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Accelerated Articles

Consecutive Infrared Multiphoton Dissociations in a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer

D. Scott Tonner and Terrance B. McMahon*

Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

Consecutive infrared multiphoton dissociations (IRMPD) may be observed in a Fourier transform ion cyclotron resonance mass spectrometer (FTICR). This is the IRMPD equivalent of previous MS^n experiments using CID. This work presents a versatile technique, using a bistable shutter to gate ON and OFF a continuous-wave (CW) CO_2 laser for multiple irradiation periods of 0.1−1000 s duration. Consecutive photodissociations, up to MS^4, are demonstrated for the proton-bound dimer of diethyl ether and the resulting fragment ions. The photoproducts are formed close to the center of the FTICR cell, resulting in high product ion recovery efficiency. This differs from CID products, which are formed throughout the FTICR cell causing reisolation/detection problems. The fragmentation resulting from the use of low-intensity, CW, infrared laser radiation is shown to be much more energy selective than CID. Photodissociation of $C_2H_5OH_2^+$ ion produces the lowest energy product ion exclusively, even though the two product channels differ only by ~5 kcal/mol. Low-energy CID, however, produces a mixture of $C_2H_5^+$ and H_3O^+ products in the ratio of 1.3:1. Hence, the *higher* energy pathway ($C_2H_5^+$) is substantially favored. The current results indicate that this IRMPD MS^n technique may be successfully applied to large biomolecules prepared by electrospray or MALDI.

Tandem mass spectrometry (MS/MS) and multiple mass spectrometry (MS^n) experiments have proven extremely powerful for identification and structural characterization of ions.[1−3] Additionally, it has been demonstrated that MS^n techniques can be applied to large (kDa), extensively protonated ions produced from electrospray ionization (ESI) because multiple protonation renders the ions more susceptible to dissociation. The use of the fragmentation information provided by MS^n techniques to directly sequence DNA and large protein molecules has gained much attention.[4,5] Of the various methods that exist for inducing fragmentation of ions, collision-induced dissociation (CID) is, perhaps, the most widely used. MS/MS experiments, incorporating CID, have been shown to provide substantial, and sometimes complete, sequence information for oligopeptide ions generated by ESI.[6]

CID of small molecules in a Fourier transform ion cyclotron resonance (FTICR) spectrometer was demonstrated nearly two decades ago by Freiser and co-workers.[7] Translational excitation of the ions is accomplished by application of a radio frequency (rf) electric field at a frequency resonant with the ion cyclotron motion. Upon colliding with either a static[8] or pulsed[9] pressure of target gas, the ions undergo fragmentation. CID in a FTICR spectrometer differs from tandem or multisector instruments in that fragment ions are separated from parent ions temporally rather that spatially. Ion beam mass spectrometers use one electric, magnetic, or quadrupole sector to separate the desired ionic species from the extraneous ions, and another sector to detect the fragmentation products. Hence, a MS^n experiment requires "*n*" analyzer stages. In contrast, MS/MS experiments can be performed in a single FTICR spectrometer because ion

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isolation, excitation, collision, and detection of the fragments all occur in the same FTICR cell. Extension of the FTICR experiment to produce consecutive CID events is then easily achieved, without additional hardware, simply by changing the pulse sequence in the FTICR software such that fragment ions are isolated and redissociated.[10] Quadrupole ion trap mass spectrometers (ITMS) are capable of producing MS/MS spectra by similar modifications in the pulse timing; however, these instruments require much higher operating pressures. Cooks and co-workers first demonstrated the MS/MS capability of ITMS in 1987.[11] An excellent comparison of the two techniques of trapped ion MS/MS is given by McLuckey et al.[4]

The efficiency of the FTICR MS^n experiment is limited by several factors: (1) A low target gas pressure ($<10^{-6}$ Torr) is necessary to maintain optimum ion detection conditions ($<10^{-8}$ Torr if high resolution detection is required). (2) The maximum translational energy imparted to the precursor ions is limited by the magnetic field and the size of the cell. (3) Rf excitation takes the ions off-axis and, consequently, forms fragmentation products away from the center of the cell, thereby decreasing the efficiency with which the ions can be reisolated or detected.[12] Various improvements on the basic CID technique such as multiple excitation collisional activation (MECA),[13] sustained off-resonance irradiation collisionally activated dissociation (SORI-CAD),[14,15] very low energy collisionally activated dissociation (VLE-CAD)[16,17] and resonant amplitude-modulated collisionally activated dissociation (RAM-CAD)[5] have been demonstrated. Using SORI-CAD, dissociation efficiencies greater than 90% have been reported for protonated ethyl acetate[15] and greater than 92% for large, multiply charged ions.[5] Even though these techniques yield high dissociation efficiencies for an individual CID event, it is still difficult to obtain multiple MS events due to the production of fragment ions far from the center of the cell.

