

SOLUBILITIES OF SOME β -AMINO ACIDS

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Interest in β -amino acids has increased markedly over the past decade. It has been found that certain β -amino acids enter into the composition of vitamins and antibiotics, but, at the same time, there do not exist in the literature, papers on research into the physico-chemical properties of these compounds. β -Alanine has attracted most attention on the part of investigators; its solubility in water and in water-alcoholic solutions [1] has been determined, as well as its acid and alkaline dissociation constants [2,3].

A task of the present work has been a quantitative study of the solubility of the β -amino acids in water in relation to their molecular structures, and the effect of hydrogen ion concentration upon the solubility of poorly-soluble acids. β -Phenylalanine, β -(α -naphthyl)- β -alanine and N-methyl- β -phenylalanine were taken as objects of investigation. All of these compounds were obtained according to the method of Rodinov [4, 5, 6].

EXPERIMENTAL

β -Phenylalanine [4] was recrystallized twice from water, m.p. 213-214°; β -(α -naphthyl)- β -alanine [5] was washed with ether, m.p. 214-215°; N-methyl- β -phenylalanine [6] was recrystallized from alcohol, m.p. 169°.

Solubility was determined by the gravimetric method. Amino acid excess was treated with water, or with the investigated solution in the flask which was placed in a thermostat at $25 \pm 0.1^\circ$. The flask was shaken in a special apparatus until equilibrium was reached. Stirring time was determined individually for each amino acid, for which purpose one sample was preheated to 50° and placed in the thermostat, a second sample being placed immediately in the thermostat. Solubility was in good agreement provided shaking was maintained for not less than 35 hours.

Separation of saturated solution from the precipitate was carried out in the thermostat. A pipette was lowered into the flask, on the lower end of which was threaded a funnel into which was sealed a glass filter. The solution was sucked into the pipette and at the same time was filtered. The resulting filtrate was divided into two parts—in one was determined the amino acid concentration, and in the other concentration of hydrogen ions. The sample in which amino acid concentration was determined was weighed in a weighing tube, dried in the oven at 70-90°, and weighed again. Solubility was determined several times in both water and in various hydrogen ion concentrations.

Hydrogen ion concentration was determined by means of a hydrogen electrode. A saturated calomel electrode was used as the reference electrode. The saturated calomel electrode potential was verified before measurement by standardization against the hydrogen electrode immersed in standard acetate buffer solution at pH 4.62.

β -Amino acid solubilities in water at 25° were determined as given in Table 1. Solubility of β -alanine was taken from the literature [1].

TABLE 1

Solubility of β -Amino Acids in Water at 25°

β -Amino acid	Density	Solubility		Number of measurements
		g/100 g of solution	moles/liter	
β -Alanine	1.1581	—	6.125	
β -Phenylalanine	1	1.982	0.1200	5
β -(α -Naphthyl)- β -alanine	1	0.553	0.00226	8
N-Methyl- β -alanine	1.0636	29.625	1.759	3

The resulting data indicate that the carbon chain structure has the greatest effect upon solubility of the β -amino acids. Introduction of a phenyl group into the chain decreases solubility; the solubility of β -phenylalanine is 51-fold less than the solubility of β -alanine. Introduction of the naphthyl radical produces an even greater decrease in solubility. The solubility of α -(α -naphthyl)- β -alanine is 2710-fold less than the solubility of β -alanine.

Introduction of an alkyl radical into the amino group has an opposite effect. The solubility of N-methyl- β -phenylalanine is greater than the solubility of β -phenylalanine by 14.6-fold.

The effect of hydrogen ion concentration upon solubility was investigated, using β -(α -naphthyl)- β -alanine as an example. Solutions with various hydrogen ion concentrations were obtained from chemically-pure HCl, with which the amino acid gives a soluble salt.

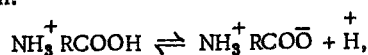
TABLE 2

Relationship of β -(α -Naphthyl)- β -alanine Solubility to Hydrogen Ion Concentration

Concentration (moles/liter $\cdot 10^2$)	pH of initial HCl solution	Solubility (S) moles/liter $\cdot 10^2$	pH of the saturated solution
0.87	2.06	0.86	2.69
1.269	1.89	1.244	2.47
2.231	1.65	1.518	2.07
4.31	1.37	2.49	1.71
16.10	1.22	2.956	1.49

From the pH values for an equimolecular hydrochloride solution of β -(α -naphthyl)- β -alanine and its solubility, it is possible to determine one of its dissociation constants. The resulting constant will express the alkaline dissociation of the NH_2 group, provided it be assumed that the neutral form of β -amino acid consists of undissociated molecules. If, however, the neutral form of the amino acid is a bipolar ion, then the constant found will characterize the acid properties of the COO^- group [9]. At present it can be considered to be proved that an aliphatic amino acid exists in aqueous solution in the form of amphoteric ions.

