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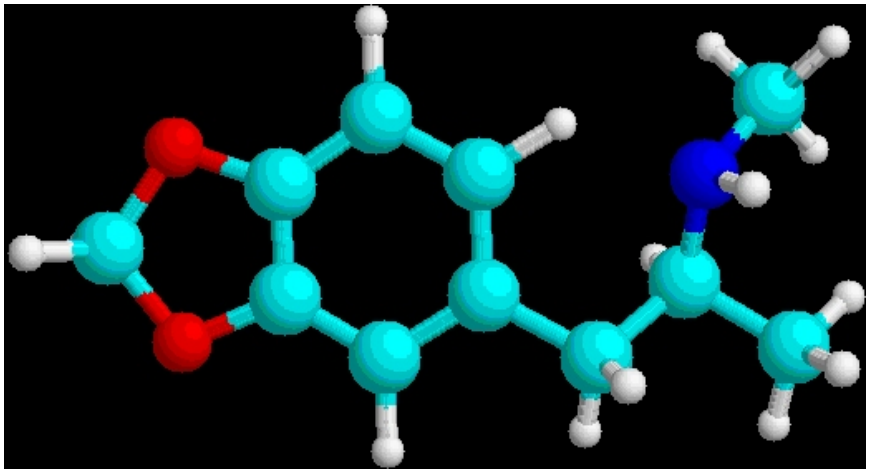

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Cover Art: "Ball and Stick" Model of 3,4-Methylenedioxymethamphetamine (MDMA) (Courtesy of Patrick A. Hays, DEA Special Testing and Research Laboratory, Dulles, VA).

Analysis and Characterization of Designer Tryptamines using Electrospray Ionization Mass Spectrometry (ESI-MS)

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[Presented in Part at the ASMS 17th Sanibel Conference on Mass Spectrometry - Mass Spectrometry in Forensic Science and Counterterrorism, Clearwater Beach, FL (January 28 - February 1, 2005).]

ABSTRACT: The analysis and characterization of 12 "designer" tryptamines by electrospray ionization mass spectrometry (ESI-MS) are presented. Molecular weights were confirmed based on the experimental observation of protonated and deprotonated pseudo-molecular ions in the positive and negative ion modes, respectively. Standard tandem mass spectrometry (MS^2) experiments were also performed, and the results provided for the characterization of various fragmentation signatures, useful for the future analysis of currently unknown, similar compounds. The fragmentation spectra obtained from collision-induced dissociation (CID) experiments (35 eV) were also compiled as part of an in-house mass spectral library. Results from selected MS^3 experiments are presented and their use in structural elucidation is discussed. For comparison, the gas chromatography/mass spectrometry (GC/MS) data for the tryptamines are also included and discussed.

KEYWORDS: Tryptamines, Analogues, Designer Drugs, Electrospray Ionization-Mass Spectrometry, ESI-MS, Pseudo-molecular Ion, Fragmentation, Tandem Mass Spectrometry, Collision-Induced Dissociation, GC/MS, Forensic Chemistry.

Introduction

"Designer drugs" are compounds with structures that are very similar to controlled substances, but that are not specifically controlled. Also known as "analogues," most of these compounds have never been previously encountered or characterized, and so are not present in commercial spectral libraries; therefore, they can represent unusual challenges for forensic laboratories. Definitive identification of such drugs usually requires in-depth analysis using multiple and complementary techniques, including infrared spectroscopy (IR), gas chromatography/mass spectrometry (GC/MS), and nuclear magnetic resonance (NMR).

For decades, the use of electron ionization (EI) mass spectrometry as a detector for gas chromatography (GC) instruments has been a main step in the structure elucidation process used by analytical and synthetic chemists [1]. Electron ionization mass spectra of many thousands of compounds, normally collected under 70 eV of energy, are readily available from reference databases [2] and instrument manufacturers [3]. The capabilities of mass spectrometry, however, have greatly expanded with the more recent development of specialized ionization techniques like electrospray ionization (ESI) [4] and atmospheric pressure chemical ionization (APCI) [5], making possible the analysis of many polar and thermally labile compounds that were not amenable to GC/MS analyses. Through the ESI process, ions in solution are transported into the gas phase by a series of solvent evaporation and Coulomb explosion steps, preserving the original intact ions and introducing them into the

vacuum-housed mass analyzer without significant fragmentation. As a result, this process produces singly and/or multiply charged ions which, upon mass spectrometric analysis, provide direct molecular weight information - a critical step in identification.

In addition, the interface of an ESI source to an ion-trap mass spectrometer provides for not only molecular weight determinations, but also for tandem and MS^n fragmentation analysis of intact gas-phase ions via collision-induced dissociation (CID) experiments using a target gas [5]. By performing these experiments under controlled conditions, further structural elucidation can be accomplished, and the additional spectra generated can be collected and stored as part of a laboratory-generated library.

Certain synthetic tryptamines produce hallucinogenic effects in humans [6,7]. These properties can be expected based on the structural similarities between these tryptamines and some naturally occurring hallucinogenic tryptamines such as psilocybin, psilocin, dimethyltryptamine, bufotenine, and ibogaine. By slightly modifying the structures of these latter substances, synthetic chemists have developed novel "designer" tryptamines with very similar, new, modified, unusual, and/or otherwise desirable psychedelic properties. Synthetic details for the preparation of some designer tryptamines have been available to the public for many years [8]. However, the scientific literature provides only very limited information regarding their analysis and characterization [9-13]. Most of the available literature focuses on the extraction of the naturally available tryptamines from their source [14], or the analysis and detection of their metabolites in animal biological fluids [15-18].

This paper presents the analysis of various designer tryptamines using ESI-MS. Figure 1 illustrates the core structure of a tryptamine-type molecule, while Table 1 lists the 12 compounds investigated in this work, along with details of their structure. Some of these compounds were recently acquired during a law enforcement investigation targeting their open sale on the internet. Initially, these compounds were identified at this laboratory using IR and NMR spectroscopy, GC, and GC/MS techniques. Analysis by ESI-MS provides complementary information that is valuable for the full characterization of these compounds. For most of the compounds, the generation of pseudo-molecular ions via ESI provides molecular weight information that is not available via GC/MS analysis. MS^n fragmentation experiments further complement the structural information obtained from GC/MS, and allow the additional characterization of thermally labile compounds.

Experimental

Solutions for each of the analogues were prepared by dissolving the appropriate amount in methanol to obtain a final concentration of approximately 10 μg/mL. Solutions were introduced into the mass spectrometer using a ThermoFinnigan Surveyor autosampler. Sample injections (10 μL) were loaded into a methanol constant flow (200 μL/min) provided by a ThermoFinnigan Surveyor solvent pump.

Mass spectra were collected using a ThermoFinnigan LCQ Advantage MAX ion-trap mass spectrometer equipped with an ESI source and operated using the Xcalibur software (Version 1.4) provided by the manufacturer. The ESI voltage needle was kept at 5.0 kV, generating a spray current of approximately 0.3 μA. The sheath and auxiliary sweep gas flows (nitrogen) were operated at 40 and 10 units, respectively. Conditions inside the source were as follows: Capillary temperature 300 ^{O}C; capillary voltage ±30 V; and tube lens voltage ±15 V. Negative and positive mass spectra were collected in the centroid mode for the *m/z* range of 50 to 550. Each scan collected was composed of three microscans using a maximum ion injection time of 50 milliseconds. During ion storage, the trap was operated with the automatic gain control (AGC) set point at 5×10^7 ions and Helium (99.999% purity) was used as the trapping gas. After sequential ejection from the trap, ions were detected using a conversion dynode (±14.7 kV) and electron multiplier (-750 V) assembly. The pressure within the mass analyzer region was kept at 6.7×10^{-6} Torr by using a turbomolecular pump. MS^n experiments were performed by isolating the desired precursor ions using an isolation window of 3.0 *m/z* units. The isolated ions were then subjected to normalized collision energies between 25 and 35 eV (%) in order to generate characteristic fragmentation.

Helium (99.999% purity) was used as the collision gas, and ions were activated during 30 milliseconds using an activation q value of 0.25.

Experimental data were analyzed using the qualitative analysis program provided within the Xcalibur software suite. In addition, all MS^2 fragmentation data were incorporated into an in-house spectral library.

For the GC/MS data (displayed in the Appendices), solutions of the 12 analogues were prepared in methanol at a concentration of 1 mg/mL. Samples were introduced into the gas chromatograph using 1.0 μL injections. The GC oven program used was: initial temperature: 90 ^{O}C (1 minute hold), ramp to 300 ^{O}C (20 ^{O}C/minute); final temperature: 300 ^{O}C (5 minute hold). Helium was used as the carrier gas at a constant flow of 1 mL/minute. Mass spectra were obtained using a ThermoFinnigan PolarisQ ion-trap mass spectrometer controlled by the Xcalibur software.

Results and Discussion

ESI-MS Experiments

Figures 2a and 2b present the positive and negative ion mode full-scan electrospray mass spectra obtained for 4-acetoxy-*N,N*-diisopropyltryptamine (**9**). The singly protonated and singly deprotonated pseudo-molecular ions are clearly represented at *m/z* 303 and 301, respectively, indicating a molecular weight of 302 for this compound. Similar spectra were obtained for the other 11 analogues. The molecular weights determined from all of these experiments are included in Table 1. Under the experimental conditions utilized, the signal intensity for the negative ion spectra was observed to be somewhat dependent on the structure of the compounds, specifically the presence of methoxy, acetoxy, or hydroxy groups. Molecules containing such functionalities were observed to produce more intense deprotonated pseudo-molecular ions.

Full-scan electrospray analysis was especially useful for the identification and differentiation of 4-hydroxy-*N,N*-diisopropyltryptamine (**7**) and 4-acetoxy-*N,N*-diisopropyltryptamine (**9**). The latter compound is thermally unstable, and standard GC/MS analyses gave highly similar spectra (see Appendices). However, ESI-MS provided accurate molecular weight information, allowing a easy identification.

ESI-MS^2 Experiments

In addition to collecting full-scan ESI spectra for these molecules, ESI-MS/MS (ESI-MS^2) fragmentation experiments were also performed in order to obtain structural information that would complement the information previously obtained via GC/MS experiments. ESI-MS^2 fragmentation experiments were performed in the positive ion mode at various normalized collision energies. The spectra generated at 35 eV were considered to be the most useful, and so were exported and compiled into an in-house library for future use in the identification of unknowns.

The Appendices contain the ESI-MS^2 spectra obtained for each of the tryptamines investigated using a normalized collision energy of 35 eV (top panels) . After isolation of the singly protonated pseudo-molecular or parent species, the ions were subjected to collisions with the target gas Helium, producing fragments that partially characterize the original molecule. For all the tryptamines investigated, major fragments observed correspond to two types of dissociations, both due to cleavages on the aliphatic side chain of the molecule. The first cleavage is between the nitrogen and the *alpha* carbon, with the second cleavage occurring between the *alpha* and *beta* carbons (see Figure 1). The former process results in the production of ammonia or a neutral primary or secondary amine (depending on the amine substituents), with the charge being transferred to the indole-containing fragment. The latter fragmentation process produces a charged amine, along with a neutral indole-containing fragment. Both of these fragmentation processes are illustrated in Figure 3 for the case of 5-methoxy-*N,N*-methylisopropyltryptamine (**11**).

For compounds with the same molecular weights, the fragmentation patterns obtained from the ESI-MS^2 experiments were useful for elucidating structures. For example, 4-acetoxy-*N,N*-methylisopropyltryptamine (**8**) and 5-methoxy-*N,N*-diisopropyltryptamine (**10**) have the same molecular weight of 274. In both cases, the protonated and deprotonated pseudo-molecular ions are observed at *m/z* 275 and 273, respectively, not allowing for their distinction. However, ESI-MS^2 analysis produced different fragmentation patterns for these compounds (see Appendices 8 and 10). For 4-acetoxy-*N,N*-methylisopropyltryptamine (**8**), the major fragment is observed at *m/z* 202, with additional fragments at *m/z* 160 and 86. Whereas for 5-methoxy-*N,N*-diisopropyl-tryptamine (**10**), the major fragment is observed at *m/z* 114, with additional fragments at *m/z* 102 and 174.

The usefulness of ESI-MS^2 analyses can also be illustrated by again comparing 4-hydroxy-*N,N*-diisopropyl-tryptamine (**7**) and 4-acetoxy-*N,N*-diisopropyltryptamine (**9**). As mentioned before, these compounds are virtually undistinguishable by GC/MS analysis, due to the thermal instability of the 4-acetoxy group. In addition to providing direct molecular weight information, ESI-MS^2 analysis produces distinctive fragments that allow their specific characterization. For 4-acetoxy-*N,N*-diisopropyltryptamine (**9**), fragments are produced at *m/z* 202, 160, 114, and 102. Whereas for 4-hydroxy-*N,N*-diisopropyltryptamine (**7**), fragments are produced at *m/z* 160, 114, and 102. While the fragment at *m/z* 202 is the predominant fragment produced from the former compound, it is absent from the latter, providing a marker ion for differentiation.

GC/MS Experiments

The Appendices also include the standard EI spectra obtained using a GC/MS system (bottom panels). Not surprisingly, most of the spectra are characterized by the presence of one major peak (base peak) corresponding to fragmentation of the *sigma* bond between the *alpha* and *beta* carbons. For 10 of the compounds (tryptamines 2 - 11), *alpha* cleavage [19] results in retention of the charge by the amine group. For *alpha*-methyltryptamine (**1**) and 5-methoxy-*alpha*-methyltryptamine (**12**), the most favorable fragmentation, inductive cleavage [19], results in charge migration to the indole-containing fragment, producing peaks at *m/z* 130 and 160, respectively. An additional hydrogen rearrangement process is also involved, resulting in major peaks at *m/z* 131 and 161, respectively. GC/MS experiments also produce characteristic signatures for the different amine substituents. For example, *m/z* 86 is characteristic of the *N,N*-methylisopropyl and *N,N*-diethyl groups, while *m/z* 114 is characteristic of the *N,N*-dipropyl or *N,N*-diisopropyl functionalities. For these two latter isomeric species, the additional presence of ions at *m/z* 72 and 86, respectively, provides a useful distinguishing factor.

Additional bond cleavages in the tryptamine molecule result in the generation of specific fragmentation signatures. These can be of great use when interpreting data generated by either GC/MS or ESI-MS^2 techniques. For example, the presence of an ion at *m/z* 144 is indicative of a non-substituted indole moiety, after cleavage of the *sigma* bond between the amine nitrogen and the *alpha* carbon. The same type of cleavage leads to the generation of peaks at *m/z* 174 and 202 for compounds where the indole contains a methoxy or acetoxy functionality, respectively. As previously mentioned, cleavage between the *alpha* and *beta* carbons produces a high-intensity peak at *m/z* 86, due to the generation of the $C_4H_{10}{}^+N{=}CH_2$ fragment, which can be produced from the diethylamine or methylisopropylamine group. Through the same process, the generation of *m/z* 114 is indicative of the $C_6H_{14}{}^+N{=}CH_2$ fragment, consistent with either dipropylamine or diisopropylamine. For dimethyltrypamine compounds, the analogous peak will appear at *m/z* 58 due to $C_2H_6{}^+N{=}CH_2$. As observed for *alpha*-methyltryptamine (**1**) and 5-methoxy-*alpha*-methyltryptamine (**12**), cleavage between the *alpha* and *beta* carbons also produces signature ions at *m/z* 130 and 160, characteristic of an unsubstituted and a methoxy-substituted indole, respectively.

ESI-MS^n Experiments

The use of ESI combined with a quadrupole ion trap as the mass analyzer provides the enhanced capabilities of MS^n experiments. To illustrate the usefulness of this type of analysis, ESI-MS^3 experiments were performed on *N,N*-dipropyltryptamine (**5**) and *N,N*-diisopropyltryptamine (**6**). As illustrated in the Appendices, the fragments

generated during MS^2 analysis do not allow for the unambiguous differentiation of these two compounds, although the presence of *m/z* 86 (5 % intensity) for *N,N*-dipropyltryptamine (**5**) does suggests that they are in fact different. By isolation and fragmentation of the singly protonated pseudo-molecular ions at *m/z* 245 using a collision energy of 25 eV (%), major ions at *m/z* 144 and 114 are generated for both compounds. However, further isolation and fragmentation of the *m/z* 114 ion, using a collision energy of 30 eV, leads to significantly different MS^3 spectra (see Figures 4 and 5). Fragmentation of the *m/z* 114 ion produces a fragment at *m/z* 86 for *N,N*-dipropyltryptamine and a fragment at *m/z* 72 for *N,N*-diisopropyltryptamine. These ions correspond to the loss of the neutral fragments $CH_2{=}CH_2$ and $CH_2{=}CHCH_3$, respectively. These losses are characteristics of the dipropyl and diisopropyl groups, respectively, providing a tool for differentiation. In fact, further fragmentation (MS^4) of the *m/z* 86 ion, produced from the fragmentation of *N,N*-dipropyltryptamine, generates a peak at *m/z* 58, consistent with the loss of a second $CH_2{=}CH_2$ molecule from the remaining dipropyl chain (data not shown). Similar MS^4 analysis of the *m/z* 72 ion from *N,N*-diisopropyltryptamine would produce additional information regarding the second diisopropyl group; however, the additional loss of 42 would produce an ion at *m/z* 30, which is below the observable low-mass limit of 50 for this instrument. These ESI-MS^3 results are in agreement with the GC/MS data obtained for *N,N*-dipropyltryptamine and *N,N*-diisopropyltryptamine, where the presence of *m/z* 86 and 72 is a basis for differentiation.

The complementary value of the GC/MS and ESI-MS data can be better illustrated using *alpha*-methyltryptamine (**1**). The GC/MS spectrum does not provide molecular weight information, but it does indicate the presence of a non-substituted indole (*m/z* 130), with the presence of a phenyl group further confirmed by *m/z* 77. Although the ion at *m/z* 44 is indicative of C_2H_6N, no confirmation of the $-NH_2$ group is obtained. By direct observation of the pseudo-molecular ion, the ESI-MS data provide a direct determination of molecular weight at 174. The ESI-MS^2 spectrum, while simple and only containing one major ion at *m/z* 158, provides a signature indicating the loss of ammonia from the protonated intact molecule, confirming the presence of a $-NH_2$ substituent.

Conclusions

The analysis of 12 tryptamine analogues using ESI-MS has been presented. Full scan analysis in the positive and negative ionization modes allowed the observation of singly protonated and singly deprotonated ions, providing molecular weight information. MS^2 experiments allowed the fragmentation of pseudo-molecular ions to be investigated under controlled experimental conditions, providing structural information for each one of the compounds, and making possible the observation of fragmentation signatures. Particularly useful was the generation of ESI full-scan and MS^2 spectra for the thermally-labile compound 4-acetoxy-*N,N*-diisopropyl-tryptamine, which provided a distinction from 4-hydroxy-*N,N*-diisopropyltryptamine. MS^3 experiments were also performed and results provided an additional technique for the differentiation of compounds with the same molecular weight and similar MS^2 spectra. The results presented are in agreement with those obtained using standard GC/MS techniques, and show the utility of ESI-MS as a complementary analytical technique that can be used in conjunction with GC/MS, NMR, and IR spectroscopy in the structural characterization of tryptamines.

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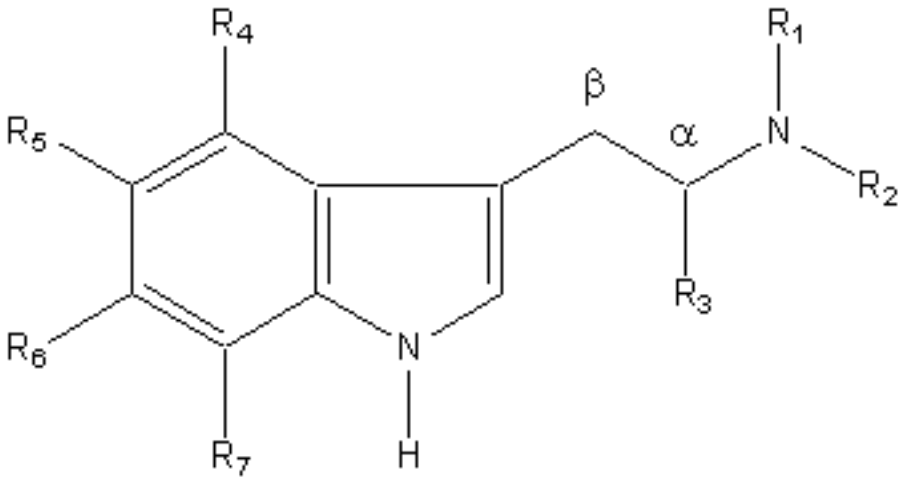
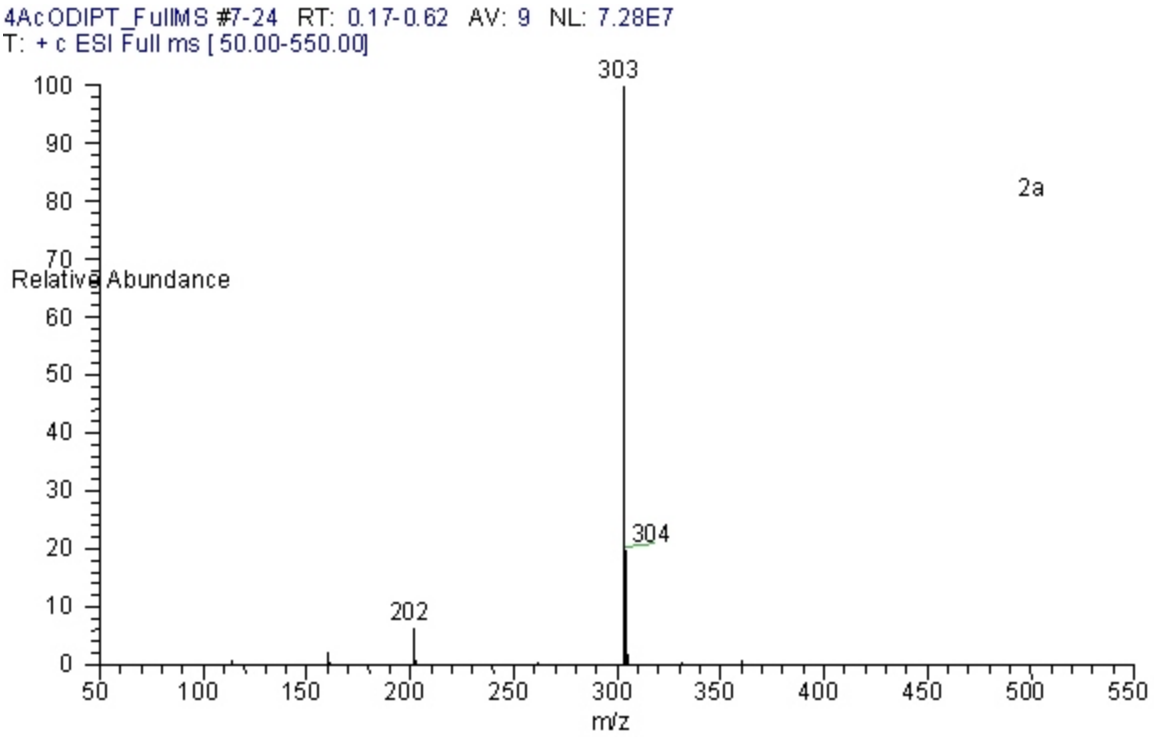


Figure 1. Core Structure of the Tryptamines

Table 1. Designer Tryptamines Investigated (Refer to Figure 1 for Substituent Positions).

No.	Compound	R1	R2	R3	R4	R5	MW
1	*alpha*-Methyltryptamine	H	H	-Me	H	H	174
2	*N,N*-Dimethyltryptamine	-Me	-Me	H	H	H	188
3	*N,N*-Diethyltryptamine	-Et	-Et	H	H	H	216
4	*N,N*-Methylisopropyltryptamine	-Me	-iPr	H	H	H	216
5	*N,N*-Dipropyltryptamine	-Pr	-Pr	H	H	H	244
6	*N,N*-Diisopropyltryptamine	-iPr	-iPr	H	H	H	244
7	4-Hydroxy-*N,N*-diisopropyltryptamine	-iPr	-iPr	H	-OH	H	260
8	4-Acetoxy-*N,N*-methylisopropyltryptamine	-Me	-iPr	H	-OAc	H	274
9	4-Acetoxy-*N,N*-diisopropyltryptamine	-iPr	-iPr	H	-OAc	H	302
10	5-Methoxy-*N,N*-diisopropyltryptamine	-iPr	-iPr	H	H	-OMe	274
11	5-Methoxy-*N,N*-methylisopropyltryptamine	-Me	-iPr	H	H	-OMe	246
12	5-Methoxy-*alpha*-methyltryptamine	H	H	-Me	H	-OMe	204



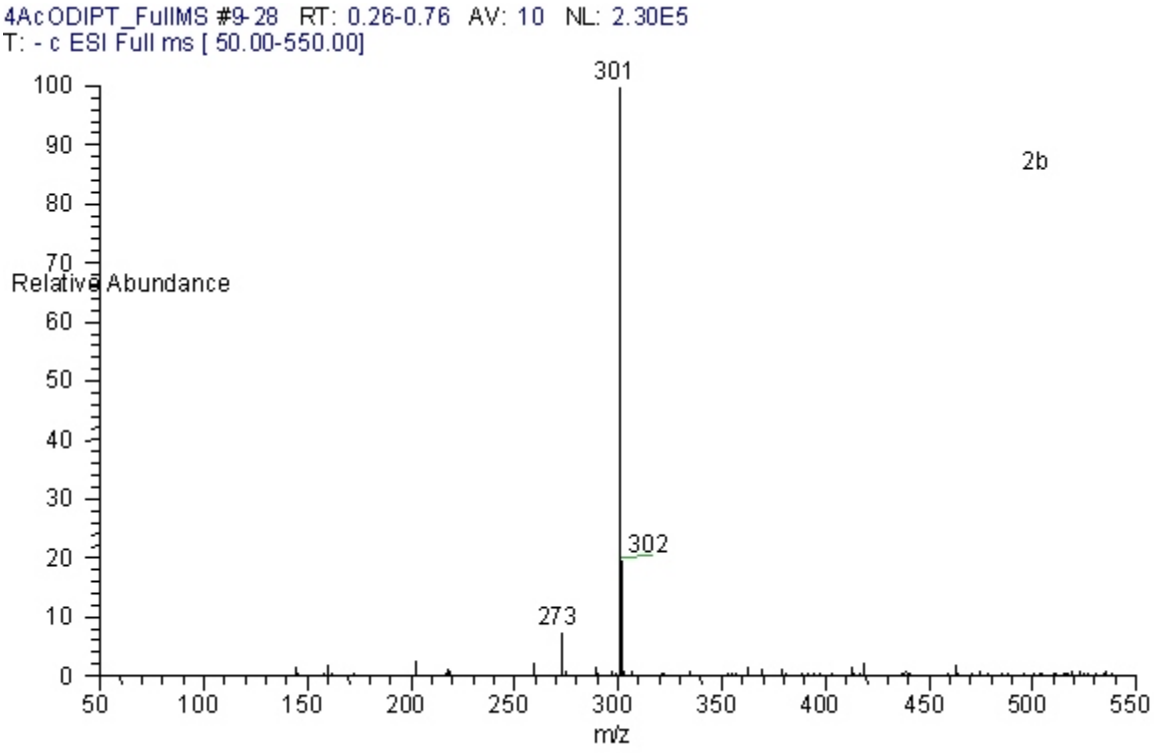


Figure 2. (a) Positive and (B) Negative Ion Mode Electrospray Ionization Mass Spectra for 4-Acetoxy-*N,N*-diisopropyltryptamine (**6**).

