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Thermodynamics of the hydrolysis of 3,4,5-trihydroxybenzoic acid propyl ester (*n*-propylgallate) to 3,4,5-trihydroxybenzoic acid (gallic acid) and propan-1-ol in aqueous media and in toluene

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Equilibrium measurements at several temperatures between 293 K and 308 K have been performed on the tannase catalyzed reaction: 3,4,5-trihydroxybenzoic acid propyl ester(sln) + H_2O(sln) = 3,4,5-trihydroxybenzoic acid(sln) + propan-1-ol(sln), where sln = aqueous phosphate buffer, aqueous acetate buffer, and toluene. The change in binding of the hydrogen ion $\Delta_r N(H^+)$ for this biochemical reaction in aqueous solution was calculated both from an equilibrium model for the biochemical reaction and from the dependence of the apparent equilibrium constant on pH. Calorimetric measurements were also performed for this biochemical reaction in aqueous phosphate and 2-(*N*-morpholino)ethanesulfonic acid (MES) buffers. Standard transformed thermodynamic quantities for the overall biochemical reaction as well as standard thermodynamic quantities for chemical reference reactions that involve specific chemical species have been calculated from the experimental results. It was found that the equilibrium yield of 3,4,5-trihydroxybenzoic acid propyl ester is significantly enhanced by carrying out the reaction in toluene rather than in the aqueous buffered solutions. The standard molar enthalpy change $\Delta_r H_m^\circ$ for the dissociation reaction $MES^{\pm}(aq) = MES^-(aq) + H^+(aq)$ has been measured calorimetrically and is $-(15.0 \pm 0.1)\,kJ\cdot mol^{-1}$ at the temperature T = 298.15 K and ionic strength $I = 0$.

1. Introduction

Tannase (Enzyme Commission number 3.1.1.20)[(1)] catalyzes the hydrolysis of 3,4,5-trihydroxybenzoic acid propyl ester to 3,4,5-trihydroxybenzoic acid and propan-1-ol. The 3,4,5-trihydroxybenzoic acid propyl ester and 3,4,5-trihydroxybenzoic acid will be referred to, respectively, as *n*-propylgallate and gallic acid (see figure 1 for the structures of these two substances). In aqueous

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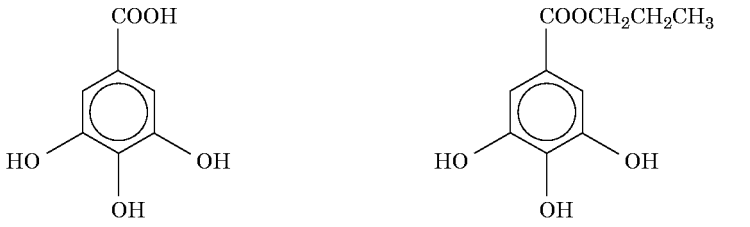

3,4,5-trihydroxybenzoic acid (gallic acid)

3,4,5-trihydroxybenzoic acid propyl ester (*n*-propylgallate)

FIGURE 1. Structures of gallic acid and *n*-propylgallate.

solution, the direction of reaction is predominantly toward the formation of gallic acid and propan-1-ol. However, when the reaction is carried out in a non-aqueous solvent, the direction of reaction has been reported[2] to be toward the synthesis of *n*-propylgallate. This finding is potentially valuable for the synthesis of *n*-propylgallate, an antioxidant useful in the food industry. Thus, there is a practical importance in having a quantitative understanding of the position of equilibrium of this reaction and how it can change with solvent. Because of this, and to gain a further understanding of the thermodynamics of biochemical reactions in non-aqueous media,[3–8] we have undertaken an investigation in which equilibrium constants for this reaction were measured both in aqueous media and in toluene, an organic solvent that has a relative permittivity much less than that of water. Calorimetry was also used to measure the molar enthalpy change for this reaction in aqueous media.

Central to the presentation and analysis of the results in this study is the distinction between the usual chemical reaction, that necessarily balances both numbers of elements and charges, and what is designated herein as an (overall) biochemical reaction. In the biochemical reaction, the molalities or concentrations of certain elements are fixed (e.g. constant pH) and the numbers of these elements are not balanced when writing the reaction. The equilibrium constant K for a chemical reaction contains the appropriate ratio of the molalities of chemical species. However, the apparent equilibrium constant K' for a biochemical reaction consists of the appropriate ratio of the molalities of biochemical reactants. These latter molalities are equal to the sums of the molalities of the pseudoisomer species that comprise a reactant. For example, the gallate ion and the neutral gallic acid species are pseudoisomers when the pH is specified. Analogous to the usual standard molar Gibbs free energy change $\Delta_r G_m^\circ$ is the standard molar transformed Gibbs free energy change, $\Delta_r G_m'^\circ = -RT \ln K'$, where R is the gas constant and T is the thermodynamic temperature. Other transformed thermodynamic quantities ($\Delta_r H_m'^\circ$, $\Delta_r S_m'^\circ$, $\Delta_r C_{p,m}'^\circ$) can also be used for biochemical reactions. These matters and recommendations for nomenclature and for reporting experimental results in biochemical thermodynamics are dealt with in recent IUPAC-IUBMB recommendations.[9]

2. Experimental

Pertinent information on the principal substances used in this study is given in table 1. Tannase (CAS registry number 9000-92-4), in the form of a powder, was obtained from Enzeco. The gallic acid and *n*-propylgallate were found to be "pure" on the basis of the chromatographic procedure described below. The vendor stated that, based upon gas chromatographic (g.c.) analysis with a flame-ionization detector, the mole fraction purity of the propan-1-ol was >0.999. A coulometrically analyzed[10] aqueous solution of HCl {molality $m = (0.050002 \pm 0.000007)\ \mathrm{mol \cdot kg^{-1}}$, molar mass $M = 0.036461\ \mathrm{kg \cdot mol^{-1}}$} was provided by Dr Kenneth Pratt of the National Institute of Standards and Technology. All uncertainties given in this paper are, unless indicated otherwise, based on two estimated standard deviations of the mean. Standardized NaOH(aq) {concentration $c = (2.00 \pm 0.02)\ \mathrm{mol \cdot dm^{-3}}$, $M = 0.039997\ \mathrm{kg \cdot mol^{-1}}$} was obtained from Fisher Scientific; the vendor stated that, based upon turbidity measurements, the mole fraction $n'(\mathrm{carbonate})/n(\mathrm{NaOH})$ in this solution was <0.01, where $n'(\mathrm{carbonate})$ is $\{n(\mathrm{CO_3^{2-}}) + n(\mathrm{HCO_3^-}) + n(\mathrm{CO_2})\}$. The sample of 2-(*N*-morpholino)ethanesulfonic acid, designated herein as MES, was stated by the vendor to have a mole fraction purity of 0.995 based upon titration with NaOH.

