'Ferral model (see e.g.[5]) speaks about a greater C-I bond breaking level in comparison with C-H in the transition state, which turns out to be "loose". It should be pointed out that in the studied alkyliodides , the transition state of substitution reactions is also "loose"[4].

As to the values of the observed effects, it should be taken into consideration that the alkyl group also increases steric screening of the reaction center, i.e., the inductive and steric effects are directed oppositely. So, the acceler-

ation during the hydrogen substitution for alkyl in the α-position in comparison with the ß-position would be greater without the presence of the steric effect. The interaction of these effects also explains the decreased i-AmI reactivity compared with that of n-PrI. A slowing - down of the reaction rate conditioned by a greater steric screening of the reaction center in case of the ß-isopropyl group exceeds the acceleration caused by the strengthening of the electron-donor properties.

The nucleophile's structural effect on the ratio of the channel rates and the elimination reaction rate was studied more thoroughly (Table 2). In case of the i-BuI, the contribution of the elimination channel changes if the structural variation of amine is not more than ten times, not exceeding 40% in case of i-Pr_2NH. The alkene yield in case of i-PrI changes 100 times amounting to 92% in case of i-Pr_2NH. s-BuI, being a sterically complicated substrate almost completely reacts with such rather slightly complicated amines as Et_2NH and Me_2BuN via elimination.

The constants of substitution and elimination rate for i-BuI and i-PrI with a series of aliphatic amines (Table 2) were processed according to Eq.(2):

$$\log k = \log k_o + \rho^{*}\Sigma\sigma^{*} + \delta E_N \qquad (2)$$

where $\Sigma\sigma^{*}$ is the sum of induction constant substituents at the nitrogen atom in amine;
E_N is the steric effect of the entire molecule of amine,
ρ^{*} and δ denote the coefficients of susceptibility to the corresponding factors[7].

The analysis of the obtained correlation parameters (Table 3) reveals that in the case of elimination reaction, the susceptibility to the amine structure is smaller than in case of substitution reaction. It becomes especially evident in case of i-PrI (cf. the ρ^{*}_{nat} and δ_{nat} coefficients for substitution and elimination reactions). A more remarkable decrease in the susceptibility to the amine's steric structure that is observed during the transition from the substitution

Table 3

Correlation Parameters of Constants of Substitution and Elimination Rate of i-BuI and i-PrI with Aliphatic Amines in Acetonitrile at 25°

Substrate	Reaction type, N(number of points)	log k_o	ρ^*		δ		s_{total}	R
			nat.	norm.[a]	nat.	norm.[a]		
i-BuI	Substitution N = 12	-2.10± ±0.12	-2.27± ±0.13	-1.10± ±0.06	1.08± ±0.04	1.52± ±0.06	0.112	0.993
	Elimination N = 12	-3.58± ±0.22	-2.09± ±0.24	-1.35± ±0.16	0.75± ±0.08	1.42± ±0.15	0.205	0.955[b]
i-PrI	Substitution N = 12	-1.88± ±0.19	-2.16± ±0.20	-0.77± ±0.07	1.39± ±0.07	1.44± ±0.07	0.173	0.990
	Elimination N = 12	-4.67± ±0.22	-1.68± ±0.23	-1.45± ±0.20	0.49± ±0.08	1.21± ±0.20	0.201	0.925[b]

[a] See[6] for the application of the normed coefficients in order to compare the contribution of the corresponding effects.

[b] Small correlation coefficient values obtained in case of the data treatment according to the elimination rates refer to their comparatively narrow variation range.

to the elimination is most probably connected with a looser transition state of the reaction.

It results in a situation where (unlike the substitution reaction with a predominanting steric effect,) in case of the elimination reaction, the inductive effect of the substituents in amines becomes to play the leading role. (cf. the corresponding $\rho^{*}_{norm.}$ and $\delta_{norm.}$ coefficients.).

Experimental

Acetonitrile, amines and alkyliodides were purified according to the known methods. In case of sterically complicated amines, the measurements were carried out by the method of high initial rates. With the reactive amines (Me_3N, piperidine), the reaction was conducted to its termination and the ratio of the alkene and quaternary ammonium salt was determined. The gross rate constant of the two channels was determined by means of potentiometric argentometric titration of the forming iodide ion. The alkene concentration was determined as described earlier[4].

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Organic Reactivity
Vol. 23 3(83) 1986

MECHANISM OF DEHYDROHALOGENATION OF ALKYLHALOGENIDES INDUCED BY ALIPHATIC AMINES

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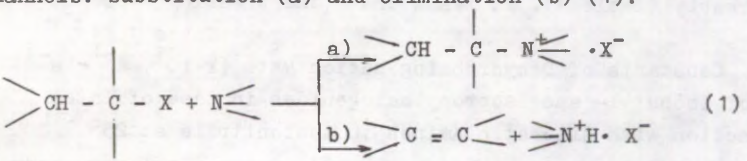
A conclusion has been made on the basis of the studies of the influence of the nucleophile concentration and structure, the leaving group nature and the deuterium kinetic isotopic effect on the rate of the dehydrohalogenation reaction of alkylhalogenides that the reaction proceeds according to the E2 elimination mechanism.

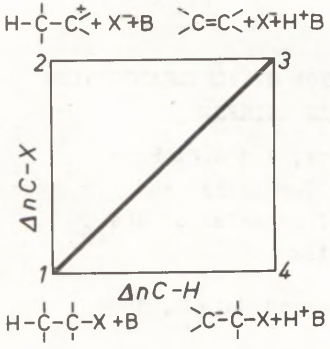
According to the earlier report[1] the interaction of alkyliodides and aliphatic amines proceeds according to two channels: substitution (a) and elimination (b) (1):



(1)

The mechanism of the first channel, i.e. the Menshutkin reaction has been thoroughly discussed in literature but the data on the latter channel is rather scarce. Therefore the present paper is aimed at studying the mechanism of the elimination reaction.

According to the Thornton-O'Ferral approach (see, e.g.[2]), the type of the mechanism of elimination reaction is determined by the ratio of breaking level of the C-X and C-H bonds and the formation of the C=C double bond in the tran-



Scheme 1

sition state (Scheme 1).

In order to estimate these values, we have examined the data concerning the influence of the amine's structure and concentration, and the leaving group nature on the reaction rate as well determined the primary deuterium kinetic isotopic effect in halkylhalogenide.

The rate of dehydrohalogenation reaction of various substrates (EtI, n-PrI, i-AmI, i-BuI, i-PrI, s-BuI), i-BuBr, i-PrBr (Table 1) obeys the second order reaction (the first order for each of the reagents) and depends on the structure of the amine, which is characteristic to the E2 and E1cB elimination mechanisms (region 1-3-4 in Scheme 1). If the nature of the leaving group X is changed (during the transition from I to Br derivatives), the constant of interaction range will drop remarkably (Table 1). It means that the breaking of the C-X bond takes place in the rate-determining stage, i.e. the orders of this bond considerably differ in the transition and initial states.

Table 1

Constants of Dehydrohalogenation Rate ($k \cdot 10^7$, $M^{-1} \cdot s^{-1}$) of Isobutyl- and Isopropylhalogenides in Case of Interaction with Aliphatic Amines in Acetonitrile at 25°

	i-BuI[1]	i-BuBr	k_I/k_{Br}	i-PrI[1]	i-PrBr	k_I/k_{Br}
Me_3N	143±10	4.45±0.40	32	45.0±3.5	1.10±0.09	41
$MeEt_2N$	55.5±4.0	1.38±0.12	40	19.0±1.1	0.79±0.07	24

Comparison of the reactivities of i-PrI ($k = 3.26 \pm 0.06) \cdot 10^{-6} M^{-1} \cdot s^{-1}$) and i-$Pr_{D7}I$ ($k = 4.72 \pm 0.08) \cdot 10^{-7} M^{-1} \cdot s^{-1}$) with piperidine and acetonitrile at 25° in the elimination channel refers to the existence of a significant primary isotopic effect ($k_H/k_D = 6.9 \pm 0.2$). Since the primary deuterium kinetic isotopic effect reaches its peak values 8-10 (only in rare cases higher) in case of a symmetrical transition state[3], the obtained k_H/k_D value evidences about the fact that in the transition state of the reaction, the expectancy of breaking of the C-H bond is 50%.

The available data help us to choose between the E2 and E1cB mechanism. The E1cB mechanism (route 1-4-3 in Scheme 1) can function in two limiting cases: (these are the nonequilibrium and pre-equilibrium variants (see, e.g.[4]). In the former case, the proton is slowly splitting off the substrate affected by the base and a rapid decomposition of the forming carbanion is observed. The susceptibility to the leaving group nature is negligible, contradicting the obtained data (Table 1). In the latter case, an insignificant (≤ 1.5) thermodynamic isotopic effect should be traced at a rapid reversible proton splitting and a slow breaking of the C-X bond, which does not correspond to the experiment either.

Taking into consideration the aforesaid, a conclusion can be drawn that the reaction of dehydrohalogenation of the studied alkylhalogenides in case of their interaction with the aliphatic amines proceeds according to the mechanism of synchronous (E2) elimination.

Experimental

Alkylhalogenides and aliphatic amines were purified according to the known methods. Isopropyliodide D_7 was obtained from the isopropyl alcohol D_8 via the reaction with iodine and triphenylphosphate[5]. The rate constants of the elimination channel were determined as described in[1].

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Organic Reactivity
Vol. 23 3(83) 1986

ELECTRONIC EFFECT OF STRUCTURE OF MORPHOLINE ON ITS NUCLEOPHILITY

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Correlation dependencies of nucleophilic reactivity of aliphatic amines (the inductive ($\Sigma\sigma^*$) and steric (E_N) effects were taken into consideration separately) in various reaction series were used in order to quantitatively estimate the effective inductive influence of morpholine structure on its activity. The found value $\Sigma\sigma^*_M = 0.62\pm0.03$ considerably differs from the calculated value which was obtained, summing up the σ^* for the substituents in the vicinity of the nitrogen atom (1.16) because of the nonadditivity of the inductive substituent effect caused by the formation of the intramolecular hydrogen bond between the hydrogen atom of the aminogroup and the oxygen atom.

It is known (see, e.g.[1].) that the nucleophilic reactivity of aliphatic amines is conditioned by two factors mainly: by the inductive substituent effect at the nitrogen atom and the accessibility of this atom. For the quantitative analysis of these effects[2] the following two parameter equation proved to be the most appropriate one:

$$\log k = \log k_o + \rho^*\Sigma\sigma^* + \delta E_N \qquad (1)$$

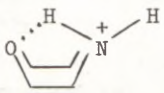
Here $\Sigma\sigma^*$ characterizes the inductive effect of the radicals joining the nitrogen atom; E_N is the steric accessibility of the atom, ρ^* and δ denote the susceptibility of the reaction series to these effects.

Still, the nucleophilic reactivity of morpholine (if values* are used) $\Sigma\sigma^*$= 1.16 and E_N= -0.79) does not obey Eq.(1). Usually, morpholine reacts 20-200 times faster than might be expected according to the correlation dependence made up for a number of other aliphatic amines, using these constants[4-7]. Supposedly, this effect is caused either by formation of the intramolecular hydrogen bond between the oxygen atom and hydrogen atom of the N-H group in the transition state when the nitrogen atom acquires a positive charge[2], or by the additional electronic effects[4].

It should be pointed out that the morpholine basicity in nitromethane does not obey[8] the Taft equation (2), which is also connected with[8,9]

$$pK_a = pK_a^o + \rho^*\Sigma\sigma^* \qquad (2)$$

the effect of intramolecular solvation of the cation by the oxygen atom according to type I:



I

Strictly taking, this effect can be considered a modification of Eq. (1), if a third parameter, characterizing the intramolecular hydrogen bond is used. Still, the formation of the intramolecular hydrogen bond can also be considered as a factor changing the inductive substituent effect at the nitrogen atom. It is quite acceptable, as far as it is supposed that the effect of all substituents is additive in character, when determining the inductive substituent effect at the nitrogen atom ($\Sigma\sigma^*$). In the case of morpho-

* The $\Sigma\sigma^*$ value is calculated taking the sum of the σ^* values for radicals H - and O$\begin{smallmatrix}\diagup CH_2CH_2- \\ \diagdown CH_2CH_2-\end{smallmatrix}$ equalling +0.49 and +0.67, respectively[3]. The E_N value is equal to that of piperidine[2].

line the additivity is hindered by the H-bond formation. Therefore it is quite correct to determine the values of the inductive substituent effect in morpholine by simply finding the σ^* sum for H(+0.49) and the radical $\diagup CH_2CH_2-$ (+0.67) $O\diagdown CH_2CH_2-$ The change of the inductive substituent effect at the nitrogen atom because of the formation of the intramolecular H-bond is actually similar to that in case of the proton transfer (e.g. in case of transition from - NR_2 to $^{+}NHR_2$ and from -OH to $-O^-$).

In order to quantitatively estimate such an effective inductive substituent effect at the nitrogen atom in morpholine ($\Sigma\sigma^*_M$)., a variety of data[4-7,10-17] concerning the reactivity of aliphatic amines, morpholine included, was analyzed (see Table).

The $\Sigma\sigma^*_M$ values for various reaction series were calculated according to Eq.(1) on the bases of the obtained data (log k_o, ς^* and δ) and the rate constants of the corresponding reactions with participation of morpholine, taking into account the value E_N= -0.79. These values (see Table) turned out to coincide well, being mostly in the range of 0.59-0.64*. Consequently, if Eq.(1) is modified by introducing the third parameter, the susceptibility of various reaction series to it will be approximately similar. The constancy of the calculated values enables us to suggest the value $\Sigma\sigma^*_M$= 0.62±0.03) (Calculated as an arithmetic mean for series 1a - 5a, 7-14) for morpholine.

