Chromatographic and Mass Spectral Studies on Methoxymethcathinones Related to 3,4-Methylenedioxymethamphetamine

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Abstract

The methoxymethcathinones are uniquely regioisomeric with the controlled drug substance 3,4-methylenedioxymethamphetamine (3,4-MDMA) or Ecstacy. The various isomeric forms of the methoxymethcathinones have mass spectra essentially equivalent to 3,4-MDMA. They all have a molecular weight of 193 and major fragment ions in their electron ionization mass spectra at *m/z* 58 and 135/136. Differentiation by mass spectrometry was only possible after formation of the perfluoroacyl derivatives, pentafluoropropionylamides (PFPA), and heptafluorobutrylamides (HFBA). Gas chromatographic separation on nonpolar stationary phases successfully resolved the three methcathinones from 2,3- and 3,4-MDMA as the PFPA and HFBA derivatives.

Introduction

Previous studies (1–4) in this series have shown the 10 direct regioisomeric substances, 3,4-methylenedioxymethamphetamine or Ecstasy (3,4-MDMA) and its nine regioisomeric equivalents, have mass spectral fragments of equivalent mass and identical molecular weights (M_w). Therefore, analysis of the underivatized regioisomers by electron ionization mass spectrometry (MS) does not provide data for the specific differentiation and identification of one of these regioisomers (specifically the drug of abuse Ecstacy or 3,4-MDMA) to the exclusion of all the other isomers. All 10 compounds of M_w = 193 showed major fragment ions for the imine at *m/z* 58 and the benzyl fragment at *m/z* 135/136. The 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 (GC) retention properties; indeed, 3,4-MDMA was found to coelute with one of its nondrug regioisomeric equivalents (1) under common conditions used to identify 3,4-MDMA in forensic drug samples. Additional studies (2) have shown that all 10 compounds can be resolved using the more polar GC stationary phases and specific temperature programming conditions. Additional background information on the structures of these 10 regioisomeric substances, as well as their individual mass spectra and chromatographic properties, can be found in the literature (1,2).

A recent report (4) showed that 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 10 direct regioisomers of MDMA. The present work focuses on preparation and analytical evaluation of the ortho-, meta-, and para-methoxymethcathinone series of indirect regioisomers related to 3,4-MDMA. These regioisomeric methoxymethcathinones are expected to yield mass spectral fragments of the same structure for *m/z* 58 as that observed for 2,3- and 3,4-MDMA. Additionally, the structure of the *m/z* 135 ion resulting from the methoxymethcathinones, the methoxybenzoyl $(C_8H_7O_2)^+$ fragment, is indirectly regioisomeric with the methylenedioxybenzyl $(C_8H_7O_2)^+$ fragment observed in the MDMAs. The three methoxymethcathinones will be compared with 2,3- and 3,4-MDMA; all five of these compounds (see Figure 1) have the same side chain structure for the *m/z* 58 ion, which is the base peak in the electron ionization mass spectrum for the underivatized amines. Regioisomer differentiation is a significant issue in forensic drug chemistry and has been addressed in a number of drug categories (5–7).

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 in the electron impact (EI) mode using an ionization voltage of 70 eV and a source temperature of 230°C. Samples were dissolved in high-performance liquid chromatography-grade acetonitrile (Fisher Scientific, Fair Lawn, NJ) and manually introduced (1 µL), individually and in a physical

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mixture, using a 10-µL Hamilton syringe (Hamilton Co., Reno, NV).

The separation was carried out on a 30-m × 0.25-mm i.d. column coated with 0.25-µm 100% dimethyl polysiloxane (Rtx-1) and 30-m × 0.25-mm i.d. column coated with 0.25-µm 95% dimethyl–5% diphenyl polysiloxane (Rtx-5), both 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 and held at 180°C for 2 min, then ramped to 200°C at a rate of 10°C/min. Program 2 [used to separate the heptafluorobutrylamides (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- and 2,3-MDMA were synthesized as previously described (1). Other laboratory reagents and chemicals were obtained from Aldrich Chemical Company (Milwaukee, WI) or Fisher Scientific (Atlanta, GA).

