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Thermodynamics of the hydrolysis of penicillin G and ampicillin

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Abstract

Apparent equilibrium constants and calorimetric enthalpies of reaction have been measured for the β-lactamase catalyzed hydrolysis of penicillin G(aq) and ampicillin(aq) to penicillinoic acid(aq) and to ampicillinoic acid(aq), respectively. High-pressure liquid-chromatography and microcalorimetery were used to perform these measurements. The results for the reference reactions at $T = 298.15$ K and $I_m = 0$ are: $K^\circ = (9.4 \pm 3.1) \times 10^{-7}$, $\Delta_r G^\circ = (34.4 \pm 1.0)$ kJ mol^{-1}, $\Delta_r H^\circ = -(73.7 \pm 0.4)$ kJ mol^{-1}, and $\Delta_r S^\circ = -(363 \pm 4)$ J K^{-1} mol^{-1} for penicillin G$^-$(aq) + H_2O(l) = penicillinoic acid^{2-}(aq) + H^+(aq); $K^\circ = (6.0 \pm 3.0) \times 10^{-6}$, $\Delta_r G^\circ = (29.8 \pm 1.7)$ kJ mol^{-1}, $\Delta_r H^\circ = -(70.0 \pm 7.5)$ kJ mol^{-1}, and $\Delta_r S^\circ = -(335 \pm 26)$ J K^{-1} mol^{-1} for ampicillin$^-$(aq) + H_2O(l) = ampicillinoic acid^{2-}(aq) + H^+(aq). Calorimetric enthalpies of reaction for the β-lactamase catalyzed hydrolysis of cephalosporin C have also been measured but the reaction products have not been identified and the measured enthalpies cannot be assigned to a specific reaction. Acidity constants for ampicillin, penicillin G, ampicillinoic acid, and penicillinoic acid are also reported. A strain energy of 116 kJ mol^{-1} for the β-lactam ring is obtained from thermochemical data.

Keywords: Acidity constants; Ampicillin; Apparent equilibrium constants; Calorimetry; Cephalosporin C; Enthalpy; β-lactamase; Penicillin G; Thermodynamics

1. Introduction

The enzyme β-lactamase (EC 3.5.2.6, also called penicillinase) catalyzes the following biochemical reactions [2]:

penicillin G(aq) + H_2O(l) = penicillinoic acid(aq), (1)

ampicillin(aq) + H_2O(l) = ampicillinoic acid(aq). (2)

The hydrolysis of cephalosporin C is also catalyzed by β-lactamase, but the reaction products have not been characterized. Structures of these substances are shown in fig. 1. In reactions (1) and (2), the terms penicillin G, penicillinoic acid, ampicillin, and ampicillinoic acid are used to represent the total amounts of the various charged and uncharged species formed from the ionizations of these substances in solution. Bacteria that are resistant to penicillin G produce β-lactamase as a defense against penicillin G. Since ampicillin and other semi-synthetic penicillins undergo β-lactamase catalyzed hydrolysis more

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[2] The Chemical Abstract Services registry numbers of the principal substances in this study are: penicillin G (also called benzylpenicillin), 61-33-6; ampicillin, 69-53-4; cephalosporin C, 61-24-5; penicillinoic acid, 13057-98-2; and ampicillinoic acid, 32746-94-4.

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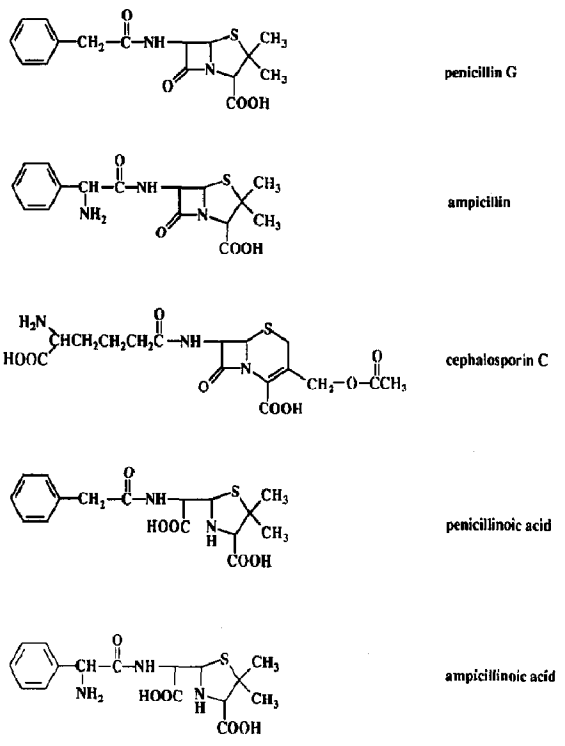

Fig. 1.

slowly than penicillin G, they are more effective against penicillin resistant strains of bacteria. In addition to the kinetic aspects, it is also of interest to know what the positions of equilibrium and the enthalpies of these reactions are and how they vary with pH, temperature, and ionic strength. Grime and Tan [1], as a part of an analytical study, have reported the results of calorimetric measurements of these hydrolysis reactions. No equilibrium studies of these reactions have been reported.

2. Experimental

The molar masses of the substances used in this study are: monosodium penicillin G ($C_{16}H_{17}N_2O_4SNa$), 0.35638 kg mol^{-1}; monosodium ampicillin ($C_{16}H_{18}N_3O_4SNa$), 0.37139 kg mol^{-1}; monopotassium cephalosporin C ($C_{16}H_{20}N_3O_8SK$), 0.45351 kg mol^{-1}; acetic acid ($C_2H_4O_2$), 0.060053 kg mol^{-1}; H_3PO_4, 0.097995 kg mol^{-1}; KCl, 0.074551 kg mol^{-1}; K_2HPO_4, 0.17418 kg mol^{-1}; sodium acetate ($NaC_2H_3O_2$), 0.082034 kg mol^{-1}; and water, 0.0180153 kg mol^{-1}. The monosodium salt of penicillin G, the monosodium salt of ampicillin, the monopotassium salt of cephalosporin C, and β-lactamase were obtained [3] from Sigma; acetic acid, phosphoric acid, and KCl were from Baker; K_2HPO_4 was from Fisher; and sodium acetate was from Mallinckrodt. Moisture contents for the following substances were determined by Karl-Fischer analysis with resulting mass fractions: penicillin G, 0.0465; ampicillin, 0.0585; cephalosporin C, 0.0692. Moisture contents for the following salts were determined by drying in an oven at $T = 413$ K with resulting mass fractions: KCl, 0.00036; and K_2HPO_4, 0.0080. The moisture content of the sodium acetate was found to be 0.369 mass fraction by drying over P_2O_5. The purity of the penicillin G, ampicillin, and cephalosporin C were determined chromatographically (see below) with both a refractive index and an ultraviolet detector. Only one peak was observed in each case.

