Proton Affinity of Canavanine and Canaline, Oxyanalogues of Arginine and Ornithine, from the Extended Kinetic Method

Erica J. Andriole, Kathryn E. Colyer, Elizabeth Cornell, and John C. Poutsma*

Contribution from the Department of Chemistry, The College of William and Mary in Virginia, Williamsburg, Virginia 23187-8795

Received: May 19, 2006; In Final Form: August 17, 2006

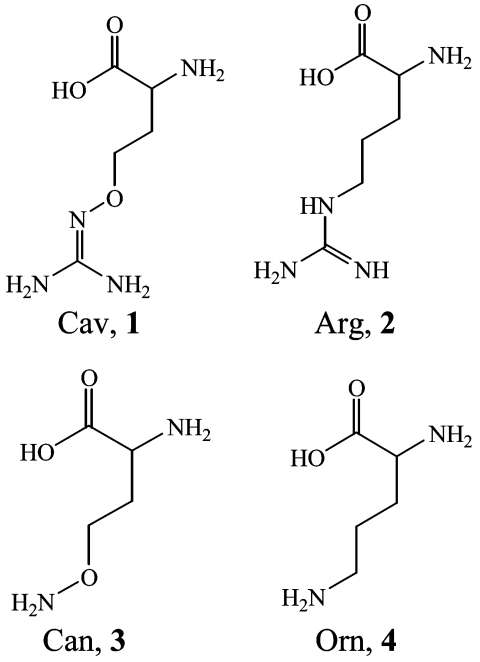
The absolute proton affinities of the nonprotein amino acids canavanine and canaline have been determined using the extended kinetic method in an electrospray ionization quadrupole ion trap instrument. Canavanine results from the substitution of an oxygen atom for the δ-CH_2 group in the side chain of the protein amino acid arginine, whereas canaline results from a similar substitution at the δ-CH_2 group in the side chain of ornithine. Absolute proton affinities of 1001 ± 9 and 950 ± 7 kJ/mol are obtained for canavanine and canaline, respectively. For canaline, this proton affinity is in excellent agreement with theoretical predictions obtained using the hybrid density functional theory method B3LYP/6-311++G**//B3LYP/6-31+G*. For canavanine, theory predicts a somewhat larger proton affinity of 1015 kJ/mol. Oxygen atom substitution in these nonprotein amino acids results in a decrease in their proton affinities of 40−50 kJ/mol compared to arginine and ornithine.

Introduction

As the building blocks of peptides and proteins, amino acids are vitally important biochemical species. A detailed knowledge of their chemical properties is essential to understanding the complex interplay between amino acid structure and biological function. Although there are hundreds of naturally occurring amino acids, only 20 protein amino acids (PAAs) play a role in human protein and peptide synthesis. The advent of soft ionization sources such as electrospray ionization (ESI)[1] and matrix-assisted laser desorption−ionization (MALDI)[2] have allowed powerful mass spectrometry techniques to be applied to amino acids and other species of biological interest. A wide variety of intrinsic gas-phase thermochemical properties of the PAAs have been investigated including proton affinities,[3−9] gas-phase acidities,[10−12] and alkali[13−26] and transition metal ion affinities.[27]

Recently, we have been studying the effects of systematic substitutions on the gas-phase thermochemical properties of amino acids by determining the intrinsic properties of nonprotein amino acids (NPAA).[28−30] The NPAAs[31] are ubiquitous in the plant and fungi kingdoms and serve a wide range of functions ranging from nitrogen storage to defense from predation.[32−35] Many NPAAs are structurally similar to one or more of the PAAs; therefore, they can compete with them in a variety of biological pathways[35−38] or be misincorporated into peptides and proteins.[36,39,40] For example, the nonprotein amino acid canavanine (**1**) results from a simple substitution of an oxygen atom for the δ-CH_2 group in the side chain of the PAA arginine (**2**). Certain insects that feed on canavanine-rich seeds have incidences of substitution of one-third of available arginine residues by canavanine,[40] while others seem to have developed a means to avoid this misincorporation.[34,41] Once misincorporated into a protein, the NPAA can cause changes in structure due to changes in acid/base properties or in hydrogen bonding capability.[36,39] This property has lead researchers to investigate the possibility of using canavanine as an anticancer therapy.[42,43]

Arginine is the most basic of the protein amino acids (pK_a of 12.5 in solution).[44] The proton affinity of arginine was measured using the kinetic method as 1051 ± 8 kJ/mol.[45] Substitution of the oxyguanido group in canavanine for the guanido group in arginine results in a decrease in basicity of 5 pK_a units in solution, with the N-terminus as the most basic site.[36] Arginine is metabolized through the urea cycle to ornithine (**4**) and then to citrulline and argininosuccinate. Species of insects that can tolerate ingestion of canavanine have developed a separate pathway to metabolize it. The first metabolite in this pathway is canaline (**3**), an oxyanalogue of ornithine. Canaline is a potent antimetabolite and appears to be unique among naturally occurring amino acids in its possession of the aminooxy group on the side chain.[46] Oxyanalogues of each metabolite in the urea cycle have been observed.



In addition to their biological relevance, the NPAAs serve as attractive candidates to study the subtle interplay between

10.1021/jp063081f CCC: $33.50
Published on Web 09/16/2006

the structure and energetics of amino acids. We have been using a combined experimental−theoretical approach using the extended kinetic method in a quadrupole ion trap and high-level density functional theory calculations to determine various thermochemical properties of NPAAs. For example, we found a correlation between the proton affinity and ring size in proline and its four- and six-membered ring analogues, azetidine-2-carboxylic acid, and pipecolic acid.[28] In a recent study on the proton affinity of PAA lysine and its NPAA homologues ornithine (**4**), 2,4-diaminobutanoic acid, and 2,3-diaminopropanoic acid, we found a monotonic decrease in both the proton affinity and derived entropy term (vide infra) with chain length.[29]

In this manuscript we report the first experimental determination of the gas-phase proton affinity of canavanine (**1**) and canaline (**3**), oxyanalogues of arginine (**2**) and ornithine (**4**). Also, the results of high-level hybrid density functional theory calculations are presented which give indications for the preferred sites of protonation in these species as well as the relative basicities of other sites. These studies indicate that oxygen atom substitution results in a lowering of the basicity of the side chains of these amino acids on the order of 40 kJ/mol.

