GC–MS Analysis of Acylated Derivatives of The Side Chain and Ring Regioisomers of Methylenedioxymethamphetamine

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Abstract

The perfluoroacyl derivatives (pentafluoropropionylamides and heptafluorobutrylamides) of the primary and secondary regioisomeric amines, related to the controlled drug substance 3,4-methylenedioxymethamphetamine, are prepared and evaluated in GC–MS studies. These derivatives show excellent resolution on nonpolar stationary phases, such as RTX-1 and RTX-5, with elution order differences from those of the underivatized amines. The mass spectra for these derivatives are significantly individualized, and the resulting unique fragment ions allow for specific side-chain identification. The individualization is the result of fragmentation of the alkyl carbon–nitrogen bond, yielding hydrocarbon fragments and other unique ions. The heptafluoro butrylamides derivatives offer more fragment ions for molecular individualization among these regioisomeric substances.

Introduction

Previous reports (1–5) in this series have described the analytical properties of a group of compounds that have unique regioisomeric equivalence to the drug of abuse 3,4-methylenedioxymethamphetamine (3,4-MDMA). Mass spectral (MS) studies (1) on the 10 compounds (Figure 1) have shown that all major fragment ions and the molecular ion occur at equivalent masses. These results illustrate that the MS alone cannot be used to identify an individual compound within this group to the exclusion of all others. This was further illustrated by the observations (1,3) that other compounds in this series coeluted with 3,4-MDMA (3,4-MDMA is compound 3 in Figure 1) when using some common gas chromatographic (GC) stationary phases and conditions. The individual MS for the 10 uniquely regioisomeric compounds of molecular weight 193 and major fragment ions at *m/z* 58 and 135/136 are available in a previous publication (1). Additional studies (3) have identified capillary GC phases and conditions for the complete resolution of compounds 1–10. Optimum separation was obtained using a 35% phenylmethylsilicone phase (DB35MS) and temperature programming conditions determined by retention modeling software. Although these studies have shown that all 10 compounds can be resolved, the lack of MS specificity makes the specific identification of 3,4-MDMA (with the exclusion of all other regioisomers) a significant challenge. The lack of available reference samples for all 10 of these regioisomeric molecules further complicates the individual identification of any one of these substances. When other compounds exist with the potential to produce the same or nearly identical MS as the drug of interest, the identification by GC–MS must be based primarily upon the ability of the chromatographic system to separate the "counterfeit substance" from the actual drug of interest. If not, those substances coeluting with the target drug in chromatographic systems

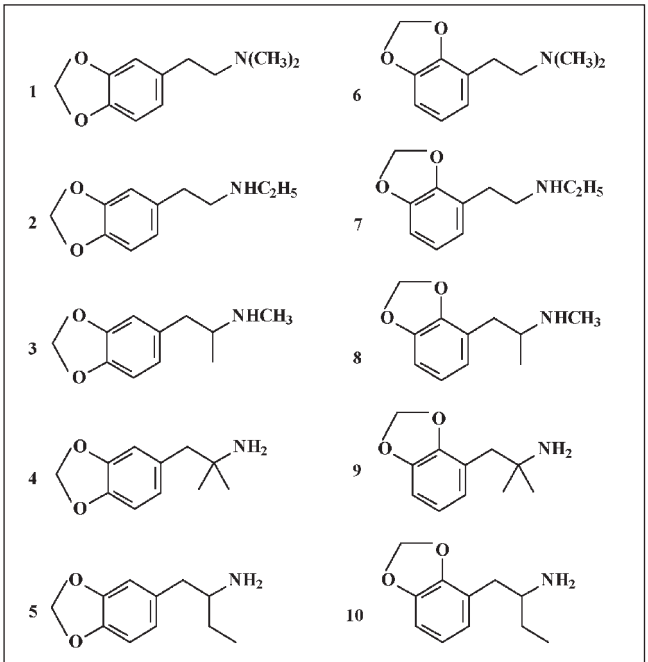

Figure 1. Structures of the regioisomeric 3,4- and 2,3-MDMA; molecular weight 193; and *m/z* 58, 135/136.

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could be misidentified as the target drug. Without the appropriate standards, a thorough method validation is not possible, and thus coelution of drug and nondrug combinations would remain a possibility. Furthermore, the ability to distinguish between these regioisomers directly enhances the specificity of the analysis for the target drugs of interest.

There are nine other methylenedioxy-substituted phenethylamines with the potential to produce an MS essentially the same as 3,4-MDMA (Figure 1), and the precursor substances exist to prepare all nine of these "counterfeit molecules". Some of these compounds are pharmacologically inactive and others have unknown pharmacological properties, yet all have the strong possibility of being identified as 3,4-MDMA by some commonly used analytical methods. In this project, the perfluoroacylated derivatives of the eight primary and secondary amines (Figure 1, compounds 2–5, 7–10) will be prepared and evaluated for their ability to individualize the GC–MS properties of the compounds in this uniquely regioisomeric series. Of course, the two tertiary amines (compounds 1 and 6) would not form stable amide derivatives. The compound numbers shown in Figure 1 have been used in all previous publications on this series of compounds and will be continued in this report (1–3).

