THE REACTIVITY OF N-[(2-OXOINDOLIN-3-YLIDENE)-2-OXIAACETYL] AMINO ACIDS

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The reactivity of N-[(2-oxoindolin-3-ylidene)-2-oxiacetyl] amino acids has been investigated in the reversible conditions by studying their acid-base properties in the binary solvent of dioxane – water (60 volume % of dioxane) at 25°C. The experimental compounds have been proven to be weak dibasic acids. Their pKa values have been determined by Noyes method. The correlation of these values to both of the reactive sites (COOH- and OH-groups) has been performed. It has been shown that each CH₂-prolongation step of the polymethylene chain reduces acidity of compounds at both reactive sites of ionization. Hammett correlation equations (pKa₁ – pKa₂, f(σ)) have been calculated for N-[(2-oxoindolin-3-ylidene)-2-oxiacetyl] amino acids, and it allows to predict the acid-base properties of compounds of the given homological series. The low sensitivity of the reactive sites towards polymethylene chain prolongation has been found. The results obtained are used for mathematical modeling of QSAR-analysis of the compounds of the isostructural series.

N-[(2-oxoindolin-3-ylidene)-2-oxiacetyl] amino acids and their derivatives possess various types of the biological activity [6-9]. That is why this class of compounds is intensively used for purposeful search of active pharmacophores. The pharmacological activity depends on the ability of a pharmacophore to form complexes with biological receptors, and it, in turn, is determined by the pharmacophore reactivity, in particular its acid-base properties. Therefore, the study of the reactivity of homologous series of these biologically active substances is of great scientific and practical interest in connection with the possibility of optimization of their targeted synthesis and modeling of the active pharmacophores. Data describing the reactivity of oxoindole derivatives are absent in the chemical and biological scientific literature sources.

Materials and Methods

The synthetic studies and physico-chemical characteristics of the experimental series of N-[(2-oxoindolin-3-ylidene)-2-oxiacetyl] amino acids were presented in our previous works [3].

The ionization constants of compounds I-VI (see Scheme) were determined by the potentiometric titration method [1]. The titrant was 0.05 M aqueous solution of potassium hydroxide free of CO₂. The concentration of the solutions to be titrated was 0.005 M at the point of semineutralization. The measurements were performed on an EV-74 ionomer using two electrodes: a glass (ESP 43-074) indicator one, as well as a saturated chlorine-silver electrode. The latter was applied as a reference electrode. Determinations were carried out at 25°C in triplicates. The accuracy of the results obtained was estimated by methods of mathematical statistics of small samples (confidence probability – 0.95) [4].

The mixed solvent of dioxane – water (60 volume % of dioxane) was prepared from freshly bi-distilled water free of CO₂ and 1,4-dioxane (very pure) without additional purification.

Results and Discussion

The reactivity of N-[(2-oxoindolin-3-ylidene)-2-oxiacetyl] amino acids was studied in the reversible conditions on the model of the acid-base equilibria (Scheme). Ionization constants (pKa₁ and pKa₂) of the corresponding acid-base equilibria (1) and (2) of the compounds studied are given in Table.

The preliminary analysis of the titration curves of compounds I-VI obtained by the electrometric method has demonstrated that these substances are dibasic acids, which ApKa(pKa₁ – pKa₂) is less than 4. Therefore, the classic Noyes method was chosen for calculation [1]. The pKa ionization values of ethyl esters of the corresponding N-[(2-oxoindolin-3-ylidene)-2-oxiacetyl] amino acids previously obtained and described in our works [2] were thoroughly analyzed to make correlations of the pKa values obtained to the certain reactive sites of acidic ionization. The high pKa values were referred to enolic hydroxyl ionization (equilibrium 2). This fact corresponds to the literature data concerning a higher acidity of the carboxyl group compared to the enolic one [10].
From the table data it follows that with each CH$_2$-prolongation step of the polymethylene chain length in the amino acid molecular fragment the ionization degree of both reactive sites of the molecules in the compounds studied decreases. However, the degree of the isolating effect of methylene groups on the reactive sites is different:

1. acidity alteration of COOH-site: $\Delta pK_a = pK_{a,n-1} - pK_{a,n} = -0.26$;
2. acidity alteration of OH-site: $\Delta pK_a = pK_{a,n-1} - pK_{a,n} = -0.29$.

This fact indicates that sensitivity of the carboxylic group to the induction effect transmission is higher than that of the enolic hydroxyl.

