Comparison between Protonation, Lithiation, and Argentination of 5-Oxazolones: A Study of a Key Intermediate in Gas-Phase Peptide Sequencing

Christopher F. Rodriguez, Tamer Shoeib, Ivan K. Chu, K. W. Michael Siu, and Alan C. Hopkinson*
Department of Chemistry, York University, Toronto, Ontario, Canada M3J 1P3
Received: January 5, 2000; In Final Form: March 27, 2000

Molecular orbital calculations at B3LYP/6-31++G(d,p) are reported for bases 2-(aminomethyl)-5-oxazolone, 2-(aminomethyl)-4-methyl-5-oxazolone, 2-phenyl-5-oxazolone, and 2-phenyl-4-methyl-5-oxazolone and for the cations formed by protonation of these bases on their imino nitrogens. Structures and relative energies for isomers generated by protonation at each of the four heteroatoms of 2-(aminomethyl)-5-oxazolone are reported. Lithium and silver cations both add to 2-(aminomethyl)-5-oxazolone, but unlike the proton, they bind with two heteroatoms simultaneously. For both the lithiated and argentinated 2-(aminomethyl)-5-oxazolone cations the lowest energy isomers have the metal coordinated with the two nitrogen atoms. Proton affinities of these bases are in the range 217.0–221 kcal mol\(^{-1}\), with the methyl group at C4 increasing the proton affinity by \(\sim 3\) kcal mol\(^{-1}\). Single-point calculations were performed at MP4(fc)/6-311++G(2df,p)//B3LYP/6-31++G(d,p) for 2-(aminomethyl)-5-oxazolone, diketopiperazine, glycine, and alanine and their conjugate acids. The proton affinities from this level of theory are lower by as much as 2.7 kcal mol\(^{-1}\) than those calculated at B3LYP/6-31++G(d,p). Enthalpies of formation calculated at B3LYP/6-31++G(d,p) from isodesmic reactions for glycine, alanine, and their conjugate acids are all within 1 kcal mol\(^{-1}\) of the experimental values, but those calculated at MP4 deviate by as much as 4.8 kcal mol\(^{-1}\). Enthalpies of formation from atomization reactions at the MP4 level are in larger disagreement with experimental values.

Introduction

The predominant mechanism for fragmentation of a collisionally activated protonated peptide in the gas phase under laboratory energies \(<100\) eV is through cleavage of a C–N bond adjacent to the charge site.\(^1\)–\(^9\) In peptides that do not have a basic side chain, the terminal amino group has the highest proton affinity (structure 1), followed by the carbonyl oxygen atoms of the amide groups (structure 2). Protonation at these two sites, however, does not permit facile cleavage of the amide bond; for this to occur protonation must be on the nitrogen of the amide bond (structure 3).\(^1\)–\(^12\) Heterolytic fission of the C–N bond of 3 should initially result in an acylium ion,\(^9\)\(^,\)\(^13\) but there is considerable evidence, supported by theory, that these ions are not formed in the fragmentation.\(^11\)\(^,\)\(^14\)–\(^18\) Rather the N-protonated amide is subject to internal nucleophilic attack on the carbon atom of the amide group by either the carbonyl oxygen or the nitrogen atom of the amino group on the adjacent amino acid residue, thereby displacing the C-terminal amino acid or peptide. If attack is by the oxygen then the ion formed is a protonated 2-substituted-5-oxazolone. Subsequent proton transfer to the peptide may occur as both the oxazolone and the peptide have similar proton affinities.\(^19\) In the usual nomenclature used in peptide analysis, the oxazolone is referred to as a \(b_1\) ion;\(^20\) the \(b_2\) ion formed from a tripeptide has structure 4, where \(X = \text{CHR'NH}_2\), with a proton added to one of the nitrogen atoms.

