GC and Mass Spectral Studies on Acylated Side Chain Regioisomers of 3-Methoxy-4-methyl-phenethylamine and 4-Methoxy-3-methyl-phenethylamine

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Abstract

The side chain regioiomers of the 3-methoxy-4-methylphenethylamines and 4-methoxy-3-methyl-phenethylamines have mass spectra essentially equivalent to the controlled drug substance 3,4-methylenedioxymethamphetamine (3,4-MDMA), all have molecular weight of 193 and major fragment ions in their electron ionization mass spectra at *m/z* 58 and 135/136. Furthermore, the compounds in this study have ring substitutions in the same relative positions as 3,4-MDMA. The nonequivalence of the substituents (methoxy and methyl) yields two sets of compounds, 3-methoxy-4-methyl- and 4-methoxy-3-methylphenethylamines. The perfluoroacyl derivatives (pentafluoropropionylamides and heptafluorobutrylamides) of the primary and secondary regioisomeric amines were prepared and evaluated in gas chromatography–mass spectrometry studies. The mass spectra for these derivatives are significantly individualized and the resulting unique fragment ions allow for specific side chain identification. The heptafluorobutrylamide derivatives offer more fragment ions than the pentafluoropropionylamides for molecular individualization among these regioisomeric substances. These acylated derivatives show excellent resolution on a dimethyl polysiloxane stationary phase such as Rtx-1.

Introduction

Early studies (1–4) in this series have shown the ten direct regioisomeric substances, 3,4-methylenedioxymethamphetamine (3,4-MDMA, Ecstasy) and nine regioisomeric equivalents, have identical molecular weights and mass spectral fragments of equivalent mass-to-charge ratios. This unique set of substances is made up of five regioisomeric side chains and two ring substitution patterns (2,3- and 3,4-), yielding a total of ten compounds. Analysis of these regioisomers by electron ionization mass spectrometry does not provide data for the specific differentiation and identification of one of these regioisomers (specifically the drug of abuse, 3,4-MDMA) to the exclusion of all the other isomers. All ten compounds of MW = 193 show major fragment ions for the imine at *m/z* 58, and the substituted benzyl fragment at *m/z* 135/136. Further studies have demonstrated that some of these compounds have very similar gas chromatographic (GC) retention properties (1).

All ten of the direct regioisomeric substances can be resolved using some of the more polar GC stationary phases and specific temperature programming conditions (2). Background information on these ten regioisomeric substances as well as their individual mass spectra and chromatographic properties can be found in the literature (1,2). Recent work has shown (4) the perfluoroacyl derivatives of the eight primary and secondary amines provide unique mass spectral fragment ions to differentiate among the side chain substitution patterns for the direct regioisomers of 3,4-MDMA. The preparation and analytical evaluation of the ring substituted methoxy methyl methamphetamines, a series of isobaric compounds (identical mass but different elemental composition) related to 3,4-MDMA, has been described (5). The ten methoxy methyl methamphetamines were compared to 2,3- and 3,4-MDMA; all 12 of these compounds have the same side chain structure generating the same structure for the *m/z* 58 ion, the base peak in the electron ionization mass spectrum for these amines. Mass spectral differentiation of 3,4-MDMA from some of the methoxy methyl methamphetamines was possible after formation of the perfluoroacyl derivatives. GC separation on non-polar stationary phases successfully resolved subsets of the methoxy methyl methamphetamines, based on ring position of the methoxy group, from 2,3- and 3,4-MDMA as the perfluoroacyl derivatives.

Combination of the five possible side chains and the ten different ring methoxy methyl substitution patterns yields fifty isomeric compounds all of MW = 193 and mass spectra of probable equivalence to 3,4 MDMA. This report describes the MS and GC behavior of a set of selected compounds having nonequivalent ring substituents at the 3- and 4-position of the aromatic ring. Thus, these ten compounds represent all the possible regioisomeric methoxy methyl phenethylamines having

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the same 3,4 substitution pattern as 3,4-MDMA. The overall goal of our current efforts is to evaluate an entire side chain series for at least one methyl group ring substitution pattern for each of the three methoxy group substitutions 2-, 3-, and 4- relative to the alkylamine side chain (5–7). Differentiation of regioisomers and isobaric substances is a significant issue in forensic drug chemistry and has been addressed in a number of drug categories (5–13).

Experimental

Analytical

Analytical studies were conducted using an Agilent Technologies (Santa Clara, CA) 7890A GC and an Agilent 7683B auto injector coupled with a 5975C VL Agilent mass selective detector. The mass spectral scan rate was 2.86 scans/s. The GC was operated in splitless mode with a carrier gas (helium grade 5) flow rate was 0.7 mL/min and a column head pressure of 10 psi.

The mass spectrometer was operated on the electron impact (EI) mode using 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 mass spectra reported were obtained by background subtraction and are the average of at least five scans. Samples were diluted in HPLC-grade acetonitrile (Fisher Scientific, Fairlawn NJ) and introduced via the auto injector as individual solutions and in a physical mixture.

The chromatographic separations (and collection of retention data) were carried out on a 30 m × 0.25 mm-i.d. fused silica column coated with 0.25 µm 100% dimethyl polysiloxane (Rtx-1) purchased from Restek corporation (Bellefonte, PA).

