I. INTRODUCTION

Amino acid is a type of important building blocks of life. Gas-phase studies on these molecules are essential to acquire thermochemical information which are crucial to understand fragmentation patterns [1] in proteomic [2] and genomic [3] sequencing experiments. Hence, quantitative studies on the various dissociations of amino acid are clearly desirable. Photoionization of neutral amino acids is of general interest not only for analytic mass spectrometry but also for matter wave experiments. These experiments aim at either an improved understanding of quantum coherence and decoherence [4–6] or precise measurements of molecular properties [7, 8].

Up to now, many studies of photoionization and dissociation of gas-phase amino acids in the vacuum ultraviolet (VUV) region are reported. Leach et al. measured synchrotron radiation (SR) photoionization mass spectroscopy and photoion yield curves of glycine (-h5 and -d5), α-alanine, β-alanine, α-amino isobutyric acid, α-valine, and explained the VUV photodissociation channels in detail [9]. Ahmed’s group used nano-particle thermal spray method to produce gas-phase amino acids to obtain free fragments photoionization mass spectra of several amino acids and β-carotene [10]. From the photoionization efficiency (PIE) spectra, ionization energies (IEs) were obtained to be 8.2±0.1 eV (histidine), 9.3±0.1 eV (glycine), 7.3±0.2 eV (tryptophan), 8.6±0.1 eV (phenylalanine), and <7.0 eV (β-carotene), respectively. Plekan et al. measured the photofragmentation spectra of guanine, cytosine, leucine, and methionine with noble gas resonance radiation at energy from 8.43 eV to 21.2 eV [11]. VUV photon-induced ionization and fragmentation of sarcosine and β-alanine were investigated with infrared laser desorption/tunable SR VUV photoionization mass spectrometry (PI-MS) and theoretical calculations [12, 13].

However there are few ionization studies on isoleucine in the gas phase. The IE of isoleucine was proposed to be 9.5 eV using general electric analytical mass spectrometer equipped with a heated crucible ion source by Junk et al. [14]. However, Klasinc et al. measured the IE value for isoleucine to be 8.66 eV by photoelectron spectroscopy (PES) [15], which was a little smaller than the calculated value of 8.96 eV using united atom approach method [14]. Armirrotti et al. demonstrated the possibility to distinguish leucine from isoleucine in several tryptic peptides by electrospray ionization ion trap multiple-stage mass spectrometric [16]. In the photoionization experiment [17], isoleucine was thermally placed into the gas phase and expanded into a vacuum system for access by time-of-flight mass spectroscopy (TOF-MS) and IR spectroscopy in the energy range of 2500–4000 cm⁻¹. Although appearance energies (AEs) of some fragment ions from isoleucine were also determined using electron impact (EI), and formation ways
were discussed by Junk et al. [14] and Armirotti et al. [16], the accuracy of the derived thermodynamic properties is not very high due to the low energy resolution of the electron beams. In addition, the AEs determined by EI ionization are normally larger than those obtained from photoionization [18]. To our knowledge, SR VUV photoionization and photodissociation of isoleucine with TOF-MS have not been reported in the literature.

The VUV photodissociation technique, especially performed with the SR source, has been proven to be a powerful tool for studying the energetics and dynamics of ionization and dissociation of organic and biocompounds [19]. In this work, we report the PIE curves and appearance energies of fragment ions from the photoionization of isoleucine. Combining these results with high-level ab initio calculations, the dissociative photoionization channels of isoleucine can then be established.

II. EXPERIMENTS

The details of the experimental setup and the computational method employed in this work were described in previous literatures [20–28]. Hence only a brief account is given below.

