ELSEVIER

International Journal of Mass Spectrometry 190/191 (1999) 265–279

International Journal of Mass Spectrometry

Waveform activated rearrangement of *n*-butylbenzene molecular ions during tandem mass spectrometry in the quadrupole ion trap

Timothy R. Croley, Ronald Zemribo, Bert C. Lynn, Jr.*

Department of Chemistry and Mississippi State Chemical Laboratory, Mississippi State University, Mississippi State, MS 39762, USA

Received 30 September 1998; accepted 9 February 1999

Abstract

The "molecular thermometer," *n*-butylbenzene, when analyzed by tandem mass spectrometry, produced an unusual product ion mass spectrum. Instead of the expected tandem mass spectrum containing fragments at *m/z* 91 and *m/z* 92, an unexpected base peak at *m/z* 119 was observed. Tandem parameters such as q_z, collision-induced dissociation (CID) voltage, trap temperature, and space charge were examined. When *n*-butylbenzene was analyzed by other ion trap mass spectrometers in our laboratory and other laboratories, analogous results were obtained. This phenomenon was investigated and found to be associated with the Varian protocol used in ion isolation during the tandem mass spectrometry experiment on commercial instruments. Studies were performed in an attempt to cool the precursor ion prior to activation with no change in the mass spectrum. Comparison of *n*-butylbenzene with other butylbenzene isomers gave evidence that rearrangement was taking place during the isolation of the precursor in the tandem experiment. Thorough investigation of the commercial protocol was undertaken that linked the cause of the rearrangement to the broadband waveform used in the precursor ion isolation step of the tandem mass spectrometry experiment. (Int J Mass Spectrom 190/191 (1999) 265–279) © 1999 Elsevier Science B.V.

Keywords: *n*-butylbenzene; Tandem mass spectrometry; Quadrupole ion trap; Rearrangement; Broadband waveforms

1. Introduction

In 1980, Brown described a method of determining relative activation energies for competing rearrangements and simple cleavages [1]. The premise of this report is that comparing the ratio of ion intensities would give data about the competitive processes that occur during activation. An example of such a competitive process is the fragmentation of *n*-butylbenzene. During activation in a tandem experiment, the molecular ion from *n*-butylbenzene (*m/z* 134) undergoes rearrangement to form *m/z* 92 or α-cleavage to produce *m/z* 91 (Fig. 1). The rearrangement to *m/z* 92 is the low energy process occurring with 1.1 eV of activation energy. The higher energy process is an α-cleavage requiring 1.7 eV to yield *m/z* 91. The simplicity of this fragmentation and the well-characterized energetics of this process position *n*-butylbenzene to be considered a "molecular thermometer" to determine energetics of activation processes and made this compound popular for mass spectral study [2–17].

Beynon et al. used a modified commercial reversed-geometry ZAB-2F mass spectrometer to monitor photodissociation of *n*-butylbenzene [2,3]. By

* Corresponding author. E-mail: blynn@ra.msstate.edu

Dedicated to J.F.J. Todd and R.E. March in recognition of their original contributions to quadrupole ion trap mass spectrometry.

PII S1387–3806(99)00025-1

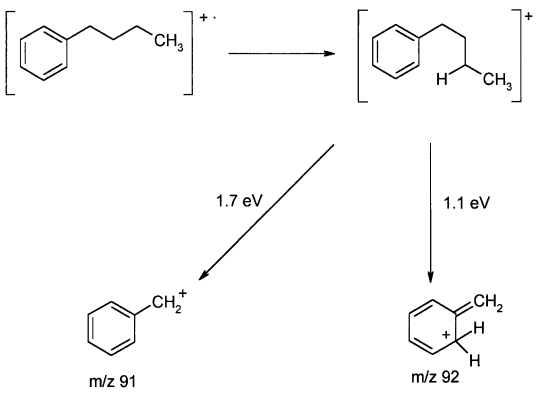

Fig. 1. The reported literature pathway for the fragmentation of *n*-butylbenzene. The rearrangement product requires 1.1 eV of energy whereas the α-cleavage requires 1.7 eV.

fitting a quartz window to a special magnet flight tube, radiation from an argon–ion laser excited mass selected ions in the second field-free region. Changing the wavelength of the photoexcitation energy produced changes in the mass spectra, i.e. the base peak was either *m/z* 92 or *m/z* 91. These data were then used to calculate the abundance of these ions relative to one another based upon their internal energy [3]. Levsen et al. described the rearrangement process that yields the methylene cyclohexadiene ion (*m/z* 92) occurring upon collisional activation and charge stripping excitation of *n*-butylbenzene [4] in a magnetic sector mass spectrometer. A comparison between collision-induced dissociation (CID) and previous photodissociation experiments was performed in a triple quadrupole mass spectrometer by Dawson and Sun [5]. Insight into the dissociation and fragmentation mechanics at low energies ($\leq$100 eV) is obtained by comparing the energies of the fragment ions at *m/z* 91 and *m/z* 92. In 1983, Harrison and Lin reported revised critical energies of formation for the fragment ions of *n*-butylbenzene [6], repeating the experiments of Dawson [5] and Beynon [2,3]. *n*-Butylbenzene was examined by Dunbar et al. using an ion cyclotron resonance mass spectrometer to determine that the energy of the *m/z* 91 formation was 0.6 eV greater than formation of *m/z* 92 [7]. Harrison used a ZAB-2F mass spectrometer to perform charge exchange mass spectrometry on *n*-butylbenzene [8]. By comparing the data obtained for these experiments with CID results, the average kinetic energy converted into internal energy at collision was determined. Welch et al. reported the source temperature dependence for determining the internal energies in photodissociation experiments involving *n*-butylbenzene [9]. In 1987, Cooks et al. investigated the fragmentation of *n*-butylbenzene in the quadrupole ion trap [10] to monitor the energy deposition during the tandem experiment when ac voltage, q_z, and β_z values were changed. Baer et al. used photoelectron photoion coincidence (PEPICO) with time of flight (TOF) mass spectrometry to determine the dissociation dynamics of *n*-butylbenzene [11]. Vettori et al. used the ratio *m/z* 91 and *m/z* 92 from *n*-butylbenzene to compare CID efficiency using a boundary activated dissociation versus a tickle voltage (resonant excitation) [12]. *n*-Butylbenzene was used as a model compound for fast collisional activation mass-analyzed ion kinetic energy spectrometry (CA-MIKES) experiments utilizing a ZAB-EQ tandem hybrid (BEqQ geometry) and a ZAB-4F tandem double focusing mass spectrometer ($B_1E_1E_2B_2$ geometry) by Thibault et al. [13]. March et al. published the first evidence that butylbenzene may fragment via different pathways in the quadrupole ion trap [14]. In their energy deposition experiments *m/z* 134 was activated to the two product ions at *m/z* 92 and *m/z* 91; *m/z* 92 is then isolated and activated in an MS^3 experiment yielding *m/z* 91 as the product. Tabet et al. utilized *n*-butylbenzene in the ion trap to investigate the tandem parameters during an MS/MS experiment [15]. Kim et al. determined that the photodissociation dynamics of *n*-butylbenzene occur competitively on a nanosecond time scale [16]. The only evidence for other possible products from *n*-butylbenzene were reported in pyrolysis of butylbenzene by Freund and Olmstead independent of a mass spectrometer [17]. This survey of the available literature revealed no publications with reference to *m/z* 119 as the base peak in the product ion spectrum of *n*-butylbenzene and that *n*-butylbenzene unequivocally fragments simply via a two channel pathway.

A study of the relative CID energetics of nonyl phenol and nonylphenyl acetate prompted an evaluation of *n*-butylbenzene, the “molecular thermometer.”