If the terminal amino group were to attack the carbonyl carbon, then the resulting ion would be an N-protonated diketopiperazine (5 with a proton added to one of the nitrogen) and, as proton transfer from this cation to the amino group of the C-terminal peptide is exothermic, then the protonated peptide, the y ion, would be formed.\(^14\)\(^,\)\(^19\) Alternatively, the protonated diketopiperazine could be formed by rearrangement of the protonated 5-oxazolone.\(^19\) In the product ion spectra of protonated tripeptides, e.g., glycylglycinylglycine (GGG) and glycyclyglycinylalanine (GGA) the intensities...
of both the b_{2} and y_{1} ions are abundant. Furthermore, the a_{2} ion, an ammonium ion, is also typically intense. This product ion is believed to form via elimination of carbon monoxide from the b_{2} ion.11,14,16

The collision-induced fragmentations of peptides in the presence of Li^{+}, Na^{+}, and Ag^{+} ions have been investigated extensively.21–28 These metalated peptides produce similar fragmentation patterns, although the relative abundances of the different product ions vary with the metal. Tripeptides are the smallest peptides that can fragment to form an oxazolone or a diketopiperazine. Here we use molecular orbital theory to examine the protonated and metalated oxazolones derived from the computationally least-expensive tripeptides, GGG and GAG, and in the case of GGG, we have also examined the protonated and argentinated diketopiperazine. In particular, we were interested in the sites of protonation and metalation of the oxazolones and also in the relative proton affinities of an oxazolone and its isomeric diketopiperazine. One of the difficulties in establishing the identity of the b_{2} ions in peptide fragmentation stems from the unavailability of 2-(aminomethyl)-5-oxazolone, its conjugate base (4) with R = H and X = CH_{2}NH_{2}) shows that protonation has a profound effect on the geometry of the ring. In the neutral 2-(aminomethyl)-5-oxazolone molecule, the bond angles in the planar five-membered ring are all in the range 105.1–106.3°, with the exception of angle NCO, which is 117.1°; i.e., the constraints imposed by the ring have resulted

### Table 1: Bond Lengths in 2-X-5-oxazolones

<table>
<thead>
<tr>
<th></th>
<th>O=C_{2}</th>
<th>C_{2}=N_{3}</th>
<th>N_{3}=C_{4}</th>
<th>C_{4}=C_{3}</th>
<th>C_{3}=O_{1}</th>
<th>C_{1}=O</th>
<th>C_{1}=R</th>
<th>C_{1}=X</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Oxazolones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>R = CH_{2}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Oxazolones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>O=C_{2}</th>
<th>C_{2}=N_{3}</th>
<th>N_{3}=C_{4}</th>
<th>C_{4}=C_{3}</th>
<th>C_{3}=O_{1}</th>
<th>C_{1}=O</th>
<th>C_{1}=R</th>
<th>C_{1}=X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protonated 5-Oxazolones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

"Bond lengths are in angstroms. "

### Results and Discussion

**A. Structures of Protonated 2-Substituted-5-Oxazolones.**

Some structural details of the 2-substituted-5-oxazolones and their imino-nitrogen protonated isomers are given in Table 1. (a) 2-(Aminomethyl)-5-oxazolone, 4a. Protonated 2-(aminomethyl)-5-oxazolone (6a with R = H and X = CH_{2}NH_{2}) is the b_{2} ion formed in the collision-induced dissociation of protonated

**Experimental Section**

Cooks’ kinetic method40 with Fenselau’s correction41 was employed for estimating the proton affinities of 2,5-diketopiperazine and 2-phenyl-4-methyl-5-oxazolone on a triple quadrupole mass spectrometer (PE-SCIEX API 3000 prototype).

**Computational Methods**

All molecular orbital calculations were performed using Gaussian 98.29 Structure optimizations were carried out using the density functional theory (DFT) hybrid method at the B3LYP level.30–33 The 6-31+G(d,p) basis set34–37 was used for structure optimizations on all protonated and lithiated ions and the DZVP basis set38 on the argentinated ions. All critical points were characterized by harmonic frequency calculations.39

