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Proton Affinity of β-Oxalylaminoalanine (BOAA): Incorporation of Direct Entropy Correction into the Single-Reference Kinetic Method

Joshua J. Wind, Lindsay Papp, Maria Happel, Karen Hahn, Erica J. Andriole, and John C. Poutsma
Department of Chemistry, The College of William and Mary, Williamsburg, Virginia, USA

A new version of the single-reference–extended kinetic method is presented in which direct entropy correction is incorporated. Results of calibration experiments with the monodentate base pyridine and the bidentate base ethylenediamine are presented for which the method provides proton affinities in excellent agreement with published values and reasonable predictions for the protonation entropies. The method is then used to determine the proton affinity and protonation entropy of the non-protein amino acid β-oxalylaminoalanine (BOAA). The PA of BOAA is found to be 933.1 ± 7.8 kJ/mol and a prediction for the protonation entropy of -39 J mol^{-1} K^{-1} is also obtained, indicating a significant degree of intramolecular hydrogen bonding in the protonated form. These results are supported by hybrid density functional theory calculations at the B3LYP/6-311++G**//B3LYP/6-31+G* level. They indicate that the preferred site of protonation is the α-nitrogen atom (PA = 935.0 kJ/mol) and that protonated BOAA has a strong hydrogen bond between the hydrogen on the α-amino group and one of the carbonyl oxygen atoms on the side chain. (J Am Soc Mass Spectrom 2005, 16, 1151-1161) © 2005 American Society for Mass Spectrometry

As the building blocks of peptides and proteins, amino acids are of fundamental importance to chemists and biologists alike. Many of their physical properties, including the pKas of various sites, have been determined in solution [1]. With the advent of soft ionization sources such as MALDI [2] and ESI [3], it has become possible to determine the intrinsic gas-phase properties of amino acids and other compounds of biological relevance in modern mass spectrometers [4–11]. While it is now relatively straightforward to form protonated/deprotonated amino acids with these soft ionization sources, generating sufficient quantities of neutral amino acids for gas-phase equilibrium studies is still a challenge [6, 12, 13]. On the other hand, forming proton-bound dimer ions containing an amino acid and a reference compound for kinetic method experiments has become routine [5, 11, 14–22]. Consequently, the extended kinetic method [17, 23–26] has become very popular for use in the determination of thermochemical properties of amino acids and other compounds of biological interest.

While many gas-phase properties of the protein amino acids (PAA), the twenty amino acids that are used for building peptides and proteins, have been established, there are still some discrepancies between recent studies and the evaluated values of Hunter and Lias [27] that are listed on the NIST website [28]. For example, the proton affinity of proline, as listed in the NIST compilation [27], is nearly 16 kJ/mol lower than recent redeterminations from our group [20] and from others [18]. Because of these discrepancies, we have avoided using the amino acids as reference bases in our kinetic method experiments. Instead, we use simple amines for which equilibrium experiments have been performed and a consensus has been reached on their proton affinities [27].

We have recently been studying the consequences of simple substitutions on the thermochemical properties of amino acids by determining the gas-phase acidities and basicities of the so-called nonprotein amino acids (NPAAs) [20, 22, 29]. Ubiquitous in nature, NPAAs are found as secondary metabolites in a variety of plants and fungi [30]. Because many of them have structures that are similar to one or more of the PAAs, they make attractive candidates for investigating substituent effects on amino acid thermochemistry. Herein, we describe the combination of two different forms of the extended kinetic method and the application of this method to the determination of the gas-phase proton

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Address reprint requests to Dr. J. C. Poutsma, Department of Chemistry, The College of William and Mary, Williamsburg, VA 23187-8795, USA. E-mail: jcpout@wm.edu

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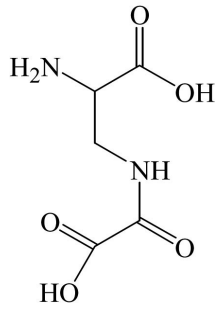
affinity of the NPAA β-oxalylaminoalanine (BOAA, **1**). BOAA is found in the chickling pea, a food staple in India and parts of Africa, and is an agonist of glutamic acid.



BOAA

In addition, BOAA is thought to play a role in the neurological disorder neurolathyrism [31, 32].

In its simplest form, the kinetic method (KM) relies on of the dissociation of proton- (or other ion-) bound dimer ions of the form B_i-H^+-A, where A is the analyte species of unknown proton affinity and B_i is one of a series of reference bases of known proton affinity [23, 33]. The proton-bound dimer ion is dissociated through either collision-induced dissociation or metastable ion decomposition to give the protonated monomer ions AH^+ and B_iH^+ with intensities I_a and I_b according to eq 1. The natural logarithm of the

$$AH^+ + B_i \overset{k_A}{\leftarrow} A - H^+ - B_i \overset{k_B}{\rightarrow} B_iH^+ + A \quad (1)$$

dissociation rates is related to the difference in critical energies and transition state partition functions for the two dissociation channels as shown in eq 2 [34].

$$\ln\left(\frac{k_B}{k_A}\right) \approx \ln\left(\frac{I_B}{I_A}\right) \approx \ln\left(\frac{Q_B^*}{Q_A^*}\right) + \frac{\Delta\varepsilon_0}{RT_{eff}} \quad (2)$$

Under the assumption that there is no reverse activation barrier to dissociation through either channel, the difference in critical energy can be approximated by the difference in PA of A and B and the partition function term can be replaced by the difference in entropies to give the familiar form of the standard kinetic method equation as shown in eq 3.

$$\ln\left(\frac{I_B}{I_A}\right) \approx \frac{PA(B) - PA(A)}{RT_{eff}} + \frac{\Delta S(B) - \Delta S(A)}{R} \quad (3)$$

The earliest studies using the kinetic method involved using reference compounds with structures that were similar to the analyte so that the entropic term in eq 3 is negligible. An alternate approach was to choose a set of reference compounds that have similar structure to each other but not to the analyte. This method was used in the electron affinity studies of Wenthold and Squires [35] and the gas-phase acidity work of O'Hair and coworkers [5]. In this method, a calibration curve is generated using a single reference compound (Compound A) and a series of Compounds B_i. In a separate experiment, the analyte compound (Compound C) is paired with the single reference Compound A, and the thermochemical property of interest is obtained from interpolation.