Another powerful technique for dissociating ions in a mass spectrometer is photodissociation. Very early in the history of ICR spectrometers, the ion-trapping capabilities of these instruments indicated their potential for photochemical studies of ions. Brauman and Smyth first demonstrated negative ion electron photodetachment in an ICR spectrometer in 1969.[18] Two years later, Dunbar performed the first photodissociation experiments in an ICR spectrometer using a monochromated xenon arc lamp to remove a chlorine atom from CH_3Cl^+ ion.[19] In 1978, Beauchamp and co-workers first showed that low-intensity (<100 W/cm^2), continuous-wave (CW), CO_2 laser radiation could produce infrared multiphoton dissociations (IRMPD) of ions trapped at low pressure in an ICR cell.[20,21] A novel method of dissociation of weakly bound molecules by ambient black body radiation termed zero-pressure thermal radiatively induced dissociation (ZTRID) was demonstrated experimentally by McMahon and co-workers,[22,23] with theoretical modeling provided by Dunbar.[24] Very recently, Williams and co-workers have extended this same technique to the higher temperatures necessary to achieve black body induced radiative dissociation (BIRD) with biomolecule ions as large as ubiquitin.[25] Applications of UV,[26] IR,[27] and BIRD photodissociation techniques to large biomolecules have, recently, been investigated as alternative methods to CID in MS/MS experiments.

To determine unimolecular reaction pathways (required for the sequencing of biomolecules) or dissociation rates, the unambiguous assignment of the observed fragmentation products to the correct parent ion(s) is required. For consecutive reactions, mechanistic uncertainties may exist. For example, does a single-step, $A \rightarrow C$ reaction occur in parallel with a consecutive $A \rightarrow B \rightarrow C$ reaction? Since strong deviations from first-order kinetics have been observed in FTICR photodissociation experiments,[28,29] it may be difficult to extract the reaction mechanism of a consecutive reaction from the observed kinetics. This process is greatly simplified if there is only one species present in the FTICR cell when the fragmentation event is initiated. The IRMPD MS^n technique presented here is a powerful tool for the characterization of these reactions, because the dissociating ion in each step is isolated before the irradiation event. In a sense, this technique provides the ability to "stop" the reaction sequence at an arbitrary point, observe the progress of the reaction, and/or instantly "purify" the resulting product(s) for further study. Similarly, the IRMPD MS^n experiment could be used to produce and trap a highly reactive species via photolysis of a stable ionic species. The reactive species, which perhaps could not be generated by other ionization methods, can then be isolated and fragmented or reacted bimolecularly with the introduction of a pulsed reactant gas to the FTICR cell.

This study demonstrates a true, IRMPD analogue of previous MS^n experiments using CID. A bistable shutter (i.e., one TTL pulse to open, another to close) is used to gate the low-power, CW laser beam before it enters the FTICR cell. Separating the "laser ON" periods by delays and/or ejection events facilitates multiple-stage, photodissociation experiments, with the extent of dissociation during each step being determined by the length of the irradiation period. Using this experimental procedure, we are able to observe MS^n spectra of externally generated $[(C_2H_5)_2O]_2H^+$ ions at low pressures. Isolation and redissociation spectra up to

