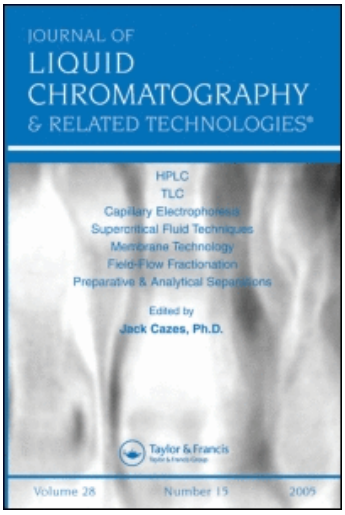

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LIQUID CHROMATOGRAPHIC AND MASS SPECTRAL ANALYSIS OF N,N-DISUBSTITUTED 3,4-METHYLENEDIOXYAMPHETAMINES

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ABSTRACT

The tertiary amine N,N-disubstituted derivatives of 3,4-methylenedioxyamphetamine (MDA) were prepared and their liquid chromatographic and mass spectral properties compared to other analogues of 3,4-methylenedioxyamphetamine. The N-methyl-N-ethyl, N-methyl-N-i-propyl and N-methyl-N-n-propyl MDA derivatives were separated in a reversed-phase system consisting of a C_{18} stationary phase and a mobile phase of pH 4 phosphate buffer-acetonitrile (55:45). The mass spectral fragmentation of these amines is similar to that observed for the secondary amine MDA derivatives and can be used for their specific structural identification.

INTRODUCTION

A number of N-substituted derivatives of 3,4-methylenedioxyamphetamine (MDA) continue to be encountered by forensic laboratories in North America [1-3]. The N-methyl derivative, also known as MDMA or "Ecstasy", appears to be the most widely used drug of this series due to its empathy-enhancing effects and its ability to reduce fear and anxiety [4]. In recent years several N-substituted "designer" analogues of 3,4-methylenedioxyamphetamine, including the N,N-dimethyl, N-ethyl and N-hydroxy derivatives have appeared as street drugs [5,6]. These compounds typically are prepared from commercially available 1-(3,4-methylenedioxyphenyl)-2-propanone via reductive amination with the appropriate amine [6]. The continued interest in designer analogues of the MDA-type, and the ease of synthesis of these compounds, suggests the potential for the appearance of additional derivatives. One possible series of compounds may be based on alkylation of MDMA to yield tertiary amines such as N-methyl-N-ethyl, N-methyl-N-i-propyl and N-methyl-N-n-propyl MDAs. These compounds have not been observed in street samples at this point in time, however, the precursor secondary amines are commercially available.

In this study the tertiary amine N-alkyl derivatives of MDMA have been prepared and their analytical profiles compared to those of similar secondary amine MDA analogues.

EXPERIMENTAL

Instrumentation The liquid chromatograph (LC) consisted of a Waters Associates Model 6000 A pump, U6K injector, Model 440 UV

detector and a Houston Instruments OmniScribe dual pen recorder. Infrared spectra were recorded on a Perkin-Elmer Model 1710 Fourier transform infrared (FTIR) spectrophotometer. The electron impact mass spectra were obtained using a Hewlett-Packard 5970B mass selective detector. The ionization voltage was 70 eV and the source temperature was 220 ^{o}C. The individual samples were dissolved in methanol (1 mg/mL) and 0.5 uL was introduced into the mass spectrometer via the gas chromatograph (GC) equipped with a 12 m x 0.31 mm i.d. fused-silica column with a 0.52 um thickness of OV-1. The column temperature was programmed from 70^{o}C to 150^{o}C at a rate of 15^{o}C/min and from 150^{o}C to 250^{o}C at a rate of 25^{o}C/min. The split ratio for the GC was 10:1 and all samples eluted in approximately 7 min.

