Chromatographic and Spectroscopic Methods of Identification for the Side-Chain Regioisomers of 3,4-Methylenedioxyphenethylamines Related to MDEA, MDMMA, and MBDB

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Abstract

Three regioisomeric 3,4-methylenedioxyphenethylamines having the same molecular weight and major mass spectral fragments of equivalent mass have been reported as components of clandestine drug samples in recent years. These drugs of abuse are 3,4-methylenedioxy-*N*-ethylamphetamine, 3,4-methylenedioxy-*N*,*N*-dimethylamphetamine, and *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine. These three compounds are a subset of a total of ten regioisomeric 3,4-methylenedioxyphenethylamines of molecular weight 207, yielding regioisomeric fragment ions of equivalent mass (*m/z* 72 and 135/136) in the electron impact mass spectrum. The specific identification of one of these compounds in a forensic drug sample depends upon the analyst's ability to eliminate the other regioisomers as possible interfering or coeluting substances. This paper reports the synthesis, mass spectral characterization, and chromatographic analysis of these ten unique regioisomers. The ten regioisomeric methylenedioxyphenethylamines are synthesized from commercially available precursor chemicals. The electron impact 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. Thus, the ultimate identification of any one of these amines with the elimination of the other nine regioisomeric substances depends heavily upon chromatographic methods. Chromatographic separation of these ten uniquely regioisomeric amines is studied using gas chromatographic temperature program optimization.

Introduction

The methylenedioxyamphetamines [such as 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyethylamphetamine (MDEA)] are novel psychoactive compounds with structural similarities to both amphetamine and the psychedelic phenethylamine, mescaline. The methylenedioxy derivatives of amphetamine and methamphetamine represent the largest group of designer drugs. MDA, MDMA, and MDEA have all been reported to produce very similar central and peripheral effects in humans with differences only in potency, time of onset, and duration of action. The central effects are described as an easily controlled altered state of consciousness, with a heightened sense of well being, increased tactile sensations, increased perception of an inwardly focused experience, and a strong desire to be with and converse with people, without significant perceptual distortion or hallucinations (1).

It has been suggested that 3,4-methylenedioxyphenylalkylamines may represent a novel class of pharmacological agents, labelled entactogens (2). These compounds, which include MDA, MDMA, 3,4-methylenedioxy-*N*,*N*-dimethylamphetamine (MDMMA), MDEA, and *N*-methyl-3,4-methylenedioxy-phenylbutanamine (MBDB), do not fit the pharmacological profile of either phenethylamine hallucinogens or psychomotor stimulants. The term entactogen is defined as producing a "touching within", which is in reference to the drugs' ability to promote inward reflection and positive self-assessment.

The ability to distinguish between regioisomers directly enhances the specificity of the analysis for the target drugs of abuse (2–5). The mass spectrum is often the confirmatory piece of evidence for the identification of drugs of abuse in the forensic laboratory. Although the mass spectrum is often considered a specific "fingerprint" for an individual compound, there may be other substances that are capable of producing very similar or almost identical mass spectra. For major drugs of abuse, such as the amphetamines (4,5) and MDMAs (5–7), there may be many positional isomers (regioisomers) in the alkyl side-chain or the aromatic ring substitution pattern that yield the same mass

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spectrum. Methamphetamine and phentermine (5) are regioisomeric, based on the substitution of a methyl group on nitrogen or carbon of the side-chain. The street drug 4-bromo-2,5-dimethoxyphenethylamine ("Nexus") would have several regioisomers based on other aromatic ring substitution patterns (8). Although NMR can be a very useful method for regioisomer differentiation, it is not a technique with direct application for all areas of forensic drug chemistry and not readily available in most forensic laboratories. Thus, the analysis of street drug samples and analytical toxicology must depend heavily upon chromatographic methods as well as mass spectrometry (MS).

When other compounds exist that have the potential to produce the same or nearly identical mass spectrum as the drug of interest, the identification by gas chromatography (GC)–MS must be based primarily upon the ability of the chromatographic system to separate the "nondrug regioisomers" from the actual drug of abuse. Those substances coeluting with the drug of abuse in chromatographic systems could be misidentified as the drug of abuse. Without the appropriate standards, thorough method validation is not possible, and thus coelution of drug and nondrug combinations would remain a possibility. The ultimate concern then is: if the forensic scientist has never analyzed all of the nondrug substances, how can he or she be sure that these compounds would not coelute with the drug of abuse? The significance of this question is related to many factors, chief among these are the separation power of the chromatographic system and the number of possible counterfeit substances. Furthermore, the ability to distinguish between these regioisomers directly enhances the specificity of the analysis for the target drugs of abuse.

