Liquid Chromatographic and Mass Spectral Methods of Identification for Regioisomeric Dimethoxyamphetamines and Brominated Dimethoxyamphetamines

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Abstract

The six regioisomeric dimethoxyamphetamines are prepared from the commercially available dimethoxybenzaldehydes. The dimethoxyamphetamines show very similar mass spectra, and chromatographic methods must be used to differentiate the positional isomers. Bromination of the six isomeric dimethoxyamphetamines yields a monobromination product as the major component in all cases except for 3,5-dimethoxyamphetamine, which yields the 2,6-dibrominated species as the major product. Mass spectrometric analysis readily divides the regioisomeric bromodimethoxyamphetamines into two groups of three compounds each. Only those isomers having a bromine substituent "ortho-" to the alkylamine side-chain show a major fragment at *m/z* 194 from loss of bromine from the molecular ion. The major drug of abuse 4-bromo-2,5-dimethoxyamphetamine (DOB) is one of three compounds that do not yield the *m/z* 194 ion. Though the mass spectra for the three "non-*m/z* 194" isomers show some subtle differences, these compounds are best differentiated by a reversed-phase liquid chromatographic system.

Introduction

The major focus of designer drug activity in recent years has been in the area of ring-substituted phenalkylamines. A number of clandestinely produced drugs of abuse from this category have appeared as street drugs including 3,4-methylenedioxymethamphetamine (1), *N*-hydroxy-3,4-methylenedioxyamphetamine (2), *N*-methyl-3,4-methylenedioxy-2-butanamine (3), and 4-bromo-2,5-dimethoxyphenethylamine (4). The recent appearance of 4-bromo-2,5-dimethoxyphenethylamine (Nexus) as a street drug has created a renewed interest in the development of definitive methods for the identification of individual regioisomers of substituted phenethylamines (5). Although nuclear magnetic resonance (NMR) is a very reliable method for such regioisomer differentiation, this is not a common tool in forensic laboratories, and NMR is clearly not a technique with application for biological sample analysis. Thus, NMR is of little value in the identification of a drug or drug metabolite from a biological sample in a toxicological investigation. The analysis of street drug samples and analytical toxicology must depend heavily on chromatographic methods as well as mass spectrometry (MS).

The Schedule I hallucinogen 4-bromo-2,5-dimethoxyamphetamine (DOB) is another example of a ring-substituted phenalkylamine that appears from time to time in street drug samples. DOB is a hallucinogen (6) several times more potent than Nexus, and the growing interest in these types of compounds suggests that DOB will again appear as a street drug. The

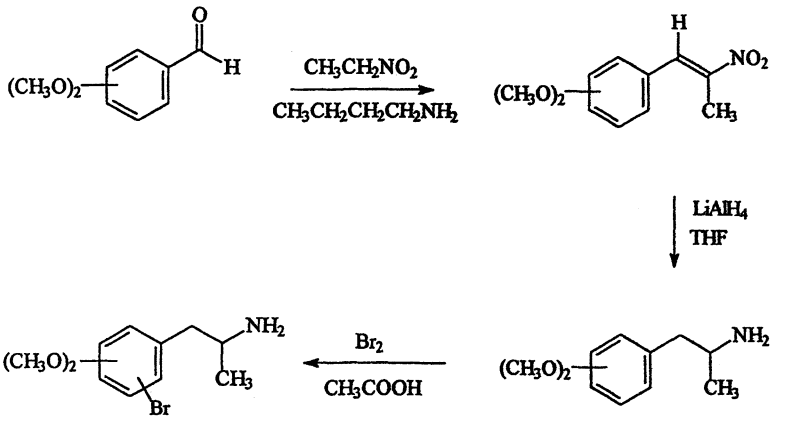

Figure 1. Synthesis of regioisomeric brominated dimethoxyamphetamines.

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most likely method for the synthesis of DOB as well as Nexus is via nitroalkane condensation with 2,5-dimethoxybenzaldehyde followed by hydride reduction and ring bromination (Figure 1). All six regioisomeric dimethoxybenzaldehydes are commercially available and uncontrolled, thus any one of the six regioisomeric dimethoxyamphetamines can be prepared by the same synthetic methodology. Bromination of the six individual dimethoxyamphetamines should yield six or more monobrominated dimethoxyamphetamines. This paper describes the mass spectral properties of the regioisomeric dimethoxyamphetamines and brominated dimethoxyamphetamines as well as chromatographic methods for their individual identification.

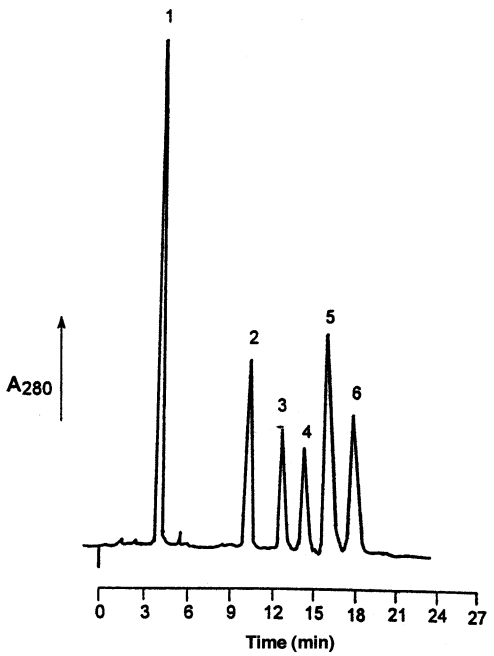

Figure 2. HPLC separation of the six regioisomeric dimethoxyamphetamines. Peaks: 1, 3,4-dimethoxyamphetamine; 2, 2,5-dimethoxyamphetamine; 3, 2,3-dimethoxyamphetamine; 4, 2,6-dimethoxyamphetamine; 5, 3,5-dimethoxyamphetamine; 6, 2,4-dimethoxyamphetamine.