Synthesis of methoxymethcathinones

A solution of the appropriately substituted methoxybenzaldehyde in dry diethylether was added to a flask and maintained under an atmosphere of dry nitrogen. Ethyl magnesium bromide solution in diethylether was added drop-wise, and the reaction mixture was stirred at –20°C for 2 h. The resulting substituted methoxyphenyl-propan-1-ol in methylene chloride was stirred overnight at room temperature with pyridinium chlorochromate (PCC) and Celite. The reaction mixture was filtered on a pad of silica gel, and the organic layer was evaporated to yield the appropriate substituted methoxypropiophenones, which were purified using Kugelrohr distillation. Methoxypropiophenones in carbon tetrachloride, *N*-bromosuccinimide, and a catalytic amount of benzoyl peroxide were refluxed overnight, filtered, and the solvent evaporated under reduced pressure. The resulting, appropriately substituted methoxy-2-bromo-propiophenones were purified using Kugelrohr distillation, dissolved in acetonitrile, and added drop-wise to a mixture of methylamine hydrochloride and sodium bicarbonate in acetonitrile. The mixture was stirred at room temperature, filtered, and the solvent was evaporated under reduced pressure. Isolation of the basic fraction gave light yellow oils, which were converted to the corresponding methoxymethcathinone hydrochloride salts using gaseous HCl.

Derivatization procedure

Each perfluoroamide was prepared individually from the hydrochloride salts of the regioisomers by dissolving approximately 0.3 mg (1.33×10^{-6} mole) of each amine in 50 µL of ethyl acetate, followed by addition of large excess (250 µL) of the appropriate derivatizing agent (pentafluropropionic anhydride or heptaflurobutric anhydride), and the derivatization reaction mixtures were incubated in capped tubes at 70°C for 20 min. Following incubation, each sample was evaporated to dryness under a stream of 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.

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). The general procedure for the synthesis of these compounds begins with 2,3-methylenedioxybenzaldehyde and 3,4-methylenedioxybenzaldehyde (piperonal) as starting materials. The preparation of 2,3-methylenedioxybenzaldehyde was reported previously (1).

The three methcathinones were prepared (see Figure 2) by reacting the appropriate anisaldehyde with ethyl magnesium bromide under a nitrogen atmosphere at –20°C. The resulting crude alcohols were stirred overnight at room temperature with PCC and Celite. The resulting methoxypropiophenones were purified using Kugelrohr distillation followed by treatment with *N*-bromosuccinimide and a catalytic amount of benzoyl peroxide, yielding the appropriate methoxy-2-bromo-propiophenones, which were purified using Kugelrohr distillation. The methoxy-2-bromo-propiophenones were treated with methylamine hydrochloride and sodium bicarbonate in acetonitrile, and the products were converted to the hydrochloride salt with gaseous HCl. Structure verification of the intermediates and end products was carried out by ^{1}H NMR and MS.

Table I. GC Retention Data for Compounds 1–5*

Compound no.	Rtx-1† derivatives			Rtx-5‡ derivatives		
	Underivatized§	HFBA derivatives**	PFPA derivatives§	Underivatized**	HFBA derivatives**	PFPA derivatives**
1	1.0 (11.108 min)	1.0 (13.556 min)	1.0 (9.390 min)	1.0 (11.679min)	1.0 (13.932 min)	1.0 (13.546 min)
2	0.889	0.927	0.932	0.878	0.929	0.926
3	0.848††	0.873	0.875	0.833††	0.978	0.870
4	0.848††	0.894	0.892	0.833††	0.898	0.886
5	0.925	0.967	0.964	0.920	0.970	0.967

* Results are the average of three experiments.
† 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 20°C/min, hold at 180°C for 2 min ramp to 250°C at 10°C/min.
** 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 250°C at 10°C/min.
†† Compounds co-elute.