**Experimental Section**

Cooks’ kinetic method40 with Fenselau’s correction41 was employed for estimating the proton affinities of 2,5-diketopiperazine and 2-phenyl-4-methyl-5-oxazolone on a triple quadrupole mass spectrometer (PE-SCIEX API 3000 prototype). Measurements were performed under constant center-of-mass energies (E_{cm}) at several E_{cm} values; for 2,5-diketopiperazine, E_{cm} = 1.0, 1.5, 2.0, and 2.5 eV, whereas for 2-phenyl-4-methyl-5-oxazolone, E_{cm} = 0.90, 0.95, 1.00, and 1.05 eV.32 Reference bases were cycloheptanone, cyclooctanone, p-tolualdehyde, 5-nonanone, cyclopentylethanone, and acetylphenone for 2,5-diketopiperazine and 1-propanamine, 1-butanamine, 2-methyl-1-propanamine, 1-hexamamine, and 1-octanamine for 2-phenyl-4-methyl-5-oxazolone.
in these angles being smaller than the ideal values, and the deviation is particularly large at the carbonyl carbon. The bond distances within the ring are close to those in acyclic molecules, except for those associated with the oxygen. The two C–O distances in all the 2-substituted-5-oxazolones are almost identical at a distance of ~1.395 Å, perhaps consistent with the fact that the oxygen is attached to two trivalent carbon atoms (Figure 1). These are intermediate between the bond lengths in all the 2-substituted-5-oxazolones are almost identical at a distance of ~1.395 Å, perhaps consistent with the fact that the oxygen is attached to two trivalent carbon atoms (Figure 1). These are intermediate between the bond lengths in an acyclic ester; for example, the experimental values in methyl formate are 1.334 Å for the HCO–OCH₂ bond and 1.437 Å for the HCOO–CH₃ bond.\(^{43}\) The C=O distance of 1.274 Å in the ring is almost identical with the bond in methylenimine (1.273 Å).\(^{45}\) The nitrogen atom of the aminomethyl side chain is coplanar with the five-membered ring and is cis to the C=O bond. The amino hydrogens adopt a staggered conformation about the C=O bond with the lone pair on the nitrogen atom oriented away from the double bond.

The most basic site on molecule 4 is the imino nitrogen. The energetics of electrophilic addition at other potential sites will be discussed in section B. The structure of protonated 2-(aminomethyl)-5-oxazolone, ion 6a, has the conformation of the amino group in the side-chain rotated 180° about the CH₂NH₂ bond relative to that in the unprotonated base (Figure 1). In this conformation the amino protons eclipse the protons of the adjacent CH₂ group, but this energetically unfavorable arrangement is offset by a favorable interaction between the lone pair on the amino nitrogen and the proton that has been added to the imino nitrogen. Other geometric features, the NH₂...HN distance of 2.303 Å, the N–H distance of 1.022 Å, and the H–N=C angle of 119°, however, all indicate that there is minimal interaction between the lone pair and the protonated imine.

The most dramatic structural changes upon protonation of 2-(aminomethyl)-5-oxazolone, 4a (with X = CH₂NH₂ and R = H), are in the bond distances around the oxygen atom in the ring. The C=O distance in 6a is 1.480 Å, compared with 1.396 Å in 4a and the O–C₂ distance is 1.313 Å compared with 1.393 Å. Furthermore, the C=N bond in 6a is 1.305 Å compared with 1.274 Å in 4a and the C=O distance of 1.174 Å is shorter than that in 4a (1.198 Å). These changes indicate that imino-protonation of the oxazolone ring greatly weakens the C=O bond and that resonance structure 6a’ is a significant contributor to this ion. Structure 6a’ is, of course, the elusive acylium ion that would be expected to lose carbon monoxide to form the immonium ion, 42.

(b) 2-(Aminomethyl)-4-methyl-5-oxazolone, 4b. Examination of the structural data in Table 1 shows that replacement of one of the hydrogens at C₄ by a methyl group, thereby creating 4b and 6b, has virtually no effect on the geometric parameters of the oxazolone ring. Also, the C₄=CH₃ distance in 6b is insignificantly longer (by 0.001 Å) than that in the neutral compound, 4b, indicating that the methyl substituent does not participate strongly in delocalizing the positive charge.

(c) 2-Phenyl-5-oxazolones, 4c and 4d. Replacement of the aminomethyl group by a phenyl group at the 2-position in the oxazolone ring has essentially no effect on the geometric parameters in the oxazolone ring; i.e., molecules 4a, 4b, 4c, and 4d all have almost identical geometries in the ring. The phenyl groups in 4c and 4d are coplanar with the oxazolone ring and the Ph–C₂ distance of 1.466 Å is indicative of some double bond character between the two rings. From a natural bond orbital (NBO) calculation\(^{45}\) the phenyl group in 4c is calculated to carry a positive charge of +0.055e.

