GC–MS Studies on Side Chain Regioisomers Related to Substituted Methylenedioxyphenethylamines: 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). A series of seven additional side chain regioisomers have mass spectra essentially equivalent to the three controlled drug substances, all have molecular weight of 207 and major fragment ions in their electron ionization mass spectra at *m/z* 72 and 135/136. The trifluoroacetyl, pentafluoropropionyl, and heptafluorobutryl derivatives of the primary and secondary regioisomeric amines were evaluated in GC–MS studies. The mass spectra for these derivatives were significantly individualized, and the resulting unique fragment ions allowed for specific side chain identification. The trifluoroacetyl and heptafluorobutryl derivatives provided more specific fragment ions for molecular individualization among these regioisomeric substances. These perfluoroacyl derivatives showed reasonable resolution on the polar stationary phase Rtx-200.

Introduction

The methylenedioxyamphetamines such as 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyethylamphetamine (MDEA) are psychoactive compounds producing very similar peripheral and central effects in humans with only 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 (4,5). MBDB and MDMA are reported to be generally similar in effect with slight differences in potency (4,5). These compounds, MDA, MDMA, 3,4-methylenedioxy-*N*,*N*-dimethylamphetamine (MDMMA), MDEA, and *N*-methyl-3,4-methylenedioxy-phenylbutanamine (MBDB) have a unique set of biological activities and do not fit the pharmacological profile of either phenethylamine hallucinogens or psychomotor stimulants (4).

Mass spectrometry is usually the confirmatory piece of evidence for the identification of drugs in forensic and other regulatory laboratories. While the mass spectrum is considered a fingerprint for an individual compound, there are many compounds with essentially equal mass spectra among the substituted phenethylamines. Thus their differentiation is a challenge

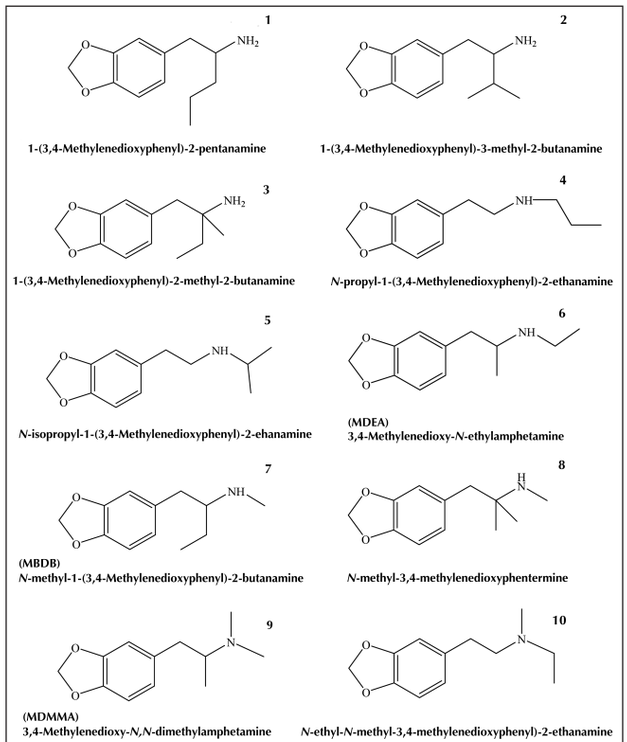

Figure 1. Structures of the 10 compounds involved in this study.

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in many analytical situations. These compounds are usually positional isomers (regioisomers) in the alkyl side chain or in the aromatic ring substitution pattern, often yielding identical mass spectra.

Regioisomeric differentiation is a significant issue in forensic drug chemistry and has been addressed in a number of drug categories (5–12). There are an additional seven side chain regioisomeric phenethylamines related to MDEA, MDMMA, and MBDB. The structures of this set of compounds are shown in Figure 1. All these phenethylamines have molecular weight of 207 with the potential to produce a mass spectrum with major fragment ions at *m/z* 135 for the 3,4-methylenedioxy benzyl fragment and *m/z* 72 for the imine fragment (Figure 2). 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. 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.

