GC–MS Analysis of Acylated Derivatives of a Series of Side Chain Regioisomers of 2-Methoxy-4-Methyl-Phenethylamines

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Abstract

Five side chain regioisomers of 2-methoxy-4-methylphenethylamine constitute a unique set of compounds having an isobaric relationship with the controlled drug substance 3,4-methylenedioxymethamphetamine (3,4-MDMA or Ecstasy). These isomeric forms of the 2-methoxy-4-methyl-phenethylamines have mass spectra 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. Mass spectral differentiation of 2,3 and 3,4-MDMA from primary and secondary amine regioisomeric side chains of 2-methoxy-4-methyl-phenethylamines was possible after formation of the perfluoroacyl derivatives, pentafluoropropionamides (PFPA) and heptafluorobutyrylamides (HFBA). The mass spectra for these derivatives are 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 unique hydrocarbon fragments of varying mass. Gas chromatographic separation on relatively non-polar stationary phases gave essentially base line resolution for these compounds.

Introduction

Regioisomeric differentiation in the methylenedioxyphenethylamine series of drugs has been an issue in forensic drug chemistry for many years (1,2). Soine et al. (1) was among the first to report the differentiation of 2,3-methylenedioxyamphetamine from 3,4-methylenedioxyamphetamine. Previous studies (3–6) in this series have shown that the ten direct regioisomeric substances, 3,4-methylenedioxymethamphetamine (3,4-MDMA, Ecstasy) and its nine regioisomeric equivalents, have identical molecular weights and mass spectral fragments of equivalent mass-to-charge ratios. Therefore direct analysis of these regioisomers by electron ionization mass spectrometry does not provide data for the specific differentiation and identification of one of these regioisomers to the exclusion of all other isomers. All ten of the direct regioisomeric compounds of MW=193 showed major fragment ions for the imine at *m/z* 58 and the substituted benzyl fragment at *m/z* 135/136. Thus, specific identification must be based on a combination of mass spectral data as well as chromatographic resolution of these regioisomeric substances. Further studies have demonstrated that some of these compounds have very similar gas chromatographic retention properties (3). 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 MDMA (6). Additional background information on the structures of these ten regioisomeric substances as well as their individual mass spectra and chromatographic properties can be found in references 3 and 4.

A previous report (7) described the preparation and analytical evaluation of the ring substituted methoxy methyl methamphetamines, a series of isobaric compounds related to 3,4-MDMA. The ten methoxy methyl methamphetamines were compared to 2,3- and 3,4-MDMA; all twelve of these compounds have the same side chain structure generating the same *m/z* 58 ion, the base peak in the electron ionization mass spectrum for these amines. The results of this study suggested that some substitution patterns may provide unique fragment ions to differentiate methylenedioxyphenyl-isomers form methoxy methylphenyl-isomers following perfluoroacyl-derivatization. The variation in fragmentation pattern appeared to be based upon the ring substitution pattern of the methoxy group relative to the alkylamine side chain.

The present work focuses on the preparation and analytical evaluation of an entire side chain series with the methoxy group in the 2-position relative to the alkylamine side chain. The 2-methoxy-4-methyl phenethylamines (Figure 1) have the same nominal mass but different elemental composition (isobaric substances), yet they are expected to yield major mass spectral fragments of equivalent mass to those observed for 2,3- and 3,4-MDMA (Figure 2). This report describes a second study with an overall goal (7) of evaluating 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

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side-chain. The first report (8) of this series described the analytical properties of the side chain regioisomers of the 4-methoxy-3-methyl phenethylamines.

Experimental

Analytical

Analytical studies were conducted using two gas chromatography–mass spectrometry (GC–MS) systems. System 1 consisted of an HP-5890 GC coupled with a HP-5970 mass selective detector (Hewlett Packard, Palo Alto, CA). The mass spectral scan rate was 1.2 scan per second. The GC was operated in splitless mode with a flow rate of 1.37 mL/min and a column head pressure of 10 psi using helium grade 5 as carrier gas. Samples were injected manually using a 10 µL Hamilton syringe (Hamilton Co., Reno NV).