In the mid 1990s, Wu and Fenselau [17], and Cerda and Wesdemiotis [24], independently developed an extended kinetic method (EKM) that utilizes measurements of ion ratios at several effective temperatures, allowing the separation of enthalpic and entropic contributions to the decomposition of the proton-bound dimer. Armentrout recently suggested modifications to their approaches that correctly account for correlation between the slope and intercept in the first kinetic method plot (see below) [25]. The working equation for this method is shown in eq 4, where PA_{avg} is the average

$$\ln\left(\frac{I_{B_i}}{I_A}\right) \approx \frac{PA(B_i) - PA_{avg}}{RT_{eff}} - \frac{PA(A) - PA_{avg}}{RT_{eff}} + \frac{\Delta S(B_i)}{R} - \frac{\Delta S(A)}{R} \quad (4)$$

PA of the set of *i* reference bases. A plot of ln (I_{Bi} /I_A) versus $PA(B_i)$–PA_{avg} gives a straight line with slope $1/RT_{eff}$ and intercept–{$PA(A)$–PA_{avg}}/RT_{eff} + δΔS/R. Separate plots are made for data taken at several effective temperatures and a second plot of the negative of the intercepts versus the slopes of these lines is generated. The slope of this line is $PA(A)$–PA_{avg} and the intercept is {$\Delta S(A)$–$\Delta S(B_i)$}/R, where this entropy term is the average difference between the entropy of A and the entropies of the individual reference bases, B_i. Typically, entropies of protonation, ΔS_p are tabulated as the difference in entropy between M and MH^+, neglecting the constant entropy of the proton (108.8 J mol^{-1} K^{-1}) [26, 27]. Unless otherwise noted, this convention is used throughout the rest of this manuscript.

Lardin et al. have recently demonstrated an extended form of the *single-reference* kinetic method in which ion ratios are generated at different effective temperatures [36]. In this approach, calibration curves are generated at several different effective temperatures. The proton-bound dimer ion between reference Compound A and the unknown Compound C is generated and allowed to decompose as governed by eq 5. If the measurements are taken at the same effective temperatures as

$$\ln\left(\frac{I_A}{I_C}\right) \approx \frac{PA(A)}{RT_{eff}} - \frac{PA(C)}{RT_{eff}} + \frac{\Delta S(A)}{R} - \frac{\Delta S(C)}{R} \quad (5)$$

the calibrations, then eq 5 can be added to eq 4 to give the required ratios for the extended kinetic method analysis as shown in eq 6.

$$\ln\left(\frac{I_{B_i}}{I_C}\right) \approx \frac{PA(B_i) - PA_{avg}}{RT_{eff}} - \frac{PA(C) - PA_{avg}}{RT_{eff}} + \frac{\Delta S(B_i)}{R} - \frac{\Delta S(C)}{R} \quad (6)$$

This method was used by Lardin et al. to determine the electron affinities of naphthyl radicals using SO_2 as a reference and was also recently used by our group to determine the proton affinity of *cis*-1,5-diaminocyclooctane using the non-protein amino acid canavanine as a reference [37].

Recently, Zheng and Cooks developed an entropy-corrected version of the EKM in which the protonation entropies of the reference bases, $\Delta S(B_i)$, are explicitly taken into account in the analysis [26]. Subtraction of $\Delta S(B_i)/R$ from both sides of eq 4 gives

$$\ln\left(\frac{I_{Bi}}{I_A}\right) - \frac{\Delta S(B_i)}{R} \approx \frac{PA(B_{i)} - PA_{avg}}{RT_{eff}} - \frac{PA(A) - PA_{avg}}{RT_{eff}} - \frac{\Delta S(A)}{R} \quad (7)$$

In this case, the protonation entropies of the individual bases are subtracted from $\ln(I_{Bi}/I_A)$ and are plotted against $PA(B_i)$–PA_{avg} to generate the first kinetic method plot. The rest of the analysis remains the same as outlined above. Cooks showed that the entropy-corrected kinetic method correctly predicts both the PA and ΔS for urea using several different reference base sets [26]. We recently applied this method to a series of lysine homologs and showed that the entropies obtained were in reasonable agreement with predictions from model compounds, the α,ω diamines$^+$ [22].

It is a simple extension to modify the extended single-reference method to explicitly account for direct entropy correction. Subtraction of $\Delta S(B_i)/R$ from both sides of eq 6 yields eq 8. The ratios I_{Bi}/I_C are obtained by multiplying the ratios

$$\ln\left(\frac{I_{B_i}}{I_C}\right) - \frac{\Delta S(B_i)}{R} \approx \frac{PA(B_i) - PA_{avg}}{RT_{eff}} - \frac{PA(C) - PA_{avg}}{RT_{eff}} - \frac{\Delta S(C)}{R} \quad (8)$$

I_{Bi}/I_A obtained from the calibration experiments by I_A/I_C at several different effective temperatures. Plots of $\{\ln (I_{Bi}/I_C)–\Delta S(B_i)/R\}$ versus $PA(B_i)$–PA_{avg} at different effective temperatures give straight lines with slopes $1/RT_{eff}$ and intercepts $–\{PA(C)–PA_{avg}\}/RT_{eff}–\Delta S(C)/R$. A subsequent plot of the negative intercept of the lines versus their slopes gives a straight line with slope $PA(C)–PA_{avg}$ and intercept $\Delta S(C)/R$.

We describe here the calibration of this method with two amines possessing known thermochemistry, pyridine (**3**), a monodentate base and ethylenediamine (**5**), a molecule that is expected to form an intramolecular hydrogen bond when protonated. We then describe the application of the method to the determination of the proton affinity of the nonprotein amino acid BOAA. We also report here the results of hybrid density functional theory calculations that support our experimental proton affinity and lend support for the use of the combined approach.