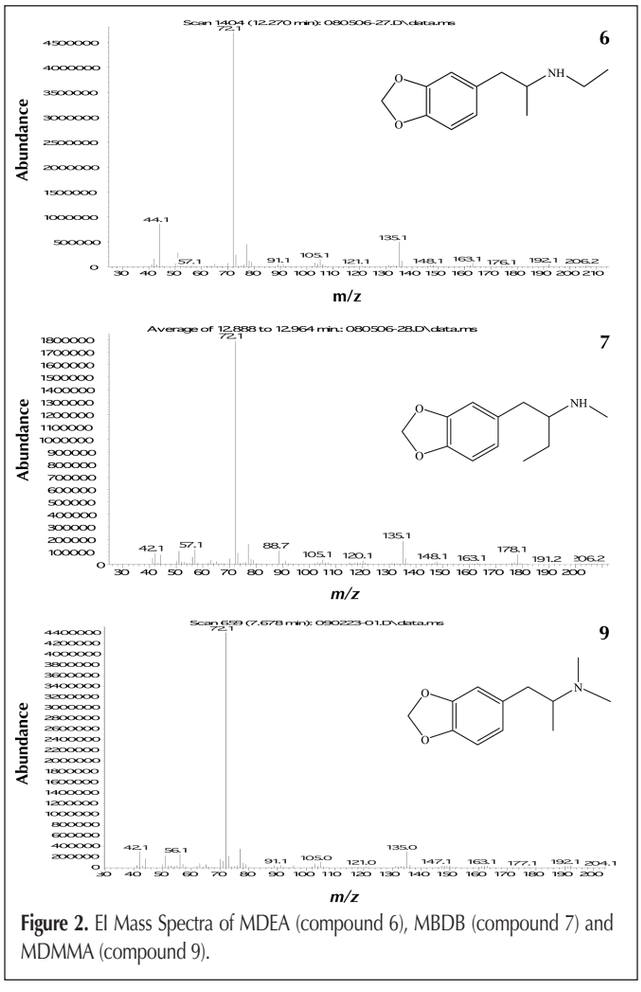

Figure 2. EI Mass Spectra of MDEA (compound 6), MBDB (compound 7) and MDMMA (compound 9).

A previous report from our research group (4) included the synthesis and mass spectral evaluation of these 10 regioisomeric compounds. The mass spectra of the underivatized compounds provided very little structural information for the specific differentiation among these regioisomers even though these regioisomers were separated by capillary gas chromatography (4).

In this study, derivatization of the primary and secondary amines was carried out in an effort to obtain more specific ions that would help in differentiating among the members of this set of compounds.

Experimental

Instrumentation

GC–MS analysis was performed on Agilent Technologies (Santa Clara, CA) 7890A gas chromatograph 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 helium (grade 5) as the carrier gas at a flow rate of 0.7 mL/min and the column head pressure was 10 psi. The MS was operated in the electron impact (EI) mode using an ionization voltage of 70 eV, and a source temperature of 250°C. The GC injector was maintained at 250°C and the transfer line at 280°C.

The GC separation was carried out on a relatively polar column (30 m × 0.25 mm i.d.) coated with 0.50 µm crossbond, trifluropropyl methyl polysiloxane (Rtx-200) purchased from Restek Corporation (Bellefonte, PA). The separation was performed using three temperature programs for each derivative studied. Program one was used to separate the trifluoroacetic anhydride (TFA) derivatives and consisted of an initial hold at 100°C for 1.0 min, ramped up to 180°C at a rate of 12°C/min, 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 rest of the run duration. The second program was used to resolve the pentafluoropropionic anhydride (PFPA) derivatives. It started at 100°C for 1.0 min, ramped up to 180°C at a rate of 7.5°C/min, 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. The third program utilized to separate the heptafluorobutyric anhydride (HFBA) derivatives had an initial oven temperature at 100°C for 1.0 min, followed by temperature increase at a rate of 9°C/min to reach 180°C, 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 the remaining of the run.

Samples were dissolved and diluted in HPLC-grade acetonitrile (Fisher Scientific, Fairlawn, NJ) and introduced via the auto injector using an injection volume of 1 µL.