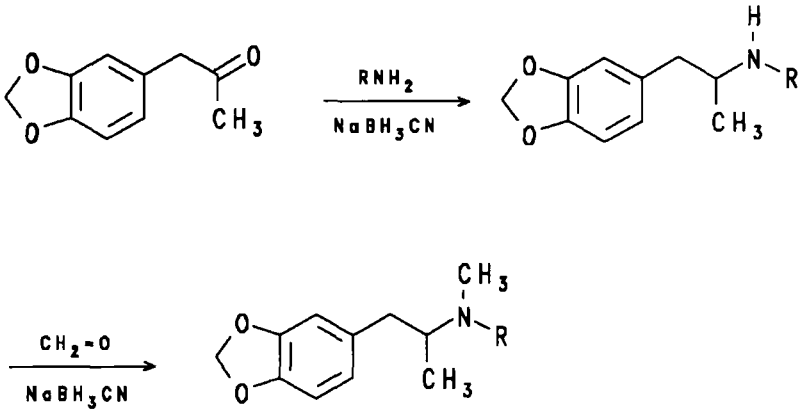
Liquid Chromatographic Procedures. The analytical columns were 15 cm X 4.6 mm i.d. packed with either Bondex C_{18} (Phenomenex, Inc.) or Deltabond C_8 (Keystone Scientific). The analytical columns were preceded by a 7 cm X 2.1 mm i.d. guard column dry packed with CO:Pell ODS (Whatman). The amines (1 mg/mL) were dissolved in HPLC-grade acetonitrile and chromatographed using a mobile phase of pH 3.0 phosphate buffer and HPLC-grade acetonitrile. The pH 3.0 phosphate buffer was prepared by mixing 9.2 g of monobasic NaH_2PO_4 in 1 L of double distilled water and adjusting the pH to 3.0 with H_3PO_4. The mobile phase flow rate was 1.0 mL/min and the column was maintained at a temperature of 40^{o}C. The detector was operated at 280 nm (0.1 AUFS) and a 15 uL aliquot of each amine solution was injected into the LC.

Synthesis of the N,N-Disubstituted 3,4-Methylenedioxyamphetamines A solution of 3,4-methylenedioxyphenylpropanone (10 mmol), alkylamine (25 mmol of ethylamine, n-propylamine or i-propylamine) and

sodium cyanoborohydride (25 mmol) in methanol (25 mL) was stirred at room temperature for 24 h. The reaction mixture was then evaporated to dryness under reduced pressure and the remaining residue was suspended in dichloromethane (50 mL). The dichloromethane suspension was extracted with 3 N HCl (2 X 50 mL) and the combined acid extracts made basic (pH 12) with sodium hydroxide pellets. The basic aqueous suspension was then extracted with dichloromethane (2 X 100 mL) and the combined organic extracts were dried over anhydrous sodium sulfate. Filtration, followed by evaporation of the filtrate solvent under reduced pressure yielded the intermediate secondary amines as the free bases. The crude bases were dissolved in a methanol solution (25 mL) containing 37% formaldehyde (10 mL) and sodium cyanoborohydride (25 mmol) and this reaction mixture stirred at room temperature for 24 h. This reaction mixture was worked-up using the acid-base extraction described above, and the resulting tertiary amine products converted to the corresponding hydrochloride salts upon treatment with ethereal HCl. The structures of the products were confirmed by ^{1}H-NMR, IR and MS.

RESULTS AND DISCUSSION

The title N,N-disubstituted 3,4-methylenedioxyamphetamines were synthesized by sequential reductive amination reactions as outlined in Scheme 1. Reaction of the commercially available 1-(3,4-methylenedioxyphenyl)-2-propanone (MDP2P) with ethylamine, i-propylamine or n-propylamine in the presence of sodium cyanoborohydride afforded the intermediate N-alkyl MDAs. These intermediates were purified by sequential acid-base extractions, and then converted to the desired N,N-disubstituted derivatives



Scheme 1. Synthesis of N-methyl-N-alkyl MDA derivatives.

upon treatment with formaldehyde in the presence of sodium cyanoborohydride. Attempts to form the disubstituted analogues directly by treatment of MDP2P with the appropriate secondary amines were unsuccessful. Complex mixtures formed during the course of these reactions, making isolation and purification of the desired compounds difficult.