A. Experimental method

All experiments were performed on the atomic and molecular physics beamline (U14A) with the supersonic expansion molecular beam or a reflection time-of-flight mass spectrometer (RTOF-MS) system by VUV SR. SR is from an undulator-based U14A beamline of 800 MeV electron storage ring at the National Synchrotron Radiation Laboratory in Hefei, China. The main apparatus consists of a molecular beam chamber, two stages differential pumping system, and a photoionization chamber. Isoleucine with purity of 99% was purchased from Aldrich Reagent Database Inc. (Shanghai, China) and used with further dryness. The isoleucine vapor was introduced into the ionization chamber by direct evaporation of solid samples in a stainless steel tube with the inner diameter of 10 mm and the length of 160 mm. To produce isoleucine monomer, the carrier gas of Ar at 101 kPa was passed through a heating tube (100 °C), then directed into the stainless steel sample cell, and through one skimmer with diameter of 2 mm. The whole tube was heated to 240 °C which provided an adequate stream of isoleucine molecules. The temperature was sufficiently low to ensure that the thermally fragile molecules remain undissociated in the gas phase. The skimmed molecular beam was ionized by the monochromatized SR VUV in the ionization region. The ions produced were mass analyzed with a RTOF-MS mounted in the direction perpendicular to the plane defined by molecular and photon beams.

In this work, we used an argon gas filter in order to suppress high energy stray light and high-order radiation. Parent and fragment ions formed by photoionization were measured using the RTOF-MS over the photon energy of 8–15 eV. The PIE curves were obtained through photon energy scanning with intervals of 50 meV. The collection time was 200 s. The PIE curves were normalized to the incident photon flux. The AEs of fragment ions were determined by linear extrapolation of the ionization efficiency curves near the photon ionization threshold.

B. Theoretical method

The dissociation pathways of isoleucine cation were calculated using the DFT methods with the Gaussian 09 program package. The geometries optimization of the reactants, products, various intermediates (INTs), and transition states (TSs) was performed at the B3LYP/6-31+G(d,p) level [29]. And vibrational analyses were carried out at the same level of theory to characterize the optimized structures as local minima or transition states, which were also used to compute the zero-point-energy (ZPE) correction. All the stationary points were positively identified for minimum or TS. Furthermore, the dissociative ionization was studied at the B3LYP/6-31+G(d,p) level.

All the calculated energies used in this work are sum of electronic energies and ZPEs which are listed in Table I. The energy of the neutral isoleucine is defined as zero, and the relative energies of all TSs, INTs, corresponding ionization and dissociation products discussed in this work are obtained with the B3LYP method. These calculations are proven to be valuable for the interpretation of the measured results and for the assignment of the fragment ions to particular ionic structures.

The optimized lowest-energy structures of neutral isoleucine and the corresponding cation at the B3LYP/6-31+G(d,p) level are shown in Fig.1. During the ionization of isoleucine, the bond length of C1–C2 is elongated from 1.540 Å to 1.694 Å, then the C1–C2 bond can cleave easily. This may indicate that the present ion of isoleucine is unstable and loses the COOH group to form C5H12N+, corresponding to the favorable channel of the dissociative photoionization of isoleucine.

III. RESULTS AND DISCUSSION

A. Photoionization mass spectra and appearance energies

We have measured the time-of-flight mass spectra from the photoionization of isoleucine in the photon energy range from its first ionization potential (∼8 eV) to 15 eV. The typical time-of-flight mass spectrum of
TABLE I Fragmentation energies ($E_0$) of isoleucine cation calculated at the B3LYP/6-31++G(d,p) level including the zero point energy. In the dissociation pathways of C$_6$H$_{13}$NO$_2$ discussed in this work, the energy of C$_6$H$_{13}$NO$_2$ is defined as zero.