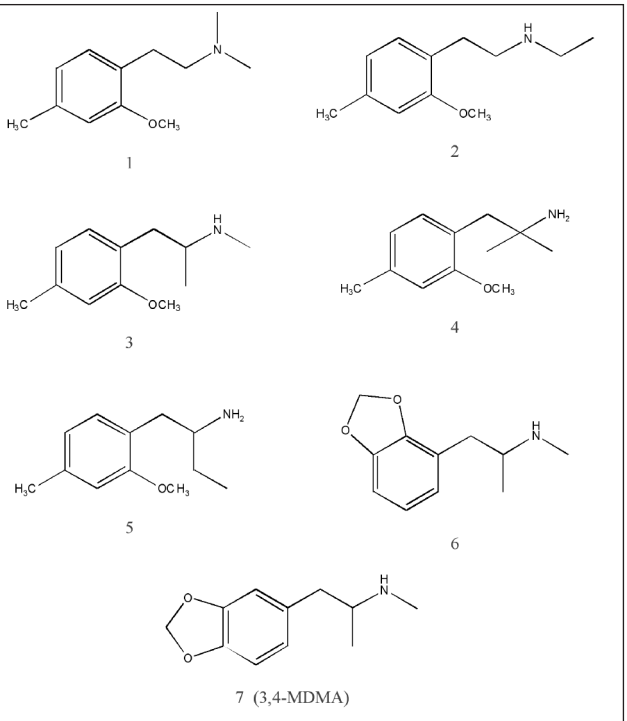

Figure 1. Structures of the side chain regioisomers of 2-methoxy-4-methyl-phenethylamines, and 2,3- and 3,4-MDMA.

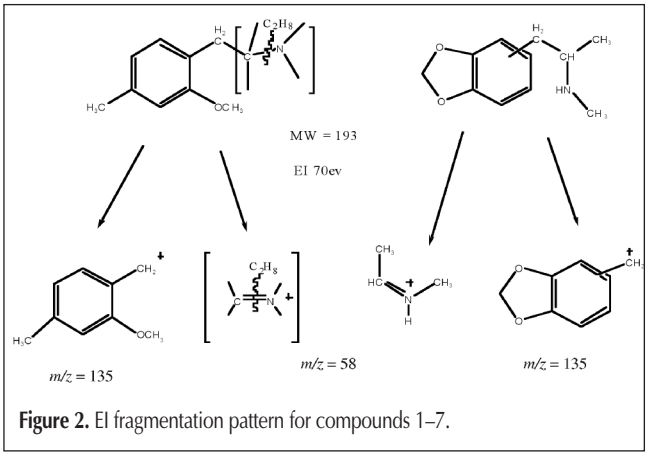

Figure 2. EI fragmentation pattern for compounds 1–7.

System 2 consisted of an 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 per second. The GC was operated in splitless mode with a carrier gas (helium grade 5) flow rate of 0.7 mL/min and a column head pressure of 10 psi (70 kPa).

In both GC–MS systems 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 NJ) and introduced (1 µL) manually (System 1) or via auto injector (System 2) as individual solutions and in physical mixture.

Retention data collection and GC separations were carried out on a 30 m × 0.25 mm i.d. column coated with 0.25 µm 100% dimethyl polysiloxane (Rtx-1) and a 30 m × 0.25 mm-i.d. column coated with 0.25 µm trifluoropropyl methyl polysiloxane (Rtx-200) purchased from Restek corporation (Bellefonte, PA). The example chromatograms in Figure 6 were generated using a temperature program consisting of an initial temperature hold at 100°C for 1 minute, ramped up to 180°C at a rate of 9°C per min followed by a hold at 180°C for 2 min then ramped to 200°C at a rate of 10°C per min.

Drugs and reagents

All laboratory reagents and chemicals were obtained from Aldrich Chemical Company 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 (3,5,8) from this laboratory. The synthetic procedures all used the corresponding ring substituted benzaldehydes as the starting precursor substance and a general synthetic method is described below.

Derivatization procedure

Each perfluoroamide 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). 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.

