Regular Article

Electron Ionization of Serine and Threonine: a Discussion about Peak Intensities

B. Farajmand and H. Bahrami*

Chemistry Department, University of Zanjan, Zanjan 45371-38791, Iran

(Received 28 April 2016, Accepted 13 June 2016)

The present study describes the fragmentation under electron ionization (EI) of gas phase serine and threonine amino acids. *Ab initio* methods were performed to calculate the fragmentation paths and interpret the mass spectra. The six lowest energy conformers of L-serine, L-threonine and L-allo-threonine were obtained with B3LYP, G3MP2 and MP2 methods. The adiabatic and vertical ionization energies of the most stable conformers are reported. The structures of fragment ions and neutral species formed by direct bond cleavages as well as rearrangement reactions were obtained. Appearance energies (AE) were calculated for the formation of particular fragment ions at 200 °C. The order of peak intensities were compared with the theoretical calculated AEs, and a complementary explanation was provided about the relative abundance of fragmentation products.

Keywords: Serine, Threonine, Mass spectrometry

INTRODUCTION

Serine and threonine are proteinogenic amino acids bearing an alcohol group in the side chains. Serine has a pivotal role in metabolism and signaling activities in living organisms [1]. It plays a crucial role as the precursor for several metabolites, including sphingolipids and folate. Serine exists in the active sites of many enzymes and has an essential role in the catalytic function of enzymes. It is also important in brain morphogenesis during embryonic development [2]. Threonine is an important constituent of collagen, elastin, and enamel protein. It promotes the proper fat metabolism in the liver [3]. Threonine contributes to a better function of the digestive tracts and its deficiency has been associated with skin disorders and weakness.

Theoretical and experimental studies have been carried out on the structure, properties and fragmentation of amino acids [4,5]. Over the past decade, some conformational investigations of the two most important hydroxyl-amino acids serine and threonine at relatively high-level theory have been reported [6,7].

─────────

*Corresponding author. E-mail: hbahrami@znu.ac.ir

Density functional theory (DFT) calculations have been performed by Miao *et al.* to investigate the gas-phase conformations of serine and its related ions [6]. The molecular structures and vibrational spectra of serine and threonine have been studied by Lakard [7]. In another study, the conformational equilibria of neutral serine were studied by experimental matrix-isolation fourier transform infrared spectroscopy in combination with DFT calculations [8]. The vertical ionization energies (VIE) of the conformers of the α-amino acids have also been calculated [9,10]. Rotational spectroscopy in combination with molecular beams and laser ablation have proved to be successful in characterizing the conformers of natural L-threonine. The experimental rotational constants have directly been compared with those predicted by *ab initio* methods to achieve the conclusive identification of seven different conformers [11].

Because of their scientific and medical interest, several academic research groups have studied the structural elucidation of amino acids using electron ionization mass spectrometry (EI-MS), but the interpretation of mass spectra has typically been performed by theoretical calculations [12-15]. Electron ionization, mainly produces positive ions and neutral radical molecules directly in the ionization

chamber. These highly reactive species can undergo additional fragmentation. The EI of the two hydroxy substituted amino acids, serine and threonine, has already been studied by Junk and Svec [16]. They discussed in detail the relative amount of the selected fragment ions including $[P-COOH]^+$, $[P-H_2O]^+$, $[P-H_2O-COOH]^+$, $[P-R+H]^+$ and $[H_2O]^+$.

Appearance energy (AE) is a valuable tool in mass spectrometry for the evaluation of thermodynamic properties of ions, neutral molecules and fragments [17-20]. The amount of energy needed to be transferred to the neutral M to detect the fragment ion m+ is called AE of that fragment ion (n denotes neutral species):

$$M \rightarrow m^+ + n + e^- \qquad AE = \Delta_r H \qquad (1)$$

The purpose of this study is to perform extensive calculations of structures and energies of ionic and neutral products with the aim of calculating the AE for each fragment ion. The comparison of experimental peak intensities and theoretical AEs can lead to better interpretation of electron ionization in mass spectrometry of amino acids. The amino acids studied in the present work only differ by their -R group, containing a primary alcohol for serine and a secondary alcohol for threonine. Therefore, comparing the fragmentation paths of these amino acids might bear promising results.

Threonine is of particular significance to study because main dissociation channels of this molecule produces fragments with the same m/z ratios. These products include $[P-COOH]^+$ and $[P-R]^+$ with m/z 74 as well as R^+ and $COOH^+$ with m/z 45. A similar behavior is observed for $[P-R+H]^+$ and $[P-CO_2]^+$ with m/z 75 and $[P-CO_2-H_2O]^+$ and $[P-R-H_2O]^+$ with m/z 57. In fact, another objective of this article is to evaluate the contribution of ions involved in the creation of each peak.

EXPERIMENTAL

Mass spectra were measured using a quadrupole spectrometer TRIO 1000, (Fisons Instruments, Manchester, England). The spectra were acquired as full scans from m/z 20 to m/z 150 under a 70-eV ionization potential. The sample was inserted into the spectrometer by the Direct Inlet Probe. Ionization source temperature was set at 200 ℃. L-serine and L-threonine was obtained from Merck (Darmstadt, Germany).

COMPUTATIONAL METHODS

All theoretical calculations were carried out using quantum computational Gaussian 09 program package [21]. Three different *ab initio* approaches were used to calculate AEs of particular ionic fragments.

The study began with geometry optimization of the structures of L-serine, L-threonine and L-allo-threonine in vacuum at the B3LYP and G3MP2 level using 6-311G basis set. The 12 more stable structures of each amino acid were used as the initial structures for the subsequent conformational analysis. They were fully reoptimized at the G3MP2, B3LYP and MP2 level using 6-311++G(d,p) basis set, yielding 6 stable conformers of each amino acid. For each optimized structure, vibrational analysis was performed to exclude any transitional states and to include the zero point vibrational energy correction. The values of enthalpies of serine and threonine isomers as well as their decomposition products were determined to be used for AE calculation.

The most stable neutral structures were also used to calculate the energies of cations by setting the charge equal to +1. The adiabatic and vertical ionization energies were taken as the energy difference between the cationic and neutral conformations.

RESULTS AND DISCUSSION

The EI-MS of the compounds studied here are shown in Fig. 1. It can be observed that a large number of positive ions are produced upon the electron ionization of serine and threonine amino acids.