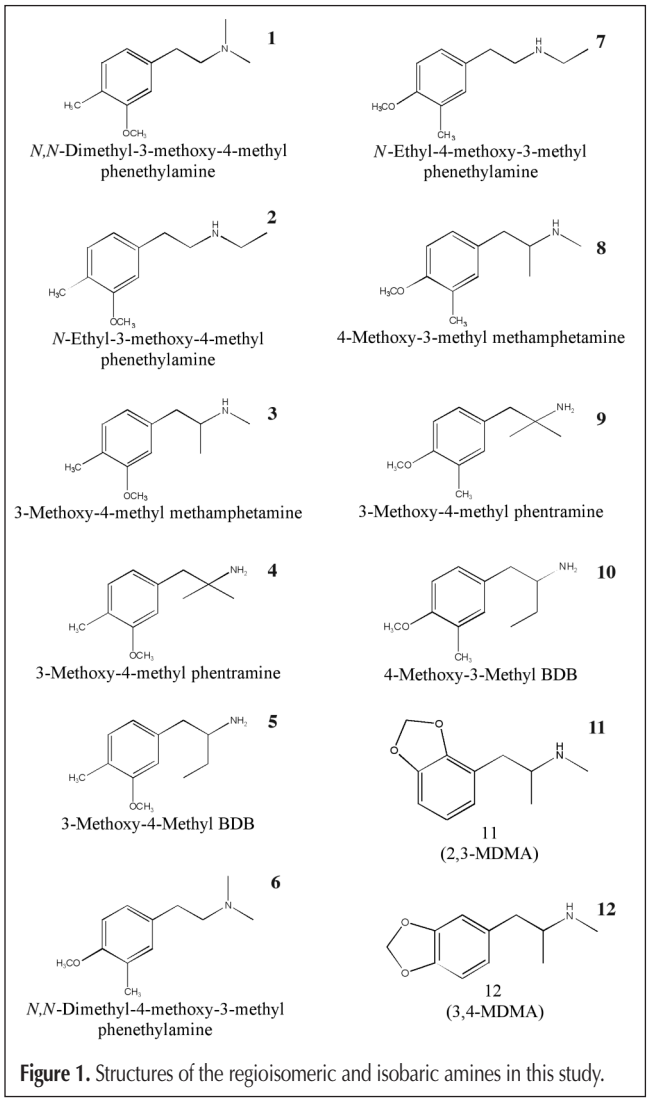

Figure 1. Structures of the regioisomeric and isobaric amines in this study.

Drugs and reagents

All laboratory reagents and chemicals were obtained either from Aldrich Chemical Company (Milwaukee, WI), TCI America (Portland, OR), or Fisher Scientific (Atlanta, GA). Pentafluoropropionic anhydride and heptafluorobutyric anhydride were purchased from UCT (Bristol, PA).

Samples of 2,3- and 3,4-MDMA and the other regioisomeric amines described in this study were synthesized as described in previous publications from this laboratory (1). The synthetic procedures all used the corresponding ring substituted benzaldehydes as the starting precursor substance.

Derivatization procedure

Each perfluoroacylamide was prepared individually from the hydrochloride salts of the regioisomers by dissolving approximately 0.3 mg (1.33×10^{-5} mole) of each amine in 50 µL of ethyl acetate followed by addition of large excess (250 µL) of the appropriate derivatizing agent (pentafluoropropionic anhydride or heptafluorobutyric anhydride) and the derivatization reaction mixtures were incubated in capped tubes at 70°C for 20 min. Following incubation, each sample was evaporated to dryness under a stream of dry air at 55°C and reconstituted with 200 µL of ethyl acetate and 50 µL of pyridine. A portion of the final solutions (50 µL) were diluted with HPLC grade acetonitrile (200 µL) to give the working solutions.

Synthesis

The methods for the preparation of the ten 2,3- and 3,4-methylenedioxy-regioisomers have been described in previous reports (1,3). 3-Methoxy-4-methylbenzaldehyde was synthesized from commercially available methyl-3-methoxy-4-methyl benzoate via RedAl reduction to the corresponding alcohol followed by selective oxidation of the resulting alcohol using pyridinium chlorochromate and celite. 4-Methoxy-3-methyl benzaldehyde is commercially available. Condensation of the ring substituted methoxy methyl benzaldehydes with a nitroalkane (nitro-methane, nitroethane, or 1-nitropropane) under basic conditions yields the corresponding 1-(methoxy-methyl phenyl)-2- nitroalkene, which upon reduction with lithium aluminum hydride (LAH) yields the primary amines. The *N*-methyl and *N*-ethyl analogues were prepared from the primary amines by acylation followed by LAH reduction. Alternately, the nitroalkenes are hydrolyzed to the corresponding ring substituted methoxy methyl phenyl ketones and reductively aminated with methyl-, dimethyl-, or ethylamine in the presence of sodium cyanoborohydride. The 1-(methoxy-methyl-phenyl)-2,2-dimethylethanamine was prepared from the

corresponding benzaldehyde via conversion to the corresponding benzylchloride and condensation with isobutyric acid. The resulting 2,2-dimethyl-3-(methoxy methyl phenyl)-1-propionic acid was treated sequentially with sodium azide, ethyl chloroformate, and benzyl alcohol followed by catalytic hydrogenation under low pressure to yield the desired 1-(methoxy-methyl-phenyl)-2,2-dimethylethanamines (1).