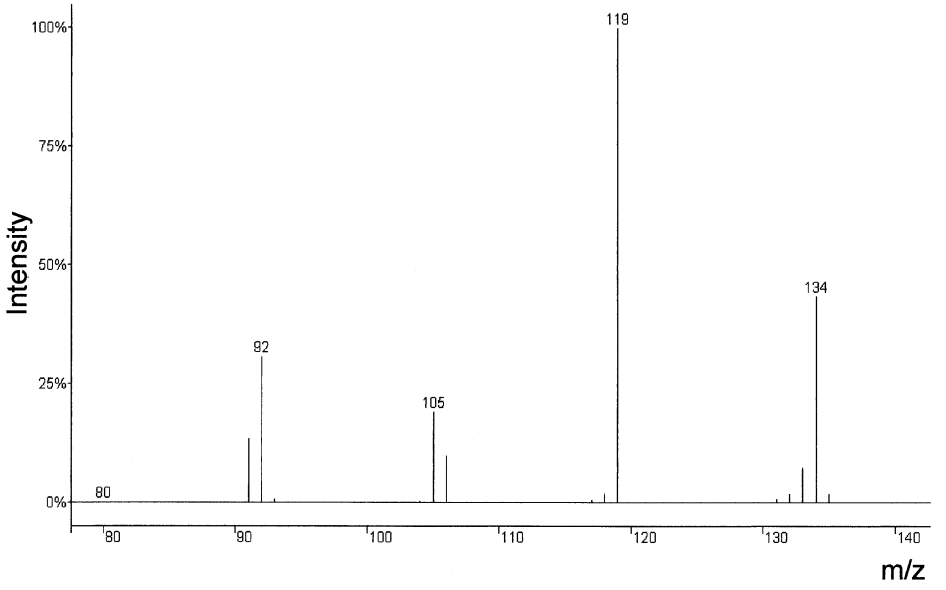

Fig. 2. Observed EI/MS/MS spectrum of *n*-butylbenzene (activation of *m/z* 134). The CID voltage was 0.4 V and the q_z was set to 0.4. Notice that *m/z* 92 and *m/z* 91 were present, however, *m/z* 119 was the base peak.

However, *n*-butylbenzene did not fragment according to the literature during the tandem experiment. In lieu of the base peak being either *m/z* 91 or *m/z* 92, the base peak was observed at *m/z* 119 (Fig. 2). The tandem experiment was repeated under different q_z values and CID energies with no change in the product ion spectrum. These results forced us to consider the CID fragmentation of *n*-butylbenzene in comparison to that reported in the literature. Using a number of mass spectrometers and tandem mass spectrometry software packages, the tandem experiment was carefully examined for *n*-butylbenzene. A thorough examination of the tandem mass spectrometry protocol on our commercial instrument was undertaken to isolate the causative agent for the unusual fragmentation.

2. Experimental

2.1. Chemicals

The mass spectrometry samples, *n*-butylbenzene, *tert*-butylbenzene, *sec*-butylbenzene, and *iso*-butylbenzene were purchased from Aldrich (Milwaukee, WI). Solvents were obtained from Burdick & Jackson (Muskegon, MI). All analytes were prepared at a concentration of 100 ng/μL in hexane.

2.2. Synthesis of deuterated n-butylbenzene

Bromoethane (2,2,2-d_3) was purchased from Cambridge Isotopes (Andover, MA). Phenylacetaldehyde was obtained from Aldrich (Milwaukee, WI). The *n*-butylbenzene (methyl-d_3) was prepared in three steps from commercially available bromoethane (2,2,2-d_3) in the following manner. Refluxing deuterated bromoethane with triphenylphosphine (1.2 equivalents) in acetonitrile for 36 h gave ethyltriphenylphosphonium bromide (methyl-d_3). Witting olefination of a phosphorous ylide, formed by lithium hexamethyldisylazide deprotonation of a phosponium salt (1 eq LiHMDS, THF, −78 °C → 0 °C) with phenylacetaldehyde (0.95 eq $PhCH_2CHO$, THF, −78 °C → 0 °C, overnight) gave 1-phenylbutene-2 (methyl-d_3), which was converted into the desired deuterated butylbenzene by Pd catalyzed hydrogenation (10

mol% Pd-on carbon, EtOH, H_2, 24 h). NMR was used to confirm the product. The following coupling constants were obtained: 1H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.35 (2H, *t*, $J = 9.3$ Hz); 1.60 (2H, *m*); 2.61 (2H, *t*, $J = 7.7$ Hz); 7.27–7.38 (5H).

2.3. GC/MS/MS

Experiments were conducted on the following instruments: three Varian 4Ds (Walnut Creek, CA) equipped with WaveBoard options, a Varian Saturn 2000 with WaveBoard options, and a Trio-3 triple quadrupole mass spectrometer. The majority of the experiments were performed using a Varian 4D ion equipped with Varian's QISMS and Revision 5.2 software and the Toolkit package. The QISMS software package allows control of the tandem experiment by introduction of user selected steps between ionization and the mass selective instability scan. Control of time, drive rf, superposition of waveforms placed on the endcaps in a dipolar fashion, and the amplitude of these waveforms is permissible. The software also has the ability to control timing of axial modulation waveform and the turn on of the electron multiplier.

A Varian 8200 AutoSampler and 1078 injector held isothermal at 260 °C operated in split mode (50:1) were used to introduce 1 μL of analyte into a Varian 3400CX GC. The analyses were performed on a 30-m DB-5MS (0.25 mm inner diameter (id), 25 μm film) capillary column (J & W Scientific, Folsom, CA). The column was operated isothermally at 120 °C for 4 min using helium as the carrier gas.

3. Discussion

To reiterate, the tandem mass spectrum of *n*-butylbenzene obtained in our laboratory on a commercial quadrupole ion trap was significantly different from that reported in the literature. When the molecular ion of *n*-butylbenzene (*m/z* 134) was activated, three major ions were observed (Fig. 2). The expected ions *m/z* 91 and 92 were observed; however, the base peak was *m/z* 119. This observation generated a number of questions that were immediately addressed including: (1) Was the *n*-butylbenzene standard contaminated with other isomers or other compounds that resulted in the appearance of *m/z* 119? (2) Could the GC conditions (such as sample introduction) cause the appearance of *m/z* 119? (3) Do other quadrupole ion traps or tandem instruments in our organization also produce this unusual ion? All of these considerations must be prefaced with the observation that this *n*-butylbenzene sample produced the correct full scan electron ionization (EI) spectrum as compared to the National Institute of Standards and Technology (NIST) library.

Other isomers of butylbenzene were analyzed to remove concerns about the quality of the *n*-butylbenzene standard used in this study. Retention time differences and full scan mass spectra were correlated with the NIST mass spectral database to confirm purity and isomer identity. The isomers were chromatographically resolved by the GC system eliminating concerns about contamination (Fig. 3). Interestingly, the unusual tandem mass spectrum obtained for *n*-butylbenzene (Fig. 2) looked very similar to the full scan spectrum of *t*-butylbenzene (Fig. 4).

Questions concerning the GC system and analyzer system were next examined. Different injector and column temperatures were evaluated with no change in the resulting mass spectrum. Injections were performed as stated in the experimental section (1 μL split at 260 °C isothermal). A range of manifold (trap) temperatures were evaluated, in each case *m/z* 119 remained the base peak. The only observable difference was the CID voltage required to fragment the precursor (i.e. higher temperature, lower CID voltage). The helium bath gas pressure was changed by adjusting the column volumetric flow rate, which allowed evaluation of collision frequency on the production of *m/z* 119. The resulting product ion spectrum changed only in the amount of CID energy required to fragment the precursor; *m/z* 119 still dominated the spectrum.