The mass fractions of water in the reagents and in the toluene solutions were measured with a Metrohm Model 633 automatic Karl Fischer titrator as previously described.[8] The molalities of *n*-propylgallate and gallic acid were determined with a Hewlett-Packard 1090 high-performance liquid chromatograph (h.p.l.c.) equipped

TABLE 1. Chemical Abstracts Services (CAS) registry numbers, empirical formulas, molar masses M, supplier, and mass fraction w moisture contents obtained by Karl Fischer analysis for the principal substances used in this study

Substance	CAS registry number	Formula	M / kg·mol^{-1}	Supplier[a,b]	w[c,d]
Gallic acid	149-91-7	$C_7H_6O_5$	0.170122	S	0.316 ± 0.010
n-Propylgallate	121-79-9	$C_{10}H_{12}O_5$	0.212202	S	0.134 ± 0.007
Propan-1-ol	71-23-8	C_3H_8O	0.060096	M	0.0044 ± 0.002
Toluene	108-88-3	C_7H_8	0.092141	M	—
Dipotassium hydrogen phosphate	7758-11-4	K_2HPO_4	0.174176	S	—
Phosphoric acid	7664-38-2	H_3PO_4	0.097995	B	—
Sodium acetate trihydrate	6131-90-4	$NaC_2H_3O_2 \cdot 3H_2O$	0.136080	B	—
Acetic acid	64-19-7	$C_2H_4O_2$	0.060053	M	—
MES[e] monohydrate	145224-94-8	$C_6H_{13}NO_4S \cdot H_2O$	0.213255	S	0.084 ± 0.005

[a] 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.

[b] B = Baker, F = Fisher, M = Mallinckrodt, S = Sigma.

[c] All uncertainties given in this paper are, unless indicated otherwise, based on two estimated standard deviations of the mean.

[d] The mass fraction moisture contents of toluene, dipotassium hydrogen phosphate, phosphoric acid, sodium acetate trihydrate, and acetic acid were not measured.

[e] MES = 2-(*N*-morpholino)ethanesulfonic acid.

with a u.v. detector set at the wavelength 254 nm and a Zorbax C_{18} column (internal diameter 4.6 mm, length 254 mm) thermostatted at $T = 311$ K. The mobile phase was a gradient of phosphate buffer $\{KH_2PO_4$ ($c = 0.02$ mol·dm^{-3}, pH = 4.65)$\}$ and methanol. At time $t = 0$, the gradient was pure phosphate buffer, and at $t = 20$ min, the gradient was pure methanol. The flow rate of the mobile phase was 0.0117 cm^3·s^{-1}. Typical retention times of gallic acid and *n*-propylgallate were 7.1 min and 17.7 min, respectively. The column was flushed with water for ≈ 5 min and was then conditioned with the phosphate buffer prior to the next injection of sample into the h.p.l.c. The molality of propan-1-ol in the toluene solution was measured with a Varian 6000 g.c. as previously described;[8] butan-1-ol was used as the internal standard.

The procedure used for immobilization of the tannase on controlled-pore glass beads was identical to the method described earlier[8] for the immobilization of α-chymotrypsin. When not in use, the immobilized tannase was stored in phosphate buffer $\{c(K_2HPO_4) = 0.1$ mol·dm^{-3}, adjusted to pH = 7.2 with $H_3PO_4\}$ at $T = 277$ K.

Equilibrium measurements were carried out by approaching equilibrium from both directions of reaction under very similar conditions. For the reaction in toluene, the substrates (*n*-propylgallate for the forward direction and gallic acid for the reverse direction) were first dissolved in ≈ 2.0 cm^3 of propan-1-ol. A volume of approximately 30 cm^3 of toluene saturated with water was then added to this solution followed by ≈ 2.5 g of immobilized tannase. The flasks containing these solutions were then placed in a shaker bath and allowed to equilibrate for 6 d to 7 d. Samples of the toluene phase were withdrawn and analyzed for the molalities of the reactants. Care was required in withdrawing samples of the toluene phase since water is bound to the immobilized enzyme and the glass beads. Thus, the reaction was, of necessity, carried out in a two phase system. The molalities of gallic acid and *n*-propylgallate in the toluene phase were measured with the h.p.l.c. as described above. The respective molalities of water and of propan-1-ol in the toluene phase were measured with Karl Fischer titration and with the g.c. method described above.

For the equilibrium measurements in aqueous solutions, the tannase was dissolved in either acetate or phosphate buffer and then added to the reaction mixtures containing the substrates dissolved in the same buffers. After an equilibration for 24 h, the molalities of *n*-propylgallate and gallic acid in the reaction mixtures were determined with the h.p.l.c. The molalities of propan-1-ol in the reaction mixtures at equilibrium were calculated from the known molalities of propan-1-ol initially present in the reaction mixtures and the extent of reactions obtained from the measured molalities of *n*-propylgallate and gallic acid. It should be noted that the rate of the reaction in water was much more rapid than the reaction in toluene; the former required 24 h while the latter required 6 d to 7 d to reach equilibrium.

The microcalorimeters were of the heat-conduction type. The sample vessels, fabricated from high-density polyethylene, contained two compartments that hold ≈ 0.55 cm^3 and ≈ 0.45 cm^3 of solution. In these experiments, the substrate solution (placed in the 0.55 cm^3 compartment) consisted of *n*-propylgallate dissolved in either phosphate (dipotassium hydrogen phosphate + phosphoric acid) or (MES + sodium hydroxide) buffers containing propan-1-ol which was needed to dissolve the

n-propylgallate. The enzyme solution (placed in the 0.45 cm^3 compartment) consisted of tannase dissolved in the same (buffer + propan-1-ol) solution used for the substrate solution. The vessels and their contents were allowed to equilibrate in the calorimeters for 1 h before the solutions were mixed. Following reaction in the calorimeter for $\approx$90 min (the time required for a nearly complete hydrolysis of the *n*-propylgallate), the reaction vessels were removed from the calorimeters and their contents were analyzed with the h.p.l.c. It was found that the mole fraction of unreacted *n*-propylgallate ranged from 0.013 to 0.028. Appropriate corrections were applied. The averages of the "blank" enthalpy changes accompanying the mixing of the substrate solution and the enzyme solution with the phosphate buffer and the MES buffer were, respectively, $-(0.45 \pm 0.43)$ mJ and $-(0.94 \pm 0.98)$ mJ. Typically, the measured enthalpy changes were approximately -41 mJ and -100 mJ for the reactions carried out in the phosphate and the MES buffers, respectively. The "blank" enthalpies were applied as corrections to the measured enthalpies of reaction. There was a change in the pH of the reaction mixture as a consequence of the reaction. Thus, for the biochemical reactions in the phosphate and MES buffers {pH(final) − pH(initial)} was -0.22 and -0.08, respectively. The negative signs indicate that protons were liberated as a consequence of the biochemical reaction.