Results and Discussion

MS

Mass spectrometry (MS) is the primary method for confirming the identity of drugs and related substances in forensic samples. The mass spectra of phenethylamines are characterized by a base peak formed from an amine initiated alpha-cleavage reaction involving the carbon–carbon bond of the ethyl linkage between the aromatic ring and the amine. In 3,4-MDMA (MW = 193), the alpha-cleavage reaction yields the substituted imine fragment at *m/z* 58 and the 3,4-methylenedioxybenzyl fragment at mass 135/136 (for the cation and the radical cation, respectively). Thus, the mass spectrum for 3,4-MDMA contains major ions at *m/z* 58 and 135/136 as well as other ions of low relative abundance (1).

The side chain regioisomers of 3-methoxy-4-methyl phenethylamine and 4-methoxy-3-methyl phenethylamine (Compounds 1–10, Figure 1) have the potential to yield a mass spectrum essentially equivalent to 3,4-MDMA. All have molecular weight of 193 and major fragment ions in their electron ionization mass spectra at *m/z* 58 and 135/136 (Figure 2). The individual mass spectra for 2,3- and 3,4-MDMA are also presented in Figure 2 (Compounds 11 and 12). The isobaric methoxy-methyl-benzyl $(C_9H_{11}O)^+$ fragments have the same mass as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at *m/z* 135. Furthermore, the *m/z* 58 ion in the ring substituted methoxy-methyl phenethylamine is regioisomeric with that obtained in the mass spectra of both 2,3- and 3,4-MDMA (Figure 3). This lack of mass spectral specificity for the isomers shown in Figure 2, in addition to the possibility of chromatographic co-elution with 3,4-MDMA, could result in misidentification in this series of drugs and drug-like substances.