<table>
<thead>
<tr>
<th>Specie</th>
<th>$E_0$/Hartree</th>
<th>Specie</th>
<th>$E_0$/Hartree</th>
<th>Specie</th>
<th>$E_0$/Hartree</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$<em>6$H$</em>{13}$NO$_2$</td>
<td>-441.523059</td>
<td>C$<em>6$H$</em>{13}$NO$_2^+$</td>
<td>-441.200619</td>
<td>TS1</td>
<td>-441.204205</td>
</tr>
<tr>
<td>TS2</td>
<td>-441.204304</td>
<td>TS3</td>
<td>-441.199230</td>
<td>TS4</td>
<td>-441.183198</td>
</tr>
<tr>
<td>TS5</td>
<td>-284.011406</td>
<td>TS6</td>
<td>-252.061658</td>
<td>TS7</td>
<td>-251.980535</td>
</tr>
<tr>
<td>TS8</td>
<td>-252.053103</td>
<td>TS9</td>
<td>-252.041129</td>
<td>INT1</td>
<td>-441.208411</td>
</tr>
<tr>
<td>TS10</td>
<td>-252.057403</td>
<td>INT3</td>
<td>-252.063055</td>
<td>INT2</td>
<td>-441.205065</td>
</tr>
<tr>
<td>COOH</td>
<td>-189.088480</td>
<td>(CH$_3$)$_3$CHCH$_2$CHNH$_2$</td>
<td>-252.121616</td>
<td>COOH$^+$</td>
<td>-188.779318</td>
</tr>
<tr>
<td>(CH$_3$)$_3$CHCH$_2$CHNH$_2$</td>
<td>-252.323784</td>
<td>C$_2$H$_7$CH$_2^+$</td>
<td>-157.428286</td>
<td>NH$_2$CHCOOH</td>
<td>-283.748550</td>
</tr>
<tr>
<td>C$_2$H$_7$CH$_3$</td>
<td>-157.693110</td>
<td>NH$_2$CHCOOH$^+$</td>
<td>-283.475698</td>
<td>NH$_2$</td>
<td>-55.866660</td>
</tr>
<tr>
<td>C$_2$H$_7$CH=CH$_2$</td>
<td>-157.137888</td>
<td>NH$_2$CH=C(OH)$_2^+$</td>
<td>-284.068304</td>
<td>C$_2$H$_5$CH(CH$_3$)CH$^+$</td>
<td>-196.105518</td>
</tr>
<tr>
<td>CH$_3$TCHCHC$_2$H$_5^+$</td>
<td>-195.519799</td>
<td>CH$_3$CH=CHCH$_3$</td>
<td>-157.137329</td>
<td>H$_2$O</td>
<td>-76.412864</td>
</tr>
<tr>
<td>NH$_2$CHCO$^+$</td>
<td>-207.618865</td>
<td>CH$_3$CH=CHCH$_3^+$</td>
<td>-195.548470</td>
<td>NH$_3$</td>
<td>-56.532726</td>
</tr>
<tr>
<td>CH$_2$=NH$_2^+$-2</td>
<td>-94.930389</td>
<td>CH$_2$=NH$_2^+$-1</td>
<td>-94.930393</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![FIG. 1 Geometries of the most stable neural isoleucine and corresponding cation at B3LYP/6-31++G(d,p) level. All distances are in Å.](image)

isoileucine at 13 eV is shown in Fig. 2. The mass peaks at $m/z=86$, 75, 74, 69, 57, 46, 45, 44, 41, 30, 28 are the fragment ions listed in Table II, along with the relative abundances of these ions at the photon energy of 13 eV. The mass peak at $m/z=18$ is ignored because it arises from the photoionization of the background water molecule in the ionization chamber. The mass peak at $m/z=86$ is the C$_6$H$_{13}$N$^+$, corresponding to the highest peak area. This indicates that the channel to form the C$_6$H$_{13}$N$^+$ is the principal dissociative photoionization channel of isoleucine. Besides, there are some other smaller mass peaks from isoleucine. The mass distribution in the SR PI-MS of isoleucine is similar to the EI mass spectrum in the NIST database [30]. It should be pointed out that all observed ions in the photoionization time-of-flight mass spectra are fragmental ions, and the parent ion C$_6$H$_{13}$NO$_2^+$ ($m/z=131$) has not been observed, indicating that rapid fragmentation of the parent ion occurs at this incident photon energy.

There is no observation of the parent ion of isoleucine in the mass spectrum, similar to that of leucine [31]. While our time-of-flight mass spectrometric experiments fail to detect the parent cation of isoleucine, the IE of this molecule is successfully measured by PES [15]. This is due to the fact that, in the PES experiments, we need only to detect the photoelectron corresponding to the formation of the parent cation, and no measurement is made on the parent cation itself. The ionization energy for isoleucine by PES [15] is obtained to be 8.66 eV, which is in good agreement with the theoretical value (8.61 eV) using DFT method at the B3LYP/6-31++G(d,p) level in this work, but a little smaller than the one (8.96 eV) calculated by the united atom approach method [11].

The PIE curves of the major fragment ions C$_6$H$_{12}$N$^+$ ($m/z=86$), C$_6$H$_7$NO$_2^+$ ($m/z=75$), C$_5$H$_9^+$ ($m/z=69$), C$_4$H$_9^+$ ($m/z=57$) and CH$_2$N$^+$ ($m/z=30$) are obtained.
TABLE II Appearance energies (AE in eV) measured in the photodissociation of isoleucine and calculated at B3LYP/6-31++G (d, p) level.