The molar enthalpy of protonation of MES was determined in calorimetric experiments in which coulometrically analyzed HCl(aq) was reacted with {MES + sodium hydroxide}(aq). The HCl was the limiting reactant in these measurements. The "blank" enthalpies (in this case, the mixing of a solution with itself in a calorimeter) were (0.38 ± 0.9) mJ and $-(0.07 \pm 0.22)$ mJ for the mixing of HCl(aq) and the mixing of the (MES + sodium hydroxide)(aq), respectively. The measured enthalpies of reaction were $\approx$0.35 J.

The calorimeters were calibrated electrically with a calibrated digital voltmeter, standard resistor, and time-interval counter. Descriptions of the calorimeters and their performance characteristics, the data-acquisition system, and the computer programs used to treat the results are given in references 11 and 12.

The pHs of the aqueous reaction mixtures were measured with a combination glass micro-electrode and an Orion Model 811 pH meter. All pH measurements were done at the temperature at which the reactions occurred. Calibration of the pH meter was performed with a standard buffer prepared from KH_2PO_4 ($m = 0.009695$ mol·kg^{-1}) and Na_2HPO_4 ($m = 0.03043$ 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 agreement to within $\pm$0.03.

3. Results and discussion

The thermodynamic formalism used herein parallels that used in our earlier study.[8] Thus, the reaction in toluene is

$$n\text{-propylgallate(sln)} + H_2O\text{(sln)} = \text{gallic acid(sln)} + \text{propan-1-ol(sln)}, \qquad (1)$$

where sln denotes the organic solvent toluene. The equilibrium constant K for reaction (1) is

$$K = m(\text{gallic acid})\cdot m(\text{propan-1-ol})/m(n\text{-propylgallate})\cdot m(H_2O), \quad (2)$$

where m is molality. Since this equilibrium constant is symmetrical, its value is independent of the choice of standard state and the measure of composition used for the solutes. Here it is assumed that each of the reactants exists in only one form, i.e. none of the substances dissociates in solution. This assumption seems reasonable since the relative permittivity of toluene is 2.38,[13] substantially less than water. In discussing the thermodynamics of the hydrolysis reaction in water, one needs to recognize that the reactants may exist in several ionic forms. Specifically, for the pH range used in this study ($5.0 \leqslant \text{pH} \leqslant 6.18$), gallic acid exists both as the gallate ion ($C_7H_5O_5^-$) and as non-ionized gallic acid ($C_7H_6O_5$) in solution. Thus, the overall biochemical reaction in water is written as

$$n\text{-propylgallate(aq)} + H_2O(l) = \text{total gallic acid(aq)} + \text{propan-1-ol(aq)}. \quad (3)$$

The apparent equilibrium constant K'_m for reaction (3) is

$$K'_m = m(\text{total gallic acid})\cdot m(\text{propan-1-ol})/m(n\text{-propylgallate})\cdot m^\circ. \quad (4)$$

The quantity $m^\circ = 1\,\text{mol}\cdot\text{kg}^{-1}$ is used in equation (4) to make the apparent equilibrium constant dimensionless. The molalities in equation (4) are the total molalities of the various charged and uncharged species that are formed from the dissociation of the various substances in solution. Since the only dissociation that is of concern here is that of gallic acid, the word total has been placed in front of it in reaction (3) to avoid confusion with reaction (1). Equation (4) also uses the convention that the activity of water $a_w = 1$. The subscript m in K'_m indicates that the apparent equilibrium constant K'_m was calculated on a molality basis. Since the value of the apparent equilibrium constant depends on the choice of standard state, if concentration c is used instead of molality in equation (4), K'_c will differ slightly ($< 0.01\cdot K'_m$) for dilute aqueous solutions at $T = 298.15$ K.

The results for the equilibrium constants for reaction (1) in toluene and for the apparent equilibrium constants for the overall biochemical reaction (3) in water are given in tables 2 and 3, respectively. The results for the calorimetrically determined molar enthalpy $\Delta_r H_m$(cal) for the hydrolysis reaction of *n*-propylgallate in aqueous phosphate buffer and in aqueous MES buffer are given in tables 4 and 5, respectively. Results of calorimetric measurements for the reaction of HCl(aq) with (MES + sodium hydroxide)(aq) are given in table 6. The latter results were obtained to insure that an accurate value for the standard molar enthalpy of protonation of the MES buffer would be available for use in the calculations used to analyze further the results given in table 5.

From the temperature dependence of the equilibrium constants given in table 2 we calculate $\Delta_r H^\circ_m = -(15.4 \pm 6.6)\,\text{kJ}\cdot\text{mol}^{-1}$ for reaction (1) in toluene at $T = 298.15$ K. In this calculation, it was assumed that the standard molar heat-capacity change $\Delta_r C^\circ_{p,m} = 0$. With this result and the equilibrium results in table 2

we obtain: $K = (0.0130 \pm 0.0023)$, $\Delta_r G^\circ_m = (10.8 \pm 0.5)\ \mathrm{kJ \cdot mol^{-1}}$, and $\Delta_r S^\circ_m = -(88 \pm 22)\ \mathrm{J \cdot K^{-1} \cdot mol^{-1}}$ at $T = 298.15$ K.

The treatment of the results follows a plan that is now outlined. First, the pertinent thermodynamic quantities (K, $\Delta_r H^\circ_m$, and, in some cases, $\Delta_r C^\circ_{p,m}$) for the acid dissociation reactions of the reactants are obtained both from the literature and from some calorimetric measurements performed as a part of this study. Then, from the measured apparent equilibrium constants for the biochemical reaction (3) and calorimetrically determined molar enthalpies of reaction (see tables 4 and 5), thermodynamic quantities (K, $\Delta_r G^\circ_m$, $\Delta_r H^\circ_m$, and $\Delta_r S^\circ_m$) are calculated for a reference reaction that involves specific species. Then, thermodynamic quantities for the reference reaction in water and in toluene are adjusted to a common basis and standard state to make a comparison of the thermodynamic quantities for the reference reaction(s). Also, information on the change in binding of the hydrogen ion $\Delta_r N(H^+)$ for the biochemical reaction (3) in aqueous solution is calculated both from an equilibrium model for the biochemical reaction and from the dependence of the apparent equilibrium constant on pH. The information obtained in this study also allows us to calculate standard transformed thermodynamic quantities as well as the equilibrium yield of *n*-propylgallate that can be obtained from reaction (3) in aqueous media and from reaction (1) in toluene.

