GC–MS Studies on the Regioisomeric Methoxy-Methyl-Phenethylamines Related to MDEA, MDMMA, and MBDB

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Abstract

Three regioisomeric 3,4-methylenedioxyphenethylamines having the same molecular weight and major mass spectral fragments of equal mass have been reported as drugs of abuse in forensic studies in recent years. These compounds are 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA), 3,4-methylenedioxy-*N*-*N*-dimethylamphetamine (MDMMA), and *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB). The mass spectra of the regioisomers (4-methoxy-3-methyl and 4-methoxy-2-methyl-phenethylamines) are essentially equivalent to the three compounds reported as drugs of abuse. This project focused on the synthesis, mass spectral characterization, and chromatographic analysis of these six regioisomeric methoxy methyl phenethylamines. Additionally, the mass spectral and chromatographic properties of these compounds will be compared to the isobaric 2,3- and 3,4-methylenedioxyphenethyl-amines of the same side chain. The six regioisomeric methoxy-methyl-phenethylamines were synthesized from commercially available starting materials. Side chain differentiation by mass spectrometry was possible after the formation of the perfluoroacyl derivatives, pentafluoropropionylamides (PFPA) and heptafluorobutrylamides (HFBA). Gas chromatographic separation on Rtx-1 was successful at resolving the perfluoroacyl derivatives of the 4-methoxy-3-methyl phenethylamines from those of the 4-methoxy-2-methyl phenethylamines. The 4-methoxy-3-methyl-phenethylamine derivatives eluted before the 4-methoxy-2-methyl-phenethylamine derivatives as both the PFPA and HFBA derivatives.

Introduction

The methylenedioxyamphetamines such as 3, 4-methylenedioxyamphetamine (MDA), 3, 4-methylenedioxymethamphetamine (MDMA) and 3, 4-methylenedioxyethylamphetamine (MDEA) are all psychoactive compounds with structural similarities to amphetamines and mescaline (a psychedelic phenethylamine). MDA, MDMA, and MDEA have all been shown to produce very similar peripheral and central effects in humans with slight differences in potency, time of onset, and duration of action (1,2). The homologous primary amine, 3,4-methylenedioxyphenylbutanamine (BDB), has both hallucinogenic and stimulant effects (3). *N*-methyl-BDB (MBDB) has been reported to have novel central nervous system (CNS) effects with neither stimulant nor hallucinogenic properties (4). MBDB and MDMA are reported to be generally similar in effect (5) with slight differences in potency.

Regioisomer differentiation is a significant issue in forensic drug chemistry and has been addressed in a number of drug categories (6–8). Three of the substances in this study have already appeared in street drug samples. In this project the analytical properties of MDEA, MDMMA, and MBDB were compared to those of the regioisomeric 2,3-methylenedioxy derivatives and the isobaric 4-methoxy-3-methyl and 4-methoxy-2-methyl-phenethylamines having the same side chains. Figure 1 shows the structure of all twelve compounds included in this study. A previous report (9) in this series described the analytical properties of the methylenedioxyphenethylamine regioisomers using the structure numbers 1–6 in Figure 1. Thus, to remain consistent and to allow for direct comparison of this report and the previous publication (9) the methoxy methyl isomers are numbered as 7–12 and compounds 1–6 are the same in this paper and in the previous report (9).

The ability to distinguish between these regioisomers directly increases the specificity of the analysis for any one of the target drugs. The mass spectrum is usually the confirmatory piece of evidence for the identification of drugs of abuse in forensic and other regulatory laboratories. There are many compounds with essentially equal mass spectra among the substituted phenethylamines making their differentiation a challenge in many analytical situations. For drugs such as the amphetamines and MDMAs, there are many positional isomers (regioisomers) in the alkyl side chain or in the aromatic ring substitution pattern, which can yield nearly an identical mass spectrum. While nuclear magnetic resonance (NMR) can be a useful method for differentiation of these regioisomers, it is not a technique with direct application for all areas of regulatory analysis. Most forensic drug samples are not of sufficient purity for direct NMR analysis and NMR is not usually applicable to the analysis of drugs in biological samples. Thus, the analysis of these drugs must depend heavily on chromatographic methods as well as mass spectrometry.

When other compounds exist with the ability to produce nearly identical mass spectra as the drug of interest, the identification by gas chromatography–mass spectrometry (GC–MS) must focus on the ability of the chromatographic system to separate the imposter molecules from the drug of interest. The

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regioisomers that coelute with the drugs of interest in chromatographic separations could be mistaken for the drug of abuse itself. Without the appropriate standards, thorough method validation is not possible, and thus coelution of the regioisomer (the non-drug) with the drug remains a possibility.