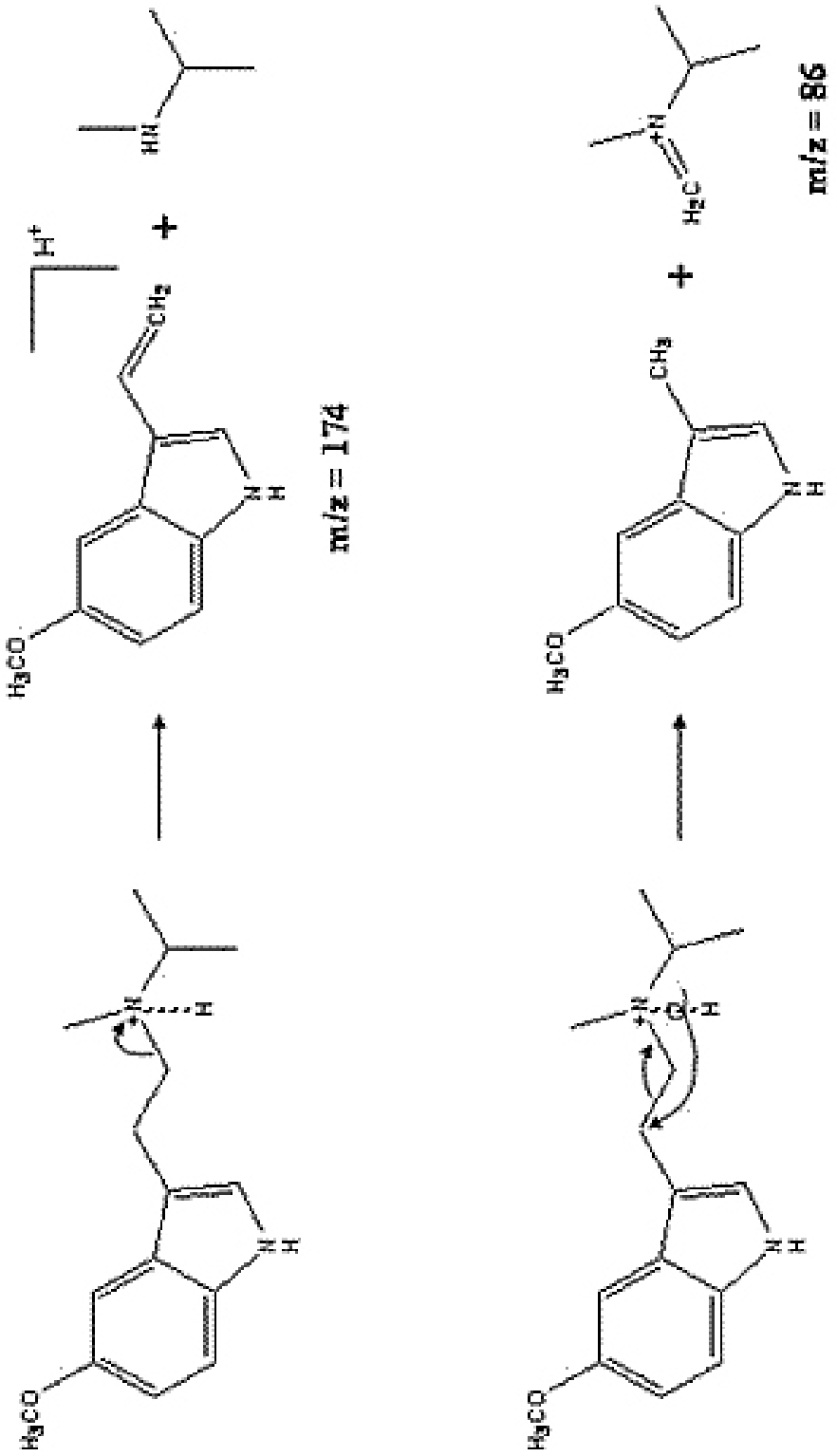


Figure 3. Chemical Diagram Illustrating the Two Main Fragmentation Processes Observed During the MS^2 Analysis of Tryptamines.

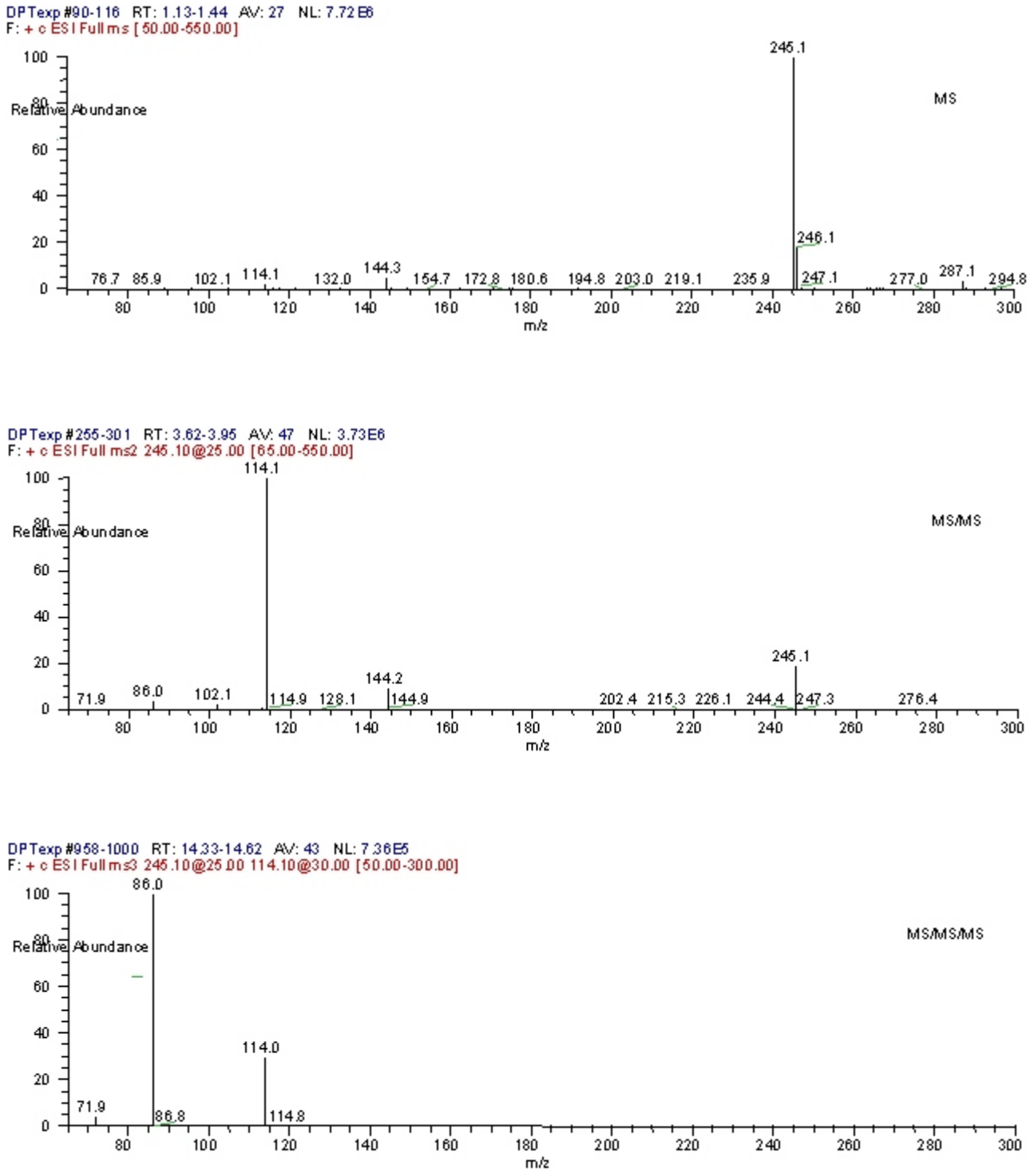


Figure 4. Full Mass, MS^2 and MS^3 Spectra Obtained for *N,N*-Dipropyltryptamine (**5**).

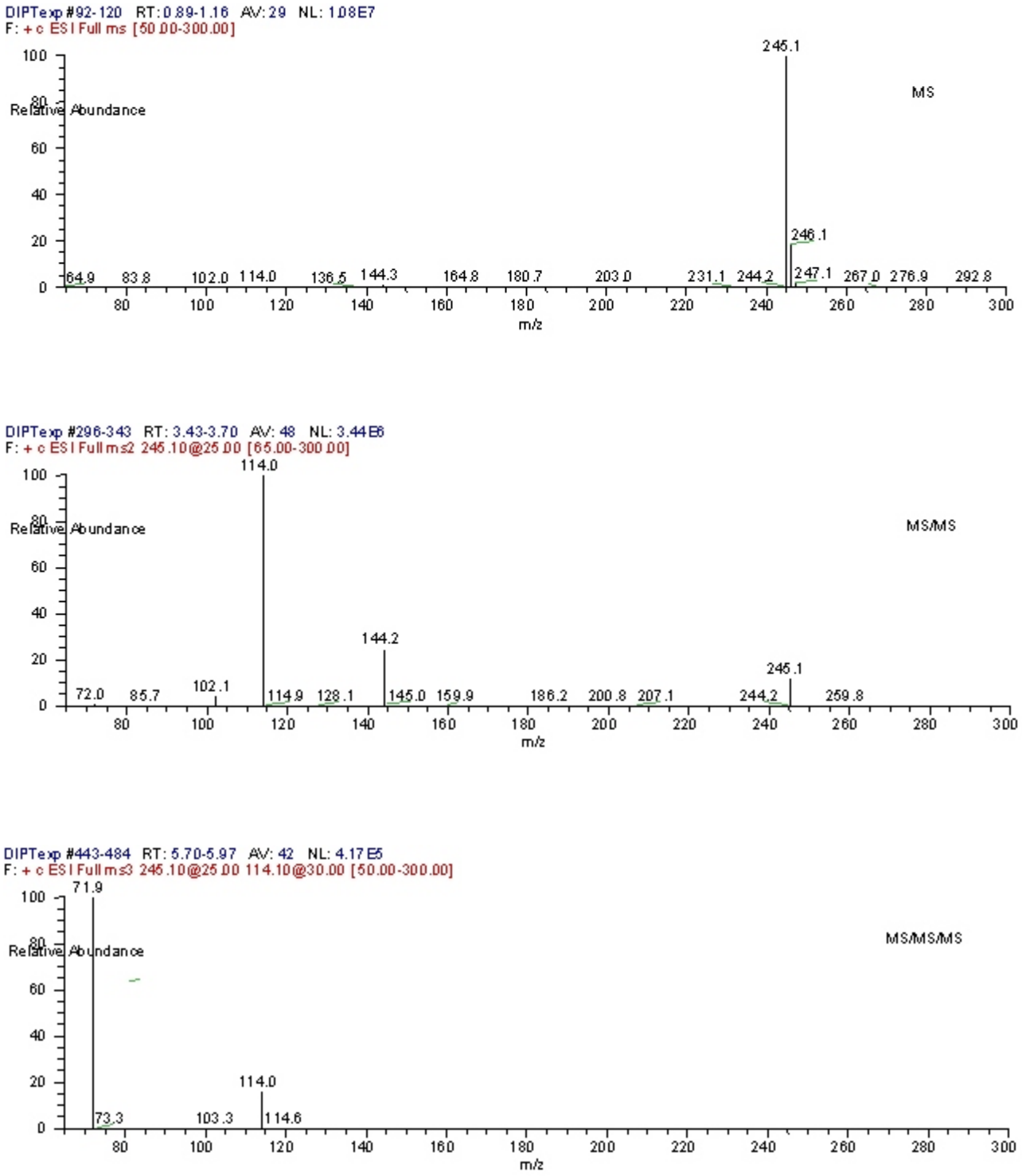
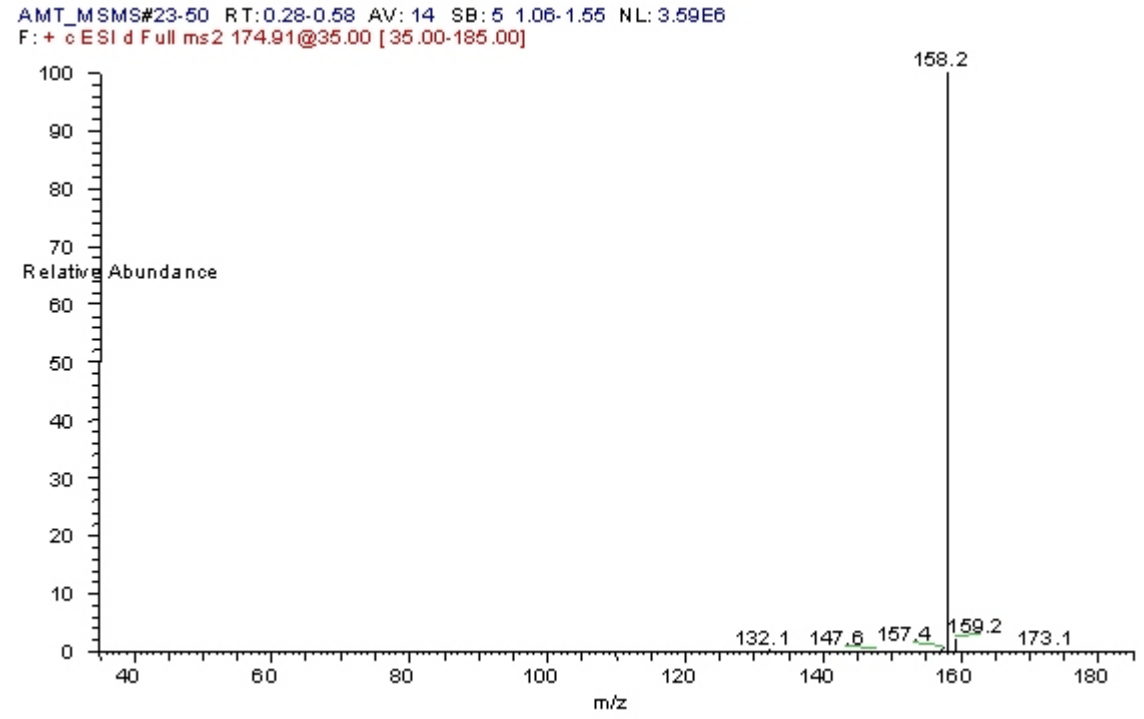
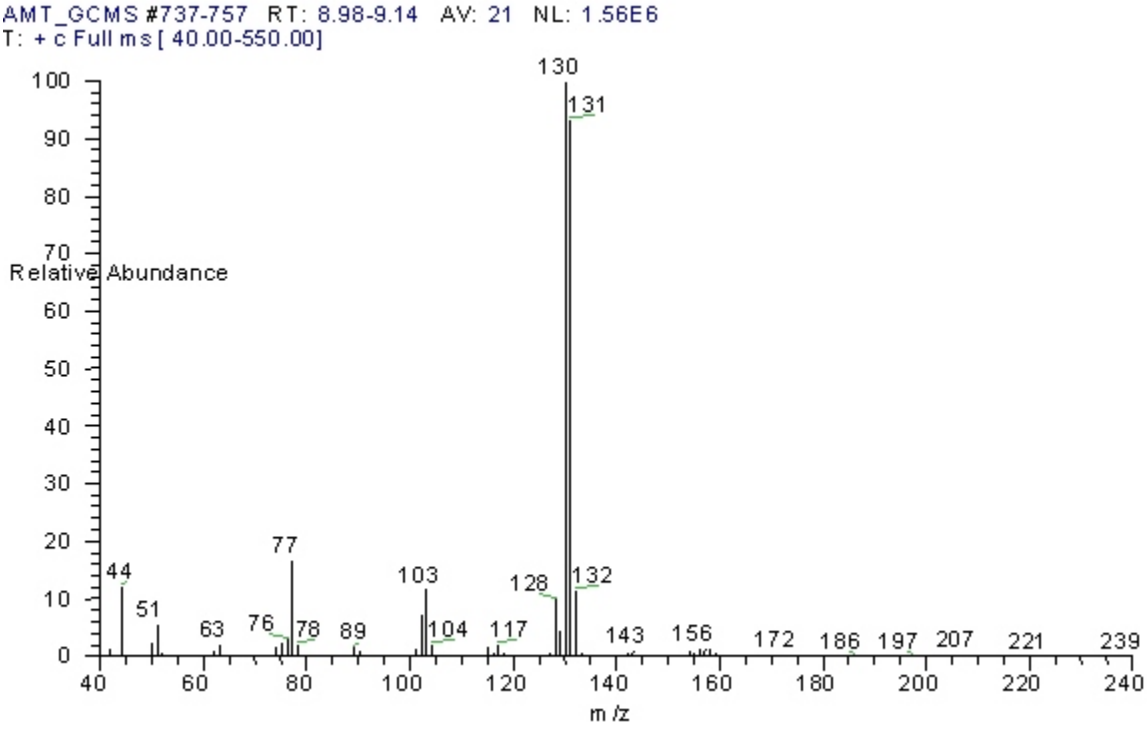
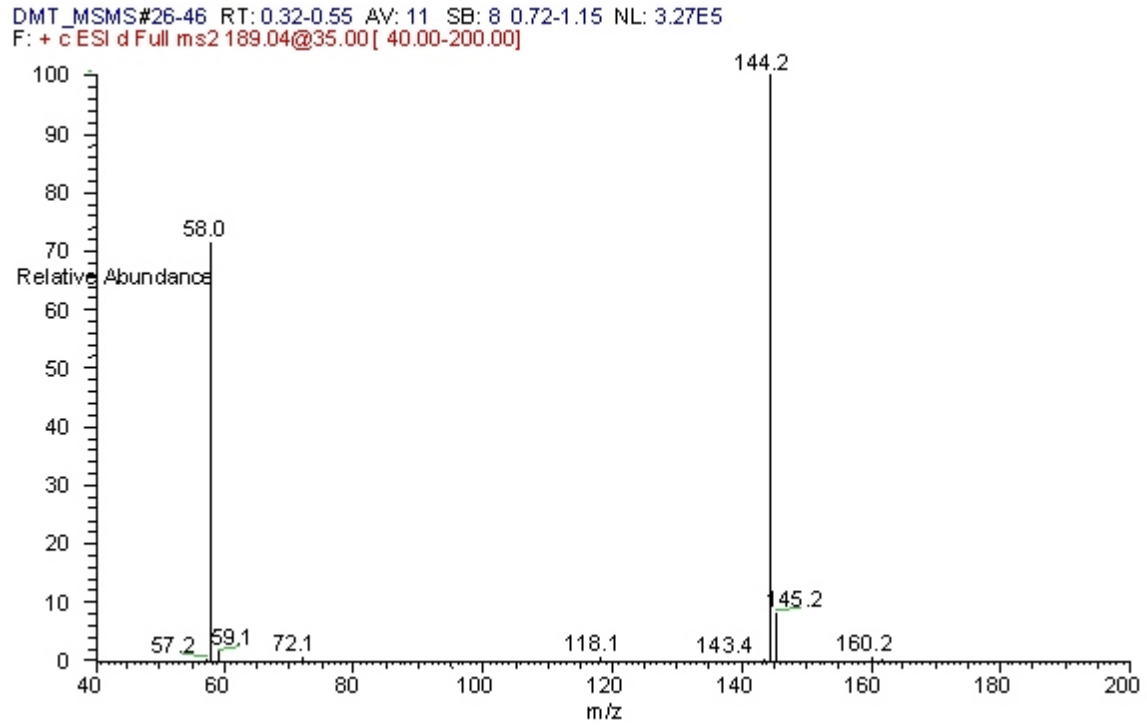


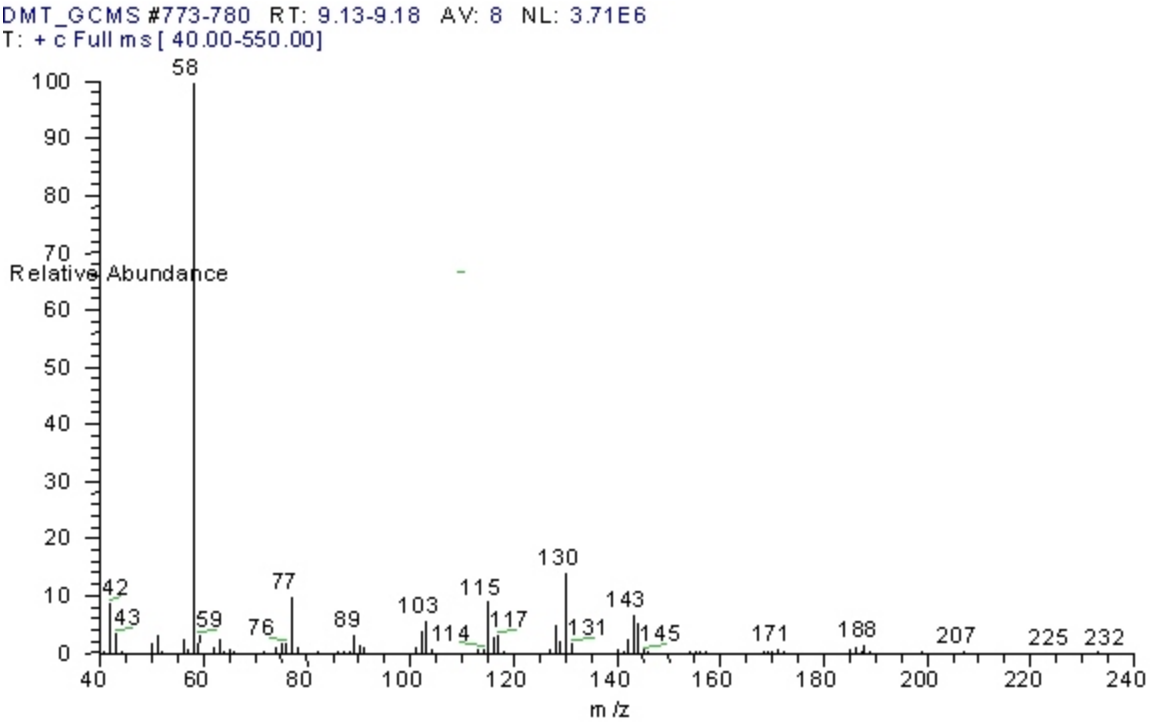
Figure 5. Full Mass, MS^2 and MS^3 Spectra Obtained for *N,N*-Diisopropyltryptamine (**6**).



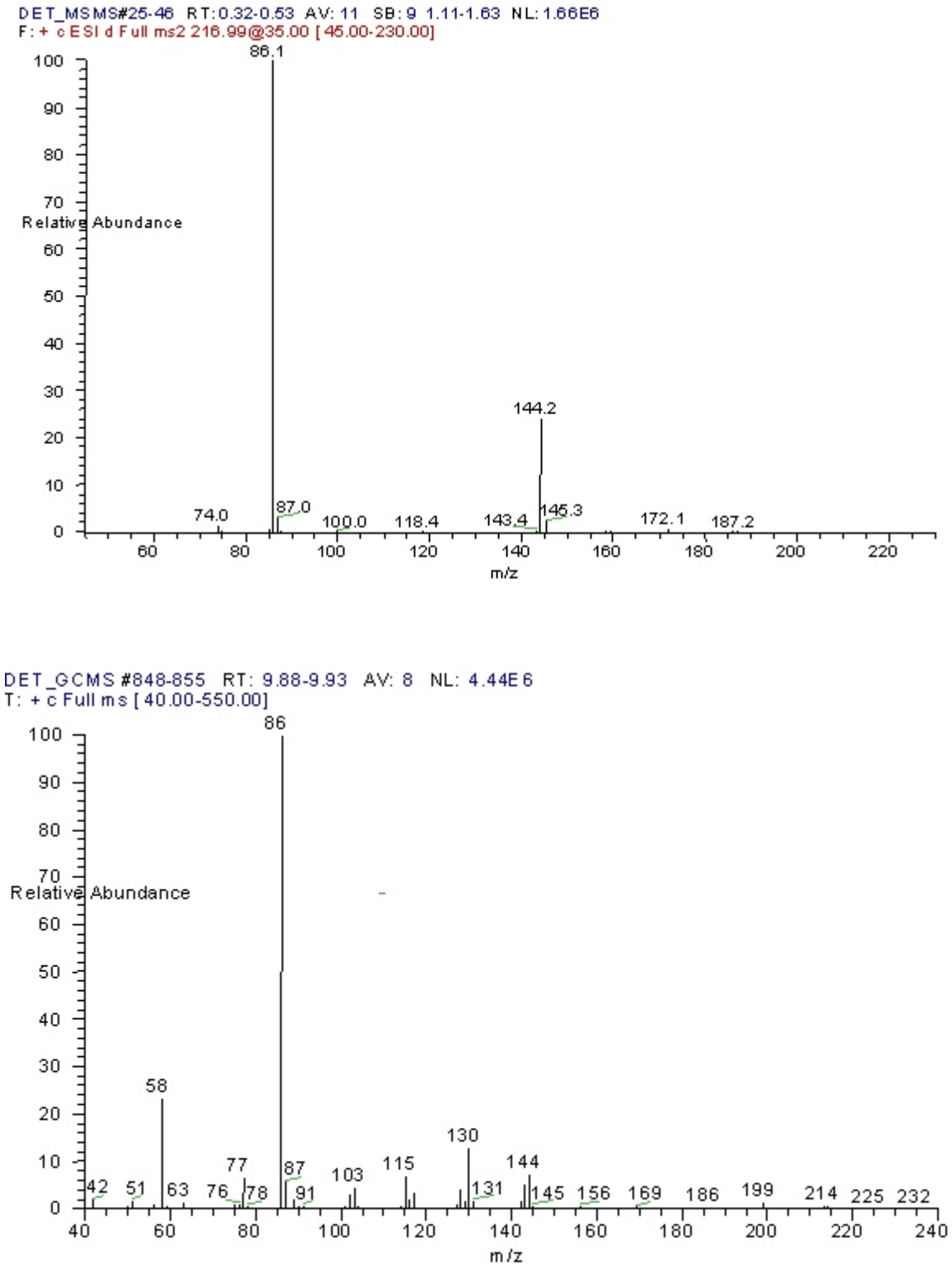


Appendix 1. *alpha*-Methyltryptamine: ESI-MS^2 (top) and GC/MS (bottom).

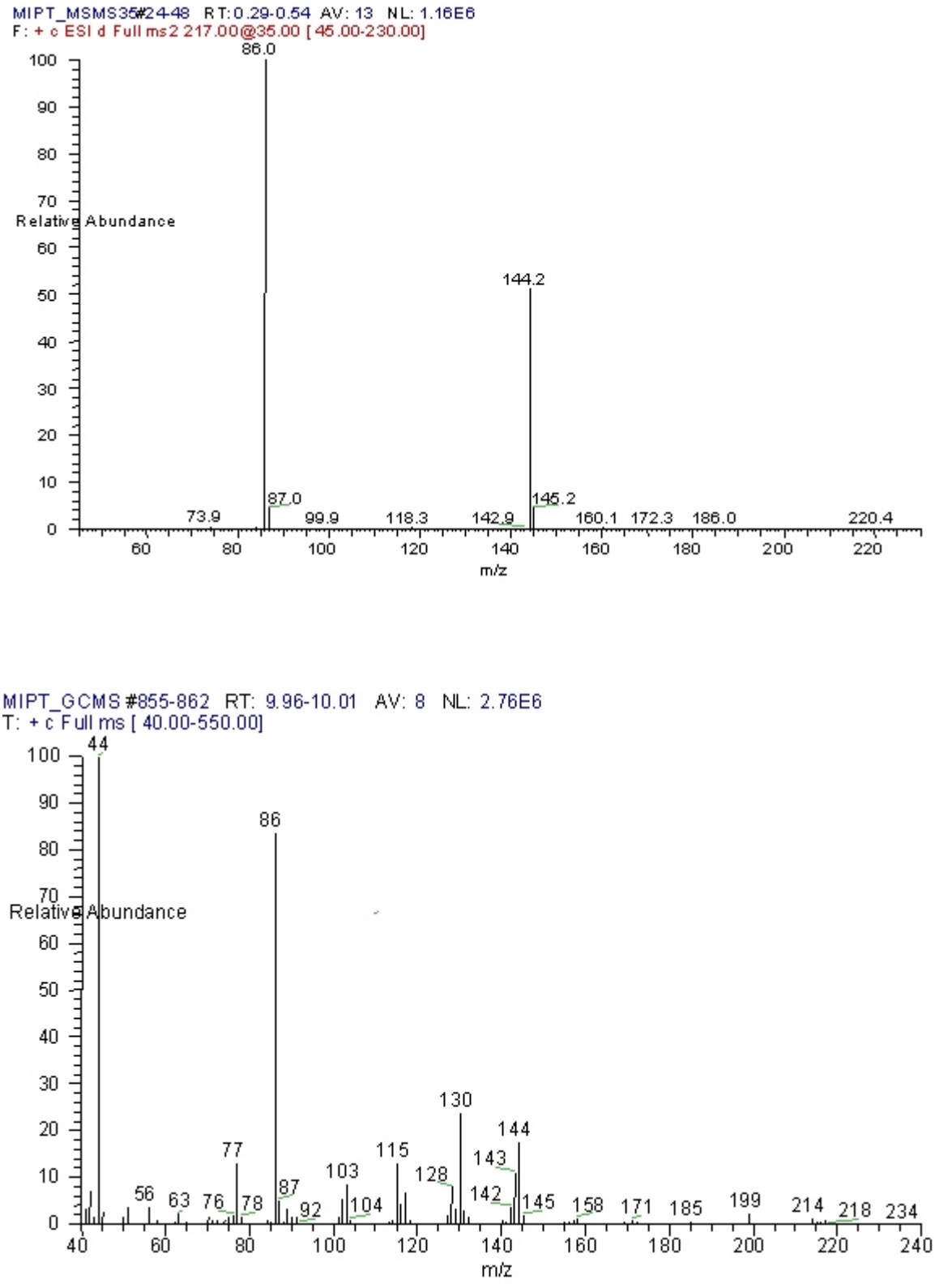




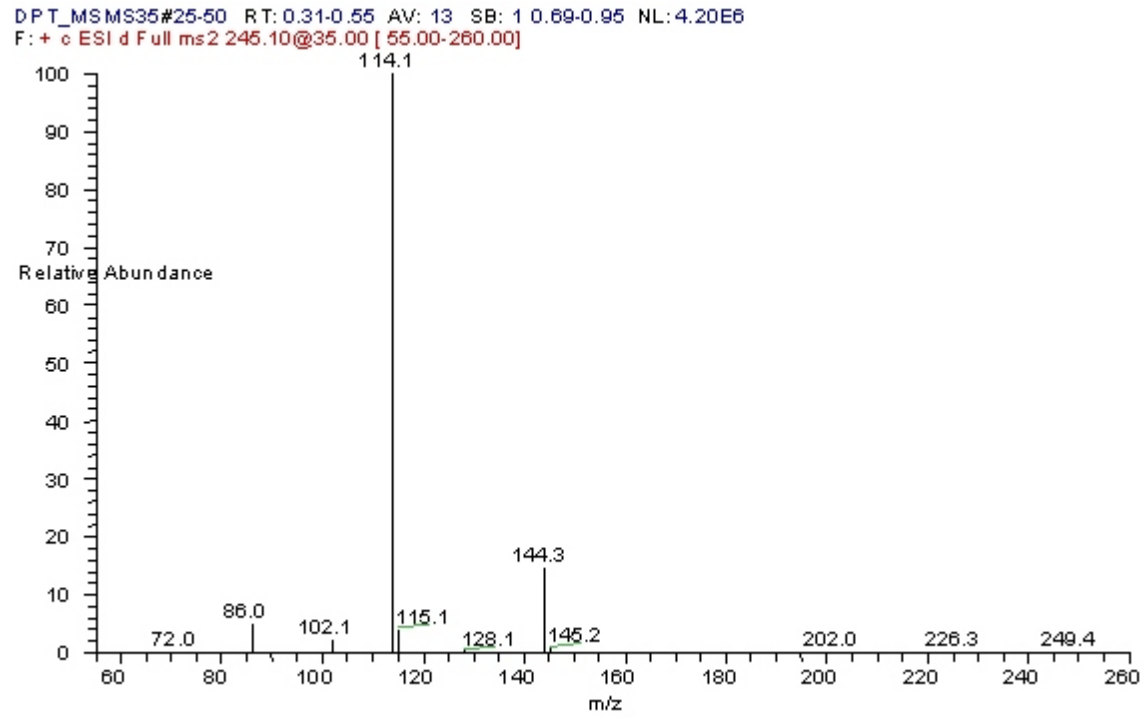
Appendix 2. *N,N*-Dimethyltryptamine: ESI-MS^2 (top) and GC/MS (bottom).