Drugs and reagents

The compounds studied were: 3,4-Methylenedioxy-*N*-ethylamphetamine (MDEA), *N*-methyl-1-(3,4-Methylene-dioxyphenyl)-2-butanamine (MBDB), 3,4-Methylenedioxy-*N,N*-dimethylamphetamine (MDMMA), and the seven other side chain regioisomeric compounds shown in Figure 1. All compounds were synthesized using methods previously reported from our laboratory (4,5). Purification (recrystallization or

distillation) was done after each synthetic step, and product purity was checked by GC–MS. The hydrochloride salt of the final desired amine was recrystallized until GC–MS analysis showed only one peak. The mass spectra at each synthetic step show fragments and MW in agreement with the expected structures. All laboratory reagents and chemicals were obtained from either Aldrich Chemical Co. (Milwaukee, WI) or Fisher Scientific (Atlanta, GA). Derivatizing agents; Trifluoroacetic anhydride (TFA), Pentafluoropropionic anhydride (PFPA), and heptafluorobutyric anhydride (HFBA) were purchased from Sigma-Aldrich, Inc. (Milwaukee, WI).

Derivatization procedure

Each perfluoroamide was prepared individually from each of the regioisomeric amines by dissolving approximately 0.3 mg (1.45×10^{-6} mol) of each amine in 50 µL of ethyl acetate, followed by addition of a large excess (250 µL) of the appropriate derivatizing agent (TFA, PFPA, or HFBA), 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 air at 55°C and reconstituted with 200 µL of ethyl acetate and 50 µL of pyridine. A portion of each final solution (50 µL) was diluted with HPLC grade acetonitrile (200 µL) to give the working solutions.

