PROTOLYSIS OF SOME α-AMINO-β-(1-PYRIMIDYL)PROPIONIC ACIDS AND THEIR ANALOGS

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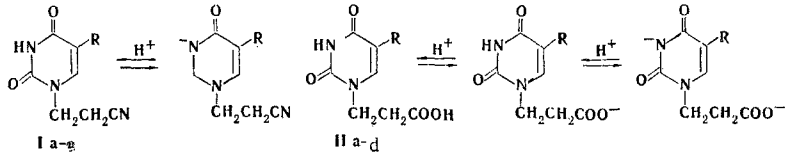
The protolysis constants of β-(1-uracilyl)propionitriles and β-(1-uracilyl)propionic α-amino-β-(1-pyrimidyl)propionic, and α-amino-γ-(1-pyrimidyl)butyric acids (willardiine analogs)* were determined. The electronic effect of substituents and of transannular interaction of the groups on the deprotonation constants is discussed.

In the course of a systematic study of the synthetic analogs of willardiine [1, 2], we determined the protolysis constants of β- and γ-pyrimidyl-α-amino acids and compared them with the protolysis constants of β-(1-uracilyl)propionitriles and β-(1-uracilyl)propionic acids. A knowledge of the numerical value of the protolysis constants makes it possible to establish and quantitatively characterize the structural peculiarities of the molecules of the studied compounds and to select the conditions for their identification and separation by electrophoresis.

In the present research we have studied the following compounds: β-(1-uracilyl)propionitriles (I), β-(1-uracilyl)propionic acids (II), α-amino-β-(1-uracilyl)propionic acids (III), α-amino-γ-(1-uracilyl)butyric acids (IV), α-amino-β-(1-cytosinyl)propionic acid (V), and α-amino-γ-(1-cytosinyl)butyric acid (VI). These compounds were synthesized by the methods in [1-5].

In compounds of the I type at pH values from 1.5 to 11.0 deprotonation of the NH group in the 3 position, which is characterized by the pK_{NH} constant, is possible, while in compounds of the II type acidic dissociation of the COOH group in the side chain (pK_{COOH}) is also possible in addition to deprotonation.



I a-g II a-d

I a R=H, b R=F, c R=CH$_3$ II a R=H, b R=F, c R=Br, d R=CH$_3$

Both deprotonation of the NH group in the 3 position and deprotonation of the amino acid grouping in the side chain ($pK_{NH_3^+}$) are possible in compounds of the III and IV types, and these two protolytic processes occur at extremely close pK_a values.

Protonation of the $N_{(3)}$ ring atom, as in cytidine [6] and cytosine [7], is also possible in addition to deprotonation of the amino acid grouping in compounds of the V and VI type. The protolysis constants of the carboxyl groups, which usually have pK_a values of 1.3 to 2.2, were not studied in the present research.

*β-(1-Uracilyl)-α-alanine (willardiine) was isolated by Gmelin from the seeds of Acacia willardiana.

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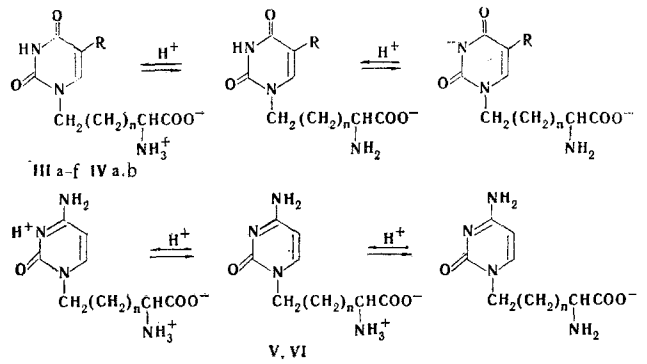

III a-f IV a,b

V, VI

III, IV a R=H; III b R=F, c R=Cl, d R=Br, e R=NO_2;
III f, IV b R=CH_3; III a-f n=0; IV a,b n=1

The constants corresponding to the above-enumerated protolytic equilibria were determined in aqueous solutions by potentiometric titration or by means of UV spectrometry and are presented in Table 1.

The degree of purity of the investigated compounds was verified from the results of elementary analysis, partition chromatography (R_f), and electrophoresis. The equivalence points on the potentiometric titration curves also characterize the purity of these compounds.

It follows from the data in Table 1 that all of the investigated compounds are close in their acid-base properties to unsubstituted uracil and its simplest N_1 and C_5 derivatives, the pK_{NH} values* of which are as follows: uracil 9.43, 5-fluorouracil 7.98, 5-chlorouracil 7.95, 5-nitrouracil 5.48, 5-methyluracil 9.87, 1-methyluracil 9.75, and 1,5-dimethyluracil 10.09.

The acidity of the carboxyl group in II is elevated as compared with unsubstituted propionic acid (pK_a = 4.87), which is explained by the certain electron-acceptor effect of the heteroaromatic ring. The possible transannular interaction of the carboxyl group with the ring cannot be the reason for the increase in acidity, since the character of the IR and UV spectra and the identical pK_{COOH} values of all of the studied II attest to the absence of the proposed interaction.