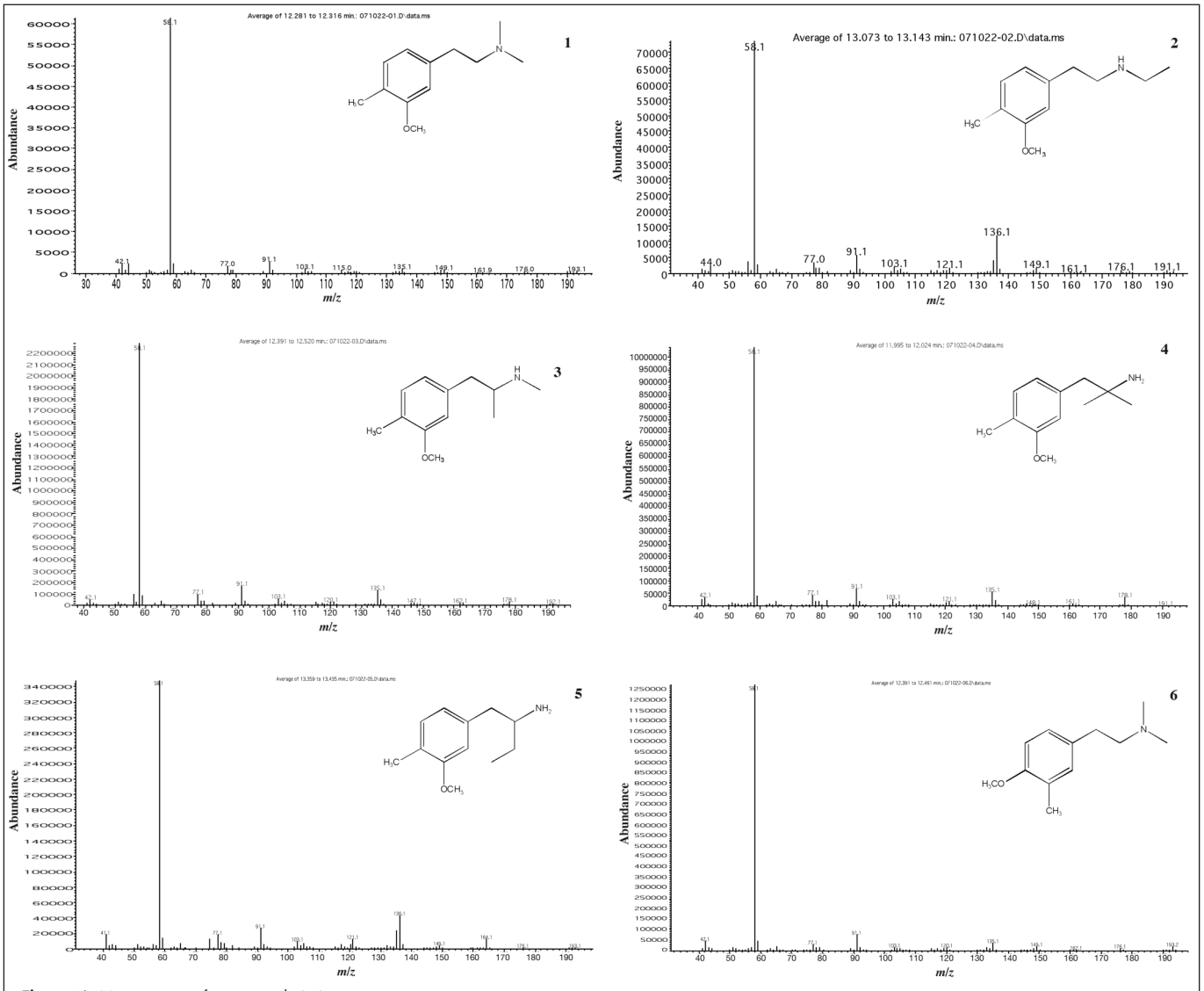

Figure 2A. Mass spectra of compounds 1–12.

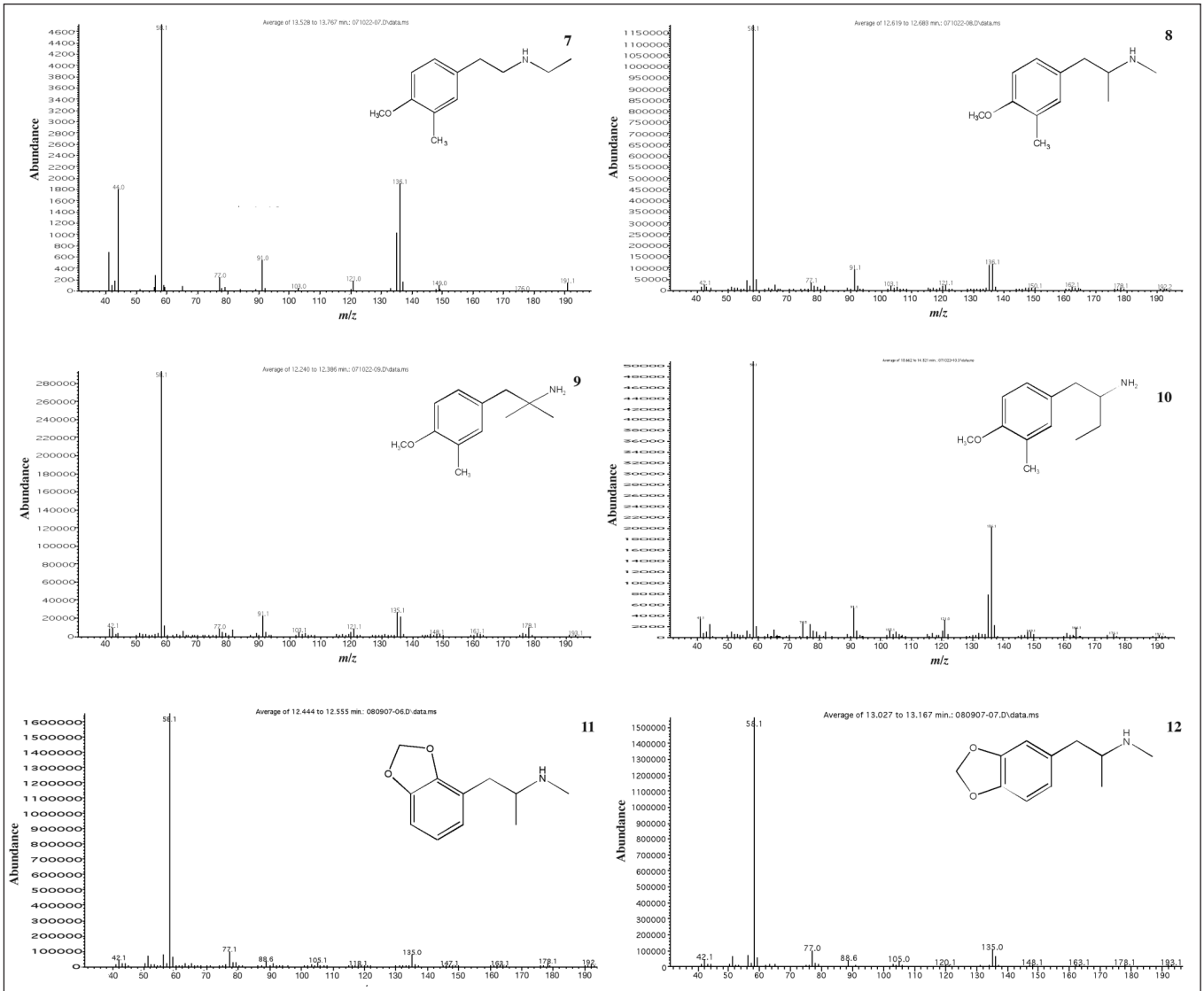

Figure 2B. Mass spectra of compounds 1–12.

