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Methods for the Differentiation of Methamphetamine from Regioisomeric Phenethylamines

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Abstract

The analytical profiles are described for five amines, methamphetamine, and four isomeric phenethylamines of MW = 149. These five amines all contain an unsubstituted benzyl moiety, thus the regioisomerism is within the carbon–carbon bond located α- to the amine moiety. Therefore these phenethylamines are regioisomeric within the imine fragment (*m/z* = 58), which is the base peak in the electron impact (EI) mass spectrum of methamphetamine. The ultraviolet absorption spectra for these compounds show the characteristic phenethylamine absorption bands in the (250 – 260 nm) range. These amines are best differentiated by chromatographic separation and are well resolved by liquid chromatographic techniques. The five regioisomeric amines are separated using an isocratic reversed-phase system consisting of a C_{18} stationary phase and a mobile phase of pH 3 phosphate buffer and methanol. The elution order under these conditions appears to parallel the length of the carbon chain attached to the aromatic ring.

Introduction

Central nervous system stimulants related to methamphetamine remain popular drugs of abuse in North America. The continued interest in drugs of this type is highlighted by the emergence in recent years of crystalline methamphetamine or "ice", as well as the appearance of analogues including *N,N*-dimethylamphetamine and *N*-ethylamphetamine in street samples (1). Also, over the past decade the scope of the substance abuse problem has broadened with the introduction of the methylenedioxyamphetamine-type drugs, MDA and MDMA (2–4), and a number of designer drug analogues of this type (5–7), as well as the narcotic analgesics (8), PCP (9), hallucinogens (10), and amphetamine-type compounds (11).

Methamphetamine ranks second only to cocaine in popularity as a substance of abuse. It is produced in clandestine labs in the U.S. by a number of methods using a variety of starting materials including the ephedrines, phenyl-2-propanone, and phenylacetic acid. A variety of methods have been developed to analyze and characterize illicitly manufactured methamphetamine (12). Mass spectrometry may be employed most commonly in forensic laboratories to identify methamphetamine in clandestine samples, but other analytical methods including infrared (IR) and ultraviolet (UV) spectrophotometry, and to a lesser extent, nuclear magnetic resonance spectrometry are also used. Several chromatographic methods have also been developed to characterize methamphetamine and other drugs of abuse (12). Frequently, accurate identification requires the use of a variety of these methods and the availability of appropriate standards. This is particularly important in cases where substances are closely related in their chemical structure. For example, the ultraviolet and mass spectra of N,N-dimethylamphetamine and N-ethylamphetamine are very similar, but the infrared and NMR spectra, as well as the chromatographic properties, differ significantly (13).

In this paper, the spectral and chromatographic properties of five phenethylamines related to methamphetamine are determined for specific identification of each of these compounds. The compounds included are (1) *N*-ethyl-2-phenethylamine, (2) *N,N*-dimethyl-2-phenethylamine, (3) methamphetamine, (4) phentermine, and (5) 1-phenyl-2-aminobutane (Scheme 1). All of these amines have the same molecular weight (149) and very similar mass spectral fragmentation patterns. Therefore a number of analytical and chromatographic methods are required to accurately distinguish between these compounds.

Experimental

Instrumentation. The liquid chromatograph consisted of a Waters Associates Model 6000A pump, U6K injector, Model 440 UV detector with a dual wavelength accessory operated at 254 and 280 nm, and a Houston Instruments OmniScribe dual pen recorder. Infrared spectra were recorded on a Perkin-Elmer Model 1710 Fourier transform infrared (FTIR) spectrophotometer. Ultraviolet spectra were recorded on a Shimadzu Instruments Model UV-265 spectrophotometer. Nuclear magnetic resonance (NMR) spectra (1H) were determined using a Varian EM-360 60 MHz spectrometer.

The electron impact (EI) mass spectra were obtained using a Hewlett-Packard 5970B mass selective detector. The ionization voltage was 70 eV and the source temperature was 220°C. The individual amine hydrochlorides were dissolved in methanol