The targets of this study were six methoxy-methyl-phenethylamines (see Figure 1) with molecular weight 207 and the potential to produce a mass spectrum with major fragment ions at *m/z* 72 for the imine and *m/z* 135/136 for the ring substituted methoxy-methyl-benzyl fragment. Additionally the chromatographic and mass spectral properties of these methoxy methyl phenethylamines will be compared to the isobaric methylenedioxyphenethylamines of the same side chain substitution pattern (9).

Experimental

Analytical

Analytical studies were conducted using an Agilent Technologies (Santa Clara, CA) 7890A gas chromatograph and an Agilent 7683B auto injector coupled with a 5975C VL Agilent mass selective detector. Each mass spectrum and chromatogram included in this study was reproduced at least three times. The mass spectral scan range was 40–500 amu and the scan rate was 2.86 scans per second. The GC was operated in splitless mode with a constant carrier gas (Helium grade 5) flow rate of 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 manual background subtraction and are the average of at least five scans. Samples were diluted in HPLC grade acetonitrile (Fischer Scientific, NJ) and introduced via the auto injector as individual solutions and in physical mixtures.

The separation was carried out on a non-polar 30-m × 0.25-mm i.d. column coated with 0.25-µm 100% dimethyl polysiloxane (Rtx-1) obtained from Restek Corporation (Bellefonte, PA). The retention data was generated using a temperature program consisting of an initial hold at 100°C for 1.0 min, ramped up to 180°C at a rate of 9°C/min and held at 180°C for 2.0 min, then ramped to 200°C at a rate of 10°C/min and held at 200°C for 20.0 min.

All reagents and chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Fischer Scientific Inc. (Atlanta, GA).

Derivatization procedure

Each perfluoroamide was prepared individually from the hydrochloride salts by dissolving approximately 0.3 mg. (1.5×10^{-6} moles) of each amine in 50 µL of ethyl acetate followed by the addition of a large excess (250 µL) of the appropriate derivatizing agent (pentafluoropropionic anhydride or heptafluorobutric anhydride). The derivatization reaction mixtures were incubated in capped vials at 70°C for 20 min. Following incubation, each sample was evaporated to dryness under a stream of air at 55°C and reconstituted with 200 µL of ethyl acetate and 50 µL of pyridine. A portion of this solution (50 µL) was further diluted with HPLC-grade acetonitrile (200 µL) and volumes of 1 µL of the resulting solutions were injected into the GC–MS for analysis.

General synthetic methods

The general methods for the preparation of the six regioisomeric 4-methoxy-3-methyl and 4-methoxy-2-methyl-phenethylamines begins with the commercially available aldehydes, 4-methoxy-3-methyl benzaldehyde and 4-methoxy-2-methyl-benzaldehyde as starting materials. The structures of all six compounds prepared and evaluated in this study are shown in Figure 1. The aldehydes were converted to the corresponding substituted 1-phenyl-2-nitroalkenes via the *n*-butylimine and the appropriate nitroalkane (nitroethane or 1-nitropropane). Reductive hydrolysis of the 2-nitroalkenes gave the desired ketones, which were converted to the desired amines by reductive amination. Combinations of the appropriate aldehyde, nitroalkane, and alkyl amine allowed for the synthesis of the amines in Figure 1 (labeled as compounds 7–12). The methylenedioxyphenyl substituted amines (compounds 1–6) were prepared in an analogous manner and were the subject of a previous report in this series (9). Compounds 1–6 are included in the chromatographic studies in this report, their mass spectra and individual chromatographic properties can be found in the literature (9).

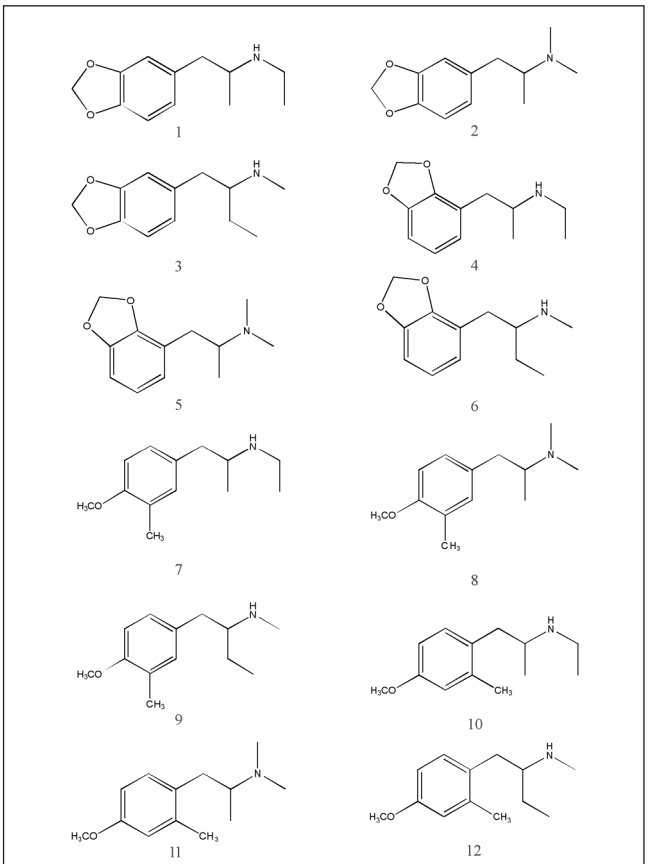

Figure 1. Structures of the regioisomeric and isobaric compounds in this study.