General synthetic methods

Methylation of 4-methyl salicylic acid afforded methyl-2-methoxy-4-methyl benzoate which upon reduction with RedAl (sodium *bis*-2-methoxy-ethoxy-aluminum hydride) yielded 2-methoxy-4-methylbenzyl alcohol. Selective oxidation of the alcohol with pyridinium chlorochromate (PCC) and celite gave 2-methoxy-4-methyl benzaldehyde. Condensation of the alde-

hyde with a nitroalkane (nitromethane, nitroethane, or 1-nitropropane) under basic conditions yields the 1-(2-methoxy-4-methylphenyl)-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 nitroalkanes are hydrolyzed to the corresponding 2-methoxy-4-methylphenyl-ketones and reductively aminated with methyl-, dimethyl-, or ethylamine in the presence of sodium cyanoborohydride. The 1-(2-methoxy-4-methylphenyl)-2,2-dimethylethanamine was prepared from 2-methoxy-4-methylbenzaldehyde following conversion to the corresponding benzylchloride and condensation with isobutyric acid. The resulting 2,2-dimethyl-3-(2-methoxy-4-methylphenyl)-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-(2-methoxy-4-methylphenyl)-2,2-dimethylethanamines (3). The methods for the preparation of the 2,3- and 3,4-methylenedioxy-isomers have been described in previous reports (1,3,5).

Results and Discussion

Mass spectrometry

Mass spectrometry is the primary method for confirming the identity of drugs and other substances of abuse in forensic samples. The five side chain regioisomers of 2-methoxy-4-methylphenethylamine (Figure 1, Compounds 1–5) have the potential to yield mass spectra essentially equivalent to 2,3-MDMA and 3,4-MDMA (Figure 1, Compounds 6 and 7). All have molecular weight of 193 and major fragment ions in their electron ionization mass spectra at *m/z* 58 and 135/136. The *m/z* 58 ion in the methoxy methyl phenethylamine is regioisomeric with that obtained in the mass spectra of both 2,3 and 3,4-MDMA. Additionally, the isobaric methoxy methyl benzyl $(C_9H_{11}O)^+$ frag-

