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Reactivity of α-Amino Acids in the Reaction with Esters in Aqueous–1,4-Dioxane Media

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Abstract—The kinetics of the reaction of a series of α-amino acids with 4-nitrophenyl acetate, 4-nitrophenyl benzoate, and 2,4,6-trinitrophenyl benzoate in aqueous 1,4-dioxane medium has been studied. Kinetics of the reactions involving 4-nitrophenyl acetate and 2,4,6-trinitrophenyl benzoate has complied with the Brønsted dependence and revealed linear correlation between rate constant logarithm and the energy difference of the frontier orbitals of α-amino acids anions.

Keywords: α-amino acids, esters, N-acylation, water–1,4-dioxane
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Acylation of α-amino acids is a point of interest due to their crucial functions in biological objects, for example, in biosynthesis of proteins, their post-translational modifications, and metabolic processes [1–7].

Acylation of amino acids is used in chemical synthesis of polypeptides for building up the polypeptide chain and protection of terminal and side functional groups of α-amino acids. The acylation products (*N*-acylamino acids) exhibit pharmacological activity: vasodilatory, neuroprotective, and analgesic actions [3, 4], inducing or inhibition of the proliferation of T lymphocytes *in vitro* [4], suppression of proliferation of cell line of rectal carcinoma [5], induction of insulin secretion [6], and prevention of skin aging [7]. Acylamino acids are low-toxic and do not cause undesirable side effects [8, 9].

N-Acylamino acids serve as constituents of hygienic and cosmetic products as well as herbicides and fungicides which can be easily degraded by environment microorganisms. At the same time, *N*-acylamino acids may be low-toxic for warm-blooded animals, non-phytotoxic, and may accelerate plants growth [10].

Kinetic data on acylation of α-amino acids in different aqueous-organic media which are optimal for industrial synthesis [10, 11] are necessary for elaboration of large-scale methods of synthesis of acylamino acids. However, the relevant data have been limited.

This study aimed to investigate the kinetics of the reactions of glycine (Gly), DL-valine (DL-Val), L-proline (L-Pro), DL-α-alanine (DL-α-Ala), DL-leucine (DL-Leu), DL-serine (DL-Ser), DL-threonine (DL-Thr), DL-methionine (DL-Met), L-tryptophan (L-Trp), and L-asparagine (L-Asn) with benzoic and acetic acids esters substituted at the phenoxide unit: 4-nitrophenyl benzoate **1**, 2,4,6-trinitrophenyl benzoate **2**, and 4-nitrophenyl acetate **3** in a water (40 wt %)–1,4-dioxane medium and to elucidate the main factors determining the reactivity of substrates.

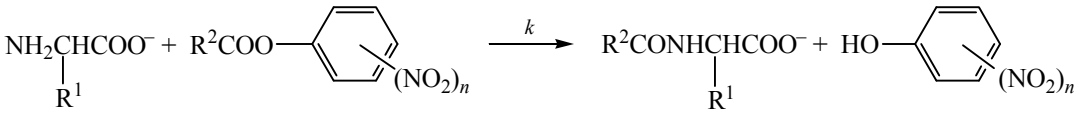
It is known that amino acids can exist in four forms in a liquid phase: cationic, anionic, zwitter-ionic, and molecular, depending on the medium acidity. Under the experimental conditions used in this study, only the anionic form is reactive. The reaction of an α-amino acid anion with esters occurs as shown in Scheme 1.

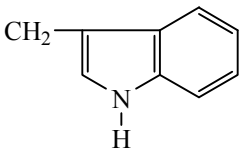
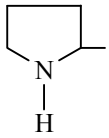
No catalytic and autocatalytic reactions were observed in the experiment.

The esters hydrolysis can accompany the *N*-acylation in aqueous-organic media. It has been shown in some reports [12, 13] that phenyl benzoates are not hydrolyzed in water; therefore, only the target reaction and alkaline hydrolysis were accounted for in the calculation of the rate constants (Scheme 2).