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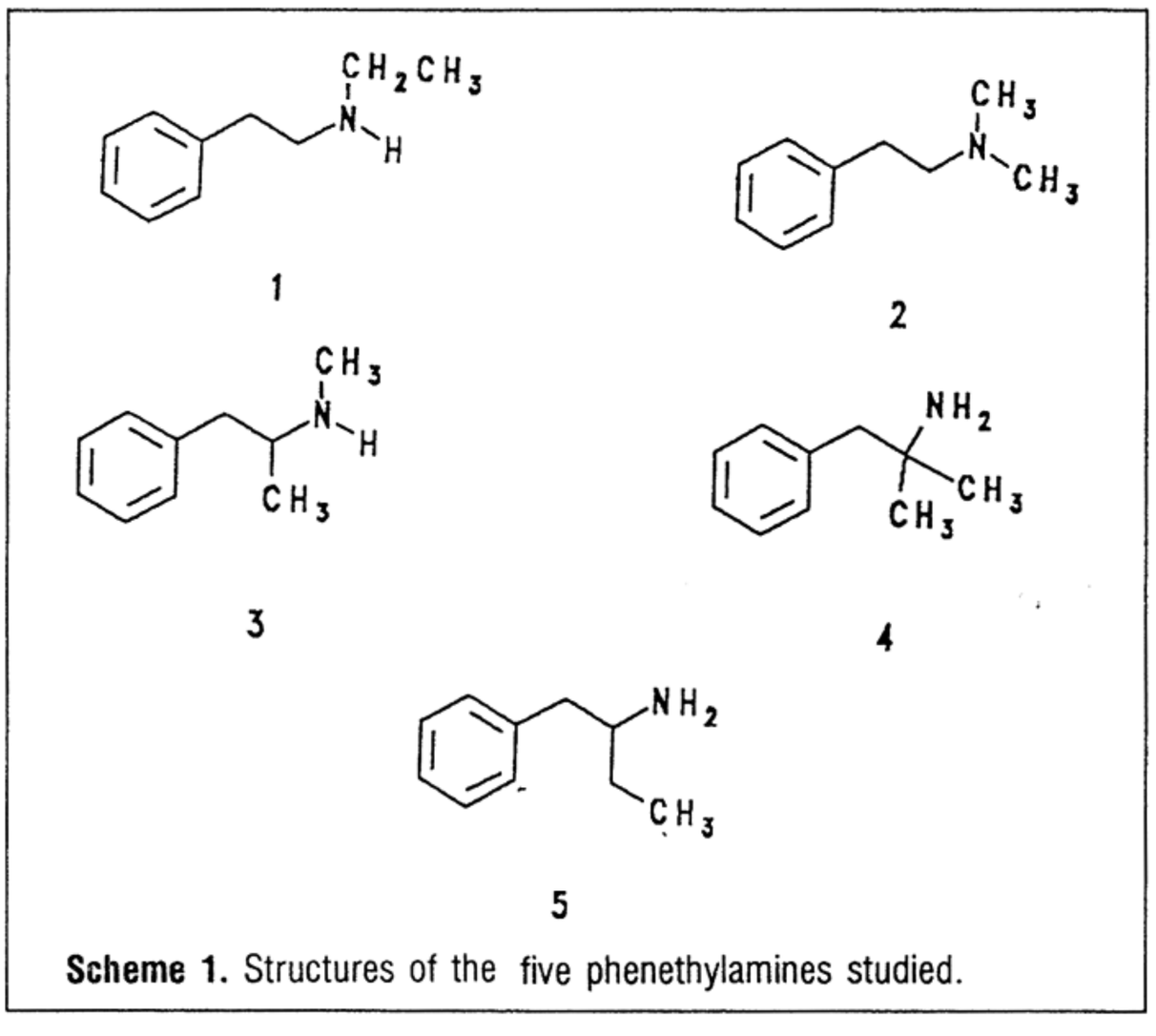
(1 mg/mL) and 0.5 μL was introduced into the mass spectrometer via a gas chromatograph equipped with a 12-m x 0.31-mm i.d. fused-silica column with a 0.52-μm thickness of OV-1. The column temperature was programmed from 70° to 150°C at a rate of 15°/min, and from 150° to 250°C at a rate of 25°/min. The split ratio for the GC was 10:1 and all sample components eluted within approximately 7 min.

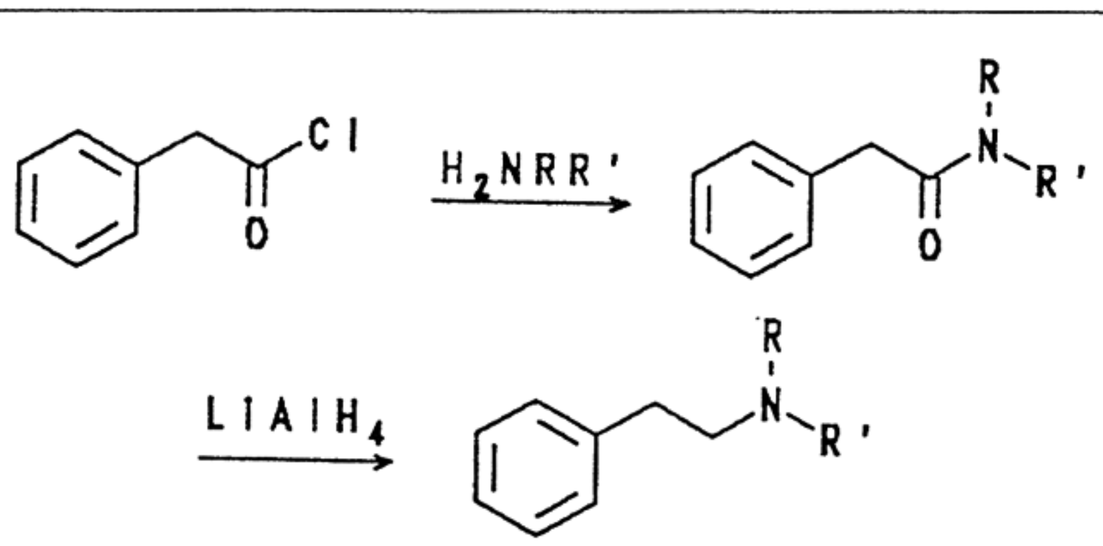
Liquid chromatographic procedures. The analytical column was 30 cm × 3.9 mm i.d. packed with μBondapak C_{18} (Waters Associates). The analytical column was preceded by a 7-cm × 2.1-mm i.d. guard column packed with CO:Pell ODS (Whatman). The amine hydrochlorides (1 mg/mL) were dissolved in HPLC grade methanol and separated using a mobile phase of pH 3.0 phosphate buffer and methanol (5:1). The pH 3.0 phosphate buffer was prepared by mixing 9.2 g monobasic sodium phosphate (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 1.5 mL/min and the detector was operated at 0.2 AUFS A 15-μL aliquot of each amine solution was injected into the liquid chromatograph.