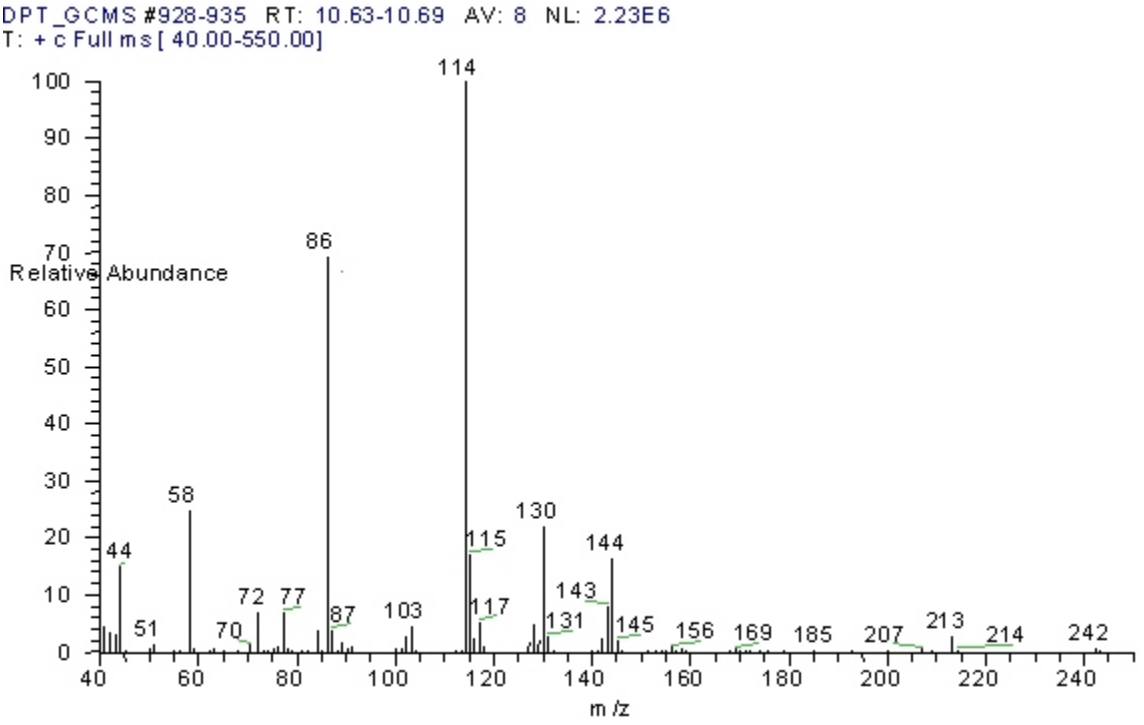


Appendix 3. *N,N*-Diethyltryptamine: ESI-MS^2 (top) and GC/MS (bottom).

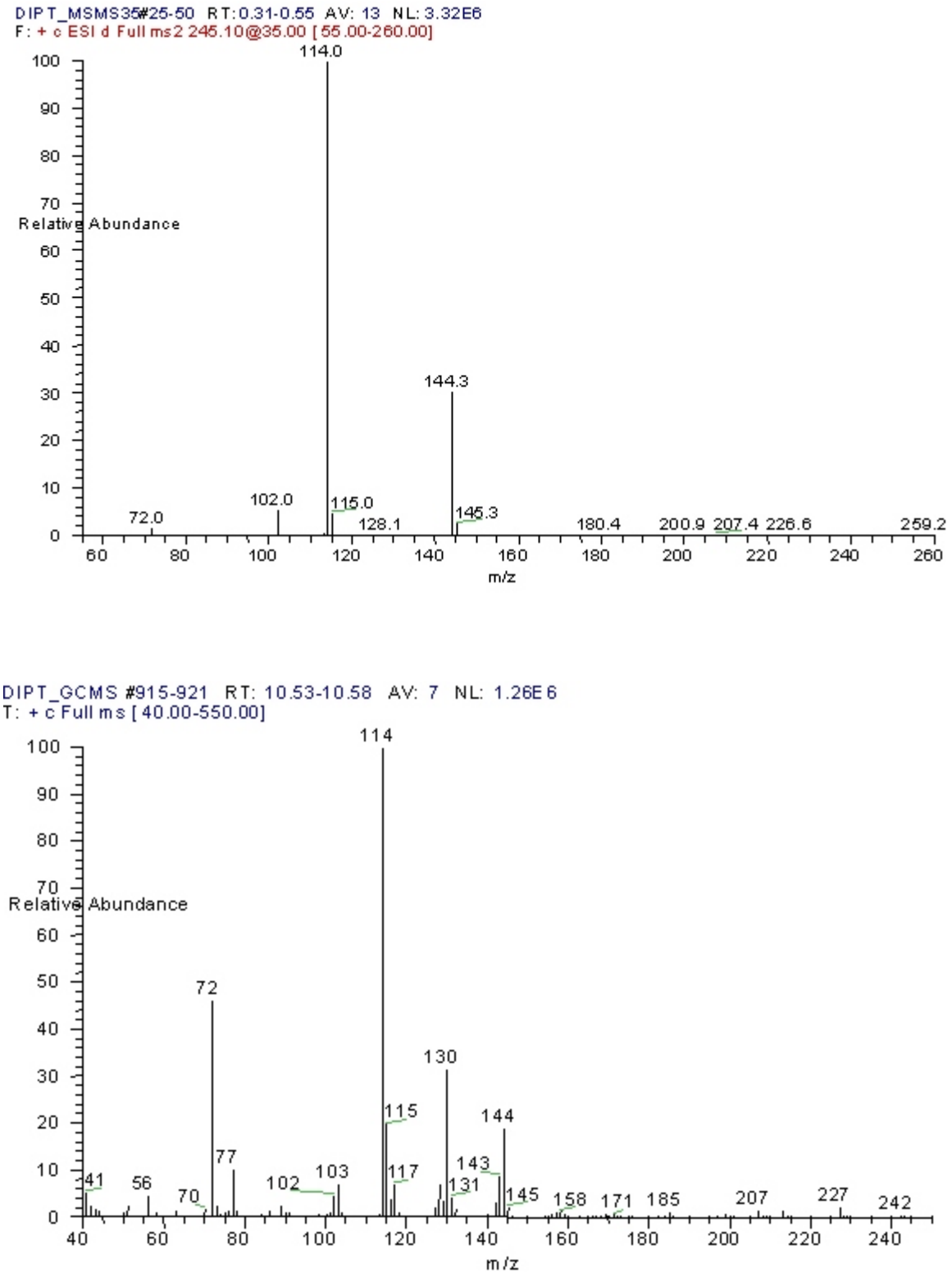


Appendix 4. *N,N*-Methylisopropyltryptamine: ESI-MS^2 (top) and GC/MS (bottom).

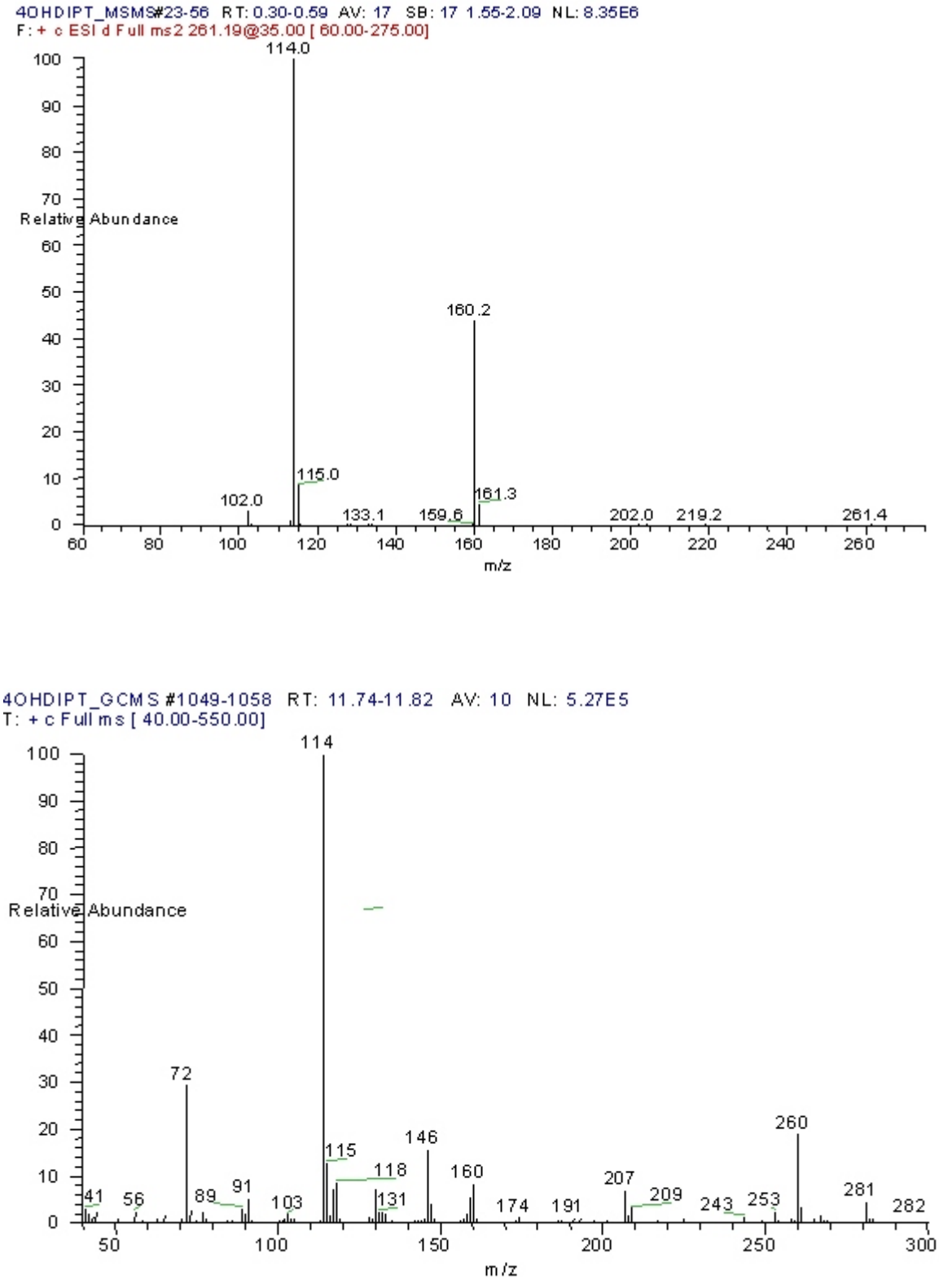




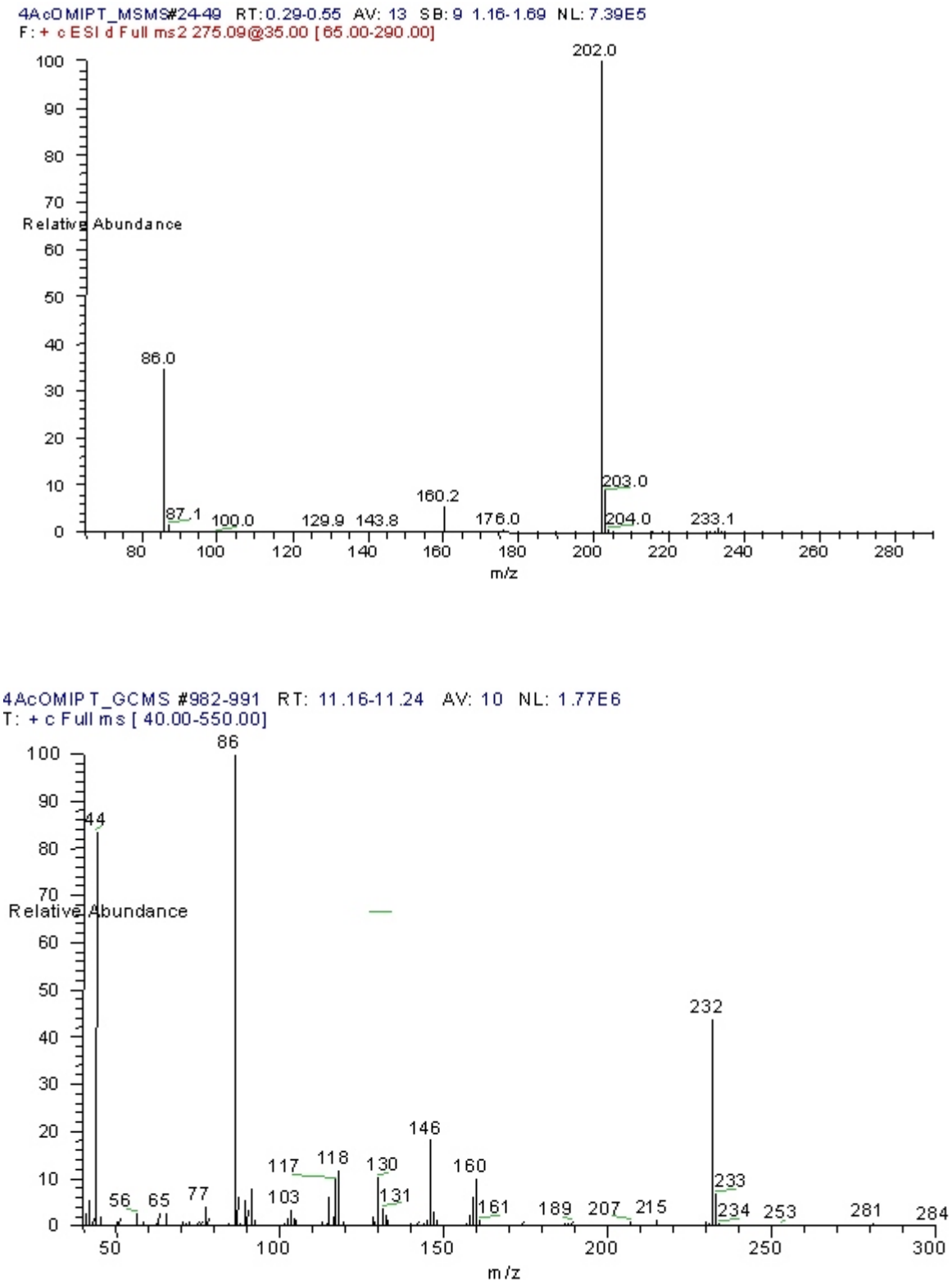
Appendix 5. *N,N*-Dipropyltryptamine: ESI-MS^2 (top) and GC/MS (bottom).



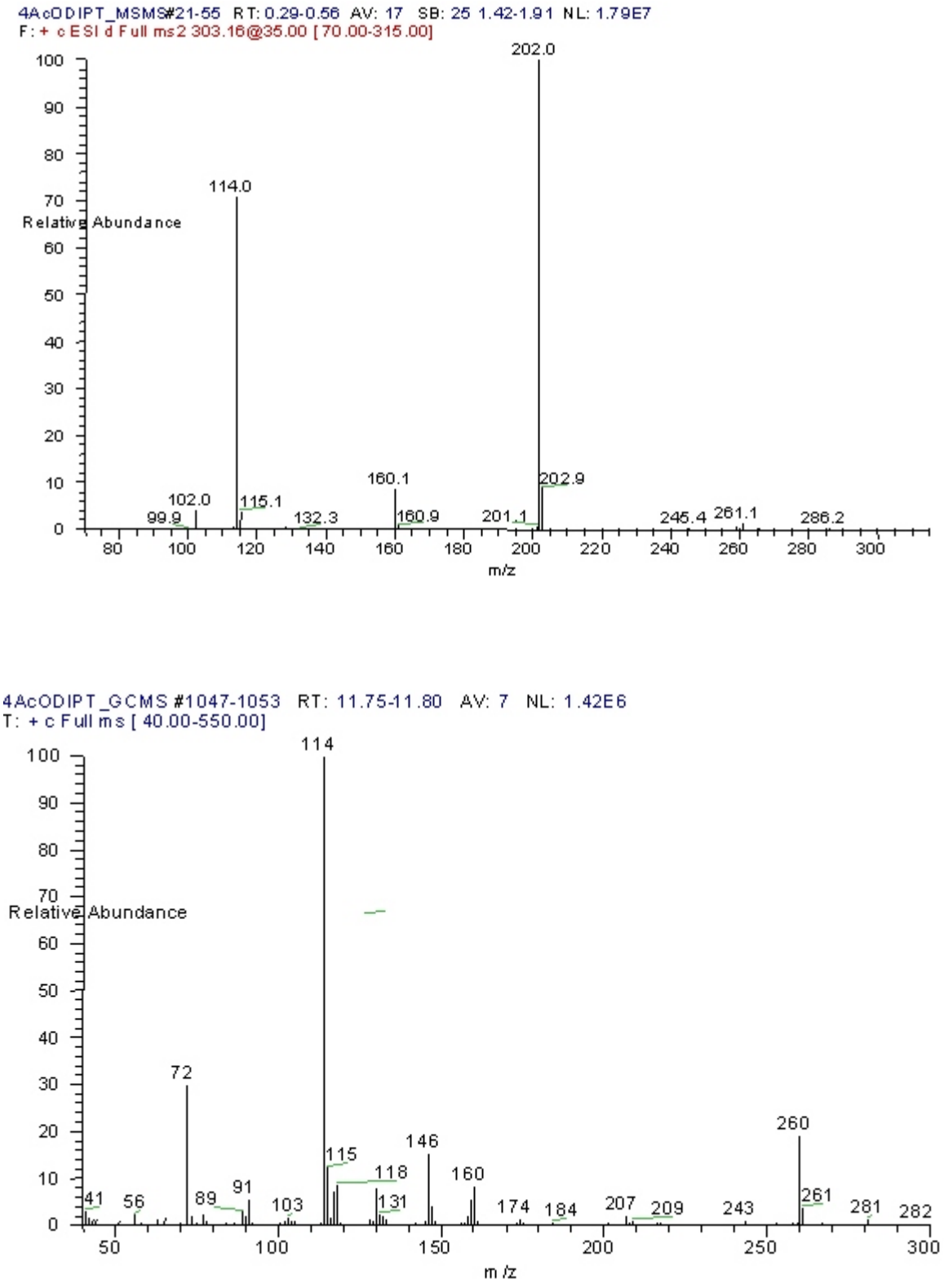
Appendix 6. *N,N*-Diisopropyltryptamine: ESI-MS^2 (top) and GC/MS (bottom).



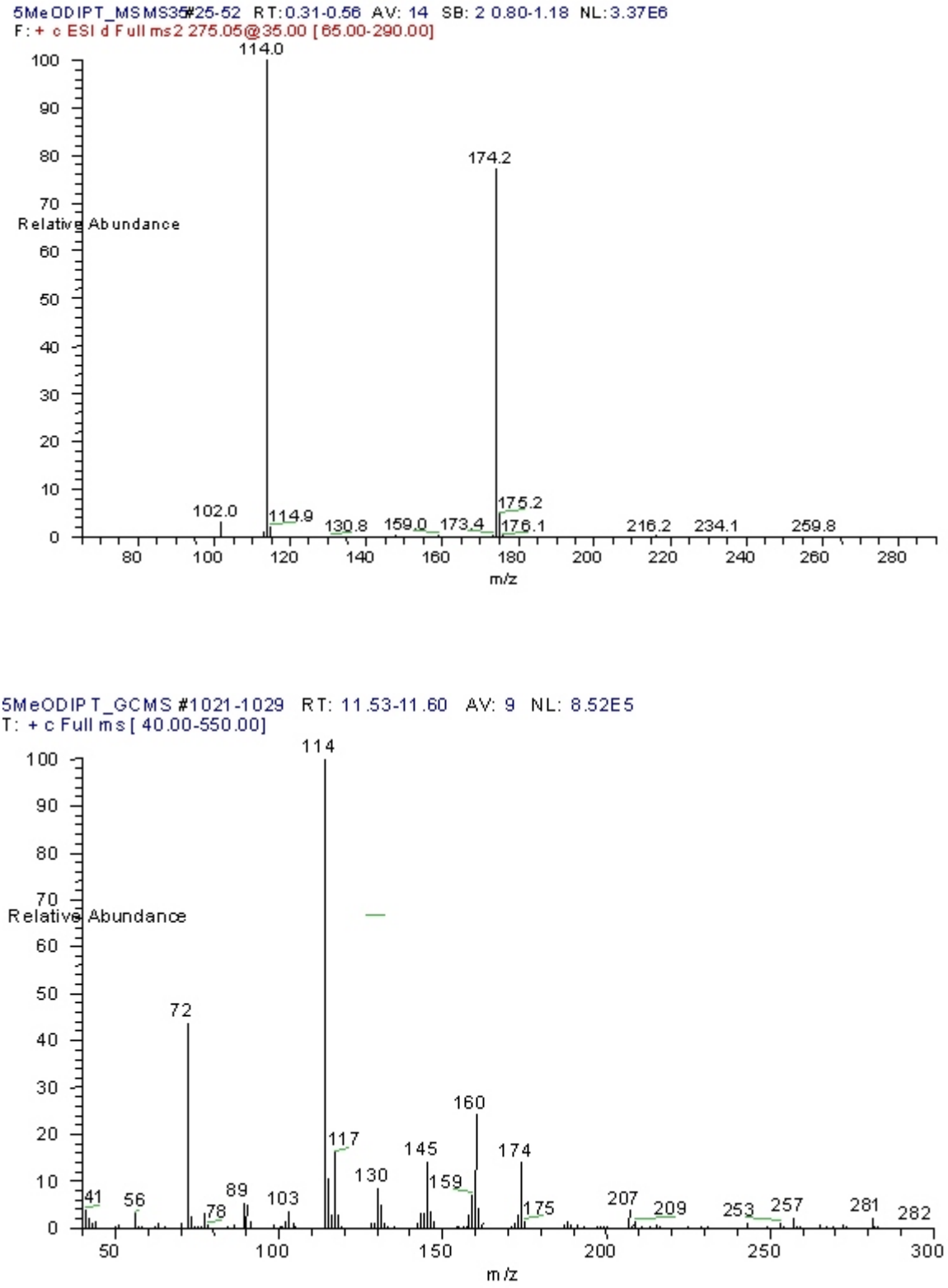
Appendix 7. 4-Hydroxy-*N,N*-diisopropyltryptamine: ESI-MS^2 (top) and GC/MS (bottom).



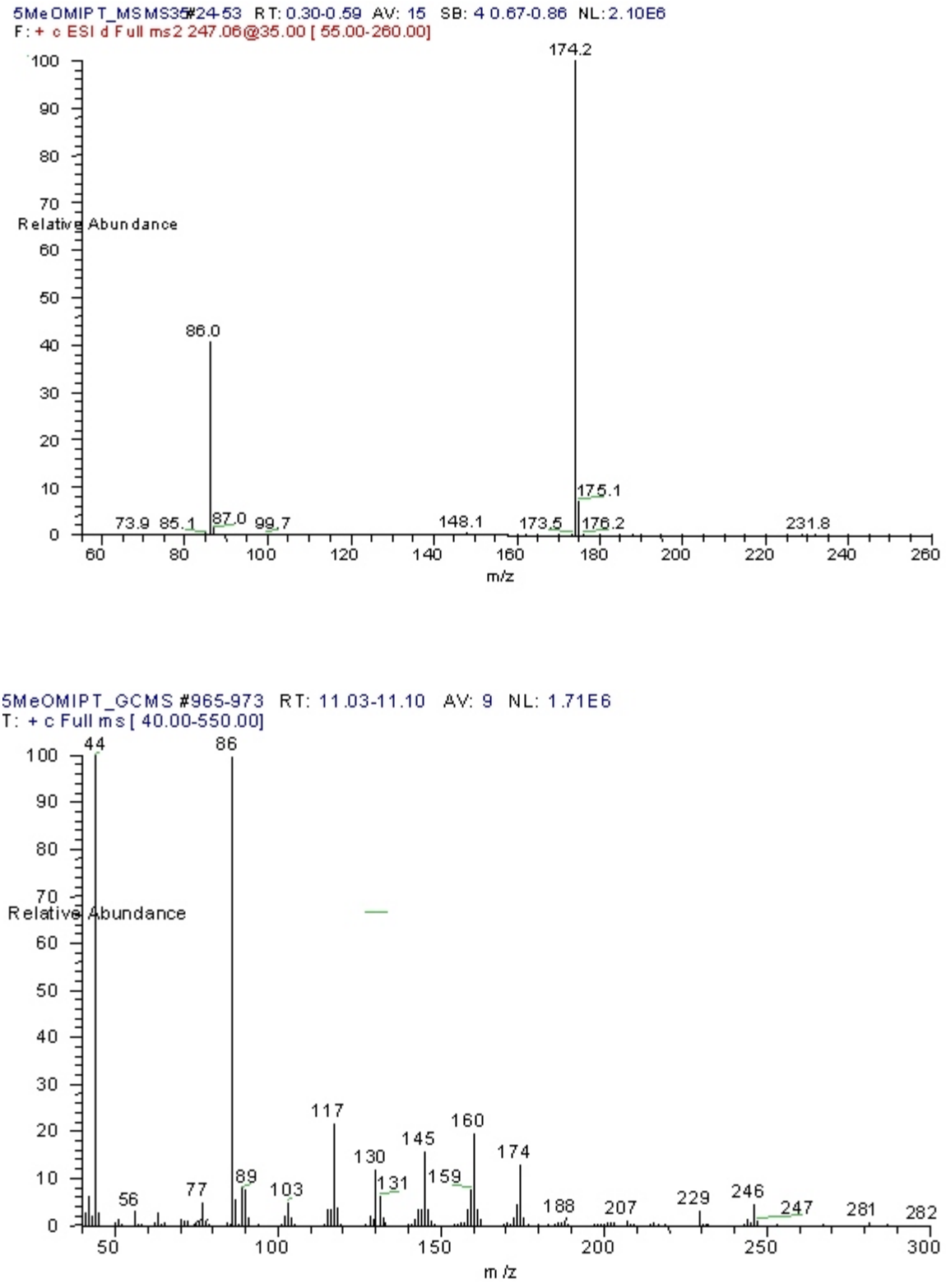
Appendix 8. 4-Acetoxy-*N,N*-methylisopropyltryptamine: ESI-MS^2 (top) and GC/MS (bottom)



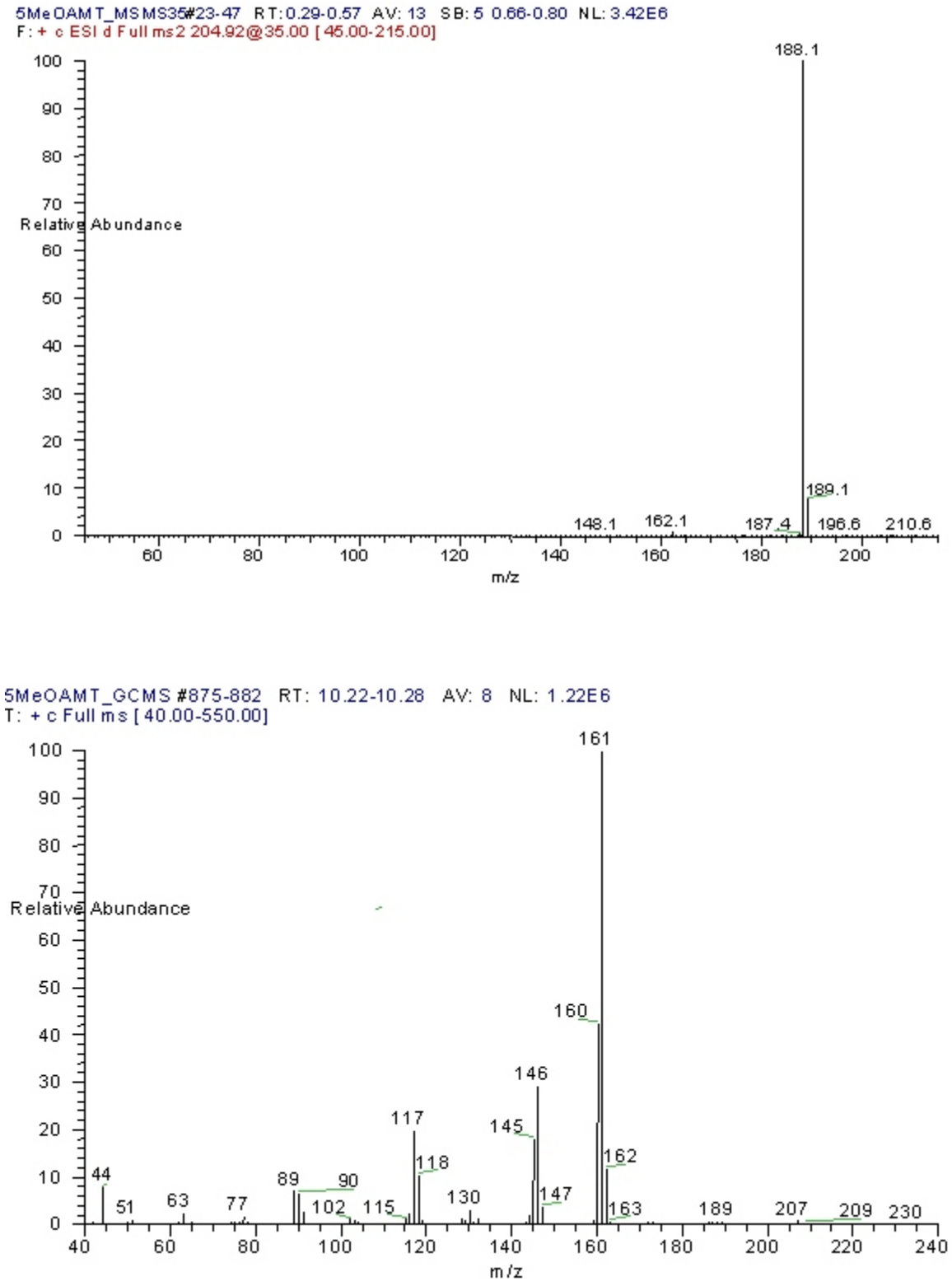
Appendix 9. 4-Acetoxy-*N,N*-diisopropyltryptamine: ESI-MS^2 (top) and GC/MS (bottom).