MS

MS is the primary method for confirming the identity of drugs and other substances of abuse in forensic samples. The mass spectrum of 3,4-MDMA is characterized by a base peak

formed by an alpha-cleavage reaction involving the carbon–carbon bond of the ethyl linkage between the aromatic ring and the amine. In 3,4-MDMA (M_w = 193), the alpha-cleavage reaction yields the substituted imine fragment at *m/z* 58, the *N*-methyl imine of acetaldehyde, and the 3,4-methylenedioxybenzyl fragment at mass 135/136 (for the cation and the radical cation, respectively). Thus, the mass spectrum for 3,4-MDMA contains major ions at *m/z* 58 and 135/136 as well as other ions of low relative abundance (1). The mass spectra for the three methoxymethcathinone regioisomers (Figure 1) also show a base peak at *m/z* 58 as seen for 2,3- and 3,4-MDMA. The major fragmentation pattern for the methoxymethcathinones is shown in Figure 3. The methoxybenzoyl $(C_8H_7O_2)^+$ fragment has the same mass and empirical formula as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at *m/z* 135. Thus, these methoxymethcathinones represent a potentially significant challenge for analytical drug chemistry. They present essentially the same mass spectrum as the drug of abuse 3,4-MDMA, and all the regioisomeric methoxymethcathinones have the same side chain structure, which fragments to yield the *m/z* 58 ion in the mass spectra for all these compounds (3).

In the next phase of this study, various perfluoroacylated derivatives of 3,4 and 2,3-MDMA and their regioisomeric secondary amines, ortho-, meta-, and para-methoxymethcathinones, 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 (4). The pentafluoropropionyl and heptafluorobutryl derivatives of methoxymethcathinones were evaluated for their ability to individualize the mass spectra and were compared with the corresponding mass spectra for derivatives of 2,3- and 3,4-MDMA.

The mass spectra for the five pentaflouropropionyl and heptaflourobutryl amides are shown in Figures 4 and 5, respectively. From these spectra, a common peak occured at *m/z* 204 and 254, which corresponds to the loss of 135 mass units from the molecular ions at 339 and 389 for pentafluoropropionylamides (PFPA) and HFBA amides. This ion at *m/z* 204 and 254 was the PFPA and HFBA imine species likely formed from the alpha-cleavage of the amide nitrogen to eliminate the 2,3- and 3,4-methylenedioxybenzyl and methoxybenzoyl radicals. Thus the *m/z* 204 and 254 in PFPA and HFBA amides were analogous to *m/z* 58 in the underivatized species because all these ions represent the $(M-135)^+$

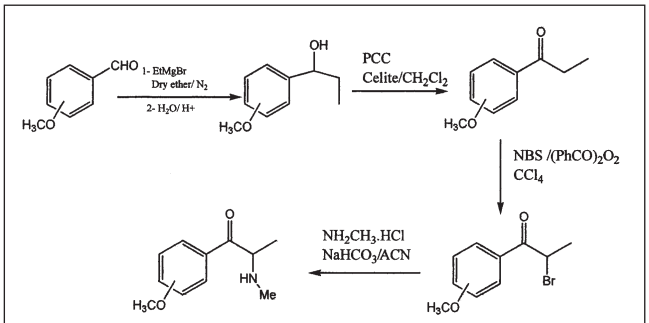

Figure 1. Synthesis of ortho-, meta-, and para-methoxymethcathinones from the corresponding anisaldehydes.