In spite of different molecular weights of serine and threonine, some common features are visible in their mass spectra. For example, m/z 74 and m/z 75 can be seen in both cases with different intensities.

Selection of Stable Conformers
Several theoretical papers on ab initio structural studies of amino acids can be found for gas phase serine and
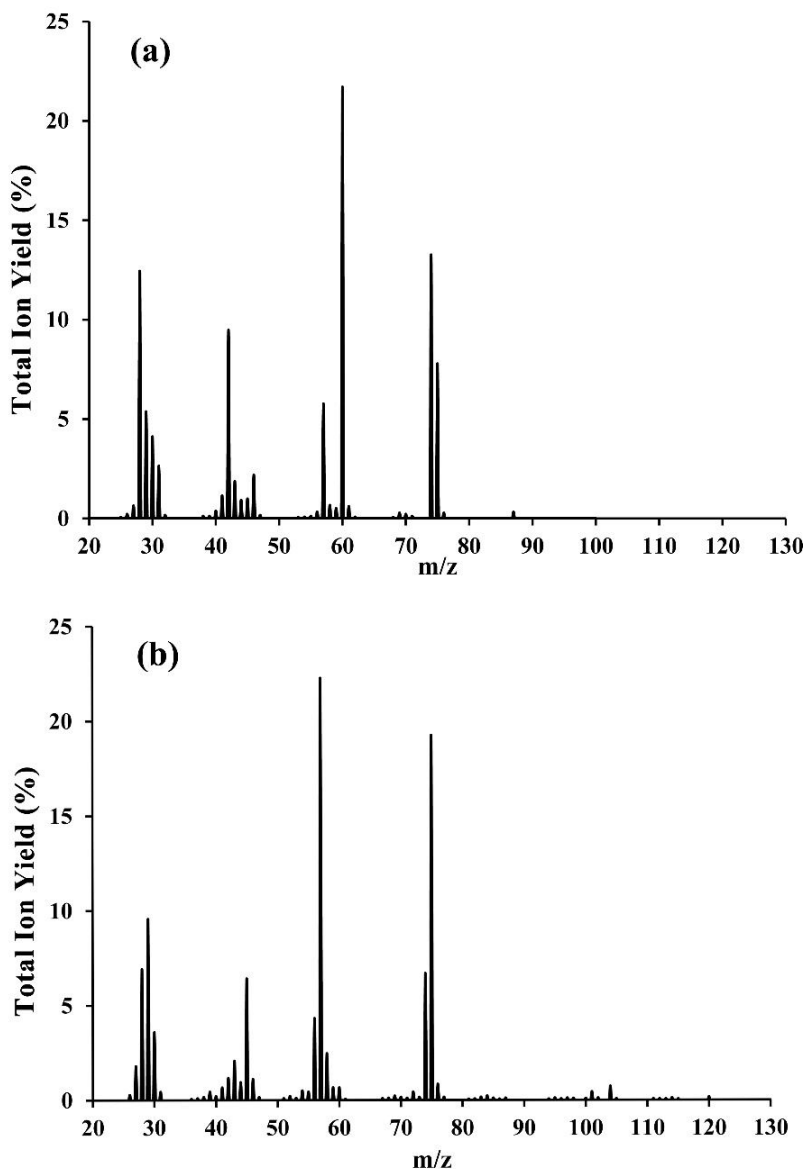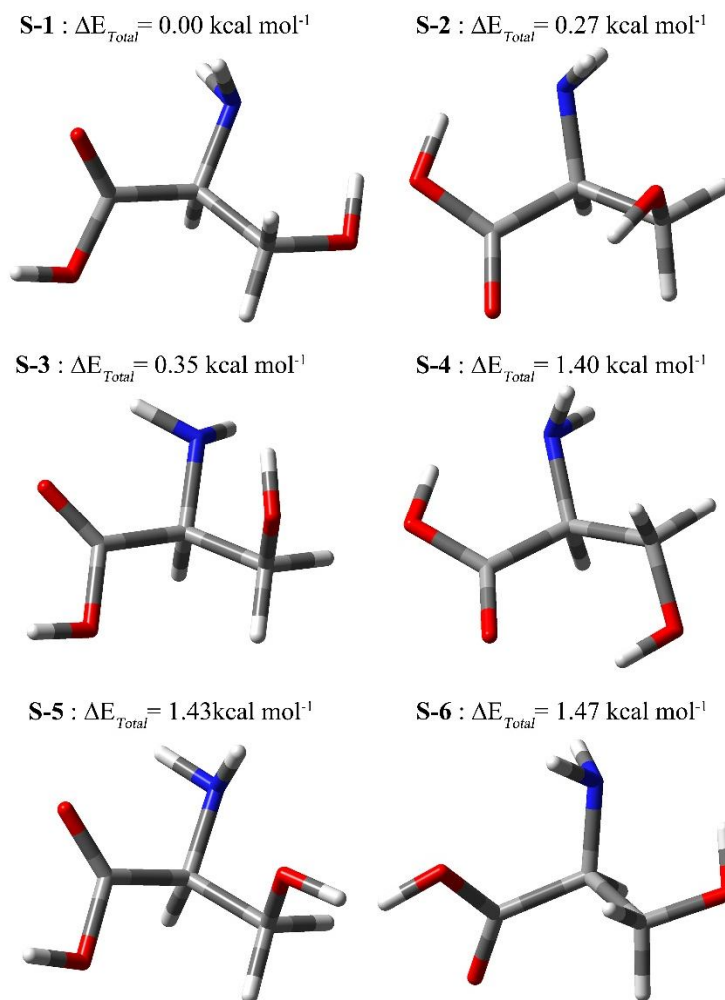
Fig. 1. EI-MS obtained for (a) L-serine and (b) L-threonine.

threonine. Three hydrogen bonding stabilization effects are known for aliphatic amino acids in gas phase; each one proportional to the corresponding amino and carboxyl groups. However, there is a level of complexity due to the presence of a hydrogen-bonding group in the side chain of serine and threonine. In their studies at the MP2/6-31+G* level, Gronert and O'Hair [22] found 51 conformations for serine. A total of 71 unique conformations were found for threonine by Zhang and Lin [3].

The six lowest energy conformers of L-serine, L-threonine and L-allo-threonine obtained from optimization at the MP2/6-311++G(d,p) level are shown in Figs. 2-4 respectively. The relative energies of those structures are listed in Table 1. All relative values were compared to that of the most stable conformer.