Synthesis of N-*ethyl- and* N,N-*dimethyl-2-phenylethylamine.* The appropriate amine (ethylamine or dimethylamine, 20 mMol) was added dropwise to a stirred solution of phenacetyl chloride (10 mMol) in chloroform (50 mL) and the mixture stirred at room temperature for 1 h. The mixture was then stirred at reflux for ca 15 min and the solvent evaporated under reduced pressure to yield an oil. The oil was partitioned between 20% potassium carbonate (50 mL) and chloroform (50 mL) and the chloroform layer separated. The chloroform solution was then washed with 10% HCl (50 mL) and evaporated under reduced pressure to yield the intermediate amide. A solution of the amide in tetrahydrofuran (THF) (40 mL) was added dropwise to a suspension of lithium aluminum hydride (1 g) in THF (10 mL) stirred under a nitrogen atmosphere. After the addition was complete, the mixture was stirred at reflux overnight. The mixture was then cooled to room temperature, filtered and the filtrate solvent evaporated under reduced pressure to yield the crude amines as oils. The oils were partitioned between 10% HCl (50 mL) and chloroform (50 mL) and the aqueous layer separated and made basic (pH 12) with aqueous sodium hydroxide. The aqueous base suspension was extracted with chloroform (50 mL) and the chloroform removed under reduced pressure to yield the product amines in free base form. Treatment of the bases with ethereal HCl afforded the desired amine hydrochlorides. The structures of the products were confirmed by IR (KBr) and ^{1}H-NMR (deuterated DMSO). The purity of the products was established by GC–MS and the LC analysis.

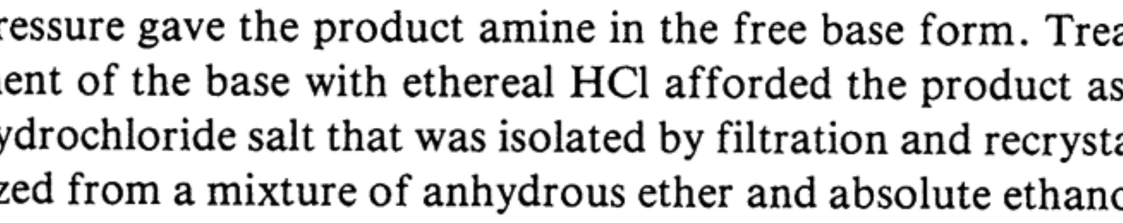
Synthesis of 1-phenyl-2-aminobutane. A solution of 1-phenyl-2-butanone (10 mMol), ammonium acetate (100 mMol) and sodium cyanoborohydride (25 mMol) in methanol (25 mL) was stirred at room temperature for 24 h. The reaction mixture was then evaporated to dryness under reduced pressure and the remaining residue suspended in dichloromethane (50 mL). The dichloromethane suspension was extracted with 3 N HCl (2 × 75 mL) and the combined acid extracts made basic (pH 12) with sodium hydroxide. The basic aqueous suspension was then extracted with dichloromethane (2 × 100 mL) and the combined organic extracts dried over anhydrous Na_2SO_4. Filtration, followed by evaporation of the filtrate solvent under reduced pressure gave the product amine in the free base form. Treatment of the base with ethereal HCl afforded the product as a hydrochloride salt that was isolated by filtration and recrystallized from a mixture of anhydrous ether and absolute ethanol.



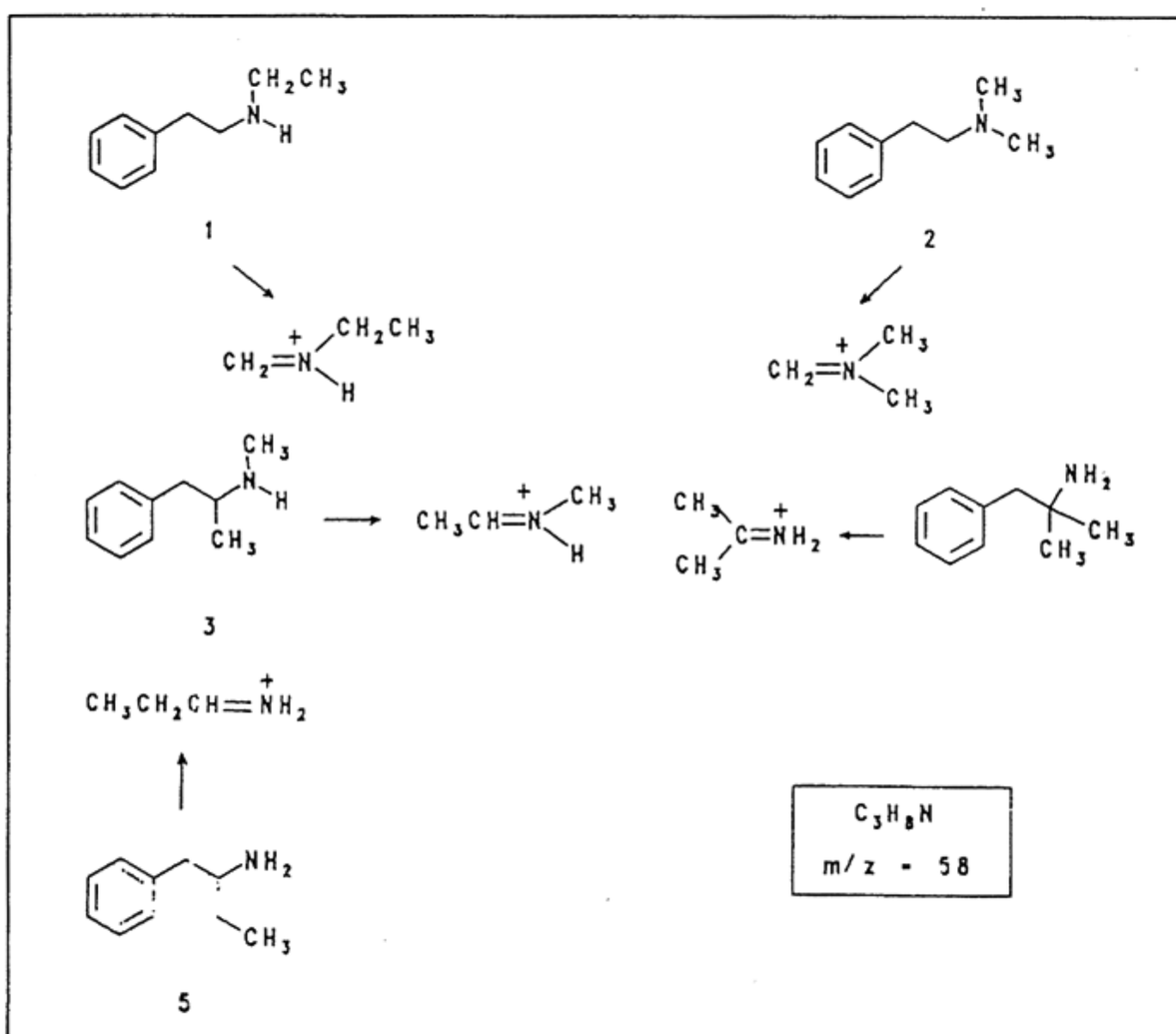
Scheme 1. Structures of the five phenethylamines studied.