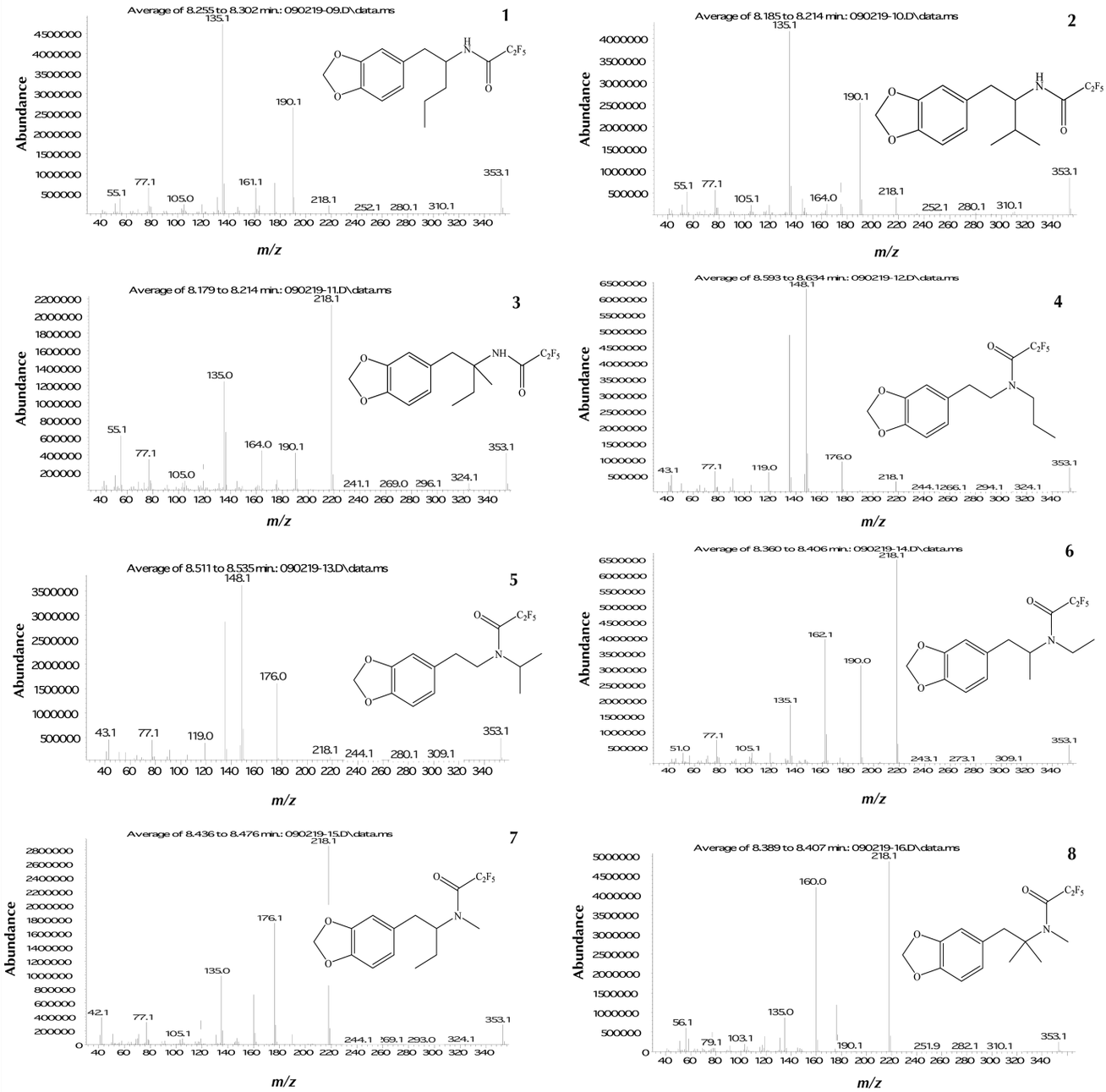

Figure 3. Mass spectra of the PFPA derivatives of compounds 1–8.

Results and Discussion

Mass spectral studies

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 2 shows representative electron impact mass spectra of the three drugs of abuse; MDEA, MBDB, and MDMMA, involved in the study (Compounds 6, 7, 9). The MS spectra of all 10 underivatized regioisomers were previously reported (4). The mass spectra of the 10 regioisomeric compounds (MW = 207) are characterized by a base peak at *m/z* 72 formed by an α-cleavage reaction involving the carbon–carbon bond of the ethyl linkage between the aromatic ring and amine nitrogen. Other less abundant peaks were observed at *m/z* 135/136 from the 3,4-methylenedioxybenzyl cation and radical cation fragments, respectively, as well as other ions of low relative abundance. The EI mass spectra of these regioisomers show some variation in the relative intensity of the major ions with only one or two minor ions that might be considered side-chain specific fragments (4). Thus, the ultimate identification of any one of these amines with the elimination of the other nine regioisomeric substances can not depend on their mass spectra alone (4). This lack of mass spectral specificity in addition to the possibility of chromatographic co-elution with any of the drugs of abuse; MDEA, MBDB, and MDMMA could result in misidentification of the target drug. Furthermore, the lack of available reference samples for the seven regioisomeric 3,4-methylenedioxyphenethylamines complicates the individual identification of any one of these substances. This constitutes a significant analytical challenge, where the specific identification by GC–MS must be based primarily upon the ability of the chromatographic system to separate the regioisomeric substance from the actual drug of interest. Additionally, the ability to distinguish between these regioisomers directly enhances the specificity of the analysis for the target drugs of interest.

Various perfluoroacyl derivatives of the regioisomeric primary and secondary amines (compounds 1–8) were prepared and evaluated in an effort to individualize their mass spectra and maintain or improve chromatographic resolution. Acylation of the amines significantly lowers the basicity of nitrogen and can allow other fragmentation pathways to play a more prominent role in the mass spectrum (6–11). The trifluoroacetyl, pentafluoropropionyl, and heptafluorobutryl derivatives of compounds 1-8 were evaluated for their ability to individualize the mass spectra through the formation of specific fragments. Compounds 9 and 10 are tertiary amines and do not form a stable amide derivative.

The mass spectra for the eight pentaflouropropionyl (PFPA) amides are shown in Figure 3. The spectra for the PFPA derivatives are representative of all the perfluoroacyl amides in this study. From these spectra, a common peak occurs at *m/z* 218, which corresponds to the loss of 135 mass units from the molecular ion at 353. This ion is the PFPA imine species, likely formed from the α-cleavage of the amide nitrogen to eliminate the 3,4-methylenedioxybenzyl radical. Thus, this ion is analogous to *m/z* 72 in the underivatized species because it represents the $(M–135)^+$ species.

The decreased role for the α-cleavage fragmentation of these amides allows the formation of more diagnostic ions for each individual isomer. Acylation, and in particular perflouroacylation, weakens the bond between nitrogen and the α-carbon of the substituted phenethyl group, allowing the formation of charged hydrocarbon species of increased relative abundance. These alkenes (radical cations) of varying mass are formed due to the transfer of a benzylic hydrogen to the ionized carbonyl oxygen followed by the loss of a neutral amide species (Figure 4). The resulting alkene radical cations (3,4-methylenedioxyphenylalkenes) significantly individualize the mass spectra and provide specific structural information. The mass spectra in Figure 3 illustrate the role of the alkene fragments at *m/z* 148, 162, 176, and 190 in identification of these regioisomers. These ions identify the number of carbons in