Protonation on the imino nitrogen of 4c produces 6c and, unlike in the bases 4a and 4c, comparison between 6a and 6c shows there to be considerable difference in the oxazolone rings. Both 6a and 6c have longer C=N distances than in the bases, with the increases on protonation being 0.031 and 0.048 Å, respectively. This larger change in C=N in 6c is accompanied by smaller changes in the C–O distances. The changes are in the same direction as in the protonation of 4a, but C=O increases by only 0.057 Å (as opposed to an increase of 0.084 Å in forming 6a) and O–C₂ decreases by 0.065 Å (as opposed to 0.080 Å). Ion 6c then has less acylium-like character than ions 6a and 6b. In ion 6c the C₂=phenyl bond is shorter than in 4c and from the NBO analysis, which gave a charge of +0.258e on the phenyl group, ~0.2 of the excess positive charge is located on the phenyl ring. The majority of this excess charge is on the para carbon and on one ortho carbon (the distant one); there are slight increases on the hydrogen atoms attached to these carbons also. By contrast, the charge on the ortho carbon adjacent to the protonated nitrogen has a slightly more negative charge in the cation. Approximately half the positive charge in the cation is located on the proton that has been added.

Substitution of a hydrogen atom at C₄ by a methyl group has very little effect on the structures of either the base or the conjugate acid; i.e., the structural parameters of 4c and 6c are almost identical to those of 4d and 6d, respectively.

(d) Other Isomers on the C₄H₃NO⁺ Surface. Nucleophilic attack by the terminal amino nitrogen on the carbonyl carbon of the second amide linkage, which is protonated on its amide nitrogen, produces an N-protonated diketopiperazine, 7, which then transfers a proton to the C-terminal amino acid to form...
Protonation of 5-oxazolone occurs preferentially at the imino nitrogen of the first amide linkage, it is believed that a nucleophilic attack can occur on the carbonyl carbon of this amide bond to form an N-protonated aziridinone, which then transfers a proton to the C-terminal fragment to produce the y2 ion.15

N-protonated aziridinone does not exist in isolation and immediately dissociates into CO and H₂C⁺.45 It is, however, possible that N-protonated aziridinone can exist in a neutral-stabilized complex. Of more importance in the current context is the formation of N-protonated diketopiperazine, 7, an ion whose formation could in principle be competitive with the formation of protonated 2-(aminomethyl)-5-oxazolone (6a).

We have optimized structures for the protonated diketopiperazines, along with those of isomers 9–11. Isomers 10 and 11 could be formed by cyclization of the central amino acid in protonated GGG, although the instability of the intermediate ring N-protonated isomer toward loss of carbon monoxide is a problem. Ions of type 9, derived from 5-oxazolones via the intermediacy of the acylim ion followed by a hydride shift, have been postulated as possible structures for the y2 ion and we therefore include ion 9 for comparative purposes.18 The total energies and relative energies of isomers 6–11 are given in Table 2. Isomers 6 and 8 have almost identical energies, with the relative energy of these ions depending on the level of theory. However, N-protonated diketopiperazine, 7, is 14 kcal mol⁻¹ above 8 and it is important to realize that in the dissociation of N-protonated GGG it is the formation of this isomer that is in competition with formation of 2-(aminomethyl)-5-oxazolone, 6a.

(e) Proton Affinities. The proton affinity of a base B is defined as being the exothermicity of the reaction given in eq 1.

\[ \text{BH}^+ \rightarrow B + H^+ \]  (1)

Molecules containing nitrogen atoms generally have high proton affinities, with the prototypical amine, ammonia, having a proton affinity of 204 kcal mol⁻¹.46 The proton affinities of the four oxazolones, 4a–4d, all fall in a very narrow range (Table 3). At B3LYP/6-31++G(d,p) 2-(aminomethyl)-5-oxazolone, 4a, and 2-phenyl-5-oxazolone, 4c, are calculated to have proton affinities of 217.4 and 217.8 kcal mol⁻¹, respectively, and in each case the introduction of a methyl group at C4 increases the proton affinity by approximately 3 kcal mol⁻¹. For 2-phenyl-5-oxazolone, 4c, 2-phenyl-4-methyl-5-oxazolone, 4d, the O-protonated form of diketopiperazine, 5, glycine, and alanine,46 there is excellent agreement with the experimental values. The experimental proton affinities of 2-phenyl-4-methyl-5-oxazolone, 4d, have never been reported and were specifically measured in this study. In a recent publication, we showed that the kinetic method with Fenselau correction is effective for estimating the proton affinity of an unknown base that binds differently (dicoordinating) from a series of reference bases that bind very similarly among themselves (monocoordinating), when data are collected under a series of different collision energy conditions.42 We expect both 2-phenyl-4-methyl-5-oxazolone and diketopiperazine would bind the proton differently from the amines, ketones, and aldehydes used as reference bases in this study; the differences, however, are unlikely to be as drastic as that in the earlier study.42 We are, therfore, expecting that our results, however, are unlikely to be as drastic as that in the earlier study.42 We are, therefore, expecting that our experimental values to have an accuracy comparable to that of the previous study (average deviation ± 1.0 kcal mol⁻¹).42