Note that the most stable conformation of threonine found by Zhang and Lin [3] is similar to L-2. However, the most stable conformers obtained here agree with the

S-1 : ΔE_{Total} = 0.00 kcal mol^{-1} **S-2** : ΔE_{Total} = 0.27 kcal mol^{-1}

S-3 : ΔE_{Total} = 0.35 kcal mol^{-1} **S-4** : ΔE_{Total} = 1.40 kcal mol^{-1}

S-5 : ΔE_{Total} = 1.43 kcal mol^{-1} **S-6** : ΔE_{Total} = 1.47 kcal mol^{-1}

Fig. 2. The six lowest energy conformers of L-serine optimized at the MP2/6-311++G(d,p) level.

characterization summarized by Schäfer and co-workers [23].

There are several types of intramolecular H-bond interaction contributing to the stabilization of amino acid conformers [22,23]. This is while the MP2/6-311++G(d,p) relative energies predicted that Fe O-H…N-H…O=C intramolecular H-bond interaction has the most significant role in the stabilization of the lowest energy conformer in both serine and threonine. These conformers (S-1, L-1, and L-allo-1) were selected for further calculations.
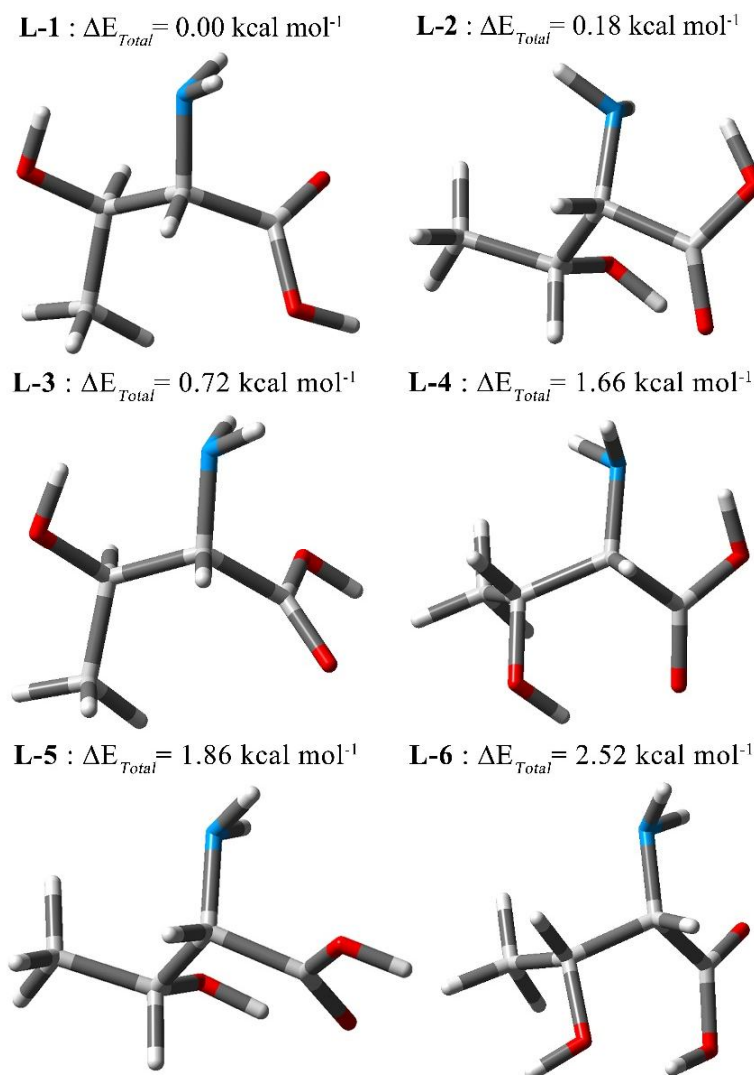
Parent Ions

The parent ions (P$^+$) of amino acids formed *via* direct ionization of neutral molecules (M) are detected rarely with very weak intensities in mass spectra.

$$e + M \rightarrow [P^+]^* + 2e \qquad (2)$$

Identification of the parent ion as the molecular ion in EI is not always simple. Soft ionization techniques facilitate this identification. These ions correspond to m/z 105 for serine and m/z 119 for threonine isomers (Fig. 1).

Ionization energy is reported in two ways. Vertical ionization energy (VIE) is estimated as the difference in total energy between the cation radical and the neutral one in the geometry of the neutral species (optimizations of the

L-1 : ΔE_{Total}= 0.00 kcal mol^{-1} **L-2** : ΔE_{Total}= 0.18 kcal mol^{-1}

L-3 : ΔE_{Total}= 0.72 kcal mol^{-1} **L-4** : ΔE_{Total}= 1.66 kcal mol^{-1}

L-5 : ΔE_{Total}= 1.86 kcal mol^{-1} **L-6** : ΔE_{Total}= 2.52 kcal mol^{-1}
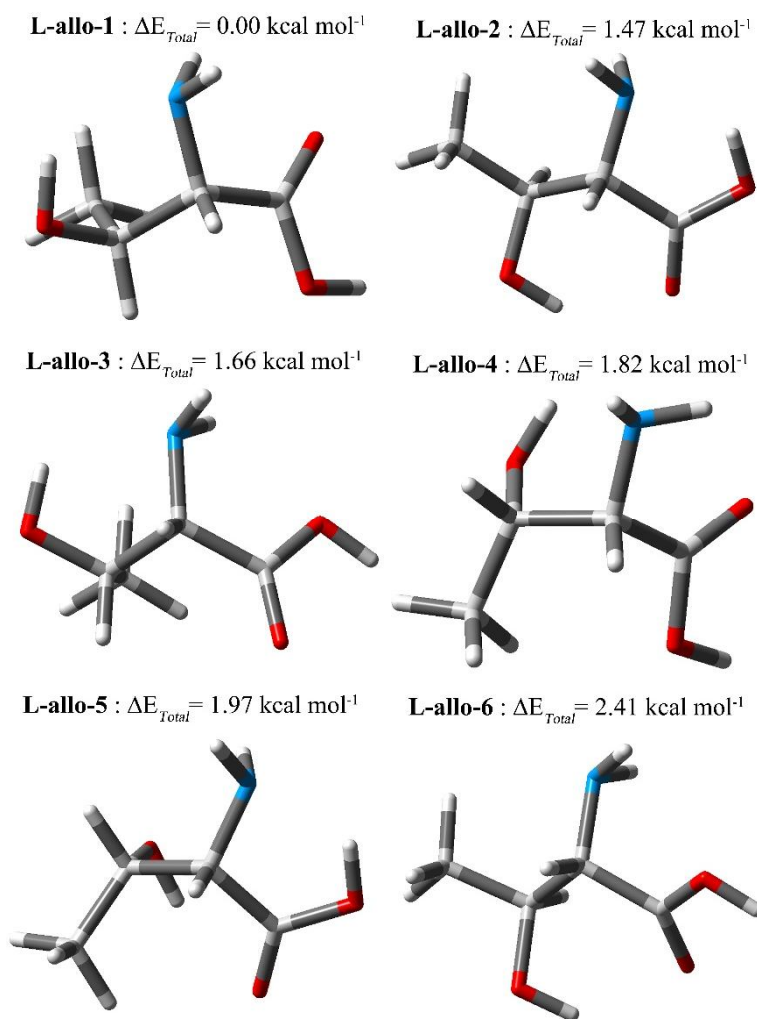
Fig. 3. The six lowest energy conformers of L-threonine (2*S*,3*R*) optimized at the MP2/6-311++G(d,p) level.