Experimental Section

Experimental Methods. All experiments were performed using a Finnigan LCQ Deca quadrupole ion trap equipped with an external ESI source. Heterodimers of the compound of interest with a reference base of known proton affinity were formed in an acidified (1% acetic acid) 49.5:49.5 methanol:water solution. Solution concentrations were varied in order to maximize the production of proton-bound dimers of the amino acid and the reference base and were usually in the range of 1×10^{-4} to 5×10^{-5} M. Solutions were directly infused into the electrospray ionization source at flow rates of 5−20 μL/min. Electrospray and ion-focusing conditions were also varied to maximize the ion count for the proton-bound heterodimer. The proton-bound dimer ions were isolated at $q_z = 0.250$ with a mass width adjusted to maximize ion signal while still maintaining isolation. The isolated ions were allowed to undergo collision-induced dissociation with the background helium atoms. For canavanine, the ratio of the protonated reference base to the protonated amino acid was obtained by performing an activation amplitude scan from 0 to 100% in steps of two. The final ion ratios are averages of at least 3 scans obtained on several different days. For canaline, instead of scanning the activation amplitude, three representative activation amplitudes were chosen.

The Extended Kinetic Method. Proton affinities and entropy contributions are obtained from the extended kinetic method that has been described in detail elsewhere.[17,47−49] The final version of the extended kinetic method takes the form

$$\ln\left(\frac{I_{B_i}}{I_A}\right) \approx \frac{\Delta H_{B_i} - \Delta H_{avg}}{RT_{eff}} - \frac{\Delta H_A - \Delta H_{avg}}{RT_{eff}} + \frac{\Delta S_{B_i}}{R} - \frac{\Delta S_A}{R}$$

from which two plots can be generated. The first plot (plot 1) is of $\ln[I(\mathrm{RefB}_i\mathrm{H}^+)/I(\mathrm{AH}^+)]$ vs $\Delta H_{B_i} - \Delta H_{avg}$, where ΔH_{B_i} is the proton affinity of reference base *i* and ΔH_{avg} is the average proton affinity of the set of 4−5 reference bases. A best-fit line to the data in kinetic method plot 1 yields a slope equal to $1/RT_{eff}$ and a *y*-intercept equal to $-[(\Delta H_A - \Delta H_{avg})/RT_{eff} + \Delta S_A/R - \Delta S_{B_i}/R]$. Each activation energy used yields a different slope and intercept. A plot of the negative of each of the intercepts vs the slopes from plot 1 gives kinetic method plot 2. From this plot the proton affinity and a prediction for the entropy of protonation can be obtained (slope $= \Delta H_A - \Delta H_{avg}$; *y*-intercept $= \Delta S_{B_i}/R - \Delta S_A/R$, vide supra).[29,49]

TABLE 1: Thermochemical Properties of the Reference Bases

base	PA[a] (kJ/mol)	1	3
cyclohexylamine	934		X
exo-2-aminonorbornane	935		X
3-picoline	943		X
4-picoline	947		X
pyrrolidine	948		X
triethylamine	982	X	
N,*N*-dimethylcyclohexylamine	984	X	
2,2,6,6-tetramethylpiperidine	987	X	
tripropylamine	991	X	
N,*N*-diisopropylethylamine	994	X	

[a] Reference 60.

The use of the intercept from plot 2, which is derived from transition-state activation entropy differences, as a quantitative measure for the thermodynamic protonation entropy has received considerable attention in the literature of late.[29,49−55] Ervin carried out Rice−Ramsperger−Kassel and Rice−Ramsperger−Kassel−Marcus (RRKM) calculations in an effort to model the derived entropy term and came to the conclusion that the entropy term is a difference in microcanonical densities of states of the two competing transition states. Recent studies by Bouchoux and co-workers of diols and amino alcohols and by Wesdemiotis and co-workers of β-alanine and α,ω-diamines[56] indicate that enthalpies obtained from extended kinetic method experiments are usually in good agreement with literature values, whereas the entropies were in poorer agreement. Vekey and co-workers performed a series of simulations using the MassKinetics program and came to the conclusion that, whereas enthalpies obtained from the kinetic method are generally in agreement with literature values, entropies are generally underestimated, and they recommended scaling the derived entropy term by 1.35.[51] Three feature commentaries on the Vekey paper were recently published in which the conclusions from the Vekey paper[51] 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 method.[52−54] Bouchoux and co-workers presented results from microcanonical simulations that demonstrate that both the enthalpic terms and entropy terms are slightly underestimated.[52] They saw underestimations in the range of 10−15% in ΔS values from the simulations while noting that certain experimental studies[57−59] have seen underestimations of 50−90%. Ervin and Armentrout[53] 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 presented a transition-state switching mechanism that helps to explain the underestimation of derived entropy values.[54] Finally, Vekey had the opportunity to comment on the commentaries[55] 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. In light of these findings, the entropy values obtained for **1** and **3** are probably underestimated and should be treated as lower limits of the true protonation entropies.

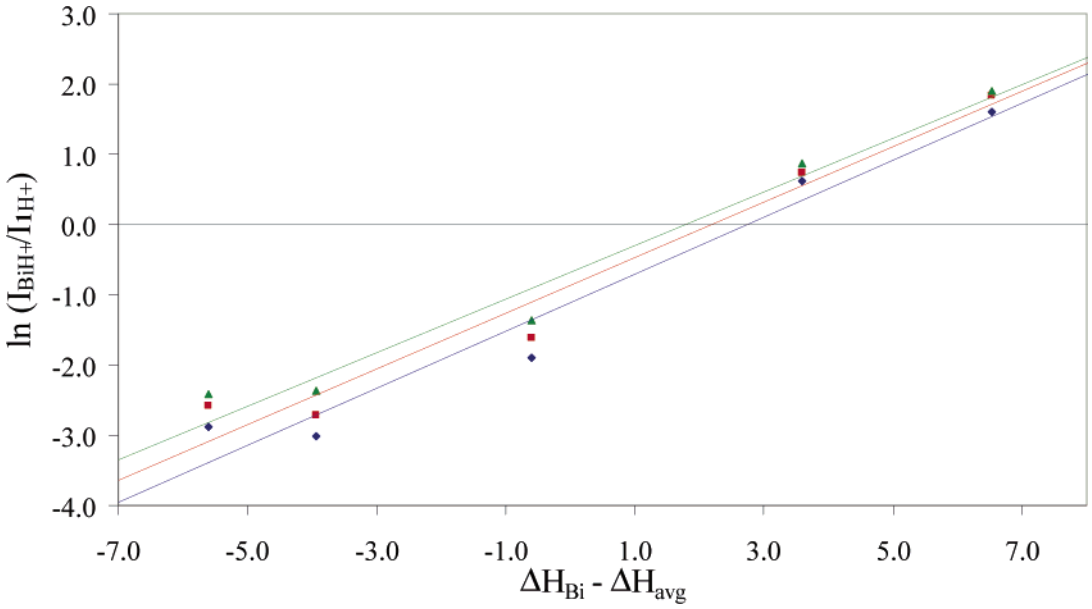


Figure 1. Plot of $\ln(B_iH^+/\mathbf{1}H^+)$ vs $\Delta H_{B_i} - \Delta H_{avg}$. Lines and symbols obtained from activation amplitudes 12% (squares), 22% (diamonds), and 38% (triangles).