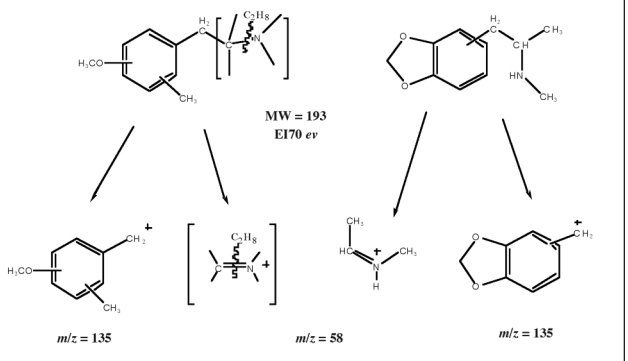

Figure 3. Structures of the major EI fragments for compounds 1–12.

The second phase of this study involved the preparation and evaluation of various perfluoroacylated derivatives of the regioisomeric primary and secondary amines, in an effort to individualize their mass spectra via formation of unique marker ions and improved chromatographic resolution. Acylation of the amines generally lowers the basicity of nitrogen and can allow other fragmentation pathways to play a more prominent role in the resulting mass spectrum (4–7,14). Of course, the tertiary amine (compounds 1 and 6) do not form a stable amide derivative.

The mass spectra for the 20 pentafluoropropionyl and heptfluorobutryl amides are shown in Figures 4 and 5, respectively. From these spectra, a common peak occurs at *m/z* 204 and 254, which corresponds to the loss of 135 mass units from the molecular ions at 339 and 389 for PFPA and HFPA amides. This ion at *m/z* 204 and 254 is the PFPA and HFPA imine species likely formed from the alpha cleavage of the amide nitrogen to eliminate the methoxy-methyl-benzyl or methylenedioxybenzyl radical. Thus the *m/z* 204 and 254 in PFPA and HFPA amides are analogous to *m/z* 58 in the underivatized species because all these ions represent the $(M-135)^+$ species (Figure 6). The 3-methoxy-4-methylbenzyl cation, 4-methoxy-3-methylbenzyl cation, and the methylenedioxybenzyl cation (*m/z* 135) are fragments common to all spectra in Figures 4 and 5. Indeed, the *m/z* 135 ion is the base peak in all the PFPA and HFBA derivatives of compounds 7–10, and this increased relative intensity may serve as an indicator ion for discrimination of the 4-methoxy-3-methyl ring substitution pattern from other ring substitution

patterns in this study. The decreased role for the alpha cleavage reaction in the fragmentation of these amides allows the formation of ions more diagnostic of each individual isomer. Acylation weakens the bond between nitrogen and the alpha-carbon allowing the formation of charged hydrocarbon species of increased relative abundance (14). These hydrocarbons of varying mass identify the number of carbons attached directly to the aromatic ring. The mass spectra in Figures 4 and 5 show hydrocarbon fragments at *m/z* 148, 162, and 176 for a two-carbon, three-carbon, and four-carbon chain attached

