value of 21.0 ± 0.1 kcal mol^{-1} for the neat phases at 25 °C and of 21.9 kcal mol^{-1} for the gas phase, which are quite different from the similarly determined earlier value of 10 kcal mol^{-1}.[22] Catalytic heats of hydrogenation measurements by Rogers et al.[23] in *n*-hexane solutions at 25 °C led to a ΔH value of 22.0 ± 0.1 kcal mol^{-1} for the N → Q isomerization or close to the earlier value of Turner et al. of 24 ± 0.9 kcal mol^{-1} in glacial acetic acid at 25 °C.[24]

A consensus of around 20–22 kcal mol^{-1} for ΔH_I thus seems to have developed in the literature, a distinctly higher value than ours of 14 kcal mol^{-1}. All of the above literature measurements have been indirect, and in general, a photocalorimetric result should be less subject to propagated error because ΔH is determined directly for the solution in question at 25 °C so that corrections needed to reduce the data to standard conditions are entirely avoided. Sensitized photolyses are intrinsically more complex than are simple ones, however, and there may be a special explanation for the discrepancy in ΔH values in this particular case. There is an indication, for example, that acetophenone may add photochemically to quadricyclene,[3g,h] and a minor component of such an exoergic reaction could account for our lower ΔH value.

It should be stressed that even if the photocalorimetric ΔH_I value is lower than the true thermochemical one for pure N_I isomerizing to pure Q_I, it is the photocalorimetric value that mimics any actual solar energy conversion scheme with acetophenone as sensitizer (and a sensitizer would have to be used if any significant portion of the insolation spectrum were to be absorbed). If there are indeed complexities in the sensitized isomerization, their effect on *f* as registered photocalorimetrically will also be present in any application of the system to practical solar energy conversion. In fact, any system for solar energy conversion should be checked photocalorimetrically and under the proposed conditions of use before a claimed efficiency can be regarded as certain.

In summary, we report here ΔH and quantum yield values for the N to Q isomerization of several substituted norbornadienes, compounds II, III, and IV. The enthalpy values for III and IV are large even though the position of the absorption band maximum has been considerably red-shifted relative to that of the parent compound. The value for compound II is somewhat higher than that found by conventional calorimetry, but at a higher temperature and in a different solvent. Our ΔH value for the parent compound, I, lies within the rather wide range of literature values but may be low because of complexities in the sensitization behavior. The method of photocalorimetry is well suited for obtaining enthalpies of clean-cut photochemical reactions, and we point out that it is particularly well suited for the determination of pragmatic efficiencies of solar energy storage systems, especially if they are at all complicated.

Acknowledgment. The authors acknowledge financial support for this work from the donors of the Petroleum Research Fund, administered by the American Chemical Society (grant to A. W.A.), the National Science Foundation (grants CHE-8210558 and INT-8309942 to C.K.), and the Science and Technology Agency, Japan (to K.Y.).

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An Equilibrium Study of the Conversion of L-Phenylalanine to *trans*-Cinnamic Acid and Ammonia

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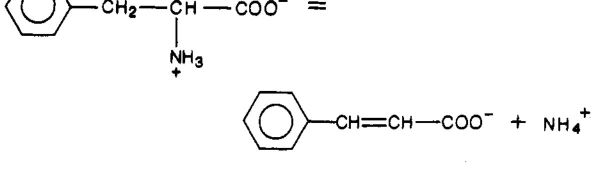
The thermodynamics of the enzymatic conversion (L-phenylalanine ammonia-lyase) of aqueous L-phenylalanine to *trans*-cinnamic acid and ammonia has been investigated by using high-performance liquid chromatography (HPLC). The reaction was carried out in 0.1 M Tris/HCl buffer containing ammonium chloride over the pH range 7.0–7.7, at ionic strengths from 1.0 to 2.1 mol kg^{-1}, and over the temperature range 285–316 K. Analysis of the HPLC data using an estimated heat capacity change of 50 J mol^{-1} K^{-1} and an "ion-size" parameter of 1.6 $mol^{-1/2}$ $kg^{1/2}$ leads to an equilibrium constant of 1.16 ± 0.3 mol kg^{-1} and an enthalpy change of 24.8 ± 2.0 kJ mol^{-1} at 298.15 K for the process L-phenylalanine$^{\pm}$(aq) = *trans*-cinnamic acid^{-}(aq) + NH_4^+(aq). The use of these thermodynamic parameters in an equilibrium model for this system allows for the prediction of values of the apparent equilibrium constant as a function of pH, temperature, and composition and also of the effects of these parameters on the optimal product yield of L-phenylalanine during its manufacture from *trans*-cinnamic acid and ammonia. The available thermochemical data for this generic type of reaction can be rationalized in terms of a scheme which views the entropy changes for related processes to be comparable and then attributes differences in Gibbs energy changes to differences in enthalpy changes which can be influenced by effects such as resonance stabilization of the double bonds which are formed.