Comparing the proton affinity of diketopiperazine, 5, (cyclic GG) with those of cyclic AA (207.9 kcal mol⁻¹) and cyclic LG (209.9 kcal mol⁻¹),19 it is apparent that there is good agreement. For oxazolones, substituting a methyl group for a hydrogen (replacing a glycyl with an alanyl residue) increases the proton affinity by approximately 3 kcal mol⁻¹. The difference of 207.9 – 201.1 = 6.8 kcal mol⁻¹ in the proton affinities of cyclic AA and cyclic GG is consistent with this trend.

Protonation of 5-oxazolone occurs preferentially at the imino nitrogen and the proton affinities for oxazolones 4a–4d are all ~13–16 kcal mol⁻¹ higher than that of methylenimine (203.8 kcal mol⁻¹).46 This is attributable to the extensive structural

TABLE 2: Energiesa of C₅H₇N₂O₃⁺ Isomers

<table>
<thead>
<tr>
<th>molecule</th>
<th>B3LYP/6-311++G(d,p)</th>
<th>MP4SDTQ(fc)/6-311++G(d,p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>-416.36765 (0)</td>
<td>-415.67444 (0)</td>
</tr>
<tr>
<td>7</td>
<td>-416.34850 (12.7)</td>
<td>76.9</td>
</tr>
<tr>
<td>8</td>
<td>-416.37070 (1.3)</td>
<td>76.7</td>
</tr>
<tr>
<td>9</td>
<td>-416.34794 (11.8)</td>
<td>74.9</td>
</tr>
<tr>
<td>10</td>
<td>-416.30906 (30.0)</td>
<td>75.8</td>
</tr>
<tr>
<td>11</td>
<td>-416.27402 (58.8)</td>
<td>74.1</td>
</tr>
</tbody>
</table>

a Total energies are in hartrees. Numbers in parentheses are energies relative to 6 (in kcal mol⁻¹). These include zero-point energies and thermal corrections. Zero-proton energies in kcal mol⁻¹. Single-point calculations using geometry optimized at B3LYP/6-31++G(d,p).

TABLE 3: Proton Affinitiesa,b

<table>
<thead>
<tr>
<th>molecule</th>
<th>B3LYP/6-311++G(d,p)</th>
<th>MP4b</th>
<th>exptl</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-(aminomethyl)-5-oxazolone, 4a</td>
<td>217.4</td>
<td>214.7</td>
<td></td>
</tr>
<tr>
<td>2-(aminomethyl)-4-methyl-5-oxazolone, 4b</td>
<td>220.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-phenyl-5-oxazolone, 4c</td>
<td>217.8</td>
<td>216.7</td>
<td></td>
</tr>
<tr>
<td>2-phenyl-4-methyl-5-oxazolone, 4d</td>
<td>220.4</td>
<td>220.7</td>
<td></td>
</tr>
<tr>
<td>diketopiperazine, 5</td>
<td>199.9</td>
<td>198.4</td>
<td>201.5</td>
</tr>
<tr>
<td>glycine</td>
<td>212.1</td>
<td>212.0</td>
<td>211.9</td>
</tr>
<tr>
<td>alanine</td>
<td>216.2</td>
<td>214.8</td>
<td>215.8</td>
</tr>
</tbody>
</table>

a In kcal mol⁻¹. b At MP4SDTQ(fc)/6-311++G(2df,p)/B3LYP/6-31++G(d,p). c Reference 19. d This study. e Reference 46.
TABLE 4: Enthalpies of Formation at 298 K