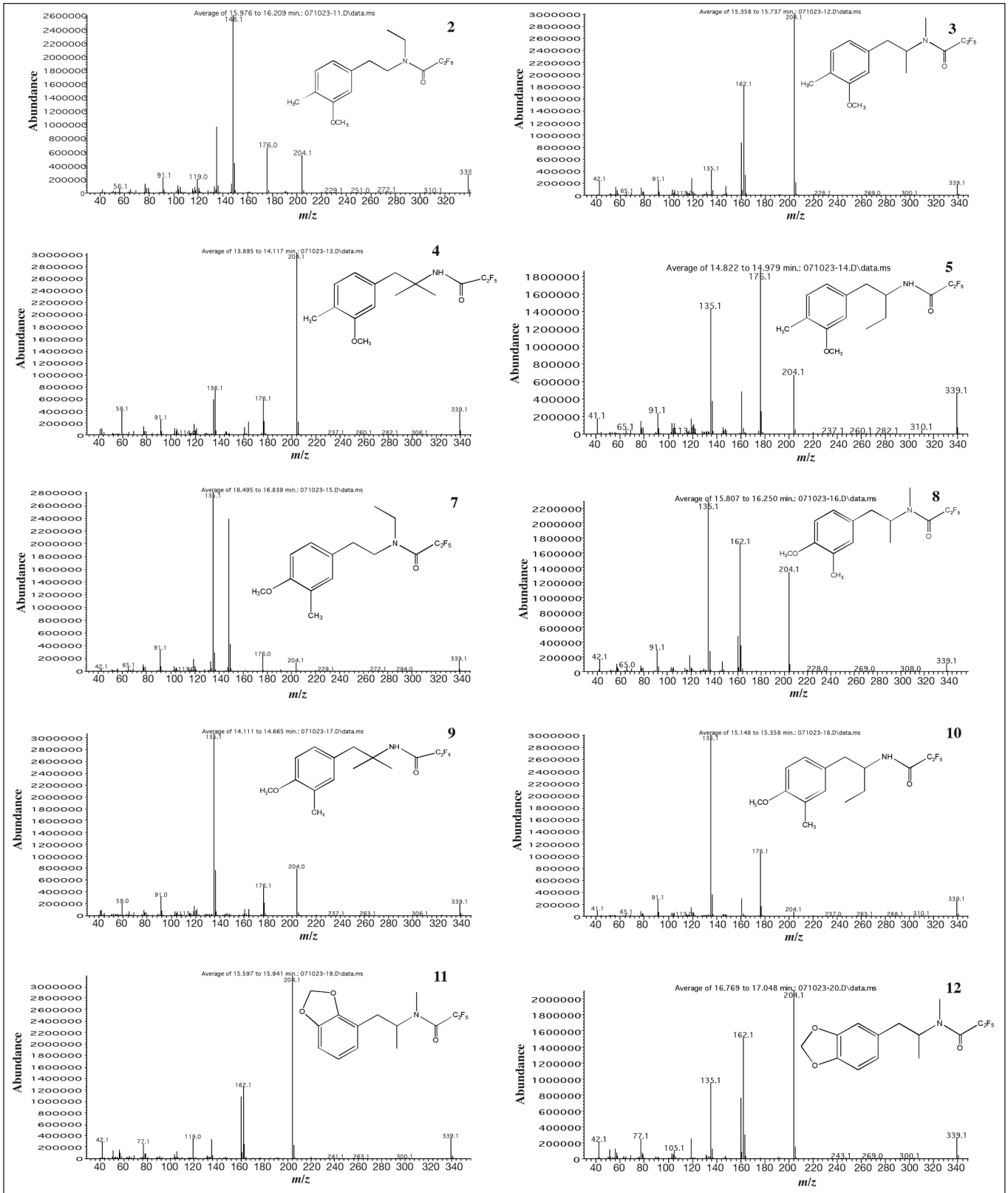

Figure 4. Mass spectra of the PFPA derivatives of compounds 2–5 and 7–12.

directly to the aromatic ring.

The spectra for the *N*-ethyl derivatives in Figures 4(2), 4(7), 5(2), and 5(7) show a base peak at *m/z* 148 corresponding to the two-carbon alkene radical cation, which occurs from hydrogen rearrangement and subsequent fragmentation of the alkyl carbon to nitrogen bond of the phenethylamine side chain. This ion at *m/z* 148 would only occur for the *N*-ethyl regioisomer. The relative abundance of both *m/z* 148 and 135 offer a clear discrimination of compound 2 from its direct regioisomer (compound 7) as well as from the other isomers. The spectra in