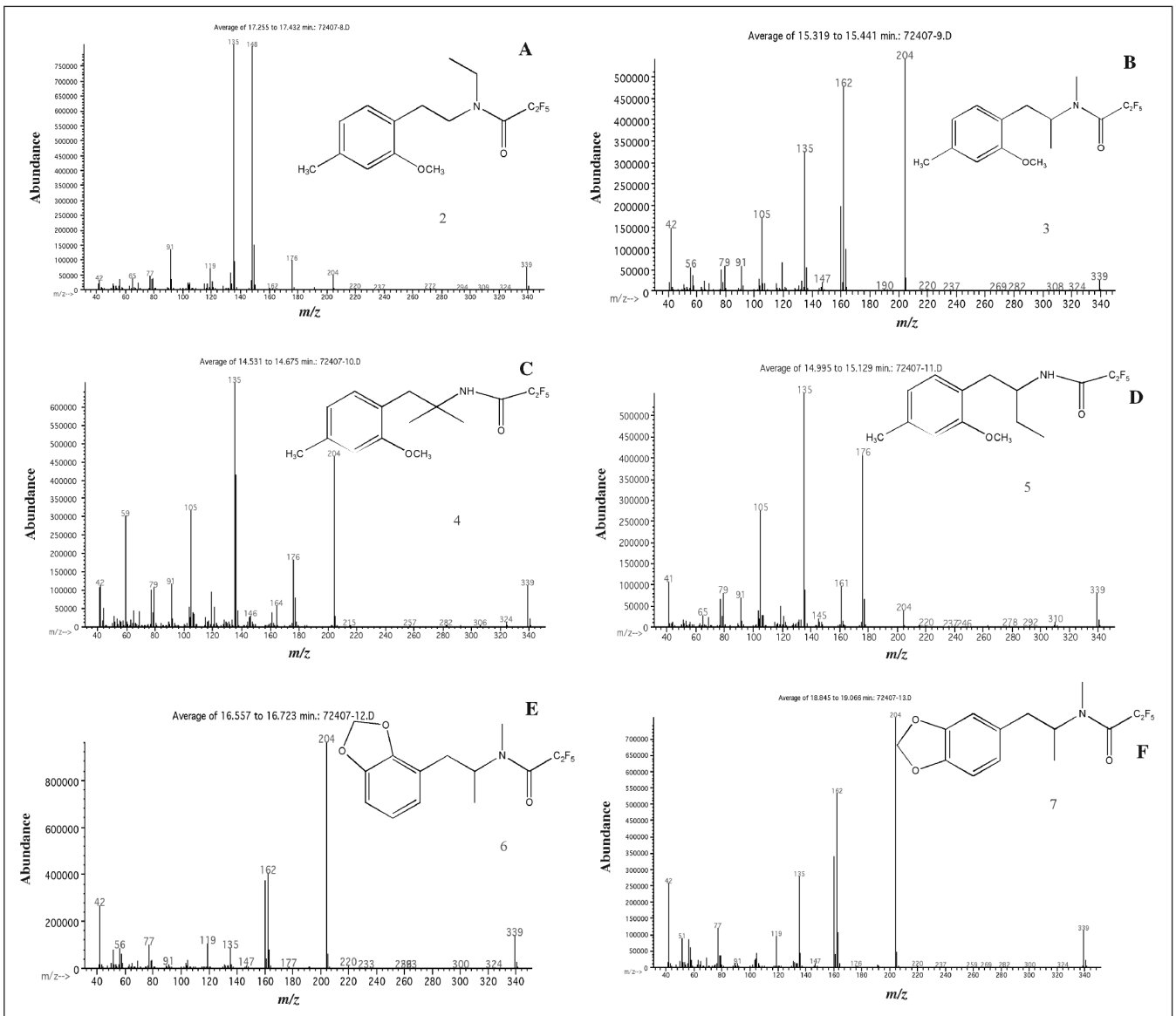

Figure 3. Mass Spectra of the PFPA derivatives of compounds 2–7.

ments have the same nominal mass as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at *m/z* 135 (Figure 2). Thus, these mass spectra do not provide any major fragment ions to differentiate among these compounds (individual spectra for the underivatized amines are not presented in this report).

In the next phase of this study, various perfluoroacylated derivatives of the regioisomeric primary and secondary amines were prepared and evaluated in an effort to individualize their mass spectra and provide unique marker ions for specific identification. Generally, acylation of the amines significantly lowers the basicity of nitrogen and can allow other fragmentation pathways to play a more prominent role in the resulting mass spectrum (9,10,11).

The mass spectra for the pentafluoropropionyl and heptafluorobutyryl amides are shown in Figures 3 and 4, 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 HFBA amides. The *m/z* 204 ions in the PFPA amides and the *m/z* 254 ions in the HFBA amides are analogous to *m/z* 58 in the underivatized species because all these ions represent the $(M\text{-}135)^+$ species. The general fragmentation pattern and structures for the *m/z* 204 and 254 ions are shown in Figure 5. The 2-methoxy-4-methylbenzyl cation (*m/z* 135) and the methylenedioxybenzyl cation (*m/z* 135) are fragments common to all spectra in Figures 3 and 4. However, the 2-methoxy-4-methylbenzyl cation at *m/z* 135 in the perfluoroacyl derivatives shows a very high relative abundance. Indeed the *m/z* 135 ion is the base peak in all the PFPA derivatives of compounds 2, 4, 5 and in the HFBA derivatives of compounds 4 and 5. The remaining two HFBA derivatives of compounds 2 and 3 and the PFPA derivative of compound 3 show the *m/z* 135 ions as a major fragment of at least 90% relative abundance. This would suggest that the perfluoroacyl derivatives of compounds 2, 3, 4, and 5 offer a distinct discrimination between the methylenedioxy and the 2-methoxy-4-methyl substitution patterns based on the difference in relative abundances