It should be stressed that regardless of the fact that the morpholine basicity in nitromethane does not obey the Taft equation (2), the value $\Sigma\sigma^*_M$ = 0.83±0.10, calculated

* For the reactions with 2,4-dinitrochlorobenzene (series 6) the $\Sigma\sigma^*_M$ value is somewhat smaller equalling 0.49. Still, the introduction of the term for morpholine ($\Sigma\sigma^*_M$ = 0.62) into the correlation dependence does not change the correlation parameters and the term for morpholine does not deviate from the dependence more than those for the other amines (benzylamine, tret-butylamine, isopropylamine, dimethylamine) in this series.

Table

Correlation Parameters of Reactivity of Aliphatic Amines According to Eq.(1) and Calculation of $\Sigma\sigma^*$ Values for Morpholine ($\Sigma\sigma^*_M$)

No	Reagent[a]	Conditions	log k	ρ^*	δ	s	R	N	$\Sigma\sigma^*_M$
1	$(n\text{-MeOPh})_3C^+$	25°, H_2O [10]	6.91±0.32	-1.60±0.33	2.12±0.19	0.30	0.951	16	0.66±0.14[b]
1a	- " -[c]	- " -	7.23±0.24	-2.06±0.27	2.22±0.15	0.21	0.979	14	0.63±0.08
2	$(PhCH{=}CHCO)_2O$	25°, CH_3CN [11]	5.63±0.38	-3.27±0.35	1.71±0.14	0.27	0.955	17	0.64±0.07
2a	- " -[d]	- " -	5.29±0.20	-2.97±0.19	1.53±0.08	0.14	0.984	16	0.64±0.04
3	 [e]	20°, H_2O [12]	-2.06±0.18	-0.98±0.19	0.58±0.09	0.15	0.945	8	0.57±0.11
3a	- " -[f]	- " -	-1.77±0.18	-1.22±0.17	0.69±0.08	0.11	0.974	7	0.62±0.09
4	 [e]	- " -	-1.97±0.16	-0.89±0.17	0.51±0.08	0.14	0.948	8	0.64±0.12
4a	- " -[f]	- " -	-1.69±0.15	-1.11±0.14	0.62±0.07	0.09	0.979	7	0.69±0.09
5	$n\text{-}NO_2PhPO_2^{2-}$ [e]	39°, H_2O [13]	-5.44±0.36	-0.79±0.43	0.62±0.14	0.18	0.972	6	0.75±0.41
5a	- " -[g]	- " -	-5.21±0.23	-1.23±0.30	0.71±0.09	0.10	0.992	5	0.61±0.15
6	$2,4\text{-}(NO_2)_2PhCl$	25°, EtOH [14]	0.52±0.33	-3.25±0.34	1.67±0.12	0.36	0.962	19	0.49±0.05
6a	- " -[h]	- " -	0.55±0.24	-3.36±0.27	1.63±0.09	0.24	0.981	17	0.49±0.04
7	BNNA	70°, H_2O [15]	0.20±0.20	-2.65±0.17	1.57±0.09	0.09	0.999	4	0.60±0.04

Table continued

No	Reagent	Conditions	log k	ρ^{*}	δ	s	R	N	$\Sigma\sigma^{*}_{M}$
8	BMDC [e]	40°, C_6H_6 [16]	-1.47±0.46	-3.48±0.55	0.68±0.14	0.28	0.976	5	0.57±0.09
9	$PhCOCH_2Br$ [i]	25°, C_6H_6 [7]	0.82±0.20	-3.24±0.20	1.38±0.07	0.20	0.979	19	0.60±0.04
10	$CH_2=CHCN$ [i]	25°, H_2O [4]	1.67±0.29	-3.94±0.31	0.84±0.14	0.26	0.963	15	0.59±0.05
10[a]	- " - [i]	25°, H_2O [6]	2.86±0.49	-4.91±0.58	1.46±0.15	0.32	0.965	9	0.61±0.07
11	n-$MeOPhCOCH=CH_2$ [i]	25°, 10%EtOH-H_2O [4]	3.58±0.33	-3.40±0.33	0.63±0.13	0.18	0.973	11	0.64±0.06
12	n-$MePhSO_2CH=CH_2$ [i]	25°, EtOH [4j]	2,07±0.28	-4.01±0.29	1.36±0.12	0.18	0.979	11	0.62±0.04
13	$H_2NCOCH=CH_2$ [i]	25°, H_2O [5]	0.37±0.31	-3.29±0.33	0.88±0.15	0.27	0.951	13	0.62±0.06
14	$PhNHCOCH=CH_2$ [i]	- " -	0.52±0.31	-3.22±0.32	0.84±0.15	0.26	0.955	13	0.63±0.06
15	PhOH [k]	25°, CCl_4 [18]	2.53±0.10	-0.60±0.07	0.27±0.03	0.11	0.937	16	0.97±0.11
15a	PhOH [l]	25°, CCl_4 [18]	2.59±0.05	-0.64±0.04	0.27±0.02	0.06	0.985	13	1.01±0.06
16	DNP [k]	25°, C_6H_6 [19]	4.84±0.31	-2.73±0.35	0.65±0.12	0.31	0.915	16	0.91±0.12
16a	- " - [m]	- " -	5.05±0.19	-3.04±0.21	0.73±0.07	0.17	0.980	13	0.86±0.06

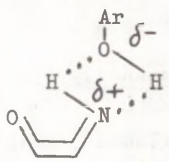
[a] Notations used : BNNA - 8-bromo-5-nitro-1-naphtoine acid, BMDC-4-bromo-4-methyl--2,6-di-tret.-butylcyclohexadiene-2,5-one, DNP-2,4-dinitrophenol. [b] The error was found from $\frac{\Delta\Sigma\sigma^{*}_{M}}{\Sigma\sigma^{*}_{M}} = \frac{\Delta\rho^{*}}{\rho^{*}}$.

Table continued

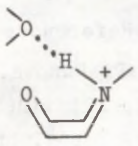
[c] Benzylamine and cyclohexylamine excluded. [d] Tret.-butylamine excluded. [e] Rate constants are given in dimensions $M^{-1}.s^{-1}$. [f] Pyrrolydine excluded. [g] Methylamine excluded. [h] Tret.-butylamine and benzylamine excluded. [i] Correlation parameters were taken from the given literature sources. [j] Correlation treatment of rate constants published in literature[17]. [k] Association coefficients $K_{ass}(M^{-1})$ are studied. [l] Isobutylamine, tri-n-butylamine and allylamine excluded. [m] Diasabicyclooctane, N,N-dimethylethylamine and diethylamine excluded.

according to equation[8,9] for the secondary amines (pK_a = = 19.36 - (4.29±0.15) $\Sigma\sigma^*$) is somewhat higher than for series 1-14. Still higher values of $\Sigma\sigma_M^*$ (0.86-1.01) are obtained from the dependences of type (1) for the constants of association of amines with phenol in CCl_4[18] and 2,4-dinitrophenol in benzene[19] (series 15 and 16). In these cases it may probably evidence about the loosening of the intramolecular bond, since the nitrogen atom charge is smaller than +1. Thus, there exists a correspondence between the charges on the nitrogen atoms and the level of formation of H-bond according to type I. Besides, in case of the constants of association with phenols, (series 15 and 16), the H-bond in I can be either weakened or broken by the hydrogen bond with oxygen atom of phenol according to type II.

The formation of intramolecular hydrogen bond of type I can also be affected by solvation of hydrogen atom of the N-H bond by the solvent (III), which should especially clearly be revealed in the protic media (water, alcohols).



II



III

In this case, the effective $\Sigma\sigma_M^*$ value depends on the strength of the hydrogen bond of each type (I-III). So, in water, where type I is practically not represented, the morpholine basicity obeys the Taft equation (2)[8,20]. At the same time, as can be seen from the data given in the Table (series 1-14), no interdependence between the medium properties and the nucleophilic reactivity of morpholine was observed. This may evidence about the fact that the role of the bond of type III is negligible in these cases. It is perhaps explained by the fact that in case of the acid-base interactions, the thermodynamic reaction parameters (K_a, K_{HB}) are examined whose value depends on the difference in the energy of the initial and final states.

But in case of the nucleophilic reactions of the amines, we must deal with the kinetic parameter (k), reflecting the difference between the energies of the initial and transition states where the intramolecular solvation of the transition state prevails the intermolecular one.

These examples illustrate well enough the dependence of the effectiveness of the electronic effect of the morpholine structure on its basicity (nucleophility) upon the acid (electrophile) nature and medium properties. It should be mentioned that the comparison of the reactivities of piperidine and morpholine as the amines having a similar steric accessibility to the nitrogen atom but different in basicity (in water their basicity differs (3 orders) often referred to in literature[17,21-24] must be treated very carefully as far as the conclusions made according to such a comparison only while a possible formation of the intramolecular hydrogen bond of Type I is not taken into consideration, may turn out to be incorrect.

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Organic Reactivity
Vol. 23 3(83) 1986

KINETIC MECHANISM OF NADH OXIDATION BY QUINOIDAL COMPOUNDS

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The thermodynamic parameters and kinetic isotope effect in the oxidation reactions of reduced nicotinamide adenine dinucleotide (NADH) by quinoidal compounds in a water medium, pH 7.0 and 25 °C were determined. It was found that an increased reactivity of O-quinones is due to a more positive $\Delta S^{\neq}$ value as compared with that of p-quinones. Single- and three-step hydride transfers in the reaction are discussed.

Oxidation reactions of reduced nicotinamide adenine dinucleotide (NADH), a cofactor of dehydrogenases, and its analogues by flavins and quinoidal compounds are intensively studied[1-9]. The main problems under investigation are the determination of specificity of various kinds of oxidizers[1-9] and the transfer mechanism of reduction equivalents[1-9]. As a results of the experiments made a number of the reaction series including the correlation relationships between the rate constant logarithm and the oxidizers redox potentials[4,6-8] or the Taft constants of the substituted NADH analogues emerged. The presence of the kinetic isotope effect in the oxidation reactions of deuterated 1,4-dehydropyridine enables one to assume that the oxidation process proceeds via the hydride-ion transfer[2,8,9]. Depending on the reaction type one-[1,2,8,9] or three-step (electron, proton, electron) hydride transfers[4,5] have been applied to describe these processes.

It was shown in Ref.[6] that a linear dependence between the logarithm of NADH oxidation rate constants and the potential of two-electron reduction of quinones exists in a water medium, and the reactivity of o-quinones is more than two orders higher than that of p-quinones. The aim of the present work is a thorough study of the reaction mechanism with deuterated NADH and the elucidation of the causes of an increased reactivity of o-quinones.

EXPERIMENTAL

NADH (Reanal, Hungary), tetracyano-p-quinodimethane (TCNQ) (Chemapol, Czechoslovakia), 2,5-dimethyl-1,4-benzoquinone (Aldrich, USA), N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) (BDH Chemicals, Great Britain) were used as received. Cation-radical TMPD ($TMPD^{+\cdot}$) was synthesized according to Michaelis method[10]. 1,4-benzoquinone, 2-methyl--1,4-benzoquinone, bromanil (tetrabromo-1,4-benzoquinone), 1,2-naphthoquinone, sodium 1,2-naphthoquinone-4-sulphonate, 9,10-phenanthrenequinone (pure) were purged in vacuum or recrystallized twice from benzene or ethanol. 4-A monodeuterated NADH was synthesized enzymatically using D_6-deuterated ethanol[11]. The deuteration degree determined according to the NMR method[12] was not less than 96 %.

The oxidation rate of NADH was measured fluorimetrically according to the fluorescence decrease at 440 nm (excitation wave length 340 nm). A MPF-4 spectrofluorimeter (Hitachi, Japan) was used in this work. The reduction rate of $TMPD^{+\cdot}$ was studied spectrophotometrically ($\Delta\varepsilon_{570}$ = 11 $mM^{-1}cm^{-1}$) on a spectrophotometer SPECORD UV-VIS (GDR). In fluorimetrical measurements the oxidizer concentration was 10-50 times greater than that of NADH (1.5-30 uM). In spectrophotometrical measurements the concentration of NADH exceeded the $TMPD^{+\cdot}$ concentration (20-100 uM) 10-30 times. For every reaction from 5 to 7 experiments were conducted with various reagent concentrations. The experiments were carried out at

15–45 °C in 0.1 M K-phosphate buffer solutions, pH 7.0 containing 1 mM EDTA. Li-phosphate buffer solution was used in the experiments with TCNQ. Some experiments were carried out in anaerobic buffer solutions prepared by bubbling with nitrogen (highest purity) for 20–30 min. Nitrogen was additionally purged through the pyrogallol solution. The data obtained were processed with a computer D 3-28.

RESULTS AND DISCUSSION

At large excess of quinones the oxidation of NADH up to a 90–95 % conversion goes in the first order. The obtained rate constants of pseudofirst order and the initial stage of the reaction are proportional to the oxidizer and NADH concentrations, respectively. So it follows, that the oxidation of NADH by quinones is second order. The reaction rate constants (k_{ox}) at 25 °C and pH 7.0 are presented in Table 1. The k_{ox} values of quinones do not respond to oxygen available in the reaction medium and k_{ox} of TCNQ in an oxygen-free medium decreases only by 5 %, as compared with that in an aerobic medium. The presence of 2-ethyl-1,4-hydroquinone (1 mM) in the reaction medium does not affect the oxidation rate of NADH by 2-methyl-1,4-benzoquinone. The reduction of NADH by a single-electron oxidizer $TMPD^{+\cdot}$ also follows a bimolecular mechanism since the initial reaction rate is proportional to the reagent concentration. However, at a comparatively low $TMPD^{+\cdot}$ reduction a linear deviation in coordinates ln A_{570} – t is observed (Fig. 1). It is due to inhibition by the reaction product TMPD whose addition decreases the initial reaction rate. The presence of oxygen does not affect markedly the reduction rate of $TMPD^{+\cdot}$. Since TMPD is oxidized by quinones its influence on the oxidation rate of NADH by quinones was not studied.