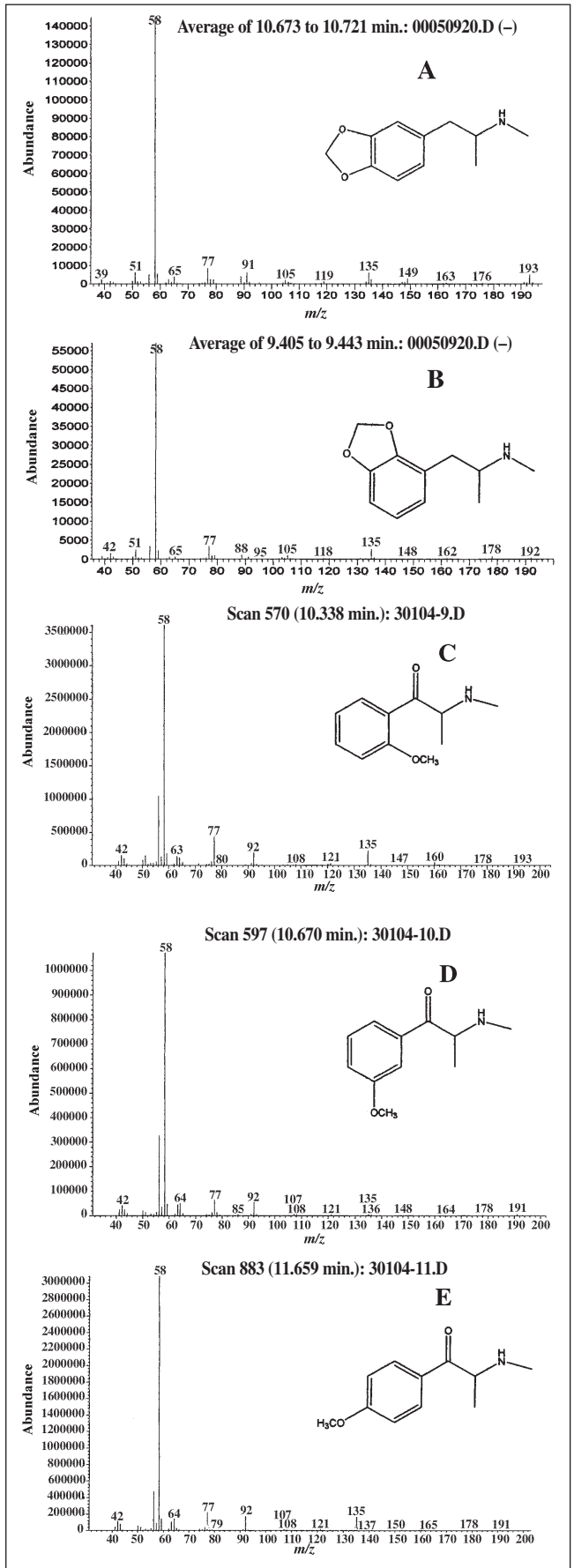

Figure 2. Mass spectra of the underivatized 3,4 –MDMA (A); 2,3-MDMA (B); ortho- (C); meta- (D); and para-methoxymethcathinone (E).

species. The general fragmentation pattern and structures for the *m/z* 204 and 254 ions were shown in Figure 6. The relative abundances for the *m/z* 204 and 254 ions are always higher in the 3,4- and 2,3-MDMAs than in the methoxymethcathinones. The methylenedioxybenzyl and the methoxybenzoyl cations at *m/z* 135 were fragments common to all the spectra in Figures 4 and 5. The relative abundance of *m/z* 135 in perfluoroacyl derivatives of methoxymethcathinones was higher than that observed for 3,4- and 2,3-MDMAs. The *m/z* 135 ion was the base peak in the mass spectra for the derivatives of the methoxymethcathinones likely because of the additional carbonyl site for initial radical cation formation in these compounds.

The decreased role of alpha-cleavage reaction in the fragmentation of these amides allowed the formation of additional diagnostic ions of each individual isomer. Acylation, perflouroacylation in particular, weakens the bond between nitrogen and the alpha-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 4 and 5 illustrate the role of the hydrocarbon fragment at *m/z* 162 in the EI mass spectral differentiation among these compounds. The spectra in Figures 4A, 4B, 5A, and 5B show the 2,3- and 3,4-methyelenedioxyphenylpropene radical cation at *m/z* 162, identifying these molecules as the PFPA and HFBA derivatives of 2,3- and 3,4-MDMAs, respectively. The formation of the *m/z* 162 ion was described in previous studies (4,8) and required the transfer of a hydrogen from the benzylic carbon (Figure 6). This fragmentation mechanism does not take place in the PFPA and HFBA derivatives of the methoxymethcathinones because of the absence of a benzylic hydrogen. One can conclude that the presence of alkene ions at *m/z* 162 can be used to identify the side chain of 3,4- and 2,3-MDMA and exclude the regioisomeric methoxymethcathinones. Conversely, the base peak at *m/z* 135, as well as the absence of the *m/z* 162 ion, would identify one of these substances as a methoxymethcathinone regioisomer.