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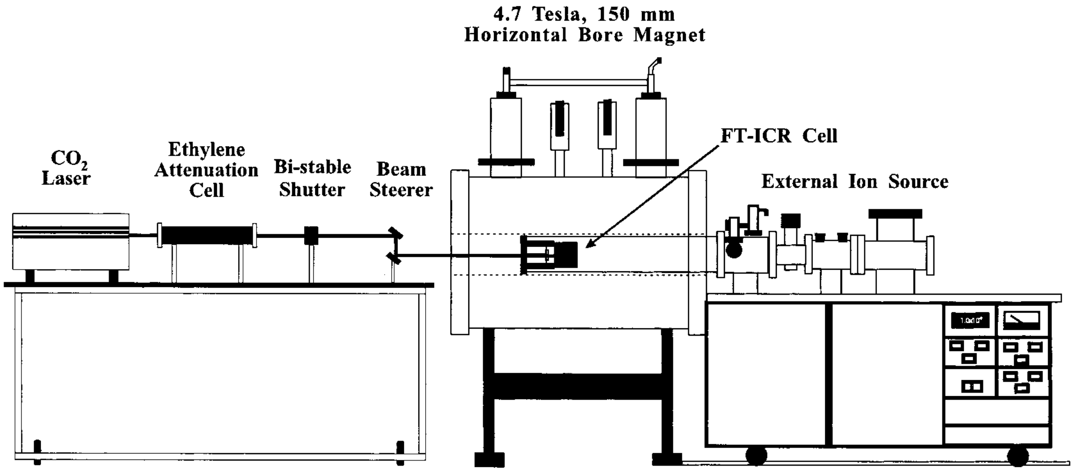

Figure 1. Schematic representation of the apparatus used for IRMPD MS^n experiments in a FTICR spectrometer.

MS^4 are demonstrated and the final dissociation reaction (3) is compared to results obtained by CID.

EXPERIMENTAL SECTION

All experiments were performed on a Bruker CMS 47 FTICR spectrometer equipped with a 4.7 T superconducting magnet and a Bruker Infinity cell. The Waterloo FTICR spectrometer has been modified by the addition of an external, high-pressure ion source similar to that found in a high-pressure mass spectrometer (HPMS). A detailed explanation of HPMS has been given elsewhere.[30] The high-pressure ion source permits the generation of weakly bound ion−molecule complexes which are thermalized by many collisions ($\sim 10^5$) before exiting the source. Details of both the CMS 47[31] and the external ion source[32] have been published previously. Differential pumping is accomplished with three Balzers turbomolecular pumps. With 3.5 Torr in the ion source, the pressure outside the source region is $\sim 2 \times 10^{-4}$ Torr while the base pressure in the FTICR cell is on the order of 1×10^{-9} Torr at 20 °C. CID experiments were performed with a static pressure ($\sim 1 \times 10^{-7}$ Torr) of either argon or methane in the FTICR cell. The $[(C_2H_5)_2O]_2H^+$ ions were generated externally from a mixture of ~1% reagent-grade diethyl ether in methane. The gas mixture flowed into the ion source at a pressure of ~3.5 Torr and was ionized by secondary electron impact ionization from 2 keV electrons. CH_5^+ and $C_2H_5^+$ ions formed by the ultrahigh CI source pressures subsequently proton transfer to the neutral diethyl ether molecules. Room-temperature ionization conditions produced almost exclusively $[(C_2H_5)_2O]_2H^+$ ions. Since the desired ion ($m/z = 149$) was so dominant initially, complete isolation was easily accomplished using standard rf ejection techniques as shown in Figure 3a. No accidental CID of the parent ion, due to near-resonant excitation, was observed.

The methods and conditions for conducting CID experiments have been described in detail previously.[7−9,33] All CID experiments used the "ion excitation" pulse provided in the standard Bruker Xmass software. Ion excitation was accomplished by application of a single-frequency rf pulse directly at the cyclotron frequency of the parent ion. The translational energy was controlled by varying the length and amplitude (controlled by attenuator settings) of the rf waveform. No off-resonant excitation experiments were performed as the purpose of the CID spectra presented here was simply to compare the fragmentation ratios with those produced by IRMPD.