There are a total of ten 3,4-methylenedioxy-substituted phenethylamines of molecular weight 207 with the potential to produce a mass spectrum with major fragment ions at *m/z* 135 and 72 (Figure 1). Three of these ten substances (9) have already appeared in street drug samples in the United States and Europe (Figure 2). Many of these compounds are pharmacologically inactive and others have unknown pharmacological properties, yet all have the strong possibility to be identified as MDEA, MDMMA, or MBDB by some analytical methods. In this project, all ten side chain regioisomers including MDEA, MDMMA, and MBDB were compared by chromatographic and spectroscopic techniques, and methods for their differentiation were explored.

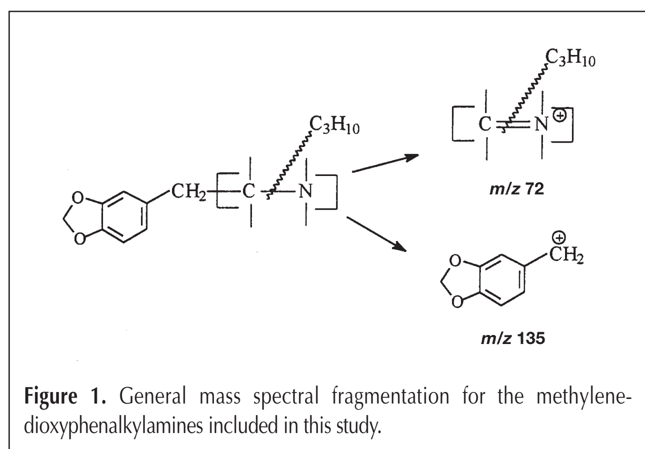

Figure 1. General mass spectral fragmentation for the methylenedioxyphenalkylamines included in this study.

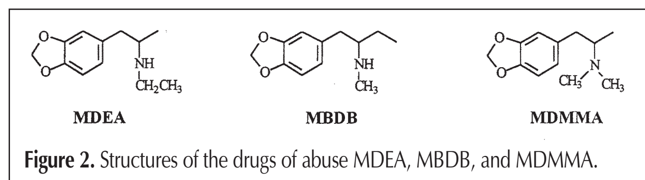

Figure 2. Structures of the drugs of abuse MDEA, MBDB, and MDMMA.

Experimental

Materials

3,4-Methylenedioxyethylamphetamine, and *N,N*-dimethyl MDA were purchased from Research Biochemicals International (Natick, MA). *N*-Methyl-3,4-methylenedioxyphenyl-2-butanamine was obtained from Lipomed (Arlesheim, Switzerland). 3,4-Methylenedioxyphenylacetone, piperonal, methylamine hydrochloride, *N*-methyl formamide, benzaldehyde, isobutyric acid, 2-methylbutyric acid, valeric acid, 1-nitropropane, hexamethylphosphoramide, diisopropylamine, ethyl chloroformate, sodium azide, and sodium borohydride were obtained from Aldrich (Milwaukee, WI).

Instrumentation

GC–MS analyses were performed with an HP 6890 gas chromatograph coupled with an HP 5973 mass selective detector (Hewlett-Packard, Little Falls, DE). The mass spectrometer was operated in the electron impact (EI) mode utilizing an ionization voltage of 70 eV and a source temperature of 230°C. The samples were dissolved in pH 8.9 trizma-base buffer (1 mg/mL), extracted with iso-octane (1 mL), and introduced (1.0 µL) into the mass spectrometer via the gas chromatograph equipped with an HP 7673 automatic injector. The separation was carried out on a 25-m (l) × 0.20-mm i.d. column, coated with 0.11 µm of 5% phenyl methyl silicone (Ultra-2) purchased from Hewlett-Packard. The injection was carried out using splitless mode with an injector temperature at 250°C and the split purge valve opened after 1 min. The injection volume was 1 µL/column. The temperature program started with 1 min isothermal held at 60°C, followed by a linear ramp (8°C/min) to 180°C, and then to the final temperature of 300°C at a rate of 30°C/min with a hold time of 10 min. The helium carrier gas was adjusted to 25 cm/s at 60°C in the constant flow mode. The sample preparation was carried out by dissolving samples in tris-buffer (pH 8.9) and extracting with iso-octane.