A comparison of the PFPA derivatives in Figure 4 with the HFBA derivatives in Figure 5 indicates unique ions at *m/z* 160 and

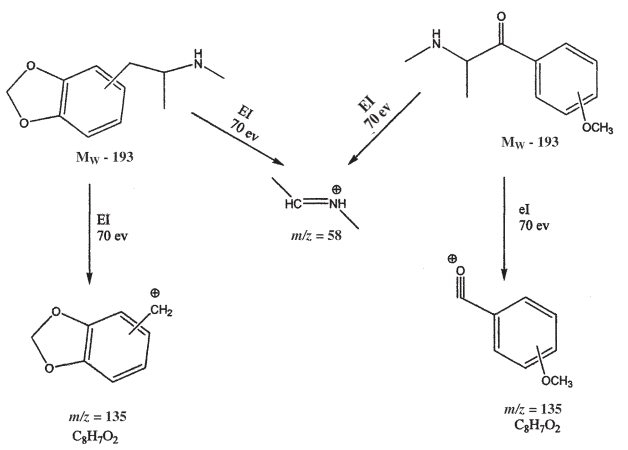

Figure 3. EI fragmentation pattern of the underivatized MDMAs and methoxymethcathinones.

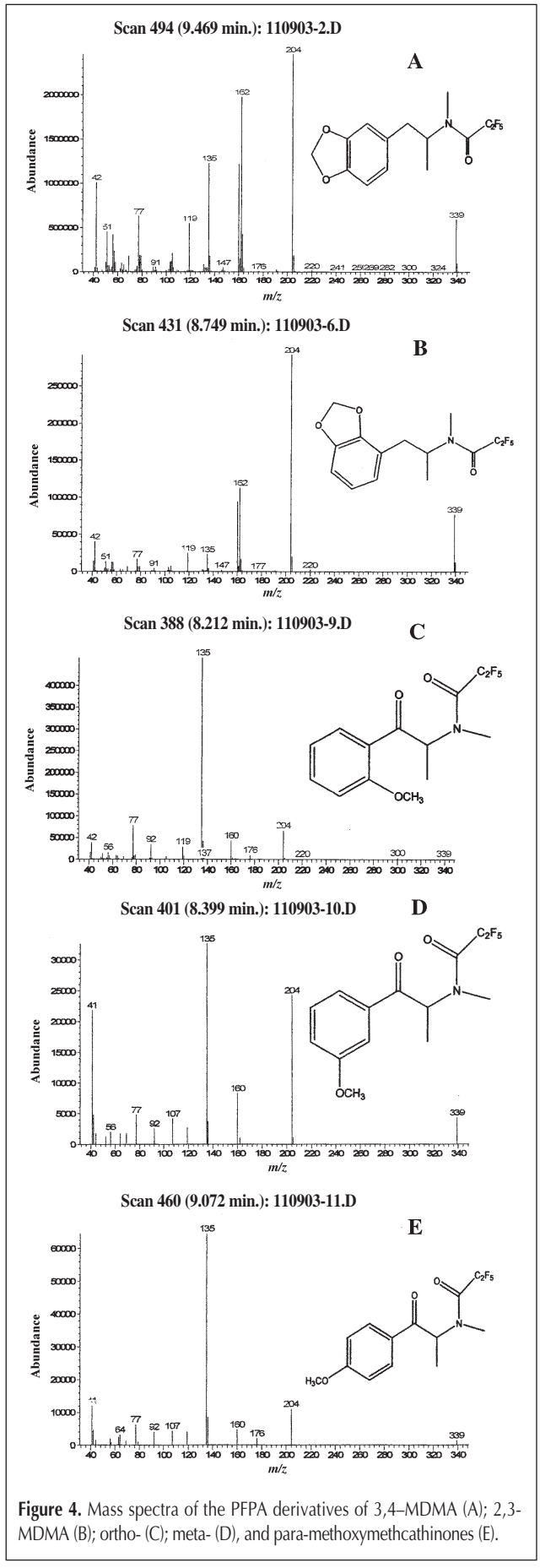

Figure 4. Mass spectra of the PFPA derivatives of 3,4–MDMA (A); 2,3-MDMA (B); ortho- (C); meta- (D), and para-methoxymethcathinones (E).