As both ethylenediamine and BOAA are expected to form intramolecular hydrogen bonds when protonated, it is prudent to assess the ability of the extended kinetic method to obtain the proton affinities of such bidentate bases. Several studies have been undertaken to evaluate the efficacy of using the extended kinetic method to study multifunctional bases, with mixed results. For example, the PA of 1,4-diaminobutane has been determined using the kinetic method in a variety of instruments including sector instruments [38, 39], triple quadrupoles [40, 41], and ion traps [22]. Equilibrium methods that explicitly account for protonation entropy effects place the PA of 1,4-diamine at 1005 kJ/mol [42]. This value is supported by recent theoretical studies by Bouchoux et al. [43]. Experimental values that are within 4–5 kJ/mol of the recommended proton affinity were obtained from our ion trap study [22], from Bouchoux's studies using the isothermal point approach [39, 41], and from Siu and coworkers' triple quadrupole work [40], provided the extended form of the kinetic method is used. In contrast, the recommended PA value of 1005 kJ/mol is 12.5 kJ/mol larger than Wesdemiotis and coworkers' value [21] and nearly 30 kJ/mol higher than Holmes and associates' value [38]. The origins of this discrepancy are unclear, but a growing consensus is that if the entropy term is not too

Table 1. Measured and calculated thermochemical values at 298 K

Measured data		
Compound	PA (kJ/mol)	ΔS (J mol^{-1} K^{-1})
BOAA	933.1 ± 7.9	−39 ± 10
Benzylamine	922.7 ± 7.8	−3 ± 10
Pyridine	936.5 ± 8.5	−7 ± 10
Pyrrolidine	950.5 ± 7.9	−8 ± 10
Ethylenediamine	954.1 ± 8.0	−43 ± 10

Calculated Data		
Compound	PA raw	PA iso
BOAA[a]	934.5	935.0
BOAA[b]	871.1	871.6
Ethylenediamine	950.9	–

[a]Data for protonation at α-nitrogen.
[b]Data for protonation at δ-carbonyl.

Table 2. Total electronic energies, zero-point energies, enthalpies at 298 K, entropies and free energies for BOAA and its protonated forms[a]

Molecule	$E_{electronic}$	ZPE	ΔH_{therm}	H_{298}
BOAA	−681.222199	0.148531	0.013051	−681.060617
$BOAAH^+$ (N-α)	−681.589123	0.162094	0.012830	−681.414199
$BOAAH^+$ (N-β)	−681.548718	0.160813	0.012815	−681.374058
$BOAAH^+(CO_{back})$	−681.519779	0.161630	0.013030	−681.347521
$BOAAH^+(CO_\delta)$	−681.563692	0.159124	0.013134	−681.390063
EDA	−190.587580	0.109154	0.006270	−190.468657
$EDAH^+$	−190.962481	0.124229	0.005993	−190.838499

[a]All values from calculations at the B3LYP/6-311 ++ G**//B3LYP/6-31 + G* level of theory in Hartrees.

large (vide infra), the derived ion affinity (enthalpy) should have systematic errors of less than a few kJ/mol [44–49].

Experimental and Theoretical Procedures

All experiments were performed in a Finnigan LCQ-DECA ion trap instrument using procedures that have been described in detail elsewhere [20]. Briefly, proton-bound dimer ions of an analyte of interest and one of a series of reference bases of known proton affinity [28] are generated using electrospray ionization from dilute solutions of slightly acidified (1%) 50:50 H_2O:CH_3OH. Electrospray ionization and ion focusing conditions are adjusted in order to maximize the production of the proton-bound dimer ions.

The proton-bound dimer ions are isolated at q_z = 0.250 and allowed to undergo collision-induced dissociation with the background helium buffer gas at several different activation energies. For these studies, an activation time of 30 ms and activation amplitudes between 15 and 85% (0.75–4.25 $V_{p\text{-}p}$, lab) were used. Total ion intensities for the protonated amino acid and protonated reference base products were obtained from signal averaging 40 scans. The final ion intensities are averages of 10–15 experiments collected over several days. Proton affinities and activation entropies for each amino acid were obtained using the entropy-corrected single-reference extended kinetic method as described above and are listed in Table 1.

Table 3. Proton affinities and protonation entropies of reference compounds

Compound	PA 298 K (kJ/mol)[a]	$\Delta S/R$[a]
n-butylamine	921.5	−0.96
Heptylamine	923.2	−0.60
Benzylamine	924.0[b]	−0.60
Hexylamine	927.5	−0.60
s-Butylamine	929.7	−0.60
t-Butylamine	934.1	−0.72
3-Methyoxypryidine	942.7	0.24
4-Vinylpyridine	944.1	0.24
4-Methylpyridine	947.2	0.24
Pyrrolidine	948.3	−0.24
Piperidine	954.0	−0.23
Ethylenediamine[c]	951.6	–

[a]Data from reference 27 unless otherwise noted.
[b]Data from Reference 38.
[c]used as isodesmic reference base for theoretical studies.

A prediction for the proton affinity for BOAA was also obtained using hybrid density functional theory. Optimized structures for neutral BOAA as well as several of its protonated forms were obtained using the B3LYP method [50, 51] with a 6-31+G* basis set using the Gaussian 98 suite of programs [52]. Table 2 shows the total electronic energy, zero-point and thermal corrections, and the derived ΔH_{298} values for BOAA, various protonated forms of BOAA, and neutral and protonated ethylenediamine, which was used as an isodesmic reference base. Stationary points were verified to be minima by the absence of negative eigenvalues in the Hessian Matrix. Zero-point energies were calculated from scaled (0.9806 scaling factor) [53] vibrational frequencies. Single-point energies were calculated at the B3LYP/6-311++G** level of theory at the optimized B3LYP/6-31+G* geometries. Total electronic energies at 0 K were converted to enthalpies at 298 K by adding the integrated heat capacity and a PV work term. The vibrational contribution to the heat capacity was obtained from scaled vibrational frequencies with a scaling factor of 0.9989 [53].

The absolute proton affinities of several different sites in BOAA were determined using the isodesmic reaction shown in eq 9, with ethylenediamine serving as the reference base, and are listed in Table 1. The enthalpy at 298 K of eq 9 was determined

$$M + NH_2CH_2CH_2NH_3^+ \rightarrow MH^+ + NH_2CH_2CH_2NH_2 \quad (9)$$

at the B3LYP/6-311++G**//B3LYP/6-31+G* level of theory and is combined with the experimental proton affinity of ethylenediamine (PA = 951.6 kJ/mol) [27, 40] to give a prediction for the proton affinity of BOAA as shown in Table 1.