Results and Discussion

Mass spectral studies

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 2 shows the EI mass spectra for the regioisomeric 4-methoxy-3-methyl phenethylamines and the 4-methoxy-2-methyl phenethylamines (Compounds 7–12). Please see the literature (9) for the individual mass spectra for compounds 1–6. The spectra in Figure 2 indicate that very little structural information is available for differentiation among these regioisomers because the major fragment ions occur at equal masses. The mass spectra are characterized by a base peak formed by an alpha cleavage reaction (*m/z* 72) involving the carbon–carbon bond of the ethyl linkage between the aromatic ring and the amine. Additionally, fragmentation initiated via initial ionization of the aromatic ring electrons yields the corresponding substituted methoxy methyl benzyl cation fragment at *m/z* 135 and/or its radical cation at *m/z* 136. The structures for these major fragment ions are shown in Figure 3. Fragments at equivalent masses and similar relative abundances are observed in the mass spectra for the 3,4-methylenedioxyphenyl substituted drugs of abuse, MDEA, MBDB, and MDMMA (9).

The *m/z* 44 ion in the spectra for compounds 7 and 10 occurs via the loss of ethylene from the *N*-ethyl group of the base peak (*m/z* 72). However, this low mass ion is present in the mass spectra for many substituted phenethylamines as well as other compounds and does not provide significant diagnostic information to individualize these mass spectra.

Acylation of amines generally reduces the basicity of the nitrogen and this often allows other fragmentation pathways to play a more prominent role in the mass spectrum of the corresponding amides (10–12). The pentafluoropropionyl (PFPA) and heptafluorobutryl (HFBA) derivatives of the methoxy methyl phenethylamines were evaluated for their ability to individualize the mass spectra and provide unique ions for compound identification and differentiation. When comparing the PFPA and HFBA derivatives of an individual amine, those fragment ions differing by mass 50 (CF_2) are likely to contain the perfluoroalkyl moiety. The mass spectra for the pentafluoropropionyl and the