neutral molecules are followed by a single point calculation on the cation) [24,25]. To obtain the adiabatic ionization energy, the cation should be also optimized. This procedure has been carried out for the most stable isomers of serine and threonine at three levels of theory (Table 2). The comparison of the experimental ionization energies from national institute of standards and technology (NIST) database [26] and VIE reported by Dehareng [10] with the present calculated values are also presented in Table 2. The present calculated values of the ionization energies are slightly lower than the data taken from NIST determined by photoelectron spectroscopy methods [26].

The B3LYP geometries of neutral L-serine, L-threonine and L-allo-threonine conformers as well as their cationic structures at three levels of theory are presented in Fig. 5. It is important to note that in the B3LYP geometries of cationic structures, side chain R has a tendency to dislodge in both serine and threonine cases. Only the G3MP2 geometries of cationic parent structures were found to be optimal in all cases.

L-allo-1 : ΔE_{Total}= 0.00 kcal mol^{-1} **L-allo-2** : ΔE_{Total}= 1.47 kcal mol^{-1}

L-allo-3 : ΔE_{Total}= 1.66 kcal mol^{-1} **L-allo-4** : ΔE_{Total}= 1.82 kcal mol^{-1}

L-allo-5 : ΔE_{Total}= 1.97 kcal mol^{-1} **L-allo-6** : ΔE_{Total}= 2.41 kcal mol^{-1}

Fig. 4. The six lowest energy conformers of L-*allo*-threonine (2*S*,3*S*) optimized at the MP2/6-311++G(d,p) level.

Direct Bond Cleavages

Electron ionization of aliphatic amino acids generates some ions including direct bond cleavages without significant rearrangement of the initial geometry [12]. These ions have been produced *via* one or more functional group loss. The structures of fragment ions and neutral species formed by direct bond cleavages are given as supplementary material. The calculated AEs of some product ions created from the most stable conformers (S-1, L-1, L-allo-1) have been listed in Table 3.

Serine. As shown in Table 3, the fragment ion [P-COOH]$^+$ with m/z 60 has the lowest calculated AE. This

cation is produced *via* cleavage of carboxylic group from the parent molecular ion. The AE correspondence to formation of COOH$^+$ counterpart (at m/z 45) is about 40 kcal mol^{-1} higher than that of [P-COOH]$^+$. It is evident from the mass spectra (Fig. 1a) that m/z = 60 is the dominant ionic product, while no significant intensity was detected for m/z = 45. Formation of m/z 60 rather than m/z 45 may be due to the presence of amine functional group in [P-COOH]$^+$ counterpart. The theoretical study showed that positive charge is mainly localized on amine group, in the optimized structure of parent cation.

A similar trend was observed when comparing peaks

Table 1. Relative Electronic Energies (ΔE), Total Energy (ΔE_{total}) and Gibbs Free Energy ($\Delta G_{(473 K)}$) in kcal mol^{-1} Computed with the B3LYP, G3MP2, and MP2 Methods for the Six Lowest Conformers of L-serine, L-Threonine, and L-Allo-threonine

Molecule	Conformer	B3LYP/6-311++G(d.p)			G3MP2			MP2/6-311++G(d.p)		
		ΔE	ΔE_{total}	$\Delta G_{(473 K)}$	ΔE	ΔE_{total}	$\Delta G_{(473 K)}$	ΔE_{MP2}	ΔE_{total}	$\Delta G_{(473 K)}$
L-Serine	S-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	S-2	-0.03	0.18	0.59	-0.31	0.07	0.83	0.04	0.27	0.90
	S-3	0.84	0.81	1.19	0.30	0.29	0.67	0.35	0.35	0.90
	S-4	0.35	0.75	1.74	0.41	0.77	1.66	0.96	1.40	2.38
	S-5	2.42	2.06	1.76	1.66	1.40	1.31	1.73	1.43	1.41
	S-6	1.57	1.56	1.05	1.72	1.65	0.50	1.42	1.47	1.27
L-Threonine	L-1	0.32	0.22	-0.06	0.40	0.14	-0.47	0.00	0.00	0.00
(2S,3R)	L-2	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.18	0.48
	L-3	1.28	1.10	0.61	1.44	1.06	0.15	0.81	0.72	0.44
	L-4	1.10	1.31	1.90	1.04	1.09	1.34	1.41	1.66	2.14
	L-5	1.75	1.65	1.53	1.44	1.16	0.68	1.95	1.86	1.77
	L-6	2.71	2.42	2.79	2.37	1.91	1.52	2.68	2.53	3.02
L-Allo-threonine	L-Allo-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(2S,3S)	L-Allo-2	-0.15	0.18	1.05	0.43	0.78	1.60	1.09	1.47	2.17
	L-Allo-3	1.59	1.59	1.39	1.77	1.76	1.21	1.61	1.66	1.66
	L-Allo-4	1.78	1.66	1.72	1.93	1.83	1.92	1.95	1.82	1.71
	L-Allo-5	0.84	1.04	1.29	1.16	1.51	2.23	1.79	1.97	2.26
	L-Allo-6	1.81	1.65	1.37	1.92	1.80	1.65	2.65	2.41	1.94

For **S-1**: E(MP2) = -249790.4008 kcal mol^{-1}; ZPE = 72.2442 kcal mol^{-1} and G(473K) = -249754.8132 kcal mol^{-1}

For **L-1**: E(MP2) = -275116.9539 kcal mol^{-1}; ZPE = 88.6206 kcal mol^{-1} and G(473K) = -274909.6265 kcal mol^{-1}

For **L-allo-1**: E(MP2) = -275116.5950 kcal mol^{-1}; ZPE = 88.5585 kcal mol^{-1} and G(473K) = -275067.0645 kcal mol^{-1}.

with m/z 74 and m/z 31, which is associated with dissociation of side chain group R. The intensity of m/z 74 is over 13%, while the intensity related to m/z 31 is less than 3%. This is in good agreement with the theoretical results, where the AE correspondence to formation of the R$^+$ counterpart (at m/z 31) is about 14 kcal mol^{-1} higher than that of [P-R]$^+$.