Activation of the molecular ions from *sec*, *iso*, and *tert* analogs provided some insight into the production of *m/z* 119 from *n*-butylbenzene. For the *sec* isomer *m/z* 105 was observed as the base peak, a result of

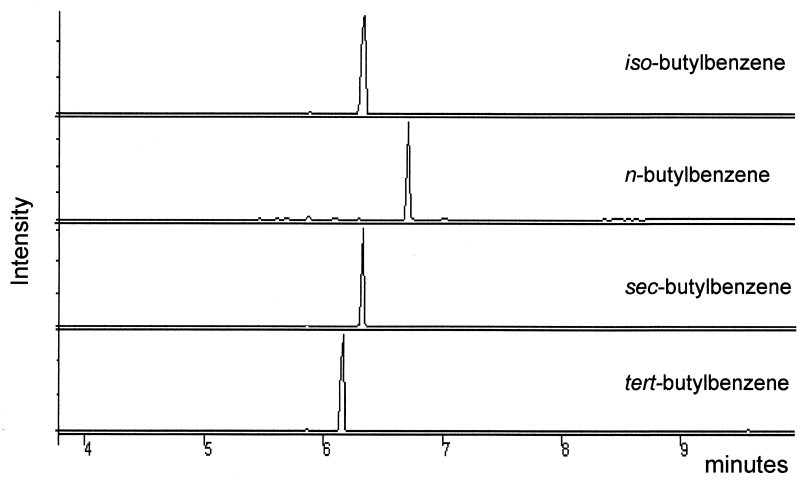

Fig. 3. GC/MS chromatograms of the four butylbenzene isomers. The isomers are chromatographically resolved, removing concerns about the purity of the *n*-BB standard material.

ethyl loss [Fig. 5(a)]. The *iso* isomer, however, produced a CID spectrum very similar to the literature spectrum of *n*-butylbenzene [i.e. *m/z* 91 from propyl loss, Fig. 5(b)]. However, activation of *m/z* 134 from *t*-butylbenzene produced exclusively *m/z* 119, which was surprisingly similar to the tandem spectrum obtained from *n*-butylbenzene on our Saturn 4D ion trap mass spectrometer [Fig. 5(c)]. Because of the similarities between the CID spectra of *n*-butylbenzene and *t*-butylbenzene, it was presumed that *n*-butylbenzene molecular ion (*m/z* 134) rearranged to some *t*-butylbenzene-like structure during the tandem experiment.

To rule out an isolated instrumental problem, other quadrupole ion trap mass analyzers were used to evaluate the response of *n*-butylbenzene during the

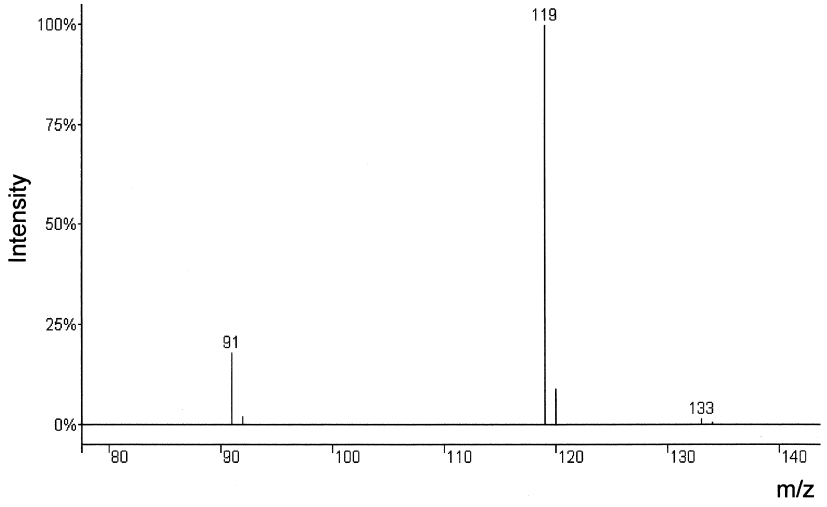

Fig. 4. EI/MS spectrum of *t*-BB. The similarities between this spectrum and that of the EI/MS/MS of *n*-BB are notable.

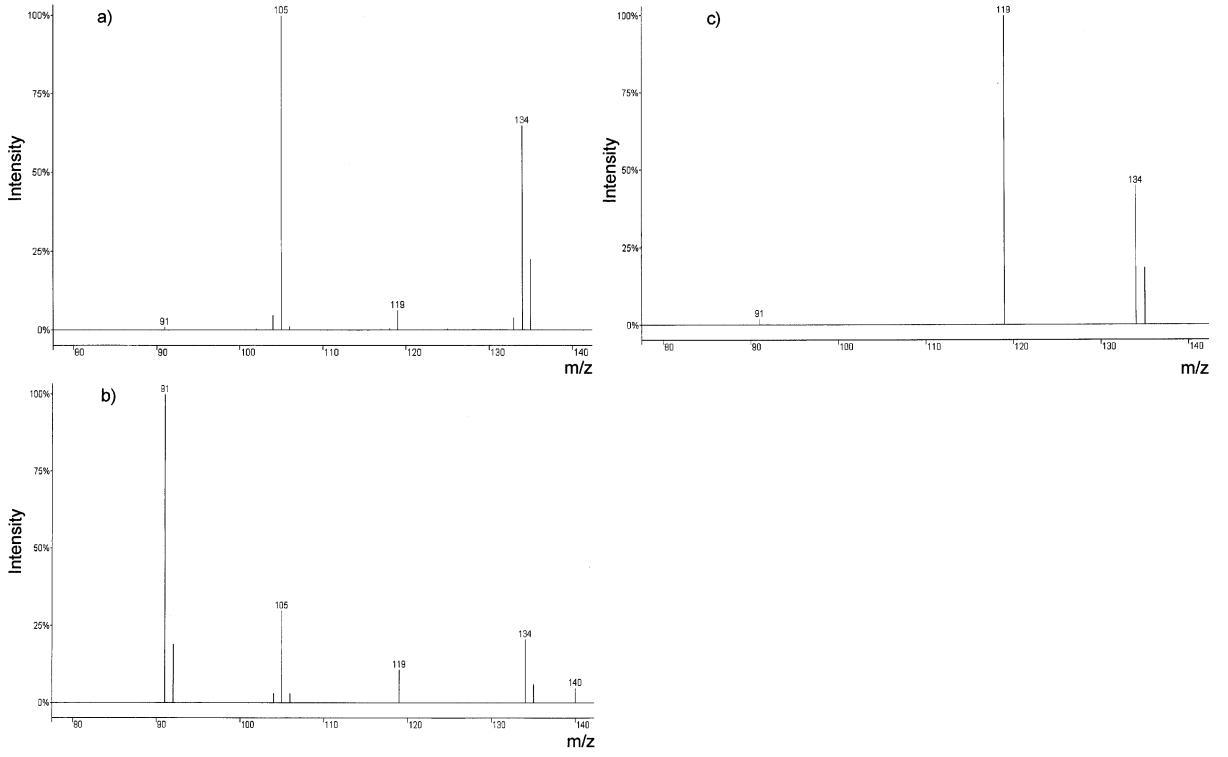

Fig. 5. MS/MS spectra of the isomers of butylbenzene (a) *sec*-BB, (b) *iso*-BB, and (c) *t*-BB. The precursor ion in each case was *m/z* 134 and a q_z of 0.4. The amplitude of the CID voltage was tailored for each isomer.

tandem experiment. Two additional Saturn 4Ds and a Saturn 2000 were evaluated in this process. The same *n*-butylbenzene standard and freshly prepared *n*-butylbenzene standards were introduced into these different GC/MS systems under similar conditions. The other 4Ds produced tandem mass spectra containing *m/z* 119 as the base peak at a q_z of 0.4 and 0.5 V CID analogous to spectra produced by the original 4D. Interestingly, the Saturn 2000 also produced the *m/z* 119 as the base peak from the tandem experiment on *n*-butylbenzene. The only difference in the observed spectra between the Saturn 2000 and the 4Ds was a change in the relative abundance of the product ions at the same CID values. The Saturn 2000 has a substantially different design than the 4Ds. The trap electrodes are independently heated, thus eliminating the temperature gradients in the elements. Varian also redesigned the three trap elements to reduce their thermal mass. These design differences were expected to have a greater impact on the tandem experiment than that observed.