GC analyses were performed with an HP 6890 gas chromatograph equipped with split/splitless inlet, HP 7683 automatic injector, and flame ionization detector (Agilent Technologies, Little Falls, DE). ChemStation software Rev. A.08.03 (Agilent Technologies) was used for data acquisition and processing. The carrier gas (hydrogen) was adjusted at 60°C to give an average velocity to the optimum of 50 cm/s. Inlet pressure was converted according to the constant flow mode and the total flow was 60 mL/min. The injection was in the split mode with an injector temperature at 260°C. The sample preparation was carried out by dissolving samples in tris-buffer (pH 8.9) and extracting with iso-octane.

The temperature program optimization was carried out using DryLab 2000 chromatography optimization software, version 3.00.06 (LC Resources, Walnut Creek, CA).

The capillary columns used are listed as follow. The conventional columns were: HP Ultra 1 (25 m, 0.2 mm, and 0.33 µm), HP Ultra 2 (25 m, 0.2 mm, and 0.33 µm), DB-35MS (25 m, 0.2 mm, 0.33 µm), HP-50+ (25 m, 0.2 mm, and 0.33 µm), DB-17MS (30 m, 0.25 mm, and 0.25 µm), and HP-1701 (25 m, 0.2 mm, and 0.20 µm).

The narrow bore columns were: HP-1 (10 m, 0.1 mm, and 0.1 µm), HP-5 (10 m, 0.1 mm, and 0.17 µm), DB-17 (10 m, 0.1 mm, 0.2 µm), and SPB-50 (10 m, 0.1 mm, 0.17 µm).

Synthesis of regioisomers

The synthesis of MDEA, MDMMA, and MBDB compounds 4, 9, and 10 was accomplished using methods previously reported (3,10–13) or obtained from commercial sources. Compounds 1 and 7 were prepared by Red-Al reduction of the *N*-isopropyl or *N*-*n*-propyl 3,4-methylenedioxyphenylacetamides (1,8,10). The intermediate primary amine 3,4-methylenedioxyphenethylamine was prepared from the base-catalyzed condensation of piperonal and nitromethane, yielding the 1-(3,4-methylenedioxyphenyl)-2-nitroethene. Reduction of the nitroethene with lithium aluminum hydride gave the desired primary amine (8).

The *N*-methyl-*N*-ethyl-3,4-methylenedioxyphenethylamine compound 5 was prepared by sequential reductive aminations of the primary amine 3,4-methylenedioxyphenethylamine according to the following procedure. A mixture of 3,4-methylenedioxyphenylethanamine (1.49 g, 0.009 mol) and benzaldehyde (2.87 g, 0.0027 mol) in benzene was refluxed overnight with the aid of a Dean Stark trap water separator. The solvent was evaporated under reduced pressure and the resulting imine reduced with sodium borohydride (3.4 g, 0.045 mol) in 2-propanol at room temperature. Isolation of the basic fraction gave *N*-benzyl-3,4-methylenedioxyphenylethanamine.

A portion of the *N*-benzyl-3,4-methylenedioxyphenylethanamine (1.7 g, 0.0067 mol) was dissolved in methanol along with acetaldehyde (0.88 g, 0.020 mol) and sodium cyanoborohydride (1.26 g, 0.020 mol). The resulting mixture was stirred for three days and the pH 7 was maintained by adding concentrated hydrochloric acid. The oily basic fraction was isolated and dissolved in ethanol along with a few drops of concentrated hydrochloric acid and 0.50 g of 10% palladium on carbon. The reaction mixture was added to a Paar flask and subjected to hydrogenation at 50 psi. The resulting debenzylated product (*N*-ethyl-3,4-methylenedioxyphenylethanamine) was dissolved in a mixture of ethanol–saturated sodium bicarbonate solution–formaldehyde and hydrogenated over 10% palladium on carbon. The product (*N*-ethyl-*N*-methyl-3,4-methylenedioxyphenylethanamine) was isolated as the hydrochloride salt in a low overall yield.

