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Thermodynamic Constants of Acid–Base Equilibria in Solutions of Penicillins

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Abstract — The constants of acid–base equilibria in solutions of benzylpenicillin, carbenicillin, and ampicillin at 20°C were determined by pH-metric titration (supporting electrolytes 0.1, 0.4, 0.7, and 1.0 M KCl and KNO_3). The sizes of the ionic species of the penicillins in their energetically favorable conformation were determined by the PM3 semiempirical method. The thermodynamic equilibrium constants were estimated by extrapolation of the concentration constants to zero ionic strength by the Hückel equation, taking into account the calculated sizes of the ions.

Ionic strength and the kind of supporting electrolyte are significant factors affecting solution equilibria. The concentration equilibrium constants K determined at a given constant ionic strength refer only to the specific experimental conditions. The thermodynamic constants K^0, which are independent of the ionic strength and supporting electrolyte, are more objective characteristics of ionic equilibria [1–3]. It seemed important to continue studies of acid–base equilibria in solutions of penicillins [4] and to determine the thermodynamic constants of acid dissociation of some compounds of this group. We chose benzylpenicillin (HBzp), carbenicillin (H_2Carb), and ampicillin (HAmp). As direct experimental determination of the thermodynamic equilibrium constant is impossible, this quantity is calculated by extrapolation of a series of concentration constants to zero ionic strength. The concentration constants of acid dissociation were determined, as in [4], by the Schwarzenbach method [5] from the pH-metric titration curves of the sodium salts of the antibiotics at 20°C. As supporting electrolytes we used KCl and KNO_3 (0.1, 0.4, 0.7, and 1.0 M). The titration was performed with 0.05 M HCl and HNO_3. The pK_a values obtained for the penicillins are listed in Table 1. They mainly coincide with those determined previously [4]. However, some constants were determined more reliably owing to the improvement of the experimental procedure (solutions of the antibiotics were prepared just before titration). The deviations from the previous data are the largest for benzylpenicillin, which is more susceptible to hydrolysis than carbenicillin and ampicillin.

Published data on the acid dissociation constants of penicillins are few [6, 7]; these data are given in Table 2.

Since the conditions (primarily temperature) at which the constants were determined differ essentially, we cannot directly compare our results to the published data; it is seen, however, that the results are fairly consistent. The somewhat higher values of pK_a that we obtained may be due to the lower solution temperatue and hence lower degree of dissociation. It should be noted also that the published data are not quite consistent. For example, the pK_a values given for benzylpenicillin (Table 2) suggest that the dissociation of its carboxy groups increases with a decrease in the temperature and ionic strength of the solution, which is improbable.

To determine the thermodynamic quantities pK_a^0, we used linear extrapolation suggested previously for determining the stability constants of the complexes [13, 14] and the dissociation constants of the acids [15].

The general expression for the thermodynamic dissociation constant of HAn is as follows:

$$K_a^0 = \frac{a_H a_{An}}{a_{HAn}} = \frac{f_H[H] f_{An}[An]}{f_{HAn}[HAn]} = K_a \frac{f_H f_{An}}{f_{HAn}}, \qquad (1)$$

where a is the activity; f is the molar activity coefficient; [HAn], [An], and [H] are the equilibrium molar concentrations of acid HAn, its dissociated species An, and hydrogen ions. The species HAn and An can be both neutral molecules and ions.

Since in the pH-metric determination of K_a [H] = a_H and f_H = 1, we obtain

$$K_a^0 = K_a \frac{f_{An}}{f_{HAn}}. \qquad (2)$$

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Table 1. Concentration constants of acid dissociation of penicillins, 20°C