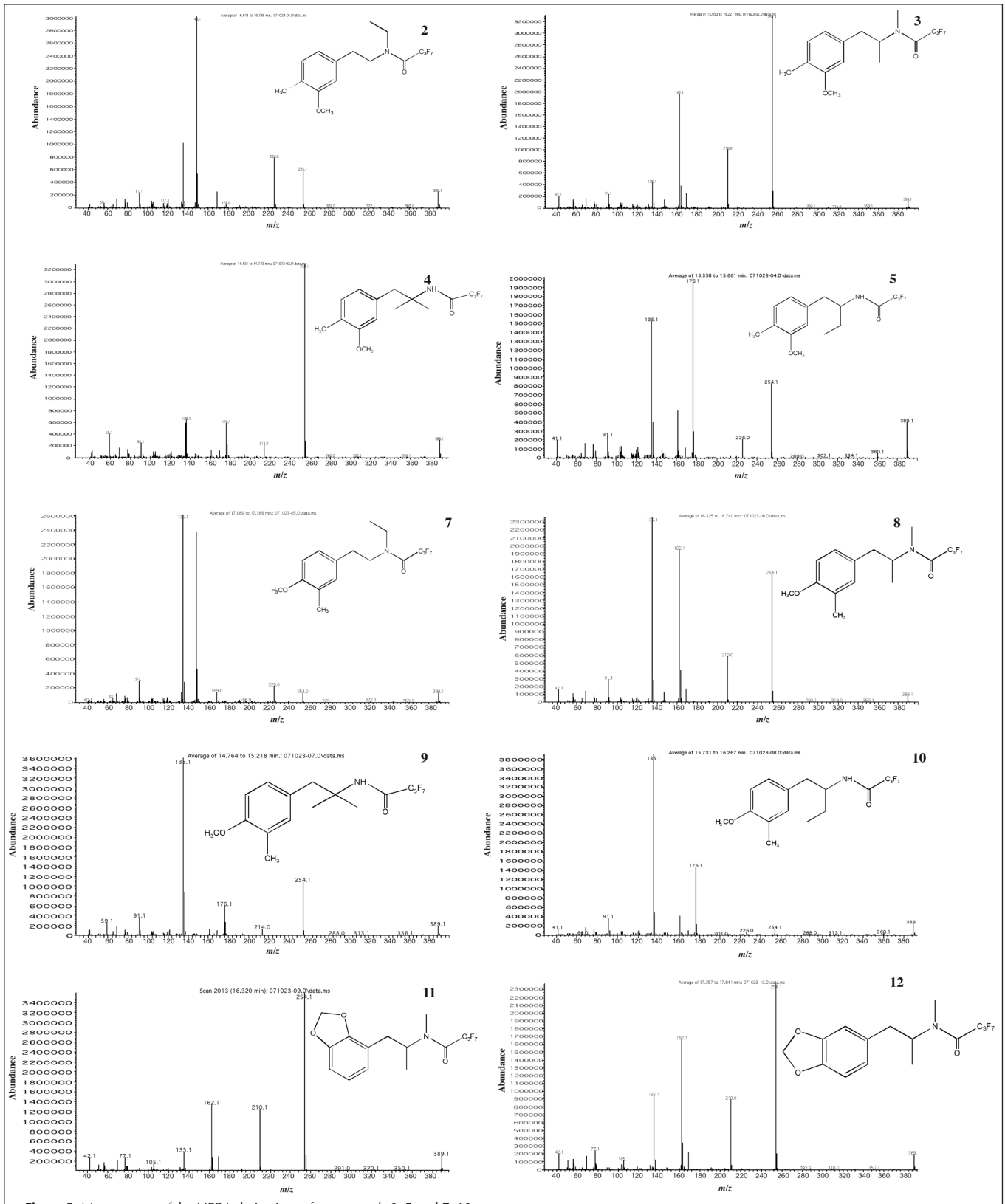

Figure 5. Mass spectra of the HFBA derivatives of compounds 2–5 and 7–12.

Figures 4(3), 4(8), 4(11), 4(12), 5(3), 5(8), 5(11), and 5(12) show the ring substituted methoxy-methyl or the methylenedioxyphenylpropene hydrocarbon ion at *m/z* 162 (the three-carbon alkene radical cation), identifying these molecules as the PFPA and HFBA derivatives containing the methamphetamine side chain, compounds 3, 8, 11, and 12, respectively. The spectra for the PFPA and HFBA derivatives of the primary amines 4, 5, 9, and 10 show ions at *m/z* 176 from the four-carbon alkene radical cation of the 3-methoxy-4-methyl- and 4-methoxy-3-methyl-phenethylamines. This *m/z* 176 results from hydrogen rearrangement and subsequent fragmentation of the alkyl carbon to nitrogen bond yielding the methoxymethyl-phenyl-butene radical cation. The lower abundance of *m/z* 176 for compounds 4 and 9 may be attributed to steric inhibition of hydrogen transfer in the alpha, alpha-dimethyl substitution pattern.

While the alkene ions at 148, 162, and 176 help to identify the side chain regioisomers, one complicating factor in the PFPA derivatives for the *N*-ethylphenethylamines [Figures 4(2) and 4(7)] is the appearance of an ion at *m/z* 176 in addition to the base peak at *m/z* 148. Based on the previous discussion, the *m/z* 176 ion suggests a four carbon chain directly attached to the aromatic ring as occurs for the alpha-ethyl- and alpha, alpha-dimethyl-phenethylamines [Figures 4(4), 4(5), 4(9), 4(10), and 5(4), 5(5), 5(9), 5(10)]. The *m/z* 176 ion in the spectra for the PFPA derivatives of the *N*-ethyl regioisomers [Figures 4(2) and 4(7)] is a rearrangement of the *m/z* 204 ion resulting in the loss of mass 28 (the *N*-ethyl group) via hydrogen transfer. This coincidental common mass from two different fragmentation pathways is confirmed by examining the mass spectra for the HFBA derivatives of the *N*-ethyl-phenethylamines shown in Figures 5(2) and 5(7). The loss of 28 mass units from the acylimine fragment at *m/z* 254 yields the equivalent fragment ion at *m/z* 226. Thus, the HFBA derivatives may offer more characteristic ions for individualization of these regioisomeric substances compared to the PFPA derivatives.

A comparison of the PFPA derivatives for compounds 3, 8, 11, and 12 [Figures 4(3), 4(8), 4(11), and 4(12)] with their HFBA derivatives [Figures 5(3), 5(8), 5(11), and 5(12)] indicates unique ions at *m/z* 160 and *m/z* 210. This mass difference of 50 (CF_2) suggests these ions contain the perfluoroalkyl group for each derivative, C_2F_5 and C_3F_7, respectively. Additional information about these ions were obtained in previous deuterium labeling studies, which confirmed that methyl group on nitrogen is a part of this resulting fragment (4,15). The remaining mass 26 would correspond to CN, and the proposed structures of *m/z* 160 and 210 are shown in Figure 6.

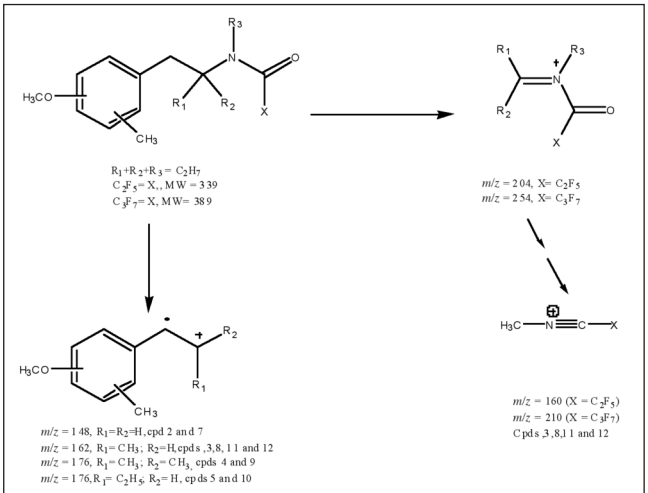

Figure 6. Structures of the major EI fragments for the perfluoroacylated compounds in this study.