Materials

BOAA was purchased from Sigma (St. Louis, MO), however as of the time of the writing of this manuscript, BOAA is no longer commercially available. Reference

Table 4. Ion ratios from extended kinetic method experiments

$\ln(B_iH^+/MH^+) - \Delta S_p(B_i)$						
		M = benzylamine (2)				
Reference (B_i)	PA-PA_{avg}	15%	25%	35%	50%	85%
n-Butylamine	−5.2	−0.1288	−0.2015	−0.2104	−0.2504	−0.3061
Heptylamine	−3.5	1.1711	1.1952	1.2108	1.2074	1.2652
Benzylamine	−2.7	0.6014	0.6014	0.6014	0.6014	0.6014
Hexylamine	0.8	1.1658	1.1255	1.1355	1.1365	1.1437
s-Butylamine	3.0	2.2871	2.1077	2.0933	2.1097	1.8283
t-Butylamine	7.4	3.5569	3.3154	3.3059	3.3204	2.9379
		M = pyridine (3)[a]				
Reference (B_i)	PA-PA_{avg}	15%	25%	35%	50%	85%
n-Butylamine	−5.2	−3.3322	−3.2959	−3.29955	−3.1310	−2.8363
Heptylamine	−3.5	−2.0324	−1.8992	−1.8744	−1.6732	−1.2650
Benzylamine	−2.7	−2.6021	−2.4930	−2.4837	−2.2792	−1.9288
Hexylamine	0.8	−2.0377	−1.9689	−1.9496	−1.7441	−1.3865
s-Butylamine	3.0	−0.9164	−0.9867	−0.9918	−0.7709	−0.7019
t-Butylamine	7.4	0.3534	0.2210	0.2208	0.4398	0.4077
		M = BOAA (1)[b]				
Reference (B_i)	PA-PA_{avg}		25%	35%	50%	85%
n-Butylamine	−5.2		1.6208	1.6149	1.5440	1.8132
Heptylamine	−3.5		3.0175	3.0365	3.0018	3.3845
Benzylamine	−2.7		2.4237	2.4267	2.3958	2.7206
Hexylamine	0.8		2.9478	2.9608	2.9309	3.2630
s-Butylamine	3.0		3.9300	3.9186	3.9041	3.9475
t-Butylamine	7.4		5.1376	5.1312	5.1148	5.0571
		M = pyrrolidine (4)				
Reference (B_i)	PA-PA_{avg}	15%	32.5%	50%	67.5%	
3-Methoxypyridine	−4.6	−2.6944	−2.6944	−2.4433	−2.4260	
4-Vinylpyridine	−3.2	−1.4486	−1.4486	−1.3044	−1.3344	
4-Methylpyridine	−0.1	−1.3039	−1.1300	−1.1471	−1.1662	
Pyrrolidine	1.0	0.2406	0.2406	0.2406	0.2406	
Piperidine	6.7	2.6819	2.5912	2.5898	2.6024	
		M = ethylenediamine (5)[c]				
Reference (B_i)	PA-PA_{avg}	15%	32.5%	50%	67.5%	
3-Methoxypyridine	−4.6	−0.1395	0.1788	0.2352	0.1952	
4-Vinylpyridine	−3.2	1.1062	1.3769	1.3741	1.2868	
4-Methylpyridine	−0.1	1.2518	1.5345	1.5315	1.4550	
Pyrrolidine	1.0	2.7955	2.9051	2.9191	2.8618	
Piperidine	6.7	5.2368	5.2557	5.2683	5.2235	
		ratios for single reference experiments				
	15%	25%	35%	50%	85%	
$\ln(3H^+/2H^+)$	3.2035	3.0944	3.0851	2.8806	2.5302	
$\ln(1H^+/2H^+)$		−1.8223	−1.8253	−1.7944	−2.1193	
	15%	32.5%	50%	67.5%		
$\ln(5H^+/4H^+)$	−2.5549	−2.6645	−2.6785	−2.6212		

[a]entries correspond to $\{[\ln(B_iH^+/2H^+) - \Delta S_p(B_i)] - \ln(3H^+/2H^+)\}$.
[b]entries correspond to $\{[\ln(B_iH^+/2H^+) - \Delta S_p(B_i)] - \ln(1H^+/2H^+)\}$.
[c]entries correspond to $\{[\ln(B_iH^+/4H^+) - \Delta S_p(B_i)] - \ln(5H^+/4H^+)\}$.

bases were purchased from Aldrich (Milwaukee, WI) and were used as supplied.

Results and Discussion

Benzylamine Calibration Curve

Several requirements are needed in order for a reference base to be used in a kinetic method experiment: (1) The reference base and analyte must form proton-bound dimers in sufficient intensity for isolation and collision-induced dissociation; (2) the reference base must have an effective basicity such that there is significant intensity from both dissociation channels; and (3) CID of the proton-bound dimer ions must only give the two desired protonated monomer products with no additional fragmentation. In the case of BOAA, only one reference

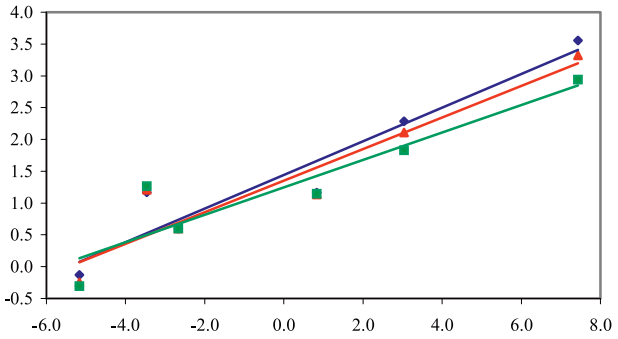


Figure 1. Plot of [ln ($B_iH^+/\mathbf{2H}^+$)–$\Delta S_{Bi}/R$] versus PA_{Bi}–PA_{avg} (kJ/mol) at activation amplitudes 15% (filled diamond), 35% (filled triangle), and 85% (filled square). Data at 25 and 50% omitted for clarity.

base, benzylamine (**2**), was found that fit all of the criteria listed above. The new single-reference variant [5, 35, 36] of the entropy-corrected [26] extended kinetic method described above was therefore used for the BOAA study with benzylamine serving as the reference base.