The reaction mixtures were analyzed with a Hewlett-Packard model 1090 high-pressure liquid-chromatograph (hplc), Zorbax C_{18} column thermostatted at $T = 311$ K, and an ultraviolet detector set at 220 nm. The mobile phase used for the analysis of the reaction mixtures was x volume percent of 0.02 mol dm^{-3} KH_2PO_4 at pH = 4.7 and y volume percent methanol. For the analysis of the (penicillin G + penicillinoic acid), x was 60 and y was 40; for the analysis of the (ampicillin + ampicillinoic acid), x was 70 and y was 30; and for the analysis of the cephalosporin C reaction mixture, x was 95 and y was 5. The flow rate was always 0.6 cm^3 min^{-1}. Typical retention times were 13 min for penicillin G and 5 min for penicillinoic acid; 10 min for ampicillin and 6 min for ampicillinoic acid; and 16 min for cephalosporin C. Solutions of penicillin G, ampicillin, and cephalosporin C were prepared daily for the determination of the re-

[3] Certain commercial materials and products are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology.

sponse factors of these substances. Solutions for the determination of the response factors of penicillinoic acid and ampicillinoic acid were also prepared daily by dissolving penicillin G and ampicillin, respectively, in 0.10 mol dm^{-3} NaOH, allowing ≈ 15 min for reaction, and then adjusting the final solution to pH = 7.0 with phosphoric acid. A problem encountered in the chromatography of this system was the continued activity of the β-lactamase in the guard column of the hplc This continued activity caused hydrolysis of penicillin G, ampicillin, and cephalosporin C in subsequent injections of these substances into the hplc. The remedy for this problem was to filter the enzyme from all solutions with Centricon concentrators (molar mass cutoff = 10 kg mol^{-1}) placed in an ultracentrifuge at 30000 rpm for ≈ 20 min prior to their injection into the hplc. The rotor of the ultracentrifuge was thermostatted at the temperature at which experiments had been performed. This procedure served both to remove the enzyme as a problem for these analyses with the hplc and to freeze the position of equilibrium of the solutions which were being analyzed.

Equilibrium measurements were performed by approaching equilibrium from both directions of reaction. Ten days were allowed for equilibration for both reactions (1) and (2). Reaction (2) was carried out at $T = 282.35$ K to minimize the thermal degradation of ampicillin which prevented an equilibrium measurement from being performed at higher temperatures. The identification of the ampicillin in the chromatogram was complicated by the presence of other peaks near to the peak attributable to ampicillin when the equilibrium was studied starting with ampicillinoic acid. Here, it was necessary to add a small amount of ampicillin to the reaction mixture following filtration of the reaction mixture with the Centricon concentrators. Comparison of the chromatogram of this "spiked" solution with the chromatogram of the reaction mixture (prior to addition of ampicillin) served to confirm the identification of the ampicillin peak. The products produced by the β-lactamase catalyzed hydrolysis of cephalosporin C have not yet been identified and we are therefore unable to report an apparent equilibrium constant.

The calorimeters were of the heat-conduction type. The sample vessels, which were fabricated from high-density polyethylene, contained two compartments holding approximately 0.55 cm^3 and 0.45 cm^3 of solution, respectively. The vessels and their contents were allowed to equilibrate in the microcalorimeters for ≈ 1 h before the solutions in the vessel were mixed. Calibration of the calorimeters was done electrically with a calibrated digital voltmeter, standard resistor, and time-interval counter. The reaction of aqueous tris(hydroxymethyl)aminomethane (Tris) with aqueous HCl (which is kept at a molality greater than that of the Tris) was used as a test reaction. The result of 31 measurements of this molar enthalpy of neutralization at $T = 298.15$ K was $\Delta_r H^\circ = -(47.475 \pm 0.058)$ kJ mol^{-1}. The uncertainty is equal to two standard deviations of the mean. This result is in excellent agreement with the result $\Delta_r H^\circ = -(47.48 \pm 0.03)$ kJ mol^{-1} reported by Öjelund and Wadsö [2]. Descriptions of the calorimeters and their performance characteristics, the data-acquisition system, and the computer programs used to treat the results are given in refs. [3,4].

Measurements of enthalpies of reaction were performed by mixing a substrate solution and an enzyme solution in the calorimeters. The substrate solution was prepared by dissolving a known amount of penicillin G or ampicillin in a buffer solution. The enzyme solution was prepared by adding the same buffer solution to the β-lactamase. The mass fractions of this enzyme in the calorimetric reaction mixtures was ≈ 0.0002. The stock enzyme solution and substrate solution were kept refrigerated at $T \approx 278$ K after preparation and were removed from the refrigerator for only a brief period of time (5 to 10 min) to load additional calorimeter reaction vessels. The extents of reaction for the reactions occurring in the calorimetric experiments were determined immediately following completion of the calorimetric measurements by chromatographic analyses of the solutions in the sample vessels. Only a small amount of unreacted penicillin G (less than 1.65 mole percent) was found for reaction (1) and less than 1.68 mole percent of unreacted ampicillin was found for reaction (2). The cephalo-

sporin C hydrolysis was essentially complete (less than 0.05 mole percent remaining). Appropriate corrections were applied for any unreacted substrate when calculating the molar enthalpies of reaction. The chromatography indicated that no side reactions occurred during the hydrolysis of either penicillin G or ampicillin. Calorimetric measurements were also performed on the β-lactamase catalyzed hydrolysis of cephalosporin C. However, the chromatograms of these reaction mixtures showed the presence of one major and two minor peaks having retention times ranging from 3 to 5 min. Since these peaks have not been identified, we are unable to assign the measured enthalpy of reaction to any specific reaction.

The calorimetric experiments typically lasted 20 to 30 min. The "blank" heats accompanying the mixing of the substrate solution and of the enzyme solution with the buffer were determined for each set of calorimetric conditions. These blank heats varied with the conditions of measurement and were −0.0039 to 0.0008 J for reaction (1), −0.0028 to 0.0024 J for reaction (2), and −0.0019 to 0.0014 J for the hydrolysis of cephalosporin C. The reproducibility of the blank heats for a particular set of conditions was in all cases within ±0.002 J. The measured enthalpies of reactions were −0.162 to −0.217 J for reaction (1), −0.111 to −0.217 J for reaction (2), and −0.116 to −0.164 J for the hydrolysis of cephalosporin C.

