Research Article

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Differentiation of cyclic tertiary amine cathinone derivatives by product ion electron ionization mass spectrometry

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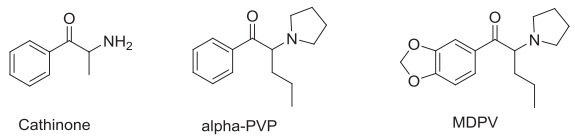
RATIONALE: A number of synthetic cathinones (aminoketones, 'bath salts') are tertiary amines containing a cyclic amino group, most commonly pyrrolidine. These totally synthetic compounds can be prepared in a number of regioisomeric designer modifications and many of these can yield isomeric major fragment ions in electron ionization mass spectrometry (EI-MS).
METHODS: A series of regioisomeric cyclic tertiary amines were prepared and evaluated in EI-MS and MS/MS product ion experiments. The cyclic amines azetidine, pyrrolidine, piperidine and azepane were incorporated into a series of aminoketones related to the cathinone derivative drug of abuse known as MDPV. Deuterium labeling in both the cyclic amine and alkyl side chain allowed for the confirmation of the structure for the major product ions formed from the EI-MS iminium cation base peaks.
RESULTS: These iminium cation base peaks show characteristic product ion spectra which allow differentiation of the ring and side-chain portions of the structure. The small alkyl side chains favor ring fragmentation in the formation of the major product ions. The higher side-chain homologues appear to promote product ion formation by side-chain fragmentation. Both side-chain and ring fragmentation yield a mixture of product ions in the piperidine and azepane series.
CONCLUSIONS: Product ion fragmentation provides useful data for differentiation of cyclic tertiary amine iminium cations from cathinone derivative drugs of abuse. Regioisomeric iminium cations of equal mass yield characteristic product ions for the alkyl side-chain homologues of azetidine, pyrrolidine, piperidine and azepane cyclic amines.

MDPV (3,4-methylenedioxypyrovalerone) is a synthetic stimulant drug with effects similar to cocaine and amphetamine.[1] It has been described as a derivative of cathinone,[2] a naturally occurring compound produced by the Khat plant native to the middle east and north Africa. MDPV can also be viewed as an aromatic ring substituted analogue of pyrovalerone, once a commercially available product in the United States and elsewhere.[3,4] A number of synthetic cathinones (aminoketones, 'bath salts') have appeared on the illicit drug market in recent years.[5–7] A variety of molecular modifications are possible for these amino ketones based on the structure of the basic cathinone molecule.



Aromatic ring substituents include bromine, chlorine, fluorine, methoxy, methyl and methylenedioxy groups arranged in varying regioisomeric patterns.[6] Alkyl side chains range from methyl to butyl and the amino groups include various small alkyl groups (methyl to butyl) as secondary or tertiary amines.[6] The tertiary amines include a number of compounds containing a cyclic amino group and drugs such as pyrovalerone, alpha-PVP (Flakka), MDPV and beta-naphyrone are in this category. The structures for cathinone, alpha-PVP and MDPV illustrate the general trends of designer modification among these compounds. Modification of the cathinone structure to extend the alkyl side chain to a propyl group and addition of the pyrrolidine group yields alpha-PVP (Flakka). Further aromatic ring alterations to yield the 3,4-methylenedioxy substituents produces the popular drug of abuse MDPV. Recent reports[8–10] indicate that designer style activity is continuing to yield new psychoactive substances in this chemical class. Legal control in some chemical classes of drugs of abuse has often provided the stimulus for clandestine development of additional designer molecules whose structures place them just outside the most recent legally established boundaries.

The stimulatory effects of the synthetic cathinones are the result of elevations in central nervous system (CNS) neurotransmitter levels for the various catecholamines.[11,12] MDPV is a potent and selective inhibitor of the monoamine reuptake transporter systems with highest affinity for the dopamine transporter and lowest affinity for the serotonin transporter.[13–15] The selectivity of MDPV for the dopamine transporter protein compared to norepinephrine and serotonin transporters is similar to the profile described for methamphetamine and cocaine.[14,16]

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The potential for structural modification within the cathinone chemical framework is quite vast and a large number of precursor substances are commercially available. These synthetic designer substances present unique challenges in forensic analysis compared to those natural product derived drugs (THC, cocaine, etc.) synthesized by a plant in an enzymatically controlled (isomeric specific) process.

Chemical derivatization procedures often yield compounds with altered or unique fragmentation pathways in mass spectrometry as well as improved chromatographic properties. Acylation of primary and secondary amines yields amides with decreased nitrogen basicity and this reduction in electron availability on nitrogen can yield different mass spectral fragmentation pathways.[17] These different fragment ions often provide additional structural information about the parent primary or secondary amine. The perfluoroacyl derivatives[17] can provide the best results among the common acylation products yielding unique fragment ions for clear structural information. Tertiary amines do not form stable acylation products in most cases and therefore the identification of regioisomeric molecules via mass spectral fragmentation patterns is a greater analytical challenge. A gas chromatography/mass spectrometry (GC/MS) method has been described for separation and differentiation of the 3,4- and the 2,3-isomers of MDPV and the infrared (IR) spectra of the 2,3-isomers were also compared with the corresponding 3,4-isomers.[18] Mixtures of closely related cathinones have been evaluated by standard ion mobility spectrometry (IMS) and this technique compared to electrospray ionization high-performance IMS methods.[19] Multiple reaction monitoring techniques in reversed-phase gradient elution liquid chromatography/tandem mass spectrometry (LC/MS/MS) have been used to screen a number of cathinone derivatives including mephedrone, butylone, MDPV, flephedrone, methylone and methedrone in forensic case samples.[20] The fragmentation properties of the cathinone derivatives have been compared by GC/EI-MS and LC/ESI-QTOFMS methods.[21] The precursor ions of these substances are hard to obtain by EI-MS, whereas the protonated molecular ions can be observed clearly by ESI-QTOFMS. Furthermore, two major characteristic cleavages are produced in the EI mode, leading to the formation of iminium and acyl ions. Secondary and tertiary fragmentations of these ions were reported to assist in molecular structure identification.[21] In the case of ESI-QTOFMS, characteristic fragments are produced via loss of water and other fragmentation pathways not observed in EI-MS. Product ion mass spectra can often provide structural information on regioisomeric compounds and this is especially useful for compounds yielding equivalent regioisomeric fragment ions. This report describes the use of product ion spectra in the analysis of regioisomeric iminium cations generated in EI-MS for a series of cathinone-type tertiary amines.