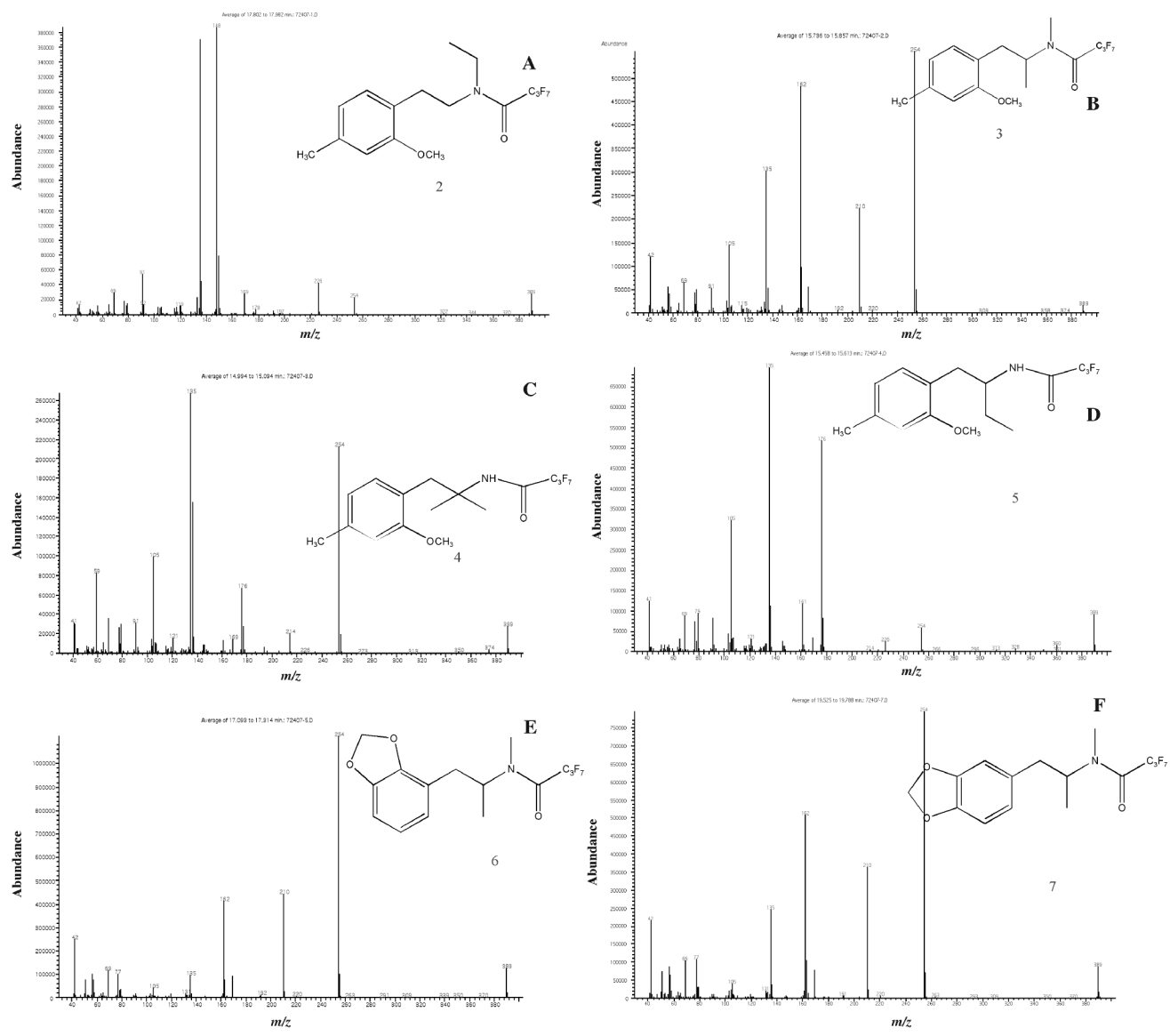

Figure 4. Mass spectra of the HFBA derivatives of compounds 2–7.

of the substituted benzyl cation at *m/z* 135.

The decreased role for the alpha cleavage reaction in the fragmentation of these amides allows the formation of more diagnostic ions of each individual isomer. Acylation weakens the bond between nitrogen and the alkyl carbon of the phenethyl side chain, allowing the formation of charged side chain specific hydrocarbon species of increased relative abundance. These hydrocarbons of varying mass individualize the mass spectra and provide specific structure information. The spectra for the N-ethyl isomer (compound 2) in Figures 3 (compound 2) and 4 (compound 2) 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 3 (compound 3), 3 (compound 6), 3 (compound 7), 4 (compound 3), 4 (compound 6), and 4 (compound 7) show the substituted phenylpropene hydrocarbon radical cation at *m/z* 162, identifying these molecules as the PFPA and HFBA derivatives of 2-methoxy-4-methylmethamphetamine and the 2,3-, 3,4-methylenedioxymethamphetamines, respectively. The spectra for the PFPA and HFBA derivatives of the primary amines (compounds 4 and 5) show ions at *m/z* 176 from the corresponding substituted phenylbutene radical cation. The lower abundance of *m/z* 176 for compound 4 may be the result of steric inhibition of hydrogen transfer in the alpha, alpha-dimethyl substitution pattern.