The intensities of m/z 60, 74, 31 and 45 are 21.7%, 13.3%, 2.6% and 1% respectively. The order of peak intensities are approved by the calculated AEs (Table 2).

Table 2. Ionization Energies (IE) from NIST Database. VIE Reported by Dehareng, and the Present Calculated Values. All Values are in eV

Amino acid	IE (NIST) [26]	OVGF [10]	B3LYP/6-311++G(d,p)		G3MP2	MP2/6-311++G(d,p)	
			VIE	AIE	AIE	VIE	AIE
S-1	10.0	9.99	9.64	8.75	9.03	10.42	8.93
L-1	10.2	9.80	9.44	8.37	8.94	10.32	8.77
L-Allo-1	-	9.80	9.48	8.38	8.93	10.30	8.79

Table 3. List of Fragmentation Products without a Considerable Rearrangement in Product Geometries, m/z of Product Ions, and Calculated (MP2) Appearance Energies in kcal mol^{-1}

|	Cation + neutral	S-1		L-1		L-Allo-1	
		m/z	MP2	m/z	MP2	m/z	MP2
(1)	[P-COOH]$^+$ + COOH	60	222.64	74	222.82	74	222.64
(2)	[P-R]$^+$ + R	74	229.26	74	230.09	74	229.95
(3)	[P-R] + R$^+$	31	243.20	45	226.54	45	226.40
(4)	[P-COOH] + COOH$^+$	45	267.21	45	267.66	45	268.73
(5)	[P-COOH-OH]$^+$ + COOH + OH	43	306.12	57	302.44	57	301.08
(6)	[P-R-OH]$^+$ + R + OH	57	327.11	57	327.98	57	327.82
(7)	NH$_2$CH$^+$ + COOH + R	29	336.82	29	339.74	29	337.51
(8)	[P-NH$_2$-COOH]$^+$ + NH$_2$ + COOH	44	372.65	58	316.48	58	314.25
(9)	[P-NH$_2$-R]$^+$ + NH$_2$ + R	58	389.66	58	390.50	58	390.35

Other direct dissociative ionization channels correspond to elimination of two neutral fragments, leading to formation of CH$_2$CHNH$_2$$^+$, NH$_2CH^+$, CH$_2$CHOH$^+$ and CHCOOH$^+$ (reactions 5-8 in Table 3). These ionization reactions have higher calculated AEs than single bond cleavage reactions. Peaks with m/z 43, 44 and 58 have been formed by simultaneous dissociation of OH + COOH, NH$_2$ + COOH and NH$_2$ + R respectively. Considering the mass spectrum (Fig. 1a), the peak intensities of these three fragments decrease with an increase in the calculated AEs.

According to the previous arguments, the intensity of m/z 29 should be lower than that of m/z 43 but the opposite trend is observed in the mass spectrum. In this case, it can be said that the NH$_2$CH$^+$ fragment ion (m/z 29) can be formed by sequential dissociation of m/z 60 and 74, with a large percentage of all ions as well as a simultaneous two-bond dissociation.

Threonine. In the case of serine, elimination of R and COOH groups led to the formation of m/z 74 and 60, respectively, while in the case of threonine isomers, both elimination channels generated only m/z 74 with an intensity about 7% in mass spectrum. Due to the less AE, it seems [P-COOH]$^+$ fragment ions have larger abundance than [P-R]$^+$ ions in the creation of m/z 74.
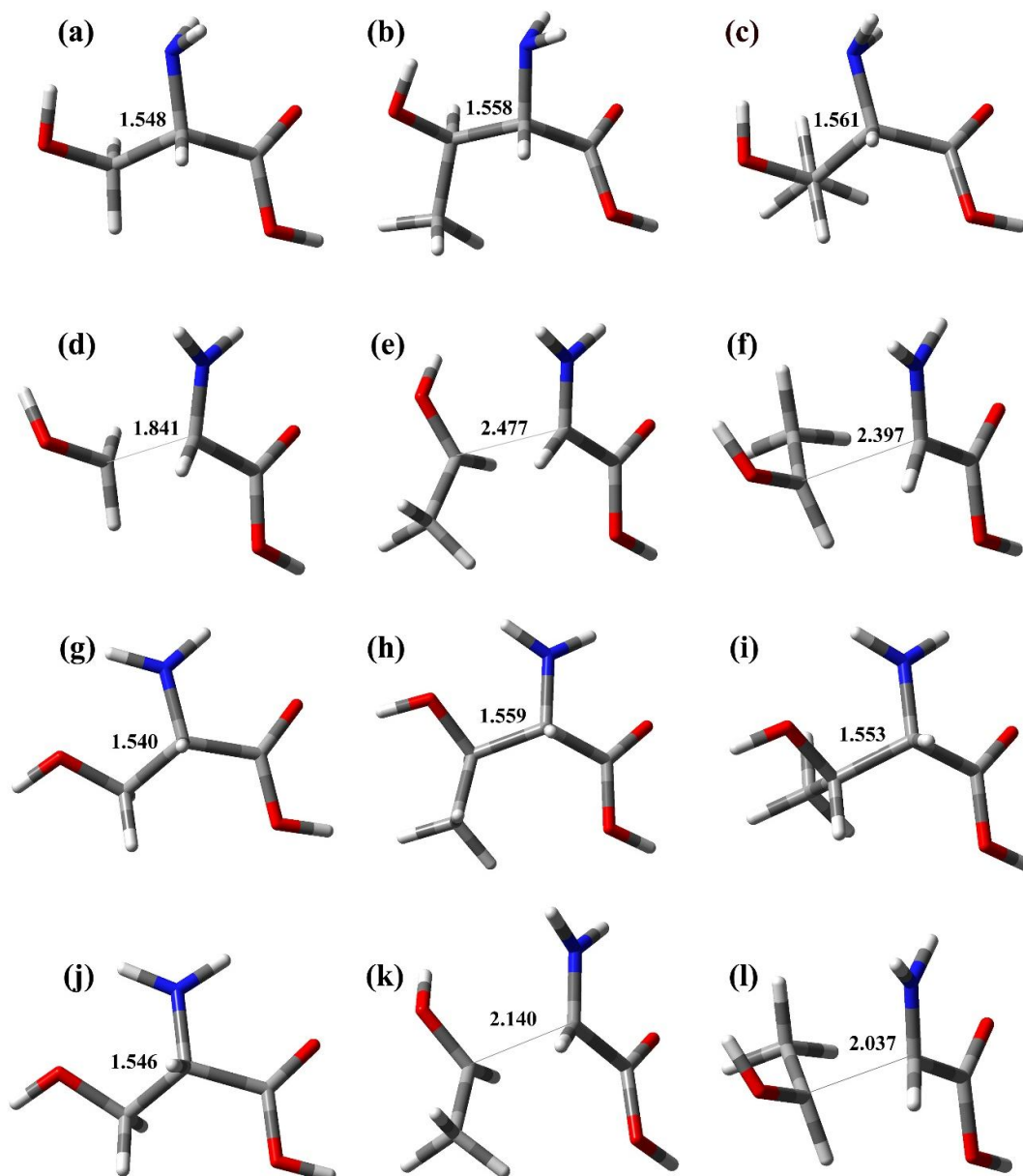
Fig. 5. Geometries of L-serine, L-threonine and L-allo-threonine conformers, in first row are the B3LYP neutral structures of (a) S-1, (b) L-1, and (c) L-allo-1, in second row the B3LYP cationic structures (d)-(f), in third row the G3MP2 cationic structures (g)-(i), and forth row the MP2 cationic structures (j)-(l) of S-1, L-1, and L-allo-1, respectively.