<table>
<thead>
<tr>
<th>molecule</th>
<th>calculated ΔHf</th>
<th>atomization</th>
<th>isodesmic</th>
<th>exptl</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-(aminomethyl)-5-oxazole, 4a</td>
<td>-68.8</td>
<td>-60.5</td>
<td>-68.3</td>
<td></td>
</tr>
<tr>
<td>diketopiperazine, 5</td>
<td>-84.0</td>
<td>-75.8</td>
<td>-79.1</td>
<td>-94.7</td>
</tr>
<tr>
<td>glycine</td>
<td>-97.4</td>
<td>-93.8</td>
<td>-93.3 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>alanine</td>
<td>-105.2</td>
<td>-99.2</td>
<td>-99.1 ± 1.0</td>
<td>-103.9</td>
</tr>
</tbody>
</table>

a In kcal mol⁻¹. b Upper limits are at B3LYP, lower limits at MP4. c Reference 57. d From proton affinity of glycine. PA = 211.9 kcal mol⁻¹. e ΔHf(H)₂98 = 365.7 kcal mol⁻¹. f From proton affinity of alanine. PA = 215.5 kcal mol⁻¹. g ΔHf(H)₂98 = 365.7 kcal mol⁻¹. h The processes in the central column are isodesmic reactions at MP4(d,p) and B3LYP/6-31++G(d,p). i At B3LYP/DZVP. j At B3LYP/6-31++G(d,p) and B3LYP/DZVP. k At B3LYP/6-31++G(d,p) and B3LYP/DZVP. l The energy of Ag⁺ is −7.28459 kcal, and at B3LYP/DZVP the energy of Ag⁺ is −5199.19815 hartrees.

The ammonium ion has to be added as one of the reactants (eq 3).

H₂NCH₂COOH + 3CH₄ → H₅CNH₂ + H₅CCH₃ + H₂CO + H₂COH (2)

For all the product molecules in eq 2 and for CH₄, experimental enthalpies of formation were taken from the compilation in ref 54. For other isodesmic reactions, the following enthalpies of formation (in kcal mol⁻¹) were used: CH₃NH₃⁺, 145.3; CH₃OH⁺, 171.7; CH₂NH₂⁺, 178.1; CH₃NH, 16.5.

In the case of protonated 2-(aminomethyl)-5-oxazolone the positive charge is formally located on the imino nitrogen and separation of the heavy atoms in this ion requires formation of both protonated methylenimine and protonated methyamine.

TABLE 5: Energies for Protonated and Metalated Ions

<table>
<thead>
<tr>
<th>molecule</th>
<th>B3LYP/6-31++G(d,p)</th>
<th>ZPE</th>
<th>MP4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>2-(aminomethyl)-5-oxazole, 4a</td>
<td>−416.01050</td>
<td>67.9</td>
</tr>
<tr>
<td>Protonated 2-(Aminomethyl)-5-oxazolone</td>
<td>−416.01050</td>
<td>67.9</td>
<td>−415.32155</td>
</tr>
</tbody>
</table>

The ΔHf values computed using isodesmic reactions at B3LYP are in remarkably good agreement with experimental values (the largest deviation, that for protonated alanine, is 0.8 kcal mol⁻¹). Those for isodesmic reactions calculated at MP4 are intermediate between the ΔHf values calculated at B3LYP and others calculated using the atomization method at MP4. From this very limited number of calculations, it appears that the B3LYP calculations using isodesmic reactions provide the most reliable enthalpies of formation.

B. Comparison of Protonation and Metalation of 5-Oxazolones. The total energies and relative energies of several isomers of protonated, lithiated, and argentinated 2-(aminomethyl)-5-oxazolones are given in Table 5, and their structures are given in Figures 1−3. The base, 4a, and the lowest energy isomers of the protonated and lithiated ions were optimized at both B3LYP/6-31++G(d,p) and B3LYP/DZVP, and, in each case, the structures are almost identical. The remainder of the protonated and lithiated structures were optimized at B3LYP/6-31++G(d,p) and the argentinated ions were all optimized at B3LYP/DZVP.

(a) Alternative Sites of Protonation. There are several potential sites for protonation of 2-(aminomethyl)-5-oxazolone. In
section A the profound effects of protonation at the preferred site, the imino nitrogen in the ring, on the geometric parameters in the ring were discussed. Here we examine the structural changes accompanying protonation at other sites (Figure 1).