Returning to the original 4D, ion times were examined to determine the impact of space charging on the appearance of *m/z* 119. A series of dilutions of the *n*-butylbenzene standard (1–0.05 ng/μl) were analyzed by tandem mass spectrometry using a fixed ion time of 11.0 ms. No differences in the relative ion ratios were observed over the concentration range examined. In every case, *m/z* 119 remained the base peak, thus removing the possibility that space charging was contributing to this unusual tandem mass spectrum of *n*-butylbenzene.

A VG Trio 3 triple quadrupole mass spectrometer was also used to examine *n*-butylbenzene. No significant *m/z* 119 ion current could be obtained from collision activation of *m/z* 134 with an argon collision gas. Regardless of the collision energy employed, *m/z* 92/91 were the most abundant product ions observed.

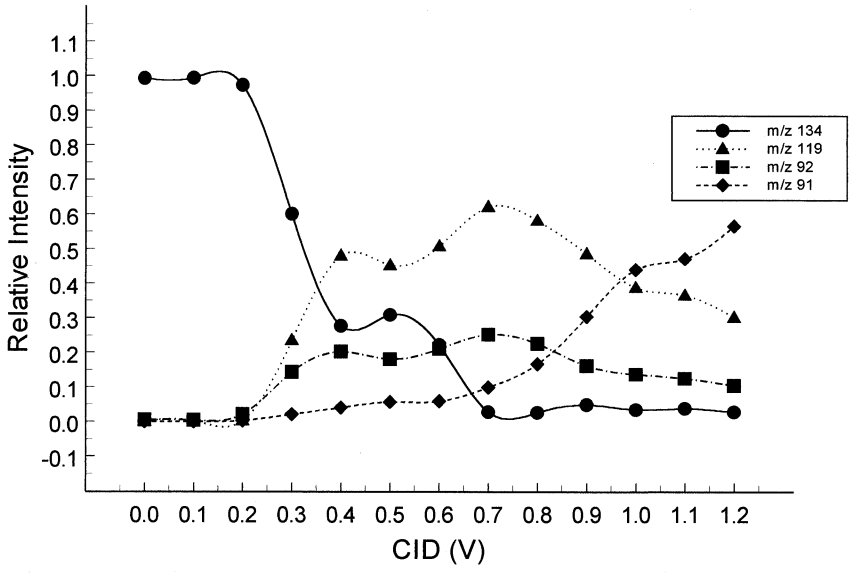

Fig. 6. A plot of the relative intensity of product ions vs. CID voltage during the tandem experiment (q_z = 0.4). From 0.4 V CID to 0.7 V, *m/z* 119 was the base peak. At 0.7 V *m/z* 91 grew to the base peak at 1.2 V.

Thus, this phenomenon appeared to be limited to the quadrupole ion trap mass spectrometers.

To insure that the observation of the *m/z* 119 base peak was not isolated to our venue, *n*-butylbenzene was independently examined offsite [18]. The only common factor between our experiment and the independent evaluation was the use of Varian ion trap instrumentation. The resulting tandem mass spectra were virtually identical to those produced in our laboratory (i.e. *m/z* 119 base peak). Based on the above discussion, we concluded that production of *m/z* 119 had little to do with the performance of our ion trap instruments, but was associated with the tandem protocol utilized by Varian in their commercial instrument. Something in the Varian tandem mass spectrometry protocol was apparently contributing to this unusual product ion spectrum.

Having isolated the production of *m/z* 119 from the CID of *m/z* 134 to Varian ion traps, the standard Revision 5.2 tandem mass spectrometry protocol on the Saturn 4D was evaluated in greater detail. Resonant experiments were conducted incrementing the amplitude of the CID voltage in 0.1 V steps from 0.0–1.1 V at a q_z of 0.4. Product ions were normalized against the total ion current and plotted as the relative intensity versus CID voltage (Fig. 6). As expected, the intensity of the precursor ion fell off with increasing CID voltage. However, instead of the expected increase in the *m/z* 92/91 ions, the intensity of *m/z* 119 increased with increasing voltage, maximizing between 0.4 and 0.9 V. The intensity of *m/z* 92 did increase following the *m/z* 119 trend but fell off as the high energy product at *m/z* 91 increased to become the base peak at 1.0 V. The formation *m/z* 119 seemed to complete with *m/z* 92. Cooling studies were implemented to explore the persistence of *m/z* 119. Using the QISMS software package, a cooling time segment was inserted between precursor isolation and the CID step. The isolated precursor (located at a q_z of 0.4) was stored for times ranging from 2–240 ms before activation at 0.4 V. The relative intensity of product ions was plotted versus cooling times (Fig. 7). As can be seen, cooling the precursor had little effect on product ion ratios and intensities. This lack of response indicated that residual energy in the precursor was rapidly removed and thus not responsible for the formation of *m/z* 119. This pointed to a possible structure modification of the precursor during the isolation phase of the experiment.

The tandem experiment as performed in other

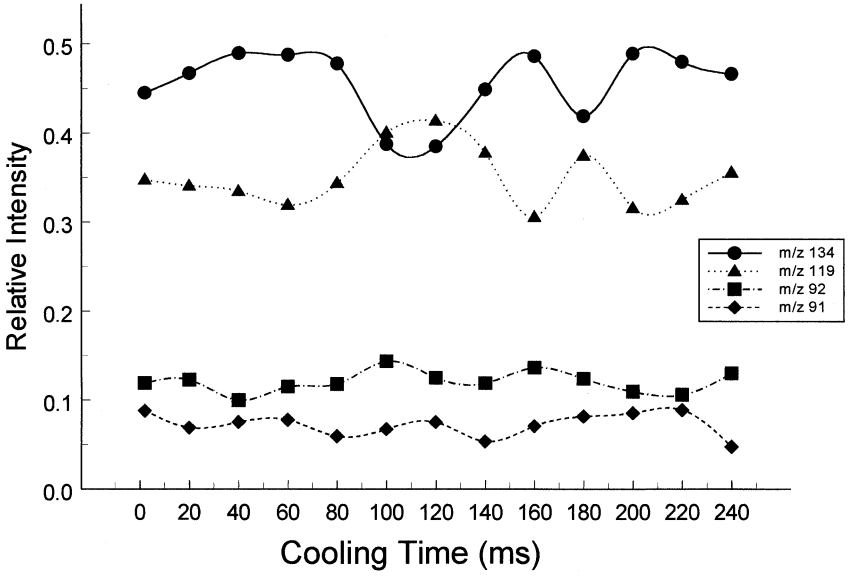

Fig. 7. Relative product ion intensity plotted vs. cooling time inserted prior to activation. Except for the perturbation between 100 and 120 ms, cooling *m/z* 134 had little effect on the product ions.

laboratories [10,13,15,16] utilizes different precursor ion isolation methods compared to that of the Varian proprietary tandem mass spectrometry isolation. Typically, in other laboratories, the rf drive frequency is adjusted so that the q_z value for a given ion is placed below the apex of the stability diagram. A negative dc potential is then applied to the ring electrode to move the ion to a new a_z value below the apex. This removes ions of lower *m/z* axially whereas higher *m/z* ions are lost radially. The dc potential is returned to zero after ~2 ms and the rf is lowered to a predetermined q_z for the subsequent activation step.