EXPERIMENTAL

Instrumentation

GC/MS System 1 consisted of a model 7890A gas chromatograph and a 7683B autoinjector coupled with a 5975C VL mass-selective detector (all from Agilent Technologies, Santa Clara, CA, USA). The mass spectral scan rate was 2.86 scans/s. The GC system was operated in splitless mode with a helium (grade 5) flow rate of 0.7 mL/min. The mass spectrometer was operated in the electron ionization (EI) mode with an ionization voltage of 70 eV and a source temperature of 230°C. The GC injector was maintained at 250°C and the transfer line at 280°C. The GC/MS analysis for the amino ketones was carried out on a column (30 m × 0.25 mm i.d.) coated with 0.10 µm film of Crossbond® 5% diphenyl, 95% dimethyl polysiloxane (Rtx-5) purchased from Restek Corporation (Bellefonte, PA, USA). The temperature program consisted of an initial hold at 70°C for 1.0 min, ramped up to 250°C at a rate of 30°C/min followed by a hold at 250°C for 15 min.

GC/MS System 2 consisted of a model 7890A gas chromatograph and a 7683B autoinjector coupled with a model 240 ion trap mass spectrometer (all from Agilent Technologies). The mass spectral scan rate was 2.86 scans/s. The GC system was operated in splitless mode with a helium (grade 5) flow rate of 0.7 mL/min and the column head pressure was 10 psi. The mass spectrometer was operated in the electron impact (EI) mode using an ionization voltage of 70 eV and a source temperature of 230°C. The MS/MS excitation amplitudes ranged from 0.2 to 1.6 V. The GC injector was maintained at 250°C and the transfer line at 280°C. The GC studies were performed on a column (30 m × 0.25 mm i.d.) coated with 0.5 µm 100% trifluoropropyl methyl polysiloxane (Rtx-200) purchased from Restek Corporation. Chromatographic analysis was done using the temperature program described for System 1 above. Samples for analysis were dissolved and diluted in high-performance liquid chromatography (HPLC)-grade acetonitrile (Fisher Scientific, Fairlawn, NJ, USA) and introduced via the autoinjector using an injection volume of 1 µL.

Synthetic methods

Precursor materials including piperonal, azetidine, pyrrolidine, piperidine, hexamethyleneimine (hexahydro-1H-azepine) and various n-alkylmagnesium halides were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) or Alfa Aesar Chemical Co. (Ward Hill, MA, USA). Samples of 2,2,3,3,4,4,5,5-pyrrolidine-D_8, 2,2,5,5-pyrrolidine-D_4, piperadine-D_{10}, and deuterated bromoalkanes were purchased from CDN Isotopes (Pointe Claire, Quebec, Canada). The synthetic methods needed to prepare the various isomeric and homologous aminoketones in this study are well established in the chemical literature and in our laboratory. The procedures used in this project were those reported by Kavanagh *et al.*[18] These desired compounds were prepared from the substituted benzaldehydes via a four-step synthetic procedure. The condensation of alkylmagnesium halides (Grignard reagents) with piperonal (3,4-methylenedioxybenzaldehyde) yields the corresponding methylenedioxybenzyl alcohols. Oxidation of these benzyl alcohols with potassium dichromate yields the methylenedioxyalkylphenones. Alpha-bromination of the ketones at the activated methylene carbon gives the alpha-bromoketones and subsequent displacement of the bromide ion by the nitrogen of the individual secondary amines yields the desired