The amino-protonated isomer 12, has a proton of the NH$_3^+$ group in plane, cis to the imino nitrogen, and the distance from this hydrogen atom to the nitrogen atom in the ring is 1.896 Å, indicating the existence of a weak hydrogen bond. This isomer is 5.7 kcal mol$^{-1}$ higher in energy than the imino-protonated isomer, 6a. Comparison of the structure of 12 with that of the base, 4a, shows the two C–O distances in the ring to have undergone large changes on protonation. These geometric changes, a decrease in C$_2$–O and an increase in C$_5$–O, are slightly smaller but in the same direction as those resulting from protonation on the imino nitrogen.

Protonation on the carbonyl oxygen gives ion 13, a cation that is energetically much less favorable than 6a (by 27.7 kcal mol$^{-1}$). Relative to 4a, 13 has slightly shorter C$_2$–N, N–C$_4$, and C$_4$–C$_5$ distances, but again the largest changes are around the oxygen atom in the ring. Here the changes are in the opposite direction from when the proton is added to the nitrogen atoms. The O–C$_2$ distance in 13 is much longer (increased by 0.196 Å to 1.589 Å); this compares with a standard C–O single bond distance of ~1.43 Å.$^{33}$ The C$_5$–O distance in 13 is 0.124 Å shorter than that in 4a. These geometric parameters suggest that resonance structure 13$^a$, an alkylated nitrile, is a significant contributor to the structure of 13a.

(b) Alternative Sites of Lithiation. The lithium ion affinity of 2-(aminomethyl)-5-oxazolone as calculated at B3LYP/6-31++G(d,p) is 58.7 kcal mol$^{-1}$ at 298 K.

Unlike the proton, lithium attaches to 2-(aminomethyl)-5-oxazolone, 4a, in a bidentate fashion, and in the structure with the lowest energy 14a, the O–C$_2$ is shorter (by 0.050 Å) and the C$_5$–O distance longer (by 0.048 Å) than in 4a (Figure 2). These changes on lithiation are in the same direction but are only slightly more than half the magnitude of those resulting from protonation of the imino nitrogen. The N–Li distances of 1.938 Å (imino nitrogen) and 2.040 Å (amino nitrogen) are comparable with a distance of 1.963 Å in CH$_2$NH$_2$Li$^+$, as calculated at B3LYP/6-311++G(d,p).

Another bidentate structure in which the amino group is lithiated involves attachment to the oxygen atom in the ring (structure 15a). This isomer lies 8.0 kcal mol$^{-1}$ above 14a. In 15a both the C–O distances in the ring are larger than in 2-(aminomethyl)-5-oxazolone and the C–NH$_2$ distance has increased by 0.041 Å as a result of lithiation.

The next highest energy isomer 16a, has the carbonyl oxygen lithiated and is 15.7 kcal mol$^{-1}$ above 14a. The difference between equivalent structures on the C$_2$H$_5$N$_2$O$_2^+$ surface, where H replaces Li, is 27.7 kcal mol$^{-1}$. The carbonyl lithiation enthalpy of 2-(aminomethyl)-5-oxazolone is 43.0 kcal mol$^{-1}$, and this compares with a value of 33.6 kcal mol$^{-1}$ for formaldehyde at the same level of theory. As in the case of protonation, lithiation results in a shortening of C$_5$–O and a lengthening of O–C$_2$, but lithiation produces much smaller structural changes.

Finally, in an attempt to determine the amount of stabilization endowed upon the lithiated imine by interaction with the amino group, we decided to examine ion 17, where the amino group is rotated away from the site of protonation. This isomer has a similar ring structure to 14a but is 22.8 kcal mol$^{-1}$ higher in energy, giving it a binding enthalpy of 35.9 kcal mol$^{-1}$. In separate calculations at MP2(full)/6-311++G(d,p) the Li$^+$ affinities of CH$_2$NH$_2$ and of CH$_2$NH were found to be 38.6 and 37.2 kcal mol$^{-1}$, respectively. Hence, in the absence of secondary interactions with another heteroatom the ability of the 5-oxazolone to delocalize the charge over the ring has little effect on the Li$^+$ affinity of the imino group in the ring. However, the binding resulting from interaction with the two nitrogens simultaneously is 17.1 kcal mol$^{-1}$ less than the sum of that with the two groups separately.