Experimental

GC–MS analysis was performed with an HP-5890 GC coupled with a HP-5970 mass selective detector (Hewlett Packard, Palo Alto, CA). The MS was operated on the electron impact (EI) mode using ionization voltage of 70 eV and a source of temperature of 280°C. Samples were dissolved in high-performance liquid chromatography-grade acetonitrile (Fisher Scientific, Fairlawn, NJ) and manually introduced (1 µL) individually and in a physical mixture using a 10-µL Hamilton syringe (Hamilton Co., Reno, NV).

The separation was carried out on a column (30-m × 0.25-mm i.d.) coated with 0.25 µm 100% dimethyl polysiloxane (RTX-1) and a column (30-m × 0.25-mm i.d.) coated with 0.25 µm 95% dimethyl–5% diphenyl polysiloxane (RTX-5) purchased from Restek corporation (Bellefonte, PA).

The retention data in Table I were generated using two temperature programs. Program 1 consisted of an initial hold at 100°C for 1 min, ramped up to 180°C at a rate of 9°C/min followed by a hold at 180°C for 2 min then ramped to 200°C at a rate of 10°C/min. Program 2 [used to separate the heptaflurobutric anhydride (HFBA) on the RTX-1 column] started with an initial hold at 70°C for 1 min, ramped up to 150°C at a rate of 7.5°C/min, held at 150°C for 2 min, and finally ramped to 250°C at a rate of 10°C/min.

Drugs and reagents

Samples of 3,4-MDMA and its regioisomers were synthesized as previously described (1). 3,4-MDMA-d_3 was prepared in an analogous manner using methylamine-d_3 obtained from Aldrich Chemical Co. (Milwaukee, WI). 3,4-MDMA-d_5 was purchased from Cerilliant (Round Rock, TX). Other laboratory reagents and chemicals were obtained from Aldrich Chemical Co. or Fisher Scientific (Atlanta, GA). Pentafluropropionic anhydride (PFPA) and HFBA were purchased from UCT (Bristol, PA)

Derivatization Procedure

Each perfluoroamide was prepared individually from the hydrochloride salts of the regioisomers by dissolving approximately 0.3 mg (1.33×10^{-5} mol) of each amine in 50 µL of ethyl acetate, followed by addition of large excess (250 µL) of the appropriate derivatizing agent (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 volume of 1 µL of each reconstituted derivative was injected into the GC.

Table I. GC Relative Retention Data for the Regioisomeric Substances

	RTX-1*			RTX-5†		
		Derivatives			Derivatives	
Compound number	Underivatized‡	HFBA derivatives‡	PFPA derivatives§	Underivatized§	HFBA derivatives§	PFPA derivatives§
2	1.021	1.017	1.033	1.044	1.023	1.021
3	1.0 (19.731 min)	1.0 (24.087 min)	1.0 (13.029 min)	1.0 (10.171 min)	1.0 (14.01 min)	1.0 (13.596 min)
4	0.923	0.936	0.877	0.984	0.887	0.872
5	0.975	0.978	0.951	1.083	0.954	0.958
7	0.972	0.983	0.967	1.079	0.959	0.966
8	0.942	0.960	0.923	0.955	0.934	0.926
9	0.885	0.903	0.833	0.918	0.828	0.861
10	0.926	0.940	0.884	0.982	0.895	0.879

* RTX-1 is a 30-m × 0.25-mm i.d. column coated with 0.25-µm 100% dimethyl polysiloxane.
† RTX-5 is a 30-m × 0.25-mm i.d. column coated with 0.25-µm 95% dimethyl–5%diphenyl polysiloxane.
‡ Temperature program used was to hold the column temperature at 100°C for 1 min, ramped to 180°C at 9°C/min, hold at 180°C for 2 min, ramp to 200°C at 10°C/min.
§ Temperature program used was to hold the column temperature at 70°C for 1 min, ramped to 150°C at 7.5°C/min, hold at 150°C for 2 min, ramp to 250°C at 10 °C/min.