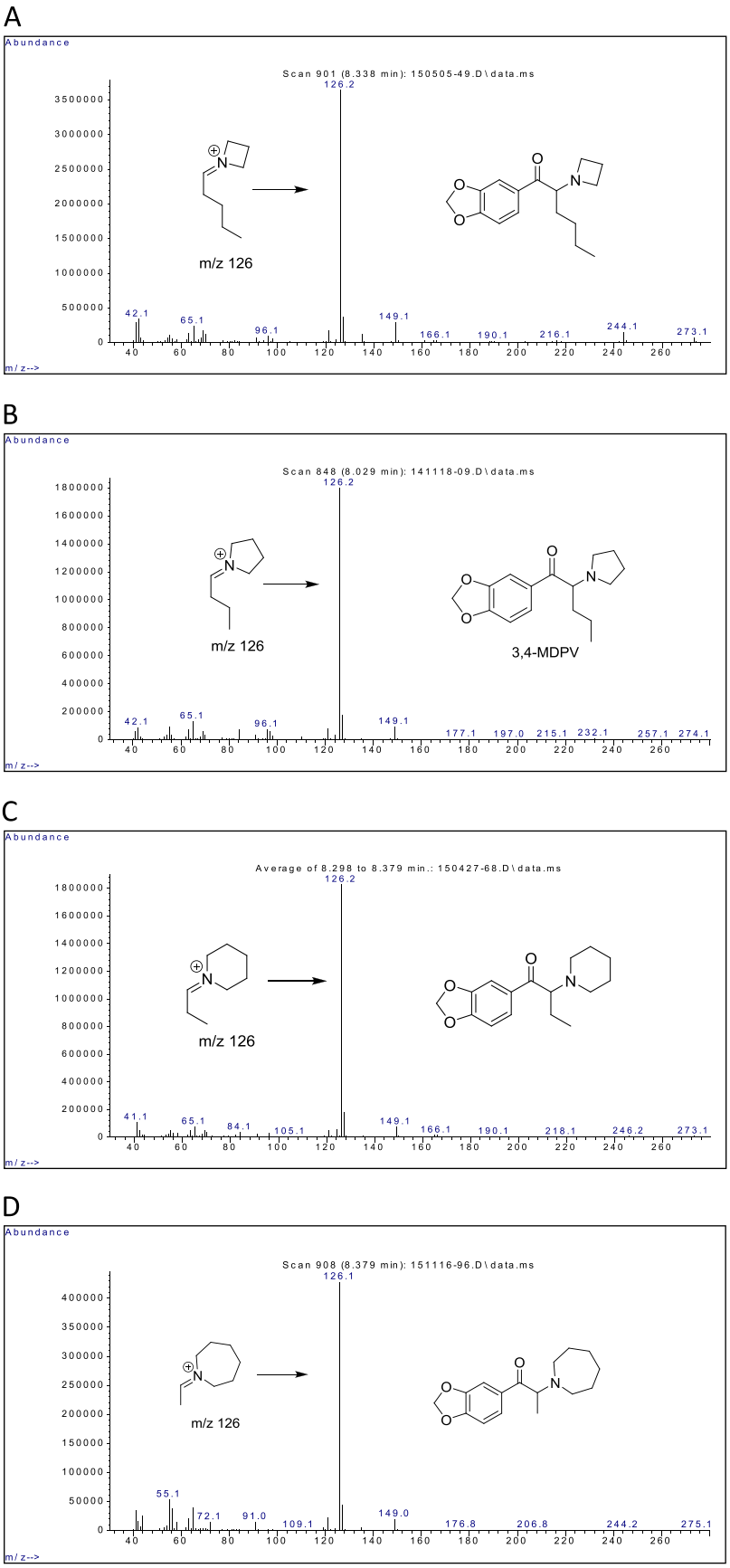

Figure 1. (A–D) EI-MS spectra for the four regioisomeric cathinone derivatives of MW = 275 and regioisomeric base peak iminium cations at *m/z* 126.

aminoketone final products. Compounds were purified using flash chromatography and in some cases preparative thin-layer chromatography (TLC).

RESULTS AND DISCUSSION

The full electron ionization (EI) mass spectra for the series of varying ring size regioisomeric amines are shown in Fig. 1. The length of the alkyl side chain varies in each of the compounds in order to yield the mass equivalent regioisomeric iminium cation species at m/z 126, the base peak for the cathinone derivative MDPV. These iminium cations are the base peaks and the only peaks of significant relative abundance in each of the four spectra shown in Fig. 1. These fragments are generated by the loss of the 3,4-methylenedioxybenzoyl radical (149 Da) from each of the molecular ions. Each of the four compounds whose spectra are shown in Fig. 1 has equivalent molecular ions at m/z 275 for the same elemental composition, $C_{16}H_{21}NO_3$. Differentiation of these compounds by GC/MS alone would be based primarily on chromatographic retention characteristics since these four compounds also yield regioisomeric major fragment ions of identical elemental composition. Such a chromatographic identification would require the availability of a number of reference samples for retention time comparisons.

In this study the MS/MS product ion spectra of the iminium cation base peaks for a variety of tertiary amines were compared in an effort to characterize the structure-fragmentation relationships in these molecules of varying ring size and alkyl side chains. This project compared the product ion MS/MS spectra of the iminium cations generated from amines containing cyclic fully saturated four-, five-, six- and seven-membered rings. The MS/MS spectra for each of the m/z 126 iminium cations from Fig. 1 are shown in Fig. 2 and each of the regioisomeric cations yield a unique product ion. These product ions provide an additional level of mass spectral differentiation for compounds containing these regioisomeric cyclic tertiary amines. The structures for a number of the cyclic tertiary aminoketones as well as their base peaks and major product ions are shown in Table 1 and the individual mass spectra are presented in the Supporting Information. The full EI-MS scans were done on System 1 in this study and the product ion spectra on System 2 in order to have the results confirmed on two different instruments.