Introduction

L-Phenylalanine is of importance for several reasons: (1) it is one of the essential amino acids, (2) it is a principal ingredient in the sweetener "aspartame", and (3) it is the common precursor from which the majority of phenolic natural products are derived.[1] A principal step in either the manufacture or use of phenylalanine is the chemical equilibrium between it and *trans*-cinnamic acid and ammonia which is catalyzed by the enzyme L-phenylalanine ammonia-lyase (E.C. 4.3.1.5). Consequently, we have undertaken a thermodynamic investigation of this process, the principal aim of which is the determination of thermodynamic parameters for the reference reaction



(A)

This study involved the use of high-performance liquid chro-

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TABLE I: Values of Thermodynamic Parameters[a] at 298.15 K Relevant to the Conversion of Phenylalanine to *trans*-Cinnamic Acid and Ammonia in Aqueous Solution[c]

process	pK or equilibrium constant	$\Delta H°$/kJ mol^{-1}	$\Delta C_p°$/J mol^{-1} K^{-1}	ref
$PhAlaH^{\pm}(aq) = tca^-(aq) + NH_4^+(aq)$	$K_A = 1.16 \pm 0.3$	24.8 ± 2.0	50[b]	this work
$PhAlaH_2^+(aq) = PhAlaH^{\pm}(aq) + H^+(aq)$	$pK_{\phi 1} = 2.20$	2.5	−140[b]	20, 21
$PhAlaH^{\pm}(aq) = PhAla^-(aq) + H^+(aq)$	$pK_{\phi 2} = 9.31$	44.6	−40[b]	20, 21
$tca^0(aq) = tca^-(aq) + H^+(aq)$	$pK_{CA} = 4.51$	11.0	−140[b]	22
$NH_4^+(aq) = NH_3^0(aq) + H^+(aq)$	$pK_N = 9.24_1$	52.22	70[b]	6
$Tris{\cdot}H^+(aq) = Tris^0(aq) = H^+(aq)$	$pK_T = 8.07_2$	47.48	−50	19, 23–25

[a] The standard state is the hypothetical ideal solution of unit molality. [b] Estimated. [c] The parameters for the ionization of $Tris{\cdot}H^+(aq)$ are needed for the treatment of the measurement data.

matography (HPLC) for the determination of equilibrium constants as a function of temperature (285–316 K), pH (7.0–7.7), and ionic strength (1.0–2.0 mol kg^{-1}). The treatment of the experimental data required the use of an equilibrium model to account for the multiplicity of chemical species present in solution and for the effects of nonideal solution behavior. The thermodynamic parameters obtained from this study together with a chemical equilibrium model allow one to predict the thermodynamic behavior of this system under a wide variety of conditions. Also, the above reaction, which involves both the formation of a double bond and the consequent release of an ammonium ion, is representative of a class of chemical reactions in solution. The thermodynamic information gained from this study will be shown to be useful in understanding the general trends in the equilibrium data for this class of reactions.

Experimental Section

Materials and their sources[30] were as follows: L-phenylalanine, *trans*-cinnamic acid, and L-phenylalanine ammonia-lyase were from Sigma; ammonium chloride was from Mallinckrodt; and Tris was from Fisher. The moisture contents of the L-phenylalanine and *trans*-cinnamic acid were determined by Karl Fischer titration and found to be 0.03 and 0.01 mass %, respectively. Using the chromatographic procedures described below, we found no indications of impurities in these materials.

Equilibrium measurements were performed starting either with L-phenylalanine and NH_4Cl in Tris/HCl buffer or with *trans*-cinnamic acid and NH_4Cl in Tris/HCl buffer. To obtain a measurable chromatographic signal due to the L-phenylalanine, it was necessary to have high molalities (1.0–2.0 mol kg^{-1}) of NH_4Cl present in solution to drive the reaction in the direction of L-phenylalanine. The L-phenylalanine ammonia-lyase was dialyzed against Tris/HCl buffer containing the appropriate concentration of NH_4Cl prior to its addition to the reaction mixture. All solutions were prepared gravimetrically.

Quantitative analysis of the reacted solutions was accomplished using a 5-μm "Spherical C_{18} Resolve Column" from Waters Associates and a Hewlett-Packard Model 1090 liquid chromatograph equipped with a diode array detector. The mobile phase consisted of 20% acetonitrile and 80% water containing 0.2% trifluoroacetic acid. The mobile phase flow rate was 0.35 mL min^{-1}. The detector was set at a wavelength of 220 nm. Under these conditions the retention times were 5.0 and 18.7 min for the L-phenylalanine and *trans*-cinnamic acid, respectively. A chromatogram of the equilibrium mixture is shown in Figure 1. Response factors for both the L-phenylalanine and *trans*-cinnamic acids were determined by using the pure compounds.

Solutions were equilibrated in a water bath thermostated to within ±0.05 K and were continuously stirred with a Teflon-coated magnet prior to injection into the HPLC. Equilibration times ranged from 1 to 4 days. The chromatograms showed no evidence of any side reactions.