The liquid chromatographic separation of the N,N-disubstituted derivatives is shown in Figure 1. This reversed-phase separation was achieved using a C_{18} stationary phase and a mobile phase consisting of pH 3 phosphate buffer and acetonitrile (55:45), maintained at 40° C. In this system, retention of the tertiary amines increases with the hydrophobicity of the N-alkyl substitutents. For example, the N-methyl-N-ethyl derivative has a significantly lower capacity factor than either the N-methyl-N-n-propyl or N-methyl-N-i-propyl analogues. Also, the N-methyl-N-i-propyl derivative elutes prior

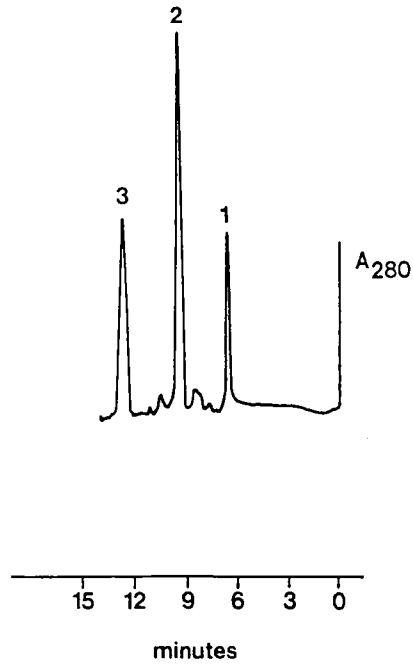


Figure 1. Reversed-phase liquid chromatographic separation of N,N-disubstituted MDA derivatives. Peaks: 1 = N-methyl-N-ethyl MDA; 2 = N-methyl-N-i-propyl MDA; 3 = N-methyl-N-n-propyl MDA.

to the N-methyl-N-n-propyl compound. The relative retention of these propyl derivatives parallels the retention observed for n-propyl and i-propyl secondary amine derivatives [3]. The reversed-phase separation of the tertiary amines in Figure 1 uses a higher percentage of organic solvent than required for the elution of most of the secondary amine N-alkyl MDA derivatives [3].

The plots in Figure 2 describe the variation in k' for representative MDA derivatives as a function of mobile phase