<table>
<thead>
<tr>
<th>m/z</th>
<th>Ion</th>
<th>Relative abundance</th>
<th>(AE_{\text{exp.}})</th>
<th>(AE_{\text{cal.}})</th>
<th>This work</th>
<th>Reference</th>
<th>(AE_{\text{cal.}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>131</td>
<td>(\text{C}<em>6\text{H}</em>{13}\text{NO}_2^+)</td>
<td>0.0</td>
<td>8.84 ± 0.07</td>
<td>8.61</td>
<td>9.5 [15]</td>
<td></td>
<td>8.61</td>
</tr>
<tr>
<td>86</td>
<td>(\text{C}_2\text{H}_5\text{CH(}\text{CH}_3\text{)}\text{CH}=\text{NH}_2^+)</td>
<td>100.0</td>
<td>9.25 ± 0.06</td>
<td>9.9 [11]</td>
<td>9.25</td>
<td></td>
<td>9.68</td>
</tr>
<tr>
<td>75</td>
<td>(\text{NH}_2\text{CH} = \text{C(}\text{OH})_2^+)</td>
<td>41.8</td>
<td>11.61 ± 0.10</td>
<td></td>
<td></td>
<td></td>
<td>10.38</td>
</tr>
<tr>
<td>74</td>
<td>(\text{NH}_2\text{CHCOOH}^+)</td>
<td>38.0</td>
<td>10.20 ± 0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>(\text{CH}_3\text{CH} = \text{CHCH}_3^+)</td>
<td>19.4</td>
<td>12.81 ± 0.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>(\text{C}_2\text{H}_5\text{CH(}\text{CH}_3\text{)}^+)</td>
<td>59.9</td>
<td>12.76 ± 0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>(\text{CH} (\text{NH}_2) \text{OH}^+)</td>
<td>13.6</td>
<td>12.85 ± 0.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>(\text{COOH}^+)</td>
<td>14.7</td>
<td>13.21 ± 0.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>(\text{NH}_2\text{CHCH}_3^+) or \text{CO}_2)</td>
<td>36.5</td>
<td>14.7 ± 0.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>(\text{CH}_3\text{CH} = \text{CH}^+)</td>
<td>23.5</td>
<td>15.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>(\text{NH}_2\text{CH}_2^+) or \text{CO}^+)</td>
<td>88.7</td>
<td>15.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>(\text{HCNH}^+)</td>
<td>15.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(\text{a}\) The calculated value is 8.96 eV using united atom approach method from Ref. [11].

FIG. 3 Photoionization efficiency curves at 240 °C for (a) \(\text{C}_5\text{H}_{12}\text{N}^+\) \((m/z=86)\), (b) \(\text{C}_5\text{H}_9\text{NO}_2^+\) \((m/z=75)\), (c) \(\text{C}_5\text{H}_9^+\) \((m/z=69)\), (d) \(\text{C}_4\text{H}_9^+\) \((m/z=57)\), (e) \(\text{CH}_4\text{N}^+\) \((m/z=30)\).

by scanning continuously the photon energy. Figure 3 shows the PIE spectra of these major fragment ions. The AE in each PIE curve is determined by the linear extrapolation method \([21, 22, 32]\). Experimental appearance energies for the principal fragment ions \(m/z=86, 75, 69, 57, 30\) are derived from the PIE curves and tabulated in Table II, along with the error ranges. Also listed in Table II are the AEs obtained using the EI technique \([11]\). Comparing these two sets of results, it can be seen that the AEs derived from the EI ex-
periments are in general higher than those obtained in this work. It is known that the EI ionization method often overestimates the AEs of fragment ions. It should be mentioned that, in measuring the AEs, a gas filter is used to eliminate the effect of higher order radiation from the grating. As shown in Table II, the AEs we have measured are close to the calculated values at the B3LYP/6-31++G(d,p) level. Because of the adoption of the continuously wavelength scanning technique and the usage of the gas filter to eliminate the higher order radiation, our photoionization onset in the PIE curve in Fig.3 appears quite sharp and clear. In addition, our experiments are carried out under supersonic cooling conditions, thereby overcoming the hot band effect and other influences on the accurate determination of the AEs. Also, the light source employed is high-intensity undulator VUV SR. We therefore believe that the AEs we have obtained are more accurate than those of the previous measurements [11, 15].