The complete apparatus for the IRMPD MS^n experiments is illustrated schematically in Figure 1. All IRMPD experiments were done with a Synrad Inc. 48-0-15W, CO_2 laser operating CW in TEM_{00} modes with frequency at or near 10.6 μm (10.55−10.65 μm).[34] Output power can be controlled up to a maximum of 10 W by changing the duty cycle from 5 to 98%. For these experiments, the duty cycle was fixed at ~40% so that the output laser power was 3.9 W. Further attenuation of laser power was accomplished by passing the beam through a static pressure (~1−10 Torr) of ethylene gas contained in an attenuation cell equipped with AR coated zinc selenide windows (>99% transmission at 10.6 μm). The beam continues through a Newport 846HP bistable shutter with a 5.6 mm aperture and a maximum incident beam power of 5 W. Positioning of the beam is accomplished with a Newport beam steerer using coated aluminum, first surface mirrors. Finally, the laser beam enters the rear of the FTICR cell through a 3 × 25.4 mm AR coated ZnSe window and passes through 6 mm restrictions at both the front and rear of the cell before being dissipated in the ion optics of the external source. Laser power was measured immediately behind the shutter with a Coherent 210 power meter. The laser beam profile, at the cell, was determined by translating a 1 mm aperture across the beam and measuring the transmitted power. The observed Gaussian beam profile had a full width half-maximum (fwhm) value of 0.66 cm. Additionally, the power at the FTICR cell was measured and found to be less than that measured behind the shutter by a factor of ~0.87. The overall infrared laser intensity was determined by dividing the corrected laser power at the cell by the beam area (fwhm). The data presented here were collected with a laser power of 3.9 W (behind the shutter), which translates into an intensity of 10 W/cm^2.

The experimental timing was controlled by the Xmass data collection software running on a Silicon Graphics Inc. Indy work station. For a MS^3 experiment, the pulse sequence presented in

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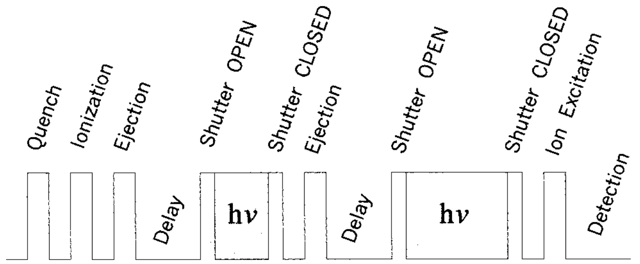

Figure 2. The FTICR pulse sequence that controls the timing of MS^n experiments. This example illustrates the MS^3 experiment used to obtain the mass spectrum shown in Figure 3d.

Figure 2 was used. The optical shutter was coupled to the Bruker data collection system via home-built electronics such that a TTL pulse from one auxiliary output would open the shutter, which remains in this position until a TTL pulse from a second auxiliary output closes the shutter. Using this design, the shutter could be opened at any point in the experimental sequence and left open for any duration. By adding additional isolation and irradiation events with the appropriate delays to the software, multiple-stage photodissociation experiments were accomplished.

RESULTS AND DISCUSSION

The proton-bound dimer ion of diethyl ether was an ideal system to illustrate the IRMPD MS^n technique because it undergoes a series of consecutive photodissociative reactions (discussed in detail in reactions 1−3). Additionally, the cross section for photodissociation of $[(C_2H_5)_2O]_2H^+$ ions was known to be large,[20] so that high conversion efficiencies for reaction 1 could be expected. Finally, photogeneration of $(C_2H_5)_2OH^+$ and $C_2H_5OH_2^+$ provides a method of making these ions which are ordinarily difficult to make in a high-pressure ion source because the equilibrium constant for formation of the respective dimers $[(C_2H_5)_2O]_2H^+$ and $(C_2H_5OH)_2H^+$ is very large and because the latter rapidly eliminates H_2O to form protonated diethyl ether. Similarly, photodissociation of $(C_2H_5OH)_2H^+$ yields exclusively protonated diethyl ether ion via water elimination[35] and, therefore, is not a viable method of producing $C_2H_5OH_2^+$ either. Ethyloxonium ion, $C_2H_5OH_2^+$, can be made in a conventional CI source, but care must be taken to minimize internal excitation of the ion from the exothermic proton-transfer reaction. In contrast, photogeneration of $C_2H_5OH_2^+$ from $[(C_2H_5)_2O]_2H^+$ is quite facile and dissociation by low-intensity photolysis has been shown to occur very nearly at threshold,[21] producing fragments with less internal energy than from corresponding pulsed laser multiphoton dissociation.[36]