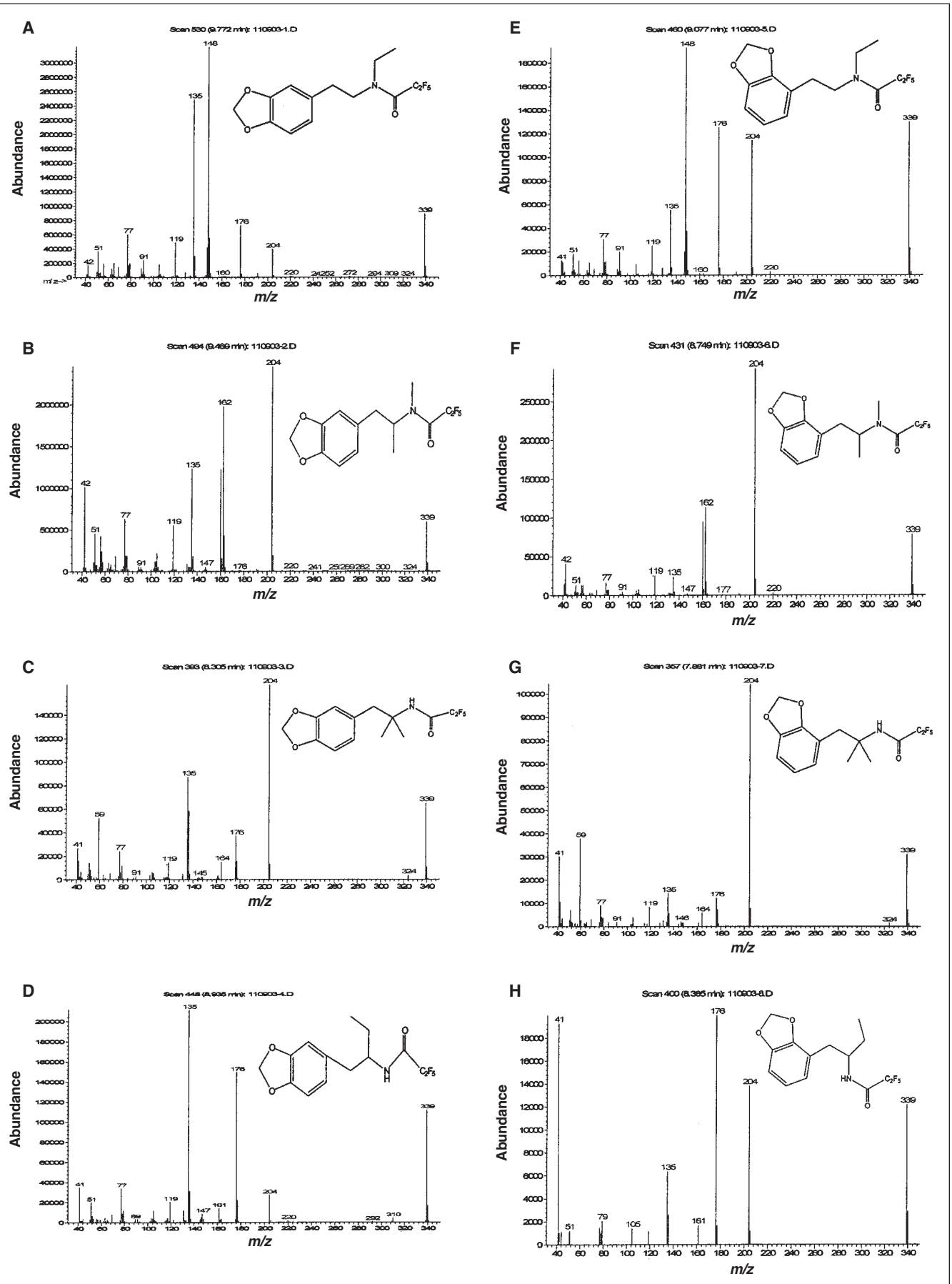

Figure 2. MS of PFPA derivatives of compounds 2–5 and 7–10.