Orthogonal Distance Regression (ODR) Analysis. Ervin and Armentrout have recently developed a new approach to fitting kinetic method plots involving an orthogonal distance regression approach.[53] In this procedure, a regression algorithm is used to force *n* best-fit lines at *m* different energies to cross at a common point. The *x*-coordinate of this crossing point corresponds to the enthalpic term of interest and the *y*-coordinate corresponds to the entropic term. Kinetic method data for **1** and **3** were fit with both the traditional and ODR procedures and the actual ion affinities and derived entropy terms are virtually identical. The improvement of the ODR method is that it gives a more realistic estimation of the uncertainty of the derived values. Final uncertainties are obtained from Monte Carlo simulations in which random noise is generated within user-defined ranges of the uncertainties in the proton affinity of each reference base and in the experimental ion ratios. For these studies, the uncertainties in the PA of the reference bases and the ln(ratio) were ±4 kJ/mol and ±0.05, respectively.

Theoretical Procedures. Theoretical values for proton affinities were obtained from hybrid density functional theory calculations using the B3LYP functional combinations.[60,61] All calculations were performed using PCModel and the Gaussian98W suite of programs.[62] A conformational search is first performed using the GMMX algorithm in PCModel and the lowest 20−30 structures for each molecule are then used as starting points for progressively increasing levels of ab initio or density functional theory calculations. Ultimately, geometries and harmonic vibrational frequencies for all amino acids and their protonated forms were calculated at the B3LYP/6-31+G* level. Total electronic energies were obtained from B3LYP/6-311++G** single-point calculations at the B3LYP/6-31+G* geometries. Enthalpies at 298 K were calculated using zero-point energy (ZPE) and thermal corrections from scaled vibrational frequencies.[63]

Predictions for the proton affinities of **1** and **3** were computed directly from calculated enthalpies at 298 K. Proton affinities were also predicted from isodesmic reaction 1 with ethylenediamine (PA = 951 kJ/mol)[64] serving as the reference base

$$AA + NH_2CH_2CH_2NH_3^+ \rightarrow AAH^+ + NH_2CH_2CH_2NH_2 \quad (1)$$

Materials. All chemicals including the nonprotein amino acids were purchased from Sigma-Aldrich (St. Louis) and used without further purification.

Results and Discussion

Canavanine. Proton-bound dimers of canavanine with a series of five reference bases were isolated in the ion trap. To probe as wide a range of effective temperatures as possible, fragmentation ratios were measured by performing an activation amplitude scan from 0 to 100%. At activation amplitudes below 12%, not enough fragmentation occurred to provide an accurate fragmentation ratio. Above 38%, the effective temperature of the collisions levels off as radiative cooling of the activated ions competes with further activation. The kinetic method analysis was therefore performed at activation amplitudes between 12 and 38%. The following compounds, with PA_{avg} = 987.6 kJ mol^{-1}, were used as reference bases: triethylamine, *N*,*N*-dimethylcyclohexylamine, 2,2,6,6-tetramethylpiperidine, tripropylamine, and *N*,*N*-diisopropylethylamine. Proton affinity values for all reference compounds are listed in Table 1. Figure 1 shows a plot of $\ln(I_{B_i}/I_A)$ vs $\Delta H_{B_i} - \Delta H_{avg}$ in which ΔH_{B_i} is the proton affinity of reference base *i* and ΔH_{avg} is the average proton affinity of the five reference bases used. A best-fit line is made for each of the activation amplitudes (only shown for 12, 22, and 38%), and each of those lines yields a slope equal to $1/RT_{eff}$ and a *y*-intercept equal to $-[\Delta H_A - \Delta H_{avg}/RT_{eff} + \Delta S_A/R - \Delta S_{B_i}/R]$. The *x*-intercepts of each of the lines give a range of apparent basicities of 989.4−990.3 kJ mol^{-1}. Figure 2 is obtained by plotting the negative of the *y*-intercepts vs the slopes of each of the lines in Figure 1. From this plot the proton affinity and entropy of dissociation can be obtained. The slope of the best-fit line in Figure 2 is 16.9 kJ mol^{-1}. Adding this to ΔH_{avg} gives a value for the proton affinity for canavanine of 1004.5 kJ mol^{-1}. To determine the entropy of dissociation for canavanine, the intercept of the best-fit line in Figure 2 (−5.8) is multiplied by the gas constant, 8.314 J mol^{-1} K^{-1}, to give a ΔS = −47.9 J mol^{-1} K^{-1}. Final values for all experimental and theoretical quantities are given in Table 2.

Fitting the data with the ODR method gives similar results for the proton affinity of **1** (1001.2 kJ/mol and −38.6 J mol^{-1}