Appendix 10. 5-Methoxy-*N,N*-diisopropyltryptamine: ESI-MS^2 (top) and GC/MS (bottom).



Appendix 11. 5-Methoxy-*N,N*-methylisopropyltryptamine: ESI-MS^2 (top) and GC/MS (bottom).



Appendix 12. 5-Methoxy-*alpha*-methyltryptamine: ESI-MS^2 (top) and GC/MS (bottom).

Technical Note

Assessment of the Volatility (Smokeability) of Cocaine Base Containing 50 Percent Mannitol: Is it a Smokeable Form of "Crack" Cocaine?

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ABSTRACT: A defendant convicted of possession and use of "crack" cocaine claimed on appeal that cocaine base containing 44 percent mannitol was not a "smokeable form," and therefore that Federal Sentencing Guidelines for "crack" cocaine were not applicable in his sentencing To investigate this claim, a sample of "crack" cocaine was made by mixing molten illicit cocaine base with an equal weight of mannitol, then cooled to form a solid "rock" that was visually consistent with typical exhibits of "crack" cocaine. A sample of this formulation was heated in a device similar to a crack pipe, and the resulting vapors were collected by dissolution into chloroform. Analysis of the resulting solution by GC/MS identified cocaine, thereby confirming that "crack" formulated from equal parts cocaine base and mannitol is smokeable.

KEYWORDS: Cocaine, Mannitol, Crack, Gas Chromatography/Mass Spectrometry, Forensic Chemistry.

Introduction

"Crack" cocaine has been a major drug of abuse since the early 1980's. The term "crack" is the most common street name used for cocaine base that is smoked. It can be in a rock-like, powder, or oil-like form, and can also be adulterated or diluted ("cut") with a virtually unlimited number of substances; some of the most common include benzocaine, procaine, mannitol, acetaminophen, aspirin, and phenacetin. "Crack" is not a scientific term; however, for sentencing purposes, "crack" has been defined as cocaine base.

Because of the violence associated with trafficking and sale of "crack" cocaine, and its unusually high potential for addiction, the U.S. Congress mandated more punitive sentences for cocaine base versus cocaine hydrochloride. Some recent judicial rulings have decreed that cocaine base exhibits must be in a "smokeable form" in order to impose Federal Sentencing Guidelines for "crack" cocaine [1]. Recently, a defendant who had been convicted for possession and use of "crack" cocaine claimed on judicial appeal that cocaine base containing 44 percent mannitol (that is, the sample he had) was not a "smokeable form" of cocaine base. Herein, we report the results of experiments designed to determine if a "crack" cocaine sample containing 50 percent mannitol is a "smokeable form" of cocaine base.

Experimental

Materials

Illicitly prepared cocaine base (m.p. 84 - 88 ^{O}C) was obtained from the reference collection of this laboratory. Pharmaceutical cocaine base and mannitol were obtained from Merck Chemical (Rahway, NJ) and

Sigma-Aldrich Chemical (Milwaukee, WI), respectively. Chloroform was a distilled-in-glass product of Burdick and Jackson Labs (Muskegon, MI).

Gas Chromatography/Mass Spectrometry (GC/MS)

Mass spectra were obtained on an Agilent Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent Model 6890 gas chromatograph. The MSD was operated in the EI mode with an ionization potential of 70 eV, a scan range of 34-700 amu, and 1.34 scans/sec. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with DB-1 (0.25 μm) (J & W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: Initial temperature, 100 ^{O}C; initial hold, 0.0 min; program rate, 6 ^{O}C/min; final temperature, 300 ^{O}C; final hold, 5.67 min. The injector was operated in the split mode (21.5:1) and a temperature of 280 ^{O}C. The auxiliary transfer line to the MSD was also operated at 280 ^{O}C.

Formation of "Crack" Cocaine Containing 50 Percent Mannitol

Illicit cocaine base (1.0 gram) was placed into a 15 mL beaker and heated on a laboratory hotplate until it melted into an oil. Mannitol (1.0 gram) was added and stirred for about 1 minute, resulting in a uniform oil. Upon cooling to room temperature, the oil solidified, after which it was broken into small, off-white to yellowish "rocks" that were visually consistent with typical exhibits of "crack" cocaine (m.p. 86 - 93 ^{O}C).

Vaporization of Crack Cocaine Containing 50 Percent Mannitol

The melting points of pharmaceutical grade cocaine base and cocaine hydrochloride are 98 ^{O}C and 195 ^{O}C, respectively [2]. Illicitly prepared cocaine base and cocaine hydrochloride have lower melting points since they contain cocaine-related impurities resulting from the crude illicit processing methodologies [3], and illicit cocaine base containing 50 percent mannitol would be expected to have an even lower melting point (in this case, however, it was actually several degrees higher than the illicit cocaine base used to prepare it).

A device similar to a "crack" pipe was fashioned from a 9-inch Pasteur pipette by inserting a tight plug of glass wool into the larger opening. The pipette was loaded with a small "rock" of the formulated "crack" (approximately 20 mg), and then fitted into a 125 mL suction filtration flask containing 5 mL of chloroform. A slight vacuum was applied to the flask and the section of the pipette containing the "rock" was gently heated with a propane flame. The "rock" vaporized within seconds (with some charring), and the produced vapors were drawn by the vacuum into the chloroform. The resulting solution was analyzed via GC/MS to determine what substances were trapped from the vapors. The chloroform was subsequently evaporated to dryness under a stream of nitrogen to provide 9 milligrams of solid material, which was also analyzed by GC/MS.

Results and Discussion

"Crack" cocaine containing 50 percent mannitol was easily prepared. Upon gentle heating with a propane flame, the mixture melted and boiled within a few seconds, giving white-colored vapors. Analysis of the chloroform solution of these vapors by GC/MS confirmed primarily cocaine and a small amount of methylecgonidine (Figures 1 – 3); mannitol was not detected. Methylecgonidine is a well-known byproduct of cocaine degradation due to heat, and has been previously documented [4]. The mass spectrum of the recovered cocaine was identical to the pharmaceutical standard. Quantitative analysis of the solid resulting from evaporation of chloroform solution confirmed that approximately 90 percent of the cocaine base present in the original "rock" was delivered into the chloroform.

Conclusions

"Crack" cocaine containing 50 percent mannitol is a "smokeable form" of cocaine base. Although beyond the scope of this study, similar results may be reasonably expected from "crack" cocaine made from any other common adulterant or diluent, regardless of their relative proportions.

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3. Casale JF, Klein RFX. Illicit production of cocaine. Forensic Science Review 1993;5(2):95-107.

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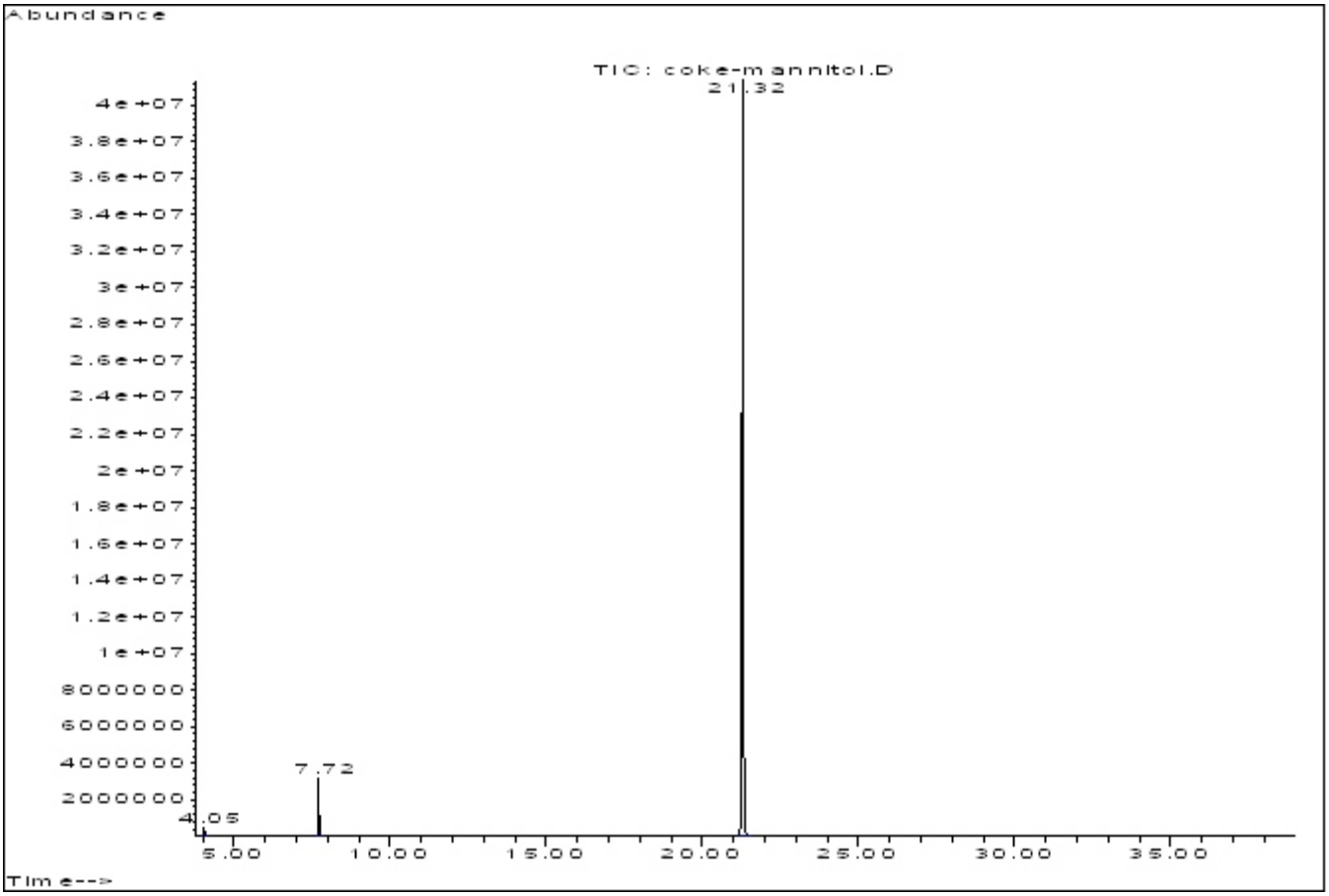

Figure 1. Total ion chromatogram of chloroform soluble vapors from heating crack cocaine containing 50 percent mannitol. Peak identification: Methylecgonidine (7.72 min) and Cocaine (21.32 min).

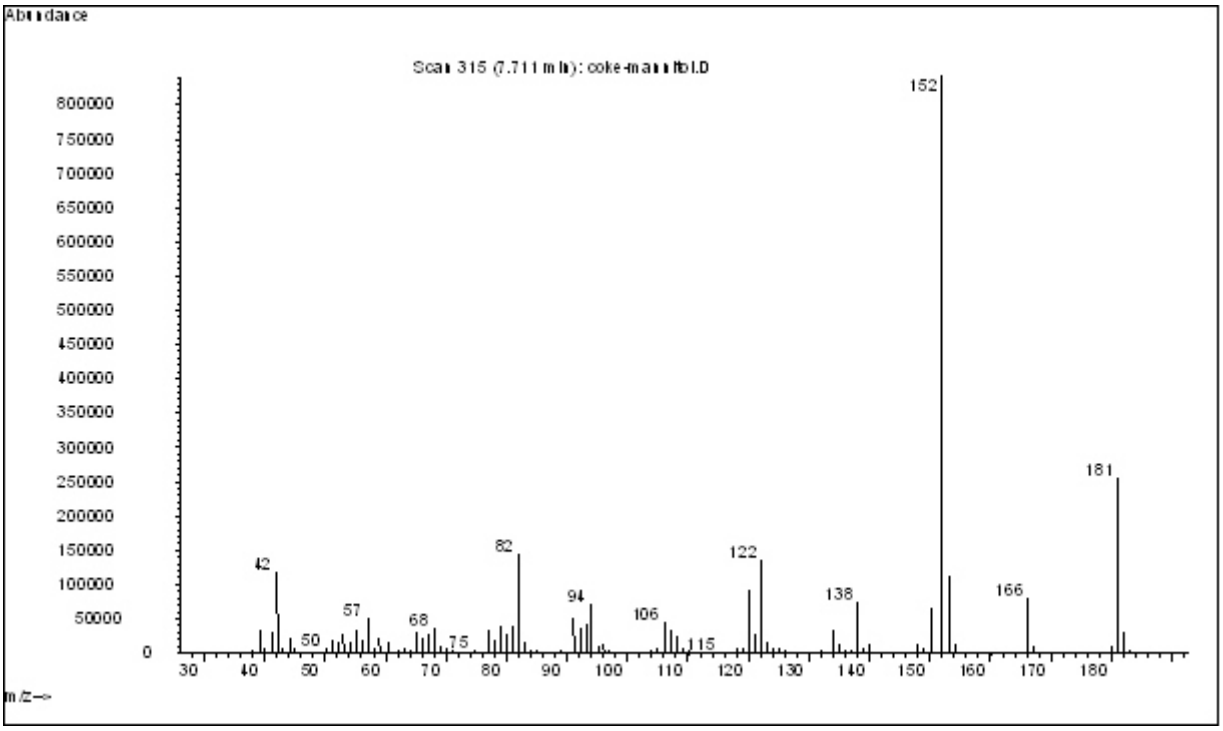

Figure 2. Electron Ionization Mass Spectrum of the Recovered Methylecgonidine.

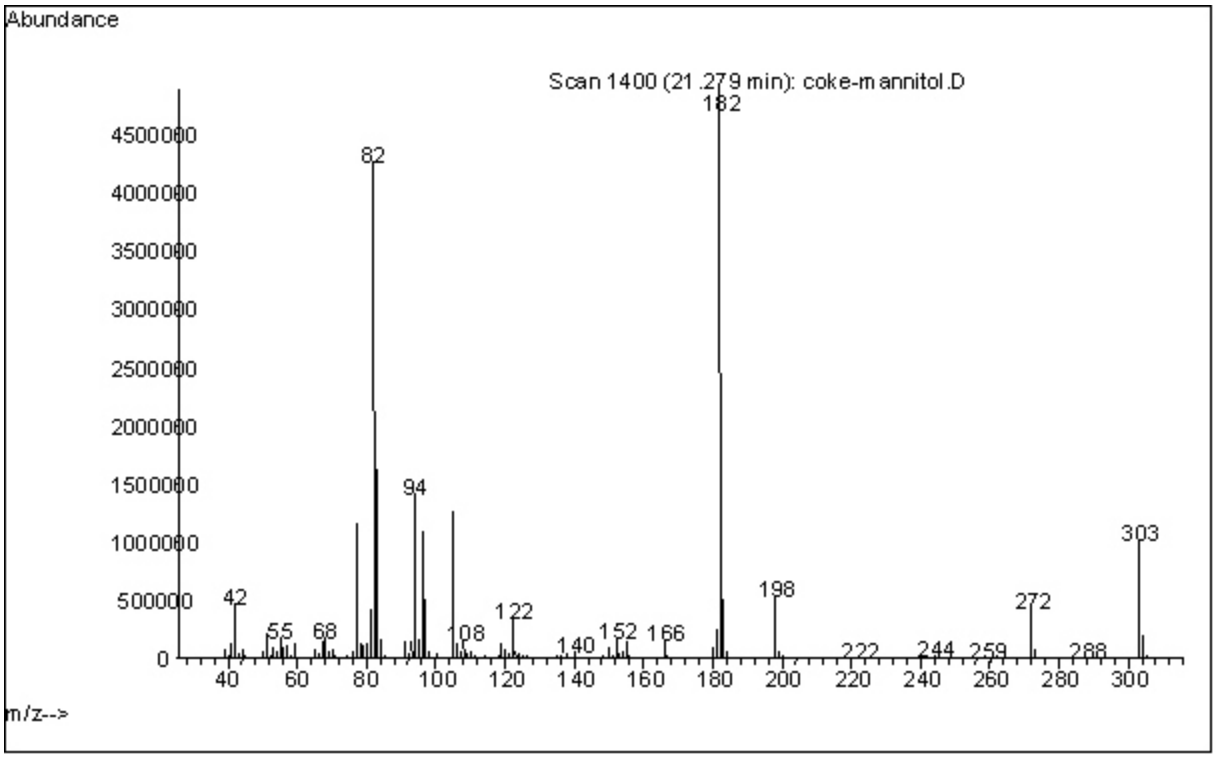

Figure 3. Electron Ionization Mass Spectrum of the Recovered Cocaine.

Technical Note

Levamisole: An Analytical Profile

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ABSTRACT: Levamisole, an antineoplactic cancer medication used in the treatment of colon cancer, has been identified in numerous submissions of illicit cocaine hydrochloride. Analytical methodologies and data (gas chromatography, capillary electrophoresis, infrared spectroscopy, mass spectroscopy, and proton nuclear magnetic resonance spectroscopy) are presented.

KEYWORDS: Levamisole, Cocaine, (l)-Tetramisole, Ergamisol, Ketrax, Solaskil, Forensic Chemistry

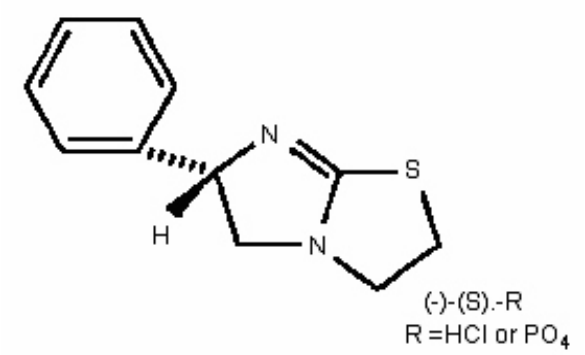

Figure 1: Structure of Levamisole

Introduction

Over approximately the past two years, this laboratory has received numerous cocaine submissions containing various amounts of levamisole, (S)-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole [1,2]. Levamisole is the *levo* enantiomer of tetramisole, and is a synthetic imidazothiazole derivative that has been widely used in the treatment of worm infestations in both humans and animals. In 1990 the U.S. Food and Drug Administration approved the use of levamisole in combination drug therapy with another cancer drug, fluorouracil, for patients to treat some advanced cases of colon cancer [3]. Analytical data for levamisole is provided.

Experimental

Levamisole: $C_{11}H_{12}N_2S$; mw = 204.3 amu [2]

Source: Sigma-Aldrich, Inc. (St. Louis, MO); Lot #073K3602

Gas Chromatography

Instrument	Agilent 6890N with a flame ionization detector
Column	HP-5, 30 m x 0.25 mm x 0.25 μm film thickness
Injector Temperature	270 ^{O}C
Oven Temperature	215 ^{O}C for 5.5 min, 45 ^{O}C to 250 ^{O}C for 1.4 min
Carrier Gas	Helium ramped flow 2.7 mL/min for 5.5 min to 5 mL/min
Split Ratio	75:1

The retention time for levamisole is 2.99 minutes under the above experimental parameters. The retention time relative to cocaine is 0.58.

Capillary Electrophoresis

Instrument	Agilent HP^{3D} CE Capillary Electrophoresis System with a diode array detector
Column	Bare fused silica capillary, 50 μm ID, 40 cm LEF
Run Buffer	Microsolve DEA Custom Chiral, Phenethylamine and Propoxyphene Buffer containing 78.8 mg/mL 2-hydroxypropy-ß-cyclodextrin
Detector	200 nm, reference 480 nm
Voltage	20 kV
Cassette Temperature	15 ^{O}C
Precondition	Flush 1.0 min 0.1 NaOH
Flush Sequence	1.0 min Water; 1.0 min Microsolve CElixir A; 2.0 min Microsolve DEA Custom Chiral, Phenethylamine and Propoxyphene Buffer containing 78.8 mg/mL 2-hydroxypropy-ß-cyclodextrin
Injection Parameters	Pressure 35.0 mbar, 2.0 sec sample vial Pressure 35.0 mbar 1.0 sec water
Injection Solvent	3.75 mM Sodium Phosphate solution, pH 3.2

Note: The above instrumental parameters enables resolution of *dextro*- and *levo*- enantiomers of tetramisole [4].

Infrared Spectroscopy

Instrument	Thermo-Nicolet Nexus 670
Number of Scans	16
Resolution	4.000 cm^{-1}
Wavenumber Range	4000 cm^{-1} to 650 cm^{-1}

Data was obtained by the use of an attenuated total reflectance (ATR) attachment on FTIR [Figure 2]. The data was not corrected.

Mass Spectrometry

Instrument	Agilent 5973
Column	HP-5 MS, 30 m x 0.25 mm x 0.25 μm film thickness
Injector Temperature	255 ^{O}C
Oven Temperature	90 ^{O}C for 1.35 min, 35 ^{O}C/min to 290 ^{O}C
Carrier Gas	Helium with a 35:1 split ratio
Scan Range	40 - 550 amu

The electron impact mass spectrum is presented in Figure 3.

Nuclear Magnetic Resonance Spectroscopy

Data was obtained using 1D proton Nuclear Magnetic Resonance on a Varian Mercury 400 MHz NMR. The sample was prepared at 25.2 mg/mL in deuterated methanol (CD_3OD) containing TMS (tetramethylsilane) as the reference at 0 ppm. The proton spectrum of the standard was obtained with 8 scans using a 1.0 second delay, 45

degree pulse, and a 2.99 second acquisition time. Data from sweep width of 6410 Hz was stored in 32K data points [Figure 4].

Results and Discussion

The presence of pharmacologically active adulterants and inactive diluents found in illicit cocaine seizures is common. Many of these adulterants cause pulmonary and systemic reactions, and therefore may contribute to the toxicity of the cocaine. However, after a brief internet inquiry concerning adulterating illicit cocaine with levamisole, it is unclear as to why this relatively expensive compound is being used.

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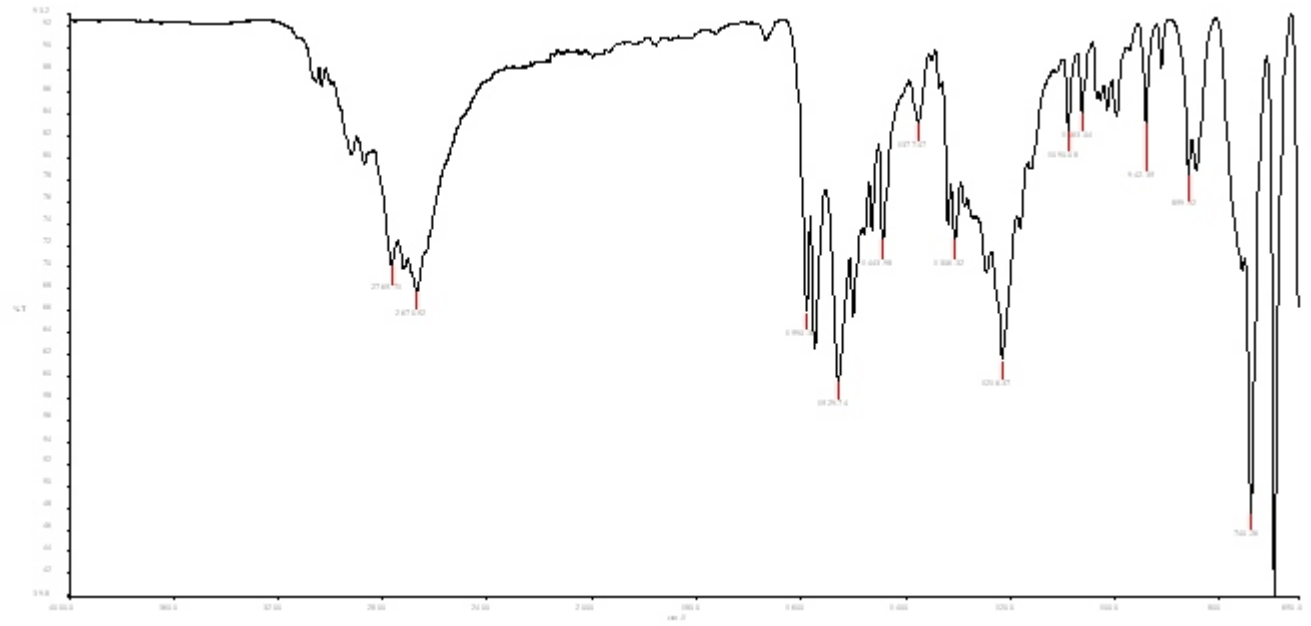

Figure 2: Uncorrected FTIR-ATR Spectrum of Levamisole Hydrochloride.

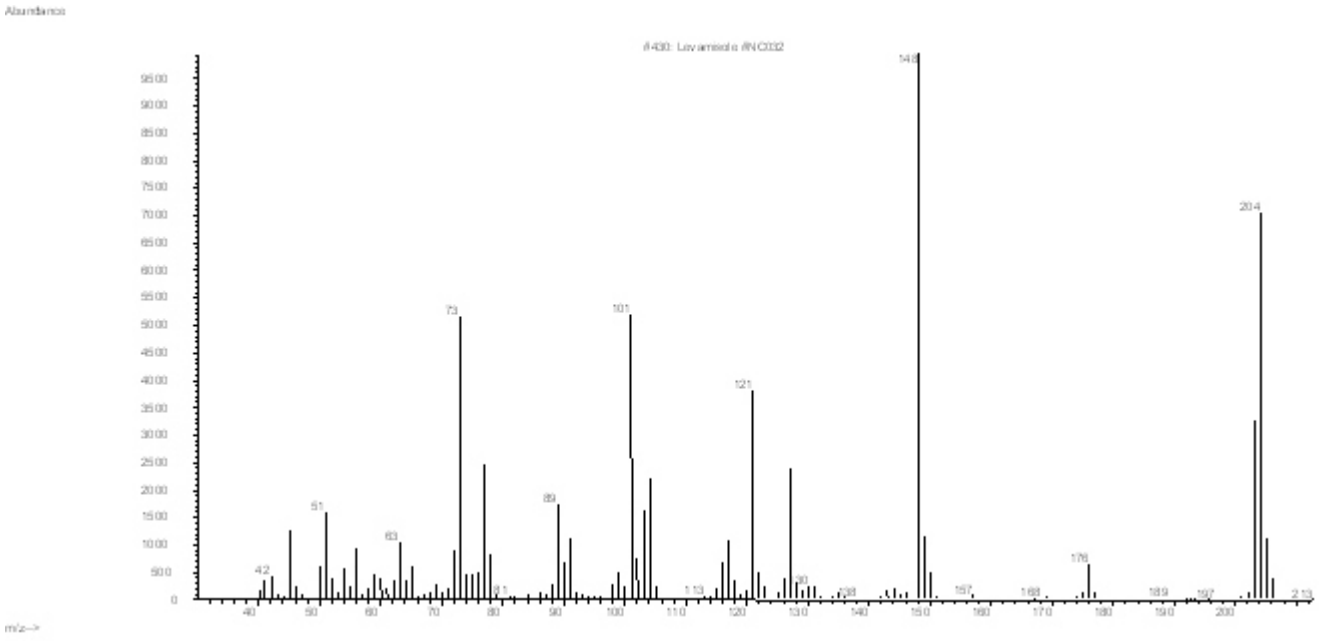

Figure 3: Electron Impact Mass Spectrum of Levamisole.