The measurement of the pH of the reaction mixtures was done with a combination glass micro-electrode and an Orion Model 811 pH meter. All measurements were done at the temperature at which the reactions occurred. Calibration was performed with a standard buffer prepared from potassium dihydrogen phosphate (0.009695 mol kg^{-1}) and disodium hydrogen phosphate (0.03043 mol kg^{-1}). These phosphates are standard reference materials 186-Id and 186-IId, respectively, from the National Institute of Standards and Technology. Intercomparisons of this "physiological" buffer against Fisher buffers certified at pH = (7.00, 8.00, and 9.00) was also done with satisfactory agreement (±0.03) in the pH of these solutions. The average of the differences between the pH of the substrate solutions in the calorimetric measurements prior to any reaction and the pH of the final reaction mixtures was 0.02 ± 0.03 for reaction (1), 0.00 ± 0.02 for reaction (2), and −(0.08 ± 0.05) for the hydrolysis of cephalosporin C. Thus, reactions (1) and (2) are considered to have occurred at essentially constant pH. The change in pH for the hydrolysis of cephalosporin C indicates a substantial release of $H^+(aq)$ as a part of the overall biochemical reaction.

Acidity constants of penicillin G and ampicillin were determined by titrating solutions of these substances with NaOH (0.106 mol kg^{-1}). A combination glass micro-electrode and Orion model 811 pH meter were used to measure the pH during the titrations. Two different molalities of HCl (0.0106 and 0.102 mol kg^{-1}) were used to initially dissolve the penicillin G and the ampicillin. The initial molalities of penicillin G and ampicillin were 0.0998 and 0.0943 mol kg^{-1}, respectively. The acidity constants of penicillinoic acid and ampicillinoic acid were determined by titration with HCl (0.102 mol kg^{-1}). The solutions of penicillinoic acid and ampicillinoic acid were prepared by addition of NaOH (0.106 mol kg^{-1}) to penicillin G and ampicillin, respectively, and allowing ≈ 15 min for the reactions to penicillinoic acid and ampicillinoic acid, respectively. The initial molalities of penicillinoic acid and ampicillinoic acid were 0.103 and 0.0978 mol kg^{-1}, respectively. Chromatographic analyses were also performed on the solutions used in these titrations. These analyses showed that both penicillin G and ampicillin were converted to penicillinoic acid and ampicillinoic acid, respectively, in alkaline solution. However, these hydrolysis reactions were not yet complete at the conclusion of the titrations which were started with penicillin G and ampicillin in acidic solution. The chromatograms also showed that only penicillinoic acid and ampicillinoic acid were present in solution following their respective titrations with HCl into the acidic region. The acidity constants were determined by solving the chemical equilibrium equations for the actual solution compositions corresponding to each result obtained from the titration. This calculation starts with assumed values of the acidity constants and yields a set of

calculated pHs. These calculated pHs are then compared with the measured pHs and the assumed acidity constants are then readjusted to yield a better fit to the measured pHs. In this way the acidity constants are determined as parameters of least-squares fits to the titration curves. In doing these calculations, the extended Debye–Hückel equation with the "ion-size" parameter set at 1.6 $kg^{1/2}$ $mol^{-1/2}$ was used to correct for non-ideality. The results obtained from these titrations at $T = 298.15$ K and at $I_m = 0$ are: for penicillin G, $pK^\circ = 2.80$; for penicillinoic acid, $pK_3^\circ = 5.54$; for ampicillin, $pK_1^\circ = 2.58$ and $pK_2^\circ = 7.29$; and for ampicillinoic acid, $pK_3^\circ = 4.76$ and $pK_4^\circ = 8.03$. The results obtained at $T = 292.55$ K and at $I_m = 0$ are: for penicillinoic acid, $pK^\circ = 5.62$; for ampicillin, $pK_2^\circ = 7.50$; and for ampicillinoic acid, $pK_3^\circ = 4.79$ and $pK_4^\circ = 8.13$. The results obtained at $T = 308.15$ K and at $I_m = 0$ are: for penicillinoic acid, $pK^\circ = 5.46$; for ampicillin, $pK_2^\circ = 7.04$; and for ampicillinoic acid, $pK_3^\circ = 4.55$ and $pK_4^\circ = 7.71$. Consideration of the uncertainties involved in the measurement of the pH, the stability of these substances, and the extrapolation to $I = 0$, leads us to believe that these pKs are reliable to $\approx \pm 0.10$. From the temperature dependencies of these acidity constants we calculate the following standard molar enthalpies of ionization: 16.9 ± 4.4 kJ mol^{-1} for Hpenicillinoic $acid^-$(aq); 49.1 ± 7.8 kJ mol^{-1} for Hampicillin^0(aq); 46 ± 15 kJ mol^{-1} for H_2ampicillinoic $acid^0$; and 27 ± 16 kJ mol^{-1} for Hampicillinoic $acid^-$(aq). These uncertainties are equal to two standard deviations of the mean.

3. Results and discussion

The apparent equilibrium constants K' for the biochemical reactions (1) and (2) are given by:

$$K'(1) = \frac{m(\text{penicillinoic acid})}{m(\text{penicillin G})}, \quad (3)$$

$$K'(2) = \frac{m(\text{ampicillinoic acid})}{m(\text{ampicillin})}. \quad (4)$$

Here, m(penicillin G) is the sum of the molalities of the various penicillin G species in solution; similar definitions hold for m(penicillinoic acid), m(ampicillinoic acid), and m(ampicillin) in equations (3) and (4). Since the reaction products of the β-lactamase catalyzed hydrolysis of cephalosporin C have not been characterized, it is not possible to assign a molar enthalpy to a given reaction or to formulate an apparent equilibrium constant at this time for the hydrolysis reaction(s) of cephalosporin C. Chemical reference reactions corresponding to the overall biochemical reactions (1) and (2) are:

$$\text{penicillin G}^-(\text{aq}) + H_2O(1) = \text{penicillinoic acid}^{2-}(\text{aq}) + H^+(\text{aq}), \quad (5)$$

$$\text{ampicillin}^-(\text{aq}) + H_2O(1) = \text{ampicillinoic acid}^{2-}(\text{aq}) + H^+(\text{aq}). \quad (6)$$

The standard equilibrium constants for reactions (5) and (6) are given in terms of the activities a of the reactants and products:

$$K^\circ(5) = \frac{a(\text{penicillinoic acid}^{2-})a(H^+)}{a(\text{penicillin G}^-)a(H_2O)}, \quad (7)$$

$$K^\circ(6) = \frac{a(\text{ampicillinoic acid}^{2-})a(H^+)}{a(\text{ampicillin G}^-)a(H_2O)}. \quad (8)$$

The thermodynamics of these reactions will be described with a model and computational procedure of the type previously used for the disproportionation reaction of adenosine 5′-diphosphate to adenosine 5′-triphosphate and adenosine 5′-monophosphate [5]. In this model, the equilibrium equations are solved to obtain the fractions of the various species in solution and the contributions of these species to the measured quantities (apparent equilibrium constants and calorimetric enthalpies of reaction) are calculated. The thermodynamic quantities which pertain to the reference reactions (5) and (6) above are then calculated as parameters in the model. The calculation is made self-consistent with respect to the ionic strength.