The $\Delta pK_a$ value ($\Delta pK_a = pK_{a,n+1} - pK_{a,n}$; where $n$ is the number of CH$_2$ – groups) of both reactive sites remains constant almost everywhere (see Table). This allowed us to perform the qualitative evaluation of dependence of pKa values of both reactive sites on the length ($n$) of the polymethylene molecular fragment using the principle of free energies linearity by the correlation analysis method.

The COOH-reactive site:

$$pK_{a_1} = (5.34 \pm 0.03) + (0.052 \pm 0.002) \cdot n$$

The OH-reactive site:

$$pK_{a_2} = (6.43 \pm 0.01) + (0.039 \pm 0.002) \cdot n$$

The electron effect of substituents was estimated by Hammett equation ($pK_a = a + \rho \cdot \sigma$). The CH$_2$-fragment constant ($\sigma$) was chosen as 0.388 according to the classic work of Palm V.A. [5]. The CH$_2$-fragment constants were calculated by the following formula:

$$\sigma(CH_2)_n = 0.388 \cdot n$$

The equations of $pK_a - f(\sigma)$ correlation for both reactive sites with statistically significant parameters were obtained.

The COOH-reactive site:

$$pK_{a_1} = (5.35 \pm 0.01) + (0.133 \pm 0.005) \cdot \sigma$$

The OH-reactive site:

$$pK_{a_2} = (6.43 \pm 0.01) + (0.105 \pm 0.006) \cdot \sigma$$

The parameters of equations (3,4) indicate weakening of the ionization degree at both reactive sites with each CH$_2$-prolongation step of the polymethylene chain length, i.e. the isolating effect of methylene groups [5], since the reaction constants $\rho_1$ and $\rho_2$ are positive. However, $\rho_1 > \rho_2$, and it indicates a somewhat higher sensitivity of the carboxylic group compared to the enolic one towards the isolating effect of the polymethylene chain. It should be noted that $\rho_1$ and $\rho_2$ values are extremely small.

Table

| Compound | $pK_{a_1}$ | $\Delta pK_{a_1} = pK_{a,n+1} - pK_{a,n}$ | $pK_{a_2}$ | $\Delta pK_{a_2} = pK_{a,n+1} - pK_{a,n}$ |
|----------|------------|------------------------------------------|------------|------------------------------------------|
| I        | 5.35±0.03  | -                                        | 6.44±0.03  | -                                        |
| II       | 5.41±0.05  | 0.06                                     | 6.47±0.04  | 0.03                                     |
| III      | 5.44±0.05  | 0.03                                     | 6.51±0.05  | 0.04                                     |
| IV       | 5.50±0.03  | 0.06                                     | 6.54±0.02  | 0.03                                     |
| V        | 5.56±0.04  | 0.06                                     | 6.60±0.04  | 0.06                                     |
| VI       | 5.61±0.03  | 0.05                                     | 6.63±0.05  | 0.03                                     |
low, i.e. attenuation of the electron effects transmission was yet insignificant.

**CONCLUSIONS**

1. The reactivity of N-[(2-oxindolin-3-ylidene)-2-oxiacetyl] amino acids has been investigated by studying their acid-base equilibria in the reversible conditions.
2. The experimental compounds have been proven to be weak dibasic acids. Their ionization process equations have been suggested.
3. The ionization constants values ($pK_a$ and $pK_b$) of six N-[(2-oxindolin-3-ylidene)-2-oxiacetyl] amino acids have been determined. It has been shown that prolongation of the polymethylene chain reduces ionization at both reactive sites (COOH and OH).
4. The influence of methylene links in the amino acid molecular fragment has been quantitatively estimated according to Hammett equation, and the low sensitivity of both reactive sites towards the polymethylene chain prolongation has been found.

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творителе диоксан-вода (60 объемных % диоксана) при 25°С. Показано, что эти соединения – слабые двухосновные кислоты. Их рКа определялись по методу Ноэса. Проведено соотнесение этих значений с двумя реакционными центрами (СООН и ОН-группы). Показано, что удлинение полиметиленовой цепи уменьшает кислотность соединений по обоим центрам ионизации. Рассчитаны корреляционные уравнения Гаммета рKа,2 – f(σ) для N-[(2-оксоиндолин-3-илиден)-2-оксиацетил]аминокислот, что позволяет прогнозировать кислотно-основные свойства соединений этого гомологического ряда. Установлена низкая чувствительность реакционных центров к удлинению полиметиленовой цепи. Полученные результаты используются для математического моделирования QSAR – анализа соединений этого изоструктурного ряда.