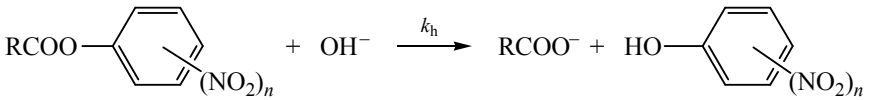
The specific pH value (8.5–9) was created to minimize the contribution of the hydrolysis into the

Scheme 1.



R^1 = H (Gly), CH_3 (Ala), $CH(CH_3)_2$ (Val), $CH_2CH(CH_3)CH_3$ (Leu), CH_2OH (Ser), $CH(CH_3)OH$ (Thr), $(CH_2)_2SCH_3$ (Met), CH_2CONH_2 (Asn),  (Trp),  (Pro); R^2 = CH_3, Ar; n = 1, 3.

Scheme 2.



process kinetics. The alkali was added to a solution of the amino acid, and the latter existed as a mixture of zwitter-ion form and the form containing non-protonated amino group capable of the acylation. Under such conditions, concentration of the molecular form of amino acids and its contribution to the reaction rate are negligibly low compared with the anionic form [14]. The earlier kinetic study has shown that if the concentrations ratio of the unreactive zwitter-ionic form ($c_{\pm}$) and the reactive anionic form (c) exceeds four ($c_{\pm}/c > 4$), the rate of alkali hydrolysis can be neglected compared with the rate of the *N*-acylation [12].

The kinetics was investigated in large excess of the amino acid (10^2–10^3-fold) with respect to the acylation agent, and the change in the acylation agent concentration (c_{ac}) could be expressed by Eq. (1).

$$-dc_{ac}/d\tau = [k_h + (k\alpha)c_0]c_{ac} = k_o c_{ac}. \quad (1)$$

Here α is the fraction of anionic form of the α-amino acid with respect to its total concentration in the solution c_0; k (L mol^{-1} s^{-1}) is the rate constant of acylation of the reactive form; k_h (s^{-1}) is the rate constant of hydrolysis of the acylation agent; k_o (s^{-1}) is the observed rate constant [Eq. (2)].

$$k_o = k_h + (k\alpha)c_0. \quad (2)$$

Concentration of the anionic form equals concentration of the added alkali ($c = c_{NaOH}$); its fraction in the solution α and the observed rate constant k_o are determined by Eqs. (3), (4).

$$\alpha = c_{NaOH}/c_0 = c/c_0, \quad (3)$$

$$k_o = k_h + kc. \quad (4)$$

Hence, in the absence of hydrolysis, the rate constant of *N*-acylation of the amino acid reactive form can be calculated using Eq. (5).

$$k = k_o/c. \quad (5)$$

The observed rate constants k_o and the rate constants k of the reactions of α-amino acids anions with compounds **1**–**3** in aqueous 1,4-dioxane calculated using Eq. (5) are given in Tables 1 and 2. The independence of the acylation rate constant k of the concentration of amino acids anions indicated the insignificant contribution of ester hydrolysis to the observed rate of the reaction.

Comparison of the data in Tables 1 and 2 showed that the rate constants of the reactions of the studied α-amino acids with compound **3** were significantly higher than those for compound **1**. The decrease in the k values in the case of acylation with ester **1** was caused by the electronic effect of the aromatic ring reducing its electrophilicity due to the conjugation with the carbonyl group, as compared with compound **3** (no conjugation). At the same time, the k values of acylation of α-amino acids with ester **2** were significantly higher than those for ester **3**. The reactivity of ester **2** was caused by two factors: the electronic influence of the aromatic π-system of the acyl part of the ester and the action of three nitro groups on the carbonyl reactive site. The second factor was prevailing, as confirmed by quantum-chemical simulations: the positive charge q(C) at the carbonyl carbon atom of ester **2** was slightly higher (0.81 a.u.) than that for ester **3** (0.775 a.u.). Furthermore, the LUMO energy of molecule of compound **2** was significantly lower