With increase in the temperature from 15 to 45 °C the reaction rate is increased, and the data on the temperature dependence upon k_{ox} linearize well in Eyring coordinates. The calculated data on the enthalpy and entropy of activation are

presented in Table 1.

When 4-A monodeuterated NADH is used the reaction rate decreases. The relationship between the rate constants of NADH and NADD oxidation at pH 7.0 and 25 °C (k_{HH}/k_{HD}) is given in Table 1.

The data given indicate some pecularities concerning the oxidation mechanism of NADH by quinoidal compounds. Firstly, for p-quinones studied the activation entropy values are similar and more negative than that of o-quinones (Table 1). So it follows, that the reaction series of p-quinones is isoentropic, and a high reactivity of o-quinones may be acounted for by a more favourable $\Delta S^{\neq}$ value. Apparently, the entropic factors determine the specificity of the reaction series of oxidizers with NADH and its analogues.

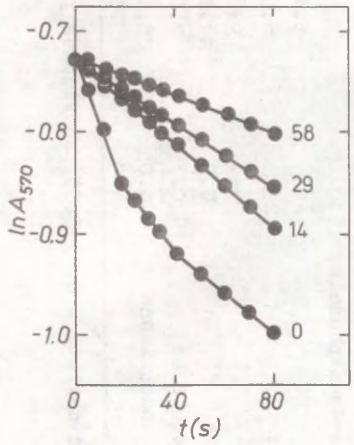

Fig. 1. The influence of TMPD on the reduction rate of $TMPD^{+\cdot}$. $TMPD^{+\cdot}$ concentration - 40 μM, NADH - 250 μM. Figures indicate the TMPD concentration (μM).

Table 1

Rate Constants, Thermodynamic Parameters and Kinetic Isotope Effects of NADH Oxidation by Quinoidal Compounds, pH 7.0, 25 °C.

Oxidizer	k_{ox}*, $l \cdot mole^{-1}s^{-1}$	$\Delta H^{\neq}$, $kcal \cdot mole^{-1}$	$\Delta S^{\neq}$, e.u.	k_{HH}/k_{HD}
Bromanil	1200	-	-	1.05 ± 0.05
TCNQ	770	4.3 ± 0.7	-31.4 ± 2.4	1.20 ± 0.06
1,2-naphthoquinone-4-sulphonate	540	-	-	1.43 ± 0.07
$TMPD^{+\cdot}$	36	5.1 ± 0.8	-33.8 ± 2.7	1.30 ± 0.10
1,2-naphthoquinone	25	8.0 ± 1.2	-25.0 ± 4.0	1.97 ± 0.05
1,4-benzoquinone	4.5	6.7 ± 1.1	-33.0 ± 4.0	1.89 ± 0.08
Phenanthrenequinone	0.9	11.3 ± 2.0	-20.7 ± 6.6	2.00 ± 0.10
2-methyl-1,4-benzoquinone	0.75	7.6 ± 1.1	-34.0 ± 4.0	1.77 ± 0.06
2,5-dimethyl-1,4-benzoquinone	0.05	8.3 ± 0.6	-36.0 ± 2.1	1.70 ± 0.10

*relative standard deviation 0.03-0.05, confidence probability 0.95.

Hydride transfer mechanism is the main problem in studying the oxidation of dehydropyridines. Single-step transfer mechanism of hydride is more probable in the reactions of dehydropyridines with pyridine and acridine ions[13]. However, in the reactions of dehydropyridines with quinoidal compounds there is the evidence for the existence of both single- and three-step transfers. Single-step transfer is more acceptable because of a considerable endothermy of a single-electron oxidation of NADH by quinones[14]. It was stated in Ref.[8] that constant k_{ox} values of quinones in the oxidation reactions of NADH over the pH interval 8.0-5.5 are due to a single-step hydride transfer in the limiting stage of the reaction since the potentials of the redox pairs Q/QH^- (E_H-) and $NAD^+/NADH$ are both dependent on pH:

$$NADH + Q \longrightarrow NAD^+ + QH^- \qquad (1)$$

Table 2 gives the standard potentials of redox pairs quinone/hydroquinone, pH 7.0 (E_o^7) for the compounds studied and the potential values E_H^7- calculated according to Refs.[8,15].

To describe the reactivity of benzoquinones at pH 7.0 in the framework of the single-step hydride transfer mechanism the correlation relationships were calculated according to Ref.[8]:

$$\log k_{ox} = (-2.12 \pm 0.71) + (16.54 \pm 3.42)\, E_H^7\text{-}, \qquad (2)$$
(p-quinones, r=0.9792),

$$\log k_{ox} = (-0.70 \pm 1.08) \pm (16.43 \pm 4.21)\, E_H^7\text{-}, \qquad (3)$$
(o-quinones, r=0.9611).

As it follows from Fig. 2 the k_{ox} values of quinones, determined in this work, satisfactorily comply with the given correlation relationships.

On the other hand, Tanaka et al.[4] showed that the oxidation of NADH analogue - 1-benzyl-1,4-dehydronicotinamide (BNAH) by quinones in acetonitrile proceeds in three stages

with the formation of radical pairs in the reaction:

$$BNAH + Q \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} BNAH^{+\cdot} - Q^{-\cdot} \xrightarrow{k_H} BNA^{\cdot} - QH^{\cdot} \xrightarrow[\text{quickly}]{} BNA^{+} + OH^{-}. \qquad (4)$$

For this reaction the k_{ox} value of quinones increases with the increase of their one-electron reduction potential[4]. For this reason, the relationship between the quinones reactivity and their one-electron reduction potentials in a water medium and the comparison of the reactivity of quinones and one-electron quinoidal oxidizers to analogous redox potentials are of great interest. The potential values of the single-electron reduction of quinoidal compounds studied at pH 7.0 (E_1^7) are presented in Table 2.

Table 2

Redox potentials of quinoidal compounds, pH 7.0

Quinoidal compounds	E_o^7 * V	E_H^7- ** V	E_1^7 *** V
TCNQ	-	-	0.36
Bromanil	0.32	0.32	0.34
1,4-benzoquinone	0.28	0.19	0.09
$TMPD^{+\cdot}$	-	-	0.27
2-methyl-1,4-benzoquinone	0.21	0.13	0.01
1,2-naphthoquinone-4-sulphonate	0.21	0.19	-
2,5-dimethyl-1,4-benzoquinone	0.16	0.07	-0.08
1,2-naphthoquinone	0.14	0.09	-
Phenanthrenequinone	0.02	-0.01	-

*data from Refs.[6,15]

**$E_H^7- = E_o^7 - 0.029\ (pK_a - 7)$ when pK_a of hydroquinone is greater than 7.0 and $E_H^7- = E_o^7$ when $pK_a \ll 7.0$

***data from Refs.[6,16,17].

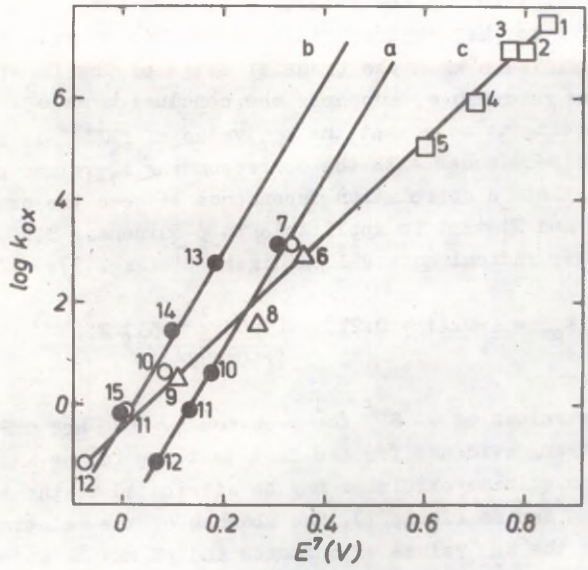

Fig. 2. The dependence of k_{ox} of quinoidal oxidizers on the redox potential, pH 7.0. Promethazine$^{+\bullet}$ (1), m-benzosemiquinone (2), chlorpromazine$^{+\bullet}$ (3), promazine$^{+\bullet}$ (4), p-methoxyphenoxyl (5), TCNQ (6), bromanil (7), TMPD$^{+\bullet}$ (8), TCNQ$^{-\bullet}$ (9), 1,4-benzoquinone (10), 2-methyl-1,4-benzoquinone (11), 2,5-dimethyl-1,4-benzoquinone (12), 1,2-naphthoquinone-4-sulphonate (13), 1,2-naphthoquinone (14), phenanthrene (15). (1,5) - data from Ref.[7], (9) - data from Ref.[6], correlation lines (a), (b), (c) are described by Eqs. (2), (3), (5).

Experimental data indicate that $TMPD^{+\cdot}$, the potential of which lies over the E_1^7 range of the quinones studied (Table 2), oxidizes NADH in a single-electron way. The reaction inhibition by the reduction product (Fig. 1), characteristic to the dehydropyridine oxidation by weak one-electron oxidizers when the transfer stage of the first electron is reversible, supports the conclusion made[2]. It is interesting to note that the k_{ox} value of $TMPD^{+\cdot}$ is close to that of p-quinones with the corresponding E_1^7 values and that there exists a correlation dependence between the oxidizers $\log k_{ox}$ and E_1^7 that is applicable to p-quinones, TCNQ, $TMPD^{+\cdot}$ and other radical quioidal oxidizers[6,7] (Fig. 2):

$$\log k_{ox} = (-0.41 \pm 0.27) + (9.05 \pm 0.53)\, E_1^7, \quad (r=0.9886). \qquad (5)$$

Similar values of $\Delta S^{\neq}$ for p-quinones, TCNQ and $TMPD^{+\cdot}$ show strong evidence for the fact that p-quinones and single-electron quinone oxidizers may be attributed to the same reaction series (Table 1). The absence of the relationship between the k_{ox} values of quinones and pH may be accounted for by the fact that the difference of redox potentials $Q/Q^{-\cdot}$ and $NADH^{+\cdot}/NADH$ does not depend on pH[7,16].

The reaction kinetic isotope effect (Table 1) may also be interpreted in the framework of the three-step hydride transfer model (Eq. 4). It is necessary to note that the k_{HH}/k_{HD} values, determined on replacing a more reactive and uncovered with an adenine ring 4-A proton in the dehydronicotinamide ring, indicate precisely a change in the primary kinetic isotope effect (k_H/k_D) as it was shown by Carlson and Miller[8]. The comparison of the data from Tables 1,2 indicates that the increase in the oxidizer reactivity decreases the isotope effect. Experimental data presented in Fig. 3 agree well with that obtained by Tanaka et al[4] where k_H/k_D in the oxidation reactions of NADH decreases from 5-6 (alkyl--substituted benzoquinones) to 1.5 (2,3-dichloro-5,6-dicyano--1,4-benzoquinone).

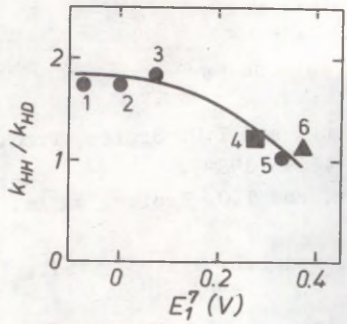

Fig. 3. The dependence of k_{HH}/k_{HD} of quinone oxidizers on the one-electron reduction potential. 2,5--dimethyl-1,4-benzoquinone (1), 2-methyl-1,4--benzoquinone (2), 1,4-benzoquinone (3), $TMPD^{+\cdot}$ (4), bromanil (5), TCNQ (6).

With the use of Eq. (4) it is possible to assume that the reaction oxidation constants are equal to k_1/k_H ($k_{-1} + k_H$). Since the pK_a values for the single-electron reduced forms of oxidizers vary from 0 to 6.5 and that of $NADH^{+\cdot}$ in a water medium equal -4 or -3.5[14,18] the k_H values for the oxidizers used must not differ significantly[19]. Obviously, in the case of low-potential oxidizers at low k_1/k_{-1} the k_{-1} value is commensurable with k_H. For this reason the k_H value, sensitive to deuterium replacement, affects the k_{ox} value. The kinetic isotope effect disappears when $k_{-1} \ll k_H$ and $k_{ox}=k_1$. It is necessary to note that the kinetic isotope effect determined by the reaction competition of proton splitting from cation-radical and a reverse electron transfer (Eq. 4)

is also recorded in a single-electron oxidation of dehydropyridines by ferricyanide[2]. Thus, the data presented in this work evidence about the three-step transfer of reduction equivalents in the oxidation of NADH by quinones in a water medium.

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THE PRIMARY SALT EFFECT ON THE DISSOCIATION OF DICARBOXYLIC ACIDS

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The primary salt effect on the pK_2 of the dicarboxylic acids is discussed on the basis of the structural theory of electrolyte solutions. The expression for the activity coefficient of the bolaform ions is derived from this theory and subsequently used in the calculation of the pK_2 dependence on the external electrolyte concentration.