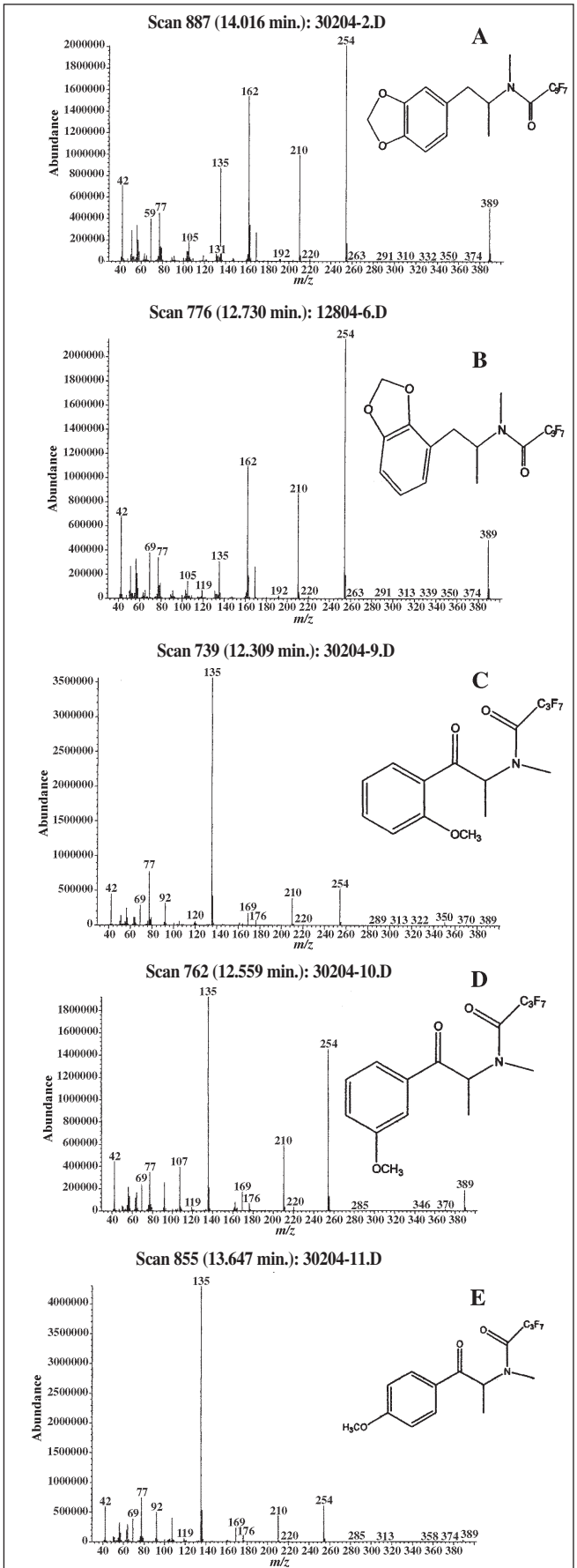

Figure 5. Mass spectra for the HFBA derivatives of 3,4–MDMA (A); 2,3-MDMA (B); ortho- (C); meta- (D); and para-methoxymethcathinones (E).

210. This mass difference of 50 (CF_2) suggested these ions contain the perfluoroalkyl group for each derivative, C_2F_5 and C_3F_7, respectively. The *m/z* 160 and 210 ions have been fully characterized (4,8) using deuterated analogs of 3,4-MDMA and methamphetamine, and these fragments are the result of a rearrangement decomposition of ions 204 and 254, respectively. The *m/z* 204 and 254 ions have the same structure, whether generated from derivatives of the MDMAs or the methoxymethcathinones. Therefore, the *m/z* 160 and 210 ions do not provide any information to differentiate between the two groups of substances, MDMAs and methoxymethcathinones.