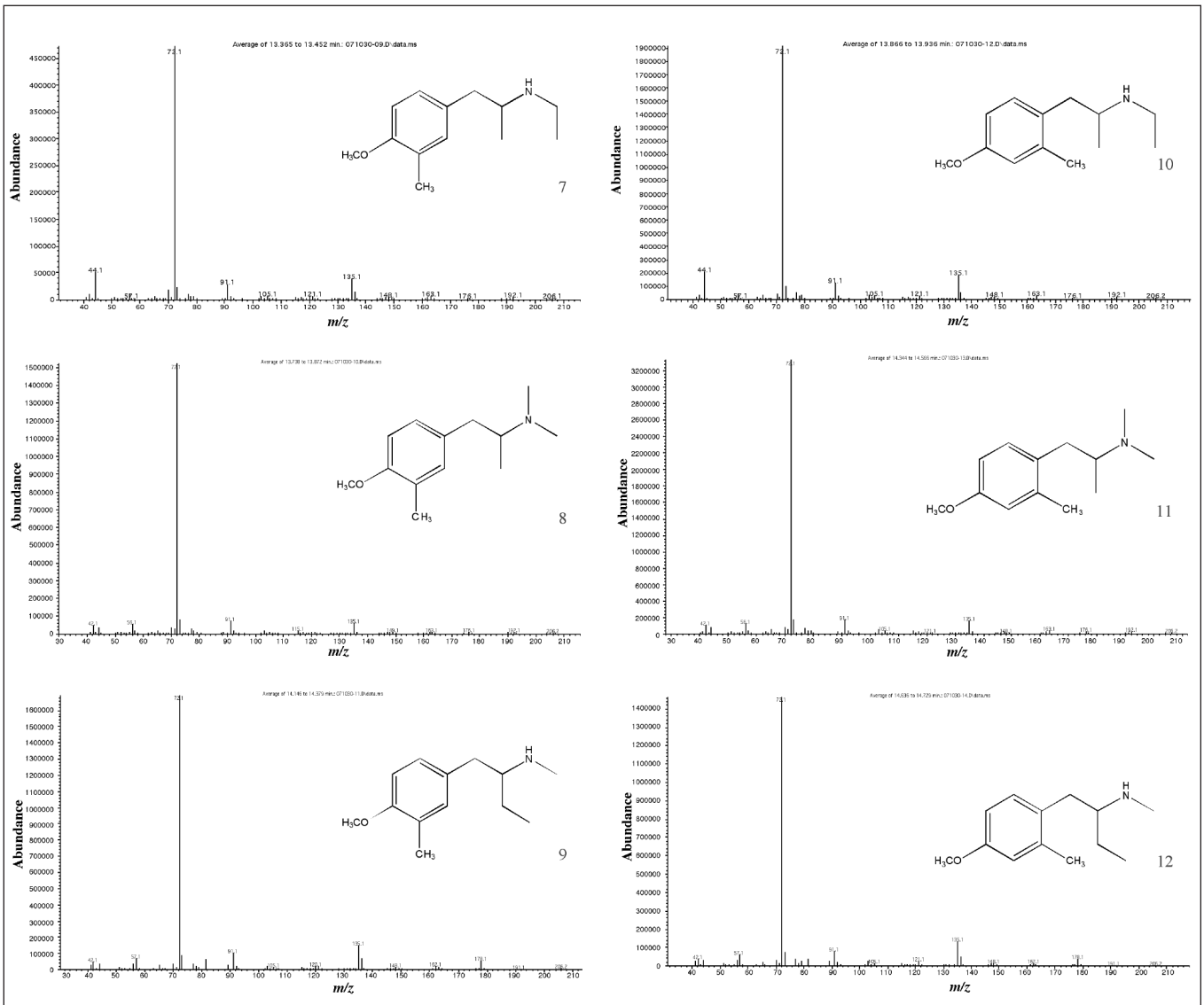

Figure 2. Mass spectra of compounds 7–12.

heptafluorobutryl amides of the secondary amines (the tertiary amines do not form stable acylation products) are shown in Figures 4 and 5. For the PFPA and HFBA derivatives, the spectra show a common base peak at 218 and 268 which corresponds to the loss of 135 mass units from the molecular ions at 353 and 403, respectively. The ions at *m/z* 218 and 268 are the PFPA and HFBA imine species likely formed from the alpha cleavage reaction of the amide nitrogen and thus these ions are analogous to the *m/z* 72 in the underivatized species. Figure 6 shows the structures of the major fragment ions for the perfluoroacyl derivatives.

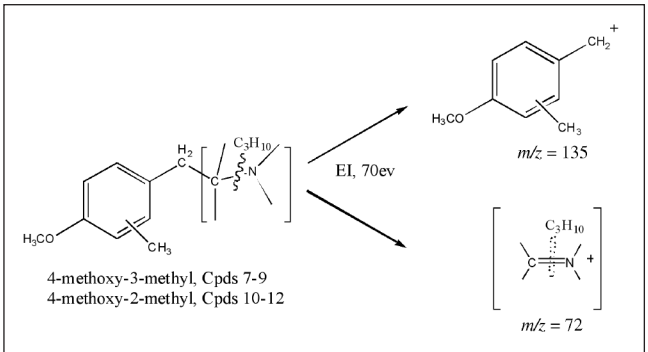

Figure 3. EI fragmentation pattern for compounds 7–12.

There are two major diagnostic pathways in the mass spectrum of the PFPA and HFBA derivatives, which allows differentiation of the side chain regioisomers. The first of these is the alkene fragment observed at *m/z* 162 and *m/z* 176; these ions occur in the spectra for both the PFPA and HFBA derivatives, indicating the perfluoroacyl moiety is not a component of these ions. The *m/z* 162 ion occurs in the mass spectrum for the PFPA and HFBA derivatives of compounds 7 and 10, the *N*-ethyl MDAs. This alkene fragment is the radical cation resulting from cleavage of the bond between nitrogen and the alkyl carbon of the hydrocarbon side chain. This bond cleavage occurs following an initial hydrogen rearrangement likely from the benzylic carbon to the carbonyl oxygen. Thus, the *m/z* 162 ion is indicative of the three carbon chain attached directly to the aromatic ring. The analogous pathway for the PFPA and HFBA derivatives of compounds 9 and 12 yields a fragment ion at *m/z* 176 indicating a four-carbon side chain attached directly to the aromatic ring.

The second diagnostic fragmentation pathway does contain the perfluoracyl group or a portion of the perfluoroacyl groups and therefore appears at different masses for the PFPA and HFBA derivatives. The loss of mass 28 (the *N*-ethyl group lost as ethylene following hydrogen rearrangement) from the base peak in the mass spectrum of compounds 7 and 10 appears at *m/z* 190 and *m/z* 240 for the PFPA and HFBA derivatives, respectively.