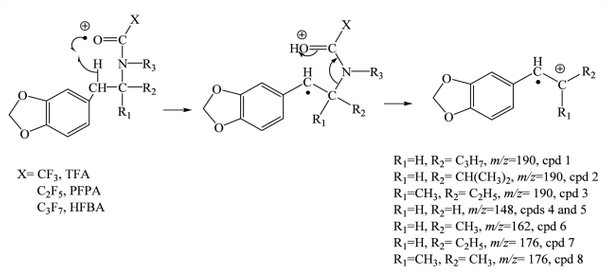

Figure 4. Formation of the alkene radical cation in the perfluoroacyl derivatives of the primary and secondary regioisomeric amines.

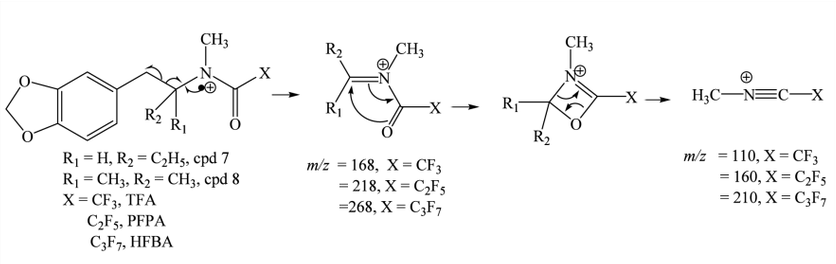

Figure 5. Formation of *m/z* 110, 160, 210 for compounds 7 and 8.

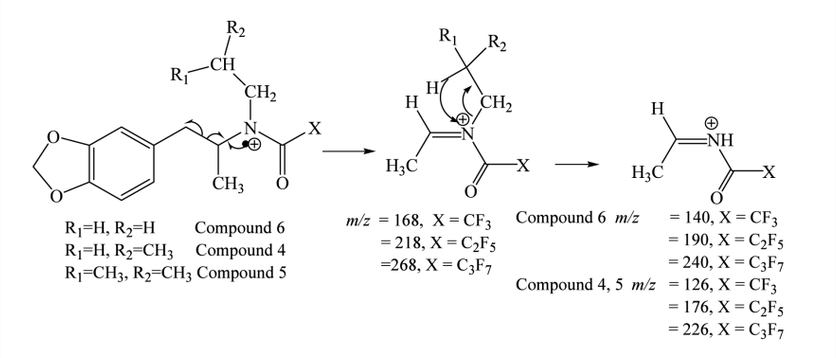

Figure 6. Formation of *m/z* 140, 190, and 240 for compound 6 and *m/z* 126, 176, and 226 for compounds 4 and 5.

the hydrocarbon chain attached directly to the aromatic ring in an uninterrupted manner.

Further examination of the mass spectra of the PFPA derivatives for the eight regioisomers (Figure 3) indicates unique ions at *m/z* 160 for both compounds 7 and 8. An analysis of the masses of the components, which make up the fragment at *m/z* 160 include C_2F_5 (119 mass units) and CH_3 (15 mass units), leaving only a mass of 26 available for the total of 160. The mass 26 would correspond to CN and the proposed mechanism for the formation of $(C_2F_5CNCH_3)^+$ is shown in Figure 5. The analogous ion occurs at *m/z* 110 and *m/z* 210 for the TFA and HFBA derivatives, respectively. These observations illustrate that the perfluo-