B. Fragmentation pathways

Isoleucine is an amino acid containing an aliphatic side chain CH(CH3)CH2. In accordance with the previous results shown in Refs.[9, 13, 16, 33, 34] and the relevant amino acid ionization properties, the bonds C1−C2, C2−C3, C2−N in the molecular ion break more easily. In this work, we take the principal fragment ions C6H12N+ (m/z=86), C6H5NO2+ (m/z=75), C5H9+ (m/z=69), C4H8+ (m/z=57) and CH4N+ (m/z=30) into accounts in detail with the aid of calculations at the B3LYP/6-31++G(d,p) level. The proposed fragmentation channels for the main product ions are shown in Figs.4−7, respectively, which display the involved energetics and geometries of TSs, INTs and fragment ions in the dissociation processes of isoleucine cation.

1. C6H12N+ (m/z=86) and CHO2+ (m/z=45)

The C6H12N+ (m/z=86) is assigned to C2H5CH(CH3)CH=NH2+, corresponding to a loss of the carboxyl group COOH by scission of bond C1−C2. This is generally valid for amino acids, as frequently the strongest fragment ion peak corresponds to the loss of a neutral COOH fragment [9, 35]. The C2H5CH(CH3)CH=NH2+ ion is the highest peak area in the PI-MS. This indicates that the channel forming the C6H12N+ is the principal dissociative photoionization channel of isoleucine, as reported previously [33], the loss of a COOH group is the favorable dissociation pathway of amino acids. In Fig.4, the length for bond C1−C2 is stretched from 1.674 Å to 1.718 Å through TS1 from mother molecule ion, indicating that this single bond has a trend to cleave. Our calculation results also show that the isoleucine parent cation loses a COOH group with a relatively small amount of internal energy (Eint=0.07 eV) above the first ionization energy. The positive charge may be localized on C1 atom to produce the ion fragment (m/z=45) appearing in Fig.1. The preferred rupture of bond with the charge remaining on the fragment containing the nitrogen and α-carbon atom is shown in the following reactions (1) and (2).

C6H13NO2+→C6H5CH(CH3)CH=NH2+COOH (1)
C6H13NO2+→C6H5CH(CH3)CH=NH2+COOH+ (2)
FIG. 5 Formation pathway of C$_2$H$_5$NO$_2^+$ (m/z=75) and C$_2$H$_3$NO$_2^+$ (m/z=57) from C$_6$H$_{13}$NO$_2^+$ calculated at B3LYP/6-31++G(d,p) level.

From Fig.3(a), the onset for the fragment ion C$_5$H$_{12}$N$_2^+$ is 8.84±0.07 eV, which is close to the calculated AE of 8.68 eV in this work. That suggests the pathway (1) is reasonable. The AE for this fragment ion lies very close to the IE value of the parent molecule, suggesting that the parent ion is very unstable and easy to break to form the fragment ion C$_2$H$_5$CH(CH$_3$)$_2$=NH$_2^+$. In Biemann’s conclusion [36], the C1−C2 bond could be easily broken, as this allowed the positive charge to be located on nitrogen, and resonance stabilized. The energy level to form COOH$^+$ is calculated to be 11.43 eV, which is much higher than that to form C$_2$H$_5$CH(CH$_3$)$_2$CH=NH$_2^+$. This may indicate that the intensity for the fragment ion COOH$^+$ is much lower than that for the fragment ion C$_2$H$_5$CH(CH$_3$)$_2$CH=NH$_2^+$.

2. C$_2$H$_5$NO$_2^+$ (m/z=75)

The amino acid molecule cations exhibit strong tendencies to rearrange one hydrogen atom upon fragmentation [11]. According to our experimental observation and theoretical calculations, the C$_2$H$_5$NO$_2^+$ ion at m/z=75 is proposed as NH$_2$CH=C(OH)$_2^+$ having two hydroxyl groups. Such carbon-centered ion, with both $\pi$ donor (NH$_2$) and $\pi$ acceptor (C(OH)$_2^+$) substituents, has been shown to be more stable than that with only one substituent [37], the extra stabilization giving rise to the so-called “captodative effect” [38]. The formation of the NH$_2$CH=C(OH)$_2^+$ ion would require structural reorganisation, including hydrogen (C6) migration to C1.