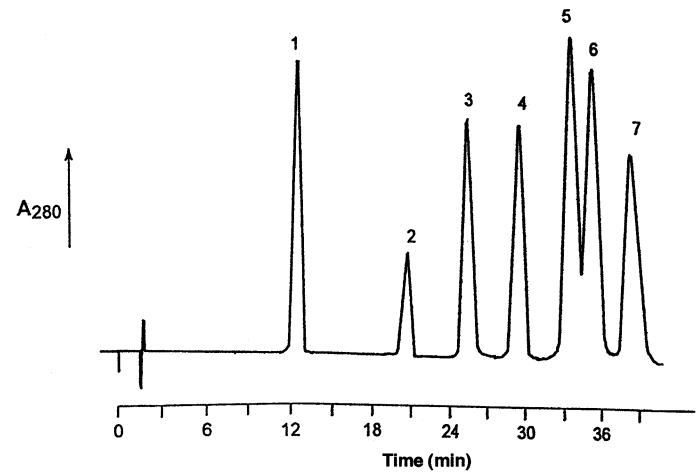

Figure 3. HPLC separation of all seven brominated dimethoxyamphetamines. Peaks: 1, 2-bromo-4,5-dimethoxyamphetamine; 2, 6-bromo-3,5-dimethoxyamphetamine; 3, 6-bromo-2,3-dimethoxyamphetamine; 4, 5-bromo-2,4-dimethoxyamphetamine; 5, 2,6-dibromo-3,5-dimethoxyamphetamine; 6, 4-bromo-2,5-dimethoxyamphetamine; 7, 3-bromo-2,6-dimethoxyamphetamine.

Experimental

Instrumentation and methods

Gas chromatographic–mass spectrometric (GC–MS) analyses were performed using a Hewlett-Packard 5970B mass selective detector (Hewlett-Packard, Palo Alto, CA). The MS was operated in the electron-impact mode with an ionization voltage of 70 eV and a source temperature of 220°C. The samples were dissolved in methanol (1 mg/mL), and 0.5 µL was introduced into the MS via a GC equipped with a 12-m × 0.20-mm-i.d. fused-silica column with a 0.33-µm film of methylsilicone (HP-1). The column temperature was held at 70°C for 2.5 min and programmed to increase to 170°C at a rate of 25°C/min and from 170 to 275°C at a rate of 12°C/min with a hold time of 6 min. The split ratio for the GC was 10:1 and the injector port temperature was 230°C. The carrier gas was ultrapure helium.

Liquid chromatographic analyses were conducted using a Waters (Milford, MA) model 590 pump, a Laboratory Data Control 3000 Spectromonitor ultraviolet (UV) detector (Laboratory Data Control, Riveria Beach, FL), a Rheodyne 7125 injector (Rheodyne, Cotati, CA), and Linear model LR 93125 recorder (Linear, Dubuque, IA). The analytical column was 15 cm × 4.6-mm i.d. Hypersil Elite C_{18} (Shandon HPLC, Cheshire, UK). The mobile phase consisted of phosphate buffer (pH 3.0) and acetonitrile (75:25) for Figure 2 and phosphate buffer (pH 3.0) and acetonitrile (85:15) for Figure 3. The pH 3.0 phosphate buffer was prepared by mixing 9.2 g of monobasic sodium phosphate in 1 L of double-distilled water and adjusting the pH to 3.0 with H_3PO_4. The UV absorbance detector was operated at 280 nm and 0.05 absorbance units full scale (AUFS). The mobile phase flow rate was 1 mL/min; the compounds were prepared as methanol solutions, and volumes of 2–10 µL were injected.

Synthesis of dimethoxyphenyl-2-nitropropenes

A mixture of the appropriate dimethoxybenzaldehyde, butylamine, and benzene was stirred at reflux for several hours using a Dean-Starke trap to remove water. The reaction mixture was evaporated under reduced pressure, and the remaining oil was dissolved in a mixture of glacial acetic acid and nitroethane. After this solution was stirred at reflux for several hours, it was cooled, and ice was added to precipitate the product. Concentrated HCl was added to adjust the pH to 2. The product was isolated by filtration, washed with water, and recrystallized from 2-propanol to yield the nitropropenes as highly colored needles.

Synthesis of dimethoxyamphetamines

A solution of the nitropropene intermediate in dry tetrahydrofuran (THF) was added to a suspension of lithium aluminum hydride (LAH) in THF stirred at room temperature. After the addition

was complete, the reaction mixture was stirred at reflux for several hours, then cooled in an ice bath. The excess LAH and LAH salts were decomposed by successive addition of water, 2N NaOH, and water. The mixture was then filtered, and the filtrate was evaporated under reduced pressure. The remaining oil was suspended in water and acidified to pH 1. The resulting aqueous solution was washed with benzene, then made basic by the addition of NaOH pellets and extracted twice with methylene chloride. The combined methylene chloride extracts were washed with water and dried over anhydrous sodium sulfate. Filtration followed by evaporation of the filtrate solvent yielded the product amines as oils. The amines were converted to their corresponding HCl salts by treatment with ethereal HCl.