All of the mass spectra presented here were collected over the mass range 14.4−300 Da. Spectra out to 1000 Da were periodically examined to ensure that no interferences from higher mass ions were observed. The initial stage of the MS^n experiment used rf ejection techniques to isolate the proton-bound dimer ion of diethyl ether produced in the external ion source. The mass spectrum presented in Figure 3a contains, almost exclusively, the isolated $[(C_2H_5)_2O]_2H^+$ ion at $m/z = 149$. Single-frequency ejection "shots" were used to remove unwanted ions after a 1 s postionization delay. The small peaks appearing at $m/z = 75.55$ and $m/z = 49.7$ are the second and third harmonics of $m/z = 149.1$, respectively. The ion appearing at $m/z = 147$, corresponding to H_2 elimination from $m/z = 149$, was not ejected both to ensure no translational excitation of $m/z = 149$ and because it did not interfere with the observed diethyl ether reactions. Hence, each of the product ions shown in Figure 3 is accompanied by a small peak 2 Da below from the photodissociation of unejected $m/z = 147$. The $[(C_2H_5)_2O]_2H^+$ ion was found to be unreactive when trapped in the FTICR cell for a period of 500 s and did not undergo black body-induced dissociation at room temperature. Irradiation of the $[(C_2H_5)_2O]_2H^+$ ions for 0.4 s at a laser intensity of 10 W/cm^2 produces the spectrum shown in Figure 3b. Photodissociation of the $[(C_2H_5)_2O]_2H^+$ ion results in the loss of a neutral diethyl ether, exclusively (reaction 1). This reaction is known, from HPMS equilibrium measurements, to be 29.1 kcal/mol[37] endothermic.

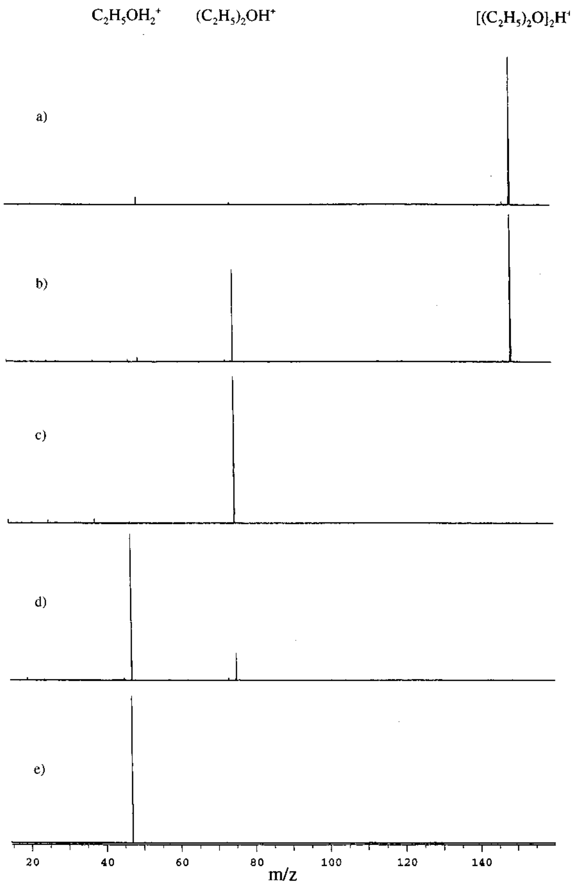

Figure 3. Series of spectra illustrating the progression of the sequential photodissociation reactions of $[(C_2H_5)_2O]_2H^+$ ion. (a) Isolation of $[(C_2H_5)_2O]_2H^+$ ion ($m/z = 149$); (b) mass spectrum obtained by irradiating $[(C_2H_5)_2O]_2H^+$ for 0.4 s with 10 W/cm^2 laser intensity; (c) isolation of the $(C_2H_5)_2OH^+$ product ion ($m/z = 75$) by rf ejection of the parent ion ($m/z = 149$); (d) MS^3 spectrum produced by irradiating the isolated $(C_2H_5)_2OH^+$ ion for 30 s with 10 W/cm^2 of laser intensity; (e) isolation of the second photoproduct ion $C_2H_5OH_2^+$ ($m/z = 47$) by rf ejection of the $m/z = 75$ precursor ion. Note: spectra a−c contain small peaks which are the second and third harmonics of the principle ion(s) in each spectrum. These peaks occur at $^1/_2$ and $^1/_3$ the m/z value(s) of the actual ion(s), respectively.