The product ion MS/MS spectrum for the iminium cation formed from the cyclic four-membered ring (azetidine) containing cathinone derivative is shown in Fig. 2(A). The m/z 126 fragment yields the m/z 70 product ion as the only major fragment and this represents the loss of 56 Da from the EI-MS base peak iminium cation. The alkyl side chain for the azetidine-containing iminium cation at m/z 126 is composed of a C_4H_9 moiety and the major product ion fragment at m/z 70 would suggest a hydrogen rearrangement in the alkyl side chain with the loss of a butene molecular fragment, C_4H_8. A series of azetidine-containing cathinone derivatives of varying alkyl side-chain length were prepared to evaluate the process of product ion formation in this group of compounds. All the alkyl group side chains yield the m/z 70 product ion whenever a four-centered H-migration to the carbon atom of the iminium species is possible. The migration

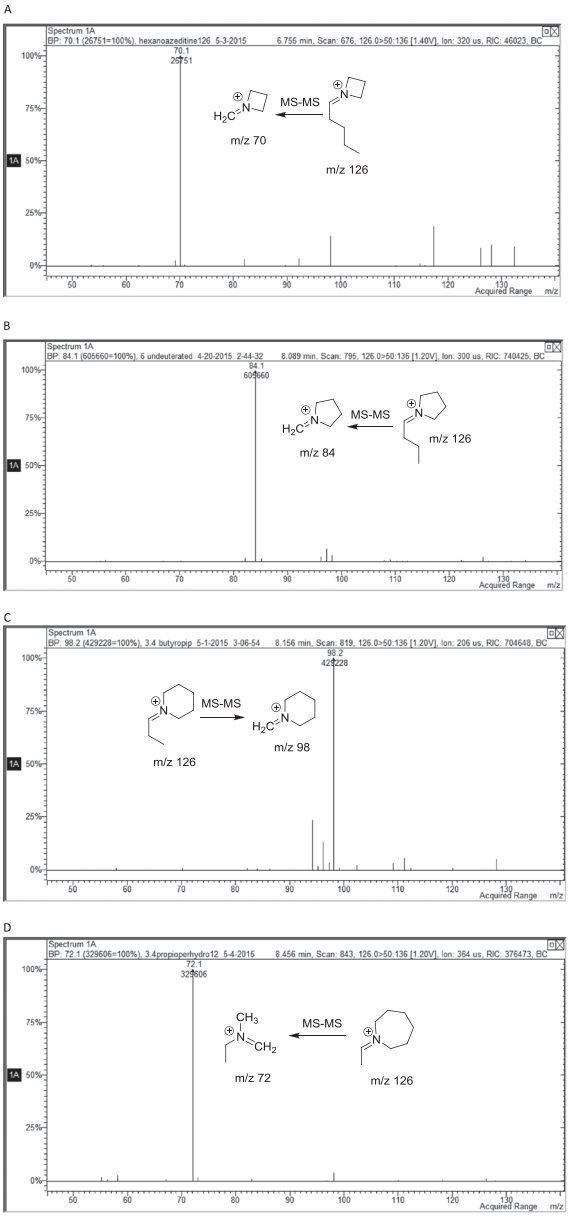

Figure 2. (A–D) MS/MS product ion spectra for the four regioisomeric m/z 126 base peak iminium cations.

of the hydrogen from the side chain was confirmed via deuterium labeling in the alkyl side chain. The full EI mass spectral scan of the propyl side chain analogue shows a base peak at m/z 112 and the major product ion for the m/z 112 base peak appears at m/z 70. The spectra for the corresponding D_8-labeled propyl side-chain analogue show the base peak m/z 120 and the major product ion occurs at m/z 72 (Table 1). The product ion spectrum for the m/z 120 ion shows the major fragment at m/z 72 indicating the addition of two deuterium atoms into this major MS/MS fragment. The results of these experiments support the proposed structure for the m/z 70 product ion shown in Table 1 and the formation of this ion via hydrogen rearrangement from the alkyl side chain. This m/z 70 fragment is the common ion observed for all the azetidine series compounds as long as a four-centered hydrogen rearrangement process is available in the side chain. In the

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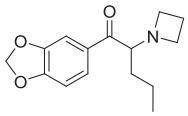
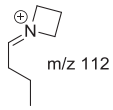
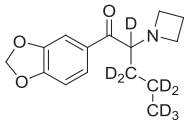
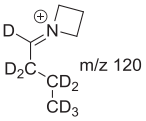
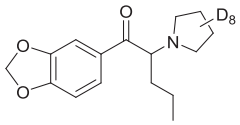
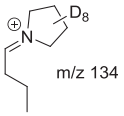
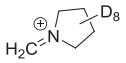
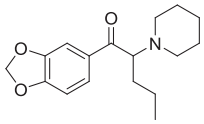
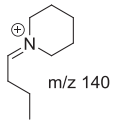
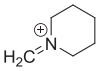
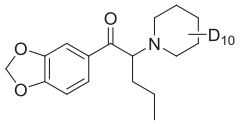
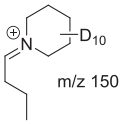
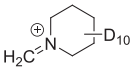
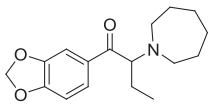
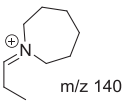
Table 1. Structures for a series of tertiary amino ketones and their EI-MS fragment ions

Parent structure	Iminium cation	Major product ion
	 m/z 112	 m/z 70
	 m/z 120	 m/z 72
	 m/z 134	 m/z 92
	 m/z 140	 m/z 98
	 m/z 150	 m/z 108
	 m/z 140	 m/z 86

case of the methyl side chain a four-centered migration is not possible and no product ion of significance was observed for the iminium cation base peaks for this homologue.