Synthesis of brominated dimethoxyamphetamines

A solution of bromine in glacial acetic acid was added over a few minutes to a solution of the amine hydrochloride in glacial acetic acid. After the addition was complete, the reaction mixture was stirred at room temperature for an hour, resulting in the formation of a yellow precipitate. The precipitate was isolated by filtration and suspended in aqueous NaOH. The aqueous basic suspension was extracted with methylene chloride, and the combined organic extracts were washed with water and dried over potassium carbonate. Filtration followed by evaporation of the filtrate solvent yielded the product amines as oils. The amines were converted to their corresponding hydrochloride salts by treatment with ethereal HCl.

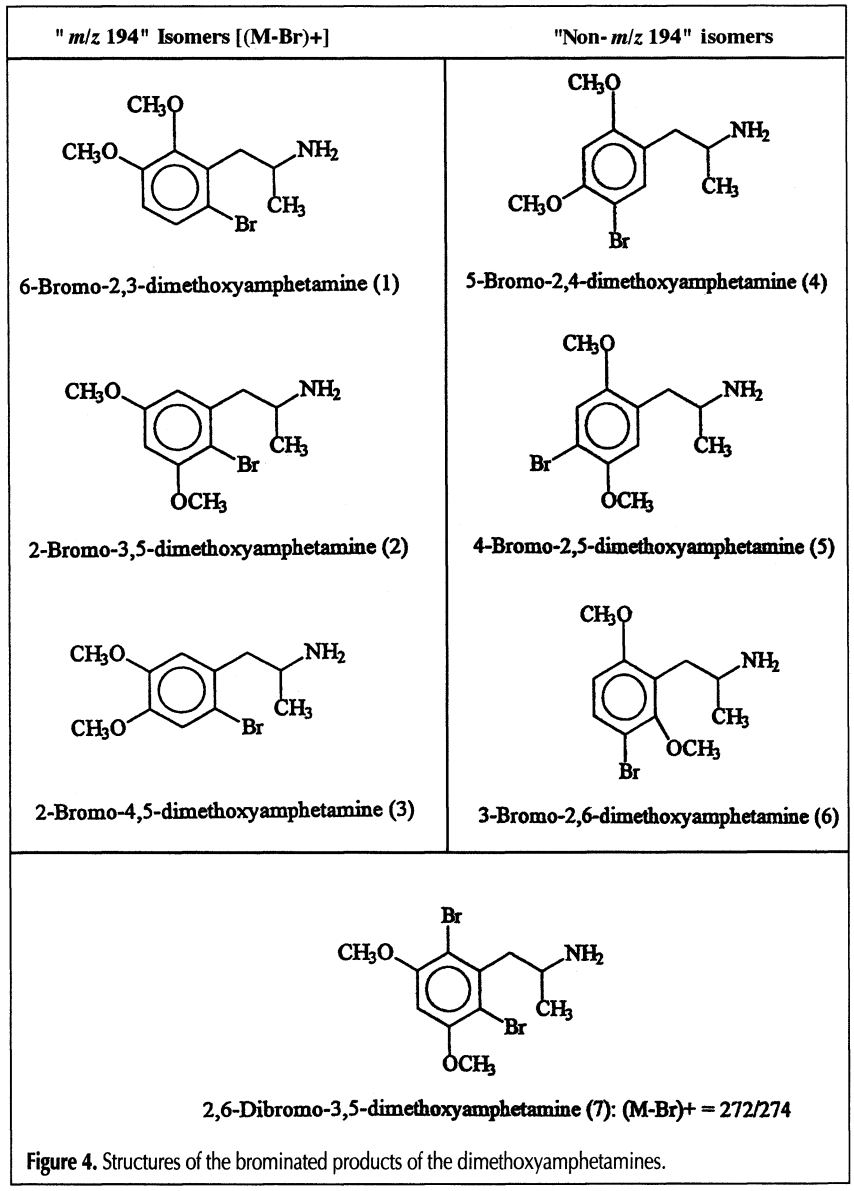

Figure 4. Structures of the brominated products of the dimethoxyamphetamines.

Results and Discussion

The synthesis of substituted phenethylamines can be accomplished by several methods. Many of the common methods begin with precursor chemicals, which are now controlled under the U. S. Precursor and Essential Chemical Act of 1989. A significant exception involves the use of readily available and uncontrolled substituted benzaldehydes. These compounds are converted to the versatile 2-nitroalkene, which is reduced to the corresponding primary amine or, in some cases, subjected to reduction hydrolysis. This general method of preparing phenethylamines is illustrated in Figure 1, which shows the synthesis of the substituted phenethylamine DOB. Commercially available 2,5-dimethoxybenzaldehyde was treated with butylamine and nitroethane to yield the nitropropene interme-