Table 1. Rate constants of acylation k_o and k of α-amino acids with esters **1**–**3** in water (40 wt%)–1,4-dioxane medium at 298 K[a]

Amino acid	pK_{II} [17]	$c\times10^3$, mol/L	$k_o\times10^3$, s^{-1}	k, L mol^{-1} s^{-1}
		1		
DL-Ala		3.40	0.111±0.002	0.0331±0.003
DL-Leu		3.40	0.652±0.008	0.0190±0.003
L-Asn		7.21	0.645±0.010	0.0089±0.002
		2		
L-Asn		7.21	5.41±0.02	0.750±0.003
DL-Ser		3.40	5.67±0.08	1.67±0.02
DL-Thr		7.21	27.1±0.6	3.80±0.08
DL-Met		7.21	22.2±0.4	3.03±0.06
L-Trp		7.21	19.2±0.1	2.67±0.02
		3		
Gly	9.78	3.55	3.73±0.030	1.05±0.01
DL-Val	9.72	3.40	2.50±0.050	0.73±0.02
L-Pro	10.64	4.26	19.9±0.500	4.67±0.01
DL-Ala	9.87	3.40	1.380±0.080	0.41±0.02
DL-Leu	9.64	3.40	0.890±0.009	0.261±0.003
L-Asn	8.80	3.40	0.153±0.007	0.045±0.002
DL-Ser	9.15	3.40	0.299±0.006	0.088±0.002
DL-Thr	9.25	7.21	0.822±0.008	0.114±0.001
DL-Met	9.21	7.21	0.728±0.004	0.101±0.001
L-Trp	9.38	7.21	1.280±0.020	0.178±0.003

[a] (c) Initial concentration of amino acid anions.

(0.32 eV) than E_{LUMO} of ester **3** (1.64 eV). The nitro groups of compound **2** in positions 2 and 6 of the phenoxide ring do not create steric hindrance to the nucleophile attack, because the phenoxide ring was rotated by 71.1° with respect to the plane of the acyl part of molecule of ester **2**. The combination of these factors explains higher reactivity of ester **2** in the reactions with α-amino acids as compared with ester **3**.

Rate constants of the reactions of α-amino acids with ester **2** (Tables 1 and 2) were 3–4 orders of magnitude lower than the k values for the reactions of the same α-amino acids with benzoyl chloride **4** [15]. The difference was due to lower nucleophilicity and higher elimination ability of the leaving group of acid chlorides compared to their esters.

The compliance with the Brønsted dependence was observed for the reactions of α-amino acids with esters **2** and **3** [Eq. (6)].

$$\log k = a + \beta_{R1} pK_{II}. \quad (6)$$

Here a is a constant parameter for the reaction series, β_{R1} is the sensibility to the basicity of nucleophile, and K_{II} is the second acid dissociation constant of the amino acid. The described dependence is described by Eqs. (7), (8) for compounds **2** and **3**, respectively.

$$\log k = (-7.5\pm0.9) + (0.85\pm0.09)pK_{II}, \quad (7)$$

$$n = 8,\ r = 0.97;$$

$$\log k = (-11.1\pm0.8) + (1.10\pm0.08)pK_{II}, \quad (8)$$

$$n = 14,\ r = 0.97.$$

Table 2. Rate constants of acylation of Gly, L-Pro, and L-Val with esters **1** and **2** in water (40 wt%)–1,4-dioxane medium[a]