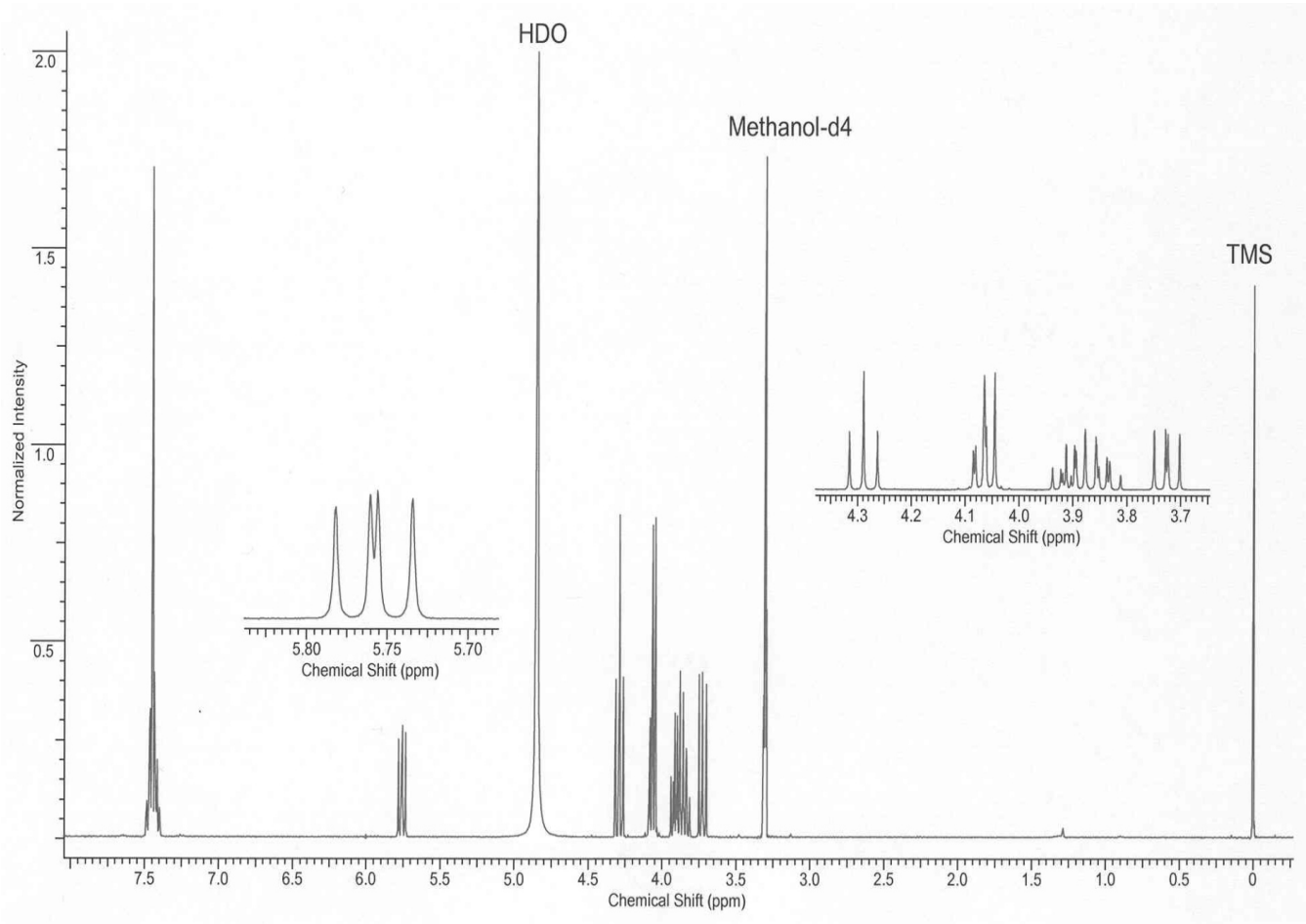

Figure 4: 400 MHz Proton NMR Spectrum of Levamisole Hydrochloride in CD_3OD.

Technical Note

Rapid Chiral Separation of Dextro- and Levo- Methorphan using Capillary Electrophoresis with Dynamically Coated Capillaries

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ABSTRACT: The chiral differentiation of the Dextro- and Levo- Methorphan is obtained in under 4 minutes with excellent peak shapes using capillary electrophoresis with dynamically coated capillaries. Dynamic coating of the capillary surface is accomplished by rapid flushes of 0.1 N sodium hydroxide, water, a buffer containing a polycation coating reagent, and a reagent containing methanol and a polyanionic coating reagent containing hydroxypropyl-*beta*-cyclodextrin.

KEYWORDS: Dextromethorphan, Levomethorphan, Chiral Analysis, Capillary Electrophoresis, Dynamically Coated Capillaries, Forensic Chemistry

Introduction

Dextromethorphan is an antitussive agent commonly found in Over-the-Counter (OTC) cough and cold pharmaceuticals (and more recently in Ecstasy (MDMA) mimic or combination tablets). Levomethorphan is a narcotic analgesic that is not commercially available, and therefore is not commonly submitted to forensic laboratories. Nonetheless, the differentiation and identification of these enantiomers is important in the United States, since Dextromethorphan is not controlled while Levomethorphan is a Schedule II controlled substance.

However, the differentiation of Dextro- and Levo- Methorphan is challenging. Methorphan is a tertiary amine which is not amenable to derivatization; therefore, the use of chiral derivatizing reagents (to form diastereomers for analysis on an achiral gas chromatography (GC) or high performance liquid chromatography (HPLC) column) is not a viable approach. Instead, relatively expensive chiral columns are required to resolve the two isomers using either GC or HPLC [1].

Capillary electrophoresis (CE) allows for the separation of enantiomers on conventional capillaries by utilizing run buffers containing chiral additives. Micellar electrokinetic chromatography (MEKC) [2], and electrokinetic chromatography (ECC) [3], both with single wavelength UV detection, and free zone capillary electrophoresis (CZE) with secondary equilibria and PDA-UV detection [4], have all been previously used to resolve the enantiomers of methorphan. However, run times in excess of 15 minutes were required.

For the separation of basic drugs at low pH, faster, more precise migration times and higher plate counts are obtained using dynamically coated versus uncoated capillaries. The use of a chiral additive (such as a cyclodextrin) imparts the additional selectivity needed for the analysis of enantiomers [5]. Using the procedure developed by Chevigne and Janssens [6], the capillary, after base hydrolysis, is sequentially coated with a polycation and then with a polyanion. The run buffer (with or without an added cyclodextrin) is the final coating

reagent. This process produces coated capillaries with a higher and more robust electroosmotic flow (EOF) at lower pH values versus uncoated capillaries. In addition, the coated capillary surface has more favorable kinetics. In the present study, the rapid chiral analysis of methorphan using a dynamically coated capillary approach is reported.

Experimental

Chemicals

Standards of Dextro- and Levo- Methorphan were obtained from the reference collection of this laboratory. Sodium hydroxide 0.1 N, CElixir A (pH 2.5), CElixir B (pH 2.5), and CElixir B (pH 2.5) with 0.95 % (w/v) hydroxypropyl-ß-cyclodextrin, were all acquired from MicroSolv Technology (Long Branch, NJ). Hydroxypropyl-ß-cyclodextrin (HP-ß-CD) was obtained from Sigma (St. Louis, MO). HPLC-grade methanol was obtained from Burdick and Jackson (Muskegon, MI). Deionized and high purity water (that is, HPLC-grade water) was obtained from a Millipore Synergy 185 water system (Bedford, MA).

Instrumentation and Procedures

An Agilent Model HP^{3D} CE Capillary Electrophoresis System fitted with a diode array detector (Waldbronn, Germany) was used for CE separations. New, bare silica capillaries were conditioned following the same procedure used for regular analyses. That is, the capillaries were first flushed with 0.1N sodium hydroxide for 1 minute, followed by water for 1 minute, then CElixir Reagent A for 1 minute, and finally the run buffer for 2 minutes. Either 2.0 mL CE glass vials or 1.0 mL polypropylene vials are used as reservoirs. For glass vials, waste vials were filled with 500 µL of water. Flush, run buffer, standard and sample vials were filled with 1000 µL of liquid (for the sodium hydroxide 0.1 N vial add 500 µL to a polypropylene vial). When polypropylene vials were used, waste vials were filled with 250 µL of water, while all others were filled with 500 µL of liquid.

Standard and Sample Preparation

The injection solvent consisted of 75 mM phosphate monobasic, adjusted to pH 2.6 with phosphoric acid, and diluted 1:20 with HPLC-grade water. Alternatively, injection solvent concentrate (MicroSolv) was diluted 1:20 with HPLC-grade water.

For standard solutions, an appropriate amount of standard Dextro- and Levo- Methorphan was weighed into an appropriate volumetric flask and diluted to volume with injection solvent, in order to obtain a final concentration of approximately 0.05 mg/mL of each component. These were sonicated for 15 minutes, then filtered. For sample solutions, an appropriate amount of powder was weighed into a volumetric flask and diluted to volume with injection solvent, in order to obtain a final concentration approximately equal to that of standard. These were also sonicated for 15 minutes, then filtered. All standard and sample solutions were filtered with 0.45 µm Nylon syringe filters (MicroSolv).

Capillary Electrophoresis Conditions

For the chiral separation either a 50 µm ID 32 cm (23.5 cm to the detector) fused silica capillary obtained from Polymicro Technologies (Phoenix, AZ) or a 50 µm ID 33 cm (24.5 cm to the detector) pre-made capillary (Agilent) were used, at 15 ^{O}C. The run buffer consisted of 15 % methanol and 85 % (CElixir Reagent B (pH 2.5) + 0.95 % HP-ß-CD). For all CE runs a 50 mbar pressure injection of 2 second duration was used, followed by a 35 mbar pressure injection of water for 1 second. For electrophoresis, an initial 0.5 minute linear voltage ramp from 0 V to the final voltage of 20 kV was used.

Results and Discussion

Dahlen and Lenz used CZE with an uncoated capillary and with added HP-β-CD to resolve Dextro- and Levo-Methorphan in under 16 minutes [4]. In the present study, using the same run buffer but on a dynamically coated

capillary, identical results were obtained in under 4 minutes (see Figure 1). Highly precise separations were obtained, as demonstrated by excellent run-to-run migration time precision (% RSD = 0.1, n = 7). Because the peaks are so narrow, identification can be difficult based on migration time alone; however, co-injection of sample and either standard eliminates any ambiguities. Relative migration time data (relative to Dextromethorphan) of solutes commonly found with Dextromethorphan is given in Table 1. The non-controlled substances are included because they and Dextromethorphan are commonly combined in various OTC pharmaceuticals. The controlled substances are included since they and Dextromethorphan are occasionally identified in Ecstasy (MDMA) mimic and combination tablets.

If needed, *n*-butylamphetamine can be used as an internal standard in this method for the quantitation of Methorphan.

The present procedure is compatible with previously reported methodology for the CE analysis of a wide variety of seized drugs using the same capillary with dynamic coatings [5]. Classes of compounds that can be analyzed using this methodology (using higher CD concentrations than used in this study) include the phenethylamines and the methylenedioxyphenethylamines. Specific compounds that can be analyzed using this methodology include propoxyphene, cocaine, oxycodone, heroin, lysergic acid diethylamide (LSD), opium, psilocybe mushrooms, *gamma*-hydroxybutyrate (GHB), and *gamma*-butyrolactone (GBL).

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6. Chevigne R, Janssens J. US Patent #5,611,903, 3/18/97.

[Table 1 and Figure 1 Follow.]

Table 1. Relative Migration Times of Solutes Commonly Found with Dextromethorphan.

Solute	Relative Migration Time *
d,l-Methamphetamine HCl	0.798
Phenylpropanolamine HCl	0.836
d,l-Pseudoephedrine HCl	0.841
MDMA HCl	0.850
d,l-Ephedrine HCl	0.851
Phenylephrine	0.874
n-Butylamphetamine HCl (internal standard)	0.919
Diphenhydramine HCl	0.973
Dextromethorphan	**1.000**
Levomethorphan	1.010
Acetaminophen	2.371
Guaifenesin	2.388

* Relative to Dextromethorphan. Note that the concentration of the cyclodextrin used in this study was insufficient to resolve the enantiomeric pairs of the listed phenethylamines and methylenedioxyphenethylamines; therefore, only one (the average) RMT is reported.

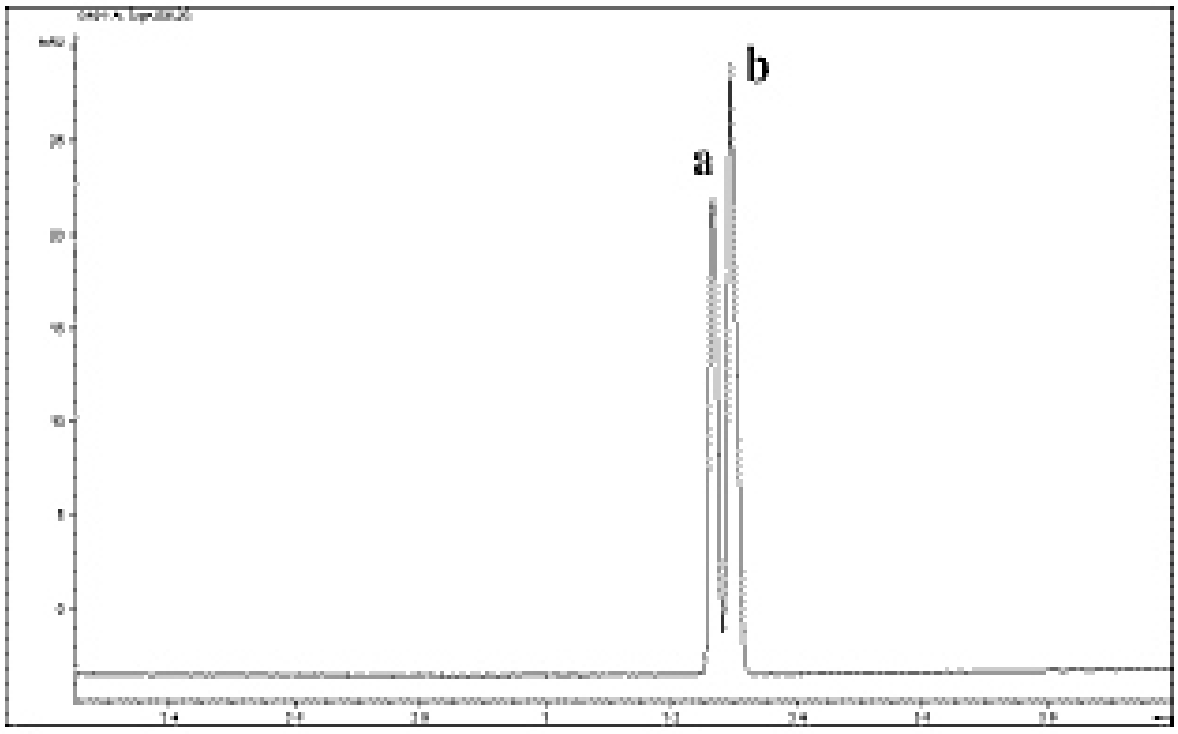

Figure 1. Electropherogram of a standard mixture of a) Dextromethorphan and b) Levomethorphan. A 33 cm (24.5 cm to the detector window) x 50 µm ID fused-silica dynamically coated capillary was used. Solute concentration of each enantiomer was approximately 0.05 mg/mL (CE conditions are described in the Experimental section).

Reduction of Phenylephrine with Hydriodic Acid/Red Phosphorus or Iodine/Red Phosphorus: 3-Hydroxy-N-methylphenethylamine

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ABSTRACT: In an effort to decrease illicit methamphetamine production within the United States, many pharmaceutical companies are now substituting phenylephrine for pseudoephedrine in many of their Over-the-Counter consumer products intended for treatment of the symptoms of the common cold, allergies, and related maladies. Because these products are the favored source of pseudoephedrine for illicit production of methamphetamine, and also because many clandestine laboratory operators are chemically naïve, it is expected that phenylephrine-containing products will occasionally be utilized in erroneous efforts to produce methamphetamine. Submission of phenylephrine to reduction conditions typically utilized in clandestine methamphetamine laboratories (hydriodic acid/red phosphorus or iodine/red phosphorus) produced 3-hydroxy-N-methylphenethylamine, commonly referred to as "Reduced Phenylephrine" or "Reduced PE." Standard analytical data for "Reduced PE" are presented.

KEYWORDS: 3-Hydroxy-N-methylphenethylamine, Phenylephrine, Reduction, Methamphetamine, Pseudoephedrine, Reduced Phenylephrine, Reduced PE, Hydriodic Acid, Red Phosphorus, Iodine, Clandestine Laboratories, Forensic Chemistry

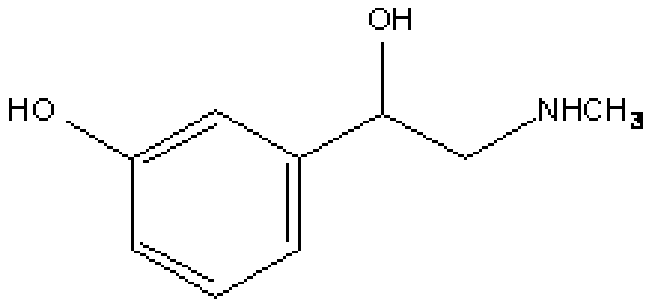
Introduction

Clandestine methamphetamine laboratories are epidemic in many areas of the United States. One of the primary synthetic methods that has been used for production of methamphetamine over the past 25 years is the reduction of ephedrine or pseudoephedrine with hydriodic acid/red phosphorus (HI/red P) [1]. In past efforts to combat illicit methamphetamine production, federal and state authorities restricted the sale of bulk ephedrine and pseudoephedrine, and closely monitored the sale and use of hydriodic acid and red phosphorus [2]. In response, clandestine laboratory operators began utilizing Over-the-Counter (OTC) consumer products containing ephedrine or pseudoephedrine, began generating hydriodic acid *in situ* with iodine and red phosphorus (I_2/red P) [3], and also turned to alternate syntheses, notably the lithium/ammonia reduction [4]. A number of states countered these initiatives with a variety of additional restrictions on the sale of ephedrine- and pseudoephedrine-containing OTC products, including purchase limits, access restrictions, and identification/signature requirements, and also further restricted sales of I_2 and red P. In turn, clandestine laboratory operators in those states resorted to so-called "road trips" to purchase ephedrine- and pseudoephedrine-containing OTC products, either traveling to states that had no restrictions, or purchasing the maximum allowable amounts at dozens or even hundreds of stores within states that had restrictions in place. In addition, as acquisition of I_2 and red P became increasingly problematic, clandestine laboratory operators turned to a variety of I_2- and red P-containing consumer products, and also began using other phosphorus compounds as substitutes for red P.

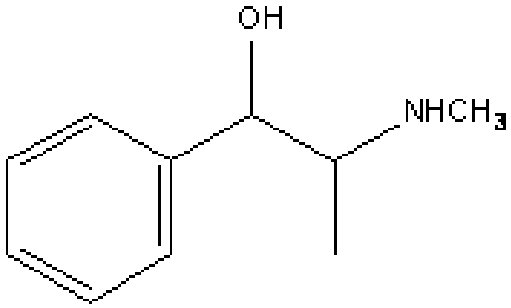
Most recently, the Combat Methamphetamine Epidemic Act (enacted in March 2006) placed federal restrictions on the sale of ephedrine-, pseudoephedrine-, and phenylpropanolamine-containing OTC products (again,

purchase limits, access restrictions, and identification/signature requirements) [5]. In total, federal and state imposed restrictions have dramatically reduced the number of methamphetamine laboratories in some states.

Initially, the pharmaceutical companies that produced ephedrine and pseudoephedrine-containing OTC products were strongly opposed to the imposition of restrictions on their sale. However, as they began to recognize the extent and increase of methamphetamine abuse, and the salient role that ephedrine- and pseudoephedrine-containing OTC products played in illicit methamphetamine manufacture, they began to create alternate formulations that contained phenylephrine (3-(1-hydroxy-2-methylaminoethyl)phenol, sometimes abbreviated as "PE"), as a substitute for ephedrine/pseudoephedrine. The structures of phenylephrine and ephedrine/ pseudoephedrine are shown below.



Phenylephrine (mw = 167.1)



Ephedrine/Pseudoephedrine

Phenylephrine-containing products have been available for OTC sale in Europe for many years. The first of these reformulated products became available to consumers in the United States in January 2005, and has gained rapid acceptance among American consumers.

However, because many clandestine laboratory operators are chemically naïve, and do not understand that phenylephrine and ephedrine/pseudoephedrine are different compounds, it is expected that these new phenylephrine-containing OTC products will eventually be utilized in erroneous efforts to produce methamphetamine. The purpose of this study is to identify the products, byproducts, and intermediates formed during the reduction of phenylephrine with HI/red P or I_2/red P.

Experimental

Reactions

Reagents were obtained from Aldrich Chemical Company. Ten grams of phenylephrine HCl, 3 grams of red phosphorus, and 30 mL of 57 % hydriodic acid were refluxed at about 120 ^{O}C in a round-bottom flask fitted with a reflux condenser. Alternately, 10 grams of phenylephrine HCl, 20 grams of iodine, 3 grams of red phosphorus, and 18 mL of water were similarly refluxed. The reactions were monitored by removal of aliquots at timed intervals, with subsequent analysis via gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The aliquots were taken before heating, once the mixture began to reflux, and every 5 minutes thereafter until the reaction was complete. The progress of the reactions was monitored as a decrease of phenylephrine and the formation of the end product. Various intermediates and byproducts were also monitored.

Gas Chromatography

Analyses were performed using an Agilent Technologies 6890N Gas Chromatograph equipped with electronic pneumatic control and a flame ionization detector. A 10.0 m x 0.32 mm i.d. fused-silica capillary column coated with 0.52 μm DB5 (Agilent Technologies) was employed. Hydrogen was used as the carrier gas, with an

average linear velocity of 40 cm/sec (constant flow). The injection port and detector were both maintained at 280 ^{O}C. For each analysis, 1 µL of the sample was injected in split mode (25:1). The oven temperature was programmed as follows: Initial temperature 130 ^{O}C, hold for 1.0 minute, then increase 25 ^{O}C per minute to 280 ^{O}C, and hold for 1.0 minute (total run time = 10.0 minutes). Relative retention times (relative to methamphetamine) are shown in Table 1.

Gas Chromatography/Mass Spectrometry

Electron impact mass spectra (70 eV) were obtained using a 5973 Agilent Technologies Mass Selective Detector equipped with a 6890N Gas Chromatograph. A 30.0 m x 0.25 mm i.d. fused-silica capillary column coated with 0.25 µm HP5-MS (Agilent Technologies) was used. Helium was used as the carrier gas with an average linear velocity of 40 cm/sec (constant flow). The injection port and ion sources were set at 240 ^{O}C and 180 ^{O}C, respectively. For each analysis, 1 µL of the sample was injected in split mode (30:1). The oven temperature was programmed as follows: Initial temperature 130 ^{O}C, hold for 1.0 minute, then increase 30 ^{O}C per minute to 280 ^{O}C, and hold for 5.0 minutes (total run time = 11.6 minutes). Mass spectra were scanned over an *m/z* range of 40 - 500.

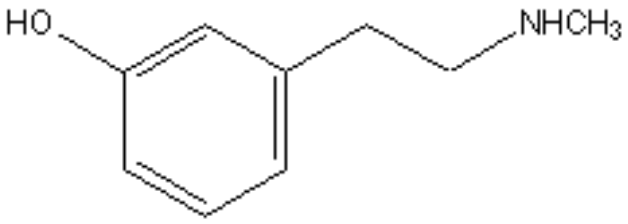
Infrared Spectrophotometry

Infrared spectra were obtained using a Nicolet Avatar 370 FTIR Spectrophotometer operated in the attenuated total reflectance (ATR) mode. Sixteen scans were collected at a resolution of 4.0 cm^{-1}.

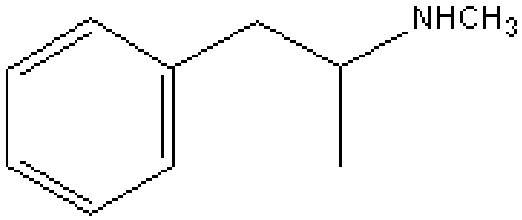
Results and Discussion

One popular preparation of the reformulated OTC "PE" allergy/cold medications contains 10 milligrams of phenylephrine hydrochloride per tablet, which is easily extracted with methanol. The base was precipitated from a saturated solution of the hydrochloride by basifying to pH 11 with ammonium hydroxide (pH control is important, see below). The infrared spectra of phenylephrine hydrochloride and base are shown in Figures 1 and 2, respectively. The full-scale and expanded mass spectra of phenylephrine are shown in Figures 3 and 4, respectively.

Based on the analogous reduction of ephedrine/pseudoephedrine [1], the HI/red P reduction of phenylephrine was expected to result in loss of the benzylic hydroxyl, thereby producing 3-hydroxy-N-methylphenethylamine, also known as "Reduced Phenylephrine" or less commonly, "Reduced PE". The structures of 3-hydroxy-N-methyl-phenethylamine and methamphetamine are shown below.



3-Hydroxy-N-methylphenethylamine
"Reduced Phenylephrine" (mw = 151.1)

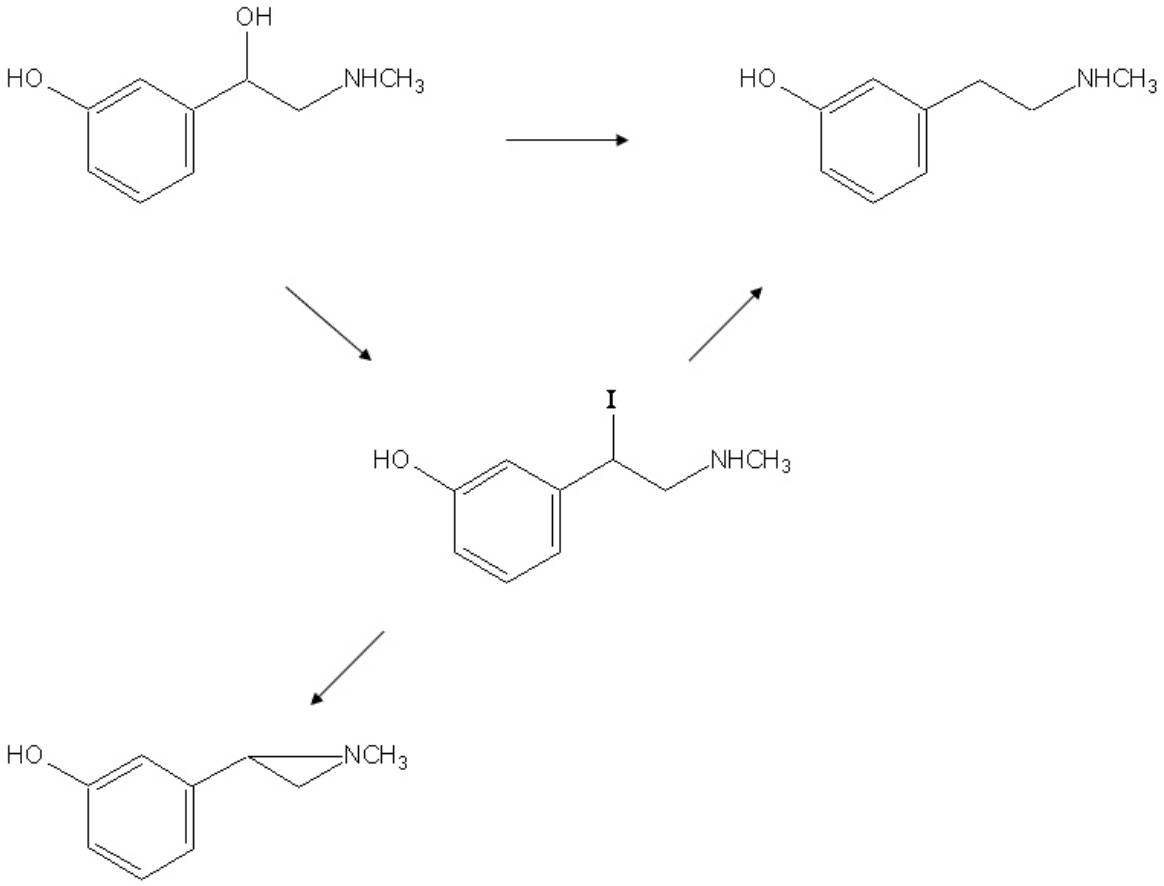


Methamphetamine

The reduction mechanism was similarly expected to parallel that of the HI/red P reduction of ephedrine/ pseudoephedrine; that is, through an iodophenylephrine intermediate that can be inferred by detection of the

corresponding aziridine. The phenolic hydroxyl was not expected to be reduced by HI/red P [6]. Similarly, phenylacetone-like and naphthalene-like compounds (that are detected as byproducts during the HI/red P reduction of ephedrine/pseudoephedrine [7]) were not expected to form during the HI/red P reduction of phenylephrine, due to the presence of the phenolic hydroxyl group.

The analytical results were consistent with these postulates. An iodophenylephrine intermediate was apparently formed, as verified by detection of the corresponding aziridine compound, 1-methyl-3-(*meta*-hydroxyphenyl)-aziridine (mw = 148.1), confirmed by mass spectrometry (see Figure 5). This iodo intermediate was in turn reduced to 3-hydroxy-N-methylphenethylamine (hereafter "Reduced PE"). The full-scale and expanded mass spectra of Reduced PE are shown in Figures 6 and 7, respectively. As expected, no phenylacetone-like or naphthalene-like byproducts were observed.