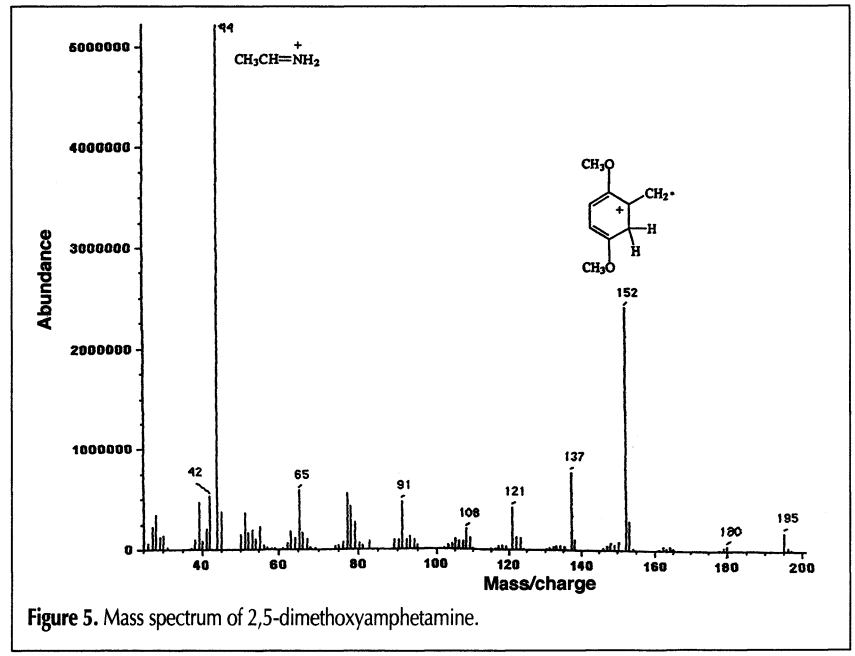

Figure 5. Mass spectrum of 2,5-dimethoxyamphetamine.

diate. Upon reduction with LAH, the phenethylamine was obtained in high yield. Bromination of the amine hydrochloride produces the 4-bromo substituted amphetamine that has the street name DOB. This analogous procedure using the appropriately substituted dimethoxybenzaldehyde was used to prepare all the isomeric amines included in this study. The structures of all products were confirmed by standard spectroscopic techniques (NMR, infrared [IR], and MS).

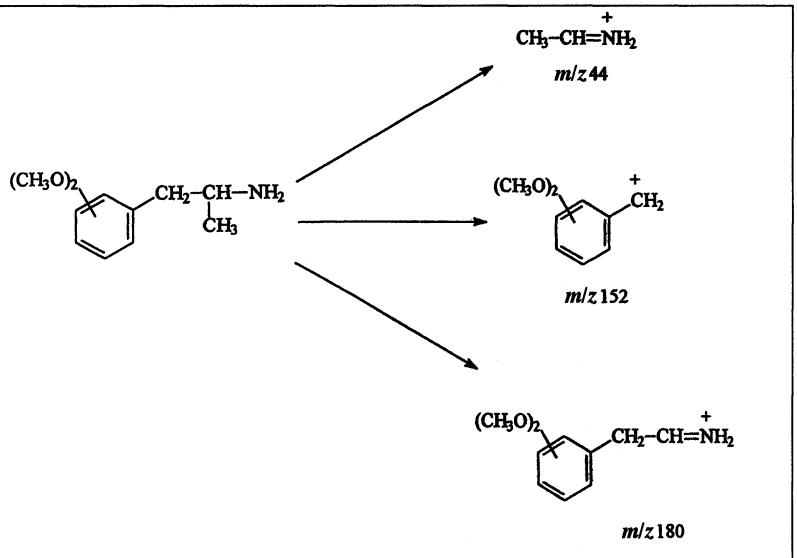

Figure 6. Major electron-impact fragmentation reactions for the dimethoxyamphetamines.

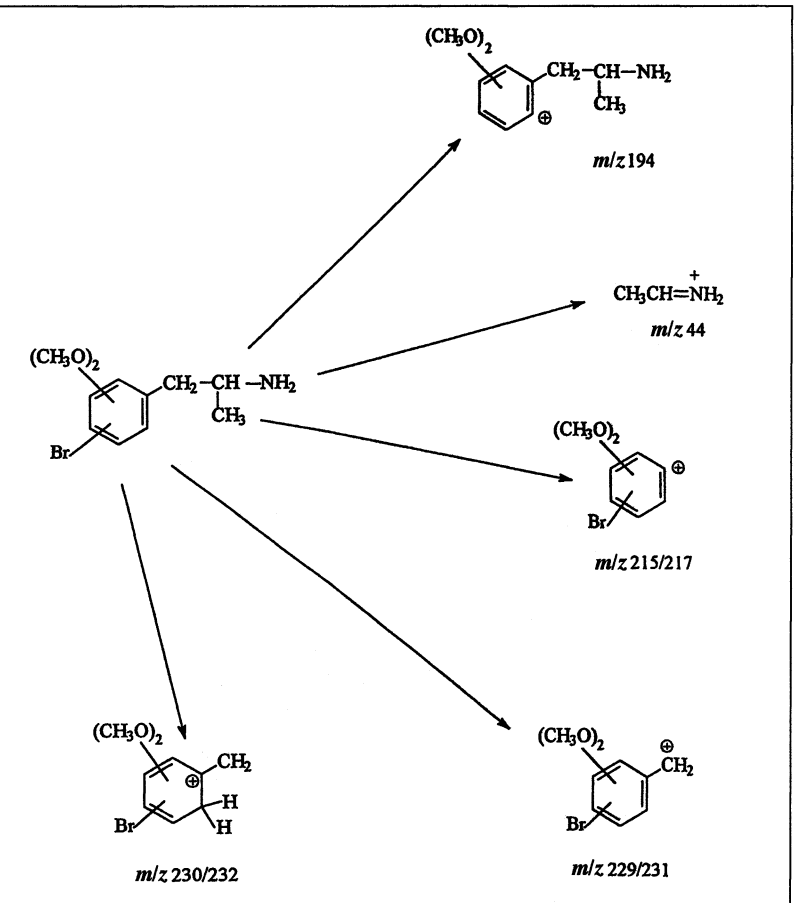

Figure 7. Major electron-impact fragmentation reactions for the bromodimethoxyamphetamines.