The product ion MS/MS spectrum for the iminium cation (m/z 126) formed from the cyclic five-membered ring amine (pyrrolidine)-containing cathinone derivative is shown in Fig. 2(B). The cathinone derivatives containing a five-membered cyclic pyrrolidine ring were compared by evaluating alkyl side-chain homologues as well as some deuterium-labeled pyrrolidine ring analogues. The alkyl side chains including methyl, ethyl and propyl as well as D_8-pyrrolidine and 2,2,5,5-D_4-pyrrolidine analogues were evaluated in this study. Figure 3 shows a series of mass spectra which serve to describe the results seen for the methyl side-chain pyrrolidine derivative. The major product ion is formed via fragmentation of the pyrrolidine ring itself to eliminate 42 Da from the EI-MS iminium cation base peak. The full scan EI-MS spectrum for the unlabeled compound in Fig. 3(A) shows the base peak for the iminium cation at m/z 98 and Fig. 3(B) shows the corresponding product ion scan with the major fragment at m/z 56. The 2,2,5,5-D_4-pyrrolidine analogue in Fig. 3(C) indicates the 4 Da mass shift for the base peak at m/z 102 as expected and Fig. 3(D) shows the m/z 58 product ion occurring from the m/z 102 iminium cation. This 2 Da mass shift observed in the m/z 58 product ion indicates that one methylene from the pyrrolidine ring remains a part of the structure for this major MS/MS product ion. This spectrum further identifies the one remaining methylene from the pyrrolidine ring as that from the 2 or 5 carbon of the ring, the carbons directly bonded to the nitrogen atom. The proposed mechanism for the formation of the product ion is shown in Fig. 3(D). The elimination of the side chain in a four-centered hydrogen rearrangement analogous to the process observed for the azetidine series occurs for the higher side-chain homologues ethyl and n-propyl groups.

The structures for the base peak in the EI-MS and product ion spectra for the D_8-pyrrolidine-containing aminoketone with the n-propyl side chain, D_8-MDPV are shown in Table 1. The EI-MS and product ion spectra for the unlabeled form of this compound are in Figs. 1(B) and 2(B), respectively. The full scan EI-MS spectrum for D_8-MDPV shows the molecular ion and the base peak (m/z 134) with an 8 Da increase in mass as expected. Furthermore, the major product ion (m/z 92) shows an 8 Da mass increase compared to the spectrum for the unlabeled analogue (Table 1). These results confirm the pyrrolidine ring remains a part of the major product ion fragment and that no significant ring fragmentation product ion is formed for this compound. In the ethyl side-chain pyrrolidine homologue (spectrum not shown) both ring (m/z 70) and side-chain (m/z 84) product ions were observed in approximately equal ion intensities.

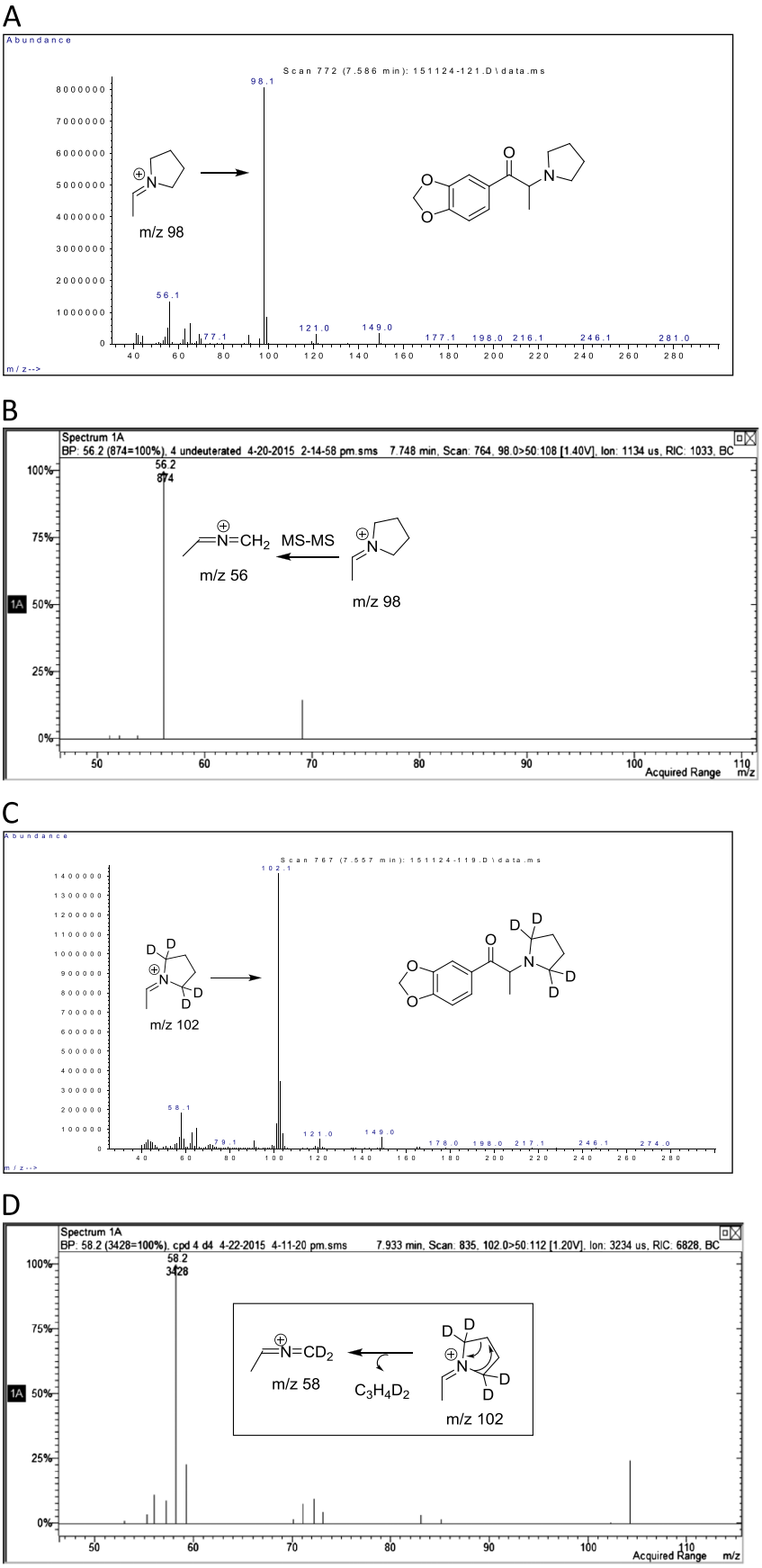


Figure 3. (A–D) EI-MS and product ion spectra for the methyl side-chain pyrrolidine isomer and the 2,2,5,5-D_4-pyrrolidine analogue.