It is necessary to know the acidity constants ($pK = -\log_{10} K$) and standard molar enthalpies ($\Delta_r H^\circ$) of ionization of the various substances

participating in these reactions in order to calculate thermodynamic quantities for the reference reactions (5) and (6). Table 1 contains the results obtained in this study for these acidity constants as well as results from the literature. The standard molar enthalpies of ionization given in table 1 were calculated from acidity constants reported at several temperatures using the equation of Clarke and Glew [12] and an assumed standard molar heat-capacity change for the reaction ($\Delta_r C_p^\circ$) of 0. From the results of Hou and Poole [10] we calculate: $\Delta_r H^\circ(T = 298.15\ \text{K}, I_m = 0) = -(4.5 \pm 1.4)\ \text{kJ mol}^{-1}$ for the ionization of H_2ampicillin$^+$(aq) and $\Delta_r H^\circ(T = 298.15\ \text{K}, I_m = 0) = -(47.3 \pm 1.2)\ \text{kJ mol}^{-1}$ for the ionization of Hampicillin^0(aq). These uncertainties are equal to two standard deviations of the mean. The results of the various studies shown in table 1 were also adjusted to $I_m = 0$ with an extended Debye–Hückel equation [5] in which the "ion-size" parameter was set at 1.6 $\text{kg}^{1/2}\ \text{mol}^{-1/2}$. In two cases, the results had to be adjusted from $T = 310.15$ K to $T = 298.15$ K. The molar enthalpies of reaction calculated from the results of Hou and Poole [10] were used to do this. In a few cases, the acidity constants were obtained at $T = 295.15$ K and $T = 296.15$ K. Here, in the absence of the standard molar enthalpy of ionization, we neglected any correction for the temperature difference.

Hou and Poole [10] report that the isoelectric point of ampicillin(aq) is 4.95. This is consistent with the reported acidity constants of ampicillin and with the charges assigned to the various ampicillin species in table 1. A report [13] of an acidity constant for penicillin G of ≈ 9.0 neglected the fact that penicillin G hydrolyzes in alkaline solution to penicillinoic acid when the titration was performed and is therefore incor-

Table 1

Acidity constants and associated molar enthalpy changes for the ionizations of penicillin G, ampicillin, penicillinoic acid and ampicillinoic acid. The results obtained at the indicated ionic strengths I_m were adjusted with the extended Debye–Hückel equation to $I_m = 0$ and where necessary to $T = 298.15$ K to obtain the results given in column 4

T (K)	I_m (mol kg^{-1})	$\Delta_r H^\circ$ (kJ mol^{-1})	pK	pK°	Ref.
Hpenicillin G^0(aq) = penicillin G$^-$(aq) + H$^+$(aq)					
298.15	≈ 0.1		2.76	2.97	[6]
295.15	≈ 0.1		2.8	3.01	[7]
298.15	≈ 0.015		2.72	2.88	[8]
298.15	0.15		2.73	2.97	[9]
298.15	0		2.80	2.80	this study
H_3penicillinoic acid$^+$(aq) = H_2penicillinoic acid^0(aq) + H$^+$(aq)					
298.15	0.15		1.76	1.76	[9]
H_2penicillinoic acid^0(aq) = Hpenicillinoic acid$^-$(aq) + H$^+$(aq)					
296.15	≈ 0.1		2.95	3.16	[6]
298.15	0.15		2.30	2.54	[9]
Hpenicillinoic acid$^-$(aq) = penicillinoic acid^{2-}(aq) + H$^+$(aq)					
296.15	≈ 0.1		5.25	5.68	[6]
298.15	?		5.32	5.64	[8]
298.15	0.15		5.19	5.68	[9]
298.15	0		5.54	5.54	this study
298.15	0	16.9			this study
H_2ampicillin$^+$(aq) = Hampicillin^0(aq) + H$^+$(aq)					
298.15	≈ 0.012		2.53	2.48	[8]
298.15	0		2.66	2.66	[10]
310.15	0.5		2.67	2.64	[11]
298.15	0		2.58	2.58	this study
298.15	0	−4.5			[10]
Hampicillin^0(aq) = ampicillin$^-$(aq) + H$^+$(aq)					
298.15	≈ 0.012		7.24	7.20	[8]
298.15	0		7.24	7.24	[10]
310.15	0.5		6.95	7.62	[11]
298.15	0		7.29	7.29	this study
298.15	0	47.3			[10]
298.15	0	49.1	this study		
H_2ampicillinoic acid^0(aq) = Hampicillinoic acid$^-$(aq) + H$^+$(aq)					
298.15	0		4.76	4.76	this study
298.15	0	46			this study
Hampicillinoic acid$^-$(aq) = ampicillinoic acid^{2-}(aq) + H$^+$(aq)					
298.15	0		8.03	8.03	this study
298.15	0	27			this study

Table 2

Thermodynamic quantities at $T = 298.15$ K, $I_m = 0$ and $p = 0.1$ MPa for the reference reactions for the hydrolysis of penicillin G to penicillinoic acid and for ampicillin to ampicillinoic acid and the acidity constants of these substances. The acidity constants and related thermodynamic quantities for orthophosphate and acetic acid are also given