The position of bromination for each final product was confirmed by the coupling patterns of the aromatic protons by proton NMR (^{1}H-NMR), and the structures of these products are shown in Figure 4. As described by Sepulveda et al. (7), aromatic protons isolated by bromine substitution display no appreciable splitting ($J << 1$ Hz), whereas protons remaining on adjacent carbons exhibit typical ortho- coupling. These coupling patterns and relative integrals of the ortho-, meta-, and para- protons allow for determination of the position of bromination. The position of bromination of the dimethoxyamphetamines is dependent on the pattern of the methoxy group substitutions. Though each of the dimethoxyamphetamines have three ring carbons available for substitution, bromination typically occurs at only one of these positions. This reaction proceeds via an electrophilic mechanism, and thus substitution occurs preferentially at ring carbons that are in direct conjugation with the electron-donating methoxy groups (the so-called "ortho-" or "para-" positions). In cases in which there are multiple ring carbons in conjugation, bromination usually occurs at the least sterically hindered site. Thus, although there are three distinct ring positions available for electrophilic substitution in 2,5-dimethoxyamphetamine, bromination occurs only at the 4- position (Figure 4, structure 5). In this compound, the 4- position is in direct conjugation with the 5-methoxy (ortho-) group and is not as sterically crowded as it would be at the 6- position. Similarly with 2,4-dimethoxyamphetamine, bromination occurs at the 5- position because this carbon is in direct conjugation with the 2-methoxy (para-) and 4-methoxy (ortho-) groups and is not as sterically crowded as the other position in conjugation, the 3- carbon (Figure 4, structure 4). With 2,6-dimethoxyamphetamine, only two bromination products are possible as a result of the ring symmetry imparted by the 2,6-dimethoxy substitution. In this case, only the 3- position is brominated because it is "ortho-" to one ring methoxy group and "para-" to the other (Figure 4, structure 6). With 2,3-dimethoxyamphetamine, bromine substitution can occur at both positions 5- and 6- because both of these carbon atoms are "para-" to at least one electron-rich methoxy group. The predominant product isolated in this study was the 6-bromo derivative (Figure 4, structure 1). Similarly, bromination of 3,4-dimethoxyamphetamine yielded only the 6-bromination product. In this case, it should be noted that the addition of bromine changes the priority for ring numbering (to obtain the lowest number for substituents); thus this product is named 2-bromo-4,5-dimethoxyamphetamine instead of 6-bromo-3,4-dimethoxyamphetamine (Figure 4, structure 3). Bromination of 3,5-dimethoxyamphetamine yielded a mixture of the

2-bromo- (Figure 4, structure 2) and 2,6-dibromo- (Figure 4, structure 7) substitution products. In this case, the dibromination product predominated, even when minimal amounts of bromine were used in the reaction. The preferential formation of the 2,6-dibromo product in this reaction was consistent with other reported results (7).

The mass spectra for the six regioisomeric dimethoxyamphetamines are quite similar, showing only differences in relative intensity of some fragment ions. The mass spectrum for 2,5-dimethoxyamphetamine in Figure 5 is representative of the group. The major fragmentation reactions are summarized in Figure 6. The base peak in each spectrum was the *m/z* 44 imine ion, and the dimethoxybenzyl radical cation at *m/z* 152 was the most abundant high-mass fragment. The other high-mass fragments occurred at *m/z* 180 from the loss of a methyl group from the molecular ion and the molecular ion itself at *m/z* 195. In a previous study (8), the difficulty in differentiation among these regioisomeric compounds by standard analytical techniques was described. The mass spectra showed only the *m/z* 44 ion, a common fragment for all 1-phenyl-2-aminopropanes, ions at *m/z* 152 and 195, which occurred for all six regioisomers, and low-mass fragments of relatively low abundance. The IR spectra for these compounds did not provide any useful information concerning regioisomer differentiation due to polymorphism, salt form, and degree of hydration (8).

The six dimethoxyamphetamines were separated by reversed-phase liquid chromatography using a stationary phase of Hypersil Elite C_{18} and a mobile phase of pH 3 phosphate buffer and acetonitrile. Preliminary experiments on mobile phase composition revealed that the addition of silanol masking agents such as triethylamine did not improve the peak shape for basic amines. The Hypersil Elite stationary phase is a surface-deactivated C_{18} material that yields excellent peak shapes for highly silanophilic substances. Figure 2 shows the separation of the six dimethoxyamphetamines under isocratic conditions in approximately 20 min with baseline resolution. These isomeric amines eluted over a wide range of capacity factors displaying a variety of retention properties, and the 3,4-dimethoxyamphetamine substitution pattern displayed the lowest stationary phase affinity. This 3,4-substitution pattern in the dimethoxyamphetamines displayed a significant increase in polarity over the other regioisomers, eluting at just over 4 min, whereas the second peak, 2,5-dimethoxyamphetamine, eluted in the 10-min range. Peaks 2 through 6 eluted over an approximate 10-min time window; the 2,4-dimethoxy isomer displayed the highest capacity factor.