Information on the dissociation constants of the reactants is needed to relate the results for the biochemical reaction in water, which were obtained in terms of an apparent equilibrium constant involving sums of molalities of species, to a chemical reaction involving specific chemical species. A search of compilations of dissociation constants[14–18] yielded 18 references that contain original results for the dissociation constants of gallic acid(aq) ($C_7H_6O_5$) to gallate$^-$(aq) ($C_7H_5O_5^-$) and H^+(aq). From these compilations, we find the following ranges of values for the pKs (p$K = -\lg K$) for the respective dissociations of gallic acid: $4.1 \leqslant pK_1 \leqslant 4.4$, $8.5 \leqslant pK_2 \leqslant 9.1$, and $11.1 \leqslant pK_3 \leqslant 11.5$ at $T = 298.15$ K and for $0 \leqslant I_m \leqslant 0.5\ \mathrm{mol \cdot kg^{-1}}$. Only the studies

TABLE 2. Results of equilibrium measurements for reaction (1) in toluene (denoted as sln): *n*-propylgallate(sln) + H_2O(sln) = gallic acid(sln) + propan-1-ol(sln). The molalities of the reactants in solution at equilibrium are given in columns 3 to 6. $C_7H_6O_5$ is gallic acid, $C_{10}H_{12}O_5$ is *n*-propylgallate, and C_3H_8O is propan-1-ol. The number of measurements were: three to five for $m(C_7H_6O_5)$ and $m(C_{10}H_{12}O_5)$; two to three for $m(H_2O)$; and six for $m(C_3H_8O)$. The values of the equilibrium constant K (column 7) were calculated with equation (2). The values and uncertainties given for K (combined) were calculated as a weighted average of the values of K obtained from both directions of reaction

$\frac{T}{\mathrm{K}}$	Direction of reaction	$\frac{m(C_7H_6O_5)\cdot 10^4}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{m(C_{10}H_{12}O_5)\cdot 10^2}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{m(H_2O)}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{m(C_3H_8O)}{\mathrm{mol \cdot kg^{-1}}}$	$K\cdot 10^2$	K (combined)$\cdot 10^2$
293.25	forward	1.95 ± 0.08	1.37 ± 0.05	0.142 ± 0.003	0.131 ± 0.002	1.31 ± 0.15	1.28 ± 0.19
293.25	reverse	2.26 ± 0.07	1.49 ± 0.03	0.154 ± 0.006	0.128 ± 0.001	1.26 ± 0.12	
298.25	forward	2.49 ± 0.08	1.60 ± 0.04	0.142 ± 0.007	0.125 ± 0.001	1.37 ± 0.16	1.30 ± 0.23
298.25	reverse	1.87 ± 0.11	1.34 ± 0.03	0.145 ± 0.006	0.128 ± 0.001	1.23 ± 0.16	
303.15	forward	2.15 ± 0.10	1.70 ± 0.04	0.158 ± 0.006	0.135 ± 0.001	1.08 ± 0.12	1.12 ± 0.17
303.15	reverse	2.16 ± 0.07	1.61 ± 0.02	0.158 ± 0.007	0.137 ± 0.002	1.16 ± 0.12	
308.15	forward	1.77 ± 0.01	1.32 ± 0.02	0.148 ± 0.005	0.109 ± 0.002	0.99 ± 0.07	0.98 ± 0.13
308.15	reverse	2.21 ± 0.10	1.59 ± 0.08	0.159 ± 0.007	0.106 ± 0.001	0.93 ± 0.14	

TABLE 3. Results of equilibrium measurements for the biochemical reaction (3) in water: *n*-propylgallate(aq) + H_2O(aq) = total gallic acid(aq) + propan-1-ol(aq). The molalities of the reactants in solution at equilibrium are given in columns 11 to 13. $C_7H_6O_5$ is gallic acid, $C_{10}H_{12}O_5$ is *n*-propylgallate, and C_3H_8O is propan-1-ol. Three and four measurements of the respective molalities of gallic acid and *n*-propylgallate were performed. The molalities of propan-1-ol were calculated from the known initial molalities of propan-1-ol with corrections for the molalities of propan-1-ol produced or consumed as a consequence of the reaction. The molalities of the acetate and phosphate buffer salts and acids are given in columns 4 to 7. All molalities are equal to the sums of the molalities of the indicated substances in their various ionic forms. The mass fraction of the tannase in solution was ≈ 0.0015. The apparent equilibrium constants K'_m were calculated with equation (4). The ionic strength I_m and the equilibrium constant K_m for the reference reaction (6) at the indicated temperature and at $I = 0$ were calculated

$\frac{T}{K}$	Direction of reaction	pH	$\frac{m(NaC_2H_3O_2)}{mol \cdot kg^{-1}}$	$\frac{m(C_2H_4O_2)}{mol \cdot kg^{-1}}$	$\frac{m(K_2HPO_4)}{mol \cdot kg^{-1}}$	$\frac{m(H_3PO_4)}{mol \cdot kg^{-1}}$
293.15	forward	5.33	0.0995	0.0814	0	0
293.15	reverse	5.31	0.0997	0.0815	0	0
298.15	forward	4.99	0.0989	0.0335	0	0
298.15	reverse	5.00	0.0992	0.0336	0	0
298.15	forward	5.32	0.0991	0.0811	0	0
298.15	reverse	5.36	0.1000	0.0818	0	0
298.15	forward	5.38	0.0998	0.0816	0	0
298.15	reverse	5.39	0.0998	0.0816	0	0
298.15	forward	5.91	0	0	0.101	0.0564
298.15	reverse	5.91	0	0	0.102	0.0588
298.15	forward	6.56	0	0	0.102	0.0240
298.15	reverse	6.56	0	0	0.102	0.0240
303.15	forward	5.37	0.0998	0.0817	0	0
303.15	reverse	5.35	0.0998	0.0817	0	0
308.15	forward	5.32	0.0998	0.0816	0	0
308.15	reverse	5.34	0.0999	0.0817	0	0

$\frac{T}{K}$	Direction of reaction	pH	$\frac{m(C_3H_8O)}{mol \cdot kg^{-1}}$	$\frac{m(C_7H_6O_5) \cdot 10^2}{mol \cdot kg^{-1}}$	$\frac{m(C_{10}H_{12}O_5) \cdot 10^5}{mol \cdot kg^{-1}}$	$\frac{I_m}{mol \cdot kg^{-1}}$	K'_m	$K_m \cdot 10^4$
293.15	forward	5.33	0.6525	1.168 ± 0.013	6.04 ± 0.19	0.080	126 ± 5	4.34 ± 0.19
293.15	reverse	5.31	0.6406	1.211 ± 0.006	6.54 ± 0.15	0.080	119 ± 3	4.28 ± 0.14
298.15	forward	4.99	0.5689	1.784 ± 0.019	17.7 ± 0.18	0.052	57.3 ± 1.2	4.00 ± 0.14
298.15	reverse	5.00	0.5774	1.598 ± 0.014	15.1 ± 0.18	0.052	61.1 ± 1.3	4.19 ± 0.15
298.15	forward	5.32	0.7019	1.286 ± 0.009	7.39 ± 0.03	0.080	122.1 ± 1.4	4.26 ± 0.09
298.15	reverse	5.36	0.6717	1.224 ± 0.011	7.05 ± 0.13	0.082	117 ± 3	3.75 ± 0.12
298.15	forward	5.38	0.4173	1.710 ± 0.022	4.85 ± 0.06	0.084	147 ± 4	4.51 ± 0.14
298.15	reverse	5.39	0.4408	1.716 ± 0.025	5.92 ± 0.09	0.085	128 ± 4	3.84 ± 0.14
298.15	forward	5.91	0.5885	1.440 ± 0.026	1.95 ± 0.08	0.109	435 ± 26	4.07 ± 0.25
298.15	reverse	5.91	0.6106	1.495 ± 0.093	2.04 ± 0.05	0.111	488 ± 39	4.18 ± 0.37
298.15	forward	6.56	0.5575	1.560 ± 0.027	0.361 ± 0.019	0.135	2409 ± 170	5.04 ± 0.37
298.15	reverse	6.56	0.5255	1.610 ± 0.027	0.300 ± 0.016	0.135	2820 ± 200	5.90 ± 0.43
303.15	forward	5.37	0.6473	1.194 ± 0.011	6.38 ± 0.12	0.082	121 ± 3	3.77 ± 0.12
303.15	reverse	5.35	0.6568	1.198 ± 0.023	6.40 ± 0.12	0.081	123 ± 5	4.00 ± 0.18
308.15	forward	5.32	0.6938	1.313 ± 0.013	7.98 ± 0.14	0.081	114 ± 3	3.91 ± 0.14
308.15	reverse	5.34	0.6529	1.186 ± 0.015	6.26 ± 0.19	0.081	124 ± 5	4.08 ± 0.19