Scheme 2. Synthesis of *N*-ethyl and *N,N*-dimethylphenethylamine.



Scheme 3. Synthesis of 1-phenyl-2-aminobutane.



Scheme 4. Electron impact fragmentation pathway for the isomeric phenethylamines.

The structure of the hydrochloride product was confirmed by IR (KBr) and ^{1}H-NMR (deuterated DMSO). The purity of the products was established by GC–MS and the LC analyses.

Results and Discussion

The five amines examined in this study each contain a phenethylamine fragment and have a molecular weight of 149. The phenethylamines methamphetamine and phentermine have been used in therapeutic situations, and methamphetamine remains a popular drug of abuse. The current street drug form of methamphetamine is referred to as "ice". The *N*-ethyl and *N,N*-dimethylphenethylamines and 1-phenyl-2-aminobutane represent the remaining methamphetamine isomers having the phenethylamine skeleton with an unsubstituted benzyl moiety.

The *N*-ethyl and *N,N*-dimethylphenethylamine were prepared according to the method outlined in Scheme 2. Treatment of phenylacetyl chloride with ethylamine or dimethylamine gave

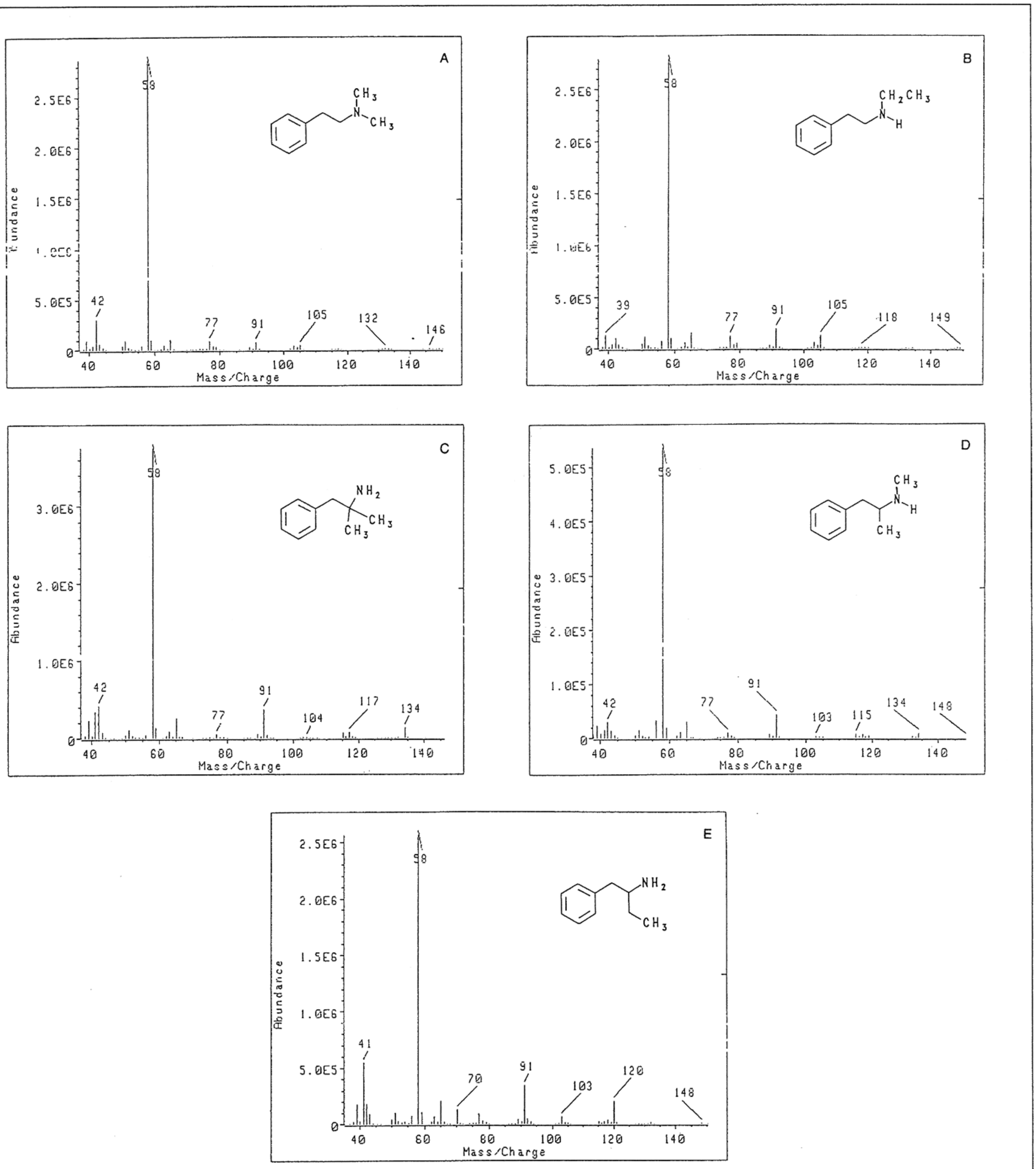
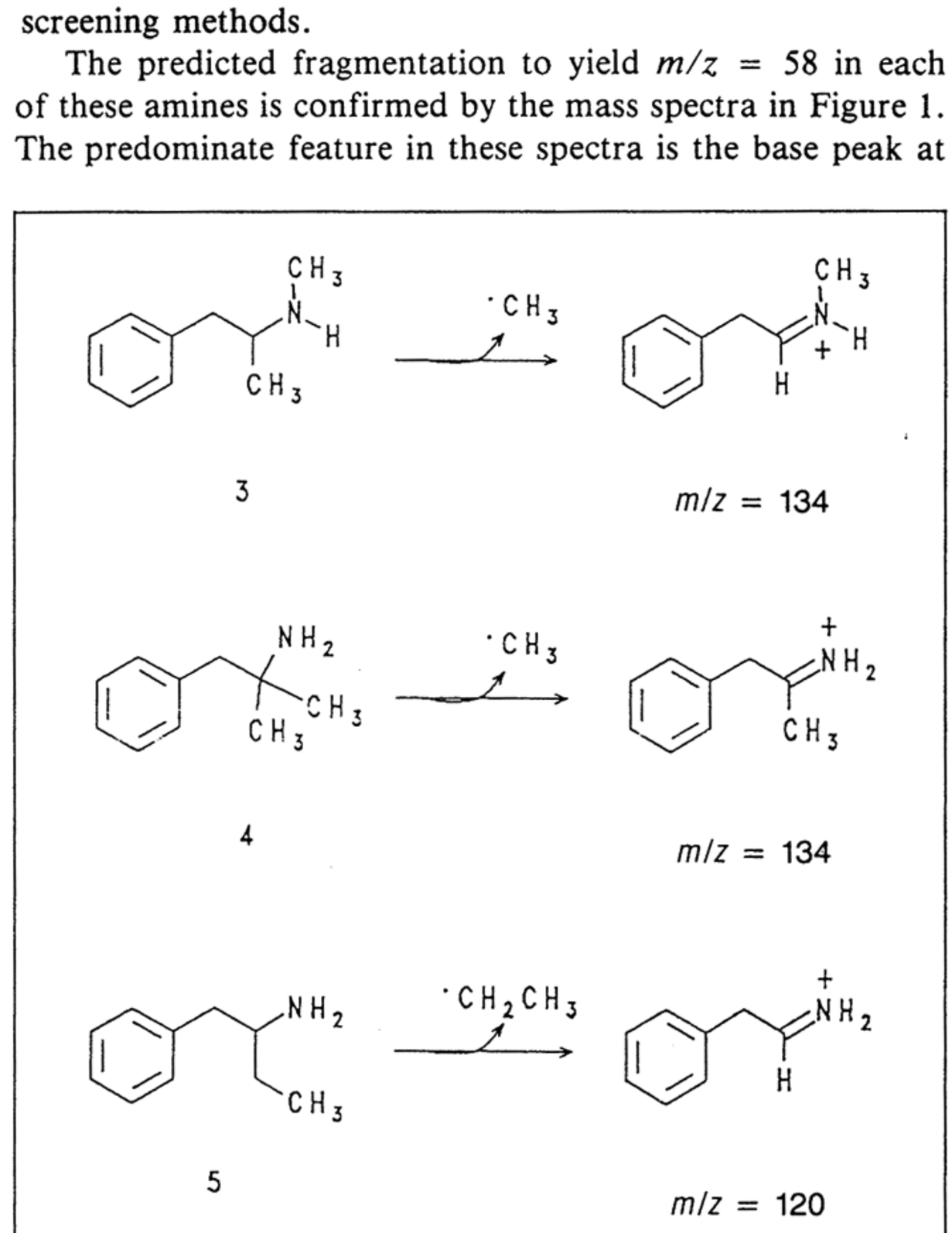


Figure 1. Mass spectra for the five isomeric phenethylamines. (A) *N,N*-dimethylphenethylamine, (B) *N*-ethylphenethylamine, (C) phentermine, (D) methamphetamine, and (E) 1-phenyl-2-aminobutane.

the *N*-ethyl and *N,N*-dimethyl phenylacetamides, respectively. The amides were reduced with lithium aluminum hydride to yield the desired phenethylamine products. The sample of 1-phenyl-2-aminobutane was prepared from 1-phenyl-2-butanone via reductive amination using ammonium acetate and sodium cyanoborohydride (Scheme 3).