For these studies, proton-bound dimer ions were generated between benzylamine(**2**) and several reference bases (B_i). The ratios ($B_iH^+/\mathbf{2H}^+$) were recorded at five different activation energies. The following bases were used to construct a calibration curve: n-butylamine, heptylamine, hexylamine, *sec*-butylamine, and *t*-butylamine. The proton affinities for the reference bases are listed in Table 3. In addition to these five bases, we included the $\ln(\mathbf{2H}^+/\mathbf{2H}^+) = 0$ point at the recently redetermined PA for benzylamine of 924.0 [38]. Measured ion ratios for all experiments described in this work are listed in Table 4. The proton affinity of benzylamine is not used in the single-reference kinetic method approach; however, in order to check the validity of the calibrations curves, a proton affinity for benzylamine was obtained using the entropy-corrected extended kinetic method approach. The first and second kinetic method plots for the benzylamine calibration are shown in Figures 1 and 2. The derived proton affinity value (Table 1) of 922.7 ± 7.8 kJ/mol is in excellent agreement with the recent redetermination by Holmes and coworkers of 924 ± 4 kJ/mol [38], and with the older value of 925.5 kJ/mol from Aue and Bowers [54]. It should be noted that the value of 913 kJ/mol listed in the NIST compilation [27] is most likely incorrect. The overall uncertainty in the PA is derived from the uncertainty in the slope of plot 2 (±0.9 kJ/mol) and the uncertainty in assigning the average to the five reference bases. We assign a conservative systematic error in the PA scale of ±7.2 kJ/mol, which is combined with the relative error of three measurements (7.2/$\sqrt{6}$ kJ/mol) to give the uncertainty of ±7.7 kJ/mol for PA_{avg}. The uncertainty in the derived proton affinity term is dominated by the uncertainty in the proton affinities of the reference base. In fact, an inspection of the ion ratios from Table 4 (and Figure 1) indicates that the recommended value for the proton affinity of heptylamine is probably between that of hexylamine and *s*-butylamine and is probably 3–4 kJ/mol too low. One advantage of the kinetic method is that the errors in the proton affinities of the individual reference bases are minimized by the statistical averaging in the construction of the first kinetic method plot.

We also used the new entropy-corrected extended kinetic method to obtain a prediction for ΔS_p for benzylamine. The derived entropy term from extended kinetic method experiments has received considerable attention recently [44–49]. Ervin used RRKM calculations to investigate entropic effects in the metastable ion decomposition version of the kinetic method and concluded that the derived entropy term is not a thermodynamic quantity, but a difference in microcanonical energy states between the two competing channels [44]. Nevertheless, Zheng and Cooks showed that the entropy-corrected EKM can reproduce the experimental protonation entropy for urea [26].

In a recent study by Drahos and Vekey, the authors used the MassKinetics program to simulate the results of kinetic method experiments [45]. In this work, the authors concluded that protonation *enthalpies* are well reproduced by kinetic method experiments, whereas the derived entropy terms are slightly underestimated. They suggest that the derived entropy terms should be multiplied by a constant factor of 1.35 [45]. Shortly after this publication, we published the results of a study of the proton affinity of a series of lysine analogs and showed that (1) the PA value for lysine agrees with previous experimental determinations and (2) the derived entropy terms from the kinetic method experiments were in the range of ΔS values for the analogous α,ω-diamines of the same chain length [22]. It was also pointed out that the experimental determinations of ΔS for the diamines (n = 2–5) varied over 30–40 J mol^{-1} K^{-1} for each diamine [27]. We chose to assign conservative error bars (±10 J mol^{-1} K^{-1}) rather than to use an arbitrary scaling factor [22].

Three feature commentaries on the Vekey paper were recently published in which the conclusions from the Vekey paper [45] were evaluated and additional data was presented in an effort to try to come to a consensus on how to handle entropy in the kinetic

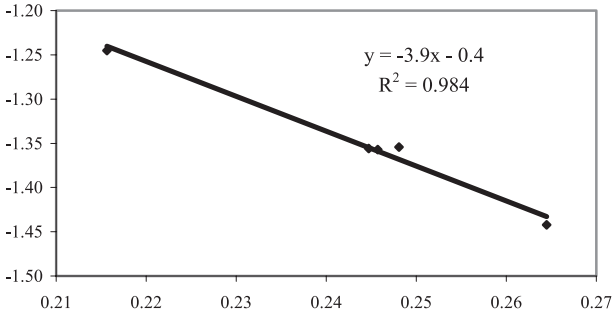


Figure 2. Plot of [(PA_2–PA_{avg})–$T_{eff}\Delta\Delta/R$]/RT_{eff} versus $1/RT_{eff}$.

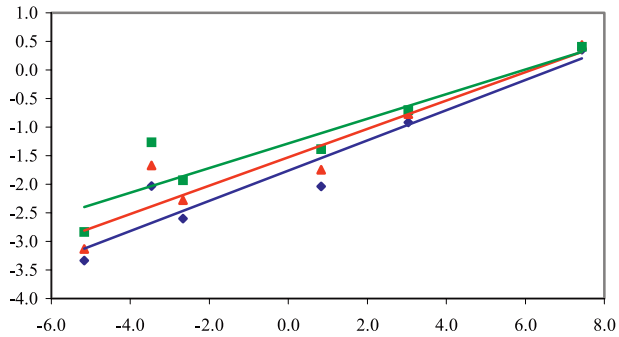


Figure 3. Plot of [ln ($B_iH^+/\mathbf{3H}^+$)–$\Delta S_{Bi}/R$] versus PA_{Bi}–PA_{avg} (kJ/mol) at activation amplitudes 15% (filled diamond), 35% (filled triangle]), and 85% (filled square). Data at 25 and 50% omitted for clarity.