Presumed Reaction Sequence for the HI/red P Reduction of Phenylephrine

Interestingly, the reduction of phenylephrine was much more facile than the corresponding reduction of ephedrine/pseudoephedrine. In fact, the aziridine was already present in the aliquot that was removed prior to heating (see Figure 8), and Reduced PE was already the major product after just 5 minutes of reflux (see Figure 9). Some trace level intermediate peaks were also noted, but were not identified. Complete conversion to Reduced PE, with essentially no byproduct formation, occurred within 30 minutes of reflux (see Figure 10). In additional experiments, phenylephrine that was added directly to 57 % HI sitting at room temperature, without applied heat or added red P, formed some of the aziridine and Reduced PE within one day. This facile conversion is most likely due to the influence of the phenolic hydroxyl group. Mass spectrometry and NMR data (not shown) also confirm that only the benzylic hydroxyl was reduced, in agreement with previous work on compounds containing both an alkyl amine and phenolic hydroxyl [6].

The extraction process for Reduced PE was more challenging than the corresponding extraction of methamphetamine. Because Reduced PE can form a phenolate salt, the extraction can only be accomplished in a narrow pH range (expected to be between the pKa's of methamphetamine hydrochloride and phenol; that is, corresponding to about pH 11). To determine the optimal extraction pH, a series of test solutions were made, each using a set amount of reaction mixture adjusted to different pH values via dropwise addition of ammonium hydroxide. The resulting solutions were then extracted with a chloroform/isopropanol (2:1) mixture, and the extracts analyzed by GC. As expected, as the pH in the respective test tubes approached pH 11 (as measured by multi-range pH paper), the amount of Reduced PE in the extracts increased, but as the pH increased past pH 11, the amount of Reduced PE in the extracts decreased.

The solvent system used for the above extractions was unusual. Reduced PE does not readily extract from a pH 11 solution into ether, hexane, or camping type fuel (extraction solvents that are typically used at clandestine methamphetamine laboratories). It is thought that a zwitterion is formed between the phenolic hydroxyl and the free amine. Since a zwitterion has salt-like characteristics, its solubility in water and polar solvents is enhanced. The chloroform/isopropanol (2:1) mixture improves the partition coefficient of Reduced PE, allowing its extraction from the aqueous solution. This solvent mixture has previously proven effective in extracting similar zwitterionic substances form aqueous solutions, including morphine, psilocybin, and lysergic acid.

The infrared spectrum of Reduced PE base (as obtained by evaporation of the above chloroform/isoproanol (2:1) extracts) is shown in Figure 11.

Conclusions

As the availability of ephedrine/pseudoephedrine-containing OTC products decrease, it is expected that some chemically naïve clandestine laboratory operators will attempt to produce methamphetamine from substitute phenylephrine-containing OTC products, thereby producing Reduced PE. It is in fact quite likely that such substitutions have already been attempted; however, it is also quite likely that these syntheses failed due to the loss of Reduced PE during the extraction procedures typically utilized in illicit methamphetamine production. Similarly, some clandestine laboratory operators will attempt to produce methamphetamine from mixtures of pseudoephedrine- and phenylephrine-containing OTC products, but these efforts will only result in lower apparent yields of methamphetamine, again due to the loss of Reduced PE during extractions.

Furthermore, the reduction of phenylephrine-containing OTC products using the lithium/ammonia process (Birch reduction) will likely be attempted, and will probably yield Reduced PE - though some other unknown product is also possible (this will be addressed in future research). Regardless of the reduction technique, however, the use of phenylephrine as a substitute for ephedrine/ pseudoephedrine in illicit methamphetamine production will probably be short-lived, since Reduced PE is believed to have no significant CNS stimulant effects [8], and clandestine laboratory operators will quickly become educated about phenylephrine through drug abuse websites, bulletin boards, and chat-rooms on the Internet, consumer complaints, and discussions with fellow laboratory operators. Nonetheless, it is necessary for forensic analysts to be able to identify these products, and understand their sources.

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8. Susan Carr, Drug Enforcement Administration, Office of Diversion Control, Arlington, VA, Personal Communication, 2005.

* Law Enforcement Restricted.

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[Figures 1 - 11 and Table 1 Follow.]

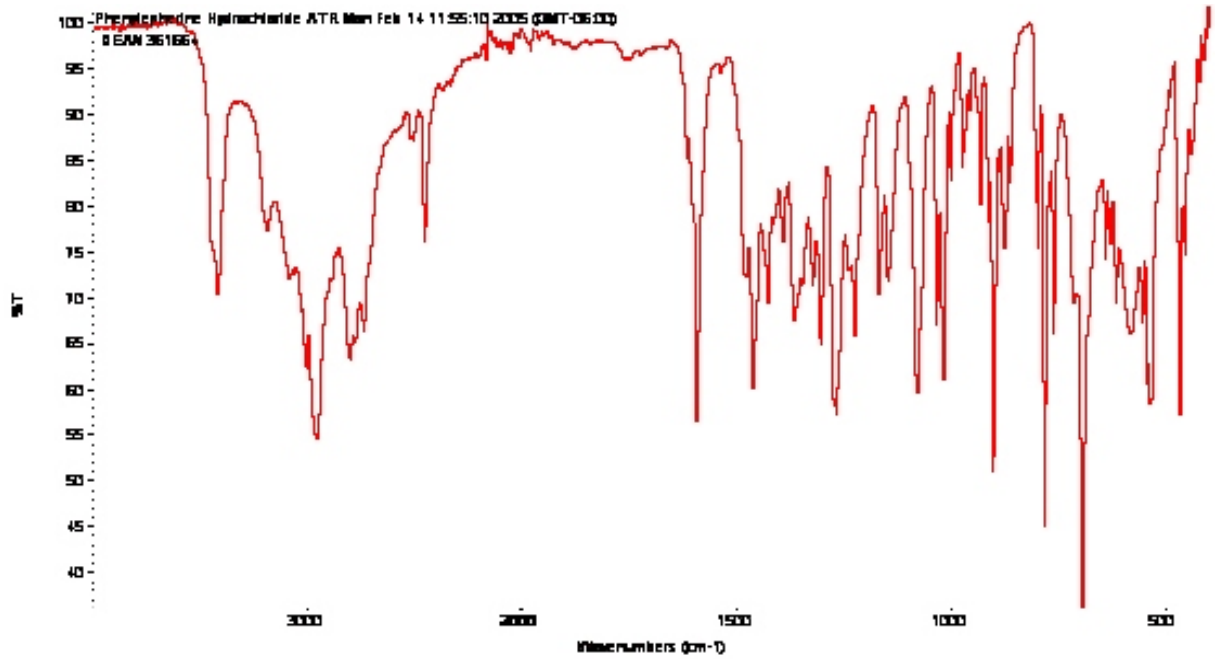

Figure 1. Infrared Spectrum (ATR) of Phenylephrine HCl.

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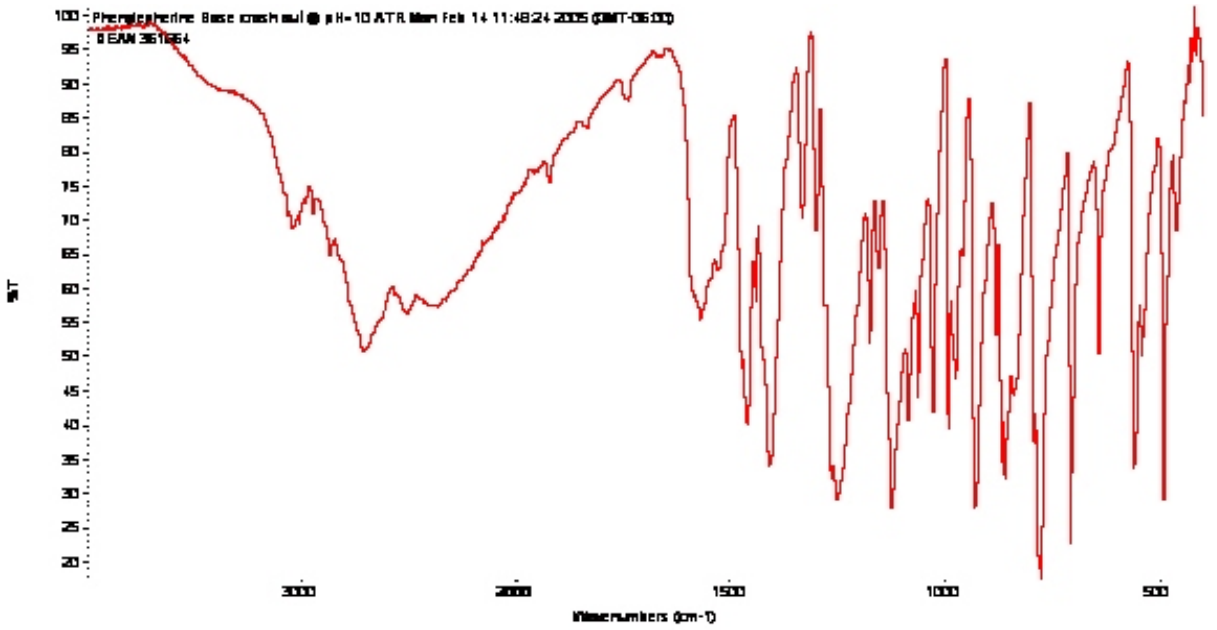

Figure 2. Infrared Spectrum (ATR) of Phenylephrine Base.

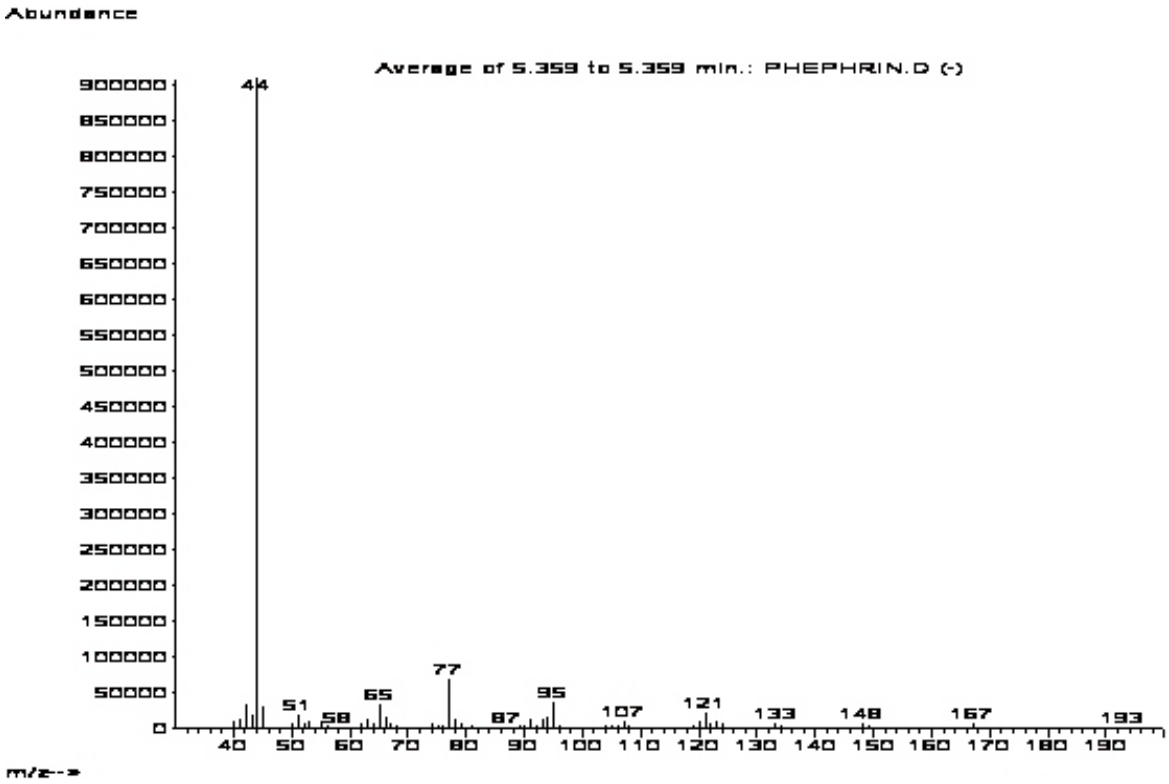

Figure 3. Mass Spectrum of Phenylephrine.

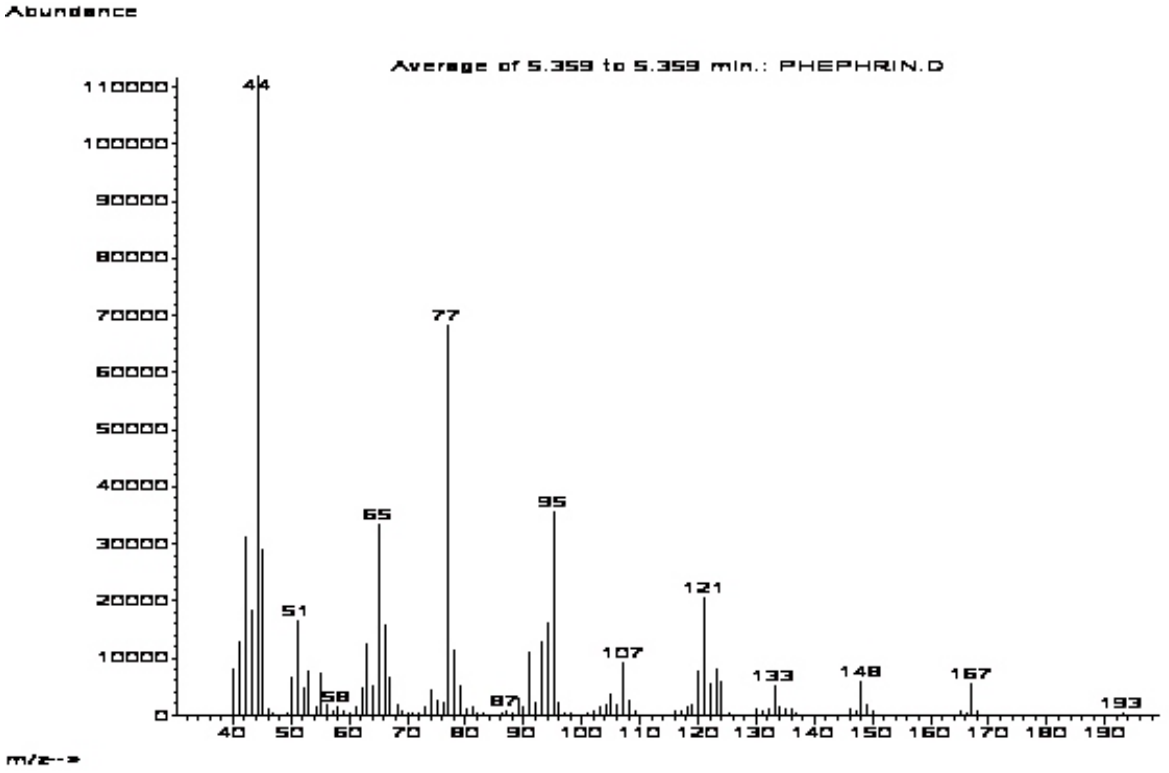

Figure 4. Expanded Mass Spectrum of Phenylephrine.

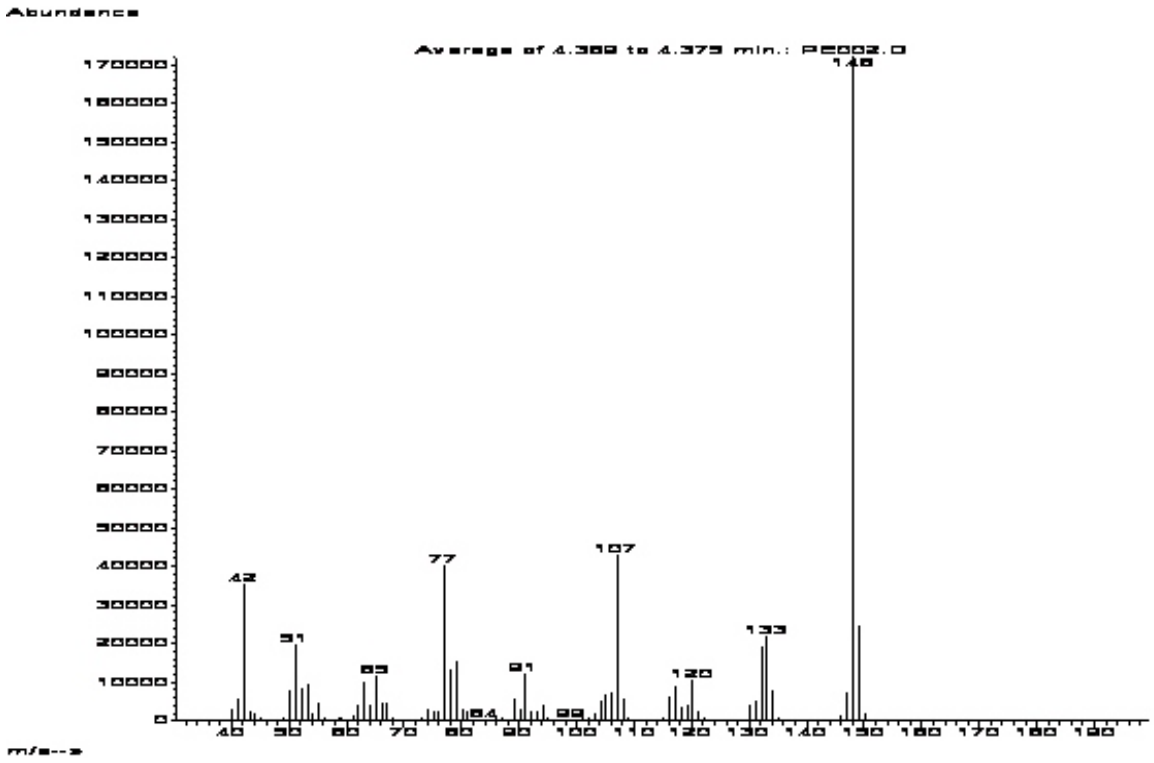

Figure 5. Mass Spectrum of 1-Methyl-3-(*meta*-hydroxyphenyl)aziridine

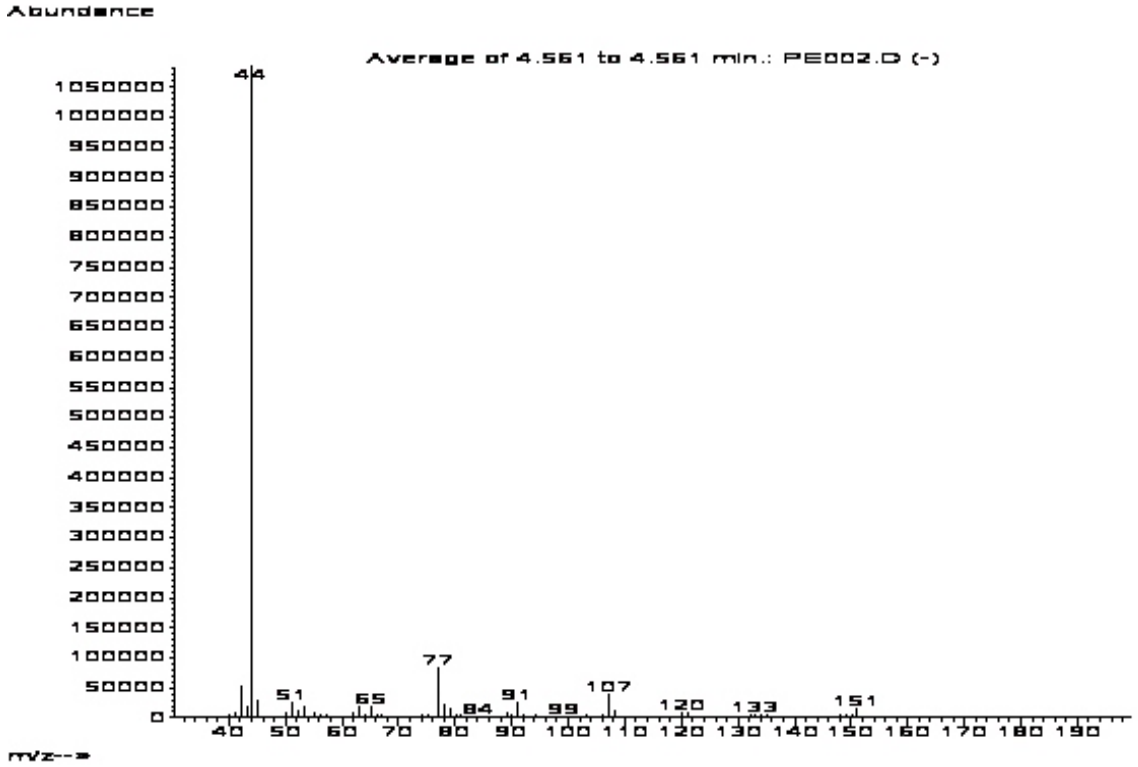

Figure 6. Mass Spectrum of 3-Hydroxy-N-methylphenethylamine (Reduced Phenylephrine).

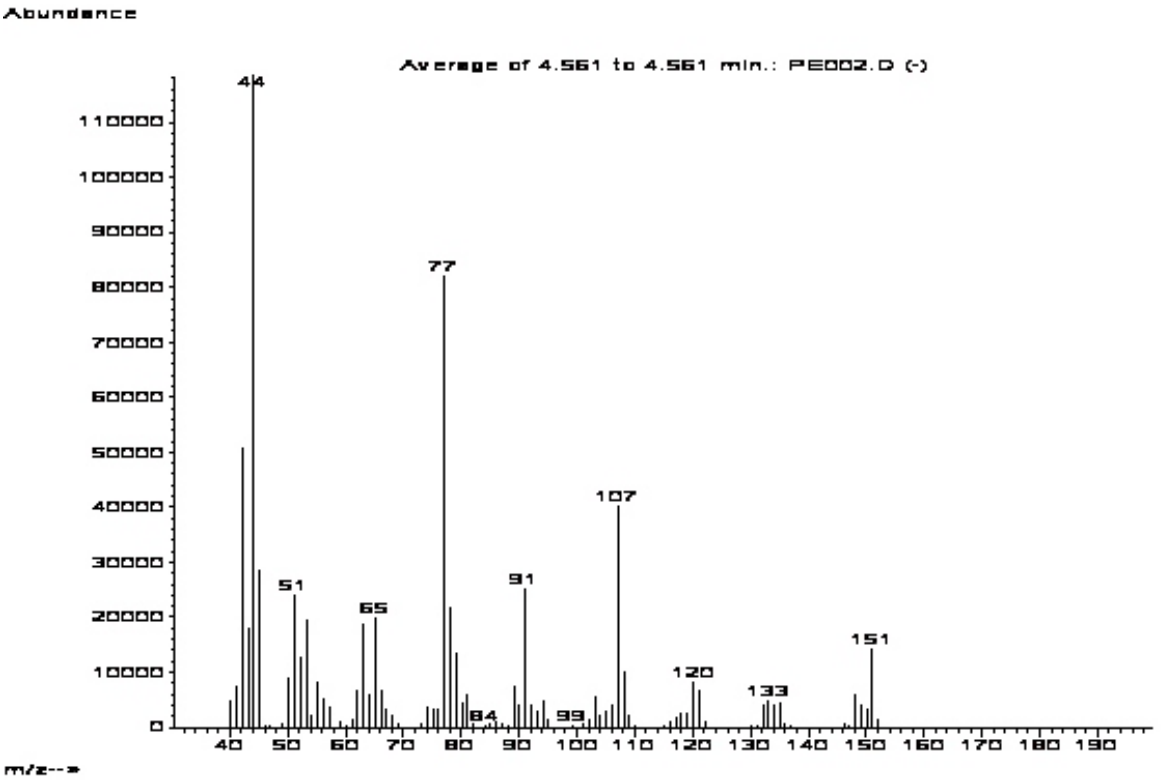

Figure 7. Expanded Mass Spectrum of 3-Hydroxy-N-methylphenethylamine (Reduced Phenylephrine).

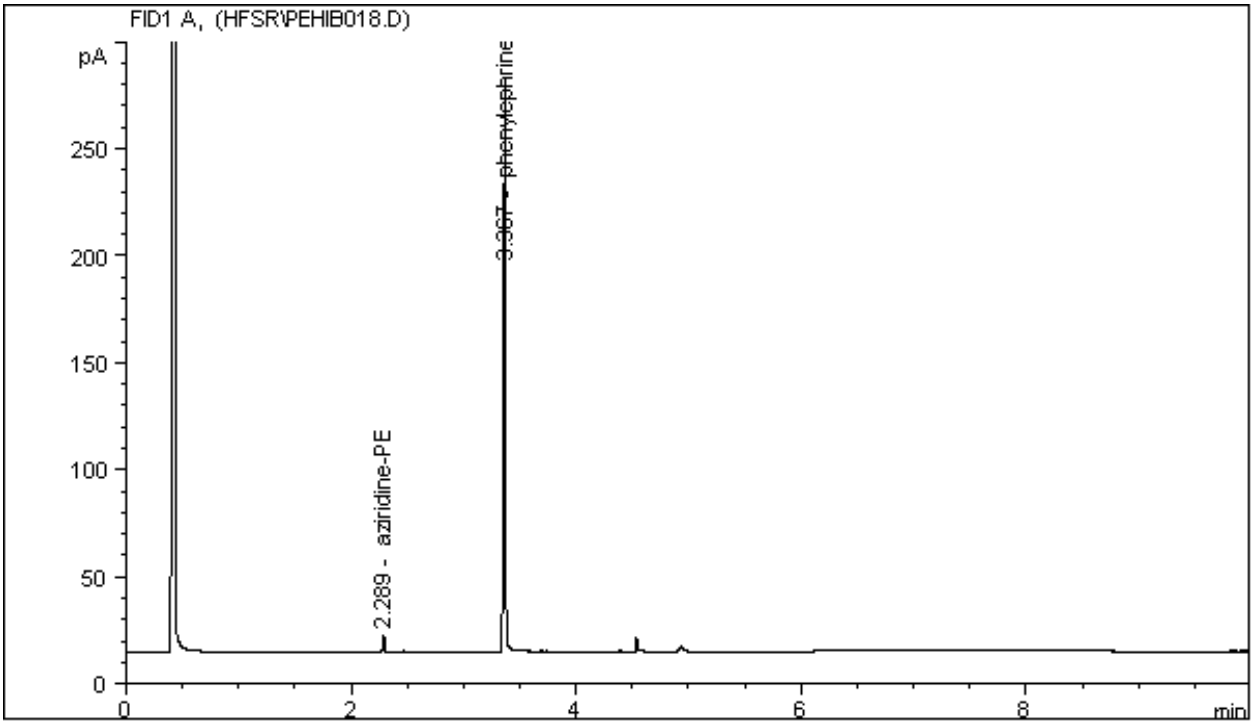

Figure 8. Gas Chromatogram of the Initial Mixture of Phenylephrine and Hydriodic Acid.

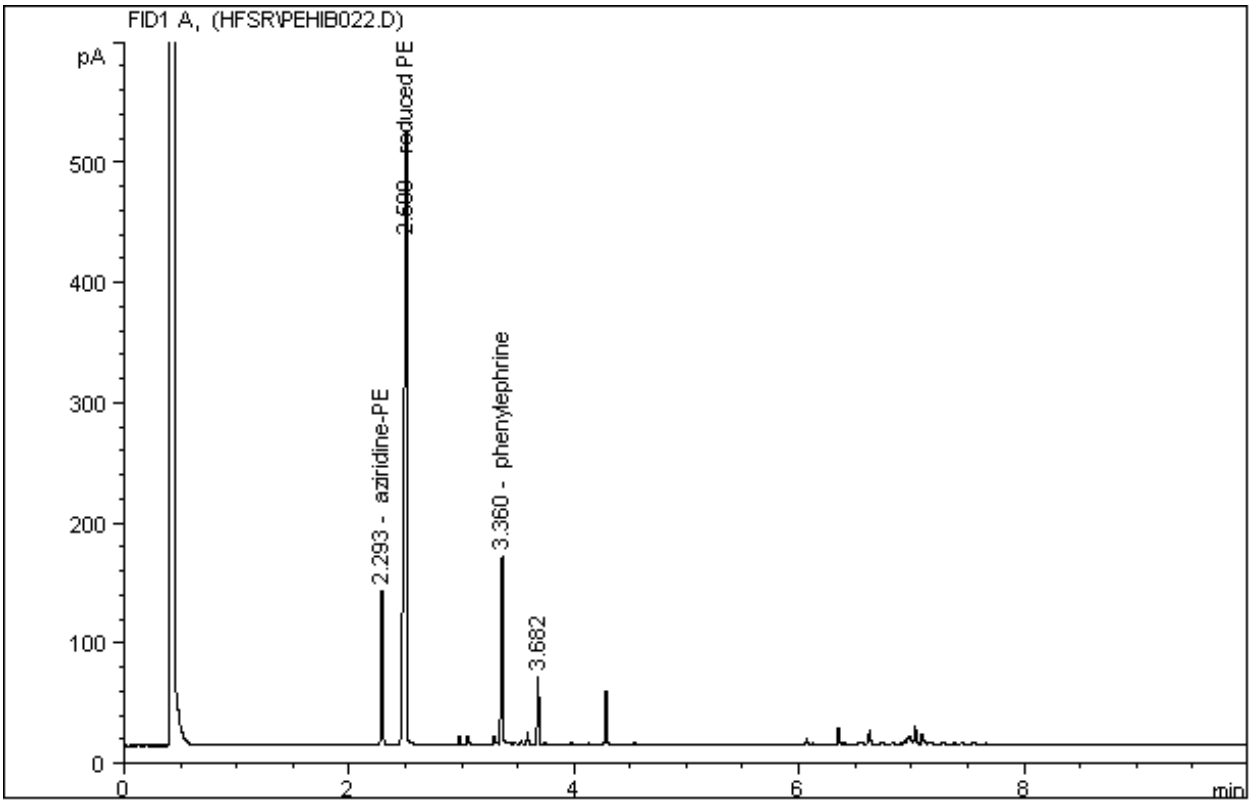

Figure 9. Gas Chromatogram after 5 Minutes of Reflux of Phenylephrine and Hydriodic Acid.