As shown in Fig.5, the parent ion firstly undergoes transition state TS2 to form the intermediate INT1. Because of the methyl group rotation on the C3 atom, one hydrogen atom on methyl approaches the carbonyl (O1 atom), and the distance between them decreases from 4.946 Å to 2.704 Å. The migration of a hydrogen atom from the methyl toward the O1 atom leads to the formation of intermediate INT1 via the transition state TS3 with an energy barrier of 0.25 eV. The bond H−C6 cleaves and the intramolecular hydrogen transfers (IHT) from methyl (C6) to O1 to form the intermediate INT2. With the bond length C2−C3 increasing to 2.102 Å in transition state TS4, the bond C2−C3 cleaves to yield the production ion NH$_2$CH=C(OH)$_2^+$ accompanied by CH$_3$CH$_2$=CH$_2$ releasing via TS4 with an energy barrier of 0.59 eV. The AE value for the NH$_2$CH=C(OH)$_2^+$ ion is 9.25±0.06 eV, which is in good agreement with the calculated value 9.25 eV in this work. The formation of NH$_2$CH=C(OH)$_2^+$ (m/z=75) is described as the reaction (3):

$$C_6H_{13}NO_2^+ \rightarrow NH_2CH = C(OH)_2^+ + CH_3CH_2CH = CH_2$$  \hspace{1cm} (3)
Then the hydrogen atom migrates to the N atom and increases to 1.453 Å with an energy barrier of 2.13 eV. In TS7, one hydrogen atom on the methyl group from C3 to C2 in the fragment ion C5H9+ (m/z=69) via TS6 to INT3. It is well known that carbocations often rearrange shifting substituent chemical groups from a carbon atom to another [39, 40]. These shift reactions, involving a migrating carbon, are known as Wagner-Meerwein rearrangements. These rearrangement processes often occur when a carbocation is involved in addition or substitution reactions. We therefore postulated a transposition of the methyl group from C3 to C2 via the corresponding TS6 and INT3 with an energy barrier of 1.63 eV for the breakdown process of isoleucine. The N−C2 and C6−C2 bonds are shorted to 1.435 and 1.594 Å in INT3, respectively. Once this rearranged ion is formed, further hydrogen shift processes drive the subsequent fragmentations. Two reactions of hydrogen atom shift via TS7 and TS8 are considered respectively. In TS7, one hydrogen atom on the methyl approaches to the N atom and N−C2 bond length increases to 1.453 Å with an energy barrier of 2.13 eV. Then the hydrogen atom migrates to the N atom and N−C2 bond breaks to lose a NH3 molecule and form the CH2=CHCHC2H5+ ion. In TS8, one hydrogen atom on the C4 atom is transferred to the N atom and N−C2 bond is elonged to 1.478 Å with an energy barrier of 0.27 eV. This rearranged ion can easily lose an ammonia to produce CH3CH=CHCH3+ via TS8. Two formation pathways from C2H5CHCH3CH=NH2+ (m/z=86) involve both the loss of NH3 in reactions (4) and (5).

\[
\text{C}_6\text{H}_{13}\text{NO}_2^+ \rightarrow \text{CH}_3 = \text{CHCHC}_2\text{H}_5^+ + \text{NH}_3 + \text{COOH} \tag{4}
\]

\[
\text{C}_6\text{H}_{13}\text{NO}_2^+ \rightarrow \text{CH}_3\text{CH} = \text{CHCHCH}_3^+ + \text{NH}_3 + \text{COOH} \tag{5}
\]

Comparing the two formation pathways for C5H9+, shown in Fig.6, the appearance energies for the two channels are calculated to be 12.24 and 10.38 eV, respectively. From Fig.3(c), the threshold for the C5H9+ is 10.20±0.12 eV, which corresponds very well with the value 10.38 eV obtained from TS8. Thus the fragment ion C5H9+ (m/z=9) is assigned to CH3CH=CHCH3+, which is in agreement with the formula from Ref.[16].