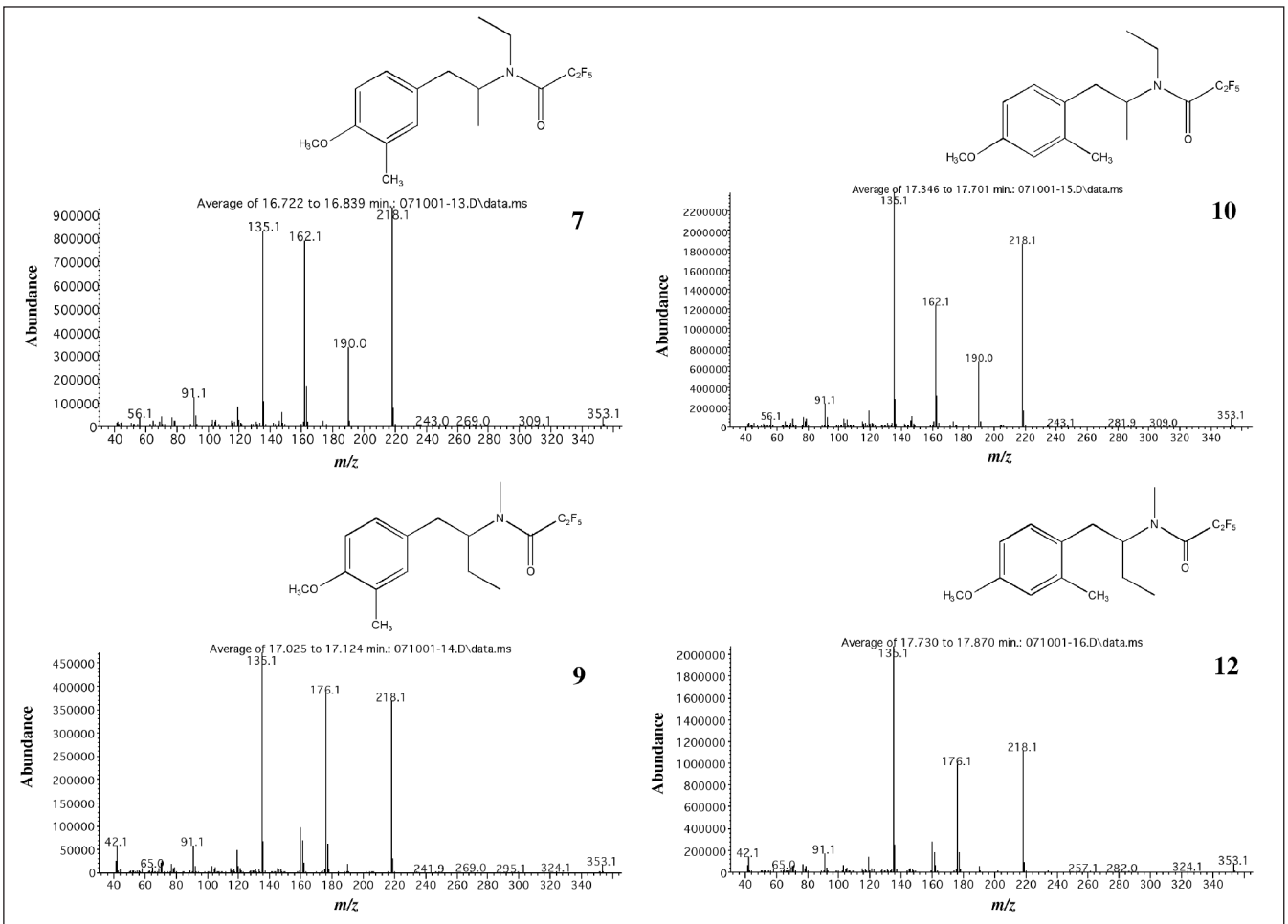

Figure 4. Mass spectra of the PFPA derivatives of compounds 7, 9, 10, and 12.

While these ions can occur in the C-ethyl regioisomers (compounds 9 and 12), the loss of ethylene from the C-ethyl amine derivatives is a much less prominent ion. When the side chain consists of an *N*-methyl group (compounds 9 and 12) the base peak undergoes a rearrangement fragmentation reaction (11) to yield the *m/z* 160 and *m/z* 210 for the PFPA and HFBA derivatives, respectively. The structure of this ion has been confirmed by deuterium labeling experiments in other series of *N*-methylphenethylamines (6,10). Thus, the mass spectra of these perfluoroacyl derivatives allow identification of the carbon side chain attached directly to the aromatic ring and identification of the alkyl group bonded to nitrogen.

Gas chromatography

Mass spectrometry alone may not provide enough information to distinguish between the isomers in this study. Therefore, the identification by GC–MS must depend heavily on the ability of the chromatographic system to separate these isomeric substances. The complete chromatographic resolution of all the underivatized amines was not accomplished in this study and is the subject of continuing efforts.

