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Acid–Base Properties of Amoxycillin

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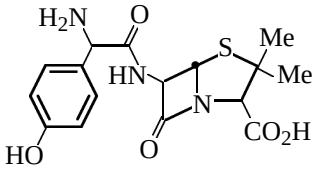
Abstract — Acid–base properties were studied by means of pH-metry. The concentration acid dissociation constants of the carboxy, ammonium, and phenolic groups (20°C, on the background of 0.1, 0.4, 0.7, and 1.0 M solutions of KCl and KNO_3) were determined. Semiempirical quanum-chemical calculations by the PM3 method were used to calculate the sizes of ionic forms of amoxycillin in energetically favorable conformations. Thermodynamic dissociation constants were obtained by extrapolation of the concentration constants to zero ionic strength according to the Huckel equation with inclusion of the calculated ionic sizes.

Preciously [1,2] we studied the acid–base properties of three widely used antibiotics of the penicillin group: benzylpenicillin, carbenicillin, and ampicillin. Along with these drugs, a growing number of uses is being found for amoxycillin (**I**), a newer and more effective antibiotic of the same chemical group [3–5].



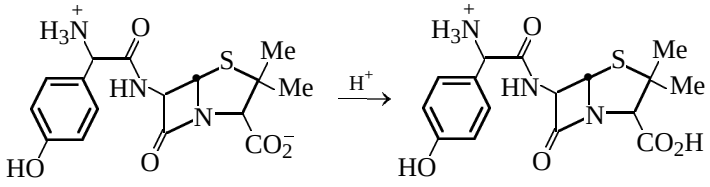
I

Almost no quantitative acid–base characteristics of amoxycillin (**I**) are available in the literature. The review [6] with reference to [7] reported two acid dissociation constants, pK_1 2.67 and pK_2 7.11, determined at 37°C and an ionic strength of 0.5. The first constant probably relates to the carboxy group and the second, ammonium. However, compound **I** also contain a phenolic group whose acidity is unknown.

It the present work we set ourselves the task to study properties of all functional groups of amoxycillin, capable of proton donor–proton acceptor interactions in aqueous solutions and to calculate their acid dissociation constants.

Aqueous solution of amoxycillin has pH 5.7. Taking this into account, as well as the presence in the molecule of amino, carboxy, and phenolic groups, we can suggest that amoxycillin would behave as a dibasic amino acid and exists in solution as dipolar ion.

Solutions of amoxycillin on the background of 0.1, 0.4, 0.7, and 1.0 M solutions of KCl and KNO_3 were titrated with acid (HCl or HNO_3, respectively) or alkali (NaOH). The titration curces in 0.1 M KNO_3 are given in Fig. 1. In the case of acid titration, the dipolar ion H_2Amx^- (**II**) is protonated and converts into the cation H_3Amx^+ (**III**).



II **III**

The titration curve of amoxycillin with alkali (Figs. 1 and 2) show two buffer portions intervened by a slight pH jump. In this case, two-step dissociation of H_2Amx^- (**II**) occurs to form anions $HAmx^{\pm}$ (**IV**) and AmX^- (**V**). Probably, consecutive dissociation of the ammonium and phenolic groups takes place.

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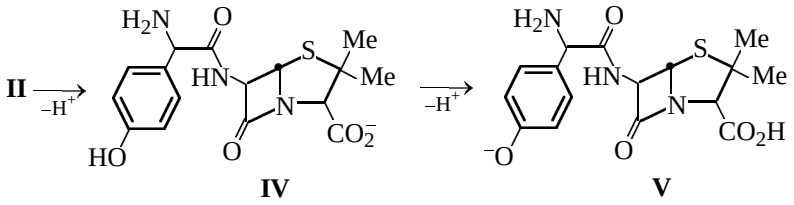

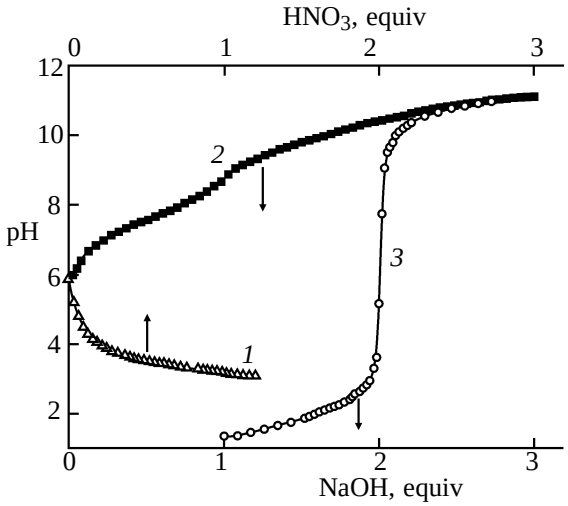

Fig. 1. pH-Metric titration curves. (*1*) Titration of 0.0023 M amoxycillin **II** with 0.0500 M HNO_3, (*2*) titration of 0.0023 M amoxycillin **II** with 0.0547 M NaOH, and (*3*) titration of 0.005 M HNO_3 with 0.0547 M NaOH (the curve is shifted by 1 equiv along the abscissa axis) [20°C, ionic strength 0.1 (KNO_3)].