Reaction	T, K	$c\times10^3$, mol/L	$k_o\times10^3$, s^{-1}	k, L mol^{-1} s^{-1}	Reaction	T, K	$c\times10^3$, mol/L	$k_o\times10^3$, s^{-1}	k, L mol^{-1} s^{-1}
Gly + **1**	298	5.77	0.523± 0.005	0.0907±0.0009	L-Pro + **1**	298	16.9	18.7±0.6	1.11±0.04
		8.66	0.870±0.009	0.101±0.001			8.46	9.31±0.01	1.10±0.01
		17.3	1.67±0.02	0.096±0.001			5.64	6.39±0.02	1.13±0.01
		16.9	1.65±0.01	0.0974±0.0004			4.23	4.79±0.05	1.13±0.01
	303	4.23	0.598±0.006	0.140±0.001	L-Pro + **2**	298	0.668	24.4±0.7	37±1
		16.9	2.36±0.02	0.140±0.001			0.634	24.2±0.6	38±1
		8.46	1.17±0.01	0.139±0.001			0.596	21.5±0.7	36±1
	308	4.23	0.846±0.006	0.200±0.001	L-Val + **1**	298	12.7	0.142±0.007	0.0112±0.0006
		8.46	1.61±0.01	0.190±0.001			15.5	0.170±0.004	0.0110±0.0003
		16.9	3.30±0.02	0.195±0.001			19.9	0.231±0.002	0.0116±0.0002
Gly + **2**	298	1.73	19.0±0.7	11.0±0.04	L-Val + **2**	298	8.63	45±6	5.2±0.7
		1.23	13.7±0.7	11.1±0.08			5.76	30±4	5.1±0.7
		1.08	11.9±0.2	11.0±0.02			4.32	22±4	5.2±0.9
	303	0.250	3.20±0.03	12.8±0.1					
		0.350	4.65±0.06	13.3±0.2					
		0.438	5.83±0.09	13.3±0.2					
		0.670	8.71±0.07	13.8±0.1					
	308	0.250	3.73±0.04	14.9±0.2					
		0.350	4.93±0.02	14.5±0.1					
		0.438	6.22±0.05	14.4±0.1					
		0.670	9.98±0.09	14.9±0.3					

[a] (c) Initial concentration of amino acid anions.

Besides the data in Table 1, the rate constants of the reactions of ester **3** with diamino- and dicarboxylic acids [16] were included in Eq. (8). The pK_{II} values in water [17] were used for derivation of the correlations, in view of the absence of available K_{II} values for the aqueous-dioxane solvent for some of the studied amino acids.

In should be noted that the correlation between logarithm of the rate constant k of the α-amino acids reactions with the corresponding acetates and benzoates was close to linear, as well as that between log k of α-amino acids acylation with the esters and compound **4** [15], the correlation coefficients being at least 0.93. The marked linear relationship and the holding the Brønsted dependence was due to the determinative influence of the amino group basicity on the rate of the α-amino acids acylation. That fact was consistent with other data on the reactions of acyl transfer [14].

We determined the values of the activation parameters of glycine reactions with esters **1** and **2**. The accessible temperature range was limited by the decrease in the solubility of the reactants and products on cooling and acceleration of the acylation agents hydrolysis on heating.

The activation energy of glycine acylation with ester **1** equaled 52±3 kJ/mol, significantly higher than for the reaction with ester **2** (22±3 kJ/mol). That fact was in good agreement with the increase in the rate constants with the increase in the number of nitro groups in the ester molecule. The determined values of

Table 3. Quantum-chemical parameters of α-amino acids anions simulated via DFT//B3LYP/6-31+G(d,p) method

Amino acid	$r(N–H^1)$, Å	$r(N–H^2)$, Å	$\angle H^1NH^2$, deg	$q(N)$, a.u.	$q(NH^2)$, a.u.	E_{HOMO}, eV	E_{LUMO}, eV
Gly	1.020	1.020	99.89	–0.462	–0.180	–0.57	3.70
Val	1.018	1.027	103.61	–0.454	–0.143	–0.98	2.95
Ser	1.015	1.019	103.34	–0.451	–0.144	–0.84	3.18
Thr	1.018	1.029	108.68	–0.477	–0.162	–1.05	2.61
Met	1.023	1.023	107.43	–0.428	–0.129	–1.30	2.50
Trp	1.017	1.018	107.57	–0.457	–0.148	–1.34	2.18
Asn	1.019	1.021	105.75	–0.451	–0.159	–0.98	2.29

activation entropy (–98±9 and –160±9 J $mol^{-1} K^{-1}$ for compounds **1** and **2**, respectively) indicated that the transition state structure was more ordered in the case of 2,4,6-trinitrophenyl benzoate as compared to 4-nitrophenyl benzoate.