Compounds 2, 3, 6, and 8 were prepared according to the general method outlined in Figure 3. Under an atmosphere of nitrogen, dry tetrahydrofuran (THF) (50 mL) and diisopropylamine (4.0 g, 0.039 mol) were added to a reaction flask, and the resulting solution was cooled with external dry ice–isopropanol. A solution of 2.5M *n*-butyllithium in hexane (17 mL, 0.043 mol) was added dropwise and the reaction mixture was allowed to warm to room temperature, stirred for 5 min, and then cooled again in the dry ice bath. The appropriate carboxylic acid (0.018 mol) was added dropwise, along with 3.7 mL of hexamethylphosphoramide. The reaction mixture was allowed to warm to room temperature, stirred for 30 min, and followed by the addition of 3,4-methylenedioxybenzyl chloride (3.0 g, 0.0176 mol), and the mixture was then stirred for three days at room temperature. Isolation of the acid fraction gave the appropriate α-substituted 3,4-methylenedioxyphenylpropionic acid in high yield.

The individual substituted 3,4-methylenedioxyphenylpropionic acid (0.0153 mol) and triethylamine (1.54 g, 0.0153 mol) were dissolved in water (4 mL) and diluted with sufficient acetone to maintain a clear solution at ice-bath temperature. A solution of ethyl chloroformate (1.74 g, 0.016 mol) in 10 mL of acetone was added dropwise to the 0°C solution, followed by the addition of a solution of sodium azide (1.11 g, 0.017 mol) in water (10 mL). Stirring was continued for 30 min at room temperature. The aqueous phase was extracted with toluene, which was washed with water and dried with magnesium sulfate. This organic solution was heated (100°C) until nitrogen evolution had ceased, which required approximately 30 min. The solvent was removed under vacuum and the residue was dissolved in 20 mL of benzyl alcohol. This solution was heated (100°C) overnight and stirred 5 days at room temperature. The excess of benzyl alcohol was removed by Kugelrohr distillation. The oily residue was dissolved in ethanol (75 mL) and 10% palladium on carbon (0.5 g) was added. The reaction mixture was hydrogenated for over 3 h (50 psi). The carbon was removed by filtration through celite. The solvent was removed under reduced pressure and the residue was dissolved in acidic water, washed with methylene chloride, and the water layer was alkalized with sodium hydroxide and extracted with methylene chloride. The solvent was evaporated under reduced pressure, and the introduction of hydrochloric acid gas in anhydrous diethyl ether gave the desired amine hydrochloride in low yield. This procedure was used to prepare compounds 2, 6, and 8.

The synthesis of *N*-methyl-3,4-methylenedioxyphentermine

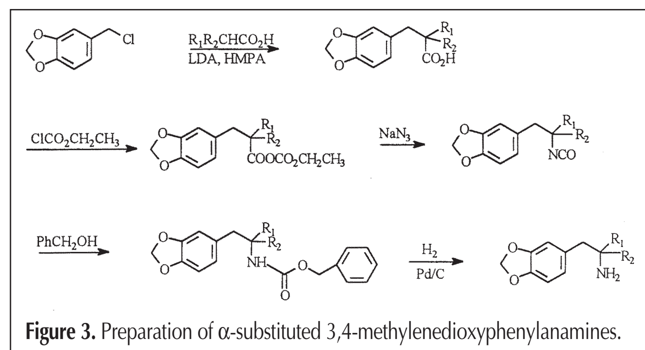

Figure 3. Preparation of α-substituted 3,4-methylenedioxyphenylanamines.

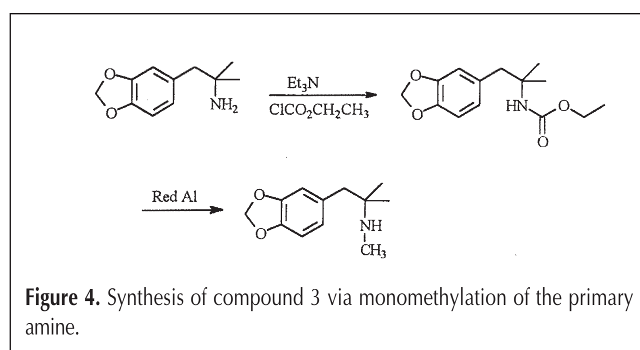

Figure 4. Synthesis of compound 3 via monomethylation of the primary amine.