of Ramaiah and Chaturvedi[19] and of Walde[20] contain information on the temperature dependence of the dissociation constant for the first dissociation (pK_1). Since Walde[20] determined dissociation constants at only two temperatures, we prefer to use the results obtained from the study of Ramaiah and Chaturvedi[19]

TABLE 4. Results of calorimetric measurements for the biochemical reaction (3), *n*-propylgallate(aq) + H_2O(aq) = total gallic acid(aq) + propan-1-ol(aq), in phosphate buffer at $T = 298.15$ K and pH = 5.61. The molalities are those after mixing of the enzyme and substrate solutions and prior to any reaction. $C_{10}H_{12}O_5$ is *n*-propylgallate and C_3H_8O is propan-1-ol. All molalities are equal to the sums of the molalities of the indicated substances in their various ionic forms. $\Delta_r H_m$(cal) is the calorimetrically determined molar enthalpy of reaction. The mass fraction of the tannase in the final solution was ≈ 0.003. The ionic strength was 0.092 mol·kg^{-1}

Experiment	$m(K_2HPO_4)$ / mol·kg^{-1}	$m(H_3PO_4)$ / mol·kg^{-1}	$m(C_3H_8O)$ / mol·kg^{-1}	$m(C_{10}H_{12}O_5)\cdot 10^3$ / mol·kg^{-1}	$\Delta_r H_m$(cal) / kJ·mol^{-1}
1	0.1017	0.05669	2.649	3.023	−8.61
2	0.1017	0.05669	2.649	2.954	−7.70
3	0.1017	0.05670	2.648	3.057	−8.26
4	0.1017	0.05670	2.649	2.868	−8.52
5	0.1017	0.05669	2.649	2.956	−8.54
				$\langle\Delta_r H_m(\text{cal})\rangle = -(8.33 \pm 0.34)$ kJ·mol^{-1}	

from which we calculate $\Delta_r G_m^\circ = (25.07 \pm 0.44)$ kJ·mol^{-1}, p$K = (4.39 \pm 0.08)$, $\Delta_r H_m^\circ = -(11.7 \pm 4.3)$ kJ·mol^{-1}, and $\Delta_r S_m^\circ = -(123 \pm 5)$ J·K^{-1}·mol^{-1} at $T = 298.15$ K and $I = 0$ for the dissociation reaction

$$\text{gallic acid(aq)} = \text{gallate}^-\text{(aq)} + \text{H}^+\text{(aq)}. \quad (5)$$

$\Delta_r C_{p,m}^\circ$ for reaction (5) was fixed at 0 in this calculation. At pH = 6.18, the highest pH at which experiments were conducted in this study, $n(C_7H_6O_5^{2-})/n(C_7H_6O_5^-) \approx 0.004$ and $n(C_7H_6O_5^{3-})/n(C_7H_6O_5^-) \approx 6\cdot 10^{-6}$. Thus, under the experimental conditions used herein, the predominant gallate species is $C_7H_6O_5^-$.

There do not appear to be any reports of the dissociation constants of *n*-propylgallate in the literature. However, dissociation constants have been reported[18] for structurally similar substances: the p*K*s of 1,2,3-trihydroxybenzene are 9.2 and 12.0 and the p*K*s of 1,2-dihydroxy-3-methoxybenzene are 9.0, 11.0, and 14.0. On this basis, the first dissociation from *n*-propylgallate ($C_{10}H_{12}O_5$) would correspond

TABLE 5. Results of calorimetric measurements for the biochemical reaction (3), *n*-propylgallate(aq) + H_2O(aq) = total gallic acid(aq) + propan-1-ol(aq), in {MES ($C_6H_{13}NO_4S$) + sodium hydroxide}(aq) buffer at $T = 298.15$ K and pH = 6.18. The molalities are those after mixing of the enzyme and substrate solutions and prior to any reaction. $C_{10}H_{12}O_5$ is *n*-propylgallate and C_3H_8O is propan-1-ol. All molalities are equal to the sums of the molalities of the indicated substances in their various ionic forms. $\Delta_r H_m$(cal) is the calorimetrically determined molar enthalpy of reaction. The mass fraction of the tannase in the final solution was ≈ 0.003. The ionic strength was 0.027 mol·kg^{-1}

Experiment	$m(C_6H_{13}NO_4S)$ / mol·kg^{-1}	m(NaOH) / mol·kg^{-1}	$m(C_3H_8O)$ / mol·kg^{-1}	$m(C_{10}H_{12}O_5)\cdot 10^3$ / mol·kg^{-1}	$\Delta_r H_m$(cal) / kJ·mol^{-1}
1	0.09978	0.05616	1.622	5.714	−20.64
2	0.09977	0.05616	1.622	5.696	−20.12
3	0.09977	0.05616	1.622	5.699	−20.13
4	0.09977	0.05615	1.622	5.539	−19.95
5	0.09977	0.05615	1.622	5.549	−19.02
6	0.09977	0.05616	1.622	5.696	−18.90
				$\langle\Delta_r H_m(\text{cal})\rangle = -(19.8 \pm 0.6)$ kJ·mol^{-1}	

TABLE 6. Results of calorimetric measurements for the reaction of HCl(aq) with {MES ($C_6H_{13}NO_4S$) + sodium hydroxide}(aq) at $T = 298.15$ K. The molalities were: $m(C_6H_{13}NO_4S) = 0.09733\ \text{mol·kg}^{-1}$; $m(\text{NaOH}) = 0.08583\ \text{mol·kg}^{-1}$; and $m(\text{HCl}) = 0.050002\ \text{mol·kg}^{-1}$. The amount of HCl(aq) reacted is given in column 2. The mole fraction $x = n(C_6H_{13}NO_4S)/n(\text{HCl})$. The measured enthalpy change is denoted by ΔH and the molar enthalpy of reaction by $\Delta_r H_m$. The pH of the {MES + sodium hydroxide}(aq) solution was 7.02