In the previous communication[1] the validity of the simple structural theory of electrolyte solutions[2,3] for the description of the concentrational dissociation constant dependence on the additional electrolyte concentration was proved for the simple monocarboxylic acids.

The value of the observable dissociation constant of the process:

$$AH \overset{K_c}{\rightleftharpoons} A^- + H^+ \qquad (1)$$

is expressed by the following formula

$$K_c = \frac{C_{A^-} C_{H^+}}{C_{AH}} \qquad (2)$$

where C_i denotes the concentration of the corresponding particles in the solution. The negative logarithm of this quantity pK_c depends on the concentration of an additional

electrolyte c as follows[1]:

$$pK_c = pK_a + 2a_t \sqrt[3]{c} + \Delta bc, \qquad (3)$$

where pK_a denotes the limiting value of the pK, a_t is a theoretical multiplier depending only on the electrolyte charge type and the dielectric properties of the solvent, and Δb is the specific ion-solvent interaction parameter for the given equilibrium. Since the parameter a_t has a theoretically calculable value, the primary salt effect on the pK_c of the simple carboxylic acids can be expressed also by the following linear relationship

$$pK_c - 2a_t \sqrt[3]{c} = pK_a + \Delta bc, \qquad (4)$$

where the left side of this equation is known, provided the pK_c is known at the given concentration. The validity of the last equation for the first step of the dicarboxylic acid dissociation

$$AH_2 \rightleftharpoons AH^- + H^+ \qquad (5)$$

dissociation constants Kd_c is demonstrated by the examples in Fig. 1 and Table 1.

The thermodynamic dissociation constant K_{a2} of the second step of the dicarboxylic acid dissociation

$$AH^- \rightleftharpoons A^{2-} + H^+ \qquad (6)$$

as defined from the concentrational constant K_{c2} :

$$K_{a2} = K_{c2} \cdot \frac{f_{A^{2-}} f_{H^+}}{f_{AH^-}} \qquad (7)$$

is complicated due to the bolaform ion

$$^-OOC - (CH_2)_n - COO^- \qquad (I)$$

activity coefficient in formula (7). It is obvious that there is no spherical symmetry in the ionic charge distribution in this particle[5,6]. Thus, neither the activity coefficient of the divalent ion nor the separate univalent ions in the form[2]:

Table 1.

The Results of the Statistical Data Treatment of the pK_{1c} of Some Dicarboxylic Acids in Aqueous Solutions[4] According to Eq. (4).

No	Acid	Electrolyte addition	pK_a	Δb	$r^{(a}$	$a^{(b}$	$s_o^{(c}$
1.	$CH_2(COOH)_2$	NaCl	$2.895^{\pm}$ 0.006	$0.229^{\pm}$ 0.004	0.9993	0.010	0.015
		KCl	$2.904^{\pm}$ 0.005	$0.246^{\pm}$ 0.003	0.9995	0.009	0.013
2.	$(CH_2)_2(COOH)_2$	NaCl	$4.263^{\pm}$ 0.005	$0.240^{\pm}$ 0.003	0.9996	0.008	0.011
		KCl	$4.272^{\pm}$ 0.004	$0.258^{\pm}$ 0.002	0.9998	0.006	0.008
3.	$(CH_2)_3(COOH)_2$	NaCl	$4.385^{\pm}$ 0.005	$0.267^{\pm}$ 0.003	0.9997	0.008	0.011
		KCl	$4.396^{\pm}$ 0.003	$0.286^{\pm}$ 0.002	0.9999	0.005	0.006
4.	$(CH_2)_4(COOH)_2$	NaCl	$4.472^{\pm}$ 0.005	$0.269^{\pm}$ 0.004	0.9995	0.009	0.013
		KCl	$4.481^{\pm}$ 0.006	$0.289^{\pm}$ 0.004	0.9995	0.010	0.013
5.	$(CH_2)_5(COOH)_2$	NaCl	$4.543^{\pm}$ 0.006	$0.277^{\pm}$ 0.004	0.9995	0.010	0.013
		KCl	$4.551^{\pm}$ 0.004	$0.297^{\pm}$ 0.003	0.9998	0.007	0.008
6.	$(CH_2)_6(COOH)_2$	NaCl	$4.573^{\pm}$ 0.005	$0.275^{\pm}$ 0.003	0.9996	0.009	0.011
		KCl	4.588^{+} 0.005	$0.299^{\pm}$ 0.003	0.9997	0.008	0.010
7.	$(CH_2)_7(COOH)_2$	NaCl	$4.598^{\pm}$ 0.005	$0.276^{\pm}$ 0.003	0.9997	0.008	0.010
		KCl	$4.604^{\pm}$ 0.003	$0.296^{\pm}$ 0.002	0.9999	0.006	0.007
8.	cis-$-(CH_2(COOH)_2$	NaCl	$1.984^{\pm}$ 0.009	$0.205^{\pm}$ 0.005	0.9979	0.015	0.026
		KCl	$1.988^{\pm}$ 0.006	$0.228^{\pm}$ 0.004	0.9992	0.010	0.016
9.	trans-$(CH)_2(COOH)_2$.	NaCl	$3.083^{\pm}$	$0.256^{\pm}$	0.9999	0.004	0.006
		KCl	$3.087^{\pm}$ 0.005	$0.256^{\pm}$ 0.003.	0.9997	0.005	0.009

(a The correlation coefficient (b The deviation
(c The relative standard deviation ($s_o = \frac{s^2}{\sigma^2}$, where σ^2 is the dispersion of the quantity to be correlated).

$$\log f_i = a_t \sqrt[3]{c} + bc \qquad (8)$$

according to the structural theory of solutions are not valid for it. Consequently, the derivation of the activity coefficient formula of the bolaform ion in electrolyte solution is needed from the very origin of the above-mentioned structural theory of electrolyte solutions.

We proceed from the principle of the electrostatic potential superposition around the ions in solution. Thus, the electrostatic excess free energy connected with the bolaform ion in solution can be divided into the following terms corresponding to the individual electrostatic interactions:

$$\Delta G_{el}(\text{bolaform ion}) = \Delta G_{bf} + 2\,\Delta G_{ii} + 2\,\Delta G_{ij} + \Delta G_{jj}, \qquad (9)$$

where

$$G_{bf} = \frac{(ze)^2}{\varepsilon a_o} \qquad (10)$$

is the electrostatic repulsion energy between the ionic charges ze separated by some distance a_o in this ion. The second term

$$\Delta G_{ii} = \frac{ze}{\varepsilon \delta} \int_o^\infty 4\pi r^2 \varrho(r)\,dr = -\frac{(ze)^2 \varkappa}{2\varepsilon} \qquad (11)$$

is the electrostatic free energy of the interaction between one ionic charge in the bolaform ion and its ion atmosphere. The function

$$\varrho(r) = \frac{ze\varkappa^2}{4\pi} \cdot \frac{e^{-\varkappa^2}}{r} \qquad (12)$$

represents the excess charge distribution along the reference ion in the solution, and the parameter $\varkappa$ is the characteristic coefficient in the well-known linearized Poisson equation for the central-field problem[7]

$$\nabla^2 \psi = -\frac{4\pi}{\varepsilon} \varrho = \varkappa^2 \psi \,, \qquad (13)$$

where ψ is the electrostatic potential along the ion and ε denotes the macroscopic dielectric constant of the solvent. The parameter $\varkappa$ is a characteristic reciprocal length in the given electrolyte solution as it follows from the definition[3].

$$\varkappa = \frac{2\sqrt{\pi}}{\bar{l}} = \alpha \sqrt[3]{c} \quad , \qquad (14)$$

where $\bar{l}$ is the average distance between the nearest-neighbor ions at their uniform distribution in solution, and α is a universal constant for the electrolytes of the given charge type. Naturally the contribution ΔG_{ii} has to be multiplied by 2 in the final formula for the bolaform ion excess free energy as there are two identical ionic charges in this particle.

This is also true about the free energy ΔG_{ij}, which takes into account the electrostatic interaction between one ionic charge with the ionic atmosphere of the other ionic charge in this particle placed at the distance a_o. Following the general equations of the electrostatic structural theory[3]

$$\Delta G_{ij} = \frac{ze}{\varepsilon a_o} \int_0^{a_o} 4\pi r^2 \rho(r) dr + \frac{ze}{\varepsilon} \int_{a_o}^{\infty} 4\pi r \rho(r) dr =$$

$$= \frac{(ze)^2}{\varepsilon a_o} \left(e^{-\varkappa a_o} - 1 \right) , \qquad (15)$$

where all the notations have the same meaning as given above.

Finally the electrostatic repulsion between the two ion atmospheres is taken into account by the following integral calculable in spheroidal coordinates[8]:

$$\Delta G_{jj} = \int_0^{\infty} 4\pi a^2 \rho(a) da \int_0^{\infty} \frac{4\pi r^2 \rho(r)}{[\vec{r}-\vec{a}]} dr = - \frac{(ze)^2}{\varepsilon a_o} e^{-\varkappa a_o} - 1 \qquad (16)$$

where a and r are the distances from the first and the second ionic charges in the bolaform ion, respectively, and $\vec{r}$ and $\vec{a}$ denote the corresponding radius-vectors.

Consequently, the following final formula is valid for the excess electrostatic free energy of the bolaform ion in solution:

$$\Delta G_{el}(\text{bolaform ion}) = \frac{(ze)^2}{\varepsilon a_o} e^{-\varkappa a_o} - \frac{(ze)^2 \varkappa}{2\varepsilon} \qquad (17)$$

At the limit of the infinitely diluted solutions ($\varkappa \to 0$) the value of ΔG_{el} :

$$\lim_{\varkappa \to 0} \Delta G_{el} = \frac{(ze)^2}{\varepsilon a_o} \qquad (18)$$

equals the interionic interaction energy inside the bolaform ion.

The electrostatic excess free energy connected with the bolaform ion interaction with the surrounding ions in solution only is therefore:

$$\Delta G_{el} = \Delta G_{el}(\text{bolaform ion}) - \frac{(ze)^2}{\varepsilon a_o} = \frac{(ze)^2}{\varepsilon a_o}(e^{-\varkappa a_o} - 1) - \frac{(ze)^2 \varkappa}{2\varepsilon} \qquad (19)$$

If to take into account the definition of the activity coefficient via the excess free energy of particle in solution[7]

$$\Delta G_{ex} = RT\ln f = 2.303RT\log f \qquad (20)$$

and to add to the electrostatic excess free energy of the bolaform ion its ion-solvent excess free energy, the following equation is obtained:

$$\log f_{A^{2-}} = \frac{(ze)^2}{2.303\,\varepsilon RTa_o}(e^{-\varkappa a_o} - 1) - \frac{(ze)^2 \varkappa}{4.606\varepsilon RT} + \frac{2\,V_{S(A^{2-})}}{2.303} c = \frac{73369 \cdot 4}{\varepsilon T a_o}(e^{-0.2993\, a_o \sqrt[3]{\nu c}} - 1) - \frac{10824.3}{\varepsilon T} \sqrt[3]{\nu c} + bc \qquad (21)$$

where ν denotes the number of ions in additional neutral electrolyte and b_i is the constant uniquely connected with the solvent-structurization volume $V_{S(A^{2-})}$ of the bolaform ion in the solution of this electrolyte.

Consequently, the primary salt effect on the second concentrational dissociation constant of the dicarboxylic acids

pK_{2c} is described by the following equation:

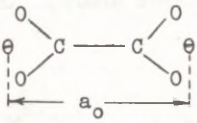
$$pK_{2c}=pK_{2a} - \frac{10824.3}{\varepsilon T}\sqrt[3]{c} + \frac{73369.4}{\varepsilon T a_o}(e^{-2993\, a_o \sqrt[3]{c}} - 1)+\Delta bc \qquad (22)$$

or in the case of 1:1 electrolytes as the additions to the aqueous solutions at 25°C:

$$pK_{2c}=pK_{2a} - 0.572\sqrt[3]{c} + \frac{3.098}{a_o}(e^{-0.3773\, a_o \sqrt[3]{c}} - 1) + \Delta bc, \qquad (23)$$

where $\Delta b = b_{A^{2-}} + b_{H^+} - b_{HA^-}$ is the characteristic constant for the given acid in the solution of the given electrolyte. The distance a_o between the ionic charges is expressed in angstroms in the last formulae.

It has to be mentioned that the final equation (23) obtained for the second dissociation constant of dicarboxylic acids in electrolyte solutions, has an essentially nonlinear dependence on the concentration of the latter. However, if to estimate the distance between the ionic charges in the bolaform ion from the molecular model



, (II)

where the negative ionic charge is localized on the bisectrice of the angle OCO in carboxylate group at the distance of 1.0 Å from the C atom, the term in the formula (23), connected with the bolaform ion activity coefficient becomes theoretically calculable. Subsequently, this equation (23) is transformed into the following two-parameter multilinear dependence

$$pK_{2c} - \frac{3.098}{a_o}(e^{-0.377\, a_o \sqrt[3]{c}} - 1) = pK_{2a} - 0.572\sqrt[3]{c} + \Delta bc \qquad (24)$$

or further to a simple linear relationship:

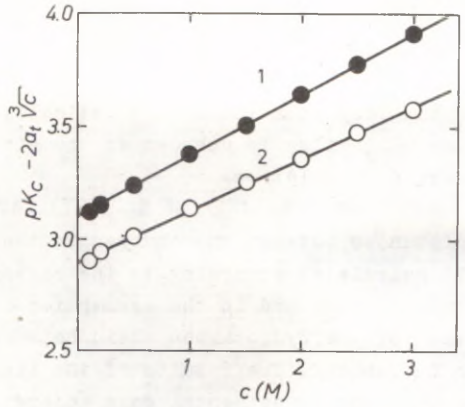


Fig. 1. The linear relationship between the function $pK_c - 2a_t \sqrt[3]{c}$ and the concentration of the additional electrolyte in aqueous solutions at 25°C [4] (1-fumaric acid in the KCl solution; 2 - malonic acid in the NaCl solution).