The pH of the reacted solutions was determined by using an Orion Model 811 microprocessor pH/millvolt meter and a Fisher combination electrode. Recommended procedures[2] for the measurement of pH were adhered to.

Attempts were made to perform calorimetric measurements on this reaction. The rate of reaction, however, was too slow to allow meaningful measurements to be performed.

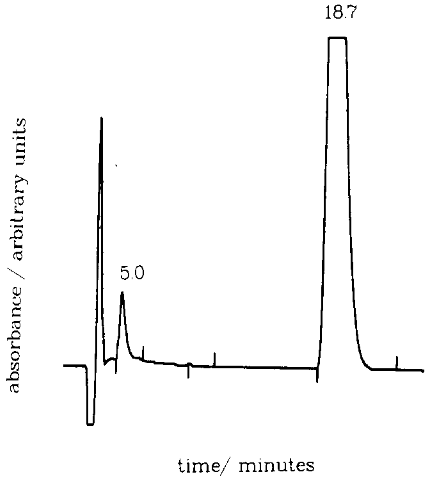

Figure 1. HPLC chromatogram of an equilibrium mixture of L-phenylalanine and *trans*-cinnamic acid. The peaks are L-phenylalanine (5.0 min) and *trans*-cinnamic acid (18.7 min).

Results and Discussion

Representation of the System. The net conversion of L-phenylalanine (PhAla) to *trans*-cinnamic acid (tca) and ammonia (amm) is represented as

$$\sum\text{PhAla} = \sum\text{tca} + \sum\text{amm} + n_H H^+ \qquad (B)$$

The $\sum$ represents the total of the different ionic states for a given substance in solution, and n_H is the number of protons produced or absorbed as a part of the reaction. A description of the net thermodynamic behavior of the system requires a knowledge of the thermodynamics of the reference reaction (process A), as well as of the ionization processes for each of the species in solution. These equilibria are summarized in Table I. The treatment of the experimental data requires a method for the calculation of the amounts of the various species in solution and their relationships to the measured or observed thermodynamic quantities. The method used to accomplish this is described in the discussion that follows.

Application of equilibrium thermodynamics to this system leads to the following:

$$f_{\text{PhAla}} = [\text{PhAlaH}^{\pm}]/[\sum\text{PhAla}] = \{1 + [H^+]/K_{\phi 1} + K_{\phi 2}/[H^+]\}^{-1} \qquad (1)$$

$$f_{\text{tca}} = [\text{tca}^-]/[\sum\text{tca}] = \{1 + [H^+]/K_{CA}\}^{-1} \qquad (2)$$

$$f_{\text{amm}} = [NH_4^+]/[\sum\text{amm}] = \{1 + K_N/[H^+]\}^{-1} \qquad (3)$$

$$n_H = f_{\text{PhAla}}\{[H^+]/K_{\phi 1} - K_{\phi 2}/[H^+]\} + f_{\text{amm}}K_N/[H^+] - f_{\text{tca}}[H^+]/K_{CA} \qquad (4)$$

$$K_{B,\text{obsd}} = [\sum\text{tca}][\sum NH_3]/[\sum\text{PhAla}] = f_{\text{PhAla}}K_A/(f_{\text{tca}}f_{\text{amm}}) \qquad (5)$$

and

$$\Delta H°_{B,\text{obsd}} = \Delta H°_A + f_{\text{amm}}K_N\Delta H°_N/[H^+] - f_{\text{tca}}[H^+]\Delta H°_{CA}/K_{CA} + f_{\text{PhAla}}\{[H^+]\Delta H°_{\phi 1}/K_{\phi 1} - K_{\phi 2}\Delta H°_{\phi 2}/[H^+]\} \qquad (6)$$

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TABLE II: Equilibrium Constants (K_{obsd} = [∑tca][∑amm]/[∑PhAla]) for the Conversion of L-Phenylalanine to *trans*-Cinnamic Acid and Ammonia[a]

		forward reaction			reverse reaction			combined	calcd from measurements	
series	T/K	$K_{B,obsd}$	pH	N	$K_{B,obsd}$	pH	N	$K_{B,obsd}$	$K_A(I = 0, T)$	$K_A(I = 0,$ 298.15 K)
1	285.65	1.55 ± 0.06	7.65	4	1.53 ± 0.09	7.71	4	1.54 ± 0.07	0.731	1.13
2	292.15	2.08 ± 0.04	7.64	5	2.10 ± 0.15	7.60	5	2.09 ± 0.03	0.993	1.22
3	298.05	2.51 ± 0.04	7.69	5	2.44 ± 0.08	7.69	5	2.47 ± 0.07	1.18	1.18
4	304.45	3.11 ± 0.07	7.61	5	3.10 ± 0.13	7.63	5	3.11 ± 0.10	1.48	1.20
5	309.95	3.52 ± 0.07	6.97	5	3.69 ± 0.05	6.98	5	3.61 ± 0.11	1.67	1.13
6	309.95	3.65 ± 0.05	7.26	6	3.83 ± 0.19	7.25	6	3.74 ± 0.16	1.74	1.18
7	309.95	3.39 ± 0.03	7.70	5	3.54 ± 0.04	7.71	5	3.46 ± 0.09	1.67	1.13
8	309.95	3.63 ± 0.19[b]	7.40	3	3.77 ± 0.10[b]	7.38	3	3.70 ± 0.16	1.73	1.17
9	309.95	3.53 ± 0.08[c]	7.27	5	3.63 ± 0.06[c]	7.28	5	3.58 ± 0.08	1.70	1.15
10	316.45	4.44 ± 0.02	7.25	4	4.51 ± 0.08	7.20	5	4.48 ± 0.07	2.08	1.15