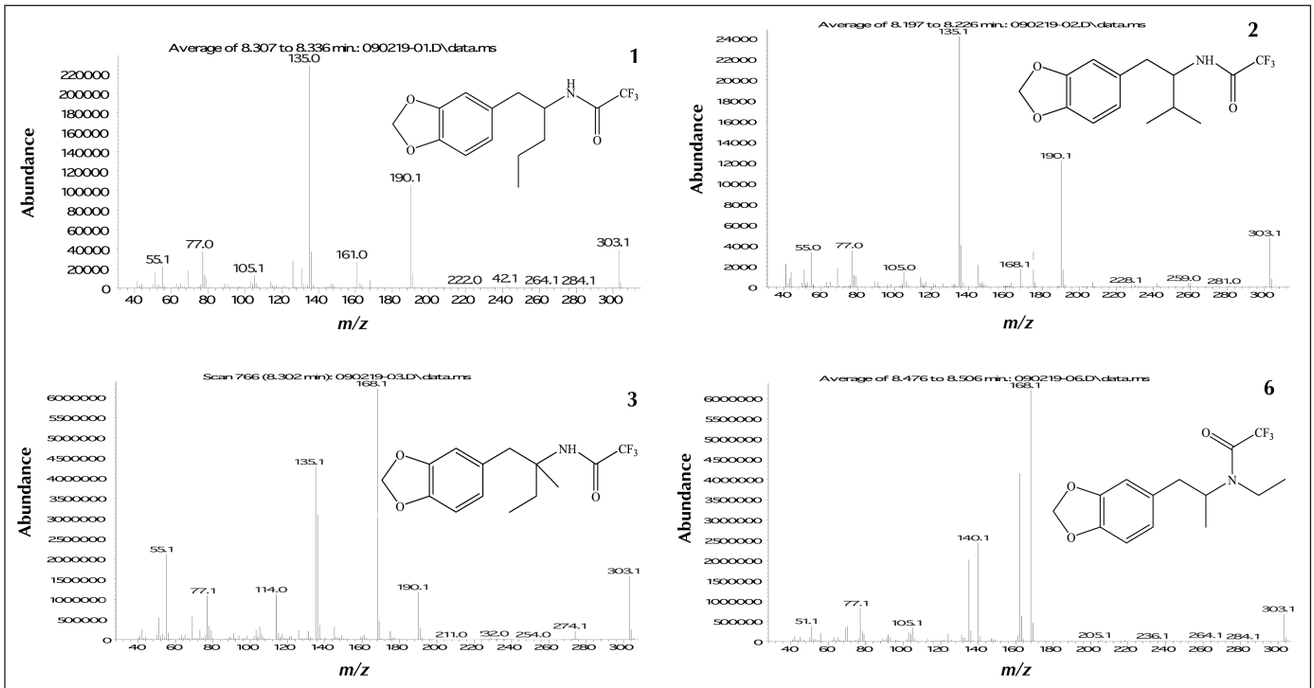

Figure 7. Mass spectra of the TFA derivatives of compounds 1–3 and 6.