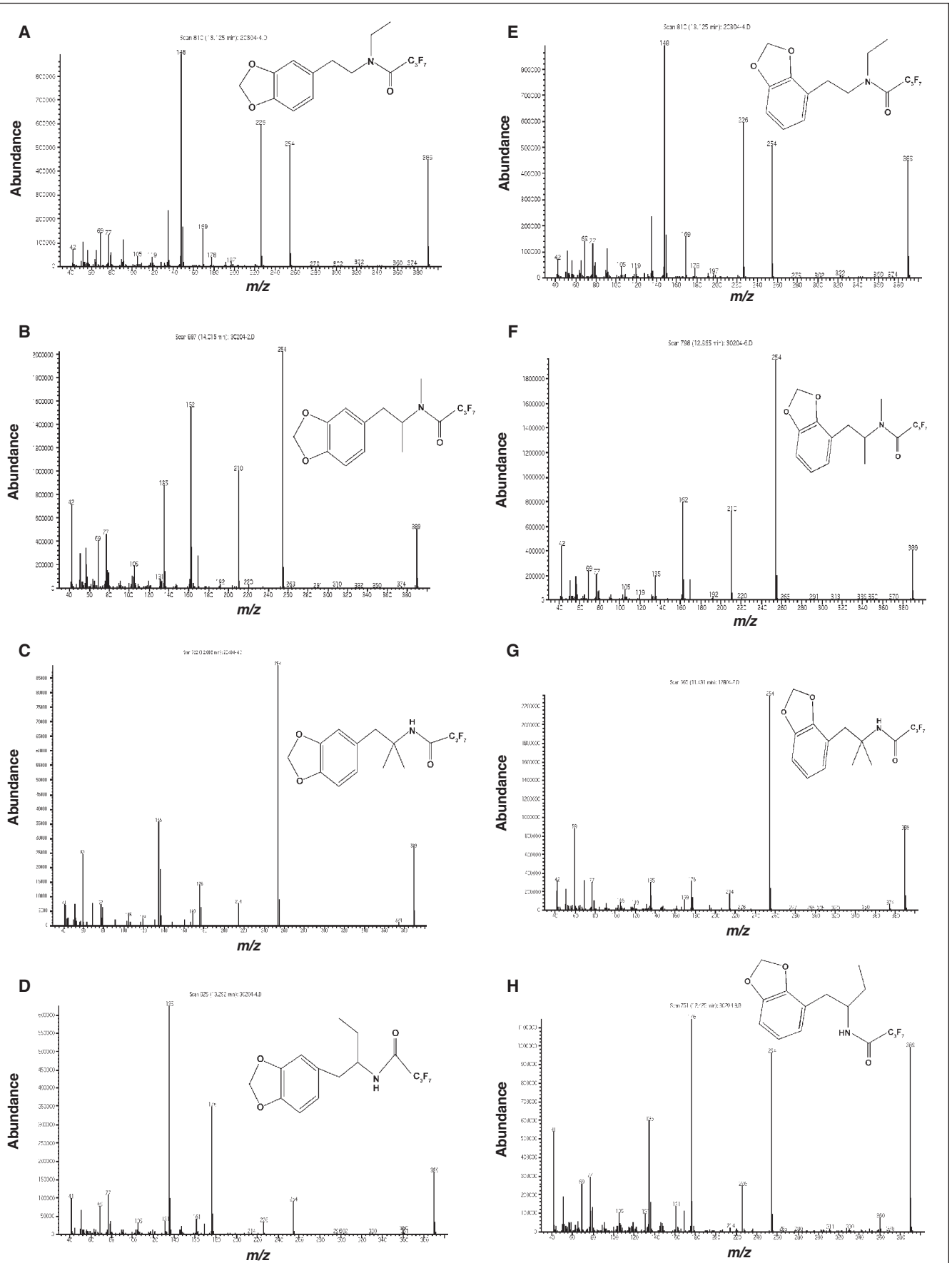

Figure 3. MS for the HFBA derivatives of compounds 2–5 and 7–10.

Results and Discussion

Synthesis

The methods for the preparation of the 10 2,3- and 3,4-methylenedioxy-regioisomers have been described in previous reports (1,3,6–8). The general procedure for the synthesis of these compounds begins with the appropriate aldehyde, 2,3-methylenedioxybenzaldehyde and 3,4-methylenedioxybenzaldehyde (piperonal), as starting materials. The preparation of 2,3-methylenedioxybenzaldehyde has been reported previously (1,8). Condensation of the appropriate aldehyde with a nitroalkane (nitromethane, nitroethane, or 1-nitropropane) under basic conditions yields the 2-nitroalkenes, which can be reduced to the primary amines or hydrolyzed to the corresponding ketones and reductively aminated. The 1-(3,4- and 2,3-methylenedioxyphenyl)-2,2-dimethylethanamines, compounds 4 and 9, were prepared in a multi-step procedure as previously described (1).

MS

MS is the primary method for confirming the identity of drugs and other substances of abuse in forensic samples. The mass spectrum of phenethylamine drugs of abuse including 3,4-MDMA is characterized by a base peak formed by an α-cleavage reaction involving the carbon–carbon bond of the ethyl linkage between the aromatic ring and amine. In 3,4-MDMA (molecular weight = 193) the α-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).

In the present study, various perfluoroacylated derivatives of the regioisomeric primary and secondary amines were prepared and evaluated in an effort to individualize their mass spectra and maintain or improve chromatographic resolution. The pentafluoropropionyl and heptafluorobutryl derivatives of compounds 2–5 and 7–10 were evaluated for their ability to individualize the mass spectra of 3,4-MDMA and the isomeric methylenedioxyphenethylamines. 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 (9).

The mass spectra for the eight pentaflouropropionyl and heptaflourobutryl amides are shown in Figures 2 and 3, 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 α-cleavage of the amide nitrogen to eliminate the 2,3- and 3,4-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. The general fragmentation pattern and structures for the *m/z* 204 and 254 ions are shown in Figure 4. The methylenedioxybenzyl cation at *m/z* 135 is a fragment common to all the spectra in Figures 2 and 3.