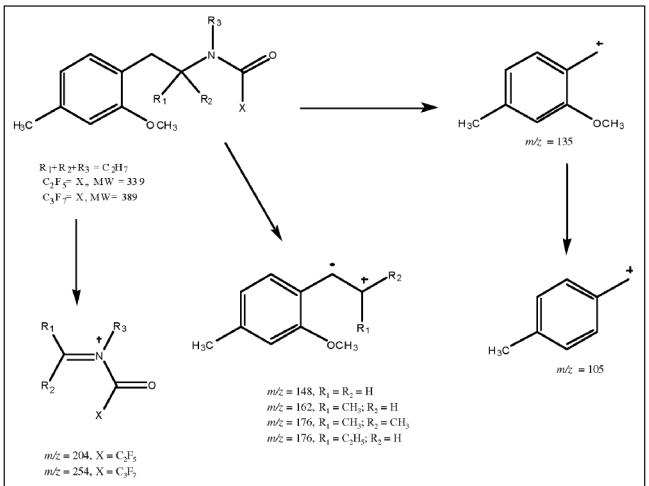

Figure 5. Mass spectral fragmentation products for the acylated 2-methoxy-4-methyl phenethylamines.

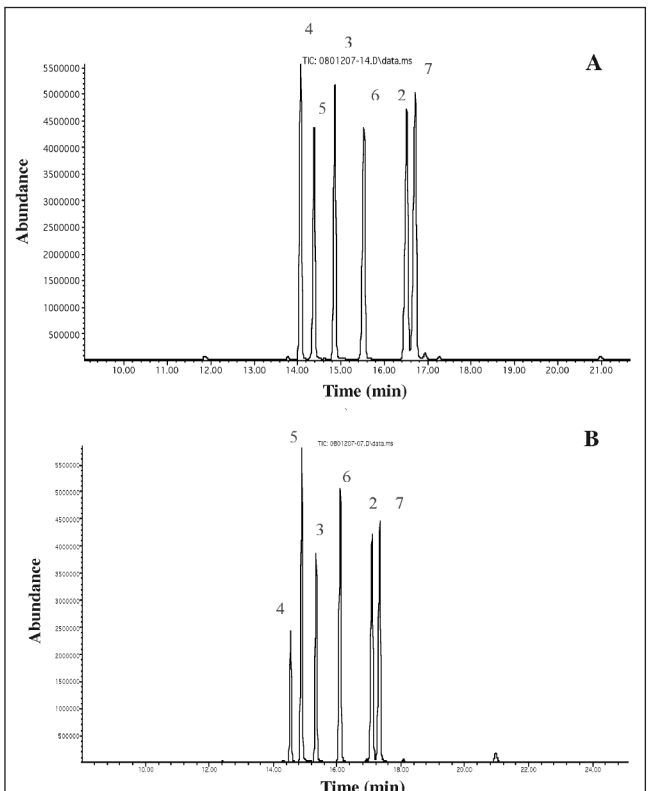

Figure 6. Chromatograms of the PFPA (A) and HFBA (B) derivatives of compounds 2–7. GC–MS system 2, Rtx-1 column.