GC

The PFPA and HFBA derivatives of the primary and secondary amine side chain regioisomers of the ring substituted methoxy methyl phenethyl amines, 2,3-MDMA and 3,4-MDMA, were compared on a 100% dimethyl polysiloxane (Rtx-1) stationary phase. Several temperature programs were evaluated and one program showing the best compromise between resolution and analysis time was used to generate the chromatograms in Figure 7. The chromatograms show that when the ring substitution pattern is held constant (i.e., 3-methoxy-4-methyl- or 4-methoxy-3-methyl-) the two secondary amides elute before the two tertiary amides. Additionally, in every case in this limited set of compounds, the branched side chain elutes before the straight chain isomer when the ring substitution pattern and the degree of amide substitution are constant, regardless of the derivatizing agent. Therefore, the alpha, alpha-dimethyl isomer elutes first followed by the alpha-ethyl isomer (both secondary amides), then the methamphetamine, and *N*-ethyl phenethylamines (the two tertiary amides).

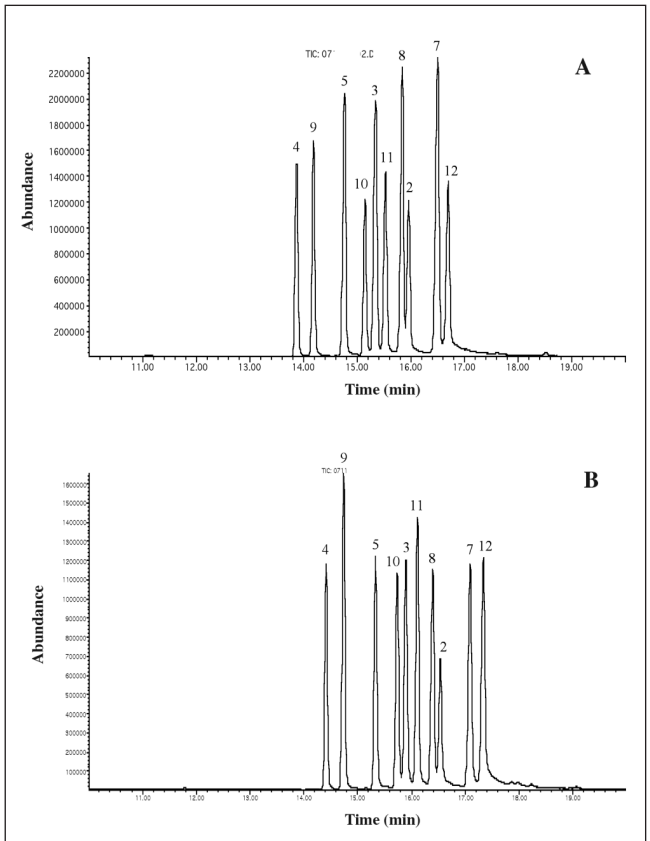

Figure 7. GC separation of the PFPA (A) and HFBA (B) derivatives of compounds 2–5 and 7–12; Rtx-1 column.

When the side chain is held constant, the 3-methoxy-4-methyl ring substitution pattern elutes before the 4-methoxy-3-methyl ring substitution pattern and this elution order is the same for both the PFPA and the HFBA derivatives. Perhaps the most useful information in these chromatograms is the relative elution of the derivatized controlled substance 3,4-MDMA and its closest eluting regioisomeric equivalents. Both the PFPA and HFBA derivatives of 3,4-MDMA elute after the *N*-ethyl-3-methoxy-4-methyl phenethylamine and 4-methoxy-3-methylphenethylamine PFPAs and HFBAs. The *N*-ethyl regioisomers show very distinct mass spectra with several characteristic ions to differentiate these compounds from the drug of abuse 3,4-MDMA. Thus, derivatization methods coupled with chromatographic and mass spectral procedures can allow for the characterization and differentiation of these ten uniquely isomeric substances. The individualization is possible without the need for reference samples of all these uniquely similar substances.

Conclusions

3,4-MDMA, 2,3-MDMA, and ten side chain regioisomers of 3-methoxy-4-methyl-phenethylamine and 4-methoxy-3-methyl-phenethylamine are a unique subset of regioisomeric and isobaric molecules. Each compound has a molecular weight of 193 and yields a base peak at *m/z* 58 in the mass spectrum from the loss of the corresponding methylenedioxybenzyl or the mass equivalent isobaric ring substituted methoxy methyl benzyl groups. Thus the traditional electron impact mass spectrum provides little structural information for differentiating among these ten compounds.

Derivatization of the eight primary and secondary amines with various acylating agents yields amides that significantly individualize the mass spectra and allow for specific identification. The individualization is the result of fragmentation of the alkyl carbon–nitrogen bond yielding hydrocarbon fragments at *m/z* 148, 162, and 176 as well as other unique fragments from these regioisomeric amides. The PFPA and HFBA derivatives are essentially equivalent for chromatographic purposes however; the HFBA derivatives offer more unique fragment ions for additional discrimination among these regioisomeric substances. Chromatographic resolution of the acylated amines was achieved on a relatively non-polar stationary phase, Rtx-1 (100% dimethyl polysiloxane).

Acknowledgement

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