[a] The starting concentrations of L-phenylalanine (for the forward reaction) and of *trans*-cinnamic acid (for the reverse reaction) were 2–3 mmol kg^{-1}. All reactions were carried out in Tris/HCl buffer (0.1 mol L^{-1}) containing, unless indicated otherwise, NH_4Cl at a concentration of 1.0 mol L^{-1}. The ionic strength is ≈1.05 mol kg^{-1} for all series except for numbers 8 and 9 which have ionic strengths of 1.55 and 2.06 mol kg^{-1}, respectively. The uncertainties refer to 95% confidence limits. N is the number of measurements performed. [b] Concentration of NH_4Cl is 1.55 mol L^{-1}. [c] Concentration of NH_4Cl is 2.06 mol L^{-1}.

In the above equations, square brackets represent molalities of substances in solution and the *f*'s are the fractions of a given substance existing in a specified ionic state.

The temperature dependencies of the Gibbs energies ($\Delta G°$), equilibrium constants (K), and enthalpy changes ($\Delta H°$) are given by

$$\Delta G°_T = -RT \ln K = \Delta H°_\theta + \Delta C_p°(T - \theta) + T(\Delta G°_\theta - \Delta H°_\theta)/\theta - T\Delta C_p°(T/\theta) \qquad (7)$$

$$\Delta H°_T = \Delta H°_\theta + \Delta C_p°(T - \theta) \qquad (8)$$

where T is the thermodynamic temperature, θ is the reference temperature of 298.15 K, and R is the gas constant (8.31441 J mol^{-1} K^{-1}). The heat capacity changes ($\Delta C_p°$) are assumed to be constant over the temperature range of interest.

Nonideal solution behavior is accounted for by the introduction of activity coefficients for each of the species in solution

$$\ln \hat{\gamma}_i = -Az_i^2\hat{I}^{1/2}/(1 + B\hat{I}^{1/2}) + \sum\lambda_{ij}\hat{m}_j \qquad (9)$$

where A is a Debye–Hückel constant,[3] z_i is the charge of the ith species, $\hat{I}$ is the ionic strength ($^1/_2\sum\hat{m}_i z_i^2$), B is an "ion-size" parameter, λ_{ij} is an interaction parameter which accounts for short-range interactions between species i and j, $\hat{m}_j$ is the molality of the jth species. The circumflex denotes a species as distinct from a stoichiometric quantity. We have assumed[4] that all of the λ_{ij} are equal and simplified the last term in eq 9 to $\lambda\sum\hat{m}_j$.

Computational Procedure. The equilibrium measurements are summarized in Table II. The aim of the data treatment was to obtain values of K_A and $\Delta H°_A$ at 298.15 K. To do this, it was necessary to use the auxiliary data given in Table I as well as estimate a value of $\Delta C_p°$ for process A and to make some reasonable assumptions about the activity coefficients of the ions in solution.

We have estimated a value of $\Delta C_p°$ = 50 J mol^{-1} K^{-1} for process A at 298.15 K. This is based upon a measured[5,6] partial molar heat capacity of 390 J mol^{-1} K^{-1} for L-phenylalanine(aq) and a value[7] of 80 J mol^{-1} K^{-1} for NH_4^+(aq). The measured difference in the partial molar heat capacities of aqueous L-phenylalanine and L-alanine is[7] 245 J mol^{-1} K^{-1}. This difference is added to the partial molar heat capacity of aqueous propionate ion[8] to obtain an estimate of 360 J mol^{-1} K^{-1} for the partial molar heat capacity of aqueous *trans*-cinnamic acid. Combination of this value with the partial molar heat capacities of NH_4^+(aq) and L-phenylalanine(aq) leads to our estimated $\Delta C_p°$ = 50 J mol^{-1} K^{-1} for process A.

Activity coefficients were estimated by using a value of the B parameter in eq 9 equal to 1.6 mol$^{-1/2}$ kg$^{1/2}$. The value is typical of values which have been found for this parameter in earlier correlations of activity and osmotic coefficient data for uni-univalent and uni-bivalent electrolytes.[9,10] The value of λ in eq 9 then becomes an adjustable parameter in the calculations. Since its value is dependent upon the assumed value of the B parameter, the value determined for λ will have little real meaning. In effect, the B and λ parameters are devices used either to extrapolate measured equilibrium constants to standard-state conditions or, conversely, to adjust the standard-state equilibrium constant to higher ionic strengths and more concentrated solutions.