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$$\underset{\mathbf{1}}{[(C_2H_5)_2O]_2H^+} + h\nu \rightarrow \underset{\mathbf{2}}{(C_2H_5)_2OH^+} + (C_2H_5)_2O \quad (1)$$

The protonated diethyl ether monomer ion **2** produced in reaction 1 was reisolated with rf ejection shots, and the resultant spectrum is given in Figure 3c. The small peaks occurring at $m/z = 37.5$ and $m/z = 25.0$ are the second and third harmonics of $m/z = 75.08$, respectively. When the $(C_2H_5)_2OH^+$ ion is subjected to a second photodissociation event (30 s, 10 W/cm^2), the spectrum shown in Figure 3d is obtained. In this case, the protonated diethyl ether monomer undergoes a rearrangement to form ethyloxonium ion **3** via ethylene elimination (reaction 2) which is 28.5 kcal/mol endothermic.[38] Since the precursor ion **2** for this reaction must be prepared through photolysis of **1**, the MS^3 pulse sequence, shown in Figure 2, represents the experimental procedure required to produce the mass spectrum shown in Figure 3d.

$$\underset{\mathbf{2}}{(C_2H_5)_2OH^+} + h\nu \rightarrow \underset{\mathbf{3}}{C_2H_5OH_2{}^+} + C_2H_4 \qquad (2)$$

Reaction 2 proceeds much more slowly than reaction 1 for the same laser intensities (the initial rate constants are $k = 0.5\ s^{-1}$ and $k = 4\ s^{-1}$, respectively). Beauchamp[21] observed that reactions 2 and 3 could only be observed with progressively higher laser intensities, but the irradiation times used were limited to less than 2 s because of back reaction of **2** with the 10^{-6} Torr background pressure of neutral diethyl ether. The use of an external ion source in this work yields a background diethyl ether pressure of $\leq 1 \times 10^{-11}$ Torr in the FTICR cell. Therefore, long irradiation times (up to 500 s) and, hence, increased laser fluences are possible. The result is the observation of low-intensity photodissociation of ions **2** and **3**.

When the ethyloxonium ion **3** is trapped and irradiated, (70 s, 10 W/cm^2) H_3O^+ ($m/z = 19$) is the only fragmentation product observed, as shown in Figure 4a. This MS^4 experiment shows that photodissociation proceeds exclusively via ethylene elimination as illustrated in reaction 3a. This is in contrast to the previous results of Bowers and co-workers in which both $C_2H_5^+$ and H_3O^+ fragment ions were produced from CID in a ratio of 1.2:1.[39,40] The dissociation of ethyloxonium ion to form $C_2H_5^+$ via elimination of water is shown in reaction 3b. When CID was performed on the ethyloxonium ion, in the Waterloo FTICR spectrometer, a mixture of products **4a** and **4b** was produced, as seen in Figure 4b. The observed ion intensities are in the ratio of 1.3:1, which is in good agreement with the results of Bowers.

$$\underset{\mathbf{3}}{C_2H_5OH_2{}^+} \rightarrow \underset{\mathbf{4a}}{H_3O^+} + C_2H_4 \qquad \Delta H = 32.5\ \text{kcal/mol} \quad (3a)$$

$$\underset{\mathbf{3}}{C_2H_5OH_2{}^+} \rightarrow \underset{\mathbf{4b}}{C_2H_5{}^+} + H_2O \qquad \Delta H = 36.8\ \text{kcal/mol} \quad (3b)$$

The literature contains mixed results regarding the products of reaction 3 observed in *unimolecular* experiments. The metastable experiments by Bowers et al. reported observation of H_3O^+ ion only,[39,40] while Smith and co-workers observed both $C_2H_5^+$ and H_3O^+ as unimolecular fragmentation products.[41] This discrepancy might be explained by the fact that the latter experiment sampled ions with greater than thermal translational energies. Reactions 3a and 3b are endothermic by 32.5 and 36.8 kcal/mol, respectively.[38] (Radom et al. have calculated the relative energetics of reactions 3a and 3b to be 31.1 and 38.2 kcal/mol, respectively.[42]) Thus, the exclusive formation of H_3O^+ from the photodissociation of ethyloxonium ion **3** shows the energy selectivity of the IRMPD MS^n technique to be on the order of 5 kcal/mol or less. The CID experimental results are clearly not energy selective since the higher energy process is favored by a factor of 1.2–1.3. The discrepancy in the reported results for the unimolecular dissociation of **3** suggests that the degree of energy selectivity for unimolecular experiments falls somewhere between low-power IRMPD and CID. The ZTRID (or BIRD) technique is very energy selective, forms exclusively the lowest energy product, but requires a large number of absorbing modes (many atoms) for dissociation of strongly bound ions. The dissociation of protonated diethyl ether (reaction 2) and ethyloxonium ion (reaction 3) are examples of reactions that cannot be observed via black body radiation (at least at $T \leq 200$ °C) and again demonstrates the utility of the current technique.