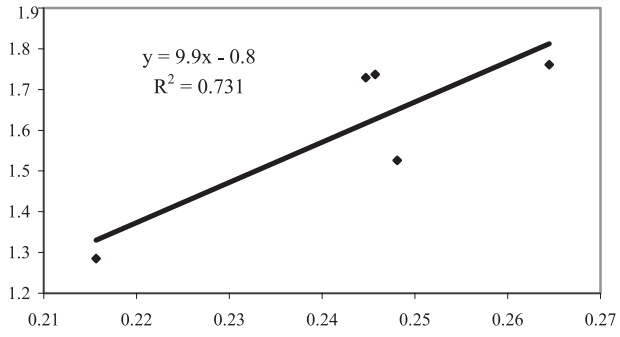


Figure 4. Plot of $[(PA_3–PA_{avg})–T_{eff}\Delta\Delta S/R]/RT_{eff}$ versus $1/RT_{eff}$.

method [46–48]. Bouchoux and coworkers presented results from microcanonical simulations which demonstrate that both the enthalpic terms and entropy terms are slightly underestimated [46]. They see underestimates in the range of 10–15% in ΔS values from the simulations while noting that certain experimental studies [38, 39, 41] have seen underestimations of 50–90%. Ervin and Armentrout [48] also presented simulations of kinetic method data using RRKM theory. They conclude that there can be systematic errors in both the derived enthalpy (±4–12 kJ/mol) and entropy terms (±9–30 J mol^{-1} K^{-1}). Wesdemiotis [47] presented a transition state switching mechanism that helps to explain the underestimation of derived entropy values. In addition, Wesdemiotis pointed out that the satisfactory agreement of our entropy terms in our lysine study [22] probably results from the fact that the dissociations are being carried out very close to threshold. Finally, Vekey had the opportunity to comment on the commentaries [49] and came to the conclusions that (1) the extended kinetic method, rather than its simpler forms, *must* be used to determine thermochemical information for all but the simplest systems, (2) when the entropy difference is less than about 35 J mol^{-1} K^{-1}, the corresponding ion affinities should be accurate, and (3) if entropy effects are large (>35 J mol^{-1} K^{-1}) it is likely that the entropy values will be underestimated. We agree with these assessments and show here that the inclusion of direct entropy effects into the single reference extended kinetic method gives similar accuracy for both enthalpy and entropy values.

The NIST compilation lists a value for ΔS_p for benzyl amine of −5 J mol^{-1} K^{-1}, based on the known entropy of protonation of methylamine. In this case, the entropy-corrected kinetic method gives a prediction of −3 J mol^{-1} K^{-1} for ΔS_p(**2**), in excellent agreement with the recommended value. The uncertainty in this value is not as straightforward as that of the proton affinity values. It contains components from the uncertainty in the intercept of plot 2, the uncertainties in the individual ΔS/R values for the reference bases, and the systematic uncertainty in using transition state entropy difference in place of thermodynamic entropies. In our previous work on the lysine analogs, we adopted conservative error limits of ±10 J mol^{-1} K^{-1} for all derived entropy terms [22]. In light of the recent commentaries [46–48], we feel that continued use of these conservative error limits is warranted.

Verification of PA and ΔS_p for Pyridine and Ethylenediamine

In order to test the validity of combining the entropy-corrected and single-reference versions of the extended kinetic method, we used the combination to re-evaluate the PAs of both a monodentate amine base and a bidentate diamine base with known thermochemistry to try to reproduce ΔS_p values over a wide range. Pyridine has a known proton affinity of 930.0 kJ/mol [27], in the same range as the reference bases used in the benzylamine calibration curve and a ΔS_p of 2 J mol^{-1} K^{-1}. Proton-bound dimer ions between benzylamine and pyridine (**3**) were generated and subjected to collision-induced dissociation at the same five activation energies as the calibration studies. The ratios ($B_iH^+/\mathbf{2H}^+$) are multiplied by the constant ratio ($\mathbf{2H}^+/\mathbf{3H}^+$) at each collision energy to generate ratios that are used to create the kinetic method plot shown in Figure 3. The second kinetic method plot (Figure 4) has a slope and intercept of 9.9 ± 3.5 and −0.8 ± 0.8, from which is

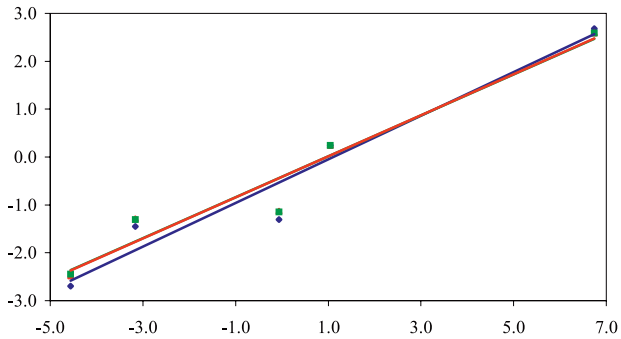


Figure 5. Plot of [ln ($B_iH^+/\mathbf{4H}^+$)–$\Delta S_{Bi}/R$] versus PA_{Bi}–PA_{avg} (kJ/mol) at activation amplitudes 25% (filled diamond), 35% (filled triangle), and 85% (filled square). Data at 50% omitted for clarity.

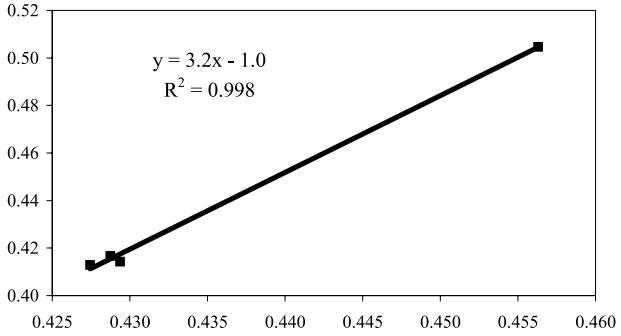


Figure 6. Plot of $[(PA_4–PA_{avg})–T_{eff}\Delta\Delta S/R]/RT_{eff}$ versus $1/RT_{eff}$.