The pK_{NH} values of all of the N_1-substituted uracils depend markedly on the character of the various substituents attached to $C_{(5)}$. The effect of substituents on the deprotonation constants of the N_3–H bond can be quantitatively expressed by the σ° constants,† and a linear correlation (Fig. 1) is observed between the pK_{NH} values and the σ° constants.

Taking the pK_{NH} values found for I-III into account, we calculated the correlation equations for the pK_{NH} values and the σ° constants of m-substituents: I: $pK_{NH} = 9.52 - 5.27\ \sigma^\circ$ ($r = 0.99$); II: $pK_{NH} = 10.03 - 4.27\ \sigma^\circ$ ($r = 0.96$); III: $pK_{NH} = 10.18 - 4.65\ \sigma^\circ$ ($r = 0.99$).

For comparison, we also calculated the correlation equation for 5-substituted uracil derivatives from the literature data: $pK_{NH} = 9.73 - 5.30\ \sigma^\circ$ ($r = 0.97$).

The increase in all of the pK_{NH} values for II-VI as compared with the corresponding values for N_1-unsubstituted uracils and I can be explained by the +I effect of the dissociated carboxyl group, and the decrease in the pK_{NH} of I as compared with the pK_{NH} for N_1-methyluracil can be explained by the –I effect of the cyano group.

As we have already pointed out, compounds of the III type have extremely close pK_{NH} and $pK_{NH_3^+}$ values. The assignment of both constants was made on the basis of an analysis of the character of the UV spectra as a function of the pH and the spectrophotometrically obtained pK_{NH} values, as well as on the basis of the effect of substituents attached to $C_{(5)}$ on the pK_{NH} values of the willardiine analogs.

In a series of willardiines, the $pK_{NH_3^+}$ values are reduced by ~1.4-2.0 units as compared with the values for such protein amino acids as α-alanine ($pK_{NH_3^+}$ 9.97), tryptophan ($pK_{NH_3^+}$ 9.39), and tyrosine ($pK_{NH_3^+}$ 9.11). This is apparently associated with both the structural peculiarities of III and with a certain electron-acceptor effect of the uracil ring. The close positioning of the NH_3^+ and $C_2 = O$ groupings in the

*The pK_a values were taken from [8, 9].
† The tabulated values of the σ° constants for substituents in the m-position of the benzene ring which according to Jaffe [10], can also be used as a characteristic of the reactivities of heteroaromatic systems of the pyrimidine type, were used in the correlation.

TABLE 1. Protolysis Constants of I-VI

Compound	Protolysis constants				Method of determination,* A^f, A^r, or B
	pK_{NH}	pK_{COOH}	$pK_{NH_3}^+$	pK_{NH}^+	
Ia	9,54±0,03	—	—	—	A^n
	9,43±0,04	—	—	—	B (263, 231nm)
Ib	7,67±0,05	—	—	—	A^o
Ic	9,87±0,02	—	—	—	A^n
IIa	10,05±0,04	4,20±0,02	—	—	A^n
	10,01±0,05	—	—	—	B (265, 231nm)
IIb	8,18±0,03	4,20±0,02	—	—	A^n
IIc	8,74±0,04	4,19±0,01	—	—	A^o
IId	10,34±0,02	4,17±0,03	—	—	A^n
IIIa	9,97±0,03	—	7,98±0,04	—	A^o
	10,02±0,05	—	—	—	B (263, 230nm)
IIId	8,76±0,04	—	7,58±0,04	—	A^o
IIIe	6,74±0,05	—	9,68±0,02	—	A^o
	6,48±0,04	—	—	—	B (325 nm)
IIIf	10,55±0,02	—	8,20±0,04	—	A^n
IVa†	10,73±0,03	—	9,02±0,03	—	A^n
	~10,05	—	—	—	B (265, 233nm)
IVb	10,47±0,05	—	9,03±0,02	—	A^n
	10,42±0,06	—	—	—	B (269, 236nm)
V	—	—	8,45±0,01	4,05±0,02	A^o
VI	—	—	8,90±0,02	4,52±0,01	A^o
			—	4,40±0,03	B (280, 241 nm)

*$\overline{A}$ indicates potentiometric titration, f is forward, r is reverse, and B indicates UV spectroscopy.

† The spectra and the character of the potentiometric titration curves attest to the presence of impurities, so that analogous compound IVb was analyzed.

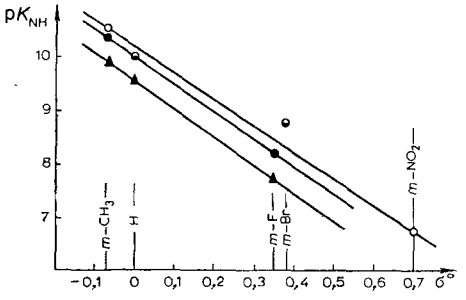

Fig. 1. Correlation of the protolysis constants of the ring imino group with the σ° substituent constants: ▲) β-(1-pyrimidyl)propionitriles; ●) β-(1-pyrimidyl)propionic acids; ○) α-amino-β-(1-pyrimidyl)propionic acids.