GC

The GC properties of the PFPA and HFBA derivatives of the 2,3- and 3,4-MDMAs and 2-, 3-, and 4-methoxymethcathinone were compared on two stationary phases using capillary columns of the same dimensions (30 m × 0.25 mm, 0.25-µm film thickness). 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). The un-derivatized compounds were not completely resolved, with 2-methoxymethcathinone coeluting with 3-methoxymethcathinone, using these common GC stationary phases and some common temperature programming conditions. The PFPA and HFBA derivatives showed improved resolution when compared with the underivatized amines. Table I shows the relative retention of these compounds compared with MDMA under identical chromatographic conditions. 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 7 and 8. The two chromatograms for the PFPA derivatives in Figure 7 were generated using two different temperature programs. The resulting elution order and resolution are quite similar. In fact, the elution order is the same for all of the chromatograms in Figures 7 and 8. In each case, the derivatized 2-methoxymethcathinone (compound 3) eluted first, followed closely by the 3-methoxymethcathinone derivative (compound 4). The third

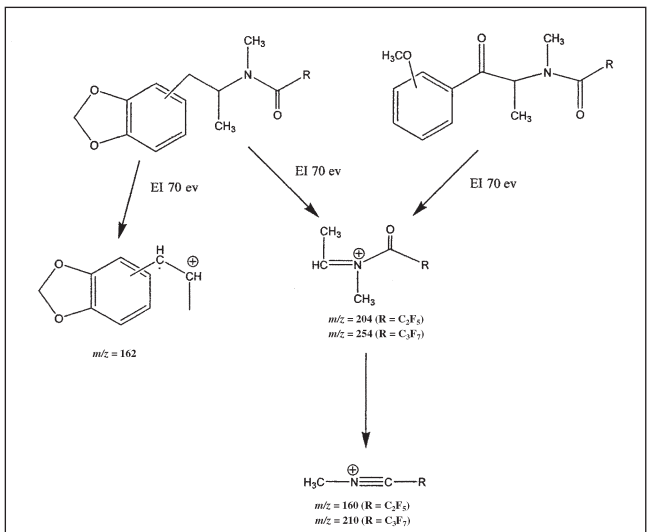

Figure 6. EI fragmentation of PFPA and HFBA of MDMA and methoxymethcathinone regioisomers.

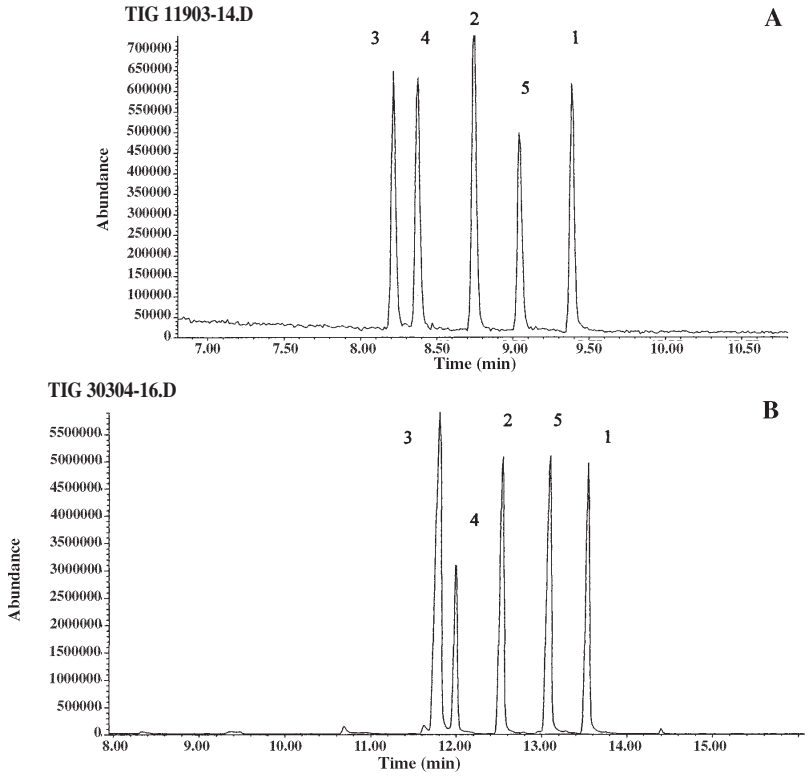

Figure 7. Capillary GC separation of PFPAs of the methcathinones and MDMAs. Columns used: Rtx-1 (A); Rtx-5 (B). Peaks: *o*-methoxymethcathinone-PFPA 3; *m*-methoxymethcathinone-PFPA 4; 2,3-MDMA-PFPA 2; *p*-methoxymethcathinone-PFPA 5; and 3,4-MDMA-PFPA 1.