4. C4H8+ (m/z=57)

Firstly, we consider the fragment ion C4H9+ (m/z=57) may derive from NH2CH=C(OH)2+ (m/z=75) by intramolecular hydrogen transfer as the following reaction:

\[
\text{C}_6\text{H}_{13}\text{NO}_2^+ \rightarrow \text{NH}_2\text{CH} = \text{CO}^+ + \text{H}_2\text{O} + \text{C}_4\text{H}_8 \tag{6}
\]
FIG. 7 Formation pathway of CH$_4$N$^+$ ($m/z=30$) from C$_5$H$_{12}$N$^+$ ($m/z=86$) calculated at B3LYP/6-31++G(d,p) level.

The NH$_2$CH$=$CO$^+$ may be obtained via the way: H transfers from O1 to O2 or from O2 to O1, then NH$_2$CH$=$C(OH)$_2^+$ release a H$_2$O molecule as shown in Fig.5. In TS5, H(O2) is closing to O1 from 3.015 Å to 1.205 Å, and the C1$-$O1 bond is elongated to 1.450 Å from 1.311 Å. Then the C1$-$O1 bond cleaves to lose a water molecule to generate the ion NH$_2$CH$=$CO$^+$. The fragment ion NH$_2$CH$=$CO$^+$ is formed via transition state TS5 with an energy barrier of 1.55 eV and the appearance energy for this ion is 10.34 eV.

Another pathway is the direct cleavage of the C2$-$C3 bond from parent ion C$_6$H$_{13}$NO$_2^+$:

\[
\text{C}_6\text{H}_{13}\text{NO}_2^+ \rightarrow \text{C}_2\text{H}_5\text{CH}(\text{CH}_3)^+ + \text{NH}_2\text{CHCOOH} \quad (7)
\]

The calculated AE for the ion C$_2$H$_5$CH(\text{CH}_3)$^+$ ($m/z=57$) yielded from the direct dissociation is 9.43 eV. This value is very close to the experimental AE 9.25±0.10 eV in Fig.3(d), thus the pathway of reaction (7), direct dissociation from mother ion, is favorable.

5. CH$_4$N$^+$ ($m/z=30$)

The fragment ion $m/z=86$ is considered to be CH$_4$N$^+$ with the structure NH$_2$−CH$_2^+$. Two proposed formation pathways, shown in Fig. 7, are taken into account. One pathway undergoes a hydrogen atom shift from C6 to C2 in C$_2$H$_4$CH(\text{CH}_3)CH$=$NH$_2^+$ ($m/z=86$) and the other involves a hydrogen atom shift from C4 to C2. Energy differences between the corresponding two TSs (TS9 and TS10) are minimal at this level of theory. The intramolecular hydrogen transfer from C6 atom to C2 atom and C2$-$C3 breaks to form NH$_2$CH$_2^+$ ($m/z=30$) via transition state TS9 with an energy barrier of 2.19 eV. Then the C2$-$C3 bond is elongated to 1.611 Å in TS9 from 1.480 Å in the $m/z=86$ ion. The other pathway has analogous process. TS10 has an energy barrier of 1.75 eV. The reactions of the two pathways are shown in reactions (8) and (9).

\[
\text{C}_6\text{H}_{13}\text{NO}_2^+ \rightarrow \text{C}_2\text{H}_5\text{CH} =\text{CH}_2 + \text{NH}_2\text{CH}_2^+ -1 + \text{COOH} \quad (8)
\]
\[
\text{C}_6\text{H}_{13}\text{NO}_2^+ \rightarrow \text{CH}_2\text{CH} =\text{CHCH}_3 + \text{NH}_2\text{CH}_2^+ -2 + \text{COOH} \quad (9)
\]

The appearance energy for the ion NH$_2$CH$_2^+$ is experimentally determined to be 11.05±0.07 eV in Fig.3(e) and the calculated AEs is 10.71 and 10.27 eV for reactions (8) and (9), respectively. Hence, the CH$_4$N$^+$ is favorably formed via reaction (8).