(c) Alternative Sites of Argentination. As with Li$^+$, silver ion preferentially binds with the two nitrogen atoms of 2-(aminomethyl)-5-oxazolone. The binding enthalpy as calculated at B3LYP/DZVP is 53.7 kcal mol$^{-1}$, i.e., slightly less than the binding enthalpy of Li$^+$. This compares with calculated values for these isolated functional groups of 40.6 kcal mol$^{-1}$ for CH$_2$NH and 43.6 kcal mol$^{-1}$ for CH$_2$NH$_2$.

We examined three isomers, two in which the silver ion is bound to the amino group, structures 14b and 15b, and one in which it is attached to the carbonyl oxygen 16b. The calculated
which “ghost” functions from the other product of the dissociation. The geometry of the complex on the dissociation products in error (BSSE) can be remedied by performing calculations at metal ion and the ligand. As a result, the enthalpies for the description of a complex than of its dissociation products, the differences from using only medium-sized basis sets result in a better description of formaldehyde (28.7 kcal mol\(^{-1}\)). The relative enthalpies of the three isomers are the same as for the corresponding isomers on the \(\text{C}_4\text{H}_6\text{N}_2\text{O}_2\text{Li}^+\) potential energy surface and the energy differences are similar (Table 5).

A comparison of structures 14b, 15b, and 16b with those of the lithiated analogues shows remarkable similarities in structural parameters with the obvious difference that Ag–ligand distances are longer than Li–ligand distances (Figures 2 and 3). Also, we were unable to locate a structure in which the silver is coordinated to only one nitrogen, as in 17 on the \(\text{C}_4\text{H}_6\text{N}_2\text{O}_2\text{Li}^+\) potential energy surface. Two minor variations are worthy of note. In structure 15a the Li–O distance is shorter than Li–N, whereas in 15b the Ag–O distance is longer than the Ag–N, reflecting the preference of lithium for oxygen and that of silver for nitrogen. Also in the carbonyl metalated structures 16, the metal–oxygen–carbon angle is much larger in the complex with lithium than in that with silver (162.2° compared with 130.5°).

(d) Basis Set Superposition Errors (BSSE). Basis set deficiencies from using only medium-sized basis sets result in a better description of a complex than of its dissociation products, the metal ion and the ligand. As a result, the enthalpies for the binding reactions are overestimated. This basis set superposition error (BSSE) can be remedied by performing calculations at the geometry of the complex on the dissociation products in which “ghost” functions from the other product of the dissociation are included. At B3LYP/DZVP BSSE corrections (in kcal mol\(^{-1}\)) are for \(\text{CH}_2\text{NHAg}^+\), 1.24; for \(\text{CH}_2\text{NHAg}^+\), 1.56; for \(\text{H}_2\text{COAg}^+\), 1.29; and for 2-(aminomethyl)-5-oxazolone, 4a, 2.36. These calculations establish that inclusion of BSSE corrections have only a minor effect in reducing binding enthalpies and will not change the relative energies discussed above.

Conclusions

The preferred site for protonation of oxazolones is on the imino nitrogen and the proton affinities of the differently substituted structures examined here are \(\approx 13\) kcal mol\(^{-1}\) greater than that of methylenimine. Furthermore, these proton affinities are largely independent of whether there is a phenyl or \(\text{CH}_2\text{NH}_2\text{Ag}^+\) substituent at the C2-position on the ring, and also of whether there is H or CH\(_3\) at the C2-position. Protonation on the imino nitrogen results in large structural changes within the ring, the largest one being lengthening of the C5–O distance, the bond that has to be broken if CO loss is to occur.

The lowest energy tautomers of protonated oxazolones are stabilized by hydrogen bonds, structures in which the proton is shared unsymmetrically between the two basic nitrogen sites. By contrast, cations in which either Li\(^+\) and Ag\(^+\) is coordinated to 2-(methylamino)-5-oxazolone, prefer dicoordination with the metal ion attached to both the amino group and either the imino nitrogen or the oxygen in the ring. The structural changes induced in the ring by adding a metal ion are similar to those produced on protonation; however, they are less dramatic.

Acknowledgment. We thank Steve Quan and Alwin Cunje for technical assistance. Financial support from the Natural Science and Engineering Council of Canada, MDS SCIEX, Canadian Foundation for Innovation, and Ontario Innovation Trust are much appreciated.

References and Notes