The Varian commercial tandem mass spectrometry protocol, as applied to *n*-butylbenzene, is composed of 10 temporally isolated steps (Fig. 8). The isolation of the precursor ion starts during ionization where a broadband high frequency waveform is applied across the endcaps to eliminate low-mass ions. This waveform reduces nonproductive ion current in the trap thus reducing space charge. Following a post ionization cooling period, two broadband waveforms are applied across the endcaps in a sequential fashion to roughly isolate the precursor ion. In steps 1 and 2 (Fig. 8), the first broadband waveform (b134), applied at 20 V_{p-p} for 3.0 ms, ejects low-mass ions with frequencies spanning from ~5 kHz above the precursor to 400 kHz. The rf is lowered from 300 digital to analog converter (DAC) steps to 297 DAC steps during the first 1.5 ms (step 1). Note: to convert from DAC to rf voltage, multiply the DAC value by 1.83 (calibration dependent), however, because DAC values are the required inputs for the QISMS software, these values will be used in this discussion. The rf is then returned to 300 DAC steps during the remaining 1.5 ms (step 2). In steps 3 and 4 the second broadband waveform (a134 also at 20 V_{p-p} for 3.0 ms) ejects high-mass ions with frequencies spanning from 20 kHz to just below the frequency of the precursor. During application of this waveform, the rf is increased from 300 DAC to 312 DAC for 1.5 ms (step 3) and then returned to 300 DAC during the remaining 1.5 ms (step 4). This rf modulation improves isolation of selected precursor ions by modulating the secular frequencies of the trapped ions, bringing them into resonance with the applied waveforms. Fine isolation of the precursor is achieved by increasing the rf to 820 DAC (~435 kHz). This ramp has two steps, involving an initial fast step to 788 DAC in 1.0 ms (step 5)

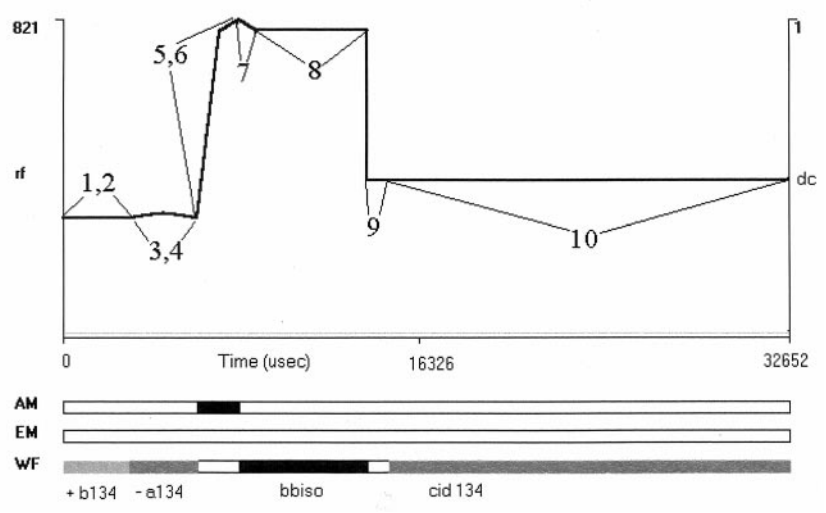

Fig. 8. A graphical representation of the MS/MS scan function created by the QISMS software. Steps 1 and 2 are implementation of the +b134 waveform; steps 3 and 4 are the −a134 waveform; steps 5 and 6 are axial modulation with the concurrent rf ramp; steps 7 and 8 are the bbiso waveform irradiation; step 9 is a cooling period before CID at step 10.

followed by a slow step to 820 DAC in 0.896 ms (step 6). In step 6, precursor ions are brought close to the ejection point, thus ejecting all ions of lower *m/z* values. Axial modulation is implemented during both steps 5 and 6 to improve the ejection process (i.e. increase resolution for the ejection of low mass ions). At the apex, 820 DAC, axial modulation is removed and a third broadband waveform is implemented that is referred to as the bbiso waveform. The bbiso waveform consists of frequencies spanning from 20–400 kHz, spaced at 500 Hz with amplitude of 30 V_{p-p}. The bbiso is on for 0.756 ms whereas the rf is lowered to 793 DAC (step 7) and maintained for an additional period of 5.0 ms at 793 DAC (step 8). The bbiso functions to remove high mass ions, completing precursor ion isolation. The bbiso irradiation between 20 and 400 kHz should have no effect on precursor ions, which have 415 kHz secular frequencies during this process. At the end of the bbiso waveform irradiation, the rf is reduced to an appropriate DAC (precursor *q*) for the CID phase of the experiment and held there for 1.0 ms to cool the precursor ion cloud (step 9). The CID waveform is then applied for 18.0 ms at a predetermined amplitude (step 10). At the end of this process, resulting product ions are scanned from the trap by a conventional mass selective instability scan. Although at first glance this process appears quite complicated and temporally costly, the entire process only takes 29.7 ms. We have used this process very effectively to conduct tandem mass spectrometry experiments on a wide variety of compounds at trace levels. This tandem mass spectrometry process has been effective and reliable, with the only anomalous result to date being that of *n*-butylbenzene.

Following the lead of March and others, a dc apex isolation was evaluated on the Saturn 4D instrument. This was accomplished by building a new tandem mass spectrometry scan function using the QISMS software. Once the dc parameters were established to isolate *m/z* 134, subsequent activation produced a product ion spectrum nearly identical to literature spectra. Thus, the production of *m/z* 119 must be linked to the Varian isolation protocol.

With the problem confined to the isolation phase of the tandem experiment, a step by step dissection of this phase of the scan function was initiated using the QISMS software package. The first experiments involved alteration of the broadband waveforms, b134 and a134, that eliminate the low mass and high mass

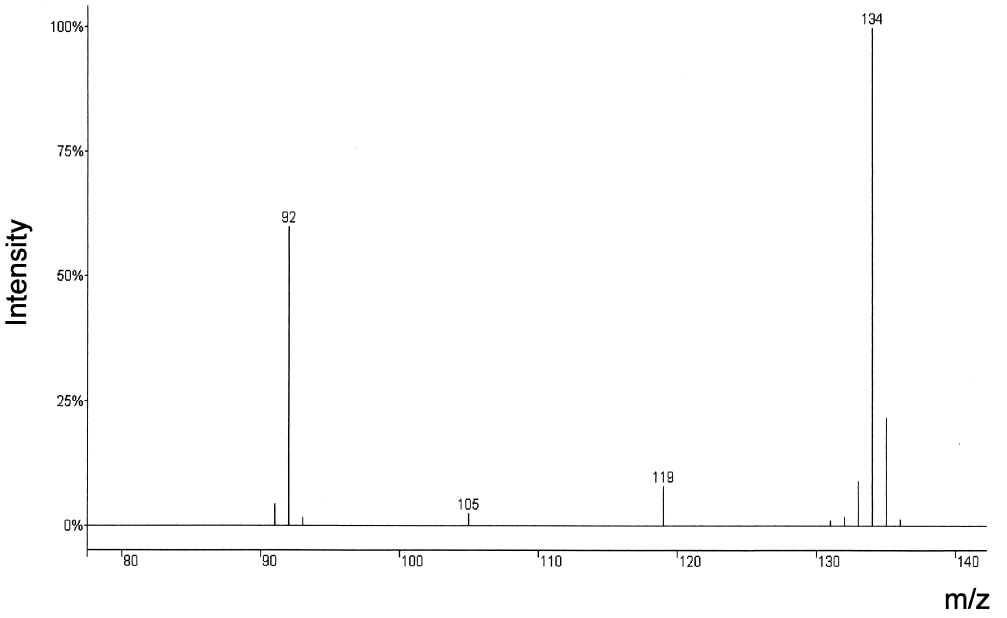

Fig. 9. EI/MS/MS spectrum of *n*-butylbenzene with the broadband isolation waveform removed. The abundance of *m/z* 92 relative to *m/z* 119 indicated a more literature-like mass spectrum.

ions, respectively. Reducing or increasing the peak to peak voltages of these waveforms did not change the MS/MS spectrum, i.e. *m/z* 119 was still observed as the base peak. When b134 and a134 waveforms were removed altogether, no change was observed in the tandem mass spectrum of *n*-butylbenzene, thus eliminating these steps from the *m/z* 119 problem. Removal of the axial modulation waveform during the rf ramp also had no effect on production of *m/z* 119. The rf ramp was evaluated by constructing a separate isolation scheme for *m/z* 134. In this new QISMS scheme *m/z* 134 was isolated simply by increasing the rf to 792 DAC for 5 ms. Low mass fragments were not axially stable, resulting in a crude isolation of *m/z* 134. Activation of the isolated *m/z* 134 produced a product ion spectrum containing only the literature predicted ions *m/z* 92 and 91. The *m/z* 119 ion was not observed. The rf levels employed in this QISMS scheme were identical to those used in the normal Varian protocol. In this case, the only difference was the absence of the bbiso waveform.