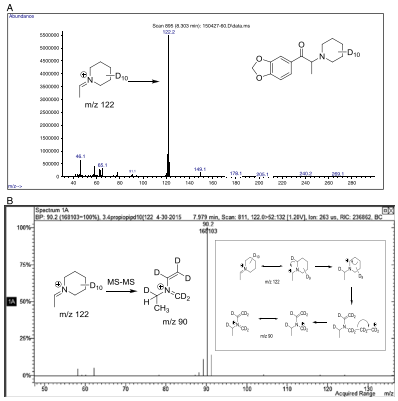


Figure 4. (A, B) EI-MS and product ion spectra for the methyl group side chain D_{10}-piperidine derivative.

The product ion MS/MS spectrum for the iminium cation (m/z 126) formed from the cyclic six-membered ring amine (piperidine)-containing cathinone derivative is shown in Fig. 2(C). The piperidine-containing compounds were evaluated via the synthesis of a series of alkyl side-chain derivatives as well as D_{10}-piperidine analogues. In this piperidine series, the alkyl side-chain homologues yield a common MS/MS product ion at m/z 98 as long as the side chain based four-centered H-migration to the carbon atom of the iminium species is possible. The structures for the full scan EI-MS base peak and major product ion for the piperidine containing n-propyl side-chain derivative and the D_{10}-piperidine analogue are shown in Table 1. The major product ion appears at m/z 98 and in the D_{10}-piperidine analogue a mass shift of 10 Da occurs as shown for the m/z 108 product ion. This mass shift to m/z 108 in the product ion spectrum confirms that the piperidine ring remains a part of the product ion and the side chain is the source of the additional fragmentation to form the observed major MS/MS fragment.

While the four-centered H-migration of the side chain yields the common m/z 98 product ion for the ethyl and propyl side chains in the piperidine series as described above, the methyl side-chain analogue undergoes ring fragmentation. Since the four-centered H-migration is not possible in the methyl side chain, an analogous migration