Reaction	K° or pK°	$\Delta_r H^\circ$ (kJ mol^{-1})	$\Delta_r S^\circ$ (J K^{-1} mol^{-1})	$\Delta_r C_p^\circ$ (J K^{-1} mol^{-1})
penicillin $G^-(aq) + H_2O(l) =$ penicillinoic $acid^{2-}(aq) + H^+(aq)$	$K^\circ = 9.4\times10^{-7}$	−73.7	−363	
ampicillin$^-(aq) + H_2O(l) =$ ampicillinoic $acid^{2-}(aq) + H^+(aq)$	$K^\circ = 6.0\times10^{-6}$	−70.0	−335	
Hpenicillin $G^0(aq) =$ penicillin $G^-(aq) + H^+(aq)$	$pK^\circ = 2.93$			
H_3penicillinoic $acid^+(aq) = H_2$penicillinoic $acid^0(aq) + H^+(aq)$	$pK^\circ = 1.76$			
H_2penicillinoic $acid^0(aq) =$ Hpenicillinoic $acid^-(aq) + H^+(aq)$	$pK^\circ = 2.85$			
Hpenicillinoic $acid^-(aq) =$ penicillinoic $acid^{2-}(aq) + H^+(aq)$	$pK^\circ = 5.64$	16.9	−51	
H_2ampicillin$^+(aq) =$ Hampicillin$^0(aq) + H^+(aq)$	$pK^\circ = 2.59$	−4.5	−65	
Hampicillin$^0(aq) =$ ampicillin$^-(aq) + H^+(aq)$	$pK^\circ = 7.24$	48	22	
H_4ampicillinoic $acid^{2+}(aq) = H_3$ampicillinoic $acid^+(aq) + H^+(aq)$	$pK^\circ < 3.0$			
H_3ampicillinoic $acid^+(aq) = H_2$ampicillinoic $acid^0(aq) + H^+(aq)$	$pK^\circ < 3.0$			
H_2ampicillinoic $acid^0(aq) =$ Hampicillinoic $acid^-(aq) + H^+(aq)$	$pK^\circ = 4.76$	27	−1	
Hampicillinoic $acid^-(aq) =$ ampicillinoic $acid^{2-}(aq) + H^+(aq)$	$pK^\circ = 8.03$	46	1	
$H_2PO_4^-(aq) = HPO_4^{2-}(aq) + H^+(aq)$	$pK^\circ = 7.21$	4.2	−124	−220
$CH_3COOH(aq) = CH_3COO^-(aq) + H^+(aq)$	$pK^\circ = 4.75$	−0.42	−92	−155

rect. Table 2 contains a summary of the thermodynamics of ionization of penicillin G, penicillinoic acid, ampicillin, and ampicillinoic acid. The quantities in this table are the averages of the respective quantities given in column 4 in table 1 with the exception of the second acidity constant of ampicillin where we have discarded the result obtained by Tsuji and Nakashima [11]. The standard entropies of ionization for the various penicillin G and ampicillin species having ionizations from an ammonium type group are all small. This is consistent with the behavior of other substituted ammonium ions (see table 1–6 in the review by Larson and Hepler [14]). The standard

Table 3

Results of equilibrium measurements for reactions (1) and (2). The molalities of the substrates (monosodium penicillin G is $C_{16}H_{17}N_2O_4SNa$, monosodium penicillinoic acid is $C_{16}H_{18}N_2O_5Na$, monosodium ampicillin is $C_{16}H_{18}N_3O_4SNa$ and monosodium ampicillinoic acid is $C_{16}H_{19}N_3O_5SNa$) given below are the averages of those determined for the final solutions. The ionic strength I_m and the standard equilibrium constants K° for the reference reactions (5) and (6) at $I_m = 0$ are calculated quantities. The equilibration times were 10 days for reactions (1) and (2). Three to four measurements were performed for each apparent equilibrium constant given in column 8. The uncertainties assigned to $K'(1)$ and $K'(2)$ are equal to two standard deviations of the mean

T (K)	pH	$m(C_{16}H_{17}N_2O_4SNa)$ (mol kg^{-1})	$m(C_{16}H_{18}N_2O_5Na)$ (mol kg^{-1})	$m(K_2HPO_4)$ (mol kg^{-1})	$m(H_3PO_4)$ (mol kg^{-1})	I_m (mol kg^{-1})	$K'(1)$	$K^\circ(5)$
reaction (1): penicillin G(aq) + $H_2O(l)$ = penicillinoic acid(aq)								
298.15 [a]	6.01	0.009678	0.01937	0.0986	0.04075	0.253	2.00±0.14	6.35×10^{-7}
298.15 [b]	6.00	0.005094	0.01941	0.0986	0.04075	0.247	3.81±0.06	1.24×10^{-6}
T (K)	pH	$m(C_{16}H_{18}N_3O_4SNa)$ (mol kg^{-1})	$m(C_{16}H_{19}N_3O_5SNa)$ (mol kg^{-1})	$m(NaC_2H_3O_2)$ (mol kg^{-1})	$m(C_2H_4O_2)$ (mol kg^{-1})	I_m (mol kg^{-1})	$K'(1)$	$K^\circ(6)$
reaction (2): ampicillin(aq) + $H_2O(l)$ = ampicillinoic acid(aq)								
282.35 [a]	5.53	0.000193	0.01874	0.1008	0.1001	0.157	97±8	6.45×10^{-6}
282.35 [b]	5.58	0.000145	0.01330	0.1008	0.1001	0.153	92±3	5.57×10^{-6}

[a] Reaction was carried out from forward direction.

[b] Reaction was carried out from reverse direction.

Table 4

Results of calorimetric measurements for reaction (1): penicillin G(aq) + H_2O(l) = penicillinoic acid(aq). The molality of the monosodium penicillin G ($C_{16}H_{17}N_2O_4SNa$) is that prior to any reaction. The pH is that of the final reaction mixture. The ionic strength I_m and the standard molar enthalpy of reaction $\Delta_r H^\circ$ at $I_m = 0$ for the reference reaction (5) are calculated quantities. Four to eight measurements were performed for each $\Delta_r H$(cal) result given in this table. The uncertainties given for $\Delta_r H$(cal) are 95 percent confidence limits. The uncertainties given for $\Delta_r H^\circ(5)$ were obtained by combining the random errors in $\Delta_r H$(cal) with estimates of possible systematic errors due to uncertainties in the quantities used in the equilibrium model used to calculate $\Delta_r H^\circ(5)$

T (K)	pH	$m(C_{16}H_{17}N_2O_4SNa)$ (mol kg^{-1})	$m(K_2HPO_4)$ (mol kg^{-1})	$m(H_3PO_4)$ (mol kg^{-1})	m(KCl) (mol kg^{-1})	I_m (mol kg^{-1})	$\Delta_r H$(cal) (kJ mol^{-1})	$\Delta_r H^\circ(5)$ (kJ mol^{-1})
298.15	6.01	0.003946	0.09380	0.06951	0.0000	0.297	−(79.45 ± 0.31)	−(73.44 ± 0.67)
298.15	6.98	0.003824	0.09673	0.01986	0.2001	0.478	−(77.11 ± 0.61)	−(72.73 ± 0.67)
298.15	7.00	0.003886	0.09655	0.02304	0.0000	0.279	−(78.35 ± 0.36)	−(73.95 ± 0.37)
298.15	7.01	0.003757	0.09694	0.01705	0.4997	0.782	−(78.89 ± 0.41)	−(74.55 ± 0.41)
298.15	7.51	0.004182	0.09739	0.00877	0.0000	0.296	−(77.66 ± 0.27)	−(73.40 ± 0.27)
304.65	6.53	0.004107	0.09681	0.04257	0.0000	0.253	−(77.08 ± 0.45)	−(73.75 ± 0.48)
310.15	6.53	0.004530	0.09681	0.04257	0.0000	0.255	−(76.5 ± 1.0)	−(74.41 ± 1.0)