The separation of the seven brominated dimethoxyamphetamine products is shown in Figure 3. This separation was obtained using the same Hypersil Elite C_{18} stationary phase and a slightly weaker mobile phase consisting of 15% acetonitrile in pH 3 buffer. The 2-bromo-4,5-dimethoxyamphetamine (the bromination product of 3,4-dimethoxyamphetamine) eluted first in the chromatogram in Figure 3 as did the unbrominated precursor amine (see Figure 2). Peak 2 in Figure 3 is 2-bromo-3,5-dimethoxyamphetamine; the monobromination product of 3,5-dimethoxyamphetamine and the addition of a second bromine substituent in 2,6-dibromo-3,5-dimethoxyamphetamine produced even greater hydrophobicity than the monobrominated product. The peak for 2,6-dibromo-3,5-dimethoxyamphetamine appears in Figure 3 at over 33 min (peak 5). Peak 3 in Figure 3 is 6-bromo-2,3-dimethoxyamphetamine, thus the first three peaks in this chromatogram were the result of monobromination of the aromatic ring at an ortho- position relative to the alkylamine side-chain. Peaks 4, 6, and 7 in Figure 3 are the monobrominated products of 2,4-; 2,5-; and 2,6-dimethoxyamphetamine, respec-

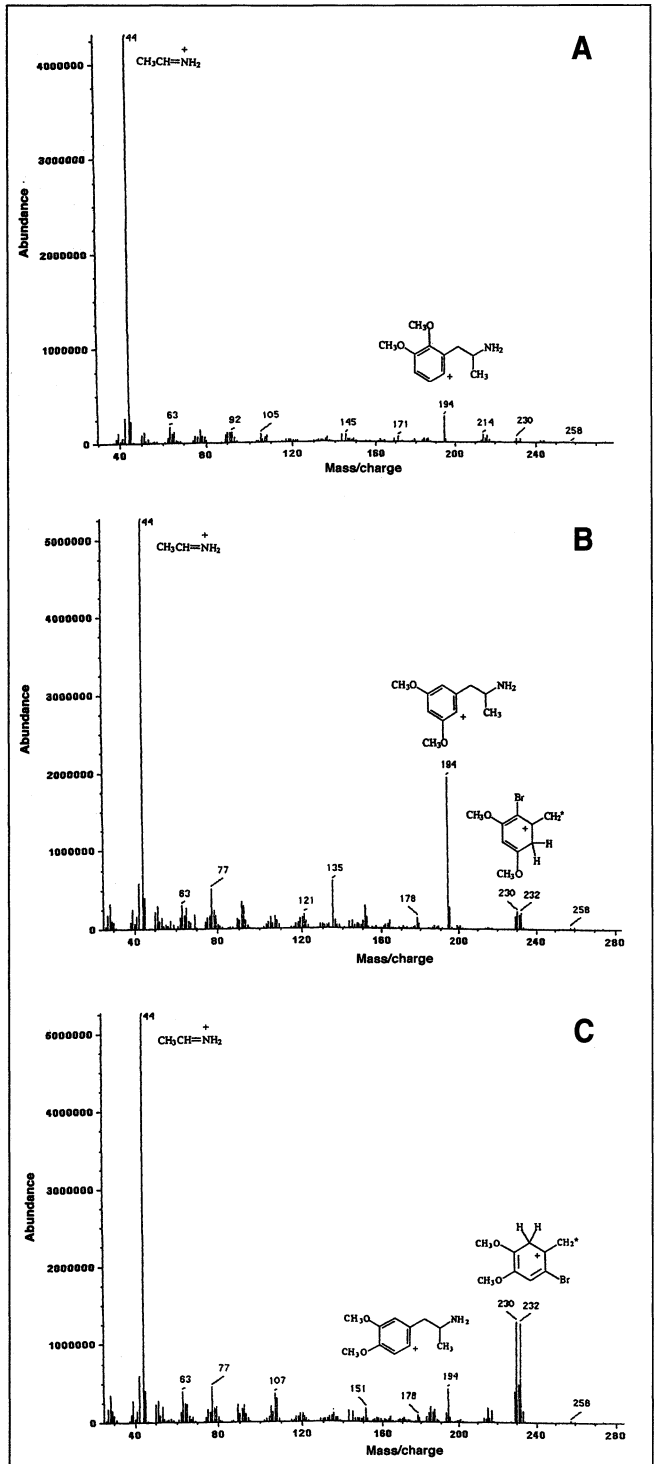

Figure 8. Mass spectra of the regioisomeric bromodimethoxyamphetamines showing the $(M\text{-}Br)^+$ major fragment. (A) Mass spectrum of 6-bromo-2,3-dimethoxyamphetamine, (B) 2-bromo-3,5-dimethoxyamphetamine, and (C) 2-bromo-4,5-dimethoxyamphetamine.

tively. These compounds contain the bromine substituent at either the 3- or 4- position of the aromatic ring. Thus, monobromination at the 3- or 4- position of the aromatic ring appeared to produce significantly greater hydrophobicity in the dimethoxyamphetamines than did bromination at the 2- position.