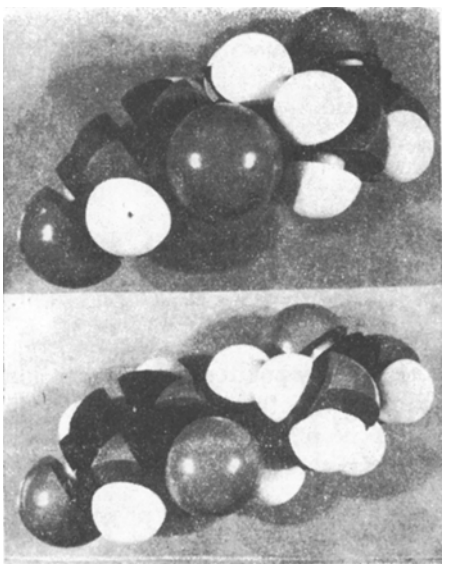

Fig. 2. Molecular models of IIIa (top) and IVa (bottom).

III molecule is seen in Stuart–Briegleb models (Fig. 2), and this makes direct transannular interaction of these groups possible. An analysis of the recorded IR and UV spectra also confirms this sort of transannular effect.

The introduction of substituents R into the 5 position of the ring causes only an insignificant change in the $pK_{NH_3^+}$ values of C_5 derivatives of willardiine, and the latter are consequently little sensitive to the distribution of the electron density in the uracil ring. An exception to this is IIIe because of the strong −I and −C effects of the nitro group on the electron-acceptor properties of the ring. What has been stated is apparently valid only in the case of the above-mentioned transannular interaction.

The basicity of the NH_3^+ grouping in IV is increased as compared with its basicity in IIIa, f, and this might have been expected, since the NH_3^+ and $C_2=O$ groups in IV are far removed from one another, and their transannular interaction is hindered.

The rather considerable (by 0.5 pK_a units) difference in the $pK_{NH_3^+}$ values of V and VI, which contain cytosine residues, can also be explained by transannular interaction of NH_3^+ and $C_2=O$, as a result of which the polarity of the carbonyl group ($C_2^{\delta+}=O^{\delta-}$) increases. A decrease in the electron density on $C_{(2)}$ in turn causes delocalization of the unshared pair of $N_{(3)}$ and, consequently, a decrease in pK_{NH^+} by 0.5 pK_a as compared with VI. It should be noted that the pK_{NH^+} of VI, in which the transannular interaction should be markedly reduced, is practically equal to the corresponding constant of 1-methylcytosine (4.55) [8].

All of the data enumerated above attest to the existence of a transannular interaction between the $\overset{+}{N}H_3$ groups of the amino acid and the ring $C_2=O$ in the series of β- and γ-pyrimidyl-1-α-amino carboxylic acids. It is completely possible that an intramolecular hydrogen bond, which facilitates deprotonation of the ammonium group of the amino acid residue, is formed between these groups. This may be one of the possible reasons for the peculiar reactivity of these amino acids with the hydrogen bond, hindering the formation of peptides from them.

EXPERIMENTAL

Potentiometric Titration. The optimum concentration for the titration of solutions of the investigated compounds proved to be 10^{-2} M. This concentration could not always be achieved for the undissociated form because of insufficient solubility in water. The fact that the solubilities of the compounds in the un-ionized form increase was used; i.e., a definite amount of alkali or acid was added, and the mixture was back titrated. The titrants were 0.1 N HCl and NaOH. The titration volume was 25 ml. A stream of argon was passed through the solutions during titration, and this ensured complete mixing of the added titrant and solution. The potentiometric titration was carried out with an LPU-01 pH meter with a glass electrode at 20 ± 1°. The pK_a calculations were performed with the formula in [11] with the Debye–Hückel correction for the ionic strength [12]. In the case of some derivatives of the III and IV type, for which the difference between the pK_a values of two functional groups proved to be less than 2.5, the Noyes method [12] with allowance for the salt effect was used in the calculations.

Spectrophotometry. The spectrophotometric determination of the pK_a values was made with the method described in [12]. Acetate ($3 < pH < 6$), phosphate ($6 < pH < 8$), and borate ($8 < pH < 12$) buffer solutions were used. The condition $0.1 < D < 1.0$, where D is the optical density of the solution, was used in selecting the concentration of the solutions. The UV spectra were first recorded with a UV-2 automatic spectrophotometer,* and the spectrophotometric measurements at the selected analytical line were then made with an SF-4 spectrophotometer. The parameters obtained from the spectral data were then introduced into the fundamental equation of the spectrophotometric method [12].

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*The UV-2 spectrophotometer was constructed in the Institute of Organic Synthesis of the Academy of Sciences of the Latvian SSR.

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