Further addition of alkali to the solution results in that the titration curve of amoxycillin (**II**) coalesces with the titration curve of a strong acid after the equivalence point, and the pH of the solution is determined by the quantity of alkali added. For comparison we give the titration curve of nitric acid under the same conditions (Figure 1, curve *3*). Thus, the other functional groups in dipolar ion **II** do not undergo acid dissociation under the experimental conditions. The titration curves of amoxycillin in KNO_3 and KCl solutions of various concentrations are quantitatively similar to each other and to the curves in Fig. 1.

Concentration acid dissociation constants of amoxycillin cation **III** (20°C)

Equilibrium	Background electrolyte	pK_a [a]			
		μ 1.0	μ 0.7	μ 0.4	μ 0.1
$H_3Amx^+ \rightleftharpoons H^+ + H_2Amx^-$	KCl	2.92	2.99	3.04	3.09
	KNO_3	2.96	3.04	3.06	3.10
$H_2Amx^- \rightleftharpoons H^+ + HAmx^-$	KCl	7.74	7.70	7.68	7.65
	KNO_3	7.84	7.78	7.73	7.66
$HAmx^- \rightleftharpoons H^+ + Amx^{2-}$	KCl	9.70	9.74	9.78	9.88
	KNO_3	9.36	9.49	9.64	9.84

[a] The confidence interval of the pK_a values is 0.01.

The concentration curves (K_a) of three-step acid dissociation of the amoxycillin cation H_3Amx^+ (**III**), corresponding to equilibria (1)–(3), were calculated by the Schwarzenbach method [8]. The resulting data are given in the table.

$$H_3Amx^+ \overset{K_1}{\rightleftharpoons} H^+ + H_2Amx^-, \quad (1)$$

$$H_2Amx^{\pm} \overset{K_2}{\rightleftharpoons} H^+ + HAmx^-, \quad (2)$$

$$HAmx^- \overset{K_3}{\rightleftharpoons} H^+ + Amx^{2-} \quad (3)$$

The resulting pK_1 and pK_2 values are higher than those reported in [6, 7]. Probably, this is explained by the lower temperature of the solution.

The thermodynamic acid dissociation constants (K_a^0) that are independent of the concentration and chemical nature of the background electrolyte were calculated by linear extrapolation of the concentration constants to zero ionic strength according to Eq. (4).

$$\xi = pK_a^0 - \delta\mu. \quad (4)$$

Here $\xi = p K_a^0 + A_f(z_{An}^2 - z_{HAn}^2)\sqrt{\mu}/(1 - B_f\alpha\sqrt{\mu})$ is the generalized variable, δ, empirical coefficient, δ, ionic strength of the solution (M), A_f and B_f, Debye–Huckel equation constants, α, ionic diameter (Å), and z_{HAn} and z_{An}, charges of the conjugate acid–base pair (HAn and An can be ions, dipolar ions, and neutral molecules). Substantiation of the procedure and detailed deduction of the equations are described in [2].

The A_f and B_f values for aqueous solution at 20°C are 0.5070 and 0.3282, respectively [9]. No experimental α values for ions **III**–**V** are available. Like in [2], these values were calculated by computer models

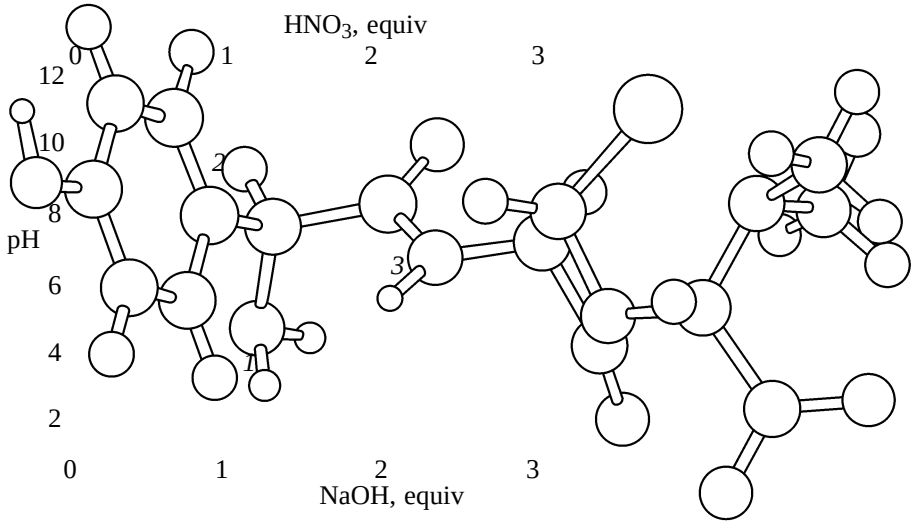

Fig. 2. Computer model of the anion $HAmx^-$.

of the ions in the least energy conformation. Figure 2 exemplifies a model of the anion $HAmx^-$. The models were calculated by the PM3 method [10] by the Chem3D program (Cambridge Soft). The ionic diameters were calculated as distances between the nuclei of the most remote atoms of the model. The following values were obtained, Å: H_3Amx^+ 12.8, $HAmx^-$ 11.6, and Amx^{2-} 12.2. In calculating ξ, for the mean α value for all ionic forms of amoxycillin (**III**–**V**) we took 12 Å.