The GC separation of the PFPA and HFBA derivatives of regioisomeric amines 7–12 is shown in Figure 7. This separation was obtained using a 30 m × 0.25 mm i.d. column with a 0.25 µm film of 100% dimethyl polysiloxane, Rtx-1 stationary phase. The dimethylpolysiloxane is a common stationary phase used in forensic drug analysis laboratories. The chromatograms in Figure 7 show that the 4-methoxy-3-methyl phenethylamines, compounds 7 and 9, elute before any of the 4-methoxy-2-methyl

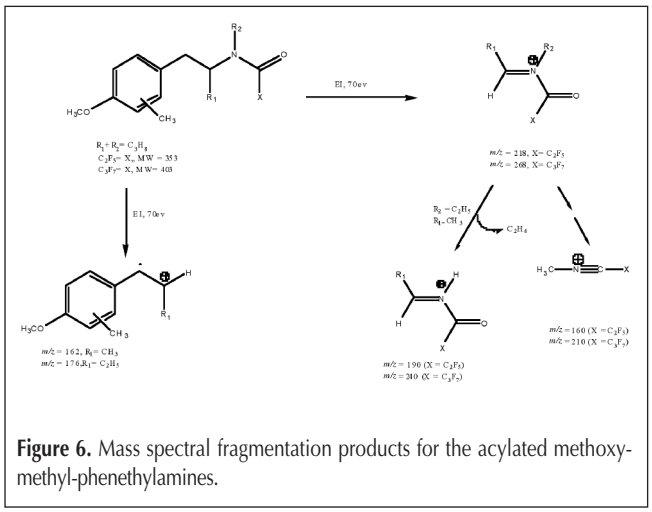

Figure 6. Mass spectral fragmentation products for the acylated methoxy-methyl-phenethylamines.

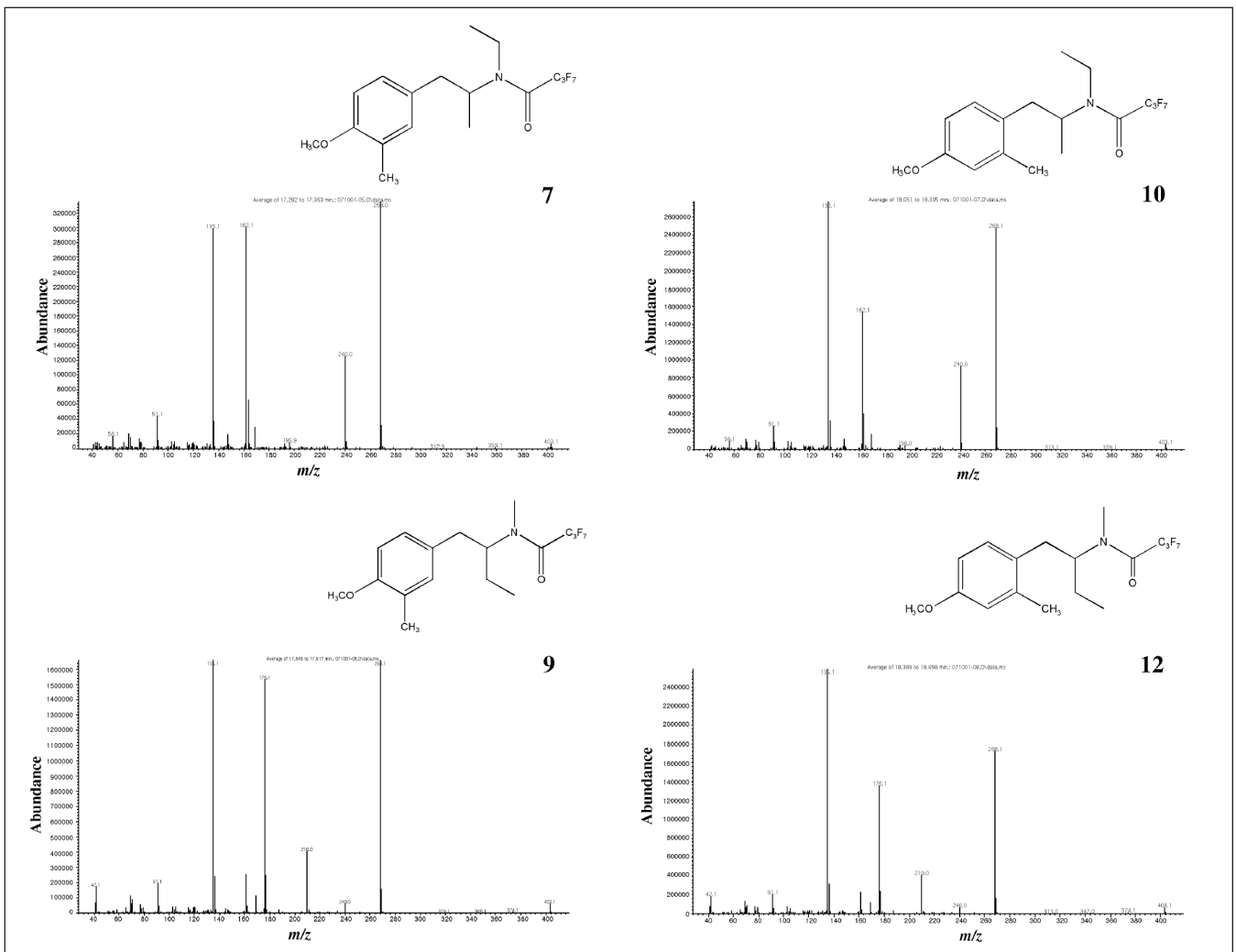

Figure 5. Mass spectra of the HFBA derivatives of compounds 7, 9, 10, and 12.