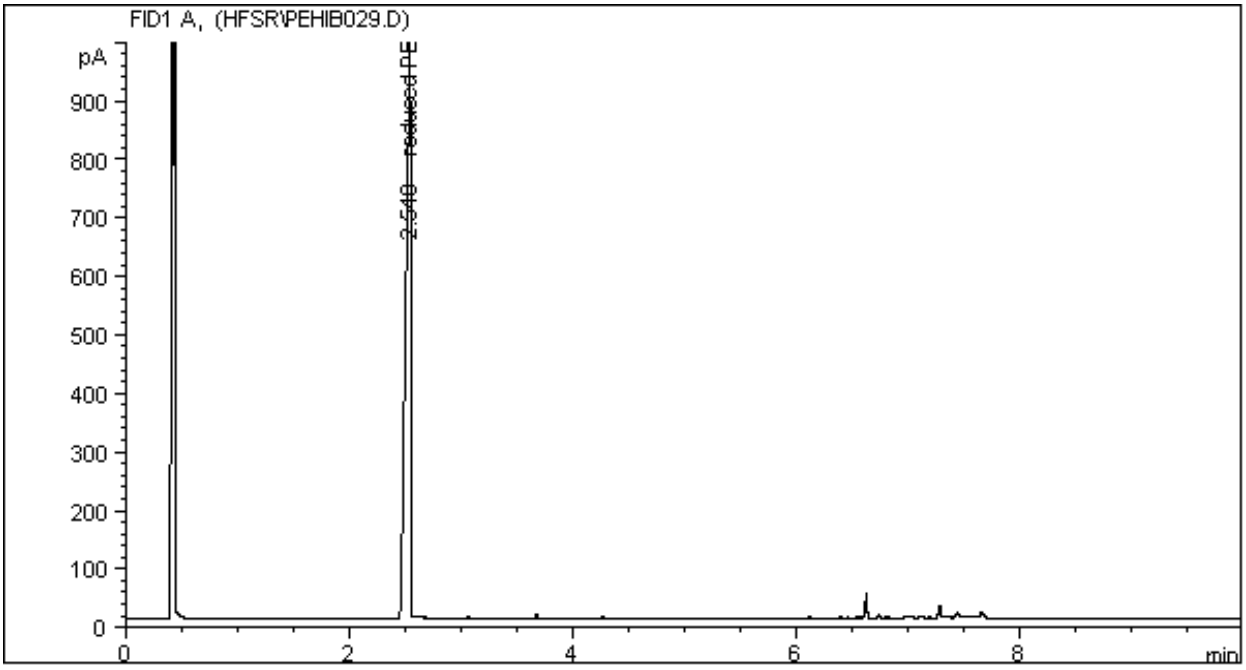

Figure 10. Gas Chromatogram after 30 Minutes of Reflux of Phenylephrine and Hydriodic Acid.

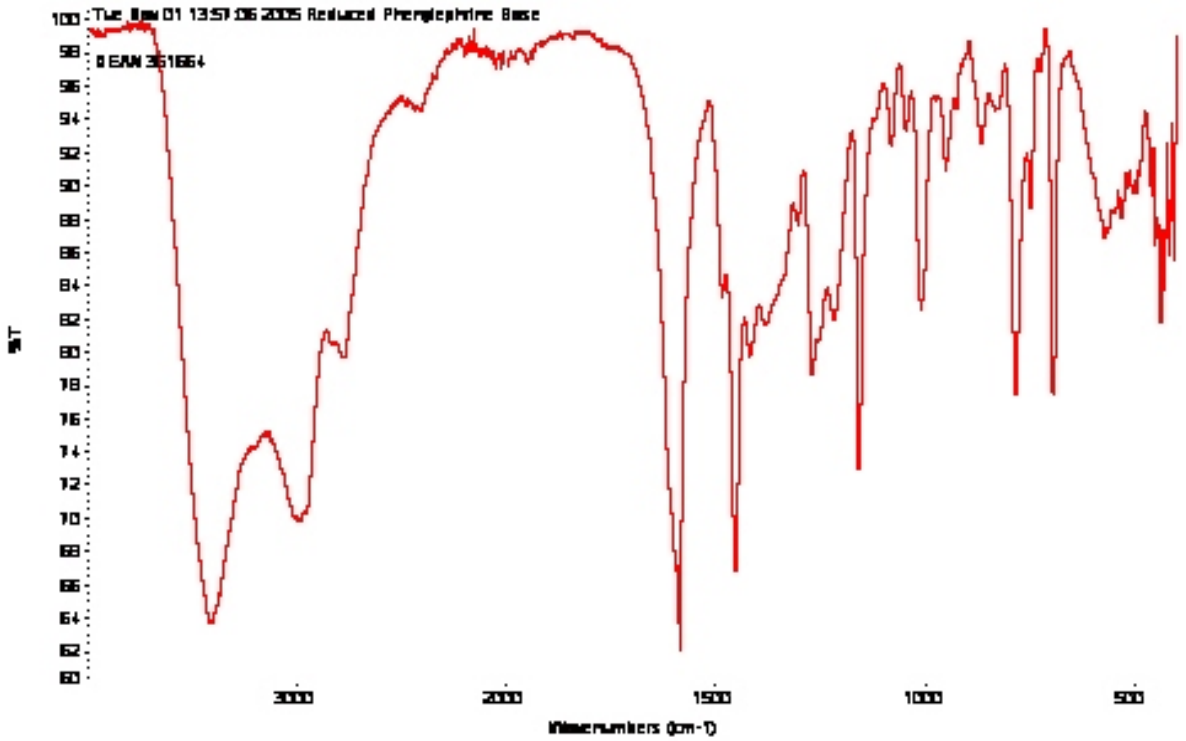

Figure 11. Infrared Spectrum of 3-Hydroxy-N-methylphenethylamine Base (Reduced Phenylephrine Base).

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Table 1. Methamphetamine Relative Retention Times (RRT's) for Gas Chromatography

Column: DB - 10 m, 0.32 mm, 0.52 µm; Temperature Profile: 130 ^{O}C for 1 Minute, Ramp at 25 ^{O}C to 280 ^{O}C, Hold 3 Minutes, 25:1 Split.

RRT	Compound
0.48	Dimethylsulfone
0.79	P2P / Amphetamine
0.83	*cis*-1,2-Dimethyl-3-phenylaziridine
1.00 (1.26 min.)	**Methamphetamine**
1.08	*trans*-1,2-Dimethyl-3-phenylaziridine
1.67	Chloroephedrine (Chloromethamphetamine)
1.69	Ephedrine / Pseudoephedrine
1.80	Aziridine Phenylephrine
1.94	Oxazolidine Pseudoephedrine
1.96	Dimethylphthalate
1.96	Reduced Phenylephrine
1.98	Oxazolidine Ephedrine
2.67	Phenylephrine
3.99	232-1
5.14	232-2

- -

Synthesis of *trans*-4-Methylaminorex from Norephedrine and Potassium Cyanate

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ABSTRACT: An unusual and previously undocumented synthesis for *trans*-4-methylaminorex was determined to be in use at a clandestine laboratory. Conventional references unanimously describe the use of norephedrine (phenylpropanolamine, 2-amino-1-phenylpropan-1-ol) and cyanogen bromide to synthesize *cis*-4-methyl-aminorex. In this case, use of norephedrine and potassium cyanate gave predominantly *trans*-4-methylaminorex. This new synthesis is explored, and its intermediates and byproducts are characterized.

KEYWORDS: 4-Methylaminorex, Oxazoline, Norephedrine, Phenylpropanolamine, Potassium Cyanate, Diastereomers, Clandestine Laboratory, Controlled Substance Analogue, Isomer, Forensic Chemistry

Introduction

In December 2004, a clandestine laboratory raid was conducted at a private residence in Ft. Lauderdale, Florida. The operator was an educated chemist (degree in Chemical Engineering). In his post-Miranda statements, he indicated that he had been synthesizing "Euphoria" (4-Methylaminorex, also known as: U4Euh, Ice*, 4-MAR, Intellex) in his home since June 2004, and stated that he was capable of producing batches as large as 1 kilogram. He also admitted to manufacturing lesser quantities of amphetamine, methamphetamine, and 3,4-methylene-dioxymethamphetamine. The chemicals and materials seized at the site supported his claims.

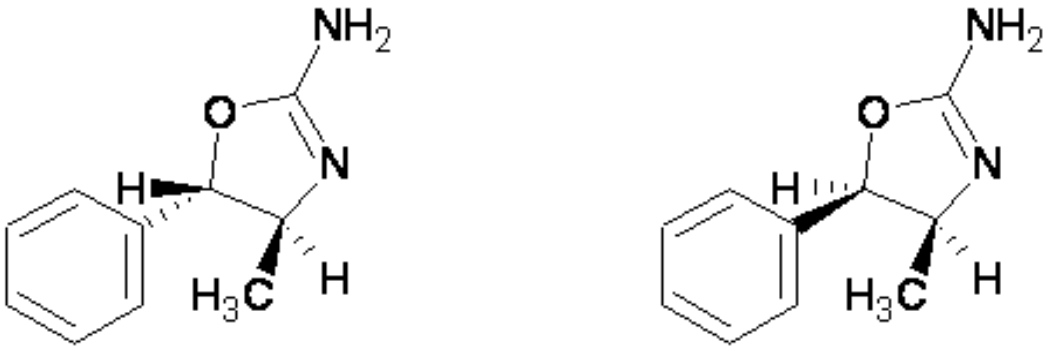
Of particular interest was 20 kilograms of a white powder alleged to be potassium cyanate (later confirmed to be a cyanate salt (cation not identified)). This compound had never been previously reported as a primary reagent at a clandestine laboratory. By the cook's account, he followed an internet recipe for synthesis of *trans*-4-methyl-aminorex from norephedrine and potassium cyanate.

This statement was surprising in that exhaustive literature searches indicated that the only reported syntheses starting with norephedrine used cyanogen bromide (not potassium cyanate) and produced *cis* (not *trans*) 4-methylaminorex [1]. The internet recipe (which had been posted on a website dedicated to drug abuse) was derived from the work of Fodor and Koczka [2], who investigated the stereochemistry of the conversion of 2-ureidoalcohols to oxazolidines. Included were the conversions of ephedrine and pseudoephedrine to the corresponding 2-ureidoalcohols, followed by their cyclization to their corresponding oxazolidines. Extrapolating from these results, the internet author theorized that the same reaction sequence could be applied to norephedrine, resulting in *trans*-4-methylaminorex [3] (Figure 1).

Klein *et al*. reported that the cyanogen bromide method is stereoselective and proceeds with retention of configuration at the benzylic carbon (C-1) of norephedrine [4]. Thus, norephedrine produces *cis*-4-methyl-

[* *In the late 1980's and early 1990's, "Ice" was a street name for 4-methylaminorex. Since then, however, it has shifted to a street name for high purity methamphetamine hydrochloride in large crystal form*.]

aminorex and norpseudoephedrine yields *trans*-4-methylaminorex (Figure 1). Therefore, if the classic synthesis (with cyanogen bromide) had been used, the *trans* isomer could only have been synthesized from norephedrine if stereochemical inversion was first performed on norephedrine to produce norpseudoephedrine [4]. This "stereoinversion" requires a tedious series of steps that is unlikely to ever be attempted in a clandestine setting. In contrast, in the potassium cyanate synthesis, *trans*-4-methylaminorex is allegedly generated directly from norephedrine without preliminary "stereoinversion" at C-1.



trans-4-Methylaminorex *cis*-4-Methylaminorex

Figure 1. The Diastereomers of 4-Methylaminorex (the Corresponding Enantiomers Have Been Omitted for Clarity).

Analysis of the seized exhibits confirmed that *trans*-4-methylaminorex was in fact the major product. Herein, the synthesis of *trans*-4-methylaminorex from norephedrine and potassium cyanate is characterized.

Legal Issues

When 4-methylaminorex was first temporarily controlled [5] in the summer of 1987, very little was known regarding the individual optical isomers of both the *cis* and *trans* forms [6]. Since the clandestine procedure employed at the time resulted in the production of the racemic *cis* isomer, there was no evidence that the *trans* isomer had any abuse potential, much less if it even existed in the clandestine market. As a result, only the *cis* isomer was explicitly controlled [7]. This marked the first and to date the only time a specific diastereomer has been listed as a controlled substance.

The production of *trans*-4-methylaminorex therefore raised an interesting legal issue. Since *cis*-4-methylaminorex is a Schedule I controlled substance, it can be inferred that "...its salts, isomers, and salts of isomers..." would also be Schedule I controlled substances. However, the term isomer, as defined in 21 CFR 1300.01(b)(21) means "...the optical isomer except as used in... [Schedules I(d) and II(b)]." *cis*-4-Methylaminorex is specifically listed as a stimulant in Schedule I(f). Since *trans*-4-methylaminorex is not an optical isomer of *cis*-4-methylaminorex, the "isomer" provision does not apply and it is not formally controlled.

Therefore, the legal issue is whether *trans*-4-methylaminorex is a controlled substance analogue. Under the Controlled Substance Analogue provision of the Controlled Substances Act, it must first be demonstrated that *trans*-4-methylaminorex has a chemical structure that is substantially similar to the chemical structure of controlled substance in Schedule I or II. The controlled substance in this case is *cis*-4-methylaminorex. The diastereomeric relationship between these two compounds clearly satisfies the requirements of the first "prong" of the provision.

Secondly, *trans*-4-methylaminorex must exhibit a stimulant effect that is substantially similar to or greater than the stimulant effect of *cis*-4-methylaminorex -OR- must be represented or intended to have a stimulant effect that is substantially similar to or greater than the stimulant effect of *cis*-4-methylaminorex. The rank order of potencies of the four enantiomers of 4-methylaminorex has been shown to be:

trans-4S,5S > *cis*-4S,5R ~ *cis*-4R,5S > *trans*-4R,5R

in several pharmacological studies [6,8-11]. One group of researchers suggested that the *trans*-4S,5S- isomer may have sufficient abuse potential to warrant its classification as a Schedule I controlled substance [10]. Thus, the second "prong" of the Controlled Substance Analogue provision is also met.

Finally, the *trans*-4-methylaminorex seized in this case was specifically stated by the clandestine chemist to be "Euphoria," which is the generic street nomenclature for 4-methylaminorex without any stereochemical (*cis* or *trans*) designation [12,13]. Therefore, all three "prongs" of the Controlled Substance Analogue provision are satisfied, and it is virtually certain that Federal prosecution of *trans*-4-methylaminorex as a "controlled substance analogue" would be successful. In this case, the clandestine chemist was convicted of manufacture of a controlled substance.

Experimental

Reagents were obtained from Sigma-Aldrich, and were used without further purification.

Gas chromatograph/mass spectrometry (GC/MS) data was obtained from an Agilent 6890 Gas Chromatograph (GC) coupled to an Agilent 5973 Mass Selective Detector (MSD) operating in electron impact (EI) mode. The mass spectral scan range was *m/z* 34 to 520. The ion source and quadrupole temperature zones were set to 230 °C and 150 °C, respectively. The interface was heated to 280 °C. The GC was equipped with a 30 meter ZB-1 column with an internal diameter of 0.25 mm and a 0.25 μm film thickness (Phenomenex). The inlet was set to 250 °C and the carrier gas was Helium with a constant flow rate of 1.3 mL/min. The oven was ramped from 100 °C - 295 °C at 35 °C/min, with a 6.43 minute hold at 295 °C, for a total run time of 12 minutes.

Fourier Transform ^{1}H Nuclear Magnetic Resonance (FT-NMR) analyses were performed on a Varian Mercury-plus spectrometer operating at 400 MHz. Eight scans were collected for each sample. Internal reference standards were not used.

Fourier Transform Infrared (FTIR) spectra were collected on a Thermo-Nicolet Nexus 470 FTIR equipped with a SensIR Technologies Durascope 3-bounce ATR attachment. The scan range was 4000 cm^{-1} to 550 cm^{-1}, with a 4 cm^{-1} resolution. 32 scans were collected for each sample.

Molecular drawings, 3-D optimizations, and IUPAC names were generated with ACD Labs ChemSketch software, version 7.0.

Preparation of *trans*-4-Methylaminorex

Because this synthesis is no longer readily accessible on the internet, experimental details have been omitted, in accordance with *Microgram Journal* policy. Law enforcement personnel with a legitimate need to know should contact the authors for further information.

Results and Discussion

It is uncommon for trained, professional chemists to produce illicit drugs. It is even more unusual that a genuinely new clandestine manufacturing process is encountered. It was helpful in this case that the cook not

only documented his reaction, batch, and scale-up information, but also was willing to discuss his "work" in a proffer hearing. As detailed above, the synthesis of *trans*-4-methylaminorex was performed using a procedure partially described on the internet and further expanded by the clandestine chemist [14]. A scaled-down version of this method was used in this study.

Analysis of the reaction mixture from the illicit method after the first 2.5 hours revealed the presence of N-(2-hydroxy-1-methyl-2-phenethyl)urea, 4-methyl-5-phenyl-1,3-oxazolidin-2-one and unreacted norephedrine (see Figure 2). No *trans*-4-methylaminorex was detected up to this point.

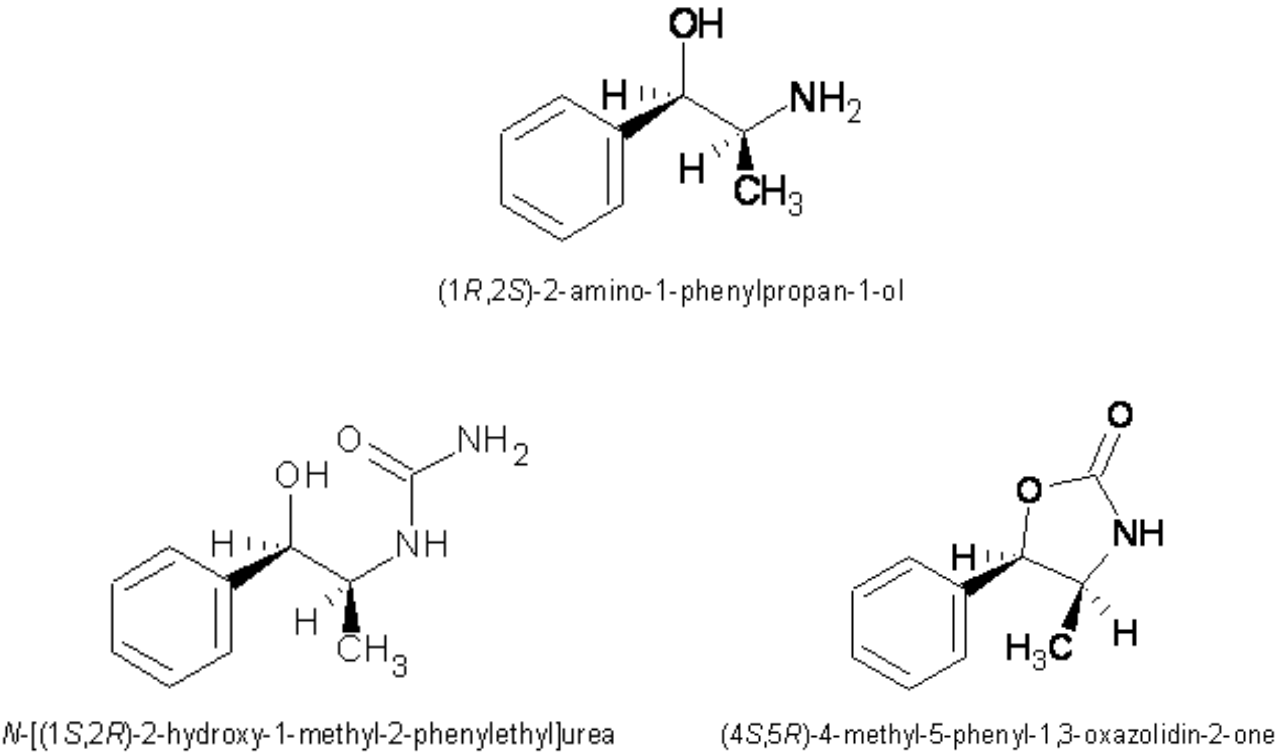


Figure 2. The Components of the Reaction Mixture from the Illicit Method after the First 2.5 Hour Reflux Period. Only One of Each Enantiomeric Pair Is Shown.

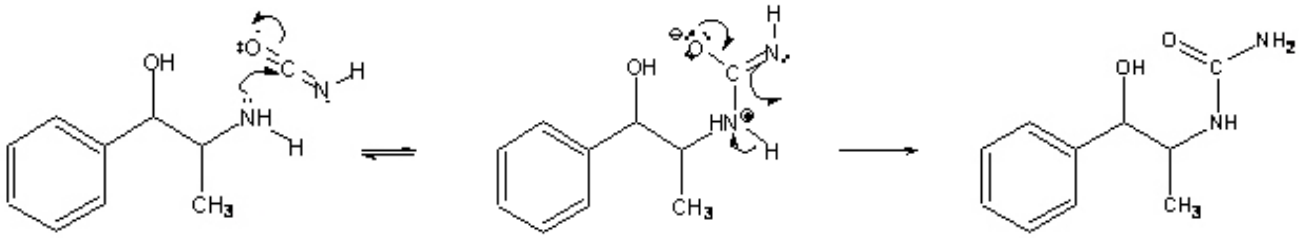
The final reaction mixture was similar in composition to the actual evidence seized from the clandestine laboratory. In addition to *trans*-4-methylaminorex, small amounts of *cis*-4-methylaminorex were also found. The crude mixture also contained 4-methyl-5-phenyl-1,3-oxazolidin-2-one, N-(2-hydroxy-1-methyl-2-phenethyl)urea, and unreacted norephedrine. For this study, this mixture was cleaned up prior to isolation of the final product.

It is likely that the synthesis occurs through the intermediate 2-ureidoalcohol *alpha*-methyl-*beta*-hydroxy-phenethylurea [15] which cyclizes to the oxazoline with inversion of configuration at C-1 [2]. Therefore, the synthesis was also conducted by the initial production and isolation of N-(2-hydroxy-1-methyl-2-phenethyl)urea intermediate. The general stoichiometric reaction is shown below:

$$\text{Norephedrine} + \text{Potassium Cyanate} + H_2O \rightarrow \text{Norephedrine-Urea} + KOH \qquad \text{(Equation 1)}$$

The pH of the solution immediately upon complete dissolution of the reactants was about 5-6. After the reaction had gone to completion, the pH of the remaining liquid was about 10-11, supporting Equation 1.

It is generally accepted that the reaction of cyanates in water does not proceed via the cyanate but via isocyanic acid [16-18]. This scenario (Scheme 1) involves nucleophilic attack by the amino group of norephedrine on the somewhat positively polarized carbon of isocyanic acid, with a subsequent proton shift from the amino group of norephedrine.



Scheme 1

This mechanism shows that the chiral centers remain unchanged during the formation of the urea intermediate. Since norephedrine produces *trans*-4-methylaminorex, inversion of configuration must occur via an intramolecular SN_2-type attack at the benzylic carbon by the carbonyl group of the urea portion of the intermediate. This can occur because N-(2-hydroxy-1-methyl-2-phenethyl)urea can achieve a favorable conformation to allow the reaction to occur [2]. The pseudo-5-membered ring conformation places the carbonyl oxygen in proximity to the benzylic carbon, enabling the SN_2 type attack. This conformation is depicted in the 3-D image shown below (Figure 3).

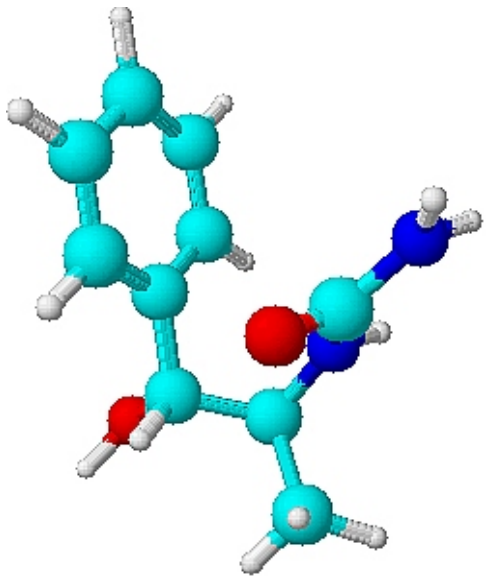


Figure 3. Three Dimensional Image of N-[(1S,2R)-(2-Hydroxy-1-methyl-2-phenethyl)]urea in a Conformation Enabling SN_2 Attack at the Benzylic Carbon (C-2) by the Ureido Carbonyl.

Note the changes in the numbering system of the various compounds. The benzylic carbon in norephedrine is designated as C-1. The same carbon is labeled as C-2 in the urea intermediate, and as C-5 in 4-methylaminorex. Thus, the (1R,2S) isomer of norephedrine becomes the (1S,2R) isomer of the urea intermediate, which is subsequently converted to the *trans*-(4S,5S)- isomer of 4-methylaminorex.

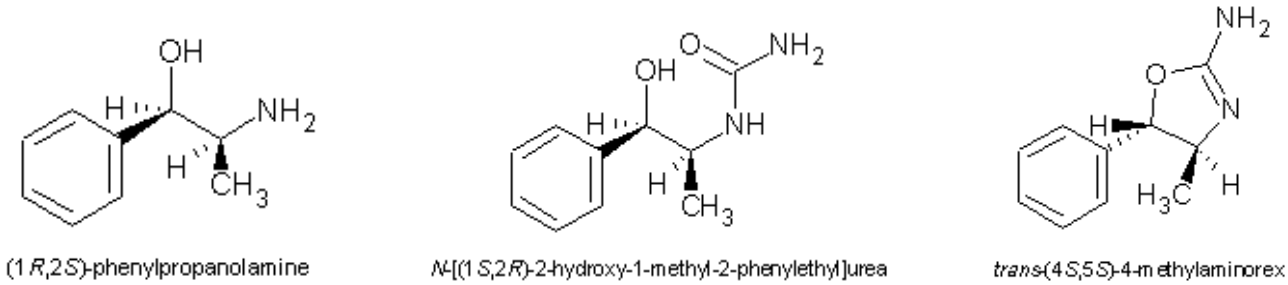


Figure 4. Summary of Absolute Configurations of Chiral Centers of Key Molecules. One of Each Enantiomeric Pair Is Shown.

The small amount of the oxazolidinone detected in the reaction mixture at the half-way point is probably formed by the attack of the benzylic hydroxyl on the carbonyl of the urea (Figure 5), liberating a molecule of ammonia into the solution (presumably as NH_4OH), which may also contribute to the already moderately high pH observed at the end of the reaction (Equation 1).

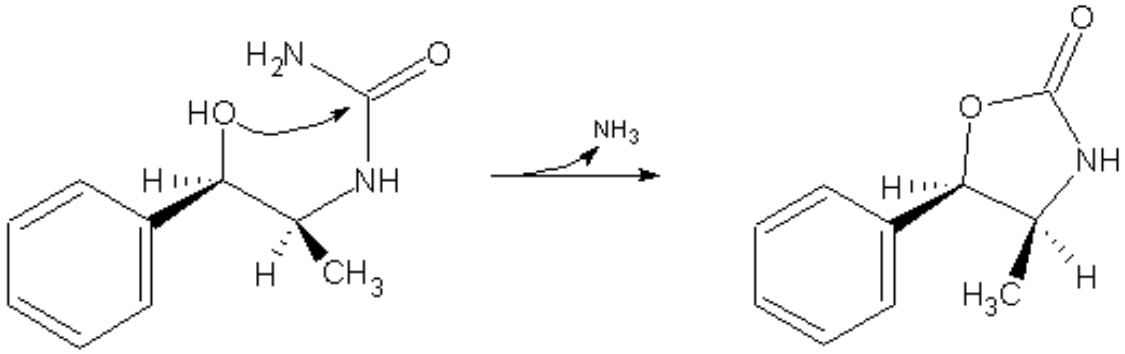


Figure 5. Formation of *cis*-4-Methyl-5-phenyl-1,3-oxazolidin-2-one from the Urea Intermediate.

This results in retention of configuration. The phenyl and methyl groups of the oxazolidinone by-product must be *cis* since no change in the configuration at C-4 or C-5 could occur. This is confirmed by NMR data. The coupling constant for the C-5 proton is consistent with a *cis* configuration ($J_{4\text{-}5}$ = 8.02 Hz). This value is comparable to literature values for the electronically similar 3,4-dimethyl-2-imino-5-phenyloxazolidine [19].