(compound 3) was accomplished as follows (Figure 4). 3,4-Methylenedioxyphentermine hydrochloride (0.5 g, 0.0022 mol) was prepared according to the previously mentioned procedure and then dissolved in 20 mL of THF. A mixture of triethylamine (0.44 g, 0.0044 mol)–ethylchloroformate (0.24 g, 0.0022 mol) in 10 mL of THF was added dropwise, and the reaction mixture was kept cool by external ice bath. The reaction mixture was allowed to warm to room temperature and stirred overnight. The carbamate was isolated by solvent extraction as a light yellow oil, which was dissolved in dry THF (5 mL) and added dropwise to the mixture of Red-Al (3 mL, 0.0088 mol)–dry THF (5 mL) under nitrogen. The mixture was refluxed overnight and quenched by the addition of a water–sodium hydroxide solution. The basic fraction was isolated by solvent extraction and the oily product converted to the hydrochloride salt.

Results and Discussion

Preparation of the regioisomers

The compounds prepared and evaluated in this study are shown in Figure 5. The methods for the preparation of many of the 3,4-methylenedioxy-regioisomers have been described in previous reports (3,8,10–13). The general procedure for the synthesis of these compounds uses 3,4-methylenedioxybenzaldehyde (piperonal) as starting material. Compounds 2, 6, and 8 were prepared from piperonal according to the methods outlined in Figure 3. The aldehyde was reduced to the alcohol and converted to the benzyl chloride, which was condensed with the appropriate carboxylic acid using butyl lithium to yield the appropriately substituted 3-(3,4-methylenedioxyphenyl)-1-propionic acid. The acid was treated sequentially with sodium azide, ethyl chloroformate, and benzyl alcohol followed by catalytic hydrogenation under low pressure to yield the desired primary amines 2, 6, and 8.

The α,α-dimethyl primary amine was also prepared according to the mentioned method, then methylated (Figure 4) using ethylchloroformate, followed by reduction with sodium bis-(2-methoxyethoxy)-aluminum hydride (Red-Al) to yield the desired secondary amine, compound 3.

The secondary amines 1 and 7 were prepared from 3,4-methylenedioxyphenylacetic acid by conversion to the acid chloride, then treatment with either isopropylamine or *n*-propylamine yielded the requisite amide. Hydride reduction of the amides with lithium aluminum hydride (LAH) gave the secondary amines 1 and 7. The tertiary amine 5 was prepared from 3,4-methylenedioxyphenethylamine produced by the condensation of piperonal with nitromethane under basic conditions. This yielded the 2-nitroethene, which upon reduction with LAH yielded the primary amine. The unsymmetrical alkylation of the