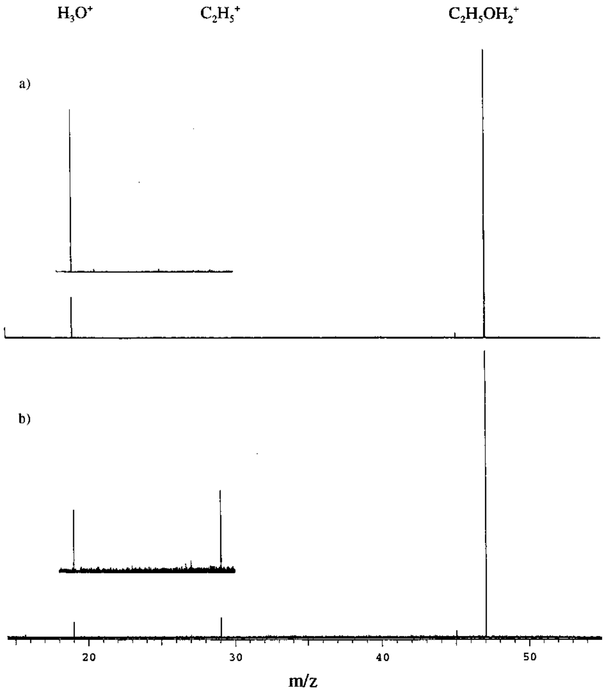

Figure 4. MS^4 spectra obtained by subjecting the isolated C_2H_5-OH_2^+ ion, from Figure 3e, to (a) 10 W/cm^2 infrared laser radiation for 70 s and (b) CID with a static pressure of 3×10^{-8} Torr argon in the FTICR cell. Inset spectra in both (a) and (b) are magnified by a factor of 4.

It can be readily seen that the signal to noise ratios in the spectra of isolated **1**, **2**, and **3** are still large. This is impressive in light of the fact that the observed maximum conversion efficiency for **1** → **2** is ~50% due to the parasitic **2** → **3** reaction

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and non-first-order kinetics. The efficient recovery of ions, throughout the sequential MS stages, can be attributed to the very strong signal in the starting ion, $[(C_2H_5)_2O]_2H^+$, and to the formation of the photofragments close to the center of the FTICR cell (along the laser axis). The difference in the S/N ratio between Figures 4a and 4b illustrates the benefit of IRMPD over CID for efficient fragmentation of ions in an FTICR spectrometer. Increasing the length and/or amplitude of the rf excitation pulse, in an attempt to increase the fragmentation efficiency of the CID experiment, results in diminished signal because ions are either ejected from the cell or moved into a larger cyclotron orbit from which they are not detected as efficiently.

CONCLUSIONS

A technique for obtaining MS^n spectra via infrared multiphoton dissociation has been demonstrated. The advantages of this method over analogous CID experiments include better selectivity of the lowest enthalpy fragmentation pathway and increased throughput of product ions from previous MS events due to formation of photofragments near the center of the FTICR cell. Additionally, the internal energy deposition is limited only by the laser power, the irradiation time (both can be increased substantially from the present experiment), and the absorption cross section of the ion at 10.6 μm (which is substantial for ions of moderate to large size) rather than by the physical dimensions of the cell and the magnetic field strength, as in CID experiments.

The superb energy selectivity, high product recovery efficiency, compatibility with external ionization sources, and potentially unlimited mass range indicate that this technique may be successfully applied to large biomolecules prepared by electrospray or MALDI. Individual exploration of multiple fragmentation pathways should generate structural and qualitative, energetic information. Price et al. have applied Dunbar's modified Tholmann theorem approach to modeling IR absorption rates to large biomolecules.[43] An extension of this treatment could produce quantitative energetic information regarding the fragmentation energetics of biomolecules observed by this technique.

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