The mass spectra for the brominated dimethoxyamphetamines show similar fragmentation patterns and are summarized in Figure 7. Most of these compounds showed only trace amounts of molecular ions; however, bromination of the dimethoxyamphetamines was obvious from the abundant fragment ions containing bromine. The brominated fragment ions are easily identified based on the nearly equal isotopic ratios of ^{79}Br to ^{81}Br. The substituted benzyl radical cation containing bromine was a major fragment for many of the compounds at *m/z* 230/232. The loss of the entire side-chain to yield the substituted benzene occurred at *m/z* 215/217. The *m/z* 199/201 ion was likely the result of loss of CH_2O from the brominated dimethoxybenzyl cation at *m/z* 229/231. The loss of bromine from the molecular ion was a major fragment for those compounds in which the position of the bromine substitution is "ortho-" to the alkylamine side-chain. This ion at *m/z* 194 was a prominent fragment in brominated 2,3-; 3,5-; and 3,4-dimethoxyamphetamines (Figure 8). Thus, the $(M\text{-}Br)^+$ ion at *m/z* 194 was a major diagnostic reference point for differentiating the "ortho-" brominated positional isomers from the "non-ortho-" brominated isomers. Although the mass spectra for 2-bromo-3,5-dimethoxyamphetamine is shown in Figure 8B, the bromination of 3,5-dimethoxyamphetamine yielded primarily the dibromo product. The GC–MS analysis of the product mixture obtained from the bromination of 3,5-dimethoxyamphetamine is shown in Figure 9. The minor component in the chromatogram (Figure 9A) eluting at 9.2 min is the monobromination product, 2-bromo-3,5-dimethoxyamphetamine, whose mass spectrum is shown in Figure 8B. The mass spectrum in Figure 9B is 2,6-dibromo-3,5-dimethoxyamphetamine, and this dibromo product showed the loss of one bromine from the molecular ion $(M\text{-}Br)^+$ as a major high-mass fragment at *m/z* 272/274.

The $(M\text{-}Br)^+$ ion was absent from the mass spectra of brominated 2,4-; 2,5-; and 2,6-dimethoxyamphetamine (Figure 10). The *m/z* 194 fragment ion then divided the six regioisomeric brominated dimethoxyamphetamines into two groups of three compounds. This subdivision based on the presence or absence of the *m/z* 194 ion simplified the challenge of distinguishing among the six isomeric amphetamines.

The mass spectra for the three positional isomers not showing the *m/z* 194 ion had some differences in the relative abundance of the high-mass fragment clusters at *m/z* 199/201, 215/217, and 230/232 (Figure 10). The brominated 2,6-dimethoxyam-

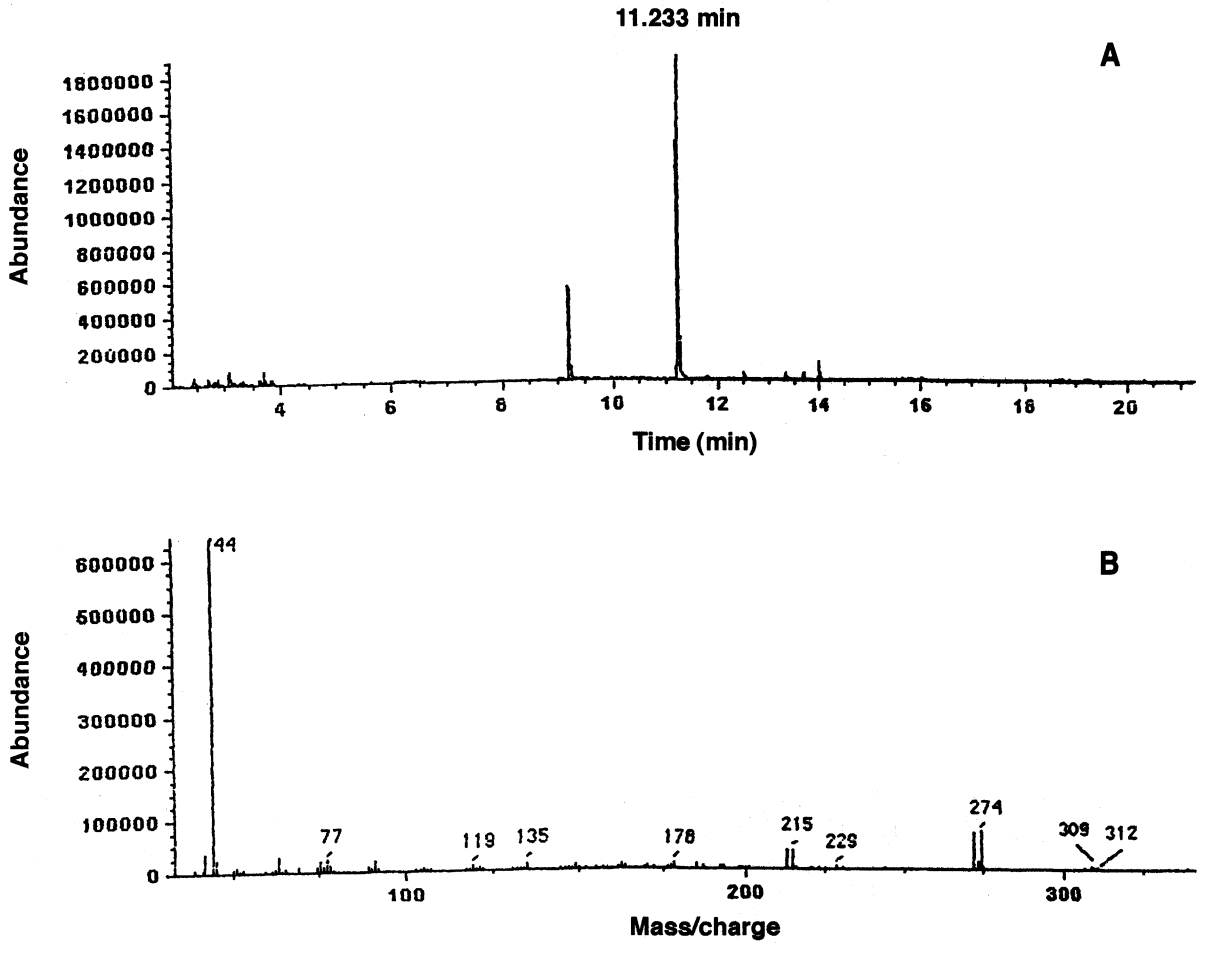

Figure 9. GC–MS analysis of the product resulting from bromination of 3,5-dimethoxyamphetamine. (A) GC separation of 2-bromo-3,5-dimethoxyamphetamine (9.2 min) and 2,6-dibromo-3,5-dimethoxyamphetamine (11.2 min). (B) Mass spectrum of 2,6-dibromo-3,5-dimethoxyamphetamine.