6. Other fragment ions

The fragment ion at $m/z=74$ may be assigned to NH$_2$CHCOOH$^+$ yielded via C2$-$C3 simple bond cleaving from C$_6$H$_{13}$NO$_2^+$. This is similar to the ionization and dissociation mechanism of alanine as proposed by Ipolyi et al.\cite{41}. The fragment ion at $m/z=46$ is possibly CH(NH$_2$)OH$^+$ ion due to rearrangement via the shift of OH from C1 to C2 from present ion: C$_6$H$_{13}$NO$_2^+$→CH(NH$_2$)OH$^+$+C$_2$H$_5$CH(\text{CH}_3)CO. The ion at $m/z=44$ may be NH$_2$CHCH$_2^+$, which is most probably formed by C2$-$C3 bond cleavages in INT3,
as shown in Fig.6. Also this fragment ion is possibly assigned as $\text{CO}_2^+$ formed via $\text{NH}_2\text{CH}_3\text{COOH}^+$ ($m/z=74$) $\rightarrow$ $\text{NH}_2\text{CH}_3+\text{CO}_2^+$. The ion at $m/z=41$ can be assigned to $\text{CH}_3\text{CH}=\text{CH}^+$ via $\text{C}_1$-$\text{C}_2$, $\text{C}_3$-$\text{C}_4$ bond cleavages of $\text{C}_2\text{H}_5\text{CH}_2\text{CH}_2\text{CH}_2\text{H}^+$ ($m/z=86$). The $m/z=28$ ion may be $\text{HCNH}^+$ or $\text{CO}^+$ as proposed by Ipolyi et al. [41], which is shown in reactions (10) and (11):

$$\begin{align*}
\text{C}_6\text{H}_4\text{NO}_2^+ & \rightarrow \text{HCNH}^+ + \text{HCOOH} + \text{CH}_2\text{H}_3\text{CH} \quad (10) \\
\text{C}_6\text{H}_4\text{NO}_2^+ & \rightarrow \text{CO}^+ + \text{OH} + \text{C}_2\text{H}_5\text{CH}(\text{CH}_3)\text{CH} = \text{NH}_2 \quad (11)
\end{align*}$$

IV. CONCLUSION

VUV-induced degradation pathways of isoleucine were investigated using SR PI-MS. PIE curves as a function of excitation energy were measured. The measurements provided fairly accurate values which are important for interpretation of dissociative photoionization pathways of isoleucine. Using theoretical calculations at the B3LYP/6-31++G(d,p) levels, the dominant fragments at $m/z=86$, 75, 69, 57, and 30 are assigned to $\text{C}_2\text{H}_5\text{CH}_2\text{CH}=\text{NH}_2^+$, $\text{NH}_2\text{CH}^-\text{C}(\text{OH})_2^+$, $\text{CH}_2\text{CH}=\text{CHCHCH}_3^+$, $\text{C}_2\text{H}_5\text{CH}(\text{CH}_3)^+$, and $\text{NH}_2\text{CH}_2^+$, respectively. The detailed formation channels of the dominant fragments from the mother ion $\text{C}_6\text{H}_{13}\text{NO}_2^+$ are discussed in detail. In summary, $\text{C}_2\text{H}_5\text{CH}_2\text{CH}=\text{NH}_2^+$ ($m/z=86$) is formed via the $\text{C}1$-$\text{C}2$ bond cleaving to lose the COOH$^+$ radical from $\text{C}_6\text{H}_{13}\text{NO}_2^+$. $\text{NH}_2\text{CH}^-\text{C}(\text{OH})_2^+$ ($m/z=75$) derived from $\text{C}_2$-$\text{C}3$ bond fission is produced via intramolecular hydrogen transfer in $\text{C}_6\text{H}_{13}\text{NO}_2^+$. $\text{CH}_2\text{CH}=\text{CHCHCH}_3^+$ ($m/z=69$) is formed from $\text{C}_2\text{H}_5\text{CH}_2\text{CH}=\text{NH}_2^+$ ($m/z=86$) with methyl group shift. $\text{C}_2\text{H}_5\text{CH}(\text{CH}_3)^+$ ($m/z=57$) is generated via the direct fission of $\text{C}2$-$\text{C}3$ bond in $\text{C}_6\text{H}_{13}\text{NO}_2^+$. $\text{NH}_2\text{CH}_2^+$ ($m/z=30$) is produced from $\text{C}_2\text{H}_5\text{CH}_2\text{CH}=\text{NH}_2^+$ ($m/z=86$) via intramolecular hydrogen transfer too.

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