phenethylamines, compounds 10 and 12. The order of side chain elution within the individual ring substitution patterns is *N*-ethyl in the arylpropylamine series followed by the *N*-methyl derivative of the butanamine series. Thus, the longest continuous hydrocarbon side chain (C4) attached to the aromatic ring shows the greater retention in this limited series of regioisomers. The HFBA derivatives (Figure 7A) show slightly greater retention on the methylpolysiloxane stationary phase than the PFPA derivatives (Figure 7B).

The third study in this project was to evaluate the compounds having identical side chains using the methoxymethyl (compounds 7–12) as well as the methylenedioxyphenethylamines (compounds 1–6). The individual mass spectra for the underivatized and derivatized forms of compounds 1–6 and their chromatographic properties have been reported previously (9). The mass spectrometry studies on the PFPA and HFBA derivatives established that specific fragment ions were available to distinguish among the different side chains. Furthermore the nature of the ring substituted methylenedioxy or methoxy-methyl showed significant and characteristic differences in fragment ion abundance. Thus, the chromatographic properties of those subsets having the same side chain are important for the ability to specifically identify each one of these substances to the exclusion of all the other amines in this study. The separations in Figures 8 and 9 were carried out on the Rtx-1 stationary phase using the same temperature program as that used to generate the chromatograms in Figure 7. For the *N*-ethyl side chains in Figure 8, the first compound to elute was compound 4 (2,3-methylenedioxy-phenyl), then compound 7 (4-methoxy-3-methyl-phenyl), followed by compound 10 (4-methoxy-2-methyl-phenyl), and finally compound 1 (3,4-methylenedioxy-phenyl). This elution order is the same for the PFPA and HFBA derivatives with the

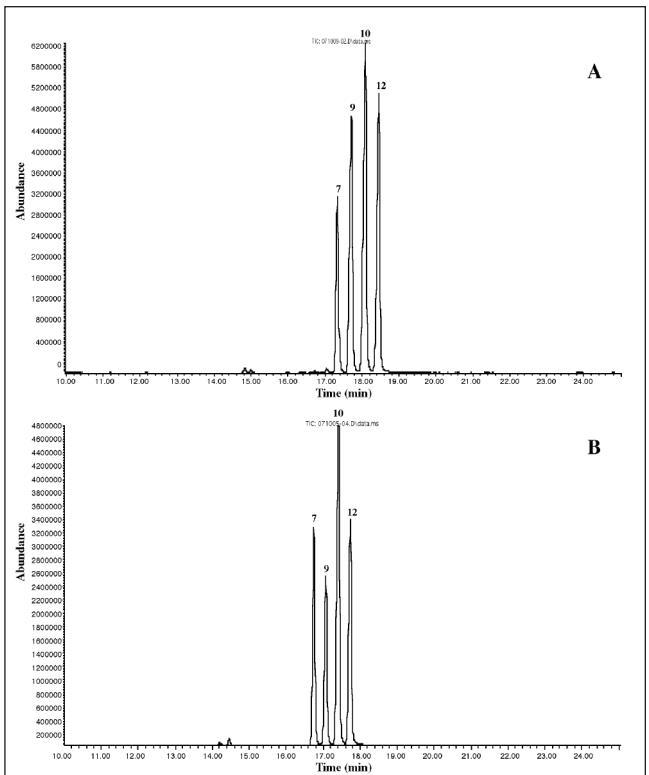

Figure 7. GC separation of compounds 7, 9, 10 and 12. HFBA (A) and PFPA (B).