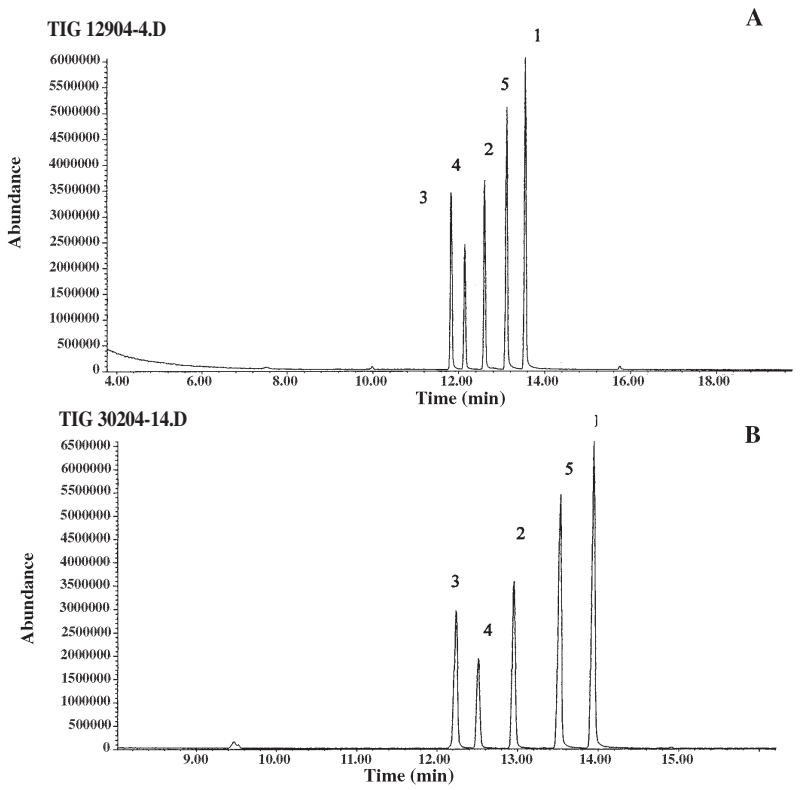

Figure 8. Capillary gas chromatographic separation of HFBAs of the methcathinones and MDMAs. Columns used: Rtx-1 (A) and Rtx-5 (B). Peaks: *o*-methoxymethcathinone–HFBA 3; *m*-methoxymethcathinone–HFBA 4; 2,3-MDMA–HFBA 2; *p*-methoxymethcathinone–HFBA 5; and 3,4-MDMA–HFBA.

compound to elute was the 2,3-MDMA derivative (compound 2), and the derivatized 4-methoxymethcathinone (compound 5) is the forth peak in each chromatogram. The derivatized form of 3,4-MDMA (compound 1) showed the greatest retention in each chromatogram.

Conclusion

3,4- and 2,3-MDMA and 2-, 3-, and 4-methoxymethcathinone are a unique subset of regioisomeric molecules; each compound has a M_w of 193 and yields a base peak for the *N*-methyl imine of acetaldehyde at *m/z* 58 in the mass spectrum from the loss of the corresponding $C_8H_7O_2$ methylenedioxybenzyl and methoxybenzoyl groups, respectively. Thus, the traditional EI-MS provides little structural information for differentiating among these five 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 the other four 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 five amines. Derivatization of these amines with various acylating agents yields amides with improved resolution compared with the underivatized amines by capillary GC on Rtx-1 and Rtx-5 stationary phases. These perfluoroacyl derivatives significantly individualize the mass spectra for these amides. The individualization is the result of fragmentation of the alkyl carbon–nitrogen bond, yielding hydrocarbon fragments at *m/z* 162 as well as other unique fragments from the MDMA amides that were not formed for the methoxymethcathinones. 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 regioisomeric substances.

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