Equilibruim	Supporting electrolyte	pK_a [a]				
		μ 1.0	μ 0.7	μ 0.4	μ 0.1	μ 0.1 [4]
$HBzp \rightleftarrows H^+ + Bzp^-$	KCl	2.97	3.01	3.04	3.11	3.04 ± 0.02
	KNO_3	3.02	3.04	3.08	3.11	–
$H_2Carb \rightleftarrows H^+ + HCarb^-$	KCl	2.46	2.55	2.65	2.79	2.79 ± 0.02
	KNO_3	2.67	2.70	2.76	2.81	–
$HCarb^- \rightleftarrows H^+ + Carb^{2-}$	KCl	3.44	3.50	3.55	3.64	3.63 ± 0.06
	KNO_3	3.49	3.54	3.57	3.65	–
$H_2Amp^+ \rightleftarrows H^+ + HAmp^\pm$	KCl	2.83	2.89	2.95	3.01	2.96 ± 0.03
	KNO_3	2.97	2.99	3.02	3.04	–
$HAmp^\pm \rightleftarrows H^+ + Amp^-$	KCl	7.28	7.27	7.26	7.25	7.22 ± 0.02
	KNO_3	7.48	7.37	7.35	7.28	–

[a] The confidence interval of pK_a is 0.01.

Table 2. Acid dissociation constants of penicillin (published data [6, 7])

Antibiotic	pK_1	pK_2	Determination conditions	References
Benzylpenicillin	2.8	–	Potentiometric method, 0.1 M KCl, temperature not given	[6], reference to [8]
	2.78	–	Potentiometric method, 0.5 M NaCl, 60°C	[7], reference to [9]
	2.75	–	Potentiometric method, 0.15 M KCl, 37°C	[7], reference to [10]
	2.56	–	Solubility method, 20°C	[7]
Carbenicillin	2.60	3.19	Potentiometric method, 0.5 M KCl, 35°C	[7], reference to [11]
	2.42	3.39	Solubility method, 20°C	[7]
Ampicillin	2.38	7.06	Conditions not given	[7], reference to [12]

By taking logarithms, we obtain

$$pK_a^0 = pK_a - \log f_{An} + \log f_{HAn}. \quad (3)$$

At a low concentration of nondissociated acid molecules in a 1–1 electrolyte solution, their activity coefficient is given by [16]

$$\log f = k_s\mu, \quad (4)$$

where k_s is the salt coefficient and μ is the ionic strength of the solution, calculated from the molar concentrations of the ions. Zwitterions behave in electrostatic interactions as neutral species [17]. Their activity coefficients are also given by (4).

Calculation of the activity coefficients of ions is a sophisticated problem. Various mathematical expressions were suggested to describe the dependences of the activity coefficients of ions on the ionic strength and kind of supporting electrolyte. The modern views on this problem are discussed in detail in [2, 3, 18]. In studies of protonation and complexation, the effect of the medium on the activity of ions at an ionic strength $\mu \leq 1$ is most frequently taken into account using the Hückel equation

$$-\log f = \frac{A_f z^2 \sqrt{\mu}}{1 + B_f \alpha \sqrt{\mu}} - D\mu, \quad (5)$$

where A_f and B_f are constants depending on the temperature and chemical nature of the solvent; at 20°C for aqueous solution A_f 0.5070 and B_f 0.3282 [19]; D is an empirical coefficient (coefficient of ionic interaction); z is the ion chanrge; and α is the effective ion diameter (Å). We can mention as example the studies made by different research teams [13–15, 20–25].

As shown in [4], the following acid–base equilibria occur in aqueous solutions of the antibiotics:

$$HBzp \rightleftarrows H^+ + Bzp^-, \quad (6)$$

$$H_2Carb \rightleftarrows H^+ + HCarb^-, \quad (7)$$

$$HCarb^- \rightleftarrows H^+ + Carb^{2-}, \quad (8)$$

$$H_2Amp^+ \rightleftarrows H^+ + HAmp^\pm, \quad (9)$$

$$HAmp^\pm \rightleftarrows H^+ + Amp^-. \quad (10)$$

For equilibria (6), (7), and (10), pK_a^0 can be expressed by substituting (4) and (5) in (3) as follows:

$$pK_a^0 = pK_a + \frac{A_f z_{An}^2 \sqrt{\mu}}{1 + B_f \alpha \sqrt{\mu}} + (k_s - D_{An})\mu. \quad (11)$$

For equilibria (8) and (9), we obtain expressions (12) and (13), respectively.