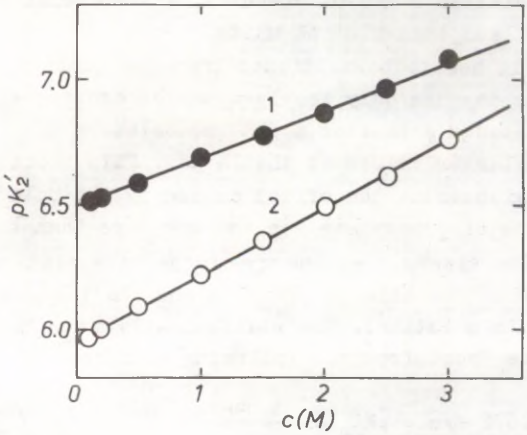


Fig.2. The linear relationship between the function pK_2' (25) and the concentration of the additional electrolyte in aqueous solution at 25°C. (1- maleic acid in the NaCl solution;2-malonic acid in the KCl solution).

$$pK_2' \equiv pK_{2c} - \frac{3.098}{a_o}(e^{-0.377\, a_o \sqrt[3]{c}} - 1) + 0.572\sqrt[3]{c} = pK_{2a} + \Delta bc \qquad (25)$$

The left side of the last equation is theoretically calculable provided the pK_{2c} value is unknown at the given electrolyte concentration in solution.

Two examples of the validity of Eq. (27) are given in Fig. 2. The distances between the charges in the bolaform dianion (II) calculated according to the carboxylate group model described above and in the assumption of the trans-configuration of the hydrocarbon chain between these groups are given in Table 2. The results of the linear regression treatment of the experimental data according to Eq. (25) are given also in this Table. It has to be mentioned that the correlation quality parameters are by no means worse than those obtained in the treatment of the pK_{1c} of the dicarboxylic acid according to the linear relationship[4] (cf. Tables 1. and 2.). The correlation coefficient is $r > 0.999$ practically in all cases, and the standard deviation $s < 0.02$ pK-units. The error of the pK_a estimate is less than 0.01 pK units.

However,it has to be mentioned that the quality of these correlations depends only weakly upon the choice of the interionic distance parameter a_o in the bolaform ion in the case of the larger values of the latter. This seems to be quite natural because the effect caused by the bolaform nature of the dicarboxylate ion is much less than the other effects on the excess free energy of the dissociation (the interionic electrostatic interaction and the ion-solvent interaction in solution). The statistically average a_o values can be found from the following equation:

$$pK_{2c} + 0.572\sqrt[3]{c} = pK_{2a} + \frac{3.098}{a_o}(e^{-0.377\, a_o \sqrt[3]{c}} - 1) + \Delta bc \qquad (26)$$

Table 2

The Results of the Linear Regression Treatment of the pK_2' Values of Some Dicarboxylic Acids in the Aqueous Solutions[4] According to Eq. (25).

No	Acid	Electrolyte addition	a_{oo}^{a} (Å)	pK_a	Δ^b	r^b	s^c	s_o^d
1	2	3	4	5	6	7	8	9
1.	$CH_2(COOH)_2$	NaCl	4.35	5.884 ± 0.007	0.239 ± 0.004	0.9990	0.012	0.018
		KCl		5.937 ± 0.007	0.274 ± 0.004	0.9993	0.012	0.016
2.	$(CH_2)_2(COOH)_2$	NaCl	5.57	5.856 ± 0.007	0.267 ± 0.004	0.9993	0.012	0.016
		KCl		5.845 ± 0.007	0.299 ± 0.004	0.9995	0.011	0.013
3.	$(CH_2)_3(COOH)_2$	NaCl	6.54	5.578 ± 0.005	0.272 ± 0.003	0.9996	0.009	0.011
		KCl		5.581 ± 0.004	0.283 ± 0.003	0.9998	0.007	0.009
4.	$(CH_2)_4(COOH)_2$	NaCl	8.20	5.539 ± 0.007	0.263 ± 0.004	0.9993	0.011	0.015
		KCl		5.574 ± 0.005	0.279 ± 0.003	0.9996	0.009	0.011
5.	$(CH_2)_5(COOH)_2$	NaCl	9.10	5.555 ± 0.008	0.257 ± 0.005	0.9990	0.014	0.019
		KCl		5.590 ± 0.005	0.273 ± 0.003	0.9997	0.008	0.010
6.	$(CH_2)_6(COOH)_2$	NaCl	10.30	5.546 ± 0.006	0.259 ± 0.004	0.9994	0.011	0.014
		KCl		5.549 ± 0.006	0.281 ± 0.003	0.9996	0.009	0.012

Table 2 continued

1	2	3	4	5	6	7	8	9
7.	$(CH_2)_7(COOH)_2$	NaCl	11.90	5.529 ± 0.008	0.252 ± 0.004	0.9991	0.013	0.018
		KCl		5.533 ± 0.016	0.281 ± 0.009	0.9967	0.027	0.033
8.	cis-$(CH)_2(COOH)_2$	NaCl	3.34	6.496 ± 0.007	0.191 ± 0.004	0.9987	0.011	0.021
		KCl		6.477 ± 0.005	0.231 ± 0.003	0.9996	0.008	0.012
9.	trans-$(CH)_2(COOH)_2$	NaCl	5.62	4.672 ± 0.004	0.253 ± 0.003	0.9997	0.007	0.010
		KCl		4.671 ± 0.003	0.279 ± 0.002	0.9999	0.005	0.006

a - a_o denotes the distance between ionic charges in the dicarboxylate ion
b - The correlation coefficient
c - The standard deviation
d - The relative standard deviation (cf. footnote[c] in Table 1.).

by the use of non-linear least-squares procedure* according to the Gauss-Newton method[9]. The constants pK_{2a}, a_o and Δb are to be found in every case. The results of such data treatment are given in Table 3.

The overall statistical fit of this treatment is comparable with the one-parameter linear approach (cf. Table 2. and 3. for the standard deviation). The accordance between the a_o values obtained from the nonlinear equation (26) and the theoretical values calculated according to the dianion model (II) is satisfactory in the case of smaller dicarboxylic acids (up to glutaric acid). In the case of the higher dicarboxylic acids the "experimental" a_o values from Table 3. are significantly smaller from the theoretical ones. This effect indicates that the assumption about the rigid trans-conformation of the hydrocarbon chain between the carboxylate groups is not longer valid for these acids and the statistically average configuration has some twisting of the hydrocarbon chain. On the other hand, the difference in the

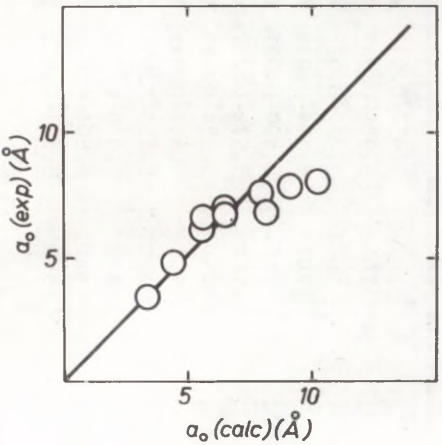


Fig. 3. The relationship between the values of the distance a_o obtained from the model estimations (II) and from the experimental data treatment according to Eq. (26).

* The treatment according to the steepest descent[10] and Marquardt[11] methods gives identical results.

Table 3.

The Results of the Nonlinear Regression Analysis of the pK_{2c} Data for the Dicarboxylic Acids in Aqueous Solutions According to Eq. (26).

No	Acid	Electrolyte addition	pK_{2a}	a_o (Å)	Δb	s^a	s_o [b]
1	2	3	4	5	6	7	8
1.	$CH_2(COOH)_2$	NaCl	5.852±0.019	4.86±0.48	0.217±0.011	0.011	0.025
		KCl	5.902±0.017	4.96±0.44	0.250±0.010	0.010	0.033
2.	$(CH_2)_2(COOH)_2$	NaCl	5.832±0.026	6.44±0.81	0.256±0.011	0.013	0.021
		KCl	5.801±0.019	6.93±0.63	0.282±0.008	0.009	0.013
3.	$(CH_2)_3(COOH)_2$	NaCl	5.590±0.021	6.32±0.65	0.278±0.009	0.011	0.016
		KCl	5.572±0.017	6.96±0.59	0.280±0.007	0.008	0.012
4.	$(CH_2)_4(COOH)_2$	NaCl	5.594±0.010	6.38±0.30	0.283±0.004	0.005	0.007
		KCl	5.604±0.019	7.23±0.64	0.290±0.007	0.009	0.012
5.	$(CH_2)_5(COOH)_2$	NaCl	5.593±0.034	7.70±1.23	0.269±0.012	0.015	0.021
		KCl	5.618±0.020	8.10±0.76	0.282±0.006	0.008	0.011
6.	$(CH_2)_6(COOH)_2$	NaCl	5.604±0.013	7.86±0.50	0.275±0.005	0.006	0.008
		KCl	5.601±0.012	8.12±0.47	0.294±0.004	0.005	0.007

Table 3 continued

1	2	3	4	5	6	7	8
7.	$(CH_2)_7(COOH)_2$	NaCl	5.601 ± 0.022	8.46 ± 0.88	0.268 ± 0.007	0.009	0.013
		KCl	5.642 ± 0.055	7.08 ± 1.87	0.309 ± 0.022	0.026	0.033
8.	cis-$(CH)_2(COOH)_2$	NaCl	6.525 ± 0.017	3.44 ± 0.36	0.213 ± 0.014	0.011	0.033
		KCl	6.505 ± 0.006	3.46 ± 0.13	0.252 ± 0.005	0.004	0.009
9.	trans-$(CH)_2(COOH)_2$	NaCl	4.649 ± 0.013	6.46 ± 0.39	0.243 ± 0.005	0.006	0.011
		KCl	4.657 ± 0.008	6.16 ± 0.24	0.273 ± 0.004	0.004	0.006

[a] The standard deviation

[b] The relative standard deviation (cf. footnote[c] in Table 1.).

standard deviations of the data treatments for these acids according to Eq. (25) assuming the trans-configuration and non-linear treatment by Eq. (26) where a_o is to be found, is small. Therefore we reach a notorious conclusion that in the case of large separations of ionic charges in multicharged particles the primary salt effect data are not very reliable for the experimental estimation of this distance.

However, this is not the case at the small interionic distances in these particles, and the results of the primary salt effect can be a powerful tool in the investigation of their charge distribution.

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THE PRIMARY KINETIC SALT EFFECT ON THE BOLAFORM AND ZWITTERION REACTIVITY

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The results of the statistical least-squares treatment of the salt effect on the rate constants of the reactions with the participation of the bolaform or zwitterions as the reagents or activation complexes are presented on the basis of the equations derived from the structural theory of electrolyte solutions. The possibility to use the kinetic primary salt effect data in the determination of the reaction mechanisms and the charge distribution in transition states are discussed.

In the previous communications,[1-3] the nature of the primary kinetic salt effect was discussed on the basis of the electrostatic structural theory of electrolyte solutions[4,5]. It has been mentioned that the participation of the particles having definitely separated ionic charges in their structure, markedly complicates the theoretical function of the corresponding equilibrium or rate constants of reactions from the concentration of electrolyte in the solution.

The activity coefficient f_i of a simple ion of charge ze in the electrolyte solution is given by the equation[5]:

$$\log f_i = a_t \sqrt[3]{\nu c} + bc , \qquad (1)$$

where

$$a_t = - \frac{5412.2}{\varepsilon T} \qquad (2)$$

is a theoretical constant depending only on the dielectric permittivity of the pure solvent ε and the temperature T (^oK), and

$$b = 0.8694\ V_s \qquad (3)$$

is the characteristic coefficient for a given ion in the solution of a given electrolyte and is proportional to its specific volume of solvent restructurization, V_s[4]. The quantity ν in Eq. (1) denotes the number of ions in the molecule of the electrolyte.

The activity coefficient of a bolaform ion fB which has by definition two ionic charges of the same sign separated at the distance a_o in its molecule is presented by the following equation[3]:

$$\log f_B = 2a_t \sqrt[3]{\nu c} + \frac{d_t}{a_o}(e^{-0.2993 a_o \sqrt[3]{\nu c}} - 1) + bc, \qquad (4)$$

where

$$d_t = \frac{73369.4}{\varepsilon T} \qquad (5)$$

denotes a theoretical constant dependent only on the solvent characteristics ε and T, and the parameters a_t and b have the same meaning as above.

Analogously to the procedure given in Ref. 3, the formula for the activity coefficient of a zwitterion which has two isolated ionic opposite charges in its molecule can be derived:

$$\log f_z = 2\, a_t \sqrt[3]{\nu c} - \frac{d_t}{a_o}\,(e^{-0.2993 a_o \sqrt[3]{\nu c}} - 1) + bc \qquad (6)$$

where the notations correspond to these given above.

Thus, it is possible to derive the equations describing the primary salt effect on the rate constants of the chemical reactions in which the bolaform or zwitterions act as the reagents or activation complexes. Starting from the above-given equations for ionic activity coefficients in

electrolyte solutions and the well-known Brønsted-Bjerrum principle[6-7], the observable rate constant k of a chemical reaction

$$A + B + C + \ldots \xrightarrow{k} [ABC\ldots]^{\neq} \longrightarrow \text{products} \qquad (7)$$

is expressed as

$$\log k = \log k_o + \log \frac{f_A \cdot f_B \cdot f_C \ldots}{f_{[ABC]^{\neq}}} \qquad (8)$$

where k_o denotes the rate constant at the standard conditions (infinitely diluted solution) and f_A, f_B, f_C, $f_{[ABC]^{\neq}}$ are the activity coefficients of the reagents and the activation complex, respectively.