By linear extrapolation of the experintal dependences $\xi = f(\mu)$ to $\mu = 0$ (Fig. 3) we obtained pK_a^0 (20°C) values.

pK_1^0 3.04 ± 0.01, δ +0.22 (KCl), δ +0.18 (KNO_3),

pK_2^0 7.71 ± 0.01, δ –0.13 (KCl), δ –0.23 (KNO_3),

pK_3^0 10.09 ± 0.01, δ +0.09 (KCl), δ +0.43 (KNO_3).

Comparing our results with those in [2] we can note quite close pKa0 values for the carboxy group in all penicillins. The amino group in amoxycillin (I) is slightly more basic than in ampicillin. The phenolic group in amoxycillin is slightly less acidic than in phenol, which is characteristic of *p*-alkyl-substituted phenols. According to [11], the pKa0 values of phenol, *p*-methylphenol, and *p*-methoxyphenol are 9.88, 10.37, and 10.45, respectively. Like with ampicillin, the dependence of pK_a^0 on μ for the ammonium group in amoxycillin is characterized by $\delta < 0$.

The resulting pK_a^0 values were used to calculate the distribution diagrams of amoxycillin forms in solution (Fig. 4).

EXPERIMENTAL

Amoxycillin trihydrate purchased from Hemofarm (Yugoslavia) was used. Working solutions of the antibiotic were prepared immediately before use to avoid undesirable chemical changes. A 500-ml flask was charged with a weighed sample of amoxycillin trihydrate and a required volume of 2 M KCl or KNO_3 for a preset ionic strength. The antibiotic was dissolved, and the solution was brought to the mark with water. A 100-ml portion of the solution was placed to

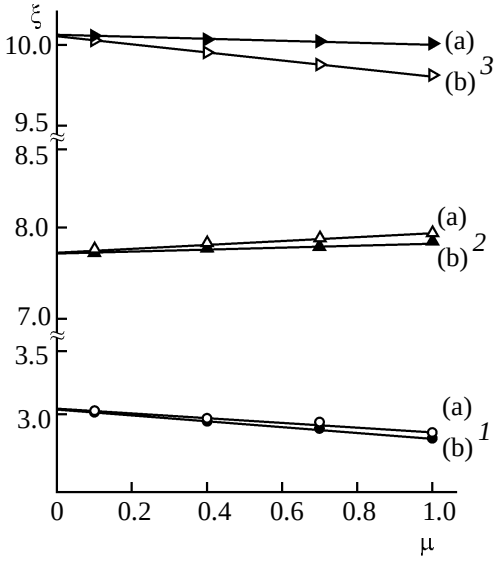

Fig. 3. (*1*)–(*3*) Linear extrapolation of the $\xi = f(\mu)$ dependences for equilibria (1)–(3) in (a) KNO_3 and (b) KCl solutions.

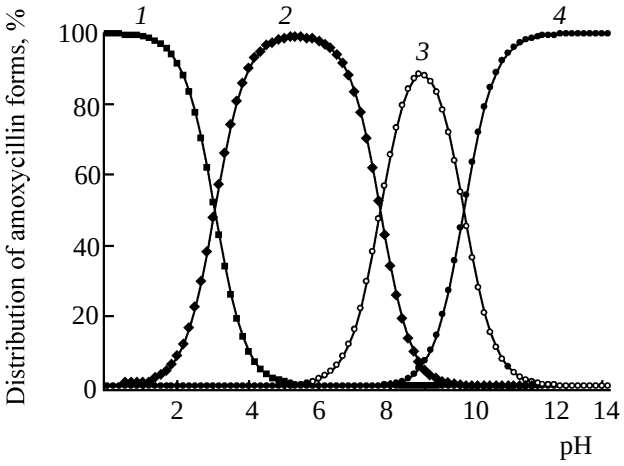

Fig. 4. Distribution of amoxycillin forms in aqueous solution vs. pH. (*1*) H_3Amx^+, (*2*) H_2Amx^-, (*3*) $HAmx^-$, and (*4*) Amx^{2-}.

the temperaturecontrolled cell at 20°C and titrated with 0.0500 M HCl or HNO_3, or 0.0543 M NaOH. All solutions were prepared with twice distilled water freed of CO_2 by boiling. The pH-meter had a glass working electrode and a saturated calomel reference electrode. The accuracy of the pH values was 0.01. All calculations, plotting, extrapolation, and statistical treatment were performed using the Origin program (Origin-Lab Corp.).

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