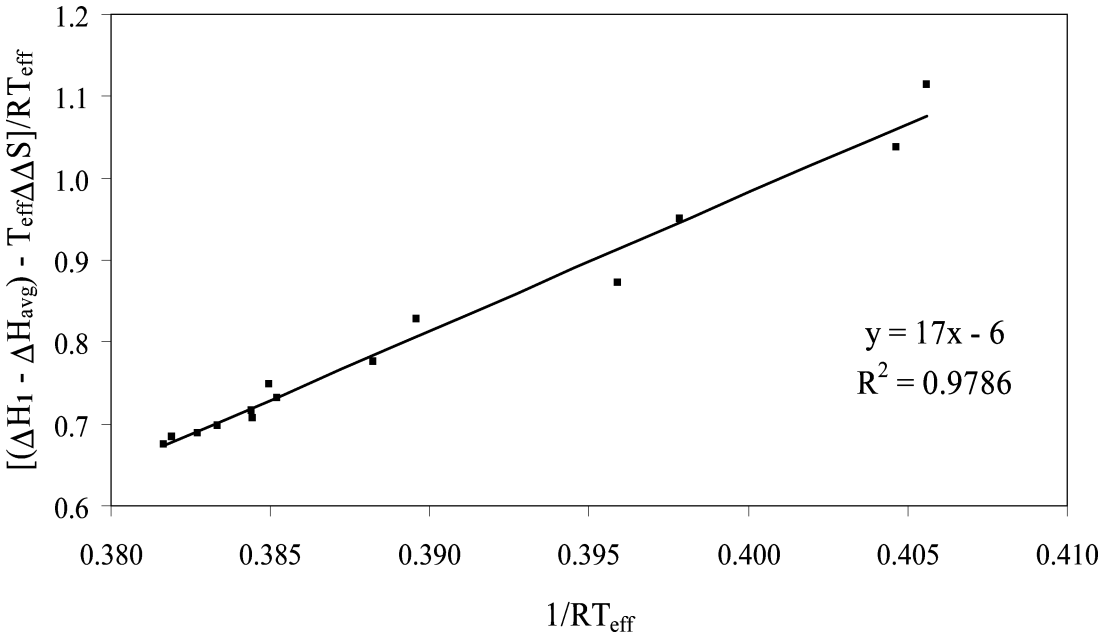


Figure 2. $[(\Delta H_1 - \Delta H_{avg}) - T_{eff}\Delta\Delta S/R]/RT_{eff}$ vs $1/RT_{eff}$. Lines and symbols are obtained from using the entropy-corrected extended kinetic method.

TABLE 2: Experimental and Theoretical Values for Canavanine and Canaline

experimental results				
molecule	PA (kJ/mol)	PA_{ODR} (kJ/mol)	ΔS (J mol^{-1} K^{-1})	ΔS_{ODR} (J mol^{-1} K^{-1})
1	1005	1001 ± 9	−48	−39 ± 21
3	949	950 ± 7	−16	−19 ± 12

theoretical results		
molecule	PA_{raw}	PA_{iso}[a]
1	1014	1015
3	960	961

[a] Calculated from isodesmic reaction with ethylenediamine as a reference.

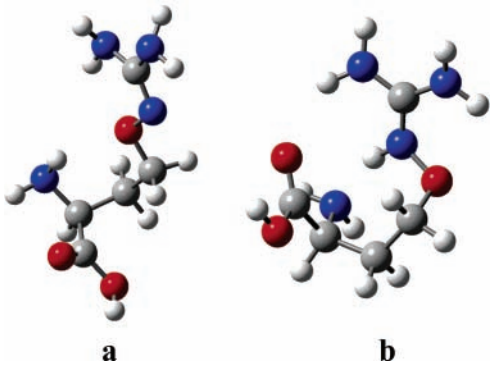


Figure 3. Lowest-energy structure of (a) neutral canavanine and (b) protonated canavanine obtained at the B3LYP/6-31+G* level of theory.

K^{-1}). The Monte Carlo simulation gives uncertainties at the 95% confidence level of ±9 kJ mol^{-1} for the proton affinity and ±21 J mol^{-1} K^{-1} for the protonation entropy. A plot of the data for Figure 1 with the ODR-derived best fit lines is shown as Figure S1 of Supporting Information. This plot shows the isothermal point with an *x*-coordinate of 13.6 kJ/mol and a *y*-coordinate of 4.6 J mol^{-1} K^{-1} as compared to 16.9 kJ/mol and −5.8 J mol^{-1} K^{-1} from the traditional method.

A theoretical value for the proton affinity of canavanine was obtained using hybrid density functional theory calculations at the B3LYP/6-311++G**//B3LYP/6-31+G* level. A conformational search for neutral canavanine was performed in PCModel using the GMMX algorithm in which a total of 50 000 conformations were investigated. The lowest 20 structures were then optimized at RHF/3-21G to give a total of nine distinct structures. These nine structures were further optimized using the B3LYP/3-21G and B3LYP/6-31+G* levels of theory. The total electronic energies for these nine neutral canavanine structures were ultimately obtained from B3LYP/6-311++G** single-point calculations at the B3LYP/6-31+G* geometries. Vibrational frequencies for three of the lowest energy structures were then calculated at the B3LYP/6-31+G* level. Predictions for the enthalpy at 298 K are obtained from the ZPE and thermal corrections to the total electronic energy. Electronic energies, ZPEs, thermal corrections, and enthalpies at 298 K for all low energy conformers of **1**, **1H**, **3**, and **3H** are listed in Tables S1 and S2 of Supporting Information. The side chain of canavanine has two low energy forms, —O—N═C$(NH_2)_2$ and —O—NH—C(NH_2)═NH. Both structures were investigated, and the lowest-energy structure with the —O—N═C$(NH_2)_2$ form (Figure 3a) was found to be approximately 34 kJ/mol lower in energy than the lowest conformer with the —O—NH—C(NH_2)═NH side chain. The lowest-energy structure for neutral canavanine is relatively extended (Figure 3a) and does not show extensive intramolecular hydrogen bonding.

Similar calculations were performed for the various protonated forms of canavanine. Protons were added at two different sites on the molecule: the amino terminal group and the side chain. Total electronic energies were obtained for 24 of the protonated canavanine structures, and vibrational frequencies were found for 12 of the lowest-energy conformers. The lowest-energy structure for protonated canavanine (Figure 3b) has significant hydrogen bonding between the side chain (ϵ-NH) and the backbone amino group. Although this interaction is significant, with a hydrogen bond length of 1.7Å, the hydrogen is formally on the side chain, indicating that this is the more basic site for canavanine in the gas phase in contrast to solution. The lowest energy structure with the proton residing formally on the backbone amino group is ca. 20 kJ/mol higher in energy. Additional stabilization for the lowest-energy structure is given by a weaker (H-bond length = 2.3 Å) interaction between a