The following reaction types may be distinguished starting from the classification of reagents and transition states.

1. The reaction between the oppositely charged ions with the formation of the zwitterion as activation complex:

$$A^+ + B^- \longrightarrow [A^+\text{-}B^-]^{\neq} \qquad (9)$$

In this case the logarithm of the observable rate constant in the electrolyte solution of the concentration c is expressed as follows:

$$\log k = \log k_o + \frac{d_t}{a_o} (e^{-0.2993 a_o \sqrt[3]{\nu c}} - 1) + \Delta bc, \qquad (10)$$

where $\log k_o$ is the logarithm of the rate constant at the infinite dilution and $\Delta b = b_{A^+} + b_{B^-} - b_{[A^+\text{-}B^-]^+}$ is the characteristic constant for the given reaction in the solution of a given electrolyte. In the dilute solutions of the latter, the Δbc term is negligible and therefore the following equation must be valid:

$$\log k = \log k_o + \frac{d_t}{a_o} (e^{-0.2993 a_o \sqrt[3]{\nu c}} - 1). \qquad (11)$$

Some model curves illustrating the latter relationship and corresponding to the different values of a_o are given in Fig. 1. If to make an estimate of the value of a_o from some model structure for the activation complex, the second term in Eq. (11) becomes calculable theoretically and therefore the function

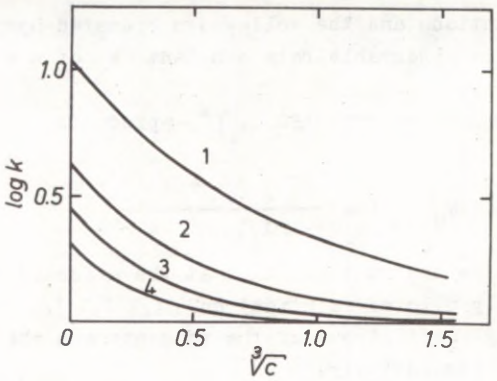


Fig. 1. Some model curves illustrating the logk dependence on the cube-root of additional electrolyte concentration for reaction (9). The curves correspond to the following values of interionic distance in activation complex (1-3Å; 2-5Å; 3-7Å; 4-10Å)

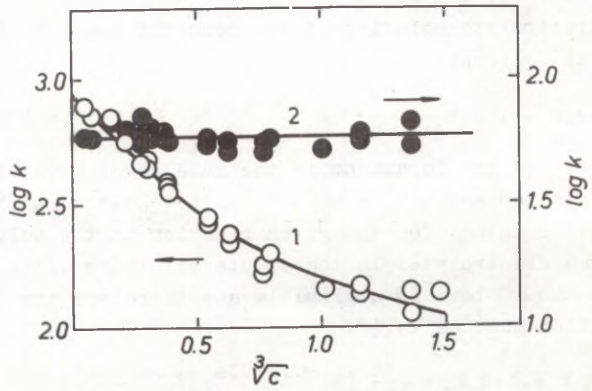


Fig. 2. The dependence of the logk for the reaction[8]
$CH_3COOCH_2\overset{+}{S}(CH_3)C_2H_5 + OH^- \longrightarrow$
on the concentration of electrolyte in solution
(1 - logk; 2 - $\log k - \frac{d_t}{a_o}(e^{-0.2993 a_o \sqrt[3]{c}} - 1)$
a_o=2.6Å assumed for the activation complex)

$$\log k' = \log k - \frac{d_t}{a_o}\left(e^{-0.2993 a_o \sqrt[3]{\nu c}} - 1\right) = \log k_o \quad (12)$$

should remain constant over the whole range of the electrolyte concentration in the solution. Some illustrative examples of such constancy are given in Figs. 2 -3. The results of the data treatment according to Eqs. (10) and (11) by the nonlinear least-squares method[10] are presented in Table 1. (Reactions 1-5).

2. The reaction between the two ions of the same charge with the formation of a bolaform ion as activation complex :

$$A^+ + B^+ \longrightarrow [A^+ - B^+]^{\neq} \quad (13)$$

or

$$A^- + B^- \longrightarrow [A^- - B^-]^{\neq}. \quad (14)$$

The corresponding logk has the following dependence or the concentration of electrolyte in solution :

$$\log k = \log k_o - \frac{d_t}{a_o}\left(e^{-0.2993 a_o \sqrt[3]{\nu c}} - 1\right) + \Delta bc \quad (15)$$

or for the diluted solutions

$$\log k = \log k_o - \frac{d_t}{a_o}\left(e^{-0.2993 a_o \sqrt[3]{\nu c}} - 1\right) \quad (16)$$

where the notations are the same as given above and a_o is the distance between two ionic charges in the activation complex. Again, if to make a suitable estimation of the latter quantity, the calculated function

$$\log k'' = \log k + \frac{d_t}{a_o}\left(e^{-0.2993 a_o \sqrt[3]{\nu c}} - 1\right) = \log k_o \quad (17)$$

must be constant in the solutions of different electrolyte concentrations. An example of this constancy is given in Fig. 4. The results of the non-linear data treatment according to Eq. (16) for the salt effects of several reactions of this type are presented in Table 1. (Reactions 6-13).

3. The reaction between the zwitterion and an ion with the formation of a simple ion as the activation complex:

$$A^+ - B^- + C^- \longrightarrow [ABC]^- \neq \quad (18)$$

or

$$A^+ - B^- + C^+ \longrightarrow [ABC]^+ \neq \quad (19)$$

The following equation is then derived for the observable logk dependence on the electrolyte concentration in solution:

$$\log k = \log k_o + 2a_t \sqrt[3]{\nu c} - \frac{d_t}{a_o}(e^{-0.2993 a_o \sqrt[3]{\nu c}} - 1) + \Delta bc \quad (20)$$

or in dilute solutions

$$\log k = \log k_o + 2a_t \sqrt[3]{\nu c} - \frac{d_t}{a_o}(e^{-0.2993 a_o \sqrt[3]{\nu c}} - 1) \quad (21)$$

where a_o denotes the distance between the ionic charges in the initial zwitterionic reagent.

If to accept the theoretical a_t value as given above for a particular electrolyte solution, the distance a_o can be found by the nonlinear least-squares treatment of the experimental logk data according to the following formula:

$$\log k - 2a_t \sqrt[3]{\nu c} = \log k_o - \frac{d_t}{a_o}(e^{-0.2993 a_o \sqrt[3]{\nu c}} - 1), \quad (22)$$

where the parameters to be estimated are $\log k_o$ and a_o. On the other hand, if to calculate the value of distance a_o from some reliable model of the ionic charge distribution in the initial zwitterion, the following linear relationship must be valid:

$$Y = \log k + \frac{d_t}{a_o}(e^{-0.2993 a_o \sqrt[3]{\nu c}} - 1) = \log k_o + 2a_t \sqrt[3]{\nu c} \quad (23)$$

where the quantity Y is fully known at a given concentration of an electrolyte, provided the logk value is known. An interesting comparison of the slope $2a_t$ values obtained from the linear regression treatment with their theoretical value can be made. The illustrative examples of linear relationships (23) for some interionic reactions of this type are presented in Fig.5. The linear regression treatment results given in Table 1. (reactions 14-15 confirm the good

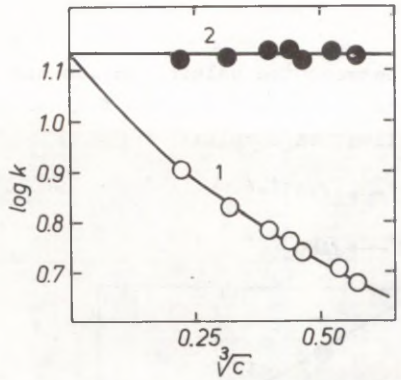


Fig. 3. The dependence of the logk for the reaction[9]

$$CH_3COO(CH_2)_2\overset{+}{N}(CH_3)_3 + OH^- \longrightarrow$$

on the concentration of electrolyte in solution (1-logk;2-logk - $\frac{d_t}{a_o}(e^{-0.2993a_o\sqrt[3]{\nu c}}-1)$ with the value a_o=3.7A assumed for activation complex)

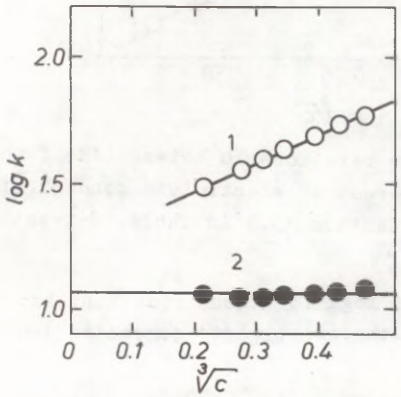


Fig. 4. The dependence of the observable logk for the reaction[14]: $^-OOC(CH_2)_3COOC_2H_5 + OH^- \longrightarrow$

on the concentration of electrolyte in solution (1-logk;2-logk+ $\frac{d_t}{a_o}(e^{-0.2993a_o\sqrt[3]{\nu c}}-1)$ with the value a_o= 7.7A assumed for activation complex)

agreement between the experimental and theoretical slopes $2a_t$.

4. The reaction between the bolaformic ion and the simple ion of an opposite charge with the formation of a single ion in the activation complex:

$$A^+ - B^+ + C^- \longrightarrow [ABC]^{+\neq} \qquad (24)$$

or

$$A^- - B^- + C^+ \longrightarrow [ABC]^{-\neq} . \qquad (25)$$

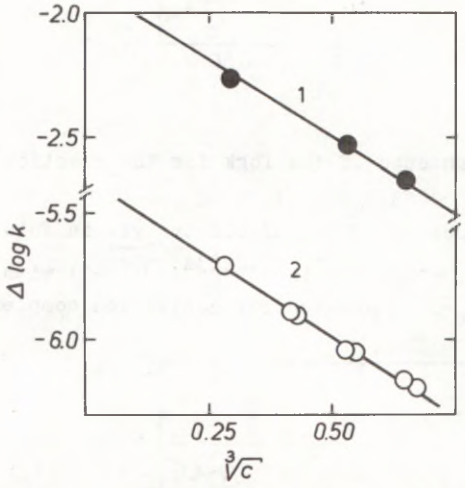


Fig. 5. The linear relationship between the function Y (23) and the cube-root of electrolyte concentration in solution (1- reaction 15.1 in Table, 2-reaction 15.4 in Table).

In this case, the logk for these reactions has the following dependence on the electrolyte concentration in solution:

$$\log k = \log k_o + 2a_t \sqrt[3]{\nu c} + \frac{d_t}{a_o}(e^{-0.2993 a_o \sqrt[3]{\nu c}} - 1) + \Delta bc \qquad (26)$$

where the notations are the same as given above. In the dilute solutions the simplified equation is valid

$$\log k = \log k_o + 2a_t \sqrt[3]{\nu c} + \frac{d_t}{a_o}(e^{-0.2993 a_o \sqrt[3]{\nu c}} - 1) \qquad (27)$$

Table 1

The Results of the Non-Linear Least-Squares Treatment of the Observable log k Data for Several Interionic Reactions in Aqueous Solutions

No	Reaction	Electrolyte added and temperature	No of equation used[a]	log k_o	a_o	$2a_t$	r^a	s^b	s_o^c	Reference
1.	$CH_3COOCH_2\overset{+}{N}(CH_3)_3 + OH^-$	KCl, 25°C	11	1.921± 0.025	2.77± 0.29	0	-	0.057	0.071	8
2.	$CH_3COOCH_2\overset{+}{S}(CH_3)C_2H_5+OH^-$	KCl, 25°C	11	2.939± 0.016	2.61± 0.18	0	-	0.052	0.041	8
3.	$CH_3COO(CH_2)_2\overset{+}{S}(CH_3)C_2H_5+OH^-$	KCl, 25°C	11	1.035± 0.011	4.97± 0.20	0	-	0.025	0.043	8
4.	$CH_3COO(CH_2)_2\overset{+}{N}(CH_3)_3+OH^-$	KBr, 25°C	11	1.263± 0.012	3.86± 0.27	0	-	0.009	0.049	9
5.	$(C_2H_5)_3\overset{+}{N}CH_2COOC_2H_5+OH^-$	KCl, 0°C	11	0.793± 0.016	4.94± 0.49	0	-	0.017	0.071	12
6.	$^-OOC\text{-}COOC_2H_5+ OH^-$	KCl, LiCl, 25°C	16	1.346± 0.006	3.48± 0.29	0	-	0.005	0.038	15
7.	$^-OOCCH_2COOC_2H_5+OH^-$	KCl, NaCl, 25°C	16	-0.215± 0.004	5.45± 0.21	0	-	0.003	0.028	15
8.	$^-OOC(CH_2)_3COOC_2H_5+OH^-$	NaCl, 25°C	16	-0.041± 0.006	5.85± 0.24	0	-	0.004	0.042	14