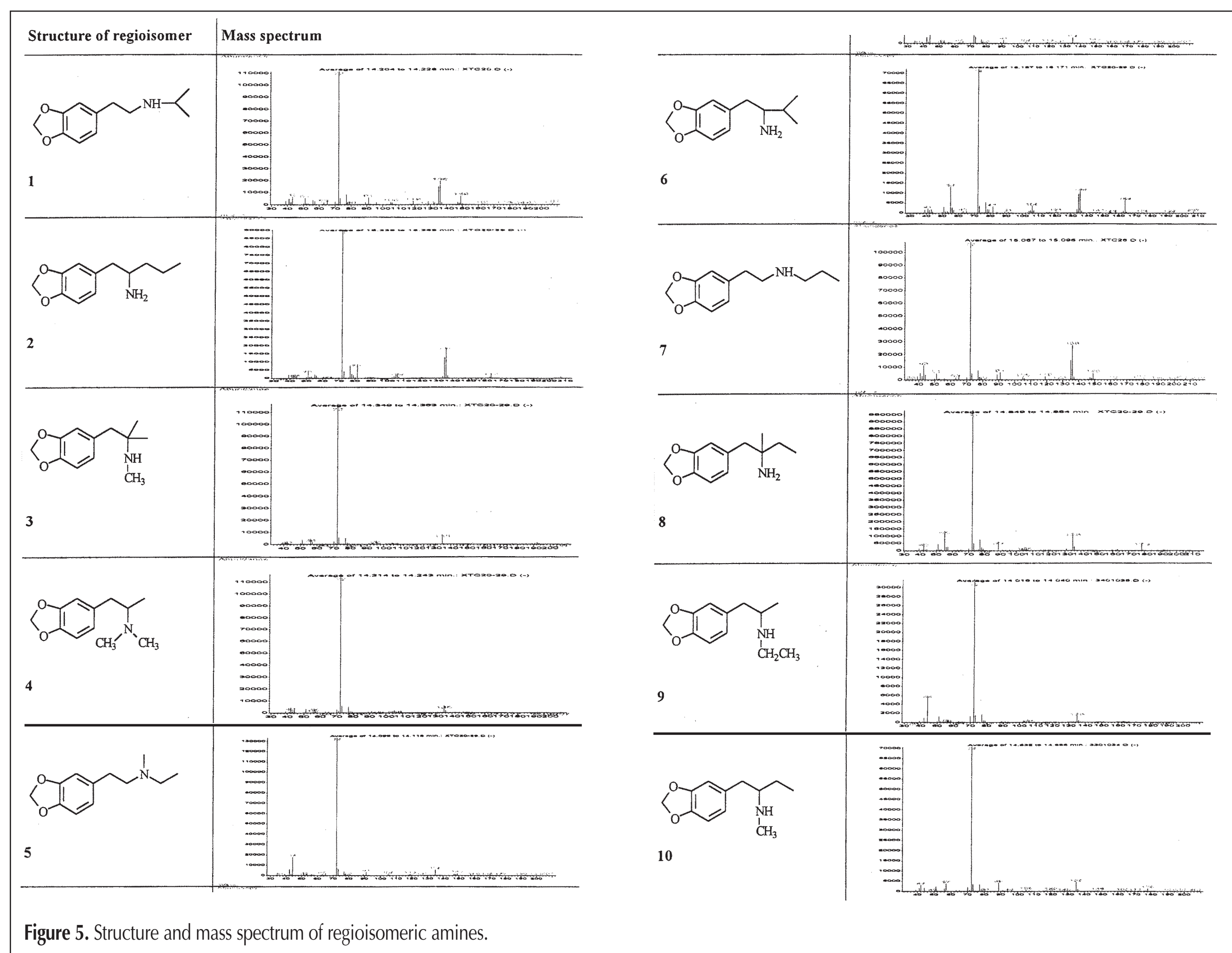

Figure 5. Structure and mass spectrum of regioisomeric amines.

primary amine was accomplished according to the method outlined in Figure 6. The primary amine was treated with benzaldehyde under dehydrating conditions and the imine was reduced with sodium borohydride. The resulting *N*-benzyl secondary amine was reductively alkylated with acetaldehyde and sodium cyanoborohydride to yield the *N*-ethyl-*N*-benzyl-phenethylamine. This amine was debenzylated by hydrogenolysis then the *N*-ethyl secondary amine was methylated with formaldehyde under catalytic hydrogenation conditions to give compound 5.

Mass spectral studies of the regioisomers

Figure 5 shows the EI mass spectra for the ten regioisomers (MW = 207) yielding the butylimine fragment (*m/z* 72) and the methylenedioxybenzyl fragment (*m/z* 135 and 136). These spectra indicate that very little structural information is available for the specific differentiation among these regioisomers because the major fragment ions occur at equivalent mass.

The *m/z* 44 ion in the spectrum of MDEA (compound 9) is a predominant fragment and this ion may occur through the loss of ethylene from the *N*-ethyl group of the base peak (*m/z* 72). Thus, one of the major differences to distinguish MDEA from MDMMA and MBDB is the relative intensity of the *m/z* 44 ion. However, the *m/z* 44 ion in the spectrum of *N*-ethyl, *N*-methyl-3,4-methylenedioxyphenylethanamine (compound 5) is of similar intensity, indicating that the relatively intense *m/z* 44 ion is not characteristic for a single regioisomer.

Some of the regioisomers showed greater relative abundance of the radical cation species at *m/z* 136. 3,4-Methylenedioxyphenyl-2-pentanamine (compound 2) and 3,4-methylenedioxyphenyl-2-isobutanamine (compound 6) are primary amines, which increased the probability of the hydrogen-rearrangement product at *m/z* 136. Additionally, the mass spectra of the unbranched phenethylamines [*N*-isopropyl-3,4-methylenedioxyphenylethanamine (compound 1) and *N*-*n*-propyl-3,4-methylenedioxyphenylethanamine (compound 7)] showed an abundant ion at *m/z* 136. This was perhaps a result of steric freedom to this hydrogen transfer rearrangement.