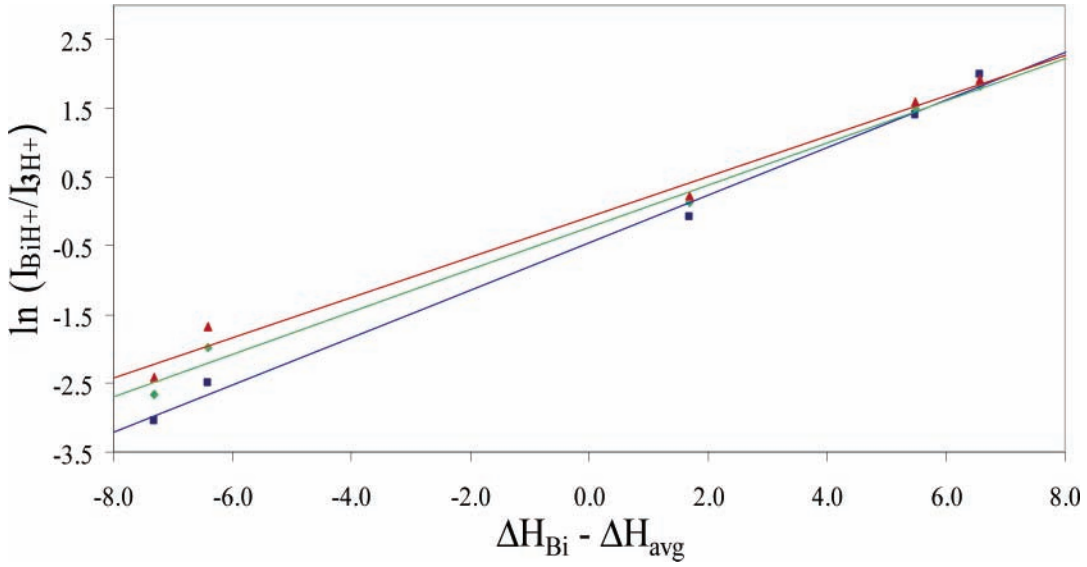


Figure 4. Plot of $\ln(B_iH^+/\mathbf{3}H^+)$ vs $\Delta H_{B_i} - \Delta H_{avg}$. Lines and symbols obtained from activation amplitudes 15% (squares), 35% (diamonds), and 50% (triangles).

hydrogen on the end of the side chain (η-NH) and the carbonyl oxygen atom. The fact that the protonated form has a high degree of intramolecular hydrogen bonding, whereas the neutral form is more extended indicates that canavanine should have a large negative entropy of protonation, in agreement with the experimental results.

A theoretical proton affinity for canavanine of 1014 kJ/mol (Table 2) was determined from reaction 1, somewhat higher than the experimentally determined value. The uncertainty of this value is not rigorously defined but is no smaller than ±10 kJ/mol. Given the relatively large error limits of the experimentally derived value, 1001 ± 9 kJ/mol, there is significant overlap between the error limits of the experimental and theoretical proton affinities. However, the agreement between theory and experiment is much poorer than for other amino acids that we have measured in our laboratory.[28−30] In previous studies, the deviation between experiment and B3LYP/6-311++G**//B3LYP/6-31+G* theoretical proton affinities was typically less than 8 kJ/mol and in most cases less than 4 kJ/mol. For comparison, we ran similar calculations on arginine and protonated arginine and derived a PA of 1057 kJ/mol, in better agreement with the experimental value of 1051 kJ/mol.[45] Given that entropic effects in this system are large, it may be that the experimental value for the proton affinity is slightly underestimated.

Nevertheless, the effect of oxygen atom substitution on the proton affinity of canavanine as compared to arginine is very large, between 40 and 50 kJ/mol. In solution, this substitution results in a pK_a change of 5 units. In fact, the side chain of canavanine is uncharged at physiological pH. Canavanine is often used as a site-specific mutagen to test for ionic interactions of specific arginine residues. In addition, canavanine has been shown to be a potent inhibitor or arginine-utilizing enzymes.[65] Misincorporation of canavanine into proteins has been shown to cause loss of function due to the loss of salt bridges and other stabilizing ionic interactions.[42,43] These results suggest that similar oxygen atom substitutions in the metabolites of canavanine should result in a significant decrease in basicity and possibly in similar toxicity.

Canaline. The proton affinity of canaline was determined in a fashion similar to that of arginine. Instead of scanning, three different activation amplitudes (15, 35, and 50%) were used for the kinetic method analysis. The following reference bases, with $PA_{avg} = 941.7$ kJ/mol, were used: cyclohexylamine, exo-2-aminonorbornane, 3-picoline, 4-picoline, and pyrrolidine. Proton affinity values and entropies of protonation for these compounds are listed in Table 1. Figures 4 and 5 show kinetic method plots 1 and 2, respectively, for canaline. The *x*-intercepts of each of the best fit lines in Figure 4 gives a range of apparent basicities of 942−943 kJ/mol. From Figure 5, the proton affinity was determined by adding the slope of the best-fit line, 7.0 kJ/mol, to $\Delta H_{avg,}$ 941.7 kJ/mol to give a PA of 948.7 kJ/mol. The intercept in Figure 5 is −1.9, which gives an entropy of dissociation of −16 eu. The ODR work up gives similar values, PA = 949.5 ± 7 kJ/mol and $\Delta S = -19 \pm 12$ J mol^{-1} K^{-1}, with the uncertainties at the 95% confidence level obtained from the Monte Carlo procedure.

Theoretical predictions for the proton affinity of canaline were also obtained using hybrid density functional theory calculations. The geometry of neutral canaline and its protonated forms were first optimized using PCModel. Protons were added at two different sites on the molecule, the backbone amino group and the side chain amino group. Total electronic energies for 10 neutral and 20 protonated canaline structures were ultimately obtained from B3LYP/6-311++G** single-point calculations at B3LYP/6-31+G* geometries. Vibrational frequencies were calculated for three of the lowest-energy neutral structures and five of the lowest-energy protonated structures. The lowest-energy structure of neutral canaline (Figure 6a) is somewhat cyclic with a weak interaction (H-bond = 2.2 Å) between the two amino groups. Upon protonation, the hydrogen bond strengthens considerably (Figure 6b) with the hydrogen bond shortening to 1.7 Å. As with canavanine, the proton is not equally shared in the hydrogen bond and the backbone amino group was determined to be the more basic site. The lowest-energy structure with the proton localized on the side-chain amino group is nearly 50 kJ/mol higher in energy than the lowest-energy conformer. This makes sense due to the electron-withdrawing nature of the substituted oxygen atom. From reaction 1, a theoretical proton affinity for canaline was determined to be 960 kJ/mol, again slightly larger than our experimental value of 950 kJ/mol but well within the combined error bars. The derived entropy term from the kinetic method experiments is smaller for canaline than for canavanine, and if