The decreased role for α-cleavage reaction in the fragmentation of these amides allows the formation of ions more diagnostic of each individual isomer. Acylation, and in particular the perflouroacylation, weakens the bond between nitrogen and the α-carbon of the substituted methylenedioxyphenethyl group, allowing the formation of charged hydrocarbon species of increased relative abundance. These hydrocarbons of varying mass significantly individualize the mass spectra and provide specific structural information. The mass spectra in Figures 2 and 3 illustrate the role of hydrocarbon fragments at *m/z* 148, 162, and 176 in the EI MS differentiation among these regioisomers.

The spectra for the *N*-ethyl derivatives in Figures 2A, 2E, 3A,

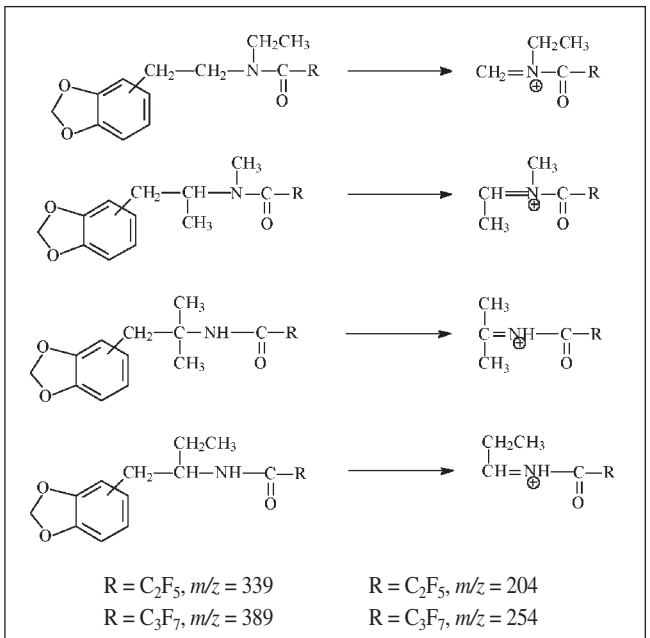

Figure 4. Formations of the $(M–135)^+$ ions in the perfluoroacyl-derivatives of the regioisomeric amines.

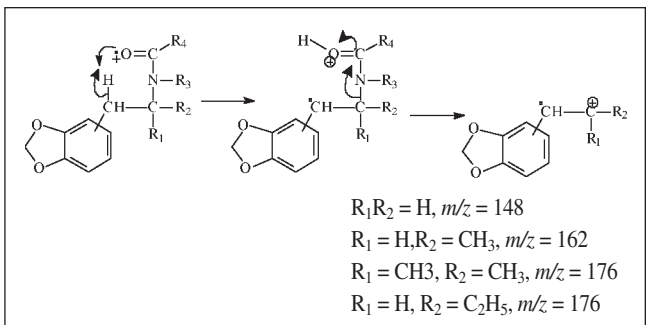

Figure 5. Mechanism for the formation of the alkene radical cation in the perfluoroacyl-derivatives of the regioisomeric amines.

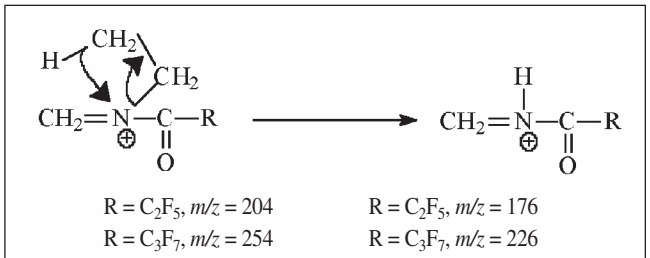

Figure 6. Mechanism for the loss of mass 28 from the perfluoroacyl-*N*-ethyl-imine cation.