Table 5

Results of calorimetric measurements for reaction (2): ampicillin(aq) + H_2O(l) = ampicillinoic acid(aq). The molality of the monosodium ampicillin ($C_{16}H_{18}N_3O_4SNa$) is that prior to any reaction. The pH is that of the final reaction mixture. The ionic strength I_m and the standard molar enthalpy of reaction $\Delta_r H^\circ$ at $I_m = 0$ for the reference reaction (6) are calculated quantities. Five to eight measurements were performed for each $\Delta_r H$(cal) result given in this table. The uncertainties given for $\Delta_r H$(cal) are 95 percent confidence limits. The uncertainties given for $\Delta_r H^\circ(6)$ were obtained by combining the random errors in $\Delta_r H$(cal) with estimates of possible systematic errors due to uncertainties in the quantities used in the equilibrium model used to calculate $\Delta_r H^\circ(6)$

T (K)	pH	$m(C_{16}H_{18}N_3O_4SNa)$ (mol kg^{-1})	$m(K_2HPO_4)$ (mol kg^{-1})	$m(H_3PO_4)$ (mol kg^{-1})	I_m (mol kg^{-1})	$\Delta_r H$(cal) (kJ mol^{-1})	$\Delta_r H^\circ(6)$ (kJ mol^{-1})
298.15	6.51	0.002589	0.09523	0.04535	0.241	−(76.42 ± 0.40)	−(67.4 ± 14)
298.15	7.06	0.003636	0.09757	0.02632	0.287	−(76.88 ± 0.28)	−(62.6 ± 12)
298.15	7.50	0.004411	0.09835	0.009465	0.296	−(83.18 ± 0.24)	−(68.1 ± 8.8)
298.15	7.96	0.003753	0.09868	0.002519	0.300	−(83.96 ± 0.14)	−(72.5 ± 4.9)
304.65	6.58	0.004162	0.09681	0.04256	0.254	−(78.04 ± 0.30)	−(67.1 ± 13)

Table 6

Results of calorimetric measurements for the hydrolysis of cephalosporin C(aq). The molality of the monopotassium cephalosporin C($C_{16}H_{20}N_3O_8SK$) is that prior to any reaction. The pH is that of the final reaction mixture. Five to nine measurements were performed for each result for $\Delta_r H$(cal) given in this table. The uncertainties given for $\Delta_r H$(cal) are 95 percent confidence limits. The reaction products have not been characterized and it is not possible to assign these results to a given reaction

T (K)	pH	$m(C_{16}H_{20}N_3O_8SK)$ (mol kg^{-1})	$m(K_2HPO_4)$ (mol kg^{-1})	$m(H_3PO_4)$ (mol kg^{-1})	$\Delta_r H$(cal) (kJ mol^{-1})
298.15	6.52	0.003184	0.09522	0.04534	−(61.77 ± 0.13)
298.15	6.93	0.003458	0.09669	0.02627	−(61.31 ± 0.38)
298.15	7.36	0.004162	0.09741	0.01055	−(62.66 ± 0.34)
298.15	7.78	0.003397	0.09778	0.02648	−(63.39 ± 0.34)
304.65	6.50	0.004042	0.09681	0.04256	−(62.05 ± 0.24)
310.15	6.52	0.003873	0.09686	0.04142	−(62.06 ± 0.30)

equilibrium constant, standard molar enthalpy change, and the standard molar heat-capacity change for the ionization of acetic acid were taken from the review of Larson and Hepler [14]. The standard equilibrium constant, standard molar enthalpy change, and standard molar entropy change $\Delta_r S^\circ$ for the ionizations of $H_2PO_4^-$(aq) were calculated from the standard formation properties given in the NBS tables [15]. The standard molar heat-capacity change for the ionization of $H_2PO_4^-$(aq) was calculated from the standard apparent molar heat capacities reported by Larson, Zeeb, and Hepler [16]. The phosphate ionizations at $pK^\circ = 2.15$ and $pK^\circ = 12.34$ [15] are neglected in the subsequent calculations as are the other ionizations that are far removed ($|pK - pH| > 2.0$) from the pH of the solutions used in this study.

Results of the equilibrium and calorimetric measurements are given in tables 3–6. In these tables, the calorimetrically determined molar enthalpy of reaction $\Delta_r H$(cal) is equal to the measured enthalpy of reaction divided by the amount of reaction. It has been shown [17] that

$$\Delta_r H(\text{cal}) = \Delta_r H'^\circ + \Delta_r N_H \, \Delta_r H^\circ(\text{buff}), \qquad (9)$$

where $\Delta_r H'^\circ$ is the standard transformed molar enthalpy of reaction, $\Delta_r N_H$ is the change in binding of H^+(aq) in the reaction, and $\Delta_r H^\circ$(buff) is the standard molar enthalpy of ionization of the buffer. The standard equilibrium constants and the standard enthalpies of reaction for the reference reactions (5) and (6), and the ionic strengths given in tables 3–5 were calculated with the equilibrium model and the auxiliary data on the acidity constants. The change in binding of H^+(aq) was also calculated and was used as an intermediate result in these calculations. The results for $K^\circ(5)$ and $K^\circ(6)$ obtained in this study were used for the calculation of $\Delta_r H^\circ(5)$ and $\Delta_r H^\circ(6)$. Since the equilibrium measurements for the hydrolysis of ampicillin were performed at $T = 282.35$ K, $\Delta_r H^\circ(6)$ was also needed for the calculation of $K^\circ(6)$. Here, the result for $\Delta_r H^\circ(6)$ obtained in this study was used and the final calculation of $K^\circ(6)$ and $\Delta_r H^\circ(6)$ were made self-consistent. From the equilibrium measurements, the averages of the results obtained from both directions of reaction are $K^\circ(5) = 9.4 \times 10^{-7}$ and $K^\circ(6) = 6.0 \times 10^{-6}$ at $T = 298.15$ K and $I = 0$. The averages of the results calculated from the calorimetric measurements are $\Delta_r H^\circ(5) = -73.7$ kJ mol^{-1} and $\Delta_r H^\circ(6) = -67.5$ kJ mol^{-1} at $T = 298.15$ K and $I = 0$. We now consider the errors associated with the treatment of the results.