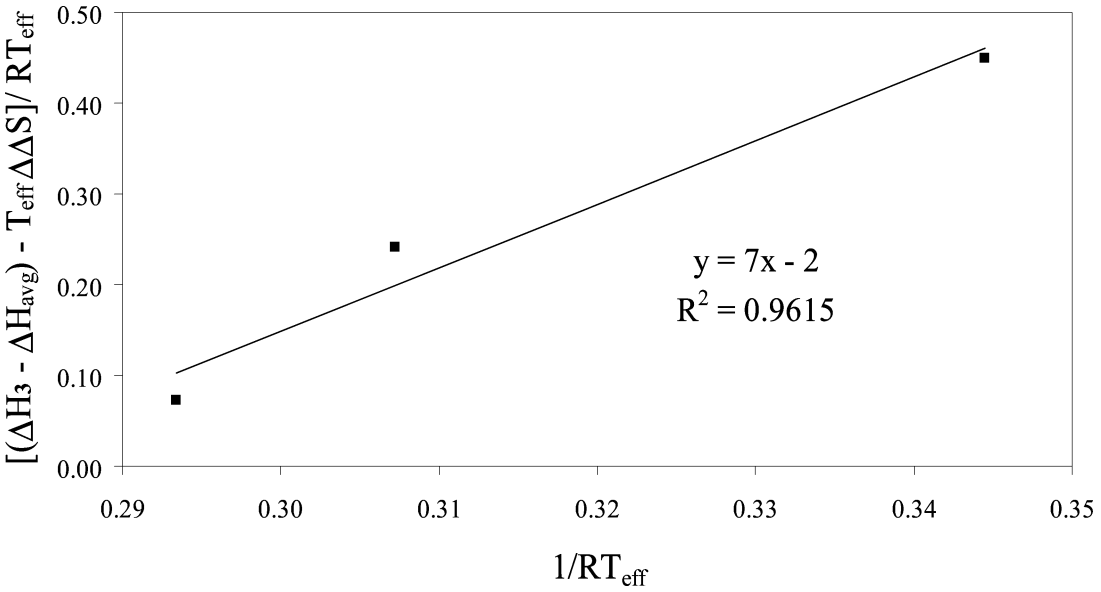


Figure 5. $[(\Delta H_3 - \Delta H_{avg}) - T_{eff}\Delta\Delta S/R]/RT_{eff}$ vs $1/RT_{eff}$. Lines and symbols obtained from activation amplitudes 16% (squares), 22% (diamonds), and 38% (triangles)

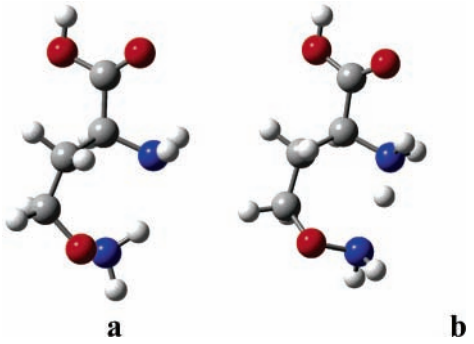


Figure 6. Lowest-energy structure of (a) neutral canaline and (b) protonated canaline obtained at the B3LYP/6-31+G* level of theory.

entropy effects are leading to an underestimation of the derived proton affinity, the effect should be smaller.

As with canavanine, oxygen atom substitution in the side chain leads to a decrease in proton affinity of more than 40 kJ/mol from ornithine (PA = 998.1 kJ/mol). Again, the side chain is significantly less basic than in ornithine and should be uncharged at physiological pH. Canaline has been shown to be a potent arginine antimetabolite and has been shown to inactivate B_6-containing enzymes by forming a covalently bound oxime species.[46] In addition, canaline has been shown to have antitumor activity against pancreatic cancer cells.[43,46] The similarity in structure allows canaline to bind to ornithine receptors, while the dramatic decrease in basicity contributes to the loss of biological function.

Canaline is unique among naturally occurring amino acids in its possession of the aminooxy group in its side chain. The substitution of an oxygen atom for a CH_2 group results in a decrease in basicity of more than 40 kJ/mol. The simplest examples of this substitution are found in hydroxylamine, NH_2-OH, and methoxylamine, CH_3ONH_2, as compared to methylamine, NH_2CH_3, and ethylamine, $C_2H_5NH_2$. We could find no experimental proton affinity values for hydroxylamine or any other aminooxy-containing molecule, and therefore the PAs of hydroxylamine, methoxylamine, methylamine, and ethylamine were calculated at the B3LYP/6-311++G**//B3LYP/6-31+G* level of theory. Substitution of an oxygen atom in hydroxylamine results in a decrease in basicity of nearly 90 kJ/mol with respect to methylamine. The substitution has less of an effect in the proton affinity of methoxylamine vs ethylamine (ΔPA $\approx$ 70 kJ/mol). One would expect the basicity difference to continue to diminish as the length of the R group bound to the oxygen atom increases, as is seen in canaline.

Conclusions

The proton affinities for canavanine and canaline have been determined using the extended kinetic method in a quadrupole ion trap mass spectrometer. Proton affinities of 1001 $\pm$ 9 and 950 $\pm$ 7 kJ/mol were determined for **1** and **3**, respectively. In addition, predictions for the protonation entropy for these species were determined and were found to be large enough to be treated as lower limits. The agreement between the experimental proton affinity value and theoretical prediction generated from hybrid density functional theory calculations for canavanine is somewhat poorer than for other amino acids previously studied in our lab under similar conditions. This could be due to entropic effects or simply due to statistical factors. Ultimately, substitution of an oxygen into the side chain of these amino acids causes a decrease in proton affinity of 40−50 kJ/mol.

Acknowledgment. Funding for this work was generously provided by the Camille and Henry Dreyfus Foundation, the Thomas F. and Kate Miller Jeffress Memorial Trust, the Research Corporation Cottrell College Research Award, ACS Petroleum Research Fund, The National Science Foundation (CAREER), and the College of William and Mary. K.E.C. acknowledges the Howard Hughes Medical Institute for summer fellowships through the Undergraduate Biological Sciences Education Program.

Supporting Information Available: A plot of the data for Figure 1 with the ODR-derived best fit lines and tables showing electronic energies, ZPEs, thermal corrections, and enthalpies at 298 K for all low energy conformers of **1**, **1H**, **3**, and **3H**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Meng, C. K.; Mann, M.; Fenn, J. B. Of Protons or Proteins. *Z. Phys. D* **1988**, *10*, 361.

(2) Karas, M.; Hillenkamp, F. Laser Desorption Ionization of Proteins with Molecular Masses Exceeding 10,000 Daltons. *Anal. Chem.* **1988**, *60*, 2299.

(3) Gorman, G. S.; Spier, J. P.; Turner, C. A.; Amster, I. J. Proton Affinities of the 20 Common α-Amino Acids. *J. Am. Chem. Soc.* **1992**, *114*, 3986.

(4) Bojesen, G. The Order of Proton Affinities of the 20 Common l-α-Amino Acids. *J. Am. Chem. Soc.* **1987**, *109*, 5557.

(5) Bojesen, G.; Breindahl, T. On the Proton Affinity of Some α-Amino Acids and the Theory of the Kinetic Method. *J. Chem. Soc., Perkins Trans. 2* **1994**, *2*, 1029.

(6) Li, X.; Harrison, A. G. A Kinetic Approach to the Proton Affinity of Amine Bases. *Org. Mass Spectrom.* **1993**, *28*, 366.

(7) Harrison, A. G. The Gas-Phase Basicities and Proton Affinities of Amino Acids and Peptides. *Mass Spectrom. Rev.* **1997**, *16*, 201.