These two regioisomeric primary amines (compounds 2 and 6) could be differentiated from the unbranched secondary phenethylamines (1 and 7). The product of the other α-cleavage in the less favored direction yielded product at *m/z* 164, which was apparent in the spectra of the primary amines (compounds 2 and 6). Additionally, the unbranched regioisomers 1, 5, and 7 showed a significantly abundant ion at *m/z* 149, which is specific for these compounds. This ion was formed by dissociation of a bond between nitrogen and the α-carbon of the phenethyl side-chain, yielding the $ArCH_2CH_2^+$ fragment.

Products from the α-cleavage in the less favored direction are also seen in the spectrum of 2-methyl-3,4-methylenedioxyphenyl-2-butanamine (compound 8) and MBDB (compound 10). Both spectra showed a relatively abundant peak at *m/z* 178. Furthermore, there was a small peak at *m/z* 192 in the spectrum of 8, which was a result of α-cleavage in the direction of the methyl group. The same α-cleavage occurred for *N*-methyl-3,4-methylenedioxyphentermine (compound 3) and *N,N*-dimethyl MDA (compound 4), but it was not very significant. Actually, the latter compounds showed few ions other than those at *m/z* 72 and 135, and were therefore very difficult to differentiate from each other. These results show that mass spectral fragmentation alone does not provide sufficient information to differentiate among these regioisomers.

GC of the regioisomers

When other compounds exist that have the potential to produce the same or nearly identical mass spectrum as the drug of interest, the separation of the "nondrug regioisomers" from the actual drug of abuse is of utmost importance. MS alone does not provide sufficient information to distinguish between the ten regioisomers in this study. Therefore, the identification by GC–MS must be based primarily upon the ability of the chromatographic system to separate the drug molecules from the nondrug regioisomers.

The regioisomeric phenethylamines were analyzed by capillary GC using first a standard screening method and second a temperature program optimization technique. The screening method used a nonpolar 5% phenyl methyl silicone column, which is commonly applied in forensic drug analysis. This method did not separate all compounds of interest. In order to overcome this problem, a computer-based temperature program optimization technique (DryLab) was applied.

Initially two runs with program rates of 2 and 20°C/min were performed. Column dimensions, efficiency, dead time, and retention times of the analytes were entered in the Drylab GC program to produce a so-called "resolution map". This is a plot that describes the relationship between a temperature program and resolution. The resolution is plotted for critical pairs (i.e., the least-resolved pairs of peaks). A resolution map is a useful tool for determining separation feasibility, which is the condition that provides the best resolution for a separation.

The nonpolar columns (Ultra 1 and 2) gave the best separation, but with relatively low minimum resolution values, and therefore no baseline separation was achieved when linear temperature program rates were used. The more polar columns showed even worse separation, especially DB-17MS, in which the coelution of

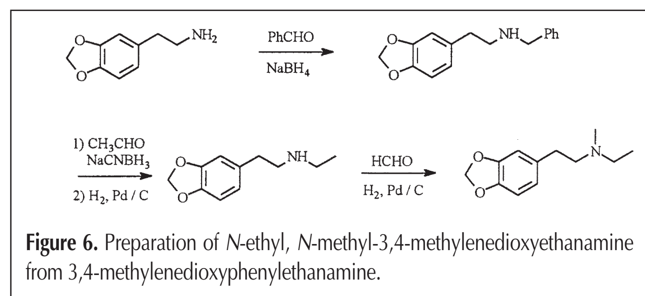

Figure 6. Preparation of *N*-ethyl, *N*-methyl-3,4-methylenedioxyethanamine from 3,4-methylenedioxyphenylethanamine.