$$pK_a^0 = pK_a + \frac{A_f (z_{An}^2 - z_{HAn}^2)\sqrt{\mu}}{1 + B_f \alpha \sqrt{\mu}} + (D_{HAn} - D_{An})\mu, \quad (12)$$

$$pK_a^0 = pK_a - \frac{A_f z_{HAn}^2 \sqrt{\mu}}{1 + B_f \alpha \sqrt{\mu}} + (D_{HAn} - k_s). \quad (13)$$

Equations (11)–(13) can be represented generally as linear dependences of certain variable ξ on μ with a slope δ:

$$\xi = pK_a^0 - b\mu. \quad (14)$$

Here for equilibria (6), (7), and (10),

$$\xi = pK_a + \frac{A_f z_{An}^2 \sqrt{\mu}}{1 + B_f \alpha \sqrt{\mu}}, \ \delta = k_s - D_{An};$$

for equilibrium (8),

$$\xi = pK_a + \frac{A_f (z_{An}^2 - z_{HAn}^2)\sqrt{\mu}}{1 + B_f \alpha \sqrt{\mu}}, \ \delta = D_{HAn} - D_{An};$$

and for equilibrium (9),

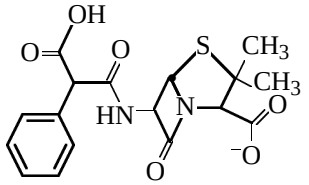
$$\xi = pK_a + \frac{A_f z_{HAn}^2 \sqrt{\mu}}{1 + B_f \alpha \sqrt{\mu}}, \ \delta = D_{HAn} - k_s,$$

Equations (11)–(13) are similar to the so-called Vasil'ev one-parameter equation [13–15]:

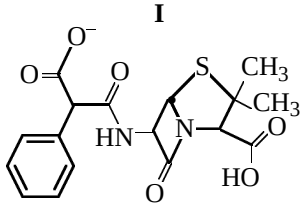
$$pK_a = \frac{A_f \Delta z^2 \sqrt{\mu}}{1 + 1.6\sqrt{\mu}} = pK_a^0 + b\mu. \quad (15)$$

However, the value α 4.9 Å assumed as parameter in the Vasil'ev equation, being adequate for many inorganic ions, is too low for such bulky species as penicillin ions. Therefore, in the subsequent calculations we used Eqs. (11)–(13) rather than (15).

Since the parameter α was not experimentally determined for penicillins, we made computer models of the ionic species of benzylpenicillin, carbenicillin, and ampicillin in the lowest energy conformation. Some of these models are shown in Fig. 1. We took into account the possible existence of two isomers of the $HCarb^-$ anion (**I**, **II**) differing in the protonation site:



I



II

The calculations were performed with ChemOffice program package (Cambridge Soft, http://www.cambridgesoft.com). The structural formulas of the ions were plotted with ChemDraw program and imported into Chem3D program, after which the conformation with the lowest energy was calculated by the PM3 semiempirical method [26] (MOPAC → Minimize Energy procedure). The parameter α was calculated as the distance between the centers of the most remote atoms of the model. In this case, such an approach to the determination of α is quite acceptable. The values of α calculated similarly for some other organic anions (benzoate, citrate) coincide with the experimental data given in [19]. As a result, we obtained the following values of α: Bzp^- 11.2, $HCarb^-$ (form **I**) 11.6, $HCarb^-$ (form **II**) 12.7, $Carb^{2-}$ 13.0, H_2Amp^+ 12.2, and Amp^- 11.0 Å. In the subsequent calculations, we took 12 Å as the mean value for all the ionic species of the penicillins. Then we calculated the parameters ξ, constructed their dependences on μ, linearized them, and extrapolated to μ 0 (Figs. 2, 3) using Origin program (OriginLab Corporation, http://www.originlab.com). As seen from Figs. 2 and 3, the pK_a values obtained by extrapolating to μ 0 the data obtained in KCl and KNO_3 solutions virtually coincide. The calculated values of pK_a^0 and δ are listed in Table 3.