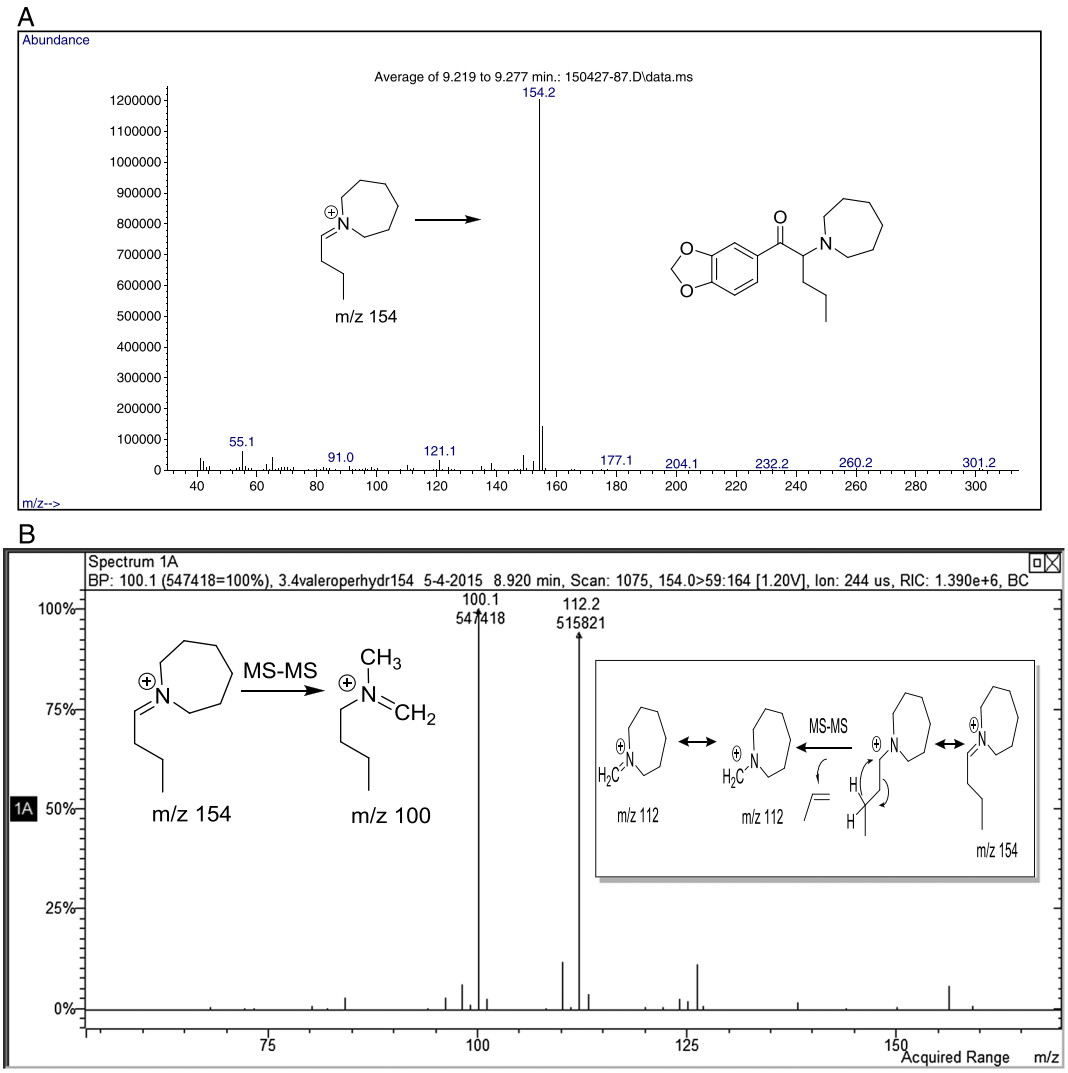


Figure 5. (A, B) EI-MS and product ion spectra for the propyl side chain isomer for the azepane series.

appears to initiate piperidine ring fragmentation for the methyl side-chain analogue. The EI-MS spectrum for the methyl side-chain analogue in the piperidine series shows a base peak at m/z 112 with the major product ion at m/z 84 (spectra not shown). Figure 4(A) shows the full scan EI-MS spectrum for the methyl side-chain D_{10}-piperidine analogue with the base peak at m/z 122 while Fig. 4(B) illustrates the product ion spectrum for the m/z 122 iminium cation showing a major fragment at m/z 90, only a 6 Da mass shift from the equivalent product ion in the unlabeled compound. The fragmentation scheme shown within Fig. 4(B) indicates the alternate four-centered H-migration which appears to operate in this methyl side-chain example. The ethyl and propyl homologues which allow the four-centered H-migration in the alkyl side chain do not show any appreciable fragments from this potentially competing H-migration from the piperidine ring. However, in the next higher ring homologue series containing the seven-membered azepane ring both the ring and side-chain H-migration processes appear to operate perhaps in competition in some compounds.

The azepane-containing compounds were evaluated via the synthesis of a series of alkyl side-chain derivatives as well as deuterium-labeled alkyl side-chain analogues. The product ion MS/MS spectrum for the iminium cation (m/z 126) formed from the cyclic seven-membered amine (azepane)-containing cathinone derivative is shown in Fig. 2 (D). The m/z 72 product ion (Fig. 2(D)) is produced from the m/z 126 base peak which consists of the azepane ring with the methyl group side chain. The homologous ethyl side chain shows a homologous product ion at m/z 86 resulting from the iminium cation base peak at m/z 140 (Table 1). These two product ions at m/z 72 and 86 represent the loss of a consistent 54 Da from the iminium cation base peaks. The most likely source for this loss would be C_4H_6, butadiene, which could only come from the azepane ring in these small side-chain homologues (methyl and ethyl).