The thermodynamic quantities for the ionizations of the penicillin G and ampicillin species (see table 2) and an estimated ion-size parameter in the extended Debye–Hückel equation were used in the calculations of the thermodynamic quantities (K° and $\Delta_r H^\circ$ at $T = 298.15$ K and $I = 0$) for the reference reactions (5) and (6). Any errors in the former quantities will affect the accuracy of the calculated quantities. Possible errors in the calculated quantities were estimated by assuming that: the pKs are reliable to ± 0.1; the enthalpies of ionization are uncertain by the amounts stated earlier in this paper (see section 2); and the ion-size parameter is uncertain by ± 0.3 kg$^{1/2}$ mol$^{-1/2}$. The errors in the acidity constants and the enthalpies of ionization of the buffers were assumed to be negligible. These assumed uncertainties were then individually used to perturb the parameters in the model. The effects of these individual perturbations were then combined in quadrature to obtain an estimate of how uncertain the calculated quantities are due to possible errors in the parameters in the model. In this way, $K^\circ(5)$ was found to be uncertain by $\pm 0.7 \times 10^{-7}$ and $K^\circ(6)$ by $\pm 3.0 \times 10^{-6}$. The results obtained for $\Delta_r H^\circ(5)$ from measurements at $T = 298.15$ K (see table 4) were found to be uncertain by $\pm(0.3$–$0.6)$ kJ mol^{-1}; the uncertainties in $\Delta_r H^\circ(5)$ were ± 0.45 kJ mol^{-1} and ± 1.0 kJ mol^{-1} for the results obtained from the measurements done at $T = 304.65$ K and $T = 310.15$ K, respectively. The results obtained for $\Delta_r H^\circ(6)$ (see table 5) were found to be uncertain by $\pm(5$–$14)$ kJ mol^{-1} with the smallest uncertainty being for the result obtained at $T = 298.15$ K and pH = 7.96. The uncertainties in the results for $\Delta_r H^\circ(6)$ are much larger than the uncertainties in the results for $\Delta_r H^\circ(5)$ primarily because the analysis of the results for reaction (2), the hydrolysis of ampicillin to ampicillinoic acid, involves the use of two acidity constants that fall in or near the

pH range in which the measurements were performed.

Reasonable assignments of random error for $K°(5)$ and $K°(6)$ at $T = 298.15$ K and $I = 0$ are $\pm 3.0 \times 10^{-7}$ and $\pm 0.5 \times 10^{-6}$, respectively. Combination of these uncertainties due to random error with the combined errors attributable to the uncertainties in the parameters used in the equilibrium model lead to the final set of results for the standard equilibrium constants at $T = 298.15$ K and $I = 0$: $K°(5) = (9.4 \pm 3.1) \times 10^{-7}$ and $K°(6) = (6.0 \pm 3.0) \times 10^{-6}$. We use weighted averages of the results (see the last columns in tables 4 and 5) for the standard enthalpies of the reference reactions at $T = 298.15$ K and $I = 0$ and obtain $\Delta_r H°(5) = -(73.7 \pm 0.4)$ kJ mol^{-1} and $\Delta_r H°(6) = -(70.0 \pm 7.5)$ kJ mol^{-1}. The standard molar Gibbs energies of reaction at $T = 298.15$ K and $I = 0$ are $\Delta_r G°(5) = (34.4 \pm 1.0)$ kJ mol^{-1} and $\Delta_r G°(6) = (29.8 \pm 1.7)$ kJ mol^{-1}. The standard entropy changes for the reference reactions are $\Delta_r S°(5) = -(363 \pm 4)$ J K^{-1} mol^{-1} and $\Delta_r S°(6) = -(335 \pm 26)$ J K^{-1} mol^{-1}. There is insufficient auxiliary data to calculate standard molar heat-capacity changes for the reference reactions.

Cephalosporin C is reported [18] to form acetic acid and a substance having a methylene group at C-3 of the aromatic ring and a cleaved β-lactam ring as in penicillinoic acid and ampicillinoic acid. This substance then decomposes further to products which do not appear to have been identified [19]. While the results cannot be attributed to a specific reaction, the calorimetric results given in table 6 are useful for thermal analytic applications (see refs. [1,20,21]) and they may become useful for thermodynamic calculations in the future after the products have been identified.

Bundgaard and Hansen [22] report that orthophosphate catalyzes the reaction of penicillin G to penicillinoic acid and the reaction of ampicillin to ampicillinoic acid and to a piperazine-2,5-dione derivative. Since, the HPO_4^{2-} ion is the most active species in this catalysis, these reactions will occur most rapidly at high pH where $m(HPO_4^{2-}) > m(H_2PO_4^-)$. Since the calorimetric measurements were complete after the substrate solutions had been at the specified temperature for ≈ 1.5 h, these phosphate catalyzed reactions are a potential systematic error in the calorimetric measurements. We now estimate this possible systematic error using the second-order rate constants for these reactions which have been reported by Finholt et al. [23] and by Yamana et al. [24] for the penicillin G reaction and by Hou and Poole [25] and by Bundgaard and Hansen [22] for the ampicillin reaction. Hou and Poole [25] also studied the kinetics of the ampicillin reaction as a function of temperature and we assume that the second-order rate constant for the penicillin G reaction has the same temperature dependency as the ampicillin reaction. We calculate that after 1.5 h in phosphate buffer, $m(\text{penicillin G})_{t=1.5\,h}/m(\text{penicillin G})_{t=0}$ is 0.996 at $T = 298.15$ K and pH = 7.51, 0.998 at $T = 304.65$ K and pH = 6.53, and 0.997 at $T = 310.15$ K and pH = 6.53. From a similar calculation for ampicillin, we find that $m(\text{ampicillin})_{t=1.5\ h}/m(\text{ampicillin})_{t=0}$ is 0.987 at ($T = 298.15$ K and pH = 7.96) and 0.993 at ($T = 304.65$ K and pH = 6.58). Since, these calculations pertain to the highest pHs used at each temperature, these represent worst case calculations for these phosphate catalyzed reactions. Since $1 - m(\text{penicillin G})_{t=1.5\ h}/m(\text{penicillin G})_{t=0}$ is either less than or approximately equal to the imprecision of the calorimetric measurements involving penicillin G, no correction is made for the phosphate catalyzed hydrolysis. While the quantity $1 - m(\text{ampicillin})_{t=1.5\ h}/m(\text{ampicillin})_{t=0}$ is greater than the imprecision of the ampicillin measurements performed at the highest pHs, it is not clear just how to correct for the formation of the piperazine-2,5-dione derivative which is also produced along with ampicillinoic acid. Consequently, we chose to make no corrections for the phosphate catalyzed hydrolysis of ampicillin and simply note that there may be a systematic error in the enthalpy measurements of ≈ 1 percent in $\Delta_r H(\text{cal})$ at $T = 298.15$ K and pH = 7.96 and at $T = 304.65$ K and pH = 6.58 for reaction (2). The systematic error in $\Delta_r H(\text{cal})$ due to phosphate catalyzed hydrolysis should be less than 0.4 percent for reaction (1). As was shown earlier, the treatment of the results to obtain standard molar enthalpy changes for the reference reactions (5) and (6) introduced larger

uncertainties than those caused by phosphate catalyzed hydrolysis.