The δ values for the dissociation of carboxy groups of the antibiotics correlate well with published data concerning the dissociation of aliphatic and aromatic carboxylic acids [3, 21, 24]. For the dissociation of the ammonium proton of ampicillin, pK_a grows with increasing μ. Such an unusual trend was observed in some cases with amines [22] and amino acids [23]; it is apparently due to the negative k_s values of the amino acids [27].

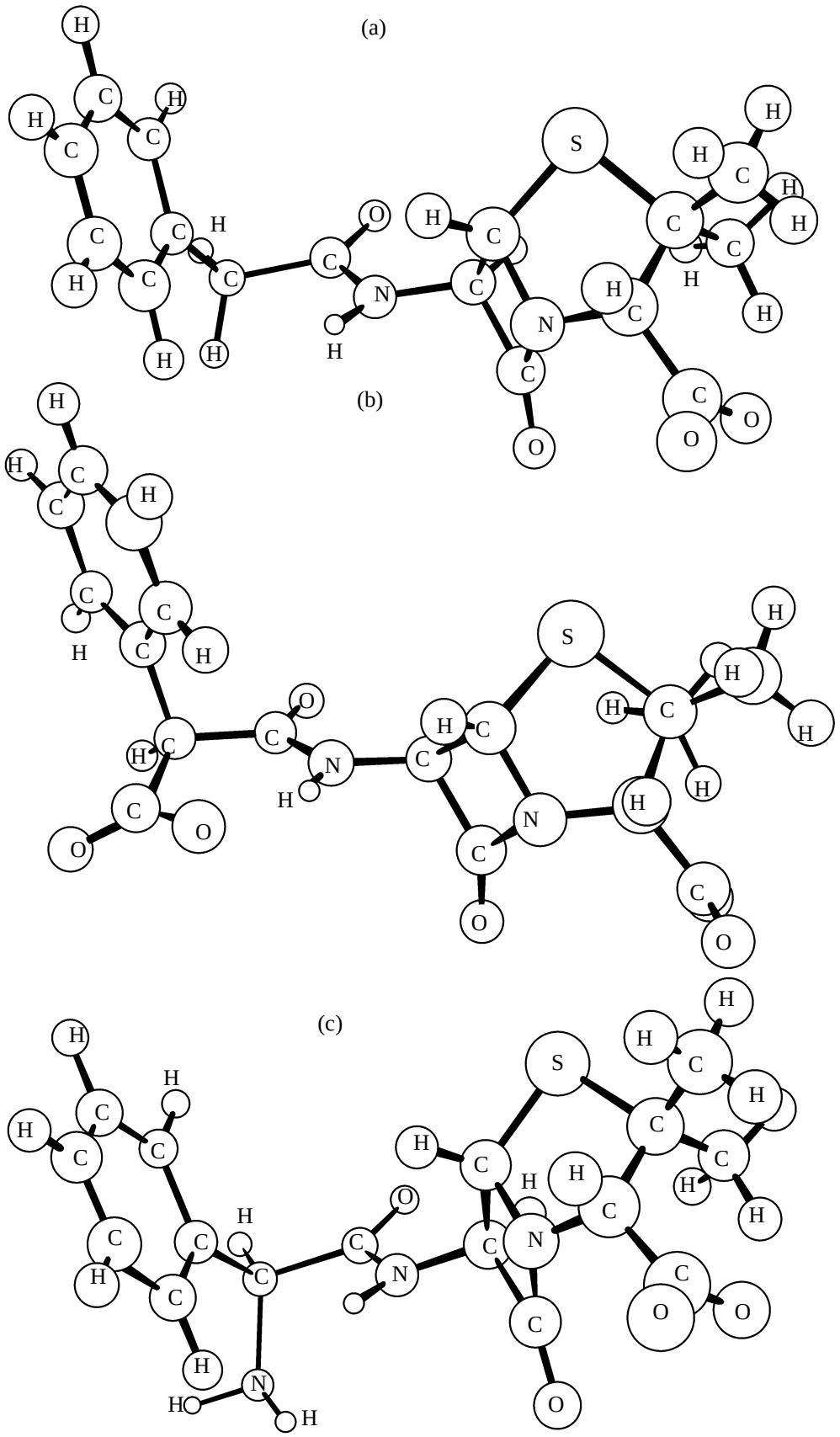


Fig. 1. Computer models of (a) Bzp^{-}, (b) $Carb^{2-}$, and (c) Amp^{-}.