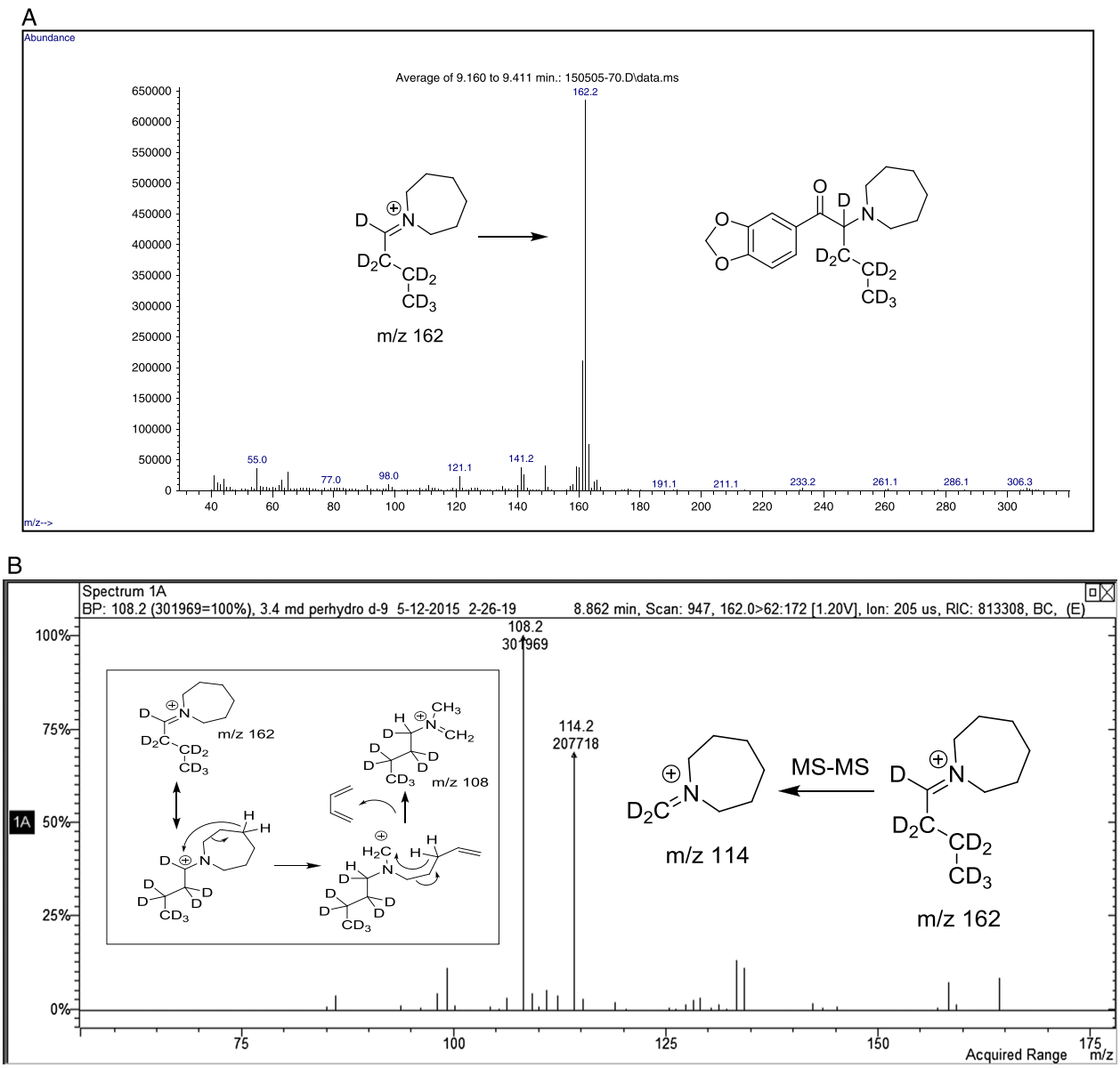


Figure 6. (A, B) EI-MS and product ion spectra for the D_8-labeled side chain for the azepane series.

The full scan EI-MS spectrum as well as the product ion spectrum for the next higher homologue, the propyl side chain, are shown in Figs. 5(A) and 5(B). The base peak in the full scan in Fig. 5(A) occurs at m/z 154 as expected from the loss of the 3,4-methylenedioxybenzoyl radical from the molecular ion. The product ion spectrum in this example (Fig. 5(B)) shows major fragments at both m/z 100 and 112. The m/z 100 product ion again represents the loss of 54 Da from the base peak while the m/z 112 ion represents the loss of 42 Da from the m/z 154 base peak. These results suggest that both side-chain and ring fragmentation are involved in the formation of these product ions. The full scan and product ion spectra for the deuterium-labeled propyl side-chain analogue are shown in Figs. 6(A) and 6(B), respectively. The base peak in Fig. 6(A) occurs at m/z 162 which represents an 8 Da shift as expected for the major iminium cation. The product ion spectrum in Fig. 6(B) indicates major fragments at m/z 108 and 114. A direct comparison of the fragment ions in Figs. 5(B) and 6(B) shows a mass shift of the m/z 100 ion to m/z 108 in the side chain labeled analogue indicating the side chain remains a part of this ion and the fragmentation occurs in the cyclic seven-membered azepane ring system. Furthermore, the 2 Da mass shift for the second major product ion from m/z 112 to m/z 114 indicates fragmentation of the side chain to eliminate the propene molecular equivalent. The next higher side-chain homologue (spectra not shown) in this azepane series having the butyl side chain shows an analogous set of ions for both side-chain and ring fragmentation of the m/z 168 base peak iminium cation. Thus, in the seven-membered cyclic tertiary amines both side-chain and ring fragmentation yield a mixture of product ions in the higher side-chain homologues while only ring fragmentation occurs in the methyl and ethyl side chains.

In summary, many of the more popular cathinone derivative drugs of abuse contain cyclic tertiary amine moieties. In most cases the cyclic amine is the five-membered pyrrolidine ring; however, other cyclic amines may appear as additional designer modifications. Product ion spectra provide useful structural information in these cyclic tertiary amines that do not form stable acylated derivatives. In this study the product ion structures allow for differentiation of the regioisomeric m/z 126 iminium cation base peaks for the cyclic tertiary amines related to the cathinone derivative MDPV. In the four-membered ring azetidine, the five-membered ring pyrrolidine as well as the six-membered ring piperidine isomers the major product ion forms via side-chain fragmentation. The seven-membered ring azepane shows ring fragmentation to yield the major product ion from the m/z 126 iminium cation base peak.

CONCLUSIONS

Product ion fragmentation of iminium cations provides useful data for differentiation of regioisomeric cyclic tertiary amines. The cyclic amines azetidine, pyrrolidine, piperidine and azepane were incorporated into a series of aminoketones related to the cathinone-type drugs of abuse. Variations in ring size and hydrocarbon side-chain length yield regioisomeric aminoketones having equivalent regioisomeric iminium cations. These iminium cation base peaks show characteristic product ion spectra which allow differentiation of the ring and side-chain portions of the structure. The azetidine series compounds all yield a common product ion at m/z 70 as long as a four-centered hydrogen-rearrangement process is available in the side chain. No significant product ion was formed for the azetidine-containing iminium cation for the methyl side-chain homologue since the analogous four-centered hydrogen migration is not possible in this compound. Product ions in the pyrrolidine series are formed via ring and side-chain fragmentation with side-chain fragmentation dominating in the higher side-chain homologues. The piperidine-containing amines undergo H-migration of the side chain to yield a common m/z 98 product ion for the ethyl and propyl side chains while the methyl side-chain homologue undergoes ring fragmentation. Both side-chain and ring fragmentation yield a mixture of product ions in the higher side-chain homologues for the seven-membered cyclic azepane tertiary amines and ring fragmentation occurs in the smaller alkyl side chains. Ring fragmentation in the pyrrolidine series results in the loss of 42 Da (C_3H_6) from the iminium cation base peak, 28 Da (C_2H_4) for the piperidine series and 54 Da (C_4H_6) for the azepane series.

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