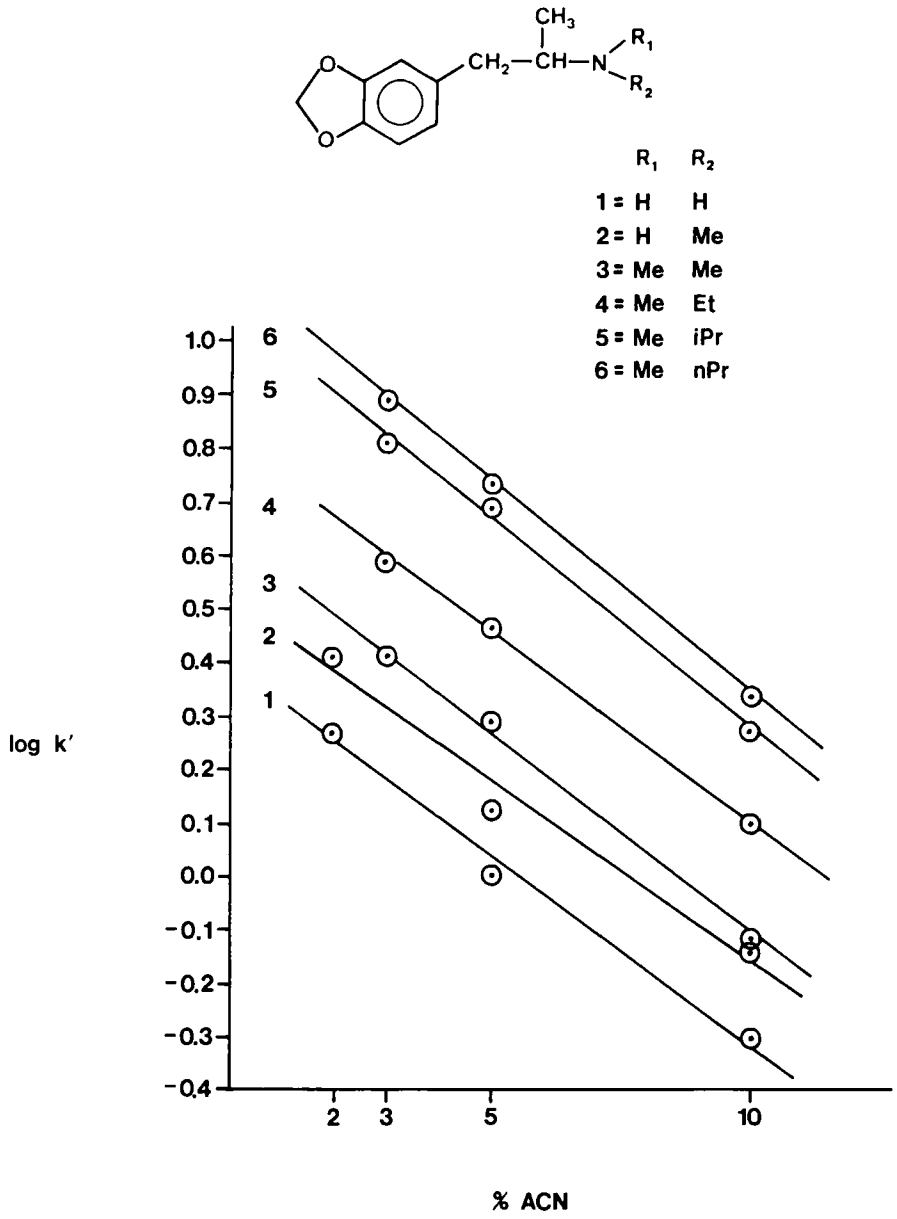


Figure 2. Plot of reversed-phase capacity factors versus acetonitrile composition in the mobile phase. 1 = MDA, 2 = MDMA, 3 = N,N-dimethyl MDA, 4 = N-methyl-N-ethyl MDA, 5 = N-methyl-N-i-propyl MDA and 6 = N-methyl-N-n-propyl MDA.

composition. These data were obtained using an octyl stationary phase specifically deactivated for the analysis of basic compounds. Amines often yield poor peak shape and inadequate resolution due to the extensive tailing on silica-based hydrocarbon phases. This phenomenom has been linked to solute interactions with the unreacted silanol sites on the silica-based stationary phase [7]. The silica surface remains weakly acidic and is, therefore, capable of producing interactions other than the hydrophobic associations between the non-polar moieties of the solute and the stationary phase. Compounds such as amines that become charged species at the chromatographic pH are capable of undergoing both ion exchange and hydrogen bonding interactions with the exposed silanol sites [8]. The degree of contribution from each of these possible interactions - hydrophobic, ion exchange and hydrogen bonding - dictates the chromatographic results and can lead to severe tailing or even irreversible adsorption of the solute to the stationary phase. This problem can be remedied by the addition to the mobile phase of silanol masking agents which act by blocking the available ion exchange and hydrogen bonding sites of the stationary phase. More recently, the availability of silica-based hydrocarbon stationary phases specifically deactivated for bases have eliminated the need for competing bases to mask this secondary retention. The plot in Figure 2 illustrates the effect of mobile phase composition on retention for a base-specific C_8 stationary phase. The amount of organic modifier is significantly lower on this phase compared to that of the separation shown in Figure 1. The significant reduction in percent organic modifier is likely the result of elimination of the secondary retention via silanol intereactions. The k' values increase with the size of the alkyl group on nitro-