In the Varian isolation protocol there are two steps that employ the bbiso waveform—the initial short 0.756 ms irradiation when the rf is reduced from 820 to 793 DAC (step 7) and the second longer irradiation of 5.0 ms at 793 DAC (step 8). When these waveforms were removed from the standard tandem mass spectrometry protocol, *m/z* 119 was not observed. In fact, the mass spectrum produced from this experiment was analogous to the above discussed dc and rf experiments and mass spectra reported in the literature (Fig. 9). Subsequent experiments indicated that the production of *m/z* 119 was related to the amplitude and irradiation time of the bbiso waveform in step 8. During these experiments another interesting observation was made: The intensity of the product ion spectrum increased as the bbiso voltage decreased. The intensity of the product ion spectrum increased over 50% when the bbiso waveform amplitude was reduced from the default setting of 30 V_{p-p} to a new setting of 20 V_{p-p}. The QISMS software was used to turn on the electron multiplier in step 8 of the standard tandem mass spectrometry protocol. When a digital oscilloscope was connected to the multiplier preamplifier circuit and time correlated to the 5.0 ms bbiso irradiation, ions were observed escaping from the trap

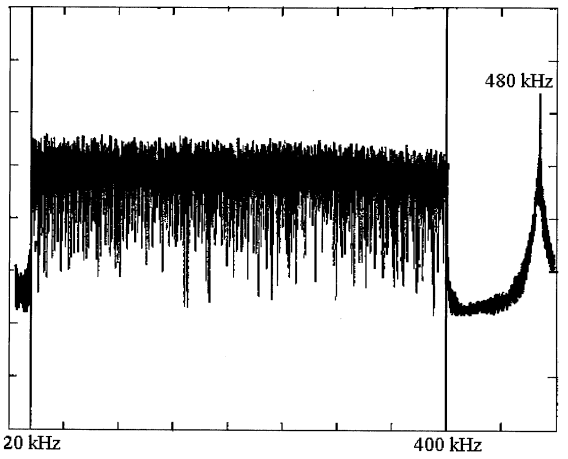

Fig. 10. Power spectrum of the broadband isolation waveform. Lines were drawn at 20 and 400 kHz. Close investigation revealed that little residual energy resided at the frequency of the precursor ion (415 kHz).

during the irradiation. This demonstrated that the ion cloud had absorbed energy from the 20–400 kHz bbiso waveform. As stated earlier, the secular frequency of *m/z* 134 during the bbiso irradiation would be 415 kHz; therefore no absorption of energy should occur.

To further elucidate the impact of the bbiso waveform, a second oscilloscope experiment was conducted. This time the oscilloscope was connected to the output of the waveform generator. The output signal was captured and Fourier transformed to produce a power spectrum of the bbiso waveform (Fig. 10). The power spectrum of the bbiso waveform indicated a prompt rise at 20 kHz and maintained a reasonably flat power output across the frequency range. The power in the waveform dropped nicely to baseline above 400 kHz with no significant contribution at the frequency of the ion, 415 kHz. Note: The resonance located at 480 kHz resulted from a small portion of the axial modulation waveform that was captured with the bbiso waveform during this experiment.

Because the problem was limited to the bbiso waveform, experiments focused on quantifying the effects of the bbiso waveform on the production of *m/z* 119. Using the Toolkit software, a tandem experiment was performed where the bbiso voltage was incremented from 0–30 V_{p-p} while the CID parameters were maintained at 0.5 V CID and a q_z of 0.4 (Fig. 11). From Fig. 11, the production of *m/z* 119 started at

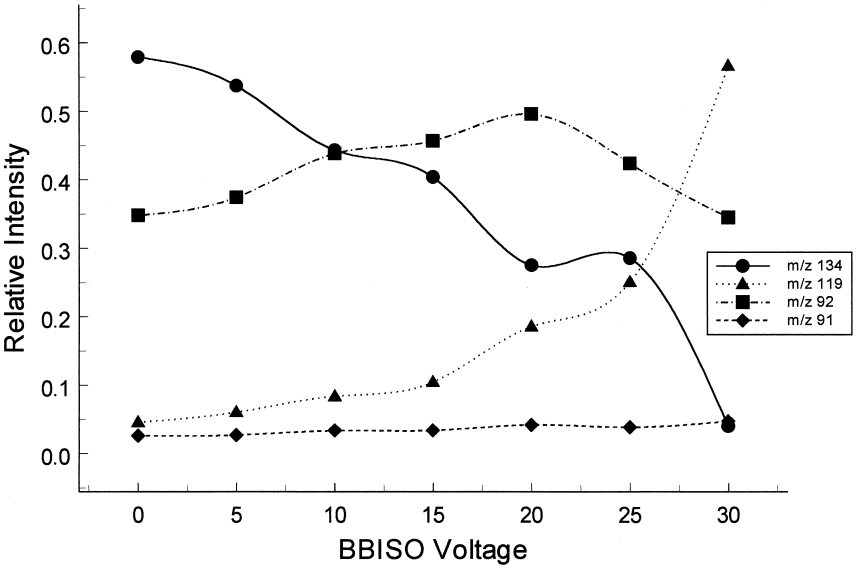

Fig. 11. A plot of relative product ion intensity vs. bbiso voltage. As the amplitude of the bbiso voltage increased, the abundance of *m/z* 119 increased exponentially.

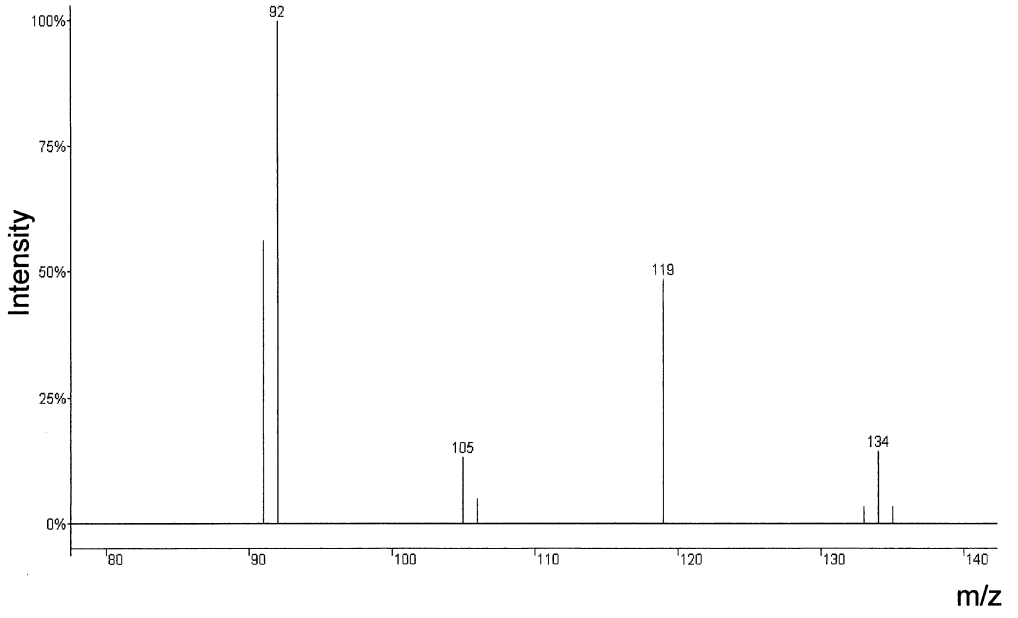

Fig. 12. Tandem mass spectrum of *m/z* 134 at a bbiso amplitude of 20 V_{p-p} (CID voltage = 0.8 V, q_z = 0.4) which was a good compromise between ion current and production of *m/z* 119.