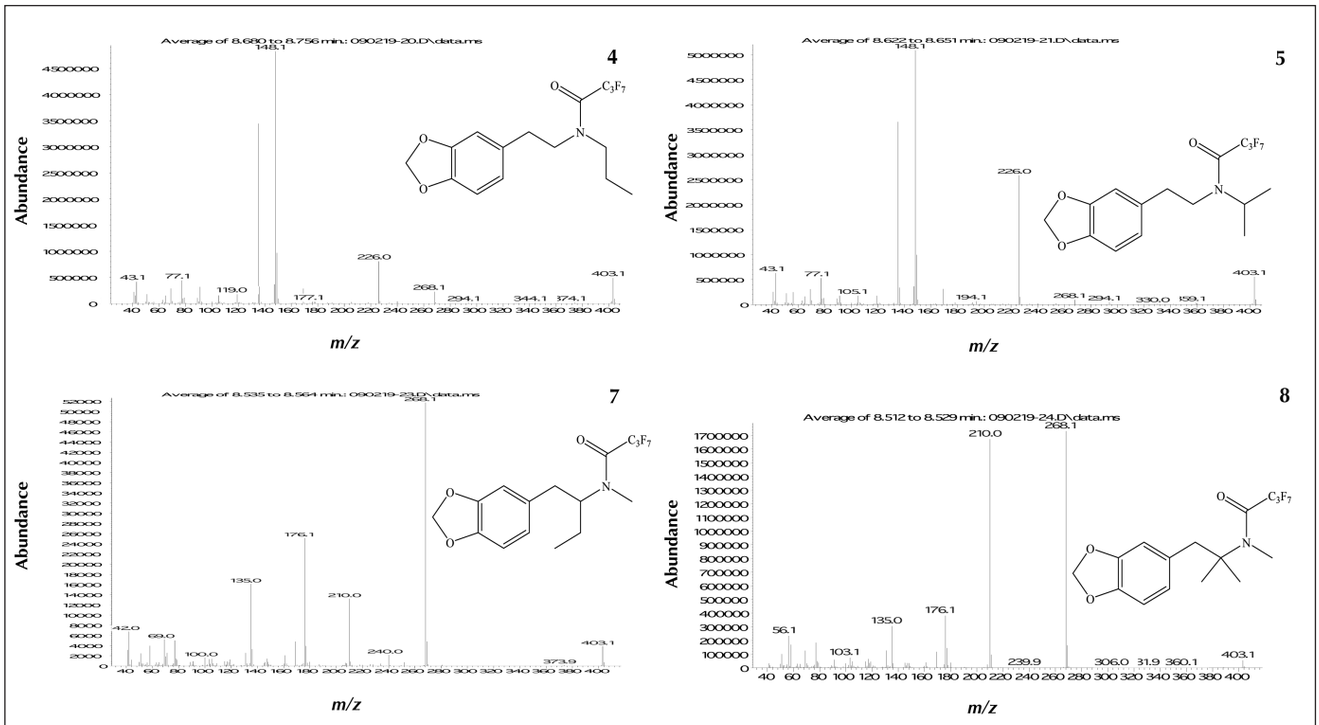

Figure 8. Mass spectra of the HFBA derivatives of the compounds 4, 5, 7, and 8.

roalkyl moiety is a component of this unique fragment. An equivalent fragmentation pathway has been previously reported (6) and this fragment appears to be characteristic of N-methyl substituted phenethylamine such as compound 7 and 8.

An important fragmentation pathway characteristic to the PFPA derivative of compound 6 (the only N-ethyl compound in this limited series) and compounds 4 and 5, which are n-propyl and isopropyl amines, is illustrated in Figure 6. This results in the formation of fragment ions at *m/z* 190 (compound 6) or *m/z* 176 (compounds 4, 5) for their PFPA amides. These ions came from the imine fragment at *m/z* 218 through hydrogen rearrangement and subsequent cleavage of the N-alkyl group on the nitrogen. This occurs only with the N-alkyl group of ethyl or larger.

Derivatization categorized this limited set of compounds into three subset groups depending on the mass recorded for the base peak. The first group includes compounds 1 and 2 having *m/z* 135 as the base peak in their PFPA derivatives. The second group comprises compounds 4 and 5 having a base peak at *m/z* 148. Finally, the third group includes compounds 3, 6, 7, and 8, which are characterized by having the fragment ions at *m/z* 218 as the base peak.

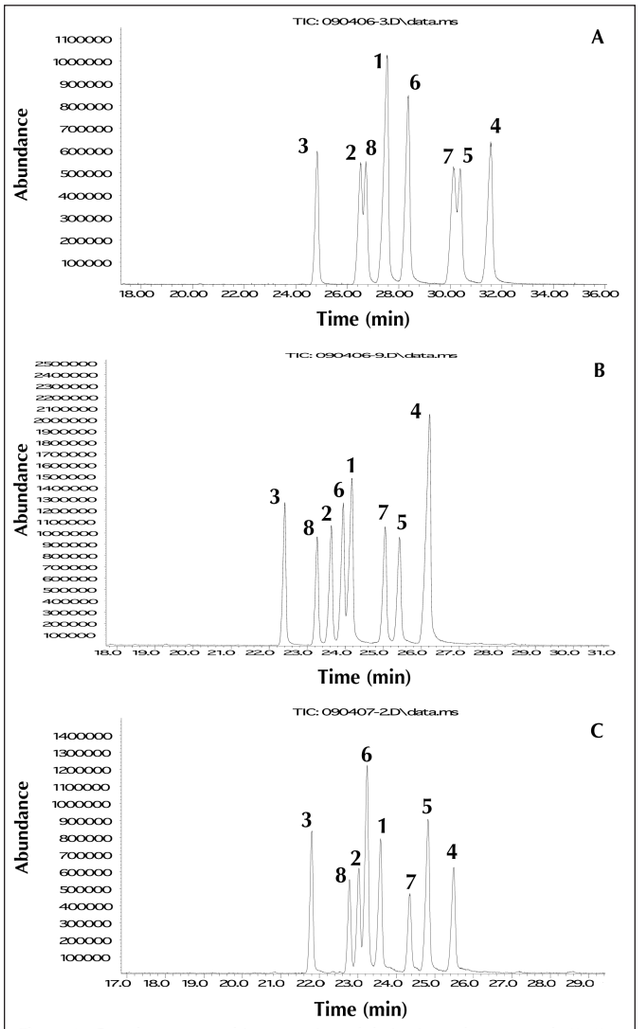

Figure 9. Gas chromatographic separation of derivatives of compounds (1–8). Chromatogram A = TFA; B = PFPA and C = HFBA. Column used; Rtx-200.