Table 1 continued

1	2	3	4	5	6	7	8	9	10	11
9.	$^{-}OOC(CH_2)_4COOC_2H_5+OH^{-}$	NaCl,KCl, LiCl, 25°C	16	0.221± 0.011	9.50± 0.81	0	-	0.007	0.107	15
10.	$H-C_6H_4-COO^{-}+H^{+}$									
	R=3-$N(CH_3)_3^{+}$	NaCl, 25°C	11	-1.165± 0.020	5.94± 0.34	0.583	-	0.035	0.031	13
	R=3-SO_3^{-}	NaCl 25°C	16	-0.031± 0.008	6.13± 0.18	0.583	-	0.013	0.021	13
	R=3,5-$(SO_3^{-})_2$	NaCl 25°C	16	0.092± 0.008	6.10± 0.14	0.583	-	0.011	0.019	13
11.	$\overset{+}{N}H_3CH_2COOC_2H_5+H^{+}$ ⟶	KCl, 34.5°C	16	-5.748± 0.021	4.79± 0.34	0	-	0.016	0.098	11
		KCl, 49.5°C	16	-5.073± 0.025	6.18± 0.56	0	-	0.022	0.148	11
12.	$\overset{+}{N}H_3CH_2CH_2COOC_2H_5+H^{+}$ ⟶	KCl, 49.5°C	16	-4.614± 0.097	5.31± 1.63	0	-	0.082	0.350	11
		KCl, 65.5°C	16	-3.948± 0.035	5.47± 0.89	0	-	0.033	0.191	11
		KCl, 77.5°C	16	-3.607± 0.021	5.46± 0.49	0	-	0.018	0.111	11
13.	$(CH_3)_3\overset{+}{N}(CH_2)_2COOC_2H_5+H^{+}$ ⟶	KBr, 49.5°C	16	-4.042± 0.034	5.19± 0.92	0	-	0.021	0.172	11

Table 1 continued

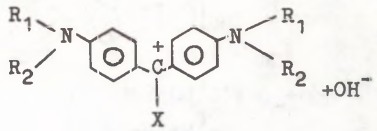
1	2	3	4	5	6	7	8	9	10	11
		KBr, 49.5°C	16	$-3.543^{\pm}$ 0.020	$5.18^{\pm}$ 0.47	0	-	0.019	0.116	11
14.										
	1) $R_1=R_2=CH_3$ X = 3-O^--C_6H_4-	$NaNO_3$, 20°C	22	$-0.829^{\pm}$ 0.014	$4.19^{\pm}$ 0.41	-0.565	-	0.010	0.067	17
			23	$-0.838^{\pm}$ 0.017	4.9	$-0.500^{\pm}$ 0.033	0.9979	0.008	0.065	17
	2) $R_1=R_2=CH_3$	KCl, 20°C	22	$-0.284^{\pm}$ 0.004	$5.36^{\pm}$ 0.20	-0.565	-	0.005	0.026	17
	X = 3-COO^--C_6H_4-		23	$-0.282^{\pm}$ 0.007	5.2	$-0.577^{\pm}$ 0.017	0.9996	0.005	0.029	17
	3) $R_1=R_2=CH_3$, X = 4-O^--C_6H_4-	$NaNO_3$, 30°C	22	$-1.871^{\pm}$ 0.016	$5.21^{\pm}$ 0.51	-0.590	-	0.010	0.089	17
			23	$-1.878^{\pm}$ 0.019	5.6	$-0.504^{\pm}$ 0.043	0.9963	0.008	0.085	17

Table 1 continued

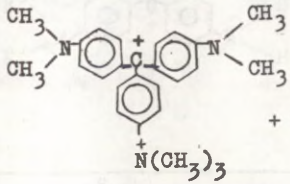
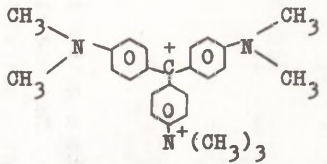
1	2	3	4	5	6	7	8	9	10	11
	4) $R_1=R_2=C_2H_5$	NaNO, 20°C	22	-2.333 ± 0.009	3.0; 5.8 [d]	-0.522 ± 0.018	0.9972	0.006	0.034	17
	$X = 2,4-(SO_3^-)_2C_6H_4-$									
15.	$R_1R_2R_3C^+ + CN^-$	KCl, $NaNO_3$, 20°C								
	1) $R_1= C_6H_5-$		23	-2.723 ± 0.017	5.1	-0.601 ± 0.043 [e]	0.9823	0.013	0.070	18
	$R_2= R_3=$ $= 3-CH_3O,4-O^--C_6H_4-$									
	2) $R_1=R_2=R_3=$ $= 3-CH_3O,4-O^--C_6H_4-$		23	-4.256 ± 0.022	5.1	-0.544 ± 0.055 [e]	0.9765	0.016	0.090	18
16.	 + OH^-	$NaNO_3$, 35°C	28	1.109 ± 0.026	6.25 ± 1.40	-0.590	-	0.033	0.158	17
			29	1.098 ± 0.025	5.1	-0.492 ± 0.067	0.9729	0.026	0.133	17

Table 1 continued

1	2	3	4	5	6	7	8	9	10	11
17.		KCl, $NaNO_3$, $20^{\circ}C$,	28	0.346 ± 0.005	4.45 ± 0.20	-0.565	-	0.005	0.027	18
			29	0.356 ± 0.007	5.1	-0.604 ± 0.017 [e]	0.9977	0.005	0.026	18.

[a] The correlation coefficient

[b] The standard deviation

[c] The relative standard deviation ($s_0 = \frac{s^2}{\sigma^2}$, where σ^2 is the dispersion of the function to be correlated).

[d] This compound has two negative charges in addition to one positive ionic charge. It is simple to modify Eq.(21) correspondingly to take into account the electrostatic effects caused by the additional negative charge in molecule. The values a_1 and a_2 denote the distances between the positive charge and either negative charges, respectively.

[e] The theoretical value of a_t is - 0.565.

which could be testified by the nonlinear least-squares method as fitting the function

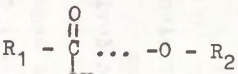
$$Z = \log k - 2a_t \sqrt[3]{\nu c} = \log k_o + \frac{d_t}{a_o}(e^{-0.2993 a_o \sqrt[3]{\nu c}} - 1) \quad (28)$$

according to the parameters $\log k_o$ and a_o (the distance between ionic charges in the initial bolaform ion). Otherwise the validity of Eq. (27) can be checked using the linear least-squares method according to function Y:

$$Y = \log k - \frac{d_t}{a_o}(e^{-0.2993 a_o \sqrt[3]{\nu c}} - 1) = \log k_o + 2a_t \sqrt[3]{\nu c}, \quad (29)$$

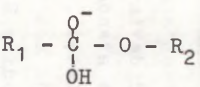
where the parameters to be found are $\log k_o$ and $2a_t$. Some model estimation of the distance a_o has to be made in this approach. The results of the statistical treatment of the experimental log k data according to Eq. (28-29) are given in Table 1. (reactions 16-17).

Finally, it has to be concluded that the model of the electrostatic excess free energy of multicharged ions used in the present work is in good accordance with the experimental primary salt effect data in aqueous solutions. Therefore it can be used as a tool for the examination of the reaction mechanism. An illustrative example of this approach is given by the results of the data treatment for the alkaline hydrolysis of aliphatic esters presented in Table 1. In case of this reaction the following two hypothetical structures are proposed for the activation complex[8]:



(III)

and



(IV)

It is obvious that the distance of the negative ionic charge on the reaction centres and that of the possible ionic charges in the substituent R_2 significantly differs in both activation complexes, whereas it is practically the

Table 2.

The Comparison of the Distances a_o Obtained from the Nonlinear Least-Squares Treatment of the Salt Effect Data for the Alkaline Hydrolysis of Aliphatic Esters in Aqueous Solutions (cf. Table 1.) with Their Model Values.

Reaction No in Table 1.	a_o(exp.) (Å)	a_o(theor) (Å)	
		Model structure III	Model structure IV
1.	2.77 ± 0.29	2.4	4.7
2.	2.61 ± 0.18	2.6	4.9
3.	4.97 ± 0.20	4.0	6.4
4.	3.86 ± 0.27	3.7	6.2
5.	4.94 ± 0.49	4.7	4.7
7.	3.48 ± 0.29	3.3	3.3
8.	5.45 ± 0.21	4.8	4.8

the same in the case if the ionic charge is localized on the substituent R_1. The data given in Table 2 strongly confirm the preferential complex III (cf. reactions 1-4), and the overall correspondence with the experimental and theoretical values of distance a_o is good in all cases. (See Fig. 6.)

In the case of the reagents or activation complexes, where the ionic charge is directly connected to the aromatic ring, the model calculations are not quite justified because of the possible resonance interaction between the ionic charge and the cycle π-electron system. This can lead to a significant charge delocalization and then an effective value of interionic distance a_o is obtained as the result of the nonlinear salt effect data treatment. However, the charge distribution in such system may be significantly different from the spherical symmetry and therefore the effective value of a_o need not always correspond to the actual situation in molecule. Thus, the use and check-up of more sophisticated charge-distribution models (e.g. according to the quantum-chemical calculations) is recommendable in such

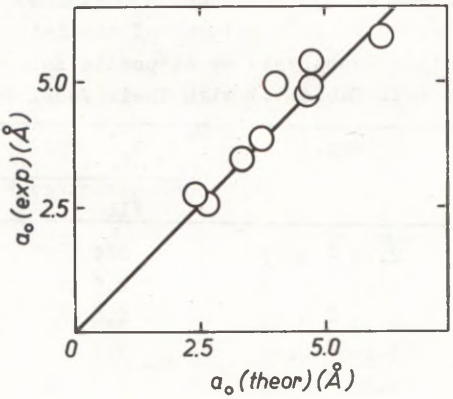


Fig. 6. The relationship between the charge distance parameters a_o obtained from the experimental data treatment (a_o(exp), cf. Table 1.) and their values calculated in accordance with the activation complex III (a_o(theor.), cf.Table 2.) for the alkaline hydrolysis of aliphatic esters.

cases. At any rate, the primary salt effect studies seem to be promising for both the reactions mechanism and charge distribution investigations in the reagent molecules or activation complexes.

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DISSOCIATION KINETICS OF ANTAGONISTS FROM COMPLEX WITH RAT BRAIN MUSCARINIC RECEPTOR

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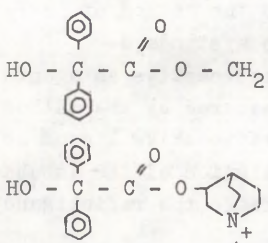
The dissociation kinetics of the muscarinic receptor complex with benzilic esters was studied . For this purpose the method for kinetic measurement of the displacement of nonradioactive antagonists from the receptor complex with L-[^{3}H]-quinuclidinylbenzilate was elaborated. The results obtained indicate that the dissociation rate of the complex does not depend on the length of an n-alkyl-substituent connected to the nitrogen atom in the alcohol part of the benzilic antagonists. Moreover, the quaternarization of the nitrogen atom leads to the loss of the sensitivity of the active site of the receptor concerning the peculiarities of the alcohol part in the ligand molecule.

Highly specific antagonists of muscarinic receptor are characterized by a low dissociation rate from complex with the receptor from different organs [1,2]. This feature must be taken into account in the experiments where the equilibrium conditions are required, e.g. those determining the equilibrium dissociation constants according to the displacement methods[3]. On the basis of the data from the literature one

can obtain the half-life time for the dissociation process of L-quinuclidinylbenzilate from the complex with rat brain muscarinic receptor $\tau_{1/2}$=9,6 hours at 25°C. [2] Under these conditions the incubation time of at least $5\tau_{1/2}$=48 hours must be used to achieve equilibrium in the displacement experiments. In the experiments of such a long duration the receptor denaturation should be taken into account. It is evident that in such circumstances for a correct measurement of equilibrium dissociation constants of potent ligands from complex with muscarinic receptor, special experimental approaches based on the investigations into kinetic behavior of the system are required.

In each case one must either separately determine the rate constants of ligand dissociation from a complex with the receptor, or establish the regularities of the influence of the compound structure on this parameter.

The aim of the present paper was to elaborate and verify the appropriate method and to determine the values of the dissociation rate constants for two series of benzilic esters with general formulae:



$\cdot I^-$, (I)

$\cdot I^-$

C_nH_{2n+I} , n = I-10. (II)

Experimental

The antagonists with the general formulae (I) and (II) were synthesized in the A.N. Nesmejanov Institute of Heteroorganic Compounds, AS USSR, as described previously[4]. L-[^{3}H]-quinuclidinylbenzilate (38 Ci/mmol) - preparation was ob-

tained from "Amersham", England. The experiments with non-radioactive and tritium-labelled preparations of N-methyl-piperidinylbenzilate were carried out in the laboratory of professor T. Bartfai at the Biochemistry department of Stockholm University and the preparations used were described in[5,6]. Atropine sulphate - preparation from "Merck",FRG,bovine serum albumin - preparation from "Reanal",Hungary,were applied. All other chemicals of analytical grade were obtained from "Reakhim", USSR, and used without an additional purification. The chemicals for the determination of radioactivity corresponded to the scintillation grade.

The isolation methods of membrane preparation of muscarinic receptor from rat brain cerebral cortex and determination of membrane-bound radioactivity by means of filtration on glassfiber filters GF/B "Whatman", England, are in detail described in[7]. The treatment of the filters and the measurement of bound radioactivity carried out on a beta-counter LS 7500 from "Beckman", USA, were described previously[7]. All experiments were carried out at 25°C in 0,05 M K-phosphate buffer, pH = 7.4 in tridistilled water. The protein concentration was determined according to the method of Lowry[8], making use of bovine serum albumin as a standard.

The dissociation kinetics of the radioactive antagonists from complex with the receptor were measured by the method of displacement with an excess of nonradioactive ligand as described in[2], after the additional dilution of the incubation mixture of 500-1000 times to decrease the radioligand concentration in the reaction mixture.