15 V and increased exponentially to a maximum at 30 V (the default peak to peak voltage). At low bbiso voltages, the abundance of *m/z* 119 was extremely low and the product ion spectra generated at these values were consistent with literature spectra. Under the CID conditions employed in this experiment, the maximum ion current produced was observed at a bbiso voltage of 20 V_{p-p}. At this voltage, the abundance of *m/z* 119 was greater than literature spectra but was determined to be a good compromise (Fig. 12).

QISMS was used to evaluate the impact of the bbiso irradiation time at 20 and 30 V_{p-p}. At 30 V_{p-p}, *n*-butylbenzene was activated at a q_z of 0.4 and at 0.5 V CID. The normalized data were plotted versus the bbiso time (Fig. 13). As can be seen, when the bbiso voltage was set at 30 V, *m/z* 119 grew to the base peak around 3.5 ms within the irradiation time of the default scan function. As expected, the intensity of *m/z* 134 decreased with time. The product ion at *m/z* 92 peaked at 2 ms, but at longer times the intensity decreased as the intensity of *m/z* 119 grew. At this low CID, the contributions from *m/z* 91 were low, as expected.

When the experiment was repeated at a bbiso voltage of 20 V the results were different. The normalized data were plotted versus bbiso time as above (Fig. 14). The precursor ion (*m/z* 134) remained the base peak for a much longer time at this lower bbiso voltage (4 ms versus <1 ms for 30 V_{p-p}) indicating that the bbiso has an additive effect in the activation of the precursor during the tandem experiment. As predicted, *m/z* 92 grew to the base peak and the intensity of *m/z* 119 was low until very long times were imposed (>6 ms). Here again, the response from *m/z* 91 was insignificant compared to the other ions.

At this stage of the project, the production of *m/z* 119 during the tandem mass spectrometry analysis of *n*-butylbenzene could now be controlled through manipulation of the bbiso waveform. An attempt was made to isolate the frequencies responsible by successive approximation. The bbiso waveform was divided into two separate waveforms, 20–200 kHz and 200–400 kHz. The low frequency bbiso did not activate the production of *m/z* 119. However, the 200–400 kHz

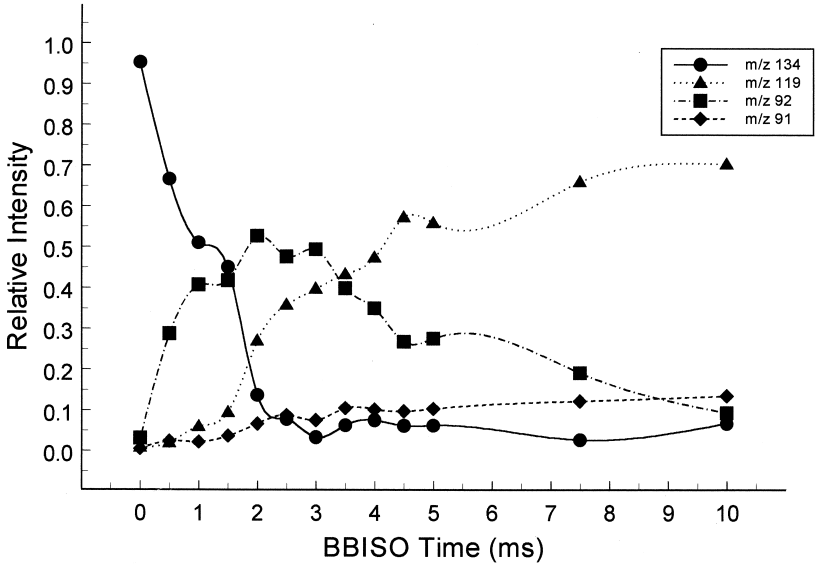

Fig. 13. A plot of relative product ion intensity vs. bbiso waveform irradiation time at a constant amplitude of 30 V_{p-p} (CID voltage = 0.5 V, q_z = 0.4). At 3.5 ms, the intensity of *m/z* 119 grew to the base peak and remained throughout the study.

bbiso waveform was active toward production of *m/z* 119. The 200–400 kHz bbiso waveform was again subdivided. This process was continued until the bandwidth of frequencies was reduced to 370–400 kHz. No finer isolation was successful because all these frequencies activated the production of *m/z* 119 to some degree. A new software control package is currently under evaluation that will provide fine

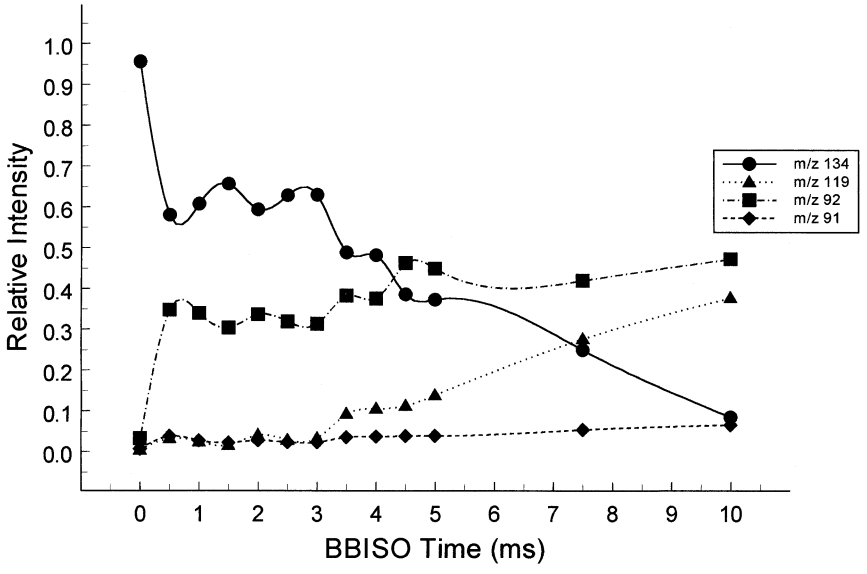

Fig. 14. Repeat of the experiment described in Fig. 14 except that the bbiso irradiation waveform amplitude was changed to 20 V_{p-p}. Contributions from *m/z* 119 were not significant for irradiation times >6.5 ms.