(8) Mirza, S. P.; Prabhakar, S.; Vairamani, M. Estimation of Proton Affinity of Proline and Tryptophan Under Electrospray Ionization Conditions Using the Extended Kinetic Method. *Rapid Comm. Mass Spectrom.* **2001**, *15*, 957.

(9) Afonso, C.; Modeste, F.; Breton, P.; Fournier, F.; Tabet, J. C. Proton Affinities of the Commonly Occurring L-Amino Acids by Using Electrospray Ionization-Ion Trap Mass Spectrometry. *Eur. J. Mass Spectrom.* **2000**, *6*, 443.

(10) O'Hair, R. A. J.; Bowie, J. H.; Gronert, S. Gas-Phase Acidities of the α-Amino Acids. *Int. J. Mass Spectrom. Ion Processes* **1992**, *117*, 23.

(11) Meot-Ner (Mautner), M. Models for Strong Interactions in Proteins and Enzymes. 1. Enhanced Acidities of Principal Biological Hydrogen Donors. *J. Am. Chem. Soc.* **1988**, *110*, 3071.

(12) Locke, M. J.; McIver, R. T., Jr. Effect of Solvation on the Acid/Base Properties of Glycine. *J. Am. Chem. Soc.* **1983**, *105*, 4226.

(13) Burlet, O.; Gaskell, S. J. Decompositions of Cationized Heterodimers of Amino Acids in Relation to Charge Location in Peptide Ions. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 461.

(14) Kish, M. M.; Ohanessian, G.; Wesdemiotis, C. The Na^+ Affinities of α-Amino Acids: Side-Chain Substituent Effects. *Int. J. Mass Spectrom.* **2003**, *227*, 509.

(15) Nojesen, G.; Breindal, T.; Andersen, U. On the Sodium and Lithium Ion Affinties of Some α-Amino Acids. *Org. Mass Spectrom.* **1993**, *28*, 1448.

(16) Andersen, U. N.; Bojesen, G. The Order of Lithium Ion Affintites for the 20 Common α-Amino Acids. Caluclation of Energy Well-Depth of Ion-Bound Dimers. *J. Chem. Soc., Perkin Trans. 2* **1997**, *2*, 323.

(17) Cerda, B. A.; Wesdemiotis, C. Li^+, Na^+, and K^+ Binding to the DNA and RNA Nucleobases. Bond Energies and Attachment Sites from the Dissociation of Metal Ion-Bound Heterodimers. *J. Am. Chem. Soc.* **1996**, *118*, 11884.

(18) Hoyau, S.; Norrman, K.; McMahon, T. B.; Ohanessian, G. A Quantitative Basis for a Scale of Na^+ Affinities of Organic and Small Biological Molecules in the Gas Phase. *J. Am. Chem. Soc.* **1999**, *121*, 8864.

(19) Armentrout, P. B.; Rodgers, M. T. An Absolute Sodium Cation Affinity Scale: Threshold Collision-Induced Dissociation Experiments and ab Initio Theory. *J. Phys. Chem. A* **2000**, *104*, 2238.

(20) Nemirovskiy, O. V.; Gross, M. L. Intrinsic Ca^{2+} Affinities of Peptides: Application of the Kinetic Method to Analogues of Calcium-Binding Site III of Rabbit Skeletal Troponin C. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 770.

(21) Ryzhov, V.; Dunbar, R. C.; Cerda, B.; Wesdemiotis, C. Cation-π effects in the complexation of Na^+ and K^+ with Phe, Tyr, and Trp in the gas phase. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 1037.

(22) Gapeev, A.; Dunbar, R. C. Cation-π Interactions and the Gas-Phase Thermochemistry of the Na^+/Phenylalanine Complex. *J. Am. Chem. Soc.* **2001**, *123*, 8360.

(23) Ruan, C.; Rodgers, M. T. Cation-π Interactions: Structures and Energetics of Complexation of Na^+ and K^+ with the Aromatic Amino Acids, Phenylalanine, Tyrosine, and Tryptophan. *J. Am. Chem. Soc* **2004**, *126*, 14600.

(24) Gapeev, A.; Dunbar, R. C. Na^+ affinities of gas-phase amino acids by ligand exchange equilibrium. *Int. J. Mass Spectrom.* **2003**, *228*, 825.

(25) Moision, R. M.; Armentrout, P. B. An Experimental and Theoretical Dissection of Potassium Cation/Glycine Interactions. *Phys. Chem. Chem. Phys.* **2004**, *6*, 2588.

(26) Moision, R. M.; Armentrout, P. B. The Special Five-Membered Ring of Proline: An Experimental and Theoretical Investigation of Alkali Metal Cation Interactions with Proline and its Four- and Six-Membered Ring Analogues. *J. Phys Chem. A* **2006**, *110*, 3933.

(27) Cerda, B. A.; Wesdemiotis, C. The Relative Copper(I) Ion Affinities of Amino Acids in the Gas Phase. *J. Am. Chem. Soc.* **1995**, *117*, 9734.

(28) Kuntz, A. F.; Boynton, A. W.; David, G. A.; Colyer, K. E.; Poutsma, J. C. Proton Affinities of Proline Analogs Using the Kinetic Method with Full Entropy Analysis. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 72.

(29) Schroeder, O. E.; Andriole, E. J.; Carver, K. L.; Poutsma, J. C. The Proton Affinity of Lysine Analogues Using the Extended Kinetic Method. *J. Phys. Chem. A* **2004**, *108*, 326.

(30) Wind, J. J.; Papp, L. D.; Happel, M.; Hahn, K.; Poutsma, J. C. Proton Affinity of β-Oxalylaminoalanine (BOAA). Incorporation of Direct Entropy Correction into the Single Reference Kinetic Method. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 1151.

(31) Hunt, S. *Chemistry and Biochemistry of the Amino Acids*; Chapman and Hall: London, 1985; Chapter 4.

(32) Bell, E. A. Non-Protein Amino Acids in Plants. *Encylc. Plant. Phys.* **1975**, 403.

(33) Simmonds, M. S. J.; Romeo, J. T.; Blaney, W. M. The Effect of Nonprotein Amino Acids from *Calliandra* Plants on the Aphid, *Aphis fabae*. *Biochem. System. Ecol.* **1988**, *16* (7/8), 623.

(34) Rosenthal, G. A.; Janzen, D. H. Avoidance of Non-Protein Amino Acid Incorporation into Protein by the Seed Predator *Caryede brasiliensis* (Bruchidae). *J. Chem. Ecol.* **1983**, *9* (9), 1353.