These isomeric amines were prepared to examine the specificity of analytical methods for the identification of methamphetamine. While these isomers clearly yield very different proton magnetic resonance spectra, this is not a very common technique employed for the small amounts of sample often found in forensic samples. Certainly NMR methods would not be directly useful for the analysis of drugs from biological samples. These five amines were chosen for their isomeric relationship to methamphetamine, with each having the same molecular weight and the predicted similarity in their mass spectra. The EI fragmentation of the five phenethylamines of molecular weight 149 should yield a propylimine major fragment (base peak) of $m/z = 58$. Thus, these five isomers are uniquely similar in that all the structural variation is on the nitrogen atom or the α-carbon of the phenethylamine moiety. This isomeric relationship produces the $m/z = 58$ via the amine-dominated α-cleavage fragmentation (Scheme 4) which eliminates the benzyl radical (mass = 91) in each compound. Other isomeric amines of molecular weight 149 would require substitution on the β-carbon or the aromatic ring and thus yield a base peak of $m/z = 44$ (ethylimine) or $m/z = 30$ (methylimine) and a substituted benzyl radical from an analogous α-cleavage reaction. Because mass spectrometry is often the method of choice or the mandated method for confirmation of drug identity, these five compounds represent a unique challenge for the specificity of analytical methods in forensic analysis and related drug screening methods.