phetamine (Figure 10C) showed a high relative concentration of the *m/z* 230/232 fragment cluster with very low abundance of the *m/z* 199/201 and 215/217 clusters. The brominated 2,4- (Figure 10A) and 2,5-dimethoxyamphetamines (Figure 10B) showed extremely similar mass spectra; the most notable differences were the relative abundance of the *m/z* 199/201 and 215/217 clusters.

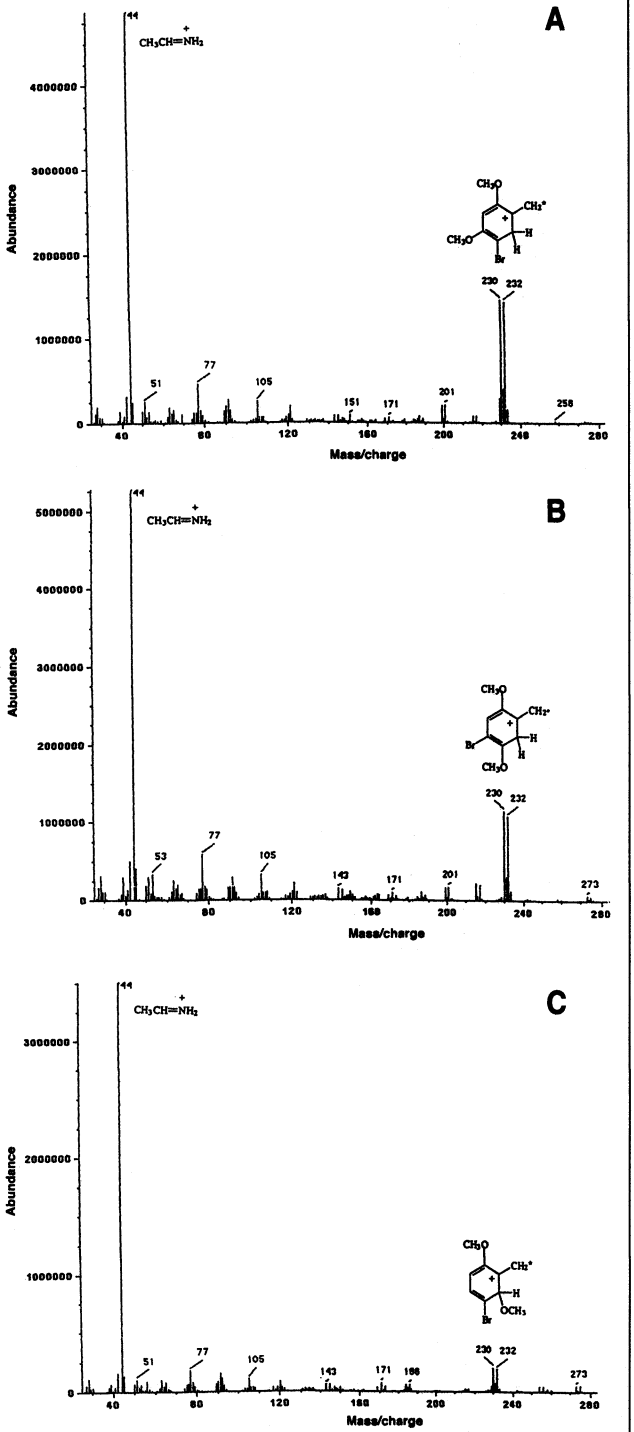

Figure 10. Mass spectra of the regioisomeric bromodimethoxyamphetamines not showing the $(M-Br)^+$ major fragment. (A) 5-bromo-2,4-dimethoxyamphetamine, (B) 4-bromo-2,5-dimethoxyamphetamine, and (C) 3-bromo-2,6-dimethoxyamphetamine.

Conclusion

In summary, the six isomeric dimethoxyamphetamines can be prepared from the commercially available dimethoxybenzaldehydes via formation of the 2-nitropropenes followed by reduction with lithium aluminum hydride. The six regioisomeric dimethoxyamphetamines showed very similar mass spectra and were separated with baseline resolution in an isocratic reversed-phase liquid chromatographic system. MS analysis readily divided the regioisomeric bromodimethoxyamphetamines into two groups of three compounds. Those isomers having a bromine substituent "ortho-" to the alkylamine side-chain showed a major fragment at *m/z* 194 due to the loss of bromine from the molecular ion. Brominated 2,4-; 2,5-; and 2,6-dimethoxyamphetamine did not show a loss of bromine from the molecular ion and did not produce the *m/z* 194 ion in their mass spectra. Thus, the mass spectra of these isomers placed the compounds into two groups depending on the presence or absence of the *m/z* 194 ion. The major drug of abuse 4-bromo-2,5-dimethoxyamphetamine is one of three compounds that does not yield the *m/z* 194 ion, and although the mass spectra for the three "non-*m/z* 194" isomers showed some subtle differences, these compounds were best differentiated by reversed-phase liquid chromatographic separation.

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