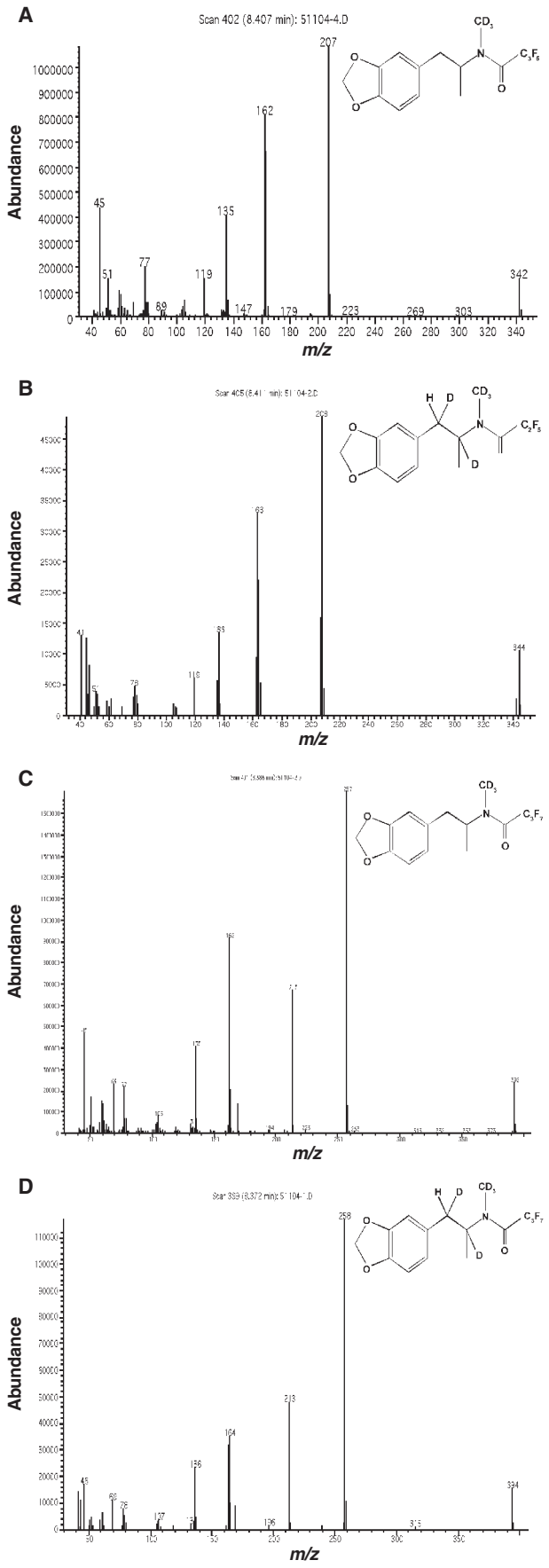

Figure 7. MS of the PFPA and HFBA derivatives for 3,4-MDMA-d_3 and -d_5.

and 3E show a base peak at *m/z* 148 corresponding to the alkene radical cation, which occurs from hydrogen rearrangement and subsequent fragmentation of the alkyl carbon to nitrogen bond of the phenethylamine side chain (see Figure 5). This ion at *m/z* 148 would only occur for the *N*-ethyl regioisomer. The spectra in Figures 2B, 2F, 3B, and 3F show the 2,3- and 3,4-methyelenedioxyphenylpropane hydrocarbon ion at *m/z* 162, identifying these molecules as the PFPA and HFBA derivatives of 2,3- and 3,4-MDMA, respectively. The proposed mechanism for the formation of the hydrocarbon fragment is illustrated in Figure 5. 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 corresponding 2,3- or 3,4-methylenedioxyphenyl alkyl radical cation. This *m/z* 176 results from hydrogen rearrangement and subsequent fragmentation of alkyl carbon to nitrogen bond. The lower abundance of *m/z* 176 for the 2,3- and 3,4-methylenedioxyphentermines (compounds 4 and 9) may be attributed to steric inhibition of hydrogen transfer in the α,α-dimethyl substitution pattern.

Although 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 2A and 2E) is the appearance of an ion at *m/z* 176, in addition to the base peak at *m/z* 148. The 176 ion suggests a four-carbon chain directly attached to the aromatic ring as occurs for the α-ethyl- and α,α-dimethyl-phenethylamines (Figures 2C, 2D, 2G, 2H, 3C, 3D, 3G, and 3H). The *m/z* 176 ion in the spectra for the PFPA

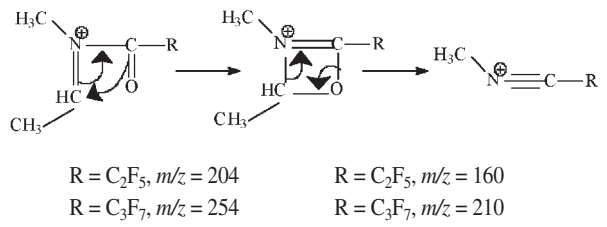

Figure 8. Mechanism for the formation of the *m/z* 160 and 210 ions in the spectrum of the perfluoroacyl-derivatives of 3,4-MDMA.