The β -amino acid investigated by the authors was an aliphato-aromatic acid with an amino group in the aliphatic radical. This made it possible to assume that the neutral form in solution is the bipolar ion, similar to amino acids of the aliphatic series. Under this condition, equilibrium in acid solutions of β -(α -naphthyl)- β -alanine hydrochloride can be expressed by the equation:



and the dissociation constant by the following ratio:

$$K = \frac{a_{\text{H}^+} + a_{\text{R}^+}}{a_{\text{R}^+}}, \quad (1)$$

where a denotes activity of the corresponding ions.

TABLE 3

Approximate Acid Dissociation Constant for β -(α -Naphthyl)- β -alanine

Amino acid concentration (moles/liter)	HCl concentration (moles/liter)	pH of the saturated solution	pK
0.00866	0.00870	2.69	3.21
0.01242	0.01269	2.47	2.94
0.0223	0.01386	2.07	3.07
0.04312	0.0249	1.71	2.98
0.06100	0.02956	1.49	2.99

Since the ionizing power of the solutions investigated by the authors is small, it can be considered, therefore, that the ionic activities are equal to their concentrations. Substituting ionic concentration for ionic activity, and introducing the pH value into equation (1), we have:

$$\text{pK} = \text{pH} - \log \frac{C_{\text{R}^+}}{C_{\text{R}^+}}. \quad (2)$$

The values C_{R^+} and C_{R^+} can be readily determined from the following ratios:

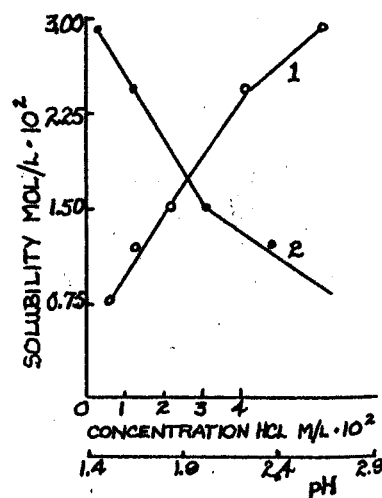
$$C_{\text{HCl}} = C_{\text{Cl}^-} = C_{\text{R}^+} + C_{\text{H}^+},$$

$$C_{\text{R}^+} = C_{\text{Cl}^-} - C_{\text{H}^+}.$$

Dry residue from hydrochloric acid solution was in the form of amino acid hydrochloride. Calculation was made for free amino acid. The experimental results are given in Table 2 and in the Figure.

It can be seen from the data obtained that solubility increases with increase in hydrogen ion concentration. However, there was not observed a simple relation between these values as occurs with some poorly-soluble α -amino acids [7, 8].

It was not possible to determine the solubility of β -(α -naphthyl)- β -alanine in alkaline solutions, since colloidal solutions were formed, difficult to filter, when the pH exceeded 8.



Effect of HCl concentration upon the solubility of β -(α -naphthyl)- β -alanine and the relationship of solubility to pH of the saturated solution.

The total amino acid concentration is known from the solubility (S) value at a given pH value. Hence, the concentration of undissociated molecules is equal to $C_{R\pm} = S - C_{R+}$. Substituting the calculated values of $C_{R\pm}$ and C_{R+} into equation (2), there is obtained the pK value. The results are given in Table 3.

As can be seen from the data obtained (Table 3), the pK value deviates sharply for solutions with low hydrochloric acid concentration where salt hydrolysis may be present, but for the other solutions deviations from the average value constitute only about 3%.

SUMMARY

1. The solubilities of β -phenylalanine, β -(α -naphthyl)- β -alanine and N-methyl- β -phenylalanine in water at 25° have been determined.
2. It has been established that introduction of an aromatic radical into the chain lowers the solubility and that introduction of an alkyl radical into the amino group increases the solubility of β -amino acids.
3. The solubility of β -(α -naphthyl)- β -alanine in solutions with varied hydrogen ion concentrations has been determined.
4. The approximate acid dissociation constant for β -(α -naphthyl)- β -alanine has been calculated.

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