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 [Figure 3 (compound 2)] 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 alpha-ethyl- (compound 5) and alpha, alpha-dimethyl-(compound 4) phenethylamines [Figures 3 (compound 4), 3 (compound 5), and 4 (compound 4), 4 (compound 5)]. The *m/z* 176 ion in the spectra for the PFPA derivatives of the *N*-ethyl regioisomers [Figure 3 (compound 2)] is a rearrangement of the *m/z* 204 ion resulting in the loss of mass 28 (the *N*-ethyl group) via hydrogen transfer (6–8). This coincidental common mass from two different fragmentation pathways is confirmed by examining the mass spectra for the HFBA derivatives of the *N*-ethylphenethylamines shown in Figure 4 (compound 2). 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 mass spectra for the PFPA and HFBA derivatives of all three ring substituted methamphetamines (Compounds 3, 6 and 7) indicates unique ions at *m/z* 160 and 210 [see Figures 3 (compound 3), 3 (compound 6), 3 (compound 7); 4 (compound 3), 4 (compound 6), and 4 (compound 7)]. 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. This ion has been described in previous studies as the cationic methylated nitrile $C_2F_5CNCH_3$ or $C_3F_7CNCH_3$ (6–8). The equivalent fragmentation pathway has been reported (10) for methamphetamine and further supported by the analysis of the mass spectra of the PFPA and HFBA derivatives of d_3- and d_5-MDMA in previous studies (6). This fragmentation pathway appears unique to the acylated *N*-methyl C-methyl pattern (methamphetamine side chain).

The presence of relatively high abundances of the *m/z* 105 ion in the spectra of the PFPA and HFBA derivatives of compound 3 allows for significant discrimination of this 2-methoxy-4-methylmethamphetamine from the two isobaric methylenedioxy-methamphetamines isomers (compounds 6 and 7). This ion at *m/z* 105 represents the loss of 30 mass units (formalde-

hyde, CH_2O) from the methoxymethylbenzyl cation at *m/z* 135. Previous reports suggested that the *m/z* 105 ion is characteristic of all *o*-methoxy substitution patterns regardless the position of the methyl group on the aromatic ring (7). However, the absence of this fragment from the spectra of the derivatives of compound 2 suggests the necessity of further structure fragmentation studies for these compounds.

Gas chromatography

The PFPA and HFBA derivatives of the six primary and secondary amines were compared on two stationary phases using two GC–MS systems, the relatively nonpolar 100% dimethyl polysiloxane (Rtx-1) and the more polar trifluoropropyl methyl polysiloxane (Rtx-200). Tertiary amides such as compound 1 do not form stable amides. Several temperature programs were evaluated and one program showing the best compromise between resolution and analysis time was used to generate the example chromatograms in Figure 6. The elution order was the same on both stationary phases and all temperature programs evaluated in this study. The chromatograms of the perfluoroacyl compounds show that the 2,3-MDMA elutes before the corresponding 3,4-isomer, which elutes last. When the ring substitution pattern is held constant (2-methoxy-4-methyl), the side chain elution order is secondary amides before the tertiary amides and in this limited set of examples, branched isomers elute before the more linear ones. Therefore, the amides of compound 4 elute first followed by the amide of compound 5 (both secondary amides), then the amides of compound 3 (the methamphetamine side chain) and finally the amides of compound 2 (the more linear of these two tertiary amides). The amides of 2,3-MDMA elute between the secondary and tertiary amides in this group of compounds. 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 and isobaric equivalents. Both the PFPA and HFBA derivatives of 3,4-MDMA elute last and the *N*-ethyl-2-methoxy-4-methylphenethylamine PFPAs and HFBAs are the closest eluted compounds in the 2-methoxy-4-methyl phenethylamine series. The isobaric acylated *N*-ethyl amides show very distinct mass spectra with several characteristic ions to differentiate it from the corresponding amides of the drug of abuse 3,4-MDMA. Thus, derivatization methods coupled with both chromatographic and mass spectral procedures can allow for the complete differentiation of the side chain substitution pattern of the 2-methoxy-4-methylphenethylamines from 3,4-MDMA and its regioisomer, 2,3-MDMA.

Conclusions

Differentiation of the side chain substitution pattern of the 2-methoxy-4-methylphenethylamines from 3,4-MDMA and its regioisomer, 2,3-MDMA was accomplished using a combination of gas chromatography and mass spectrometry.

Derivatization of the primary and secondary amines with various acylating agents yields amides with improved resolution compared to the underivatized amines by capillary gas chromatography on Rtx-1 and Rtx-200 stationary phases. Additionally, the perfluoroacyl derivatives significantly individualize the mass spectra for these amides and allow for unambiguous identification. The individualization results from fragmentation of the alkyl carbon-nitrogen bond yielding characteristic hydrocarbon fragments at *m/z* 105, 148, 162, and 176 as well as other unique fragments.

Acknowledgment

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