(35) Rosenthal, G. A. The Biological Effects and Mode of Action of l-Canavanine, a Structural Analogue of L-Arginine. *Qu. Rev. Biol.* **1977**, *52*, 155.

(36) Boyar, A.; Marsh, R. E. L-Canavanine, a Paradigm for the Structures of Substituted Guanidines. *J. Am. Chem. Soc.* **1982**, *104*, 1995.

(37) Carvajal, N.; Torres, C.; Uribe, E.; Salas, M. Interaction of Arginase with Metal Ions: Studies of the Enzyme From Human Liver and Comparison With Other Arginases. *Comp. Biochem. Physiol.* **1995**, *112*, (1), 153.

(38) Boje, K. M.; Fung, H.-L. Endothelia Nitric Oxide Generating Enzyme(s) in the Bovine Aorta: Subcellular Location and Metabolic Characterization. *J. Pharmocol. Exp. Therapeutics* **1990**, *253* (1), 20.

(39) Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; Wiley: Chicester, 1961; Vol. 1−3.

(40) Rosenthal, G. A. The Biochemical Basis for the Deleterious Effects of L-Canavanine. *Phytochemistry* **1991**, *30*, 1055.

(41) Melangeli, C.; Rosenthal, G. A.; Dalman, D. L. The Biochemical Basis for L-Canavanine Tolerance by the Tobacco Budworm *Heliothis virescens* (Noctuidae). *Proc. Nat. Acad. Sci., U.S.A.* **1997**, *94*, 2255.

(42) Bence, A. K.; Crooks, P. A. The Mechanism of L-Canavanine Cytotoxicity: Arginyl tRNA Synthetase as a Novel Target for Anticancer Drug Discovery. *J. Enzymol. Inhib. Med. Chem.* **2003**, *18*, 383.

(43) Bence, A. K.; Worthen, D. R.; Adams, V. R.; Crooks, P. A. The antiproliferative and immunotoxic effects of L-canavanine and L-canaline. *Anti-Cancer Drug* **2002**, *13*, 313.

(44) Stryer, L. *Biochemistry*, 3rd ed.; W. H. Freeman and Co.: New York, 1988.

(45) Wu, Z.; Fenselau, C. Proton Affinity of Arginine Measured by the Kinetic Approach. *Rapid Comm. Mass Spectrom.* **1992**, *6*, 403.

(46) Rosenthal, G. A. L-Canaline: A Potent Antimetabolite and Anti-Cancer Agent from Leguminous Plants. *Life Sci.* **1997**, *60*, 1635.

(47) Wu, Z.; Fenselau, C. Gas-Phase Basicities and Proton Affinities of Lysine and Histidine Measured from the Dissociation of Proton-Bound Dimers. *Rapid Commun. Mass Spectrom.* **1994**, *8*, 777.

(48) Armentrout, P. B. Entropy Measurements and the Kinetic Method: A Statistically Meaningful Approach. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 371.

(49) Zheng, X.; Cooks, R. G. Thermochemical Determinations by the Kinetic Method with Direct Entropy Corrections. *J. Phys. Chem. A* **2002**, *106*, 9939.

(50) Ervin, K. M. Microcanonical Analysis of the Kinetic Method. The Meaning of Apparent Entropy. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 435.

(51) Drahos, L.; Vekey, K. Entropy Evaluation using the Kinetic Method: Is it Feasible. *J. Mass Spectrom.* **2003**, *38*, 1025.

(52) Bouchoux, G.; Sablier, M.; Berruyer-Penaud, F. Obtaining Thermochemical Data by the Extended Kinetic Method. *J. Mass Spectrom.* **2004**, *39*, 986.

(53) Ervin, K. M.; Armentrout, P. B. Systematic and Random Errors in Ion Affinities and Activation Entropies from the Extended Kinetic Method. *J. Mass Spectrom.* **2004**, *39*, 1004.

(54) Wesdemiotis, C. Entropy Considerations in Kinetic Method Experiments. *J. Mass Spectrom.* **2004**, *39*, 998.

(55) Drahos, L.; Peltz, C.; Vekey, K. Accuracy of Enthalpy and Entropy Determination Using the Kinetic Method: Are We Approaching a Consensus? *J. Mass Spectrom.* **2004**, *39*, 1016.

(56) Hahn, I. S.; Wesdemiotis, C. Protonation Thermochemistry of β-Alanine. An Evaluation of the Proton Affinities and Entropies Determined by the Extended Kinetic Method. *Int. J. Mass Spectrom.* **2003**, *222*, 465.

(57) Bouchoux, G.; Djazi, F.; Gaillard, F.; Vierezet, D. Application of the Kinetic Method to Bifunctional Bases. MIKE and CID-MIKE Test Cases. *Int. J. Mass Spectrom.* **2003**, *227*, 479.

(58) Bouchoux, G.; Buisson, D.-A.; Bourcier, S.; Sablier, M. Application of the Kinetic Method to Bifunctional Bases. ESI Tandem Quadrupole Experiments. *Int. J. Mass Spectrom* **2003**, *228*, 1035.

(59) Cao, J. C.; Aubry, C.; Holmes, J. L. Proton Affinities of Simple Amine: Entropies and Enthalpies of Activation and Their Effect on the Kinetic Method for Evaluating Proton Affinities. *J. Phys. Chem. A* **2000**, *104*, 10045.

(60) Becke, A. D. Density Functional Thermochemistry. III. The Role of Exact Exchange. *J. Chem. Phys.* **1993**, *98*, 5648.

(61) Lee, C.; Yang, W.; Parr, R. G. Development of the Colle-Salvetti Correlation Energy Formula into a Functional of the Electron Density. *Phys. Rev. B* **1988**, *37*, 785.

(62) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, N.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, version A.9; Gaussian, Inc: Pittsburgh, PA, 1998.

(63) Scott, A. P.; Radom, L. Harmonic Vibrational Frequencies: An Evaluation of Hartree−Fock, Moeller−Plesset, Quadratic Configuration Interaction, Density Functional Theory, and Semiempirical Scale Factors. *J. Phys. Chem.* **1996**, *100*, 16502.

(64) Hunter, E. P.; Lias, S. G. Evaluated Gas-Phase Basicities and Proton Affinities of Molecules: An Update. *J. Phys. Chem. Ref. Data* **1998**, *27*, 3.

(65) Lu, X.; Li, L.; Feng, X.; Wu, Y.; Dunaway-Mariano, D.; Engen, J. R.; Mariano, P. S. L-Canavanine is a Time-Controlled Mechanism-Based Inhibitor of *Pseudomonas aeruginosa* Arginine Deiminase. *J. Am. Chem. Soc* **2005**, *127*, 16412.