Experiment	$\frac{n(\text{HCl})\cdot 10^5}{\text{mol}}$	x	$\frac{\Delta H}{\text{J}}$	$\frac{\Delta_r H_m}{\text{kJ·mol}^{-1}}$
1	2.1967	2.367	−0.33989	−15.473
2	2.2616	2.349	−0.35025	−15.487
3	2.3248	2.258	−0.35904	−15.444
4	2.3182	2.194	−0.35773	−15.431
5	2.3184	2.318	−0.35726	−15.409
			$\langle\Delta_r H_m\rangle = -(15.449 \pm 0.028)\ \text{kJ·mol}^{-1}$	

to p$K \approx 9.0$. Since all the equilibrium and calorimetric measurements done in this study pertain to pH $\leqslant$ 6.18, $n(C_{10}H_{12}O_5^-)/n(C_{10}H_{12}O_5) < 0.002$. Therefore, the dissociation of *n*-propylgallate will be neglected in the discussion and calculations to follow. Also, since the dissociation of gallic acid at pH = 8.6 was neglected, there will be a substantial cancellation of the errors caused by neglecting the dissociations corresponding to the higher pKs of *n*-propylgallate and gallic acid in the results of the chemical equilibrium modeling calculations given below. The other reactant in reaction (3) is propan-1-ol; it can dissociate only under extremely alkaline conditions. Thus, it is seen from the preceding discussion that the predominant species in solution in the pH range in which equilibrium and calorimetric experiments were performed ($5.0 \leqslant \text{pH} \leqslant 6.18$) are gallate$^-$, *n*-propylgallate, and propan-1-ol. On this basis, the chemical reference reaction corresponding to the overall biochemical reaction (3) in aqueous solution is selected as:

$$n\text{-propylgallate(aq)} + H_2O(l) = \text{gallate}^-(\text{aq}) + \text{propan-1-ol(aq)} + H^+(\text{aq}). \quad (6)$$

Here the charge number on the *n*-propylgallate and propan-1-ol is understood to be zero. The equilibrium constant for reaction (6) is

$$K_m = m(\text{gallate}^-)\cdot m(\text{propan-1-ol})\cdot m(H^+)/m(n\text{-propylgallate})\cdot(m^\circ)^2. \quad (7)$$

From the potentiometric measurements of Vega and Bates[21] we calculate $\Delta_r G_m^\circ = (35.785 \pm 0.003)\ \text{kJ·mol}^{-1}$, p$K = (6.270 \pm 0.001)$, $\Delta_r H_m^\circ = (14.62 \pm 0.04)\ \text{kJ·mol}^{-1}$, and $\Delta_r C_{p,m}^\circ = (6 \pm 6)\ \text{J·K}^{-1}\text{·mol}^{-1}$ at $T = 298.15$ K and $I = 0$ for the reaction

$$\text{MES}^\pm(\text{aq}) = \text{MES}^-(\text{aq}) + H^+(\text{aq}). \quad (8)$$

The results of Bates and Vega are extremely precise and the uncertainties (two estimated standard deviations of the mean) given for the calculated thermodynamic quantities reflect only the statistical uncertainties in the fit to the pKs determined by them as a function of temperature. In performing the calorimetric measurements in this study (see table 6), an aqueous solution containing MES ($C_6H_{13}NO_4S$) was first

adjusted with aqueous NaOH to pH = 7.02, where MES^- is the predominant species. This {MES + sodium hydroxide} solution was then mixed in the calorimeters with an HCl solution. Since $n(C_6H_{13}NO_4S)/n(HCl)$ was kept at ≈ 2.3, the HCl is the limiting reactant. We use equation (2.46) of Vanderzee,[22] which is based on Young's rule,[23] to estimate $\{H_m(sln) - H_m^\circ(sln)\}$ for the initial and final states of the solutions in these experiments. Here, the standard state of the solute is the hypothetical ideal solution of unit molality ($m^\circ = 1\ mol \cdot kg^{-1}$). In these calculations, we assumed that $\Delta_{mix}H_m = 0$ for the solutions at constant ionic strength and that the apparent molar enthalpy L_ϕ of the uni-univalent electrolyte NaMES(aq) was equal to L_ϕ of NaCl(aq). The apparent molar enthalpies of HCl(aq) and NaCl(aq) tabulated by Parker[24] were also used in these calculations. Thus, we obtain $\Delta_r H_m^\circ = -(15.0 \pm 0.1)\ kJ \cdot mol^{-1}$ for the protonation reaction, the reverse of reaction (8), at $T = 298.15$ K. The largest contribution to the estimated uncertainty given here is from the uncertainty in the dilution correction. The difference of $0.38\ kJ \cdot mol^{-1}$ between this result and that obtained from the potentiometric measurements of Vega and Bates[21] is larger than the combined uncertainties in these quantities. While, we cannot explain this difference, we believe that the statistical uncertainty assigned to the value of $\Delta_r H_m^\circ$ obtained from the results of Vega and Bates[21] for reaction (8) is fortuitously small, and that a more realistic uncertainty would be in the range $\pm$(0.2 to 0.5) $kJ \cdot mol^{-1}$. This would bring the two results for $\Delta_r H_m^\circ$ for reaction (8) into agreement.

In the equilibrium calculations to follow we adopt $pK = (4.39 \pm 0.08)$ and $\Delta_r H_m^\circ = -(11.7 \pm 4.3)\ kJ \cdot mol^{-1}$ for reaction (5) and $pK = (6.270 \pm 0.002)$, $\Delta_r H_m^\circ = (15.0 \pm 0.1)\ kJ \cdot mol^{-1}$ for reaction (8) at $T = 298.15$ K and $I = 0$; $\Delta_r C_{p,m}^\circ = 0$ is assumed for both reactions. Since the phosphate and acetate buffers used in the measurements affect the ionic strength of the solutions, information on the respective dissociations of $H_2PO_4^-(aq)$ and $C_2H_4O_2(aq)$:

$$H_2PO_4^-(aq) = HPO_4^{2-}(aq) + H^+(aq), \tag{9}$$

$$C_2H_4O_2(aq) = C_2H_3O_2^-(aq) + H^+(aq), \tag{10}$$

is also needed for the equilibrium calculations. For reaction (9) at $T = 298.15$ K and $I = 0$, we calculate $pK = 7.21$ and $\Delta_r H_m^\circ = 4.2\ kJ \cdot mol^{-1}$ from the standard molar Gibbs free energies and standard molar enthalpies of formation;[25] a value of $\Delta_r C_{p,m}^\circ = -220\ J \cdot K^{-1} \cdot mol^{-1}$ was calculated from the standard apparent molar heat capacities reported by Larson *et al.*[26] For reaction (10) at $T = 298.15$ K and $I = 0$, we obtain $pK = 4.75$, $\Delta_r H_m^\circ = 0.42\ kJ \cdot mol^1$, and $\Delta_r C_{p,m}^\circ = -155\ J \cdot K^{-1} \cdot mol^{-1}$ from the review of Larson and Hepler.[27] In the calculations to follow we shall assume the following uncertainties for the dissociation of $H_2PO_4^-$ and $C_2H_4O_2$: p*K*s, ± 0.002; $\Delta_r H_m^\circ$s, $\pm 0.2\ kJ \cdot mol^{-1}$, and $\Delta_r C_{p,m}^\circ$s, $\pm 15\ J \cdot K^{-1} \cdot mol^{-1}$.