Acknowledgements

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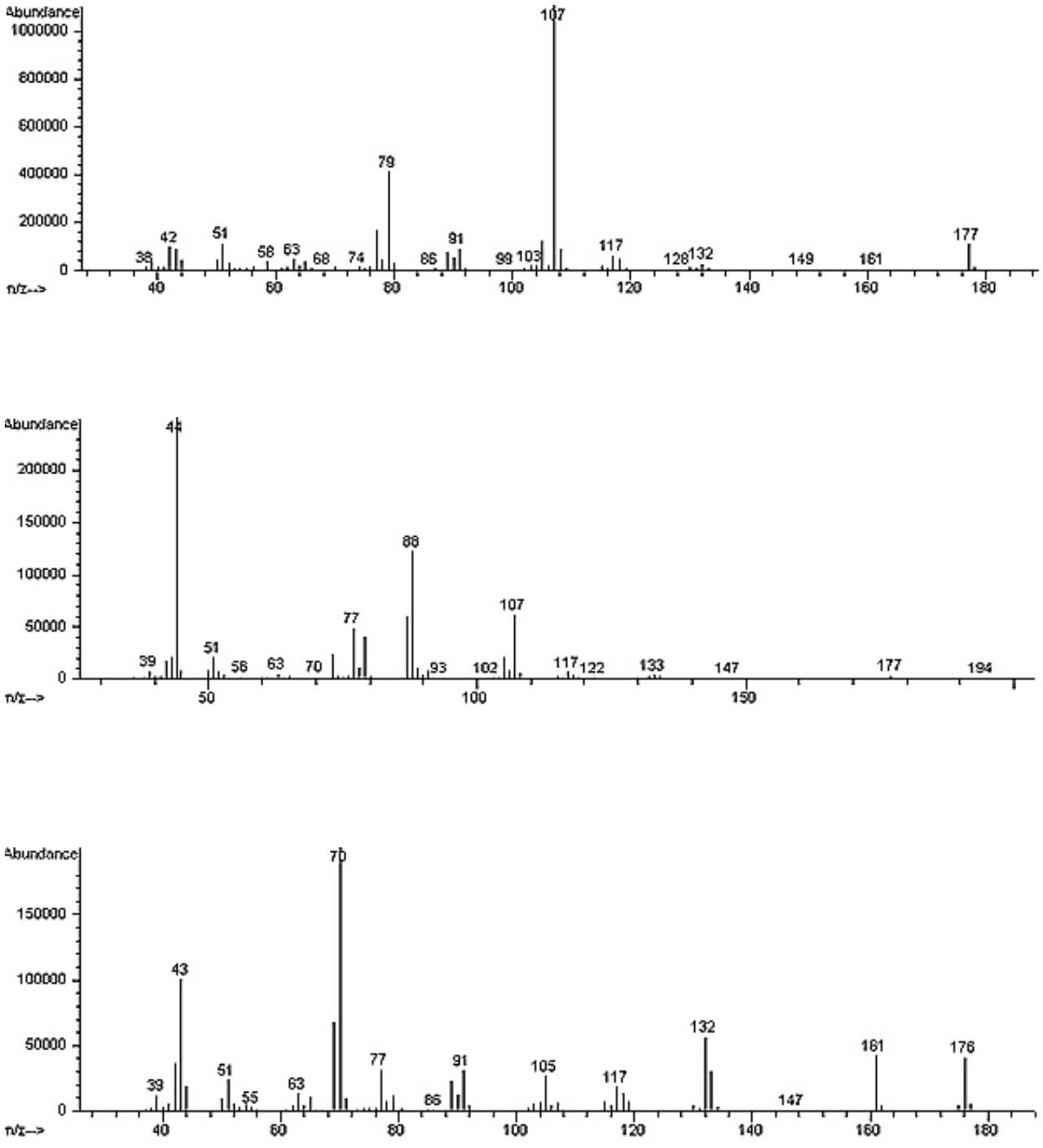

Figure 6. EI Mass Spectra. Top: 4-Methyl-5-phenyl-1,3-oxazolidin-2-one; Middle: N-(2-Hydroxy-1-methyl-2-phenethyl)urea; Bottom: *trans*-4-Methylaminorex.

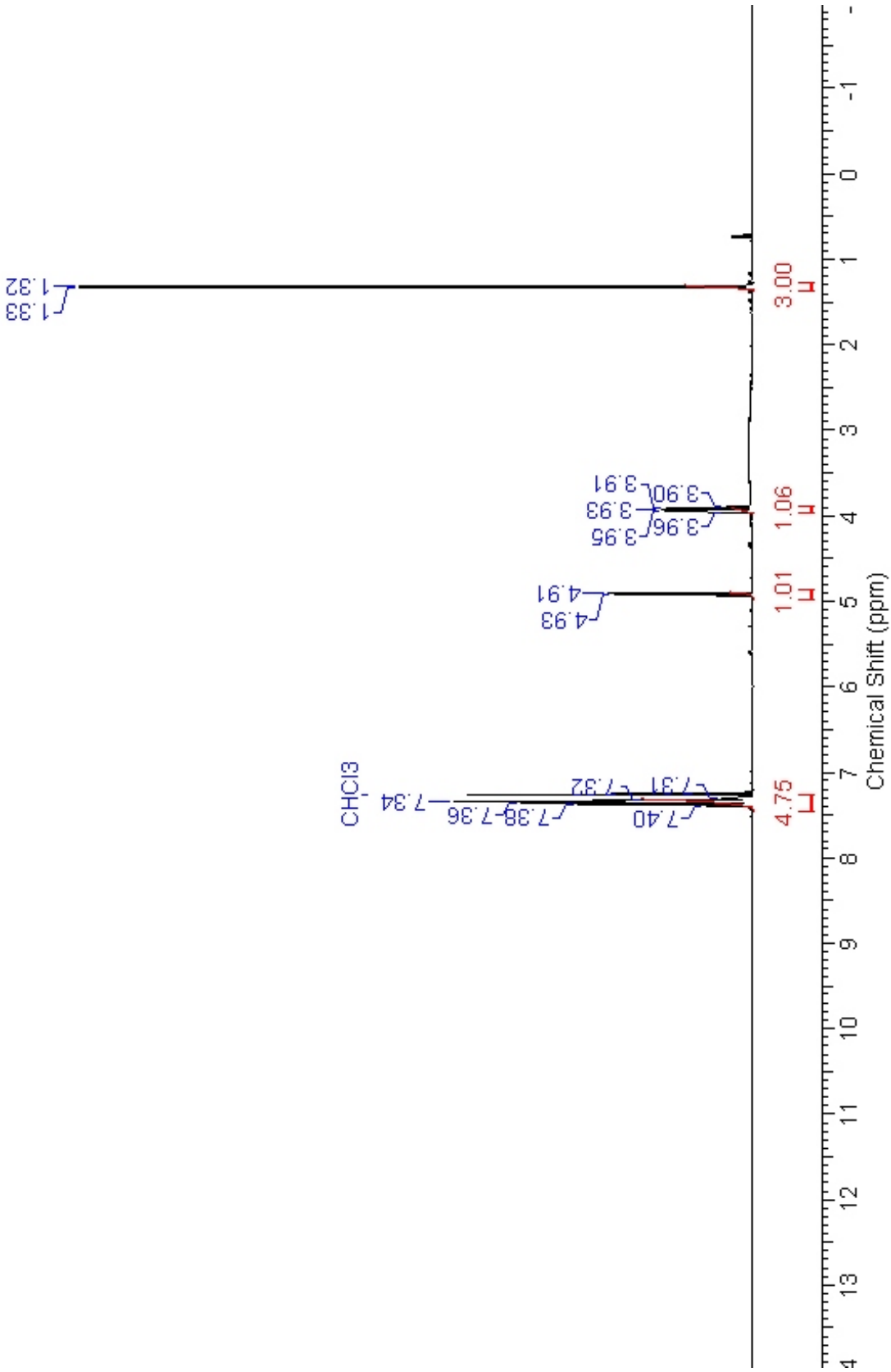

Figure 7. [1]H NMR, 8 scans, *trans*-4-Methylaminorex.

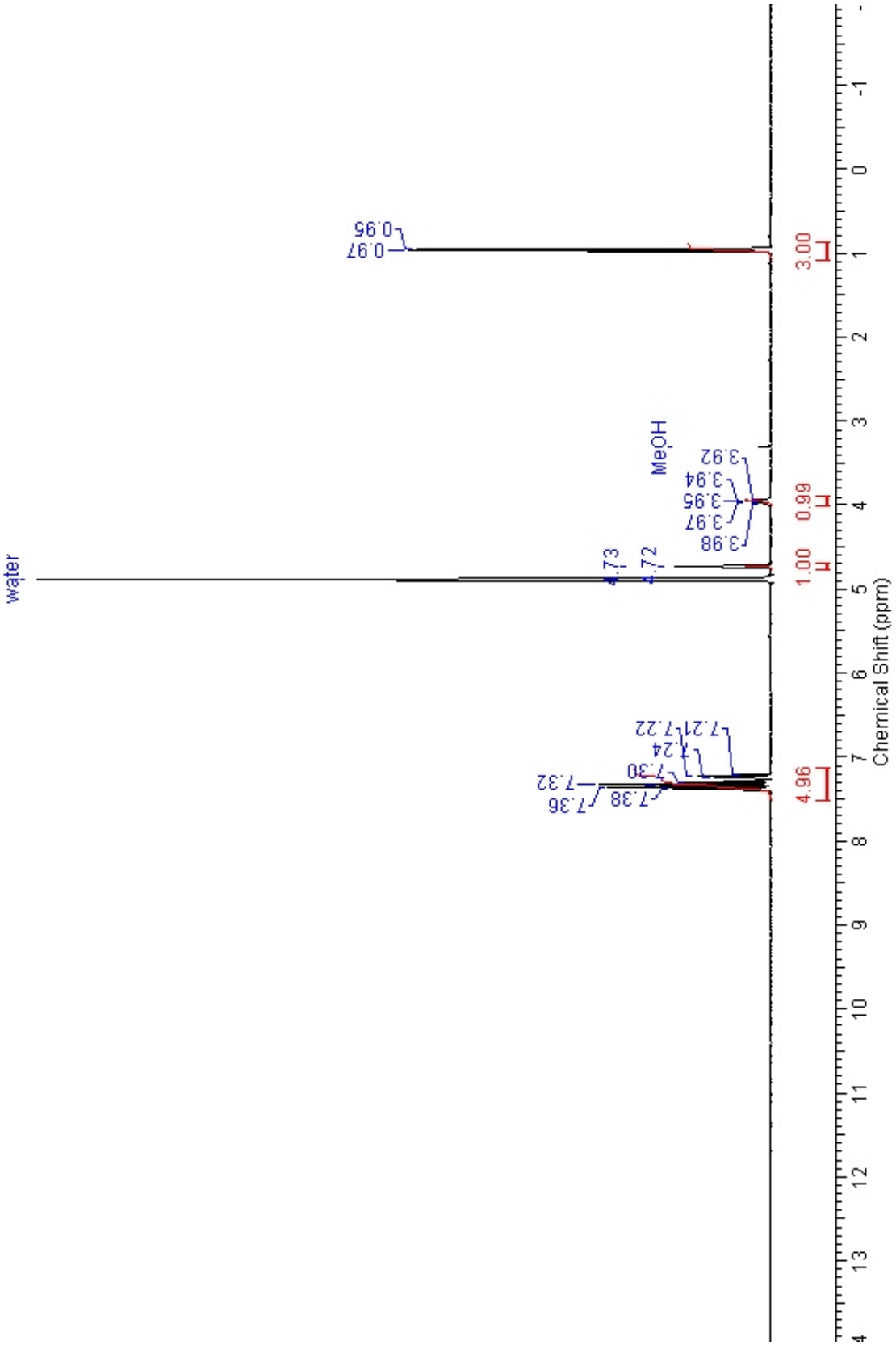

Figure 8. [1]H NMR, 8 scans, N-(2-Hydroxy-1-methyl-2-phenethyl)urea.

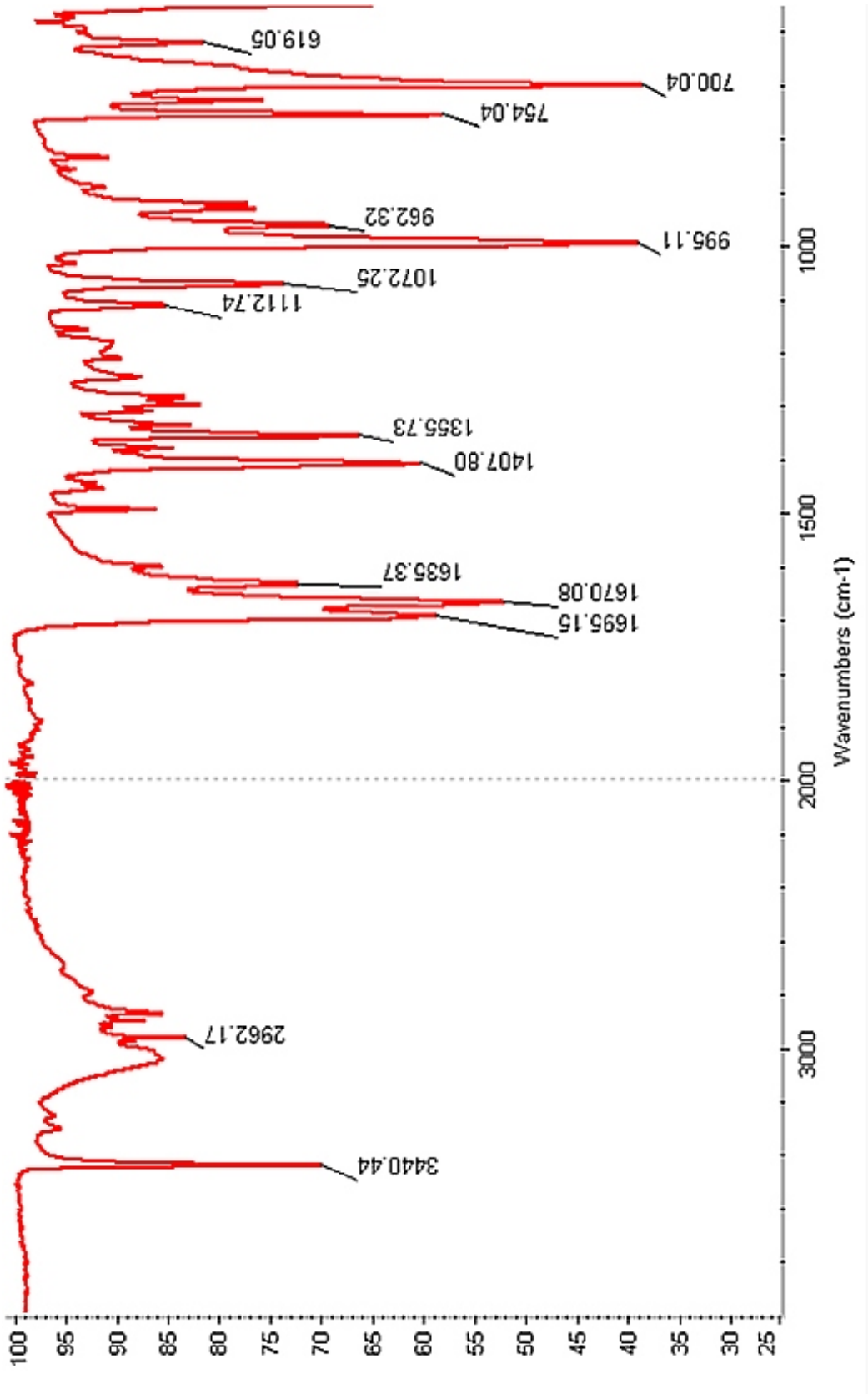

Figure 9. FTIR. 32 scans, *trans*-4-Methylaminorex.

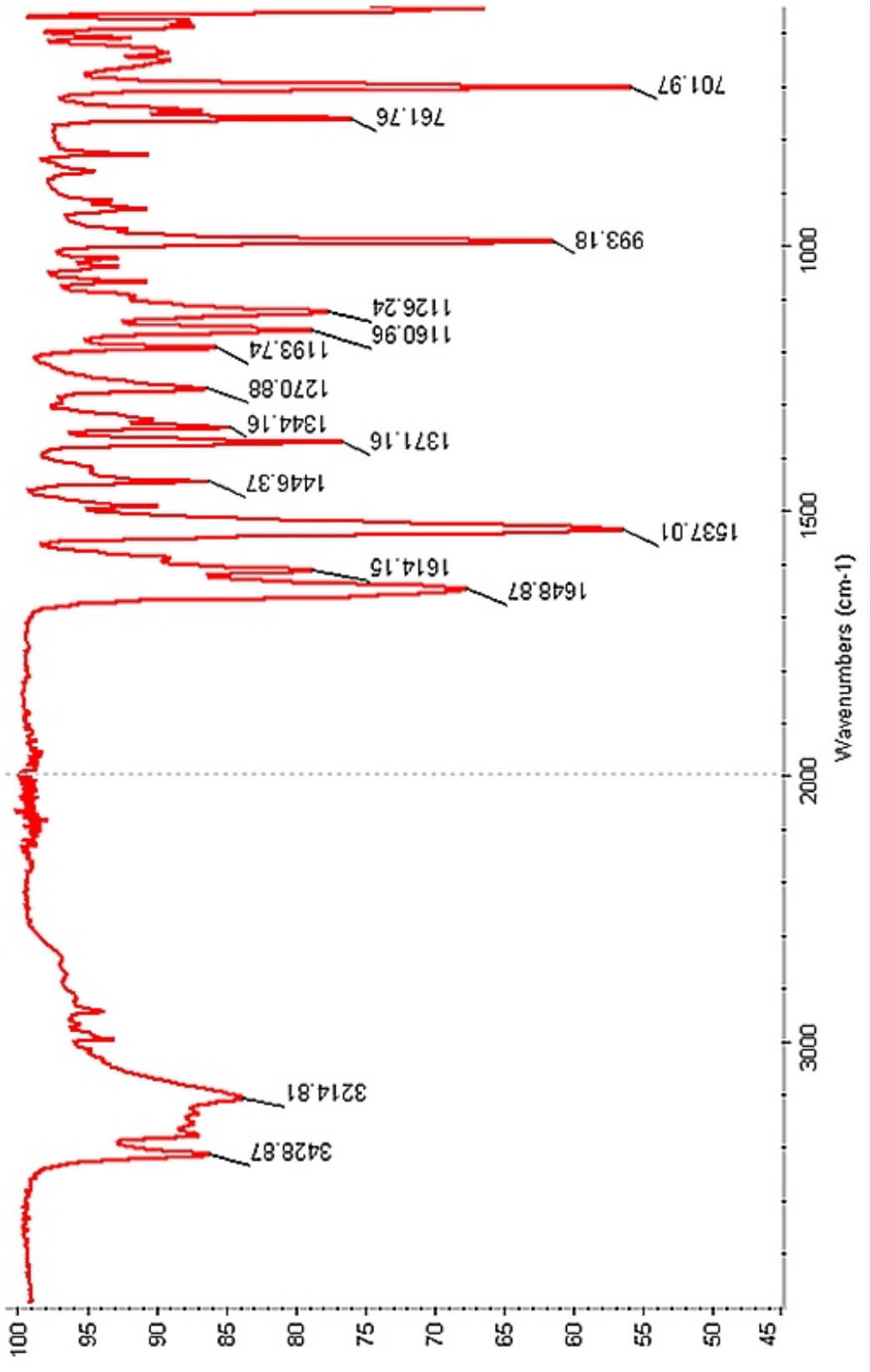

Figure 10. FTIR. 32 scans, N-(2-Hydroxy-1-methyl-2-phenethyl)urea.

Identification of a New Amphetamine Type Stimulant: 3,4-Methylenedioxy-*N*-(2-hydroxyethyl)amphetamine (MDHOET)

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ABSTRACT: 3,4-Methylenedioxy-*N*-(2-hydroxyethyl)amphetamine (MDHOET), an MDA derivative, was identified in Ecstasy-type tablets seized in France, and subsequently in exhibits seized in Austria, The Netherlands, Switzerland, and the United Kingdom. This unusual amphetamine type stimulant (ATS) was submitted as an unknown to seven European laboratories participating in a sponsored ATS profiling program. Six

of the seven laboratories successfully identified MDHOET upon initial analysis. Analytical data from gas chromatography, infrared spectroscopy, mass spectrometry, and nuclear magnetic resonance spectroscopy are presented.

KEYWORDS: 3,4-Methylenedioxy-*N*-(2-hydroxyethyl)amphetamine, MDHOET, MDMA, Ecstasy, ATS, CHAMP, Forensic Chemistry

Introduction

In December 2004, one thousand white Ecstasy-type tablets with the "Euro" logo were seized in Saint Etienne, France (see Photo 1). These tablets were subsequently determined to actually contain a combination of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxy-*N*-(2-hydroxyethyl)amphetamine (MDHOET; see Figure 1) [1,2]. Subsequent to this initial submission, six additional seizures containing MDHOET were made in Europe (see Table 1), including powders in the Netherlands (three separate submissions), white tablets with a "LOVE" logo (no photo) in Austria and Switzerland, and fragments of tablets in the United Kingdom. MDMA was present as a co-ingredient in five of the seven cases, but when present was always at a lower percentage versus MDHOET.

Photo 1

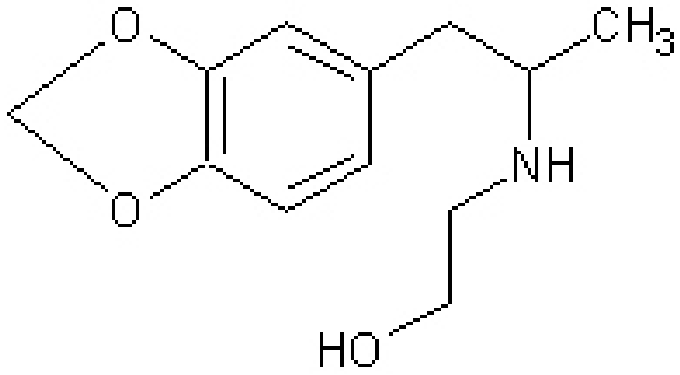

Figure 1. 3,4-Methylenedioxy-*N*-(2-hydroxyethyl)amphetamine (MDHOET)

MDHOET can be synthesized similarly to MDMA; for example, by reductive amination of 3,4-methylenedioxy-phenyl-2-propanone (piperonylmethylketone or PMK) with ethanolamine, using a reducing agent (e.g., sodium cyanoborohydride) or via catalytic hydrogenation (e.g., H_2 over Platinum) [1,2]. Not much is known about the physiological effects of this drug; it is reported to have very limited activity, presumably due to its relatively high polarity [2]. It is unknown whether the drug was intentionally synthesized as a non-controlled "designer drug," or instead was an erroneous synthesis of 3,4-methylenedioxyethylamphetamine (MDEA); that is, by mistakenly using ethanolamine instead of ethylamine.

As MDHOET had not, to our knowledge, been previously encountered in Ecstasy-type tablets or powders, it represented an ideal test compound for submission to the seven laboratories currently participating in a project entitled: "Collaborative Harmonisation of Methods for Profiling of Amphetamine Type Stimulants" (CHAMP). Exhibits of the "Euro" tablets seized in France were used as the test samples.

Experimental

Gas Chromatography - Mass Spectrometry (GC/MS)

An Agilent 6890 GC coupled to a 5973 Mass Selective Detector system (MSD) was used. The column that was used was a HP Ultra-1 (length: 12 m, inner diameter: 0.22 mm, film thickness: 0.3 μm). Helium was used as the carrier gas (1 mL/minute, split ratio 50:1). The GC oven was programmed from 110 °C (1 minute hold) to 275 °C at a rate of 40 °C/minute. The carrier gas velocity was set at 40 cm/second (1.0 mL/minute, constant flow rate) and the inlet temperature was set at 275 °C. The injected volume was 1 μL. The scan range was *m/z* 35 to 450. A solvent delay of 0.8 minute was applied. The temperature of the MS transfer line was 300 °C.

Liquid Chromatography Mass Spectrometry (LC-MS/MS)

An Agilent 1100 Series HPLC system with autosampler and an Agilent 1100 series LC/MSD Trap ion trap MS was used, with Agilent LC/MSD Trap software version 5.2 (Bremen, Germany). The column was a Phenomenex Luna C18 (3 mm x 150 mm, 3 mm). The eluent A was 0.01 M ammonium acetate with 0.1 % formic acid and eluent B was acetonitrile with 5 % 0.01 M ammonium acetate and 0.1 % formic acid, in gradient run. The gradient used was 20 - 100 % eluant B over 0 - 10 minutes and 100 % eluant B from 10 - 25 minutes. The flow rate was 0.3 mL/minute, and the column temperature was 30 °C. The injection volume was 10 μL.

The electrospray ionisation ESI technique was used, in the positive ion mode. Operating parameters of the ESI ion source were as follows: Drying gas temperature was 350 °C, drying gas flow 9.0 L/minute, nebuliser gas pressure 40 psi, end plate voltage -3500 V, and end plate offset -500 V. Ion trap parameters were as follows: Accumulation time was 43 ms and averages 5, rolling averaging off, and ion charge control on. The fragmentation amplitude was increased from 30 % to 200 % from the set value of 1.0 V. AutoMS(n) mode was used. The scan range was *m/z* 50 - 500.

Fourier Transform Infrared Spectroscopy (FT-IR)

A Thermo-Nicolet Nexus 670 was used. Scans were recorded from 4000 cm^{-1} to 400 cm^{-1}; an average of 32 scans was taken. Spectra were obtained using an attenuated total reflectance (ATR) attachment (data not corrected).

Nuclear Magnetic Resonance Spectroscopy

Analyses (see Figures 6 and 7) were performed on a Varian Mercury 400 MHz NMR using a Varian Nalorac 5 mm indirect detection, pulse field gradient (PFG), variable temperature probe, with PulseTuneTM. The sample was prepared at 26.8 mg/mL in deuterium oxide (D_2O) containing TSP (3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt) as the 0 ppm reference, and maleic acid (5 mg/mL) as the internal standard for quantitation (maleic acid exhibits a singlet at 6.4 ppm). The proton spectrum of the standard used to determine the purity of the synthesized reference compound was obtained with 8 scans using a 45 second delay, 90° pulse, 5 second acquisition time, and oversampling of 4. Confirmation of the structure of the synthesized reference compound was performed using proton, carbon, COSY, HSQC, and HMBC NMR spectra, and Advanced Chemistry Developments Structure Elucidator software program (ACD/Labs, Toronto, Canada).

Synthesis of Reference Material

MDHOET was synthesized by reductive amination of 3,4-methylenedioxyphenyl-2-propanone (PMK, 4.52 grams) with ethanolamine hydrochloride (25 grams) in isopropanol (IPA) and sodium cyanoborohydride ($NaCNBH_3$, 1.1 grams) as the reducing agent [1]. PMK was added to ethanolamine hydrochloride in IPA and vigorously stirred. $NaCNBH_3$ was added and the mixture was stirred, adjusting the pH (as needed) with HCl in IPA at room temperature. After 3 days, 300 mL of water was added, and the mixture was made strongly acidic with 37 % HCl. The resulting solution was extracted 3 times with 100 mL CH_2Cl_2, which was discarded. The remaining aqueous phase was then made basic with 25 % NaOH, then extracted 3 times with 100 mL CH_2Cl_2. The resulting organic extracts were combined and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum, yielding a clear, slightly viscous oil (2.82 grams). This oil was dissolved in IPA containing HCl and diluted with diethyl ether, precipitating 3.85 grams (68 %) MDHOET HCl as an off-white powder.

Results and Discussion

Laboratory Results

Subexhibits of the "Euro" tablets seized in Saint Etienne, France were distributed to the participating laboratories as "blind" samples (that is, with no indication that they were a test, and no instructions on recommended methods for analysis). This resulted in the use of a variety of techniques, of which GC/FID, GC/MS, FT-IR were the most frequently employed (see Table 2). The composition of the tablets (approximately 2 % MDMA (as base), caffeine, and approximately 7 % MDHOET (as base)) was an unusual complicating factor. Six of the seven partners properly identified MDHOET; the lone exception missed its presence during initial screening due to its co-elution with caffeine during the GC/FID analysis used in their laboratory. To confirm the identification, one laboratory synthesized a reference standard, a second laboratory used NMR spectroscopy (^{1}H, ^{13}C-, COSY and HETCOR), and a third laboratory did both. In addition to MDMA and MDHOET, caffeine, sorbitol and cellulose were identified by some of the laboratories (using GC, ^{1}H-NMR, and/or FT-IR).

Mass Spectrometry

All laboratories taking part in the round robin used mass spectrometry for the elucidation of the structure of the unknown analyte. Apart from GC/MS, both liquid chromatography - mass spectrometry (LC/MS) and capillary electrophoresis - mass spectrometry (CE/MS) were used. When LC/MS, CE/MS or ion-trap GC/MS was used, the M+H ion was present at *m/z* 224 (Figure 2). However, if quadrupole GC/MS was used, derivatization (for example, silylation or acetylation) was necessary to determine the molecular ion.

Upon GC/MS analysis of a non-derivatized sample, both MDMA (Retention index (RI) 1515) and MDHOET (RI 1865) were detected [3]. The mass spectrum of MDHOET is displayed in Figure 3. No molecular ion could be identified. Besides the base peak at *m/z* 88 ($CH_3CH=^{+}NHCH_2CH_2OH$),other characteristic fragment ions are visible at *m/z* 44, *m/z* 70 (-18 (H_20) from *m/z* 88), *m/z* 135 (-88 ($CH_3CH=^{+}NHCH_2CH_2OH$)) and *m/z* 163 (-60 ($H_2NCH_2CH_2OH$)). The two fragments at *m/z* 70 and *m/z* 88 both suggest the presence of an -OH functionality (that is, loss of H_2O).

Acetylation of MDHOET gave two derivatives, in agreement with the presence of two active hydrogens (NH and OH). The major compound was