The peak intensity of m/z 45 in the mass spectra of threonine is increased considerably compared to that of serine. This m/z also has two sources, R^+ and $COOH^+$. As shown in Table 3, the AE of R^+ is about 40 kcal lower than that of $COOH^+$. Therefore, it seems the m/z 45 is predominantly composed of R^+ fragment ions.

A peak with m/z 57 is the base peak in the mass spectrum of threonine. The $[P-COOH-OH]^+$ fragment ion is

Table 4. List of Fragmentation Products of Serine and Threonine Considering the Rearrangement, m/z of Product Ions, and Calculated (MP2) Appearance Energies in kcal mol^{-1}

	Cation + neutral	S-1		L-1		L-Allo-1	
		m/z	MP2	m/z	MP2	m/z	MP2
(1)	$[P-R+H]^+$ + R-H (proton migration to N)	75	221.26	75	-53.64	75	-53.78
(2)	$[P-R+H]^+$ + R-H (proton migration to O)	75	210.58	75	-64.32	75	-64.46
(3)	$[P-CO_2]^+$ + CO_2 (proton migration to N)	61	-89.83	75	-90.98	75	-91.12
(4)	$[P-CO_2]^+$ + CO_2 (proton migration to O)	61	-^{a}	75	-^{a}	75	-^{a}
(5)	$[P-R-OH]^+$ + R-H + H_2O	57	234.08	57	-40.79	57	-40.95
(6)	$[P-R-H_2O]^+$ + R + H_2O	56	281.25	56	282.28	56	282.22
(7)	$[P-R-H_2O]^+$ + R-H + OH	56	305.15	56	306.91	56	306.85
(8)	$[P-NH_2-COOH]^+$ + NH_3 + CO_2	44	-14.35	58	-72.61	58	-72.75
(9)	$[P-COOH-OH]^+$ + CO_2 + H_2O	43	-92.75	57	-98.52	57	-97.78
(10)	$[P-COOH-H_2O]^+$ + COOH + H_2O	42	271.82	56	226.97	56	228.67
(11)	$[P-COOH-H_2O]^+$ + HCOOH + OH	42	293.92	56	250.77	56	246.98
(12)	$[NH_2CH]^+$ + CO_2 + RH	29	-38.59	29	-36.95	29	-37.09
(13)	$[NHCH]^+$ + HCOOH + R	28	266.45	28	267.29	28	267.14
(14)	$[NHCH]^+$ + COOH + RH	28	267.81	28	271.54	28	271.40

*Further fragmentation was occurred to form $[P-CO_2-H_2O]^+$ *via* H_2O cleavage.

related to m/z 57. As can be seen in Table 3, there is a relatively high AE by simultaneous direct dissociation of the two bonds of threonine. This indicates a negligible contribution of direct cleavage in the formation of m/z 57. To interpret the intensity of this peak, the rearrangement of dissociation products should be considered. This issue is discussed in the next section.

Two fragment ions including $[P-NH_2-COOH]^+$ and $[P-NH_2-R]^+$ participate in the creation of m/z 58, and NH_2CH^+ fragment ion creates m/z 29. Creation of these ions by direct dissociation requires large amounts of AE (Table 3). The relative abundance of these ions lies in the rearrangement of by-products dissociation.

Rearrangement Reactions

Some ion fragments that cannot be formed by simple cleavage of a bond or bonds of the molecule are found in the electron ionization mass spectrum. Molecular rearrangement during ion formation is very common in mass spectrometry [27]. One of the effective factors on the relative abundance of a rearranged ion is relative stability of products of the rearrangement reaction, as compared to other possible reaction paths. It is well-stablished that intramolecular rearrangement of the molecular ion is the main mechanism of rearrangements [28,29]. The intensity of a peak in a mass spectrum depends on formation rate of the ions, making up this peak by fragmentation of parent ions, as well as decomposition rate for creation of smaller fragments [30].

Several products of electron ionization of serine and threonine are produced via rearrangement reactions. All the corresponding structures can be found in the supplementary material. The corresponding calculated AEs of these ions are listed in Table 4.

Serine. The m/z 75 is associated with one hydrogen atom transfer to the fragment ion and formation of formaldehyde as a neutral structure (reactions (1) and (2) in Table 4). Elimination of COOH radical, followed by hydrogen atom transfer to the fragment ion (reaction (3) in Table 4), has a negative AE, while its peak intensity is negligible in the mass spectrum (m/z 61 Th). Formation of neutral CO_2 has not been suggested by Junk and Svec [16] in EI of serine and threonine. It may be due to the steric hindrance for the hydrogen transfer from COOH to a suitable receptor site in fragment ion during elimination of COOH.