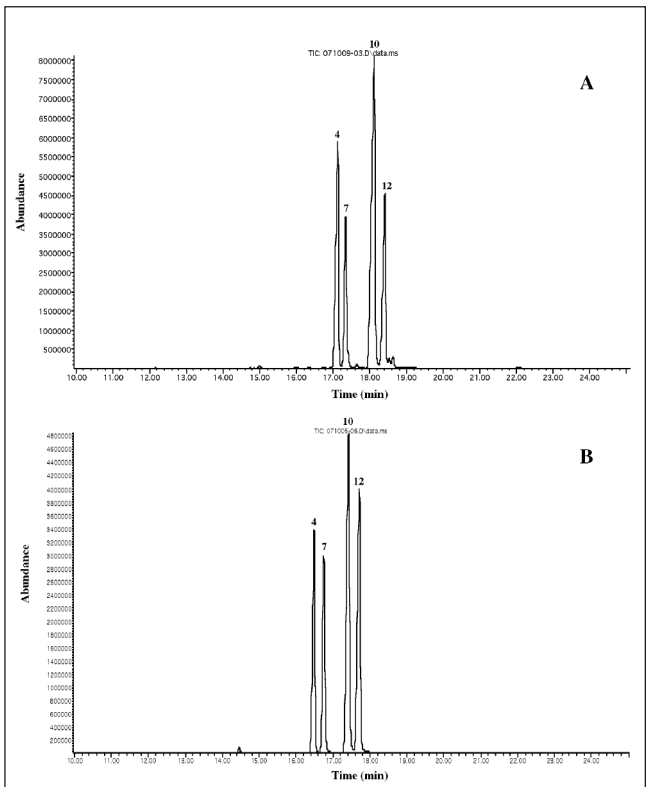

Figure 8. GC separation of compounds 1, 4, 7, and 10. HFBA (A) and PFPA (B).

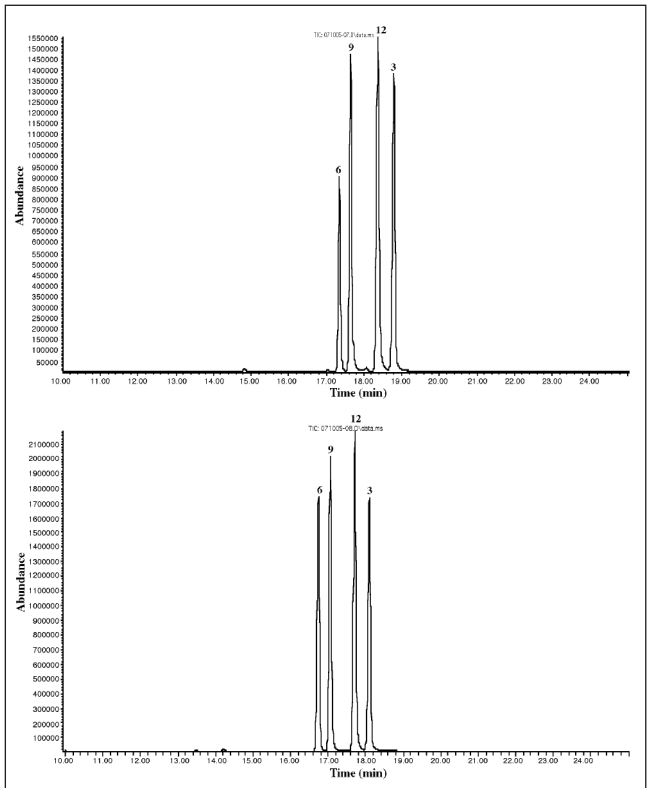

Figure 9. GC separation of compounds 3, 6, 9, and 12. HFBA (A) and PFPA (B).

PFPAs in Figure 8B having slightly lower retention.

The chromatographic properties of the compounds with the *N*-methyl, C-ethyl side chain were also evaluated and the separations obtained are shown in Figure 9A and 9B. The elution order for the various ring substituents is the same as that described for Figure 8. The 2,3-methylenedioxyphenyl elutes first and the 3,4-methylenedioxyphenyl group elutes last in this series. Thus the nonpolar dimethyl polysiloxane chromatographic phase shows base line resolution for the ring substitutents and patterns. Chromatographic separation coupled with the mass spectral fragmentation properties should allow for complete individualization among the compounds in this study.

Conclusion

In summary, six regioisomeric methoxy methyl phenethylamines of molecular weight 207 and major mass spectral fragments at *m/z* 72 and 135/136 were synthesized and compared to six methylenedioxyphenethylamines of equal molecular weight and fragment ions of equal mass. The results of this study show that the traditional EI mass spectrum provides little structural information for differentiating among these 12 compounds. Because of the unique similarity of these compounds by mass spectrometry, the specific identification of any one of these compounds requires analytical methods to eliminate the other isomers. Thus, the ultimate identification of any one of these amines with the elimination of the other isomeric substances depends heavily on chromatographic methods.

Derivatization of these amines with various perfluoroacylating agents produced amides with improved resolution compared to the underivatized amines by gas chromatography. These perfluoroacyl derivatives significantly individualized the mass spectra, especially the side chain regioisomers. The PFPA and HFBA derivatives are essentially equivalent for chromatographic purposes, however, the HFBA derivatives offer more unique fragment ions for additional mass spectral discrimination among these regioisomers. A nonpolar stationary phase consisting of 100% dimethyl polysiloxane (Rtx-1) gave good chromatographic resolution of the amide derivatives.

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