The predicted fragmentation to yield $m/z = 58$ in each of these amines is confirmed by the mass spectra in Figure 1. The predominate feature in these spectra is the base peak at $m/z = 58$ as predicted in Scheme 4. Phentermine and methamphetamine show the $m/z = 134$ fragment from the loss of an α-methyl group which is the less likely α-cleavage product. The analogous reaction in 1-phenyl-2-aminobutane results in the $m/z = 120$ from the loss of the α-ethyl group (Scheme 5). The mass spectra in Figure 1 were obtained by GC–MS analysis using an OV-1 capillary column with temperature programming. Under these chromatographic conditions the five amines eluted within a 0.65 min window from 3.1 to 3.75 min. The similar retention properties of these amines on this stationary phase again point to the potential for confusion in the analysis of these



Scheme 5. Electron impact α-alkyl cleavage fragmentation pathway for (3) methamphetamine, (4) phentermine, (5) 1-phenyl-2-aminobutane.

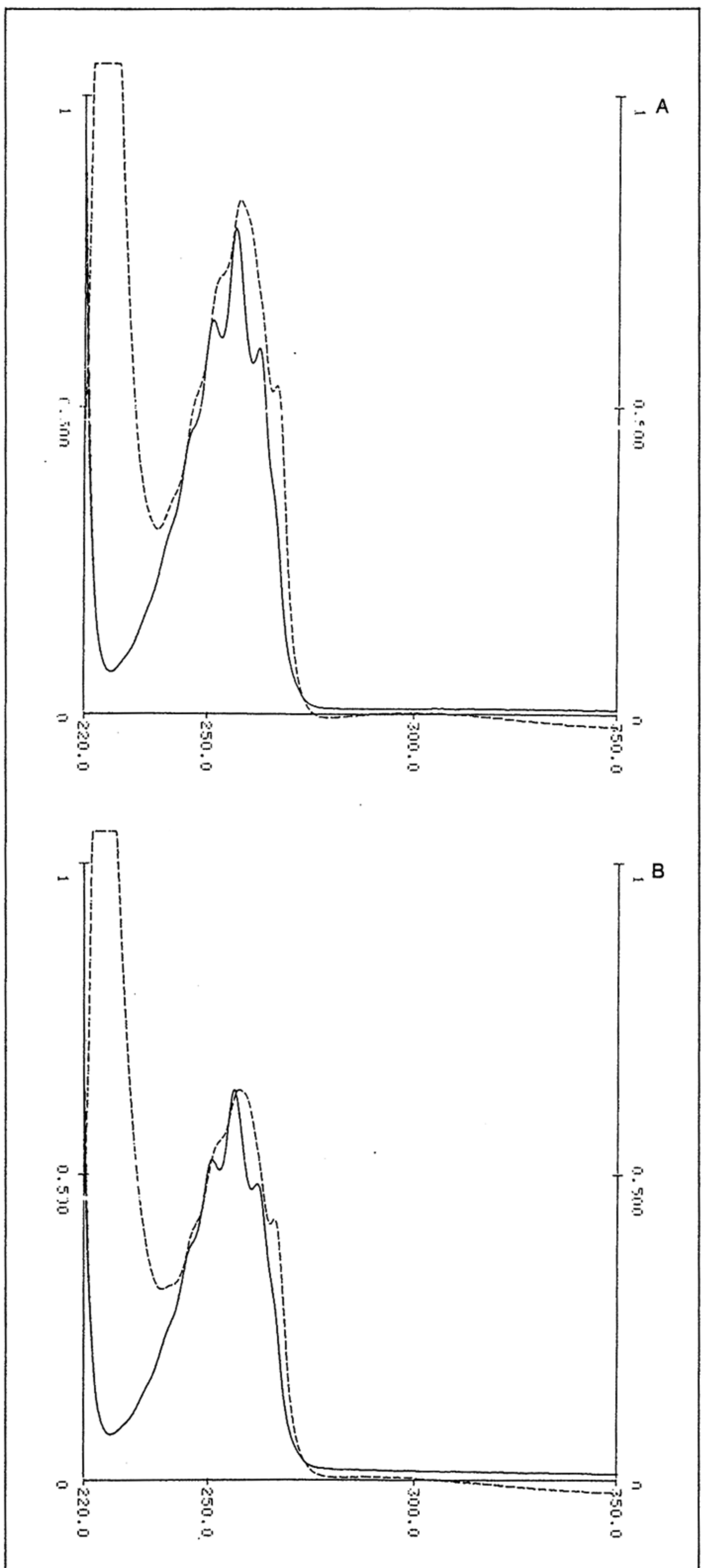


Figure 2. Ultraviolet absorption spectra for (A) methamphetamine and (B) *N,N*-dimethylphenethylamine in aqueous acid (—) and base (--).