The procedure used to obtain the values of K_A and of $\Delta H°_A$ at 298.15 K from the equilibrium measurements follows:

1. Estimate values for both B and for $\Delta C_p°$ for process A. This was done above.
2. Make initial estimates for (a) $\Delta H°_A$ at 298.15 K, (b) the value of $K_{B,obsd}$ under the actual experimental conditions, and (c) the ionic strength of the solution of interest.
3. Adjust the standard-state equilibrium constants to the temperature of interest.
4. Calculate the activity coefficients for each of the species in solution.
5. Calculate values of apparent equilibrium constants using both the thermodynamic equilibrium constants which pertain to standard-state conditions and the activity coefficients obtained in step 4.
6. Calculate the fractions of each of the species in solution using eq 1–3 as well as analogous ones for all of the other species in solution. Note that apparent equilibrium constants must be used here rather than standard-state equilibrium constants.
7. Calculate the total amounts of L-phenylalanine, *trans*-cinnamic acid, and ammonia in solution using the assumed value of $K_{B,obsd}$ and the known initial molalities of the substances in solution.
8. Calculate the molalities of each of the species in solution and the ionic strength.
9. Iterate (steps 2c to 8) until the value obtained for the ionic strength converges to within a given tolerance (0.0001 mol kg^{-1}).
10. Calculate $K_{B,obsd}$ using eq 5. As in step 6, apparent equilibrium constants must be used rather than standard-state equilibrium constants.

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11. Iterate (steps 2b to 10) until $K_{B,obsd}$ converges to within a given tolerance (0.01% of its value).

12. Using the data obtained at 310 K at three different ionic strengths, regress a value of K_A both at 298.15 K and at 310 K and of λ which "best" represent the data.

13. Using the value of λ obtained in step 12, similarly treat each of the experimental data points to obtain a value of K_A both at the temperature of measurement and at 298.15 K.

14. From the temperature dependency of the values of K_A calculate a value of $\Delta H^\circ{}_A$ (eq 7 and 8).

15. Iterate (steps 2a to 14) until $\Delta H^\circ{}_A$ converges to within a given tolerance (0.1% of its value).

In effect, the above procedure serves to regress values of K_A, $\Delta H^\circ{}_A$, and λ from the experimental data given in Table II using an equilibrium model of the system.

Results of Calculations. Since the above procedure required assumed values of both the B parameter and $\Delta C_p{}^\circ{}_A$, these calculations were also performed using values of B ranging from 1.0 to 2.2 $mol^{-1/2}$ $kg^{1/2}$ and $\Delta C_p{}^\circ{}_A$ ranging from 0 to 100 J mol^{-1} K^{-1} to access uncertainties to be associated with the regressed parameters due to uncertainties in both B and in $\Delta C_p{}^\circ{}_A$. It was found that 50% of the uncertainty in the value of K_A is attributable to a lack of definite knowledge of the value of the B parameter, while the value of $\Delta H^\circ{}_A$ was affected by only ±0.4 kJ mol^{-1} due to variations in this parameter. The perturbations in the value of $\Delta C_p{}^\circ{}_A$ caused only a moderate increase (0.2 kJ mol^{-1}) in the uncertainty assigned to $\Delta H^\circ{}_A$, and it had a negligible effect (0.03%) on the value of K_A at 298.15 K.

As a result of the calculations described above, the following values were obtained at 298.15 K: $K_A = 1.16 \pm 0.3$ mol kg^{-1}, $\Delta G^\circ{}_A = -0.37 \pm 0.57$ kJ mol^{-1}, $\Delta H^\circ{}_A = 24.8 \pm 2.0$ kJ mol^{-1}, and $\Delta S^\circ{}_A = +84.4 \pm 7$ J mol^{-1} K^{-1}. The assigned uncertainities reflect both the experimental imprecision and the results of variations in both B and $\Delta C_p{}^\circ{}_A$ described above. A value of λ equal to 0.125 mol^{-1} kg was obtained as a by-product of the calculations when a value of 1.6 $mol^{-1/2}$ $kg^{1/2}$ was assumed for B. Since the experiments were performed in such a way as to either eliminate or minimize systematic errors due to interferences, lack of chemical equilibrium, and errors in the quantitative analysis of reactants and products, we believe that the uncertainties assigned to the final results are also large enough to include possible systematic errors.

Comparison with Other Measurements. Havir and Hanson[11] used gas-liquid chromatography to study chemical equilibrium for the L-phenylalanine ammonia-lyase reaction (process B). These measurements were performed at 30 °C, at two different values of pH (6.8 and 8.5), and at ionic strengths varying from 0.3 to 1.5 mol kg^{-1}. Examination of their reported values of the equilibrium constants for process B shows them to be 2–4 times larger than either the corresponding values determined in this study or the values predicted from our equilibrium model of this system. While the effects they observed due to a change in pH are in the direction predicted by our model, the ionic strength dependence of $K_{B,obsd}$ which they determined is larger than our data and model predict.

Another possible pathway to the thermodynamics of the L-phenylalanine ammonia-lyase reaction requires the formation properties of the individual compounds combined with entropies and Gibbs energies of solution. The formation properties of $NH_4{}^+$(aq) are relatively well-known.[7] The available data on L-phenylalanine(c) include an enthalpy of combustion and formation,[12] a third law entropy,[13] and aqueous solubilities as a function of temperature.[14,15] For *trans*-cinnamic acid(c) there is only an enthalpy of combustion and formation[16] and apparently only one determination of its aqueous solubility.[17] In view of the missing gaps in thermochemical information and since the errors involved in such a cycle calculation can be large, we are unable to utilize this pathway.

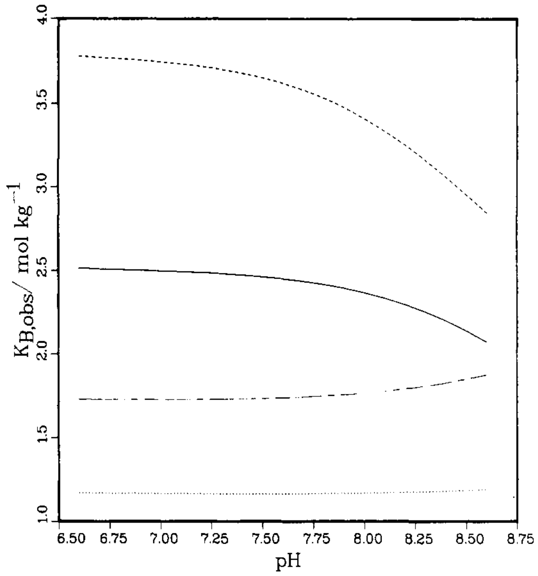

Figure 2. Calculated values of $K_{B,obsd}$ as a function of pH under the following conditions: (—) T = 298.15 K, [∑PhAla] + [∑tca] = 0.0025 mol kg^{-1}, [NH_4Cl] = 1.0 mol kg^{-1}, and [Tris] = 0.10 mol kg^{-1}; (- - -) T = 310.15 K, [∑PhAla] + [∑tca] = 0.0025 mol kg^{-1}, [NH_4Cl] = 1.0 mol kg^{-1}, and [Tris] = 0.10 mol kg^{-1}; (···) T = 298.15 K and I = 0 mol kg^{-1}; (- - -) T = 310.15 K and I = 0 mol kg^{-1}.

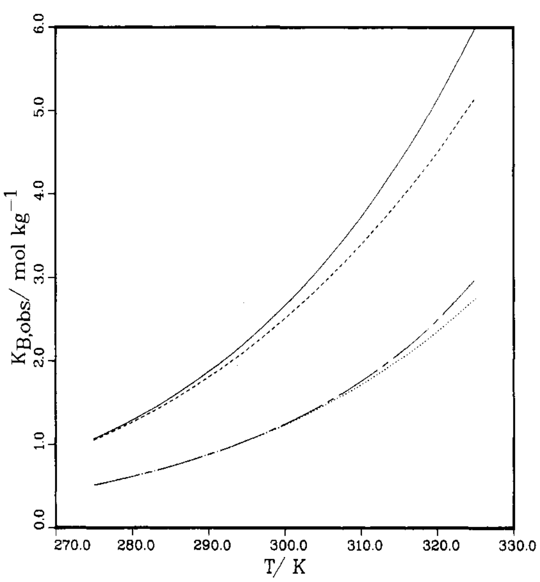

Figure 3. Calculated values of $K_{B,obsd}$ as a function of temperature under the following conditions: (—) pH 7.0, [∑PhAla] + [∑tca] = 0.0025 mol kg^{-1}, [NH_4Cl] = 1.0 mol kg^{-1}, and [Tris] = 0.10 mol kg^{-1}; (- - -) pH 8.0, [∑PhAla] + [∑tca] = 0.0025 mol kg^{-1}, [NH_4Cl] = 1.0 mol kg^{-1}, and [Tris] = 0.10 mol kg^{-1}; (···) pH 7.0 and I = 0 mol kg^{-1}; (- - -) pH 8.0 and I = 0 mol kg^{-1}.

Modeling Calculations. It is also of interest to use the thermodynamic parameters determined herein in conjunction with the equilibrium data involving ionizations of reactants and products to model the equilibrium behavior of the L-phenylalanine ammonia-lyase system as a function of temperature, pH, and solution composition. Note that since the equilibrium model uses an interaction parameter (λ) to calculate activity coefficients, ionic strength cannot be varied independently of solution composition. The results of these calculations which use the thermodynamic

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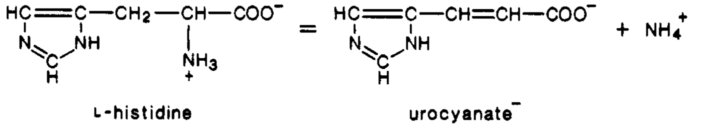
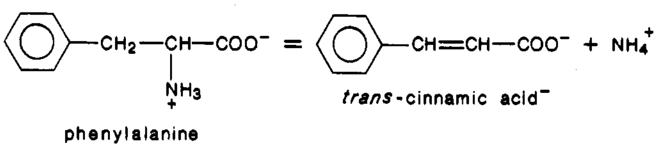
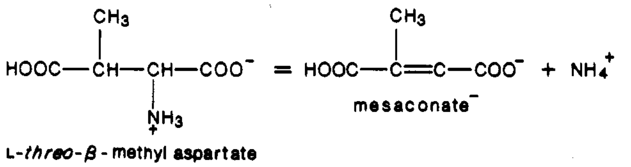
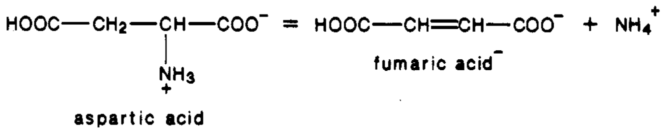
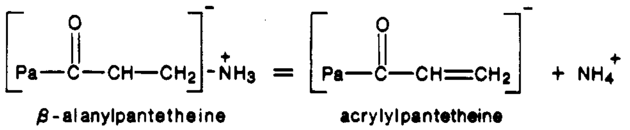
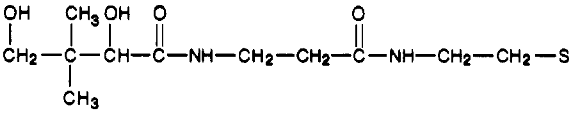
TABLE III: Thermodynamic Parameters for Reactions Involving the Elimination of an Ammonium Ion in Aqueous Solution at 298.15 K[a,b]

	process	$\Delta G°$, kJ mol^{-1}	$\Delta H°$, kJ mol^{-1}	$\Delta S°$, J mol^{-1} K^{-1}	ref
(I)		−2.7			26
(II)		−0.37	24.8	84.4	this work
(III)		3.2			27
(IV)		12.2	32.0	66.3	28
(V)		33.8			29

[a] Note that in all cases the processes have been written so that the ammonium ion is eliminated from the zwitterion to form a species containing a double bond and having a charge of minus one. [b] Pantetheine, abbreviated as Pa, is



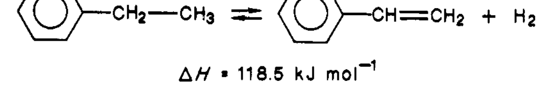
parameters in Table I together with a value of B equal to 1.6 mol$^{-1/2}$ kg$^{1/2}$ and a value of λ equal to 0.125 mol^{-1} kg are shown in Figures 2–4. Since in these calculations ammonium chloride was the major substance present in solutions, the ionic strength is primarily determined by it and is within a few percent of the molality of the ammonium chloride shown in these figures.

Structural Interpretations. In Table III are summarized available thermochemical data on processes in aqueous solution which involve both the formation of a double bond in a compound and the consequent elimination of an ammonium ion. The processes have been arranged in the order of increasing values of the Gibbs energy change. Havir and Hanson[11] have rationalized this order by considering the entropy changes for these processes to be similar. They then attributed the differences in the Gibbs energy changes to differences in the enthalpy changes which can be influenced by effects such as resonance stabilization of the double bonds which are formed. In the discussion to follow we present additional evidence which is consistent with this hypothesis.

Direct evidence for the idea of resonance stabilization of a double bond is obtained by examination of the thermodynamic data[18] for the following gas-phase reactions:

$$CH_3{-}CH_2{-}CH_2{-}CH_3 \rightleftharpoons CH_3{-}CH_2{-}CH{=}CH_2 + H_2$$

$$\Delta H = 126.1\ \text{kJ mol}^{-1}$$



$$\Delta H = 118.5\ \text{kJ mol}^{-1}$$

The difference between the enthalpy changes for the above reactions is 7.6 kJ mol^{-1}. It is very close to the difference (7.2 kJ mol^{-1}) between the enthalpy changes for processes II and IV given in Table III. Note also that the entropy changes for these two processes are comparable and thus in agreement with Havir and Hanson's hypothesis.[11] Evidence that neglect of solvation effects does not cause a serious error in these arguments comes from

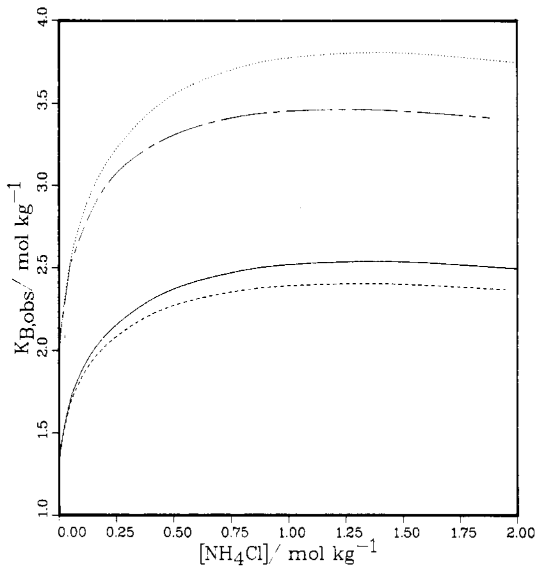


Figure 4. Calculated values of $K_{B,obsd}$ as a function of the molality of NH$_4$Cl under the following conditions: (—) pH 7.0, T = 298.15 K, [$\sum$PhAla] + [$\sum$tca] = 0.0005 mol kg^{-1}, [Tris] = 0 mol kg^{-1}; (- - -) pH 8.0, T = 298.15 K, [$\sum$PhAla] + [$\sum$tca] = 0.0005 mol kg^{-1}, [Tris] = 0 mol kg^{-1}; (···) pH 7.0, T = 310.15 K, [$\sum$PhAla] + [$\sum$tca] = 0.0005 mol kg^{-1}, [Tris] = 0 mol kg^{-1}; (–·–) pH 8.0, T = 310.15 K, [$\sum$PhAla] + [$\sum$tca] = 0.0005 mol kg^{-1}, [Tris] = 0 mol kg^{-1}.

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consideration of thermodynamic data[19] for aqueous ionization reactions at 298.15 K. Specifically, the difference between the enthalpies of ionization of acetic and benzoic acids is only 0.84 kJ mol^{-1} compared to the value of 7.2 kJ mol^{-1} observed for the ammonia elimination reactions. Also, the difference between the entropies of ionization of acetic and benzoic acids is 13.5 J mol^{-1} K^{-1}. This value is very close to the difference (18.1 J mol^{-1} K^{-1}) between the corresponding entropy changes for the ammonia elimination reactions given in Table III.

Also note that in Table III the Gibbs energy change for the ammonia elimination from L-histidine is lower than that for phenylalanine. This is consistent with the increased electronegativity of the nitrogens in the histidine ring structure. In summary, the general argument of Havir and Hanson[11] is strengthened by a variety of evidence. This hypothesis provides an approximate and qualitative scheme for ordering the thermodynamics of ammonia elimination reactions in aqueous solutions.

Industrial Use of Thermodynamic Results. A principal industrial process which utilizes this reaction is the manufacture of L-phenylalanine. This process is accomplished by using an excess of ammonia to drive process B′ to the right:

$$\sum \mathrm{tca} + \sum \mathrm{amm} = \sum \mathrm{PhAla} - n_{\mathrm{H}}\mathrm{H}^+ \qquad (\mathrm{B}')$$

The optimal product yield under a given set of conditions can be calculated from a knowledge of the equilibrium constant ($K_{\mathrm{B}'} = 1/K_{\mathrm{B}}$). Note that the calculation of K_{B} is dependent upon both the thermodynamic data given in Table I and some information on the nonideality of the solution (see eq 9). Examination of the results of the equilibrium calculations performed herein indicates that the optimal product yield of phenylalanine will increase by going to lower temperatures and low ionic strengths. The effect of pH is seen to be insignificant in the pH region 6.5–7.0. Clearly, kinetic and other factors also need to be considered in the design of any industrial process.

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Registry No. E.C. 4.3.1.5, 9024-28-6; L-phenylalanine, 63-91-2.

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Network Topology in Simulated Water

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Configurations of 216 water molecules sampled during the course of isobaric–isothermal simulations over the temperature range −25 to 100 °C at 1 atm pressure, using the TIP4P model, are analyzed to study the hydrogen-bond network topology. Results are presented for the total number of polygons of up to seven molecules and for primitive polygons, being those which have no pair of nonadjacent vertices connected by a bridge which is shorter than either of the paths between these vertices within the polygon itself. We introduce the concept of a reference network, in which each molecule is hydrogen bonded to four others, and report on the temperature dependence of its topological characteristics.

Introduction

Most discussions of the structure of liquid water emphasize the dominant influence of hydrogen bonds and, particularly since Bernal and Fowler's seminal work,[1] many have suggested that the instantaneous structure of the liquid can be modeled by a random hydrogen-bonded network. Thermal excitation of the network may be represented by broken bonds, as in the several "discrete" models, or in terms of intact but variously distorted bonds as in the "continuum" models.[2]

In this work we blur the distinction between the discrete and continuum pictures by defining a reference network in which each molecule is hydrogen bonded to exactly four others, as a limiting case of a discrete model. Our analysis takes configurations generated during the course of previously reported NTp simulations of 216 TIP4P water molecules[3] at −25, 0, 25, 60, and 100 °C. Hydrogen bonds are defined by a "saturated-energetic" criterion. That is, any pair of molecules is regarded as being hydrogen bonded if their energy of interaction is less than an arbitrary cutoff value V_{HB} but if, as a result, any molecule would have more than four hydrogen bonds only the four strongest bonds are retained. The properties of the hydrogen-bonded network which result can then be extrapolated to the state in which $V_{\mathrm{HB}} = 0$ and every molecule is hydrogen bonded to exactly four others. The topological characteristics of this "reference network" should

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