The results given in tables 3, 4, and 5 for reaction 3 in water can be used together with the above thermodynamic quantities for the dissociation constants and standard molar enthalpies of dissociation and a previously described equilibrium model[28] to obtain thermodynamic quantities for the chemical reference reaction (6). In performing these calculations, we used an estimated "ion-size" parameter of $(1.6 \pm 0.3)\ kg^{1/2} \cdot mol^{-1/2}$ in the extended Debye–Hückel equation to estimate the

activity coefficients of the aqueous species in solution. From these equilibrium calculations we obtained the values of the ionic strengths and equilibrium constants for reaction (6) given, respectively, in columns 11 and 13 in table 3.

The uncertainties given for the calculated values of the equilibrium constants for reaction (6) have two components: those from the apparent equilibrium constants (see column 11 in table 3) and those from possible errors in the quantities used in the equilibrium model. The latter were estimated by perturbing each of the quantities (pKs and $\Delta_r H^\circ_m$s for dissociation reactions and the "ion size" parameter) used in the equilibrium model by an amount equal to the uncertainties given in the preceding discussion. The effects of these perturbations on the calculated values of the equilibrium constant for reaction (6) were then combined in quadrature to obtain estimates of the uncertainties attributable to the equilibrium model calculation. These estimated uncertainties were then combined in quadrature with the experimental uncertainties in the respective apparent equilibrium constants which are based on two estimated standard deviations of the mean. A similar procedure was also used to estimate uncertainties in the calculated values (see below) of $\Delta_r H^\circ_m$ and $\Delta_r N(H^+)$. From the values of K given in column 13 in table 3, we adopt $\langle K \rangle = (4.37 \pm 0.40) \cdot 10^{-4}$ for reaction (6) at $T = 298.15$ K. From the temperature dependence of this equilibrium constant we obtain $\Delta_r H^\circ_m = -(6 \pm 10)$ kJ·mol^{-1}.

With the equilibrium model[28] and the results given in tables 4 and 5 we calculate, respectively, $\Delta_r H^\circ_m = -(4.27 \pm 0.45)$ kJ·mol^{-1} and $\Delta_r H^\circ_m = -(5.17 \pm 0.64)$ kJ·mol^{-1} at $T = 298.15$ K and at $I = 0$ for reaction (6). Thus, the values of $\Delta_r H^\circ_m$ for reaction (6) obtained from measurements with two different buffers are in agreement. We adopt the weighted average $\Delta_r H^\circ_m = -(4.6 \pm 0.4)$ kJ·mol^{-1} which agrees with $\Delta_r H^\circ_m = -(6 \pm 10)$ kJ·mol^{-1} calculated from the temperature dependence of the equilibrium constant. In summary for reaction (6) in water: $K_m = (4.37 \pm 0.40) \cdot 10^{-4}$, $\Delta_r G^\circ_m = (19.18 \pm 0.24)$ kJ·mol^{-1}, $\Delta_r H^\circ_m = -(4.6 \pm 0.4)$ kJ·mol^{-1}, and $\Delta_r S^\circ_m = -(80 \pm 2)$ J·K^{-1}·mol^{-1} at $T = 298.15$ K and $I = 0$.

The equilibrium model also yields the following changes in binding of the hydrogen ion $\Delta_r N(H^+)$ for the overall biochemical reaction (3) in aqueous solutions: $\Delta_r N(H^+) = -(0.955 \pm 0.007)$ at pH = 5.61 (the pH used for the calorimetric measurements with phosphate buffer) and $\Delta_r N(H^+) = -(0.986 \pm 0.002)$ at pH = 6.18 (the pH used for the calorimetric measurements with MES buffer). Since[29]

$$\Delta_r N(H^+) = -(\partial \lg K'_m / \partial \mathrm{pH})_{T,p,I}, \tag{11}$$

we can also use the apparent equilibrium constants given in table 3 to calculate $\Delta_r N(H^+)$. Thus, from a plot of $\lg K'_m$ as a function of pH (see figure 2) we obtain $\Delta_r N(H^+) = -(1.054 \pm 0.070)$ from the least-squares fit to the results at an average pH = 5.8. The value of $\Delta_r N(H^+)$ calculated with the equilibrium model at this pH is $-(0.970 \pm 0.005)$. Thus, the values of $\Delta_r N(H^+)$ calculated with the equilibrium model and with equation (11) are almost in agreement within the indicated uncertainties.

The values of K'_m, the standard molar transformed Gibbs free energy change $\Delta_r G'^\circ_m$, and the standard molar transformed enthalpy change $\Delta_r H'^\circ_m$ for the biochemical reaction (3) under physiological conditions are useful quantities for thermodynamic

calculations.[9] Therefore, with the equilibrium model[28] and the summary values of the thermodynamic quantities for the reference reaction (6) given above, we calculate for the biochemical reaction (3) in aqueous solutions at pH = 7.0 and $I_m = 0.25\ mol{\cdot}kg^{-1}$: $K'_m = 6.05{\cdot}10^3$, $\Delta_r G'^{\circ}_m = -21.6\ kJ{\cdot}mol^{-1}$, and $\Delta_r H'^{\circ}_m = -3.8\ kJ{\cdot}mol^{-1}$ at $T = 298.15$ K; $K'_m = 5.65{\cdot}10^3$, $\Delta_r G'^{\circ}_m = -22.4\ kJ{\cdot}mol^{-1}$, and, assuming $\Delta_r C^{\circ}_{p,m} = 0$ for reaction (6), $\Delta_r H'^{\circ}_m \approx -3.6\ kJ{\cdot}mol^{-1}$ at $T = 311.15$ K.

The literature does not appear to contain any results on the thermodynamics of the hydrolysis of *n*-propylgallate to gallic acid and propan-1-ol either in water or in any other solvents; nor are we able to calculate any of the thermodynamic quantities obtained in this study from a thermodynamic cycle calculation.