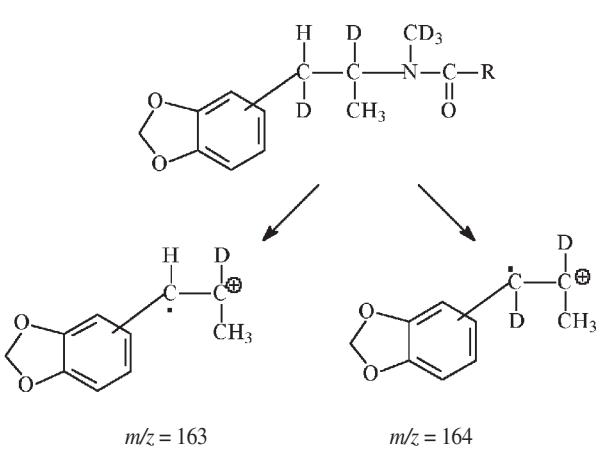

Figure 9. Formation of *m/z* 163 and 164 in perfluoroacyl-derivatives of 3,4-MDMA-d_5

derivatives of the *N*-ethyl regioisomers (Figures 2A and 2E) is a rearrangement of the *m/z* 204 ion, resulting in the loss of mass 28 (the *N*-ethyl group) via hydrogen transfer (see Figure 6). This coincidental common mass from two different fragmentation pathways is confirmed by examining the MS for the HFBA derivatives of the *N*-ethyl-phenethylamines shown in Figures 3A and 3E. 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.

A comparison of the PFPA derivatives for 3,4- and 2,3-MDMA (Figures 2B and 2F) with the HFBA derivatives (Figures 3B and 3F) indicates unique ions at *m/z* 160 and 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 can be obtained by a comparison of the MS for the PFPA and HFBA derivatives of 3,4-MDMA and NCD_3-3,4-MDMA (3,4-MDMA-d_3) in Figure 7. The corresponding ions in these spectra occur at *m/z* 163 and 213, and the equivalent ions for the derivatives of 3,4-MDMA-d_5 also occur at *m/z* 163 and 213. Thus, an analysis of the masses of the components, which make up the fragment at *m/z* 160, include for example 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 8. An equivalent fragmentation pathway has been reported (10) for methamphetamine.

The spectra for the derivatives of 3,4-MDMA-d_3 and -d_5 in Figure 7 also lend support to the proposed mechanism for the formation of the alkene fragment at *m/z* 162 (Figure 5). The exact structure for the 3,4-MDMA-d_5 is shown in Figure 9; the benzylic position contains one hydrogen and one deuterium, and the transfer fragmentation can occur to remove either species to form the alkene radical cation. Thus, the resulting alkene can yield ions at *m/z* 163 or 164, depending on the probability of transfer.

GC

The PFPA and HFBA derivatives of the eight primary and secondary amines were compared on two stationary phases using capillary columns of the same dimensions (30 m × 0.25 mm, 0.25-µm film thickness). Previous studies on the chromatographic properties of the underivatized compounds (1,3) have shown that other compounds in this series coeluted with 3,4-MDMA, using some common GC stationary phases and conditions. Table I shows the relative retention of these compounds compared with *N*-methyl-3, 4-methyelenedioxyphenyl-2-propanamine (3,4-MDMA) under identical chromatographic conditions. The stationary phases compared in this study were the relatively nonpolar phases, 100% dimethyl polysiloxane (RTX-1), and 95% dimethyl–5% diphenyl polysiloxane (RTX-5). Several temperature programs were evaluated, and the best compromises between resolution and analysis time were used to generate the data in Table I and the chromatograms in Figures 10 and 11. The two chromatograms for the PFPA derivatives in Figure 10 were generated using identical temperature programs. The resulting elution order and resolution are quite similar. In

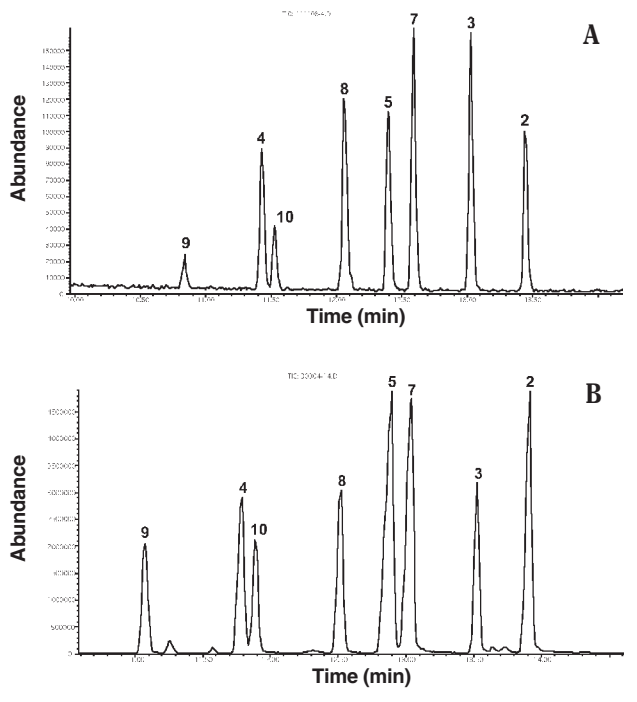

Figure 10. Capillary GC separation of PFPAs of the eight regioisomeric primary and secondary methyenedioxyphenethyl amines. Columns used: (A) RTX-1; (B) RTX-5. Peaks: (9) 2,3-phentermine-PFPA; (4) 3,4-phentermine-PFPA; (10) 2,3-BDB-PFPA; (8) 2,3-MDMA-PFPA; (5) 3,4-BDB-PFPA; (7) *N*-ethyl 2,3-methylenedioxyphenethyl amine-PFPA; (3) 3,4-MDMA-PFPA; and (2) *N*-ethyl-3,4-methylenedioxyphenethylamine-PFPA.