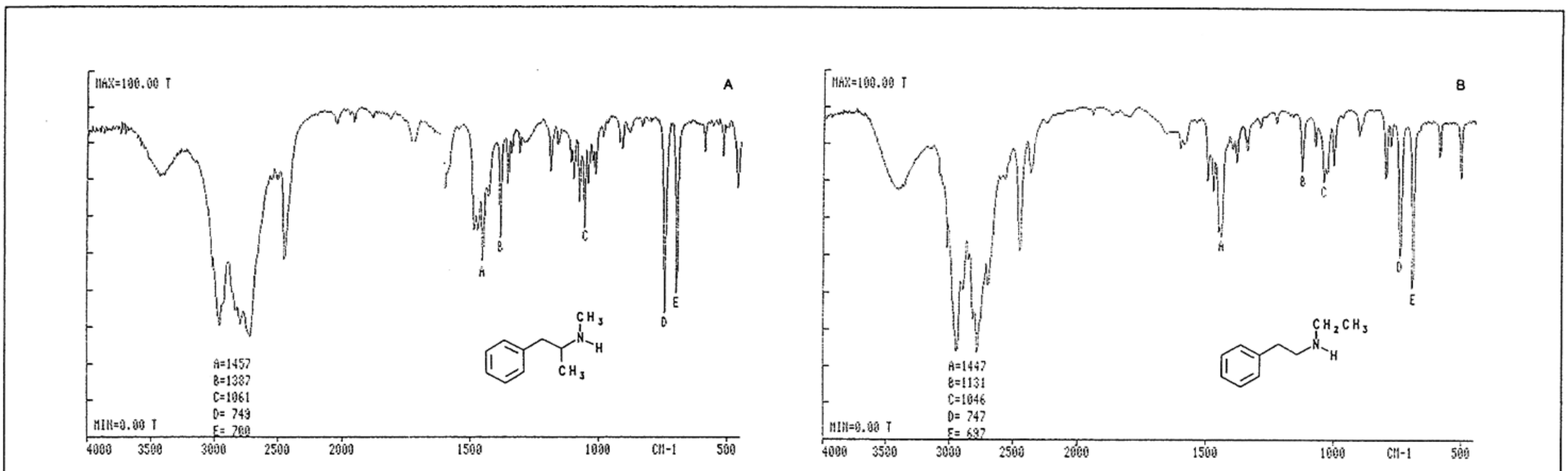


Figure 3. FT-IR spectra for the hydrochloride salts of (A) methamphetamine and (B) *N*-ethylphenethylamine.

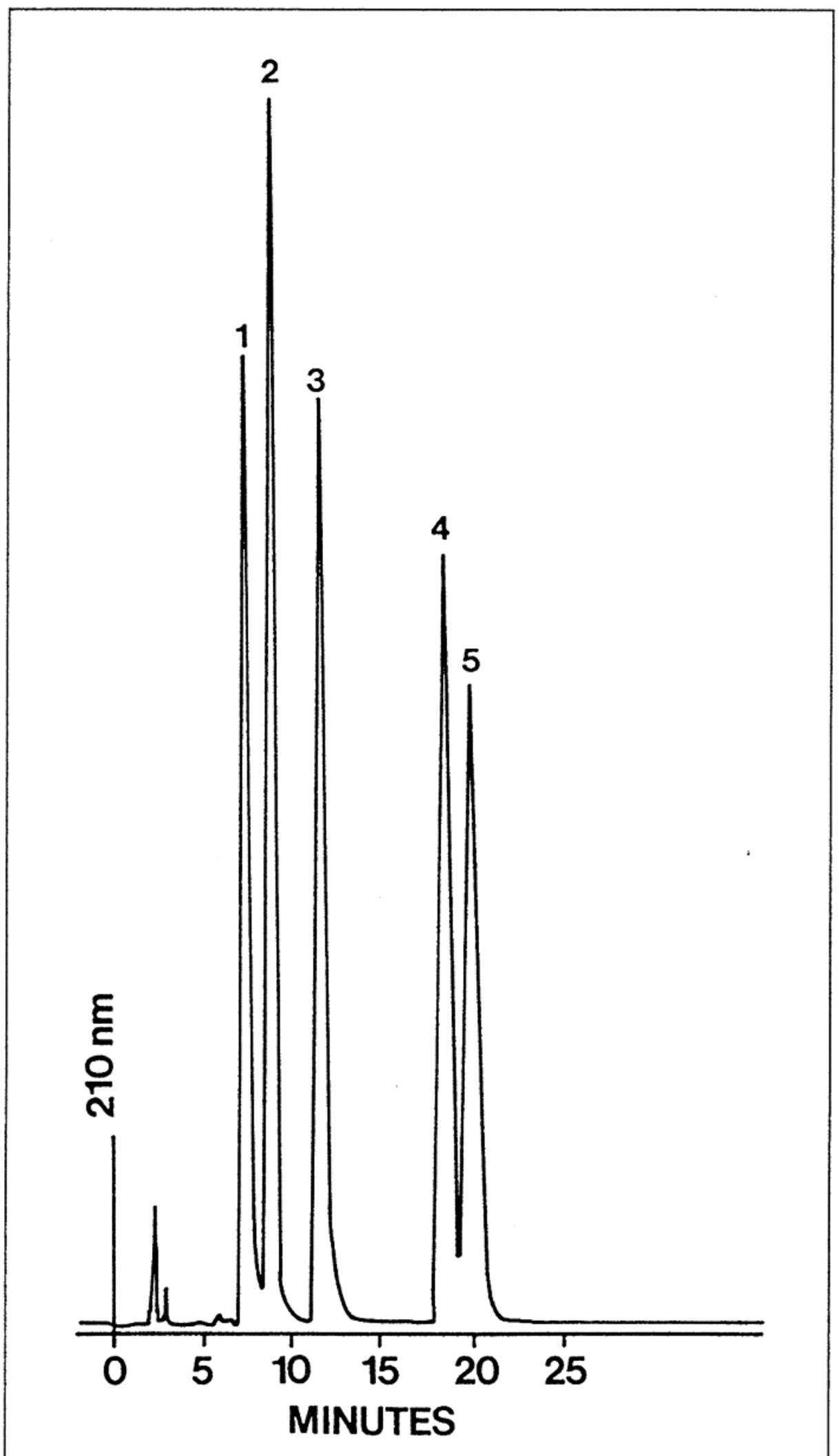


Figure 4. Liquid chromatographic separation of the isomeric phenethylamines with a μ-Bondapak C_{18} column and a mobile phase of pH 3 phosphate buffer and methanol (5:1). Peaks: (1) *N*-ethylphenethylamine, (2) *N,N*-dimethylphenethylamine, (3) methamphetamine, (4) phentermine, and (5) 1-phenyl-2-aminobutane.

materials. A substance producing the appropriate mass spectrum for methamphetamine at approximately the same retention time could lead to the identification of these structural analogs as methamphetamine.