Moreover, derivatization also enables the discrimination among some members within the same group. For the second group, compounds 4 and 5 could be differentiated by the difference in the relative abundance of the fragment ions at *m/z* 176. Among the members of the third group, compounds 7 and 8, which are the only two N-methyl compounds in this series, could be characterized by the fragment ions at *m/z* 160. In addition, they can also be differentiated by the relative abundances of fragment ions at *m/z* 160 and 176.

The fragment ion *m/z* 190 in the mass spectra of the PFPA derivative of compound 6 (shown in figure 6) can be confused with the fragment ion of equal mass found in the mass spectra of the PFPA derivatives of compounds 1–3. However, these fragments are formed by different pathways. The *m/z* 190 ion for compounds 1–3 is the alkene radical cation shown in Figure 4. The *m/z* 190 fragment for compound 6 in Figure 6 contains the perfluoroalkyl moiety because the TFA and HFBA derivatives show analogous ions at *m/z* 140 and 240. Figure 7 shows representative mass spectra of the TFA derivatives of compounds 1–3 and 6.

In an analogous manner, the ion at *m/z* 176 can be formed through two different fragmentation pathways (Figures 4 and 6) in the PFPA derivatives of compounds 4 and 5 compared to 7 and 8. The use of TFA and HFBA as derivatizing agents resolved this problem by providing characteristic peaks at *m/z* 126 and 226 for the TFA and HFBA derivatives of compounds 4 and 5. Figure 8 shows representative mass spectra of the HFBA of these four compounds. Hence, TFA and HFBA derivatives provide more structure-specific fragment ions than the PFPA analog for these compounds.

Gas chromatography

GC–MS is a powerful tool that combines the identification power of mass spectrometry with the separation power of gas chromatography. The separation of a physical mixture of the compounds 1–10 in the underivatized form was the subject of a previous report from our laboratory (4). Several stationary phases and different temperature programs were evaluated in an effort to resolve the TFA, PFPA, and HFBA derivatives in this study. The non-polar stationary phase Rtx-1 as well as the semi-polar phase Rxi-50 did not offer complete resolution for this set of compounds. Some co-elution was observed on both columns for all evaluated temperature programs. The separation of these compounds was only achieved on the polar stationary phase Rtx-200 using the described temperature programs. The Rtx-200 phase provided adequate resolution for these compounds of similar retention properties; however, different temperature programs were necessary for resolving each set of perfluoroacyl derivatives. The three chromatograms in Figure 9 show the separation of all derivatized forms (TFA, PFPA, and HFBA) of compounds 1–8. The chromatogram shows that within the derivatives of the primary amines (compounds 1–3), the most branched side chain (compound 3) elutes first and the least branched (compound 1) elutes last. For the secondary amine derivatives (compounds 4–8), the perfluoroamides elute in a similar pattern. Thus the compound of greatest side chain branching (compound 8) elutes first followed by compound 6, 7, and 5, and the most linear tertiary amide (compound 4) elutes last. The resolution of the PFPA and HFBA derivatives was better

than that of the TFA derivatives. However both TFA and HFBA derivatives provide more structurally specific ions in the mass spectrum to help in discriminating among these compounds. Hence, the HFBA derivatives are considered the best choice to discriminate by mass spectrometry and to resolve by gas chromatography this set of regioisomeric phenethylamine derivatives.

Conclusion

Three regioisomeric methylenedioxyphenethylamines (MDEA, MDMMA, and MBDB), having the same molecular weight and major mass spectral fragments of equivalent mass, have been reported as drugs of abuse. Seven additional phenethylamines have a side chain regioisomeric relationship with the controlled substances. Derivatization of the eight primary and secondary amines with various perfluoroacylating agents yields amides which significantly individualized their mass spectra and allowed for specific side chain identification. The individualization is the result of formation of unique marker ions or as a result of differences in the relative abundances of some common ions. The trifluoroacetyl and heptafluorobutryl derivatives offer more unique fragment ions than the pentafluoropropionyl derivatives. Chromatographic resolution of the perfluoroacyl amides was achieved on a relatively polar stationary phase Rtx-200.

Acknowledgement

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