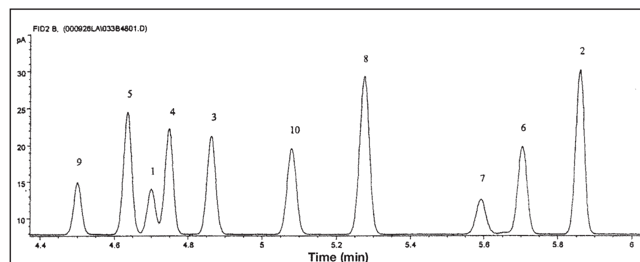

Figure 7. The separation of the regioisomers obtained with the Ultra 1 column using a segmented temperature ramp.

compounds 1 and 4 was constant. The coelution of peak pair 1/4 was critical in most of the columns, except in HP-1701, in which the critical peak pair was 9/5. In both cases the coelution was especially prejudicial because one compound in each of the peak pairs was an illicit drug, either dimethyl-MDA (4) or MDEA (9), but the other compound in each peak pair (1 or 5) was not a known drug substance.

The optimum linear temperature programming separation obtained in this study was on the 100% methyl silicone column (Ultra 1). However, the resolution of the critical pair of compounds (1/4) was 0.86 at a temperature programming rate of 9°C/min. This was not a baseline separation, and, to be able to improve the separation, a segmented temperature ramp was used. The use of a segmented temperature ramp yielded improved resolution (1.05) for the critical peak pair 1/4 and decreased the analysis time. The chromatogram is shown in Figure 7.

The elution order of the regioisomers was constant for all the stationary phases when compared using a temperature program rate of 10°C/min. The same elution order is seen in Figure 7. MDEA (9) eluted first and was followed by other branched regioisomers. The regioisomers with unbranched carbon side-chain showed greater retention and eluted last.

The application of narrow-bore capillary columns (i.d. < 0.1 mm) brings a number of advantages (14). Decreasing the column diameter of open-tubular capillaries is an effective way to speed up the separation process. Because the optimum reduced plate heights H_{min} have fixed values, a decrease of d resulted in a proportionally decreased value of H_{min} ($H = hd$). Therefore, the column length ($L = NH$) can be decreased by the same factor in order to yield the same plate number (N). For example, a 10-m × 0.10-mm i.d. capillary column has approximately the same theoretical plate number as a 25-m × 0.25-mm i.d. column. The use of narrow-bore columns improved both the analysis time and separation for this set of regioisomers. The temperature program rate of 40°C/min gave the separation of regioisomers with minimum resolution of 0.99, and the retention time of last eluting compound was 3.65 min (Figure 8).

Conclusion

In summary, three regioisomeric 3,4-methylenedioxyphenethylamines having the same molecular weight and major mass spectral fragments of equivalent mass have been reported as components of clandestine drug samples in recent years. These drugs of abuse (MDEA, MDMMA, and MBDB) are a subset of a total of ten 3,4-methylenedioxyphenethylamines of molecular weight 207, and they yield regioisomeric fragment ions of equivalent mass (m/z 72 and 135/136) in the EI mass spectrum. The results of this study show that MS alone would not allow for the specific identification of one of these compounds to the exclusion of the other nine possible molecules. The ultimate identification of any one of these amines with the elimination of the other nine regioisomeric substances depends heavily upon chromatographic methods.

The nonpolar methylsilicone stationary phases gave the best separation for these regioisomeric amines. But with relatively low minimum resolution values, no baseline separation was achieved when linear temperature program rates were used. The more polar columns showed even worse separation, especially DB-17MS, for which the coelution of compounds 1 and 4 was constant. The coelution of peak pair 1/4 was critical in most of the columns. Because one compound in the peak pairs is an illicit drug and the other compound is not a known drug substance, the ability to differentiate between these substances is a significant analytical issue.

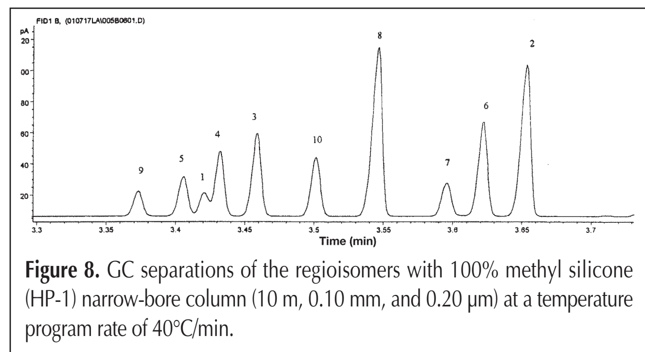

Figure 8. GC separations of the regioisomers with 100% methyl silicone (HP-1) narrow-bore column (10 m, 0.10 mm, and 0.20 µm) at a temperature program rate of 40°C/min.

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