In the experiments of displacement of L-[^{3}H]-quinuclidinylbenzilate and [^{3}H]-N-methylpiperidinylbenzilate in the receptor complex the 100 μM solution of atropine sulphate was used.

In the experiments with nonlabelled antagonists the membrane preparation of muscarinic receptor was incubated with the excess of these reagents during 5 hours. It was enough to achieve the complex, that was established extra. The samples of 20-30 μl were taken from this reaction mixture, which were added to 20 ml of the solution, consisting of

1 nM L-[^{3}H]-quinuclidinylbenzilate thermostated at 25° C. In appropriate time intervals from this reaction mixture the aliquots of 1-2 ml were taken and filtered on glass--fiber filters. The filters were washed with cold 0,1 M NaCl solution 5x4 ml. To assay the nonspecific binding of L-[^{3}H]-quinuclidinylbenzilate the experiments under the same conditions were carried out, but 100 μM atropine sulphate was added to the reaction mixture. The rate constants k_{diss} were calculated making use of the dependence of the filter-bound radioactivity on time:

$$B = B_{ns} + B_{sp} (1-e^{-k_{diss} \cdot t})$$

by the method of nonlinear least squares on a computer "Nord 100", Norway. The program used was compiled analogously to the approach described in[9].

Results and Discussion.

Dilution of the reaction mixture, consisting of the complex of the muscarinic receptor with a nonradioactive antagonist (RL) and addition to this system an excess of radioactive ligand Q, lead to the dissociation of the complex and to the binding of the radioactive label with the released active centers of the receptor.

Competition of two ligands for a receptor binding site is described by the following scheme:

$$R + L \rightleftharpoons RL \qquad (1)$$

$$R + Q \rightleftharpoons RQ \qquad (2)$$

Displacement of the system's equilibrium towards the formation of the complex RQ, that can be brought about by intense dilution of the mixture RL and adding a large excess of Q, can be presented by two consecutive processes:

$$RL \xrightarrow{k_{-L}} R + L, \qquad (3)$$

$$R + Q \xrightarrow{k_Q} RQ. \qquad (4)$$

If the rate constant of the latter process (4) is greater than the rate constant of the dissociation of the complex RL (3),

$$k_Q > k_{-L}, \qquad (5)$$

the dissociation process of L from the receptor complex can be followed by the inclusion of Q into the receptor preparation. Then we obtain

$$\frac{d[RQ]}{dt} = k_{-L} \cdot [RL] . \qquad (6)$$

Maintenance of the conditions (5) and (6) gives us a practical chance for the investigation into the receptor-ligand complex dissociation kinetics in the case of various ligands L, using only one radioactively labelled compound as a "reporter" ligand, that considerably simplifies the appro-priate experiments .

In this paper a commercial preparation of L-[^{3}H]quinuclidinylbenzilate (L-[^{3}H]-QNB) was used as a radioactive "reporter" ligand. The association kinetics of the latter with membrane-bound muscarinic receptor from the rat brain was previously studied in detail[2]. According to the data from[2], the observed binding rate constant of that ligand with receptor is equal to $k_{diss} = (6.4 \pm 0.5) \cdot 10^{-3} s^{-1}$ at 1 nM L-[^{3}H]-QNB (cf. Fig. 1.). If, however, the experiments were made using the receptor complex with nonradioactive preparations of series (I) and (II) and also with the nonlabelled N-methylpiperidinylbenzilate, the observed association rate constant of L-[^{3}H]-QNB with the receptor was considerably smaller. This decrease in the association rate is illustrated in Fig. 1. where the data for the receptor complex with cholinebenzilate are given. The decrease in the rate of L-[^{3}H]-QNB binding with the membranes evidences about the dissociation of the nonradioactive ligand from the receptor complex being the rate-limiting step in this process. This conclusion can be confirmed by two other facts. Firstly, the observed kinetics of radioactive "reporter" ligand binding does not depend upon its concentration nor

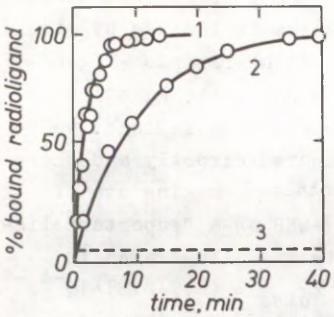


Fig.1. The kinetics of 1 nM L-[^{3}H]-quinuclidinyl-benzilate binding with membrane preparation from rat brain in 0.05 M K-phosphate buffer with pH 7.4 at 25°C: 1-natural membranes, 2-after a previous 5-hour incubation with $2.2.10^{-7}$ M choline-benzilate and dilution of this mixture for 700 times, 3-the level of a nonspecific binding.

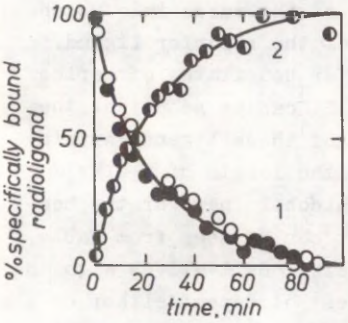


Fig.2. 1-the kinetics of ^{3}H -N-methylpiperidinyl-benzilate dissociation from the complex with rat brain muscarinic receptor: (○) - the kinetics of the dissociation initiated by the dilution of the reaction mixture, (●) - the kinetics of the dissociation initiated by dilution of the reaction medium and addition of 100 μM atropine sulphate; 2 - the kinetics of specific L-[^{3}H]-quinuclidinylbenzilate binding after previous incubation of the membranes with non-radioactive preparation of N-methylpiperidinylbenzilate in 0.05 M K-phosphate buffer with pH 7.4 at 25°C.

upon the dilution degree of the membrane preparation. Consequently, the rate of the studied process is limited by the monomolecular dissociation reaction of the receptor-ligand complex. Secondly, in the experiments made, the kinetics of the dissociation of the receptor complex with radioactive N-methylpiperidinylbenzilate was measured directly and these data were compared to the results obtained making use of the nonradioactive ligand and L-[^{3}H]-QNB as a "reporter" ligand. The results of these experiments are illustrated in Fig. 2. The values of the constants $k_{diss} = (6.7 \pm 0.8) \cdot 10^{-4} s^{-1}$ and $k_{diss} = (6.3 \pm 0.6) \cdot 10^{-4} s^{-1}$ were obtained for the radioactive N-methylpiperidinylbenzilate and the nonlabelled preparation studied making use of L-[^{3}H]QNB as a "reporter" ligand, respectively. Coincidence in these rate constants gives evidence about the applicability of this method for studying the dissociation kinetics of the muscarinic receptor-antagonist complex making use of the reporter ligand.

The obtained values of k_{diss} for benzilates of series (I) and (II) are given in Table 1. It can be seen that the rate constants of the dissociation of these ligands within the error limit do not depend upon the length of n-alkyl chain at the nitrogen atom in the alcohol part of the benzilic esters (Fig.3). Moreover, it can be seen from Table 1 that in the case of the studied n-alkyl derivatives with the quaternary nitrogen atom, k_{diss} does not depend either on the structure of the hydrocarbon backbone of the alcohol part, which separates the nitrogen atom from the ester moiety in the antagonist molecules. As it can be seen from formulae (I) and (II), the appropriate structural fragments in the derivatives of N,N-dimethyl-2-aminoethylbenzilate (I) and quinuclidinylbenzilate (II) differ from each other considerably.

If, however, the data for ligands with tertiary nitrogen atom in the alcohol part N,N-dimethyl-2-aminoethylbenzilate (I, n=0), N-methylpiperidinylbenzilate and quinuclidinylbenzilate (II , n = 0) are compared, a considerable dissociation rate dependence on the structure of hydrocarbon back-

Table 1.

Dissociation Constants, k_{diss}, of the Antagonists (I) and (II) from the Muscarinic Cholinoreceptor Complex in 0.05 M K-phosphate Buffer with pH 7.4 at 25°C.

	$k_{diss} \cdot 10^3$, s^{-1}	
n	I	II
0	3.2 ± 0.3	0.020 ± 0.004
1	1.9 ± 0.1	2.0 ± 0.3
2	2.4 ± 0.2	2.4 ± 0.5
3	2.5 ± 0.4	3.0 ± 0.4
4	2.7 ± 0.2	2.9 ± 0.4
5	3.2 ± 0.3	3.0 ± 0.2
6	2.7 ± 0.2	3.9 ± 0.2
7	3.1 ± 0.3	2.7 ± 0.2
8	3.1 ± 0.2	3.9 ± 0.3
9	3.5 ± 0.2	3.6 ± 0.3
10	2.9 ± 0.2	4.1 ± 0.5

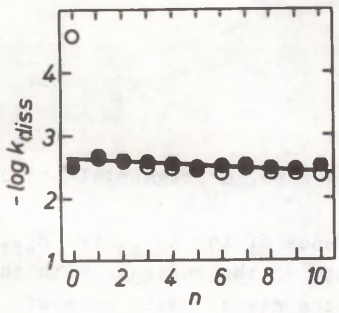
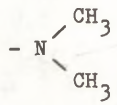


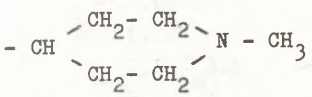
Fig. 3. The dependence of $-\log k_{diss}$ on the length of n-alkyl substituent connected to the nitrogen atom in the compounds (I) - ●, and (II) - ○ in 0.05 M K-phosphate buffer with pH 7.4 at 25°C.

bone can be observed. As the bulkiness of the structure increases from the polymethylene chain to the alicyclic and bicyclic structures, the dissociation rate considerably decreases.

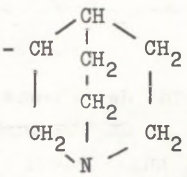
A physico-chemical factor governing this dependence can be either some geometrical characteristic of this fragment of the antagonist molecule or the hydrophobicity of the substituent. It is rather difficult to make a choice between the two variants on the basis of these data as there is no universally recognized common system for the estimation of the geometric peculiarities of bulky substituents. The effective parameter of hydrophobicity can be simply calculated, since the effect of tertiary atom of nitrogen can be neglected as this structural fragment occurs in all the following radicals:



$\pi_{eff} = 2.44,$ (III)



$\pi_{eff} = 3.11,$ (IV)



$\pi_{eff} = 3.27.$ (V)

To calculate the π_{eff} for these groups, a fragmental method was used as described in[10].

It can be seen from the dependence of log k_{diss} vs. π_{eff}, in Fig. 4 that as the hydrophobicity of the radicals with the tertiary nitrogen atom increases, the dissociation rate of the complex RL decreases:

$$\log k_{diss} = \log k^{o}_{diss} + \varphi \cdot \pi_{eff}, \quad (7)$$

where $\varphi = -2.2$ and $\log k^{o}_{diss} = 3.0$

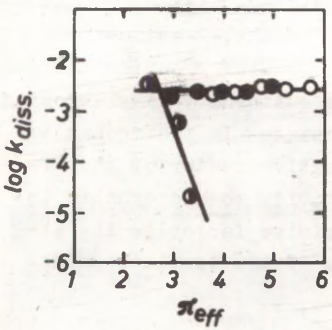


Fig. 4. The dependence of $-\log k_{diss}$ on the effective hydrophobicity of the alcohol part of the compounds (I)-(V): (◑) - the compounds with trialkylammonium atom (III,IV,V) (●) - the compounds of series I and (○) the compounds of series II with tetraalkylammonium atom.

The negative sign of φ in this equation confirms that the hydrophobic interaction of the ligand with the active center of the receptor unequally stabilizes both the initial state (i.e. RL-complex) and the activated state of the dissociation process. The appropriate effect in the initial state considerably exceeds that in the activated state. It can be concluded that some conformational transition of the receptor protein leading to the destruction of the appropriate hydrophobic regions of the active center takes place in the dissociation process of these ligands.

It is worth mentioning that the absolute value of φ-constant considerably exceeds unity, predicted by the simple extraction model of the hydrophobic interaction[11,12]. The latter fact can be explained, proceeding from the view about a considerable role of the conformational reorganization of the active center of the receptor in the interactions with ligands[11,12].

The elongation of the alkyl residue in N-alkylsubstituted antagonist molecules of series (I) and (II), brings about also the increase in the hydrophobicity of these molecules. However, the obtained data show that in this case the appropriate physico-chemical factor does not influence the rate of ligand dissociation. Besides, in the case of the derivatives of series (I) and (II) with $n \geqslant 1$ a practical

coincidence of k_{diss} can be observed, however the structure of the alcohol part of these esters differs similarly that of L-quinuclidinylbenzilate and N,N-dimethyl-2-aminoethyl benzilate (cf.Fig. 4). Consequently, passing from the tertiary alkylammonium derivatives to the quaternary alkylammonium compounds leads to the fundamental changes in the mechanism of antagonist interaction with the active center of the receptor. In spite of that, the sensitivity to hydrophobic interaction is lost. Probably, the decisive factor is the alteration of the acid-base properties of the tertiary and quaternary ammonium compounds:

$pH < pK_a$

Interestingly, the different behavior of muscarinic antagonists with tertiary and quaternary alkylammonium residues has been found also in some other studies[13,14]. However, the kinetic aspects of the interrelation between the structure and activity of these ligands are studied for the first time.

Thus, the obtained results have revealed greatly different dependences of dissociation rates on the structure of antagonists with tertiary and quaternary nitrogen atoms in the alcohol residue of the benzilic esters. For a more detailed characterization of these differences,however,a larger set of compounds should be studied, and also other kinetic parameters, such as the association and equilibrium constants of receptor-antagonist complex, should be taken under the investigation.

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