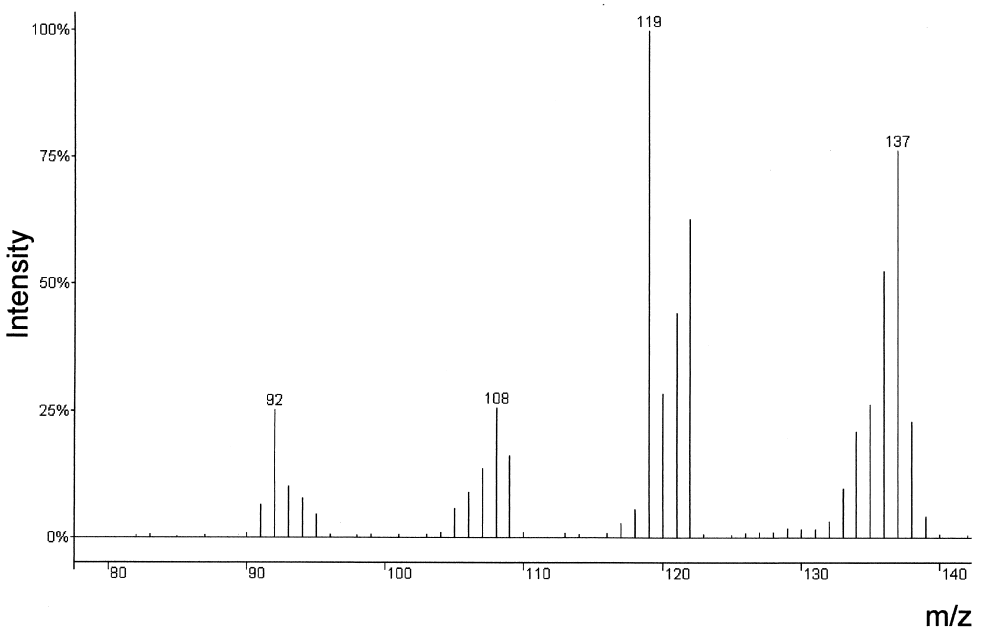

Fig. 15. Tandem mass spectrum of the deuterated *n*-BB standard. The molecular ion *m/z* 137 is activated at 0.5 V CID at a q_z of 0.4 with a bbiso amplitude of 30 V_{p-p}. The presence of the ion peaks observed at *m/z* 119, 120, 121, and 122 indicated scrambling of the deuterium label during the tandem mass spectrometry experiment that indicated rearrangement.

control of the waveboard necessary to pinpoint the causative frequency.

Having identified the instrumental parameters responsible for the production of *m/z* 119, efforts were directed toward exploring the mechanism for the production of this unusual ion. As stated earlier, the tandem mass spectrum of *n*-butylbenzene resembled closely the full scan spectrum of *t*-butylbenzene regarding the presence of *m/z* 119. To further evaluate this similarity, *t*-butylbenzene and *n*-butylbenzene were subjected to tandem and MS^3 experiments, respectively. For *t*-butylbenzene, *m/z* 119 was isolated and activated to give the product ion, *m/z* 91. When *n*-butylbenzene was analyzed by MS^3 (*m/z* 134 to *m/z* 119 to products), a product ion at *m/z* 91 was also obtained, pointing to a possible similarity in ion structure. This led to a number of questions about the structure of this unusual *m/z* 119 product ion.

A deuterium isotope labeling study was initiated to illuminate the rearrangement of *m/z* 134 from *n*-butylbenzene. A deuterium labeled *n*-butylbenzene was synthesized with the label on the delta carbon (CD_3). The labeled standard had a slightly different GC retention time than the unlabeled *n*-butylbenzene and produced the expected full scan spectrum with the correct molecular ion (*m/z* 137). The proton NMR spectrum confirmed that the deuterium label was placed on the terminal (delta) methyl group. Tandem mass spectrometry analysis of *m/z* 137 from the labeled *n*-butylbenzene using the default bbiso parameter (30 V_{p-p}) produced a cluster of peaks at *m/z* 119, 120, 121, and 122 (Fig. 15). This complex pattern of fragmentation was consistent with loss of CD_3, CD_2H, CDH_2, and CH_3, respectively. This scrambling of the deuterium label was possibly due to hydrogen shifts and methyl migrations to an ion structure similar to that of *t*-butylbenzene. Although not conclusive, this result does confirm that the radical molecular cation (*m/z* 134) from *n*-butylbenzene upon irradiation by the bbiso waveform (20−400 kHz) rearranges to a *t*-butylbenzene-like ion that subsequently fragments during the CID activation to produce *m/z* 119.

4. Conclusion

It has been determined that the bbiso broadband isolation waveform activated the molecular ion of *n*-butylbenzene to rearrange to a *t*-butylbenzene-like analog under tandem conditions. Cooling the precursor prior to CID has no effect on the isomerization mechanism. When the amplitude of the broadband isolation waveform is decreased from 30–20 V, isomerization does not occur. The exact nature of the process activated by the bbiso has not been fully elucidated. However, the possibility exists that among the many secondary motions exhibited by an ion, one may have a frequency component that resonates at around 375 kHz. This secondary motion, when excited, could lead to a different type of collision process than the traditional translational collision process and stimulate the unexpected result observed in this work.

This study has several significant consequences. If the isomerization of precursor ions in the tandem experiment can be predicted and controlled through the application of waveforms, then the differentiation of structural isomers by mass spectrometry would be possible. Secondly, since the ion current observed from a tandem experiment was directed related to the voltage of the bbiso, control of this variable could lead to more sensitive analyses than currently available. The impact of the bbiso waveform on the sensitivity of trace analysis is currently under investigation.

Acknowledgments

The authors are grateful to the U.S. Department of Interior, Fish and Wildlife Service for financial support of TRC. The authors also thank Dr. Robert D. Brittain for his independent tandem analysis of *n*-butylbenzene and Varian Chromatography Systems for their hardware support. Finally, the authors appreciate the thoughtful council of Professor Raymond E. March regarding this and other ion trap problems.

References

[1] P. Brown, Org. Mass Spectrom. 3 (1970) 1175.
[2] E.S. Mukhtar, I.W. Griffiths, F.M. Harris, J.H. Beynon, Int. J. Mass Spectrom. Ion Processes 37 (1981) 159.
[3] I.W. Griffiths, F.M. Harris, E.S. Mukhtar, J.H. Beynon, Int. J. Mass Spectrom. Ion Process 41 (1981) 83.
[4] P.C. Burgers, J.K. Terlouw, K. Levsen, Org. Mass Spectrom. 17 (1982) 295.
[5] P.H. Dawson, W-F. Sun, Int. J. Mass Spectrom. Ion Processes 44 (1982) 51.
[6] A.G. Harrison, M.S. Lin, Int. J. Mass Spectrom. Ion Processes 51 (1983) 353.
[7] J.H. Chen, J.D. Hays, R.C. Dunbar, J. Phys. Chem. 88 (1984) 4759.
[8] S. Nacson, A.G. Harrison, Int. J. Mass Spectrom. Ion Processes 63 (1985) 325.
[9] M.J. Welch, D.J. Pereles, E. White V, Org. Mass Spectrom. 20 (1985) 426.
[10] J.N. Louris, R.G. Cooks, J.E.P. Syka, P.E. Kelley, G.C. Stafford Jr., J.F.J. Todd, Anal. Chem. 59 (1987) 1677.
[11] T. Baer, O. Dutuit, H. Mestdagh, C. Rolando, J. Phys. Chem. 92 (1988) 5674.
[12] C. Paradisi, J.F.J. Todd, U. Vettori, Org. Mass Spectrom. 27 (1992) 1210.
[13] P. Thibault, A.J. Alexander, R.K. Boyd, K.B. Tomer, J. Am. Soc. Mass Spectrom. 4 (1993) 845.
[14] J.B. Plomley, F.A. Londry, R.E. March, Rapid Commun. Mass Spectrom. 10 (1996) 200.
[15] P. Liere, R.E. March, T. Blasco, J.-C. Tabet, Int. J. Mass Spectrom. Ion Processes 153 (1996) 101.
[16] S.T. Oh, J.C. Choe, M.S. Kim, J. Phys. Chem. 100 (1996) 13 367.
[17] H. Freund, W.N. Olmstead, Int. J. Chem. Kinetics 21 (1989) 561.
[18] R.D. Brittain, Varian Walnut Creek, CA, personal communication, 1998.