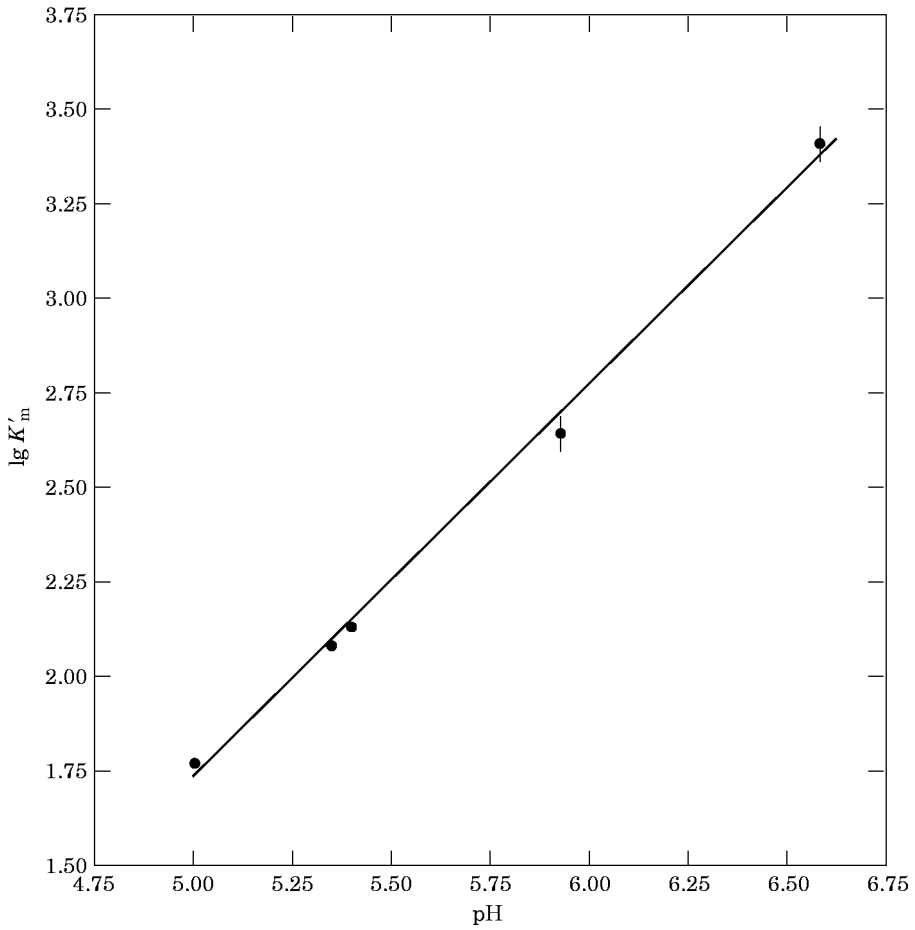

FIGURE 2. lg K'_m for the biochemical reaction (3) as a function of pH at $T = 298.15$ K. The values of K'_m obtained from forward and reverse directions of reaction at the respective pHs have been averaged to give the respective values of K'_m shown in this figure. In three cases the error bars are too small to be seen. The straight line is the least-squares fit to the results. The slope of this line is $-\Delta_r N(H^+)$ and is equal to (1.054 ± 0.070).

However, for comparison of thermodynamic quantities, we now consider an alternative to the chemical reference reaction (6), namely the chemical reference reaction involving only neutral chemical species:

$$n\text{-propylgallate(aq)} + H_2O(l) = \text{gallic acid(aq)} + \text{propan-1-ol(aq)}. \quad (12)$$

When considering reaction (12) in water, the equilibrium constant generally used is

$$K_m = m(\text{gallic acid}) \cdot m(\text{propan-1-ol}) / m(n\text{-propylgallate}) \cdot m^\circ. \quad (13)$$

Combination of the thermodynamic quantities for reactions (5) and (6) yields for reaction (12) in water at $T = 298.15$ and $I = 0$: $K_m = (10.8 \pm 2.0)$, $\Delta_r G_m^\circ = -(5.89 \pm 0.50)\ \text{kJ}\cdot\text{mol}^{-1}$, $\Delta_r H_m^\circ = (7.1 \pm 4.3)\ \text{kJ}\cdot\text{mol}^{-1}$, and $\Delta_r S_m^\circ = (44 \pm 15)\ \text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$. These thermodynamic quantities are based on the convention that the activity of water $a_w = 1$, and that $m^\circ = 1\ \text{mol}\cdot\text{kg}^{-1}$. Since the thermodynamic quantities for reaction (1) in toluene are based on the actual molality of water in the solution, we now introduce the molality of the water ($55.508\ \text{mol}\cdot\text{kg}^{-1}$) into the expression for the equilibrium constant and obtain

$$K = m(\text{gallic acid}) \cdot m(\text{propan-1-ol}) / m(n\text{-propylgallate}) \cdot m(H_2O). \quad (14)$$

We then calculate the following thermodynamic quantities for reaction (12) in water at $T = 298.15$ and $I = 0$ that are based on this convention: $K = (0.195 \pm 0.036)$, $\Delta_r G_m^\circ = (4.1 \pm 0.5)\ \text{kJ}\cdot\text{mol}^{-1}$, $\Delta_r H_m^\circ = (7.1 \pm 4.3)\ \text{kJ}\cdot\text{mol}^{-1}$, and $\Delta_r S_m^\circ = (10 \pm 15)\ \text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$. The thermodynamic quantities for reaction (1) in toluene are: $K = (0.0130 \pm 0.0023)$, $\Delta_r G_m^\circ = (10.8 \pm 0.5)\ \text{kJ}\cdot\text{mol}^{-1}$, $\Delta_r H_m^\circ = -(15.4 \pm 6.6)\ \text{kJ}\cdot\text{mol}^{-1}$, and $\Delta_r S_m^\circ = -(88 \pm 22)\ \text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ at $T = 298.15\ \text{K}$. Having reduced the thermodynamic quantities to a common basis, it is seen that the position of equilibrium of reaction (1) in toluene lies farther to the left than reaction (12) in water. Thus, there is an enhancement of the equilibrium yield of *n*-propylgallate above the first-order effect one estimates on the simple basis of the stoichiometric removal of water ($\approx 55.5\ \text{mol}\cdot\text{kg}^{-1}$) from the mixture when this reaction is carried out in an organic solvent. This is in contrast to our earlier results[8] obtained on the chemical reference reaction

$$N\text{-acetyl-L-phenylalanine ethyl ester(sln)} + H_2O(\text{sln}) = N\text{-acetyl-L-phenylalanine(sln)} + \text{ethanol(sln)}. \quad (15)$$

There it was found that the equilibrium constants, when calculated on a common basis, were comparable for this reaction in water and in toluene, dichloromethane, and carbon tetrachloride. A more quantitative understanding of the thermodynamics of these reactions in terms of solvent effects requires accurate thermodynamic quantities for the transfer of the reactants in their condensed phase(s) to the solution(s) in which the reaction occurs. Nevertheless, a particularly useful result is that the equilibrium yield of *n*-propylgallate, expressed as the quantity

$$Y = 10^2 \cdot [n'(n\text{-propylgallate}) / \{n'(n\text{-propylgallate}) + n'(\text{gallic acid})\}]$$

is significantly larger for the reaction in toluene than in water. Specifically, from tables 2 and 3, $Y \approx 98.6$ for reaction (1) in toluene in contrast to a value of $Y = 0.98$ for the reaction in water at $T = 298.15\ \text{K}$ and $\text{pH} = 5.0$.

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