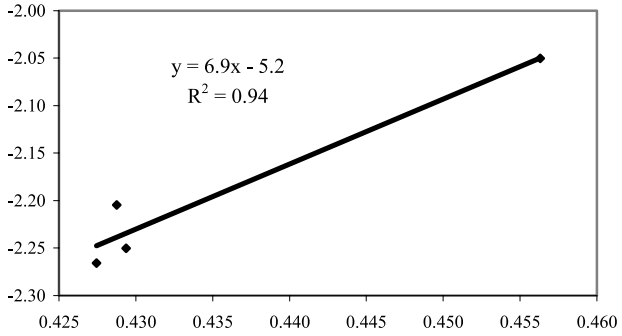


Figure 8. Plot of $[(PA_5–PA_{avg})–T_{eff}\Delta\Delta S/R]/RT_{eff}$ versus $1/RT_{eff}$.

derived a proton affinity of 936.5 ± 8.5 kJ/mol and a protonation entropy of −7 ± 10 J mol^{-1} K^{-1}. In this case, we are measuring an entropy value that is slightly too negative, although the error bars do encompass the recommended value. It should also be noted that the recommended value is based in part on a theory value from Radom and associates [55], and that one of the measured values for ΔS_p from Mautner and Sieck is −0.5 J mol^{-1} K^{-1}, which is in somewhat better agreement with our value [56]. In light of the conservative error bars, we consider the derived entropy term to be in reasonable agreement with previous determinations.

We also wanted to test the method on a bidentate base with a large negative value for ΔS_p. We attempted to use benzylamine as a single reference for the re-evaluation of the PA of ethylenediamine (**5**), but dissociation of the dimer ions produced only $\mathbf{5H^+}$. We therefore needed a different single reference compound with a PA in the same range of **5**. We chose pyrrolidine (**4**), which has a PA of 948.3 kJ/mol and a ΔS_p of −2 J mol^{-1} K^{-1} [27]. A separate calibration curve was created using 3-methoxypyridine, 4-vinylpyridine, 4-picoline, and piperidine as references and four different activation energies in the same range as those used in the benzylamine study. As with the benzylamine calibration curve, we included the $\ln(\mathbf{4H^+}/\mathbf{4H^+}) = 0$ point. Figures 5 and 6 show the first and second plots from the entropy-corrected EKM analysis of the PA of pyrrolidine. Again, the PA of **4** does not enter into the calculation of the PA of **5**, but the results obtained from the EKM analysis gives an indication of the reliability of the calibration curves. The EKM method gives values of 950.5 ± 7.9 kJ/mol and −8 ± 10 J mol^{-1} K^{-1}, in good agreement with the recommended values from Hunter and Lais [27].

Proton-bound dimer ions were generated between pyrrolidine (**4**) and ethylenediamine (**5**) for use in the single reference EKM. Figures 7 and 8 show the first and second EKM plots. The second plot has a slope and intercept of 6.9 ± 1.2 kJ/mol and −5.2 ± 0.5, which correspond to a PA of 954.1 ± 8.0 kJ/mol and a ΔS_p of −43 ± 10 J mol^{-1} K^{-1}. The derived proton affinity is in excellent agreement with the recommended value of 951 kJ/mol from Hunter and Lias [27]. In this case, the derived entropy term is much more negative than the recommended value of −22.1 J mol^{-1} K^{-1} from Hunter and Lias. However, the actual experimental values cited in Hunter's compilation vary from −19 to −55. Our value lies within this range and we consider it to be in reasonable agreement with previous studies. In our lysine study, we redetermined the PA and ΔS_p of **5** using the normal version (i.e., not single reference) of the entropy-corrected EKM to be 956.4 ± 6.5 kJ/mol and −43 J mol^{-1} K^{-1} using a slightly different set of reference bases. The excellent agreement in derived proton affinity and protonation entropies between the normal and single-reference versions of the entropy-corrected EKM indicates that the single-reference variant does not introduce any additional error.

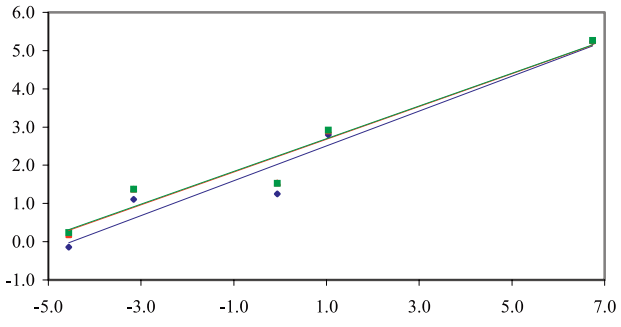


Figure 7. Plot of $[\ln(B_iH^+/\mathbf{5H^+})–\Delta S_{Bi}/R]$ versus $PA_{Bi}–PA_{avg}$ (kJ/mol) at activation amplitudes 25% (filled diamond), 35% (filled triangle), and 50% (filled square). Data at 65% omitted for clarity.

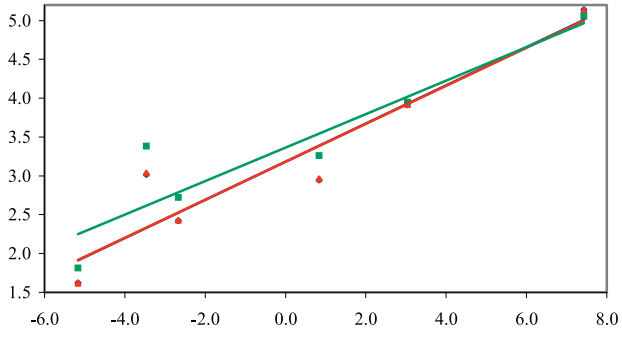


Figure 9. Plot of $[\ln(B_iH^+/\mathbf{1H^+})–\Delta S_{Bi}/R]$ versus $PA_{Bi}–PA_{avg}$ (kJ/mol) at activation amplitudes 25% (filled diamond), 35% (filled triangle), and 50% (filled square). Data at 65% omitted for clarity.