There are two paths for the formation of m/z 57 including (a) direct elimination of OH and R without rearrangement (reaction (6) in Table 3) and (b) proton transfer from R group to OH after direct bond-cleavage and formation of H_2O molecule (reaction (5) in Table 4). The AE of path (b) is less than that of path (a); therefore, the main path for the formation of m/z 57 is path (b). As seen in Table 5, the AE corresponding to the formation of $[P-R+H]^+$ fragment ion (m/z 75) is less than that of $[P- R-OH]^+$ (m/z 57) by about 20 kcal mol^{-1}, confirming the relative intensities in the mass spectrum.

As was mentioned above about m/z 61, the formation of m/z 43 and m/z 44, due to the release of CO_2, has low probabilities despite the negative AEs. m/z 42 and m/z 28 are two prominent peaks with ion yields over 9.5% and 12.5%, respectively. As shown in Table 4, the AEs are in agreement with peak intensities (reactions (10-11) and (13-14) in Table 4).

The peaks related to m/z 28 and m/z 42 are more intense than those of m/z 75 and m/z 57 in mass spectrum, however the AE values show a contrary trend. This conflict may be due to the destruction probability of m/z 60 fragment ion and formation of the ions with m/z 28 and m/z 42.

The intensity of m/z 56 is negligible (about 0.3%) in mass spectrum of serine. The predicted AE for production of ion with this m/z is in reasonable agreement with the peak intensity (reactions 6 and 7, Table 4).

Threonine. There are two channels for production of the peak with m/z 75 in the case of threonine including elimination of R-H or CO_2 from molecular ion (reaction 1-4, Table 4). According to the interpretation given in the previous section, elimination of CO_2 has a lower contribution to creation of this peak, whereas the proton transfer from detached R group to the fragment ion plays a significant role in the formation of m/z 75.

The dominant peak in the mass spectrum of threonine isomers is m/z 57. As seen in Table 4, the AE values concerning m/z 57 are relatively more positive than those of m/z 75, while the intensity of m/z 57 is more than that of m/z 75. m/z 57 can be produced by H_2O elimination from m/z 75. It seems that partial conversion is involved with high intensity of m/z 57 in mass spectrum.

In the mass spectrum of threonine, m/z 56 has a reasonable intensity (4.3%) compared to serine. Theoretical prediction shows that there are other channels with lower AEs (reactions 10 and 11, Table 4) for creation of m/z 56 for threonine.

The predicted structure for m/z 28 is $[NHCH]^+$ fragment ion. This ion can be produced by simultaneous elimination of HCOOH + R or COOH + RH from molecular ion with AEs of about 267 and 271 kcal mol^{-1}, respectively, for both isomers. The considerable intensity of this peak (6.9%) can be due to sequential dissociation of m/z 74 and 56.

CONCLUSIONS

Electron ionization of serine and threonine isomers results in several peaks in the mass spectra. These peaks appear due to the fragmentation of sample molecules as a result of collisions with high energy electrons. Theoretical calculation was carried out for interpretation of the peak intensities. Theoretical calculations at the DFT, G3MP2, and MP2 levels were used to find the optimized structure of stable conformers for serine, threonine and their corresponding ionic species. The AEs were calculated for formation of particular fragment ions. The order of peak intensities was compared with that of the calculated AEs. It is important to note that the method proposed here can be used to explain fragmentation associated with direct bond cleavage perfectly. Nevertheless, this method has limitations in describing fragmentation associated with inter-molecular rearrangements.

ACKNOWLEDGMENTS

Reaction Research Group at University of Zanjan.

REFERENCES

[1] Ros, R.; Muñoz-Bertomeu, J.; Krueger, S., Serine in plants: biosynthesis, metabolism, and functions. *Trends Plant Sci.* **2014**, *19*, 564-569, DOI: 10.1016/j.tplants.2014.06.003.

[2] Tabatabaie, L.; Klomp, L. W.; Berger, R.; de Koning, T. J., l-Serine synthesis in the central nervous system: A review on serine deficiency disorders. *Mol. Genet. Metab.* **2010**, *99*, 256-262, DOI: 10.1016/j.ymgme.2009.10.012.

[3] Zhang, M.; Lin, Z., *Ab initio* studies of the conformers and conformational distribution of the gaseous hydroxyamino acid threonine. *J. Mol. Struct. THEOCHEM* **2006**, *760*, 159-166, DOI: 10.1016/j.theochem.2005.12.008.

[4] Citir, M.; Hinton, C. S.; Oomens, J.; Steill, J. D.; Armentrout, P. B., Infrared multiple photon dissociation spectroscopy of cationized histidine: effects of metal cation size on gas-phase conformation. *J. Phys. Chem. A* **2012**, *116*, 1532-1541, DOI: 10.1021/jp209636a.

[5] Simon, S.; Gil, A.; Sodupe, M.; Bertrán, J., Structure and fragmentation of glycine, alanine, serine and cysteine radical cations. A theoretical study. *J. Mol. Struct. THEOCHEM* **2005**, *727*, 191-197, DOI: 10.1016/j.theochem.2005.02.053.

[6] Miao, R.; Jin, C.; Yang, G.; Hong, J.; Zhao, C.; Zhu, L., Comprehensive density functional theory study on serine and related ions in gas phase: Conformations, gas phase basicities, and acidities. *J. Phys. Chem. A* **2005**, *109*, 2340-2349, DOI:10.1021/jp0453919.

[7] Lakard, B., *Ab initio* study of amino acids containing hydroxy groups (serine, threonine and tyrosine). *J. Mol. Struct. THEOCHEM* **2004**, *681*, 183-189, DOI: 10.1016/j.theochem.2004.04.067.

[8] Lambie, B.; Ramaekers, R.; Maes, G., Conformational behavior of serine: An experimental matrix-isolation FT-IR and theoretical DFT(B3LYP)/6-31++G** study. *The J. Phys. Chem. A* **2004**, *108*, 10426-10433, DOI: 10.1021/jp047192v.

[9] Close, D. M., Calculated vertical ionization energies of the common α-amino acids in the gas phase and in solution. *J. Phys. Chem. A* **2011**, *115*, 2900-2912, DOI: 10.1021/jp200503z.