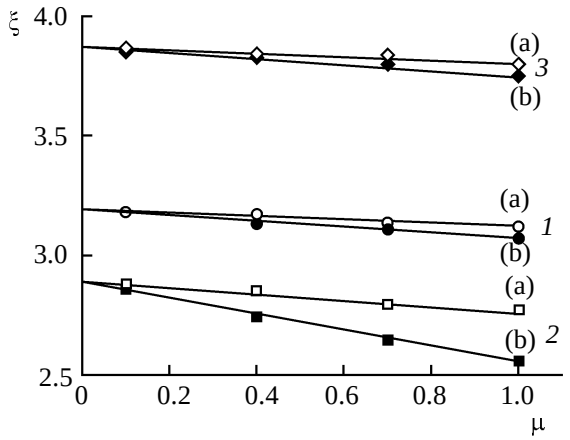


Fig. 2. Linear extrapolation of the dependences of ξ on the solution ionic strength. Equilibrium: (*1*) (6), (*2*) (7), and (*3*) (8). Supporting electrolyte: (a) KNO_3 and (b) KCl.

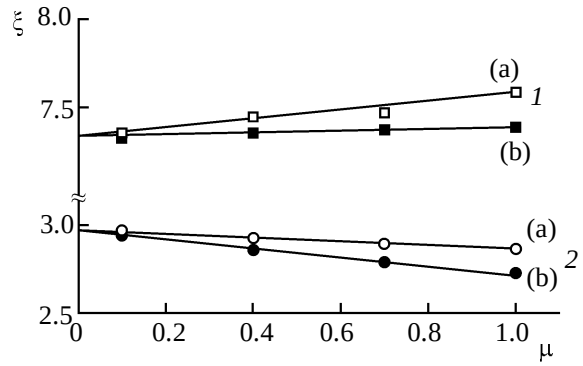


Fig. 3. Linear extrapolation of the dependences of ξ on the solution ionic strength. Equilibrium: (*1*) (9) and (*2*) (10). Supporting electrolyte: (a) KNO_3 and (b) KCl.

EXPERIMENTAL

Experiments were performed with pharmaceutical samples (powders in vials) of benzylpenicillin sodium salt (Biokhimik Joint-Stock Company, Saransk, Mordovia, Russia), carbenicillin disodium salt (Ferane, Russia), and ampicillin sodium salt (Ferane, Russia). Working solutions of the antibiotics were prepared just before the experiments, to avoid undesirable chemical and biochemical changes. To an accurately weighed portion of the antibiotic, we added the required volume of 2 M KCl or KNO_3 to provide the necessary ionic strength. The antibiotic was dissolved, and the solution was brought to the mark with water. A 100-ml portion of the working solution was placed in a temperature-controlled cell at 20°C and titrated with 0.05 M HCl or HNO_3. All the solutions were prepared in double-distilled water from which CO_2 was removed by boiling. The pH was measured with a pH meter equipped with a glass measuring electrode and a saturated calomel reference electrode (measurement accuracy 0.01 pH unit).

Table 3. Thermodynamic constants of acid dissociation of penicillins, 20°C

Equilibrium	pK_a^0	δ	
		KCl	KNO_3
$HBzp \rightleftarrows H^+ + Bzp^-$	3.19 ± 0.01	0.12	0.07
$H_2Carb \rightleftarrows H^+ + HCarb^-$	2.89 ± 0.01	0.33	0.13
$HCarb^- \rightleftarrows H^+ + Carb^{2-}$	3.87 ± 0.01	0.11	0.08
$H_2Amp^+ \rightleftarrows H^+ + HAmp^{\pm}$	2.97 ± 0.01	0.23	0.11
$HAmp^{\pm} \rightleftarrows H^+ + Amp^-$	7.33 ± 0.01	–0.07	–0.24

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