Grime and Tan [1] report $\Delta_r H(\text{cal}) = -(114.7 \pm 0.6)$ kJ mol^{-1} for reaction (1) and $\Delta_r H(\text{cal}) = -(125.8 \pm 1.2)$ kJ mol^{-1} for reaction (2) at $T = 298.15$ K. The buffer was 0.2 mol dm^{-3} Tris adjusted to pH = 7.5 with HCl. Using our chemical equilibrium model with the auxiliary information on the ionizations (see table 2) and with $\Delta_r H°(T = 298.15\ \text{K}, I_m = 0) = 47.48$ kJ mol^{-1} for the ionization of TrisH$^+$(aq) from Öjelund and Wadsö [2], we calculate $\Delta_r H°(5) = -(69.3 \pm 0.6)$ kJ mol^{-1} and $\Delta_r H°(6) = -(80.4 \pm 8.0)$ kJ mol^{-1} at $T = 298.15$ K and $I_m = 0$ from the results of Grime and Tan [1]. The uncertainties given here were obtained in the same way as was used above for the analysis of the results from our experiments. These results obtained from the measurements of Grime and Tan [1] differ by 4.4 and 10.4 kJ mol^{-1} from the results obtained in the current study for the respective hydrolyses of penicillin G$^-$(aq) and ampicillin$^-$(aq). The larger difference of 10.4 kJ mol^{-1} is probably explicable in terms of the uncertainties in the thermodynamic quantities used in the model. The smaller difference of 4.4 kJ mol^{-1} is not explained by either the reported imprecisions of the measurements or by the uncertainties in the thermodynamic quantities in the model and it is therefore attributed to systematic errors in the measurements. For example, Grimes and Tan [1] did not determine the moisture contents of their samples. This alone could cause an error of ≈ 5 kJ mol^{-1} in $\Delta_r H(\text{cal})$ for reaction (1). This error would propagate directly to $\Delta_r H°(5)$ and could easily explain the differences in the results. We are unaware of any thermodynamic results in the literature that can be used to calculate formation properties for the biochemical substances used in this study or that can be used to confirm these results with a thermodynamic cycle calculation.

The results obtained in this study permit the calculation of the apparent equilibrium constant, the standard transformed Gibbs energy change ($\Delta_r G'°$) [26,27], and the standard transformed enthalpy change ($\Delta_r H'°$) under a wide variety of conditions. Thus, at $T = 298.15$ K, pH = 7.0, and $I_m = 0.25$ mol kg^{-1} we calculate: $K'(1) = 25$, $\Delta_r G'°(1) = -8.0$ kJ mol^{-1}, $\Delta_r H'°(1) = -78.1$ kJ mol^{-1}, $K'(2) = 354$, $\Delta_r G'°(2) = -14.5$ kJ mol^{-1}, and $\Delta_r H'°(2) = -79.8$ kJ mol^{-1}. At $T = 310.15$ K, pH = 7.0, and $I_m = 0.25$ mol kg^{-1}, we calculate: $K'(1) = 8.2$, $\Delta_r G'°(1) = -5.4$ kJ mol^{-1}, $\Delta_r H'°(1) = -75.4$ kJ mol^{-1}, $K'(2) = 1000$, $\Delta_r G'°(2) = -17.8$ kJ mol^{-1}, and $\Delta_r H'°(2) = -80.6$ kJ mol^{-1}. The results obtained in this study show that the β-lactamase catalyzed hydrolyses of penicillin G and ampicillin proceed very largely in the direction of the formation of their respective products.

The reactions studied herein are characterized by the breaking of a β-lactam ring. The strain energy of this ring, referred to as a ring strain correction herein, is a quantity which can be obtained by comparison of the enthalpy changes for the following two reactions:

$$\text{N,N-dimethylacetamide} + \text{H}_2\text{O(1)} = \text{acetic acid(aq)} + \text{dimethylamine(aq)}, \quad (10)$$

$$\text{penicillin G(aq)} + \text{H}_2\text{O(1)} = \text{penicillinoic acid(aq)}. \quad (11)$$

Since the principle difference between reactions (10) and (11) is the breaking of the β-lactam ring in reaction (11), the difference in the enthalpy changes of these two reactions will yield the strain correction to be associated with the β-lactam ring. The enthalpy change for reaction (10) is obtained from the enthalpies of formation of the reactants and products. Specifically, the enthalpies of formation $\Delta_f H°$ for acetic acid(aq) and for $H_2O(1)$ are taken from the NBS tables [15] and the enthalpies of formation of *N,N*-dimethylacetamide(aq) and dimethylamine(aq) are taken from the paper by Guthrie [28]. The enthalpy change for reaction (11) is calculated from the results given in table 2 in this paper. Here, we have used the acidity constants with estimated entropy changes of zero to estimate the enthalpy changes for two of the ionizations needed for this calculation. Thus, at $T = 298.15$ K, $\Delta_r H° = 26$ kJ mol^{-1} for reaction (10), $\Delta_r H° = -90$ kJ mol^{-1} for reaction (11), and the ring strain correction of the β-lactam ring is 116 kJ mol^{-1}. In 1949, Woodward et al. [6] estimated this quantity to be

≈ 100 kJ mol^{-1} based upon some approximate enthalpies of combustion. More recently, Greenberg [29] arrived at an estimate of 127 kJ mol^{-1} for this ring strain correction. An approximate estimate can also be obtained from Domalski's tabulated ring strain corrections for organic substances [30]. Thus, for cyclobutane(g) the ring strain correction is 110.9 kJ mol^{-1} and for cyclobutene(g) the ring strain correction is 125.8 kJ mol^{-1}. Although these ring strain corrections pertain to the gas phase and to rings that differ somewhat from the β-lactam ring, a result in the range 111 to 126 kJ mol^{-1} seems reasonable for the β-lactam ring based upon Domalski's tabulation. Thus, the result of 116 kJ mol^{-1} for the ring strain correction for the β-lactam ring based upon reactions (10) and (11) is in reasonable accord with these other estimates of this quantity. For purposes of estimating enthalpy changes for biochemical reactions, this result is preferable to the earlier estimates made for the ring strain correction since it is based upon results that pertain to aqueous solutions. Since the entropy changes for reactions and (6) are quite negative and since it is ring strain that makes the principle contribution to the enthalpy changes, the Gibbs energy changes for these β-lactam hydrolysis reactions are negative primarily due to strain in the β-lactam ring.

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