[10] Dehareng, D.; Dive, G.; Vertical ionization energies of α-L-amino acids as a function of their conformation: an *Ab Initio* study. *Int. J. Mol. Sci.* **2004**, *5*, 301-332, DOI: 10.3390/i5110301.

[11] Alonso, J. L.; Perez, C.; Eugenia Sanz, M.; Lopez, J. C.; Blanco, S., Seven conformers of l-threonine in the gas phase: a LA-MB-FTMW study. *Phys. Chem. Chem. Phys.* **2009**, *11*, 617-627, DOI: 10.1039/B810940K.

[12] Papp, P.; Shchukin, P.; Kočíšek, J.; Matejčík, Š.; Electron ionization and dissociation of aliphatic amino acids. *J. Chem. Phys.* **2012**, *137*, 105101-9, DOI: 10.1063/1.4749244.

[13] Tamuliene, J.; Romanova, L. G.; Vukstich, V. S.; Snegursky, A. V., Electron-impact and thermal fragmentation of amino acid molecules: Mechanisms and structure of the molecules. *Nucl. Instrum. Methods Phys. Res., Sect. B* **2012**, *279*, 128-134, DOI: 10.1016/j.nimb.2011.10.022.

[14] Borkowski, E. J.; Cecati, F. M.; Suvire, F. D.; Ruiz, D. M.; Ardanaz, C. E.; Romanelli, G. P.; Enriz, R. D., Mass spectrometry and theoretical calculations about the loss of methyl radical from methoxilated coumarins. *J. Mol. Struct.* **2015**, *1093*, 49-58, DOI: 10.1016/j.molstruc.2015.03.007.

[15] Grimme, S., Towards first principles calculation of electron impact mass spectra of molecules. *Angew. Chem. Int. Ed.* **2013**, *52*, 6306-6312, DOI: 10.1002/anie.201300158.

[16] [16] Gregor, H.Svec, The Mass Spectra of the α-Amino Acids. *J. Am. Chem. Soc.* **1963**, *85*, 839-845, DOI: 10.1021/ja00890a001.

[17] Opitz, J., Electron-impact ionization of benzoic acid, nicotinic acid and their n-butyl esters: An approach to regioselective proton affinities derived from ionization and appearance energy data. *Int. J. Mass Spectrom.* **2007**, *265*, 1-14, DOI: 10.1016/j.ijms.2007.04.014.

[18] Głuch, K.; Cytawa, J.; Michalak, L.; Electron impact ionization of acrylonitrile. *Int. J. Mass Spectrom.* **2008**, *278*, 10-14, DOI: 10.1016/j.ijms.2008.07.001

[19] Ipolyi, I.; Cicman, P.; Denifl, S.; Matejčík, V.; Mach, P.; Urban, J.; Scheier, P.; Märk, T. D.; Matejčík, Š., Electron impact ionization of alanine: Appearance energies of the ions. *Int. J. Mass Spectrom.* **2006**, *252*, 228-233, DOI: 10.1016/j.ijms.2006.03.009.

[20] Papp, P.; Lacko, M.; Mészáros, D.; Stano, M.; Mach, P.; Matejčík, Š., An experimental and theoretical study of electron impact ionization and dissociative electron attachment to trimethyl borate. *Int. J. Mass Spectrom.* **2014**, *365-366*, 157-162, DOI: 10.1016/ j.ijms.2014.01.015.

[21] M.J., Frisch, G.W.T.H.B., Schlegel, G.E.; Scuseria, M.A.; Robb, J.R.; Cheeseman, G.; Scalmani, V.; Barone, B.; Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O.Kitao, H. Nakai, T. Vreven, J.A. Montgomery, Jr., J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, O. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian 09, Revision B.01, Gaussian, Inc., Wallingford CT, 2010.

[22] Gronert, S.; O'Hair, R. A. J., *Ab initio* studies of amino acid conformations. 1. The conformers of alanine, serine, and cysteine. *J. Am. Chem. Soc.* **1995**, *117*, 2071-2081, DOI: 10.1021/ja00112a022.

[23] Schäfer, L.; Kulp-Newton, S. Q.; Siam, K.; Klimkowski, V. J.; Van Alsenoy, C., *Ab initio* studies of structural features not easily amenable to experiment: Part 71. Conformational analysis and structural study of valine and threonine. *J. Mol. Struct. THEOCHEM* **1990**, *209*, 373-385, DOI: 10.1016/0166-1280(90)80089-7.

[24] Valadbeigi, Y.; Farrokhpour, H.; Tabrizchi, M., G4MP2, DFT and CBS-Q calculation of proton and electron affinities, gas phase basicities and ionization energies of hydroxylamines and alkanolamines. *J. Chem. Sci.* **2014**, *126*, 1209-1215, DOI: 10.1007/ s12039-014-0668-y.

[25] Boldyrev, A. I.; Simons, J.; Zakrzewski, V. G.; von Niessen, W., Vertical and adiabatic ionization energies and electron affinities of new silicon-carbon (SinC) and silicon-oxygen (SinO) (n = 1-3) molecules. *J. Phys. Chem.* **1994**, *98*, 1427-1435, DOI: 10.1021/j100056a010.

[26] Cannington, P. H.; Ham, N. S., He(I) and He(II) photoelectron spectra of glycine and related molecules. *J. Electron. Spectrosc. Relat. Phenom.* **1983**, *32*, 139-151, DOI: 10.1016/0368-2048(83)85092-0.

[27] Pihlaja, K.; Mäki, E.; Schulze, K.; Richter, C., Elimination and rearrangement reactions in the electron impact ionization mass spectrometry of 2,4,5,5-tetrasubstituted 1,2,4-triazolidine-3-thiones. *Org. Mass Spectrom.* **1991**, *26*, 844-848, DOI: 10.1002/oms.1210261007.

[28] McLafferty, F. W., Mass spectrometric analysis. molecular rearrangements. *Anal. Chem.* **1959**, *31*, 82-87, DOI: 10.1021/ac60145a015.

[29] Zubarev, R. A., Reactions of polypeptide ions with electrons in the gas phase. *Mass Spectrom. Rev.* **2003**, *22*, 57-77, DOI: 10.1002/mas.10042.

[30] Beynon, J. H.; Lester, G. R.; Williams, A. E., Some specific molecular rearrangements in the mass spectra of organic compounds. *J. Phys. Chem.* **1959**, *63*, 1861-1868, DOI: 10.1021/j150581a018.