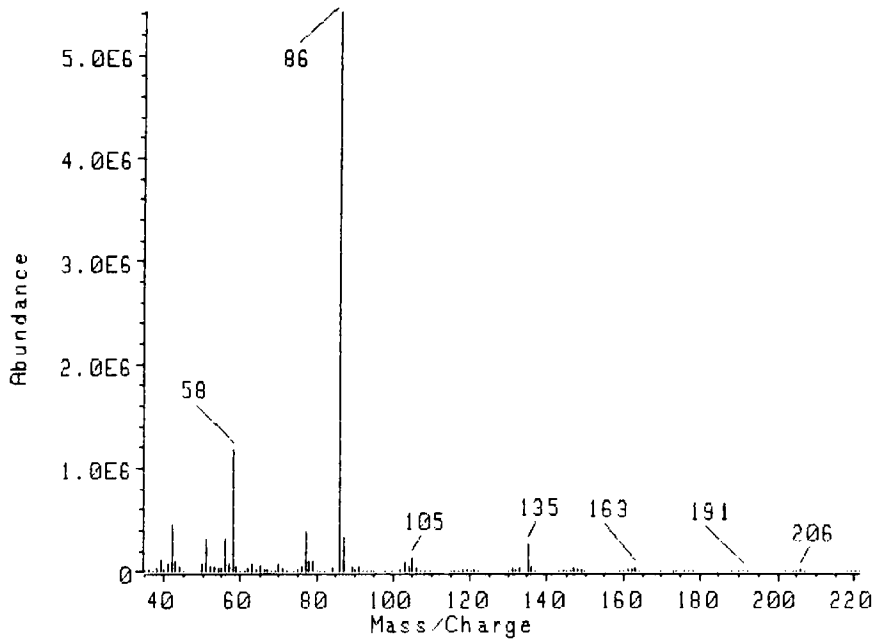
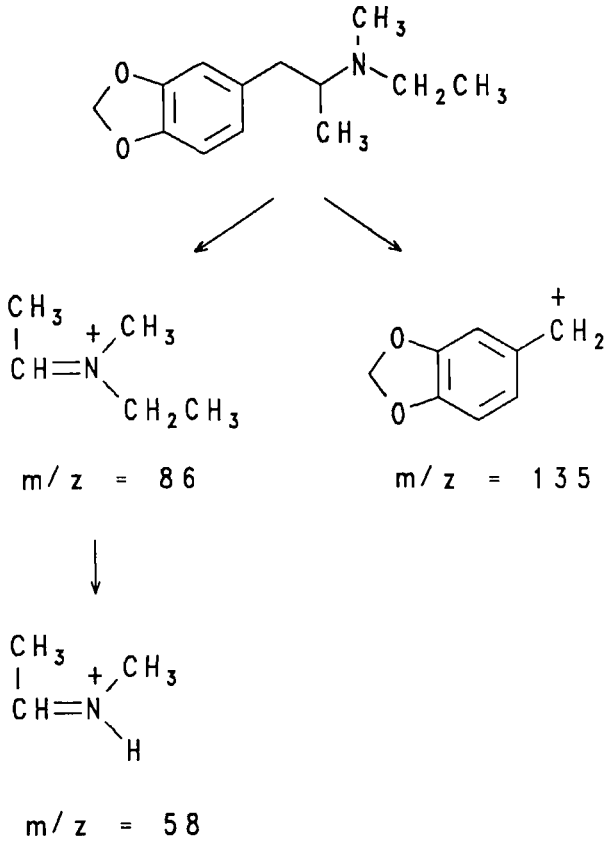


Figure 3. Electron impact mass spectrum for N-methyl-N-ethyl MDA.

gen, thus, the N-methyl-N-n-propyl MDA has the highest k' value as expected based on hydrophobic surface area. Although the plots in Figure 2 show only the various portions of aqueous acetronitrile, similar results were obtained using methanol as the organic modifier.

The electron impact mass spectra of the N,N-disubstituted MDA derivatives were determined at 70eV using a capillary GC-MS system. The column contained an OV-1 stationary phase and a temperature program was used to elute the tertiary amines within seven minutes. The spectra shown in Figure 3 demonstrate that fragmentation is extensive and therefore very little molecular



Scheme 2. Electron impact mass spectral fragmentation of N-methyl-N-ethyl MDA.

ion is observed. These compounds undergo the typical amine-dominated fragmentation with the base peak resulting from cleavage at the benzylic carbon, as is the case in the other MDA series (Scheme 2). Thus, the base peak in each spectrum represents the ammonium species with a m/z ion of 86 for the N-methyl-N-ethyl derivative and a m/z ion of 100 for the N-methyl-N-propyl analogues. The 3,4-methylenedioxybenzyl ion (m/z = 135) is also

evident in each spectrum and the base peaks for each derivative can rearrange to yield the m/z 58 ion seen in each spectrum. Thus, the fragmentation mechanism for the disubstituted derivatives is the same as that observed for MDA and secondary amine N-substituted MDAs [3]. These compounds can be distinguished based on mass differences of the base peaks and rearrangement ions. The characteristic mass 135 ion identifies these compounds as MDA derivatives and the molecular ion, the base peak (m/z of 86 or 100) and the m/z 58 ion aid in the specific identification of the individual MDA derivatives. The fragment ion (m/z 58) would be very useful in distinguishing a secondary amine MDA from a tertiary amine derivative of the same molecular weight. For example, N-n-propyl MDA and N-methyl-N-ethyl MDA would show the same molecular ion and the same base peak (m/z = 86). However, the rearrangement ion for N-methyl-N-ethyl MDA would have a m/z = 58, while the imine base peak from N-n-propyl MDA rearranges to yield m/z = 44 [3].

In summary, the tertiary amine MDMA derivatives were prepared by reductive amination and separated by reversed-phase liquid chromatography. The mass spectra of these amines can be used for their specific identification.

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