The UV absorption properties of these compounds are illustrated in Figure 2 which compares the spectra for methamphetamine and *N,N*-dimethylphenethylamine in both acidic and basic solution. It would be most difficult to differentiate between these two compounds based on their UV absorption spectra. The shape of the curves in acid and base, the relative absorbance, and the various absorption maxima as well as the wavelengths of maximum absorption are all very similar. The three other amines gave UV spectra similar to those shown in Figure 2. The UV spectra are characteristic of phenethylamines in general and these very similar regioisomers would be expected to display electronic spectra characteristic of the common molecular fragment.

The infrared absorption spectra for methamphetamine and *N*-ethylphenethylamine are shown in Figure 3. While some slight differences are obvious in the position of absorption bands and relative intensities, these spectra are also very similar. Variations from sample to sample and time to time are often observed even for methamphetamine. These variations are the result of crystalline structure, degree of hydration, and other related factors.

Other than differentiation based on the degree of nitrogen substitution, standard color reagents are not very helpful in the analysis of these materials. The modified Feigl test for amine substitution showed the usual pink color for the primary amines and the blue-purple for the secondary amines. All five amines gave the orange to brown color change with the Marquis reagent.

The liquid chromatographic separation of the five amines is shown in Figure 4. The amines were separated on a C_{18} stationary phase using an acidic (pH 3) mobile phase consisting of phosphate buffer and methanol (5:1). Under these conditions the amines would exist predominantly in the protonated form. The *N*-ethyl phenethylamine has the lowest capacity factor under these conditions followed closely by the *N,N*-dimethyl phenethylamine. Methamphetamine elutes third in the sequence and is followed by phentermine with 1-phenyl-2-aminobutane having the highest capacity factor. These five amines elute over a 12 min window in this system with retentions between 7 and 20 min. Methamphetamine (peak 3) is well resolved from both of the *N*-substituted phenethylamines (peaks 1 and 2) and the

two primary amines, having a four carbon chain attached to the aromatic ring. The elution order of the compounds parallels the length of the carbon chain attached to the aromatic ring, with the C_2 (phenethyl) compounds eluting before the C_3 compound (methamphetamine), which in turn elutes prior to the C_4 compounds. This chromatographic separation easily allows the differentiation of methamphetamine from the other four isomers, even though many of their analytical profiles are quite similar.

References

1. D.A. Cooper. Future synthetic drugs of abuse. In *Proceedings of the International Symposium on the Forensic Aspects of Controlled Substances,* U.S. Government Printing Office, Stock Number PB90-199472, 1990, pp. 79–103.
2. U. Braun, A.T. Shulgin, and G. Braun. Centrally active *N*-substituted analogs of 3,4-methylenedioxyphenylisopropylamines (3,4-methylenedioxyamphetamine). *J. Pharm. Sci.* **69:** 192–95 (1980).
3. G.H. Hayner and H. McKinney. MDMA—the dark side of Ecstacy. *J. Psychoactive Drugs,* **18:** 341–47 (1986).
4. G.P. Dowling, E.T. McDonough, and R.O. Bost. Eve and Ecstacy: A report of five deaths associated with the use of MDEA and MDMA. *J. Am. Med. Assoc.* **257:** 1615–17 (1987).
5. F.T. Noggle, Jr., C.R. Clark, A.K. Valaer, and J. DeRuiter. Liquid chromatographic and mass spectral analysis of *N*-substituted analogues of 3,4-methylenedioxyamphetamine. *J. Chromatogr. Sci.* **26:** 410–17 (1988).
6. F.T. Noggle, Jr., C.R. Clark, and J. DeRuiter. Liquid chromatographic and mass spectral analysis of 1-(3,4-methylenedioxyphenyl)-3-butanamines: Homologues of 3,4-methylenedioxyamphetamines. *J. Chromatogr. Sci.* **27:** 240–43 (1989).
7. C.R. Clark, F.T. Noggle, Jr., and J. DeRuiter. Liquid chromatographic and mass spectral analysis of N,N-disubstituted 3,4-methylenedioxyamphetamines. *J. Liq. Chromatogr.* **13:** 263–74 (1990).
8. G. Henderson. Designer drugs: The new synthetic drugs of abuse. In *Proceedings of Controlled Substance Analog Leadership Conference.* A. Church and F. Sapienza, Eds., U.S. Department of Justice, Drug Enforcement Administration, Office of Diversion Control.
9. A. Kalir, H. Edery, Z. Pelah, D. Balderman, and G. Porath. 1-Phenylcycloalkylamine derivatives. II. Synthesis and pharmacological activity. *J. Med. Chem.* **12:** 473–77 (1969).
10. J. DeRuiter, F.T. Noggle, Jr., and C.R. Clark. Reversed-phase liquid chromatographic separation of lysergic acid diethylamide (LSD) and lysergic acid methylpropylamide (LAMPA). *J. Liq. Chromatogr.* **10:** 3481–88 (1987).
11. F.T. Noggle, Jr., C.R. Clark, and J. DeRuiter. Liquid chromatographic and mass spectral analysis of methoxyamphetamines and methoxymethamphetamines. *J. Chromatogr. Sci.* **27:** 602–606 (1989).
12. F.T. Noggle, Jr., J. DeRuiter, and C.R. Clark. Methods for analysis and characterization of forensic samples containing amphetamine and related amines. *J. Chromatogr. Sci.* **28:** 529–36 (1990).
13. F.T. Noggle Jr., C.R. Clark, and J. DeRuiter, unpublished data.

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