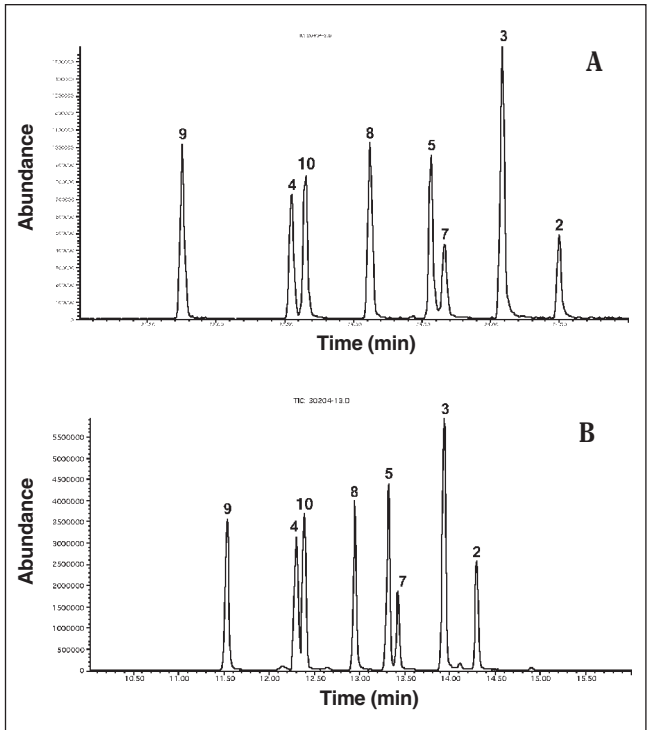

Figure 11. Capillary GC separation of HFBAs of the eight regioisomeric primary and secondary methyenedioxyphenethyl amines. Columns used: (A) RTX-1; (B) RTX-5. Peaks: (9) 2,3-phentermine-HFBA; (4) 3,4-phentermine-HFBA; (10) 2,3-BDB- HFBA; (8) 2,3-MDMA-HFBA; (5) 3,4-BDB-HFBA; (7) *N*-ethyl 2,3-methylenedioxyphenethyl amine-HFBA; (3) 3,4-MDMA-HFBA; (2) *N*-ethyl-3,4-methylenedioxyphenethyl amine-HFBA.

fact, the elution order is the same for all the chromatograms in Figures 10 and 11. The chromatograms show that the 2,3-isomer elutes before the corresponding 3,4-isomer for all the side chain regioisomers. For example, 2,3-MDMA elutes before 3,4-MDMA. When the ring substitution pattern is held constant (i.e., 2,3- or 3,4-) and the side-chain elution order is evaluated, 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. Therefore, the 2,3-phentermine-PFPA elutes first, followed by 2,3-BDB-PFPA (both secondary amides), then 2,3-MDMA-PFPA and *N*-ethyl 2,3-methylenedioxyphenethylamine-PFPA, the two tertiary amides. 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 between the *N*-ethyl-2,3- and 3,4-methylenedioxyphenethylamine PFPAs and HFBAs, both 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 both chromatographic and mass spectral procedures can allow for the complete characterization of the side-chain substitution pattern for these 10 uniquely isomeric substances.

Conclusion

3,4-MDMA and nine other methylenedioxyphenethylamines are a unique subset of regioisomeric molecules; each compound has a molecular weight of 193 and yields a base peak at *m/z* 58 in the MS from the loss of the corresponding methylenedioxybenzyl group. Thus, the traditional EI MS provides little structural information for differentiating among these 10 compounds. Because of the unique similarity of these compounds by MS, the specific identification of a compound such as 3,4-MDMA requires methods to eliminate any of the other nine isomers.

This elimination process may be accomplished on the basis of chromatography alone but would ultimately require the analyst to use reference samples of each of the 10 amines. The reference samples would be necessary to determine if any of the isomeric methylenedioxyphenethylamines coeluted with 3,4-MDMA. Derivatization of the eight primary and secondary amines with various acylating agents yields amides with similar resolution to the underivatized amines by capillary GC on RTX-1 and RTX-5 stationary phases. However the perfluoroacyl derivatives significantly individualize the mass spectra for these amides 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.

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Manuscript received August 18, 2004;
revision received April 11, 2005.