We simulated the α-amino acids anions using the DFT//B3LYP/6-31+G(d,p) method; selected results are given in Table 3. The presented data showed that the structure of amino group was not significantly changed in the series of the anions: the variation of the N–H bond length and the bond angle between the N–H bonds were as low as 0.014 Å and 8.79°, respectively.

The results of simulation of α-amino acids anions were compared with the kinetic data for their reactions with esters **2** and **3** in aqueous 1,4-dioxane (Table 1). It was found that the rate constants of amino acids acylation k were not related to the values of the charge on the nitrogen atoms $q(N)$ and α-amino groups $q(NH_2)$ of the anions. At the same time, linear correlation between log k values and the energy gap between the frontier orbitals (ΔE) of α-amino acids anions [Eq. (9)] was observed for both reaction series.

$$\Delta E = -(E_{HOMO} - E_{LUMO}). \quad (9)$$

The derived equations were as follows [Eqs. (10), (11)].

$$\log k = -(5.6\pm0.6) + (1.58\pm0.18)\Delta E, \quad (10)$$

$$n = 7,\ r = 0.96;$$

$$\log k = (0.32\pm0.12) - (0.339\pm0.034)\Delta E, \quad (11)$$

$$n = 7,\ r = 0.98.$$

In summary, rate constants of the reactions (10) of α-amino acids with substituted phenyl esters of benzoic and acetic acids were obtained in this study. The observed Brønsted dependence indicated the determining role of basicity of amino groups in the acylation kinetics. The simulation results showed that the orbital parameters of the reagents could be used to describe the reactivity of α-amino acids anions and esters in acylation.

EXPERIMENTAL

α-Amino acids [Gly ("pure" grade), DL-α-Ala ("analytical pure" grade), DL-Val ("pure" grade), DL-Leu ("pure" grade), L-Pro ("analytical pure grade), DL-Met ("analytical pure" grade), DL-Thr ("analytical pure grade), DL-Ser ("analytical pure" grade), L-Trp ("analytical pure" grade), L-Asn ("analytical pure" grade)] were dried at 373 K for 1 h and then were used without further purification. Esters **1** and **2** were obtained via acylation the corresponding nitro derivatives of phenol with benzoyl chloride. 4-Nitrophenol and acetic anhydride were used for preparation of ester **3**. Sodium hydroxide ("analytical pure" grade) was used as received. 1,4-Dioxane ("pure" grade) was kept over KOH for several days and distilled over metal sodium. Bidistilled water for the preparation of the mixed water–dioxane solvent was obtained using a DV-1 deionized water generator. Physical properties of the reagents (melting point, boiling point, refraction index, etc.) coincided with the reference data [19–22] after the purification.

Kinetics of α-amino acids reactions with the esters was investigated using indicator spectrophotometric method, the reaction products (mono- and trinitrophenols) serving as the indicators being transformed into the corresponding yellow-colored anions under the experimental conditions. The reaction progress was monitored by measuring the increasing absorbance of the solution at λ 400 nm using a SF-26 spectrophotometer equipped with a Scsh300 digital voltmeter and a

temperature-controlled cell holder as described in detail elsewhere [12].

Quantum-chemical simulation of amino acids anions and ester molecules was performed using the DFT//B3LYP/6-31+G(d,p) and HF/6-31G(d) methods, respectively, implemented in the Firefly 7.1.g software package [18]. The correspondence of the structures to true minimums in the potential energy surface during the geometry optimization was confirmed by the absence of imaginary frequencies in the vibration spectrum.

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