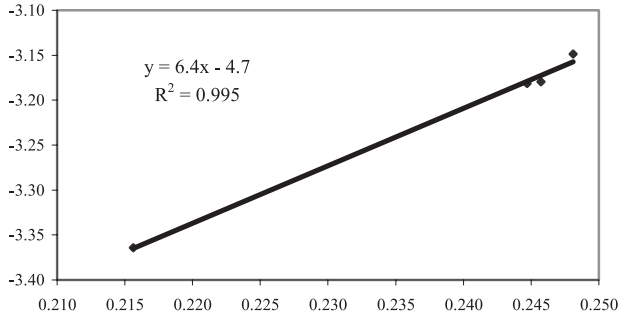


Figure 10. Plot of $[(PA_1–PA_{avg})–T_{eff}\Delta\Delta S/R]/RT_{eff}$ versus $1/RT_{eff}$.

BOAA

Given that we could reproduce the proton affinities of pyridine and ethylenediamine and that we obtained reasonable values for ΔS_p for these species, we felt confident to use the method to determine the PA and a protonation entropy for BOAA. Proton-bound dimer ions of BOAA and benzylamine were generated, isolated, and subjected to collision-induced dissociation at four of the five activation energies from the benzylamine study. The ratios ($\mathbf{2H}^+/\mathbf{1H}^+$) are multiplied by the ratios ($RefH^+/\mathbf{2H}^+$) at each collision energy to give the required ratios for the extended kinetic method analysis. Figures 9 and 10 show the two kinetic method plots for BOAA from the entropy-corrected approach. A proton affinity of 933.1 ± 7.9 kJ/mol and a protonation entropy of −39 ± 10 J mol^{-1} K^{-1} are derived for BOAA. The protonation entropy is on the same order as that of ethylenediamine and suggests that BOAA is forming an internal hydrogen bond when protonated.

Theoretical calculations at the B3LYP/6-311++G**//B3LYP/6-31+G* level of theory were carried out on BOAA and several of its protonated forms. Table 2 gives the total electronic energy, zero-point, and thermal energy corrections, and ΔH_{298} for the lowest energy structures that we found for neutral BOAA and for BOAA with a proton attached at one of four different protonation sites: (1) the α NH_2 group, (2) the β NH group, (3) the backbone C—O group, and (4) the side-chain δ-C—O group. For the cation studies, several initial structures were generated by placing a proton on one of the basic sites and performing a geometry optimization at the B3LYP/6-31 + G* level. Sometimes during the geometry optimizations, some of the higher energy forms isomerized to a lower energy form. Thermochemical quantities for the 7 neutral structures and 34 cation structures are shown in Table T1 of Supporting Information. Gaussian 98 archives containing optimized geometries for these species are found in Table T2 of Supporting Information. The lowest energy structures for BOAA and $BOAAH^+$ are shown in Figure 11. The lowest energy conformer that we located has a weak interaction between a hydrogen atom on the amino nitrogen atom and the ε-CO oxygen (r = 2.219 Å). Upon protonation, the hydrogen bond shifts to the δ-CO group, and the interaction strengthens considerably (r = 1.604 Å). This intramolecular hydrogen bond is consistent with the increased entropy of protonation of −39 J mol^{-1} K^{-1}.

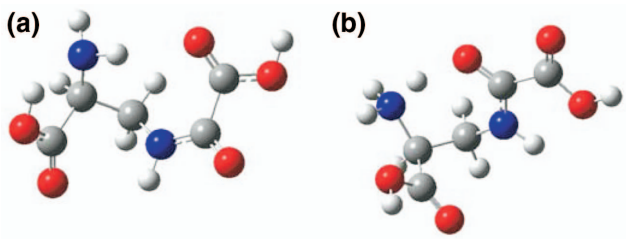
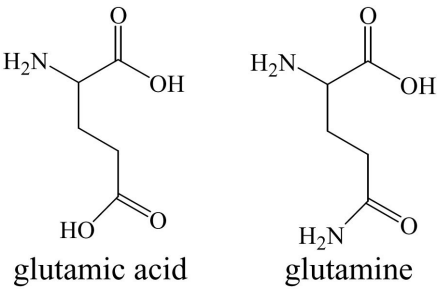


Figure 11. Optimized structures for: (**a**) BOAA and (**b**) BOAA protonated on the α-nitrogen atom from B3LYP/6-31 + G* calculations.

From the computational studies, the following basicity order was established, $N_\alpha > CO_\delta > N_\beta > CO_{back}$. The α amino group is the most basic site in BOAA with a PA of 935.0 kJ/mol predicted from isodesmic reaction in eq 9. The only other strongly basic site is the side-chain carbonyl group with a predicted PA of 871.1 kJ/mol. The uncertainty in these values is probably on the order of ±10 kJ/mol, and the agreement between the experimental PA and PA of the side-chain nitrogen protonated form is excellent.

BOAA is structurally similar to the PAAs glutamic acid and glutamine.



Both of these species can presumably form similar hydrogen bonds between the protonated amino group and the side-chain carbonyl. The difference in PA between Glu and Gln can be rationalized by the difference in hydrogen bonding ability in the cation, with the amide forming a stronger H-bond than the acid. This is supported by the recommended values from the NIST site (PA(Glu) = 913 kJ/mol, PA(Gln) = 937 kJ/mol) [27]. BOAA should have a PA between Glu and Gln as it possesses an amide functionality in the side chain that is tempered by the electron withdrawing ability of the additional COOH group. The experimental proton affinity for BOAA of 933.1 ± 6.5 kJ/mol is consistent with these arguments.

Conclusions

A new entropy-corrected version of the single-reference version of the extended kinetic method has been derived and has been shown to give correct values for the proton affinity for both the monodentate base pyridine and the bidentate base ethylenediamine. In addition, reasonable values for ΔS_p for these compounds are obtained. The method was used to determine the proton affinity and a prediction for the protonation entropy for the nonprotein amino acid BOAA. Theoretical calculations at the B3LYP/6-311++G**//B3LYP/6-31+G* level support the experimental value and provide information as to the preferred site of protonation, the alpha nitrogen of BOAA. Further experiments with known compounds are being carried out in order to develop a database of successes and failures of the entropy-corrected extended kinetic method for the prediction of protonation entropies.

Acknowledgments

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Supplementary Material

Supplementary data associated with this article can be found, in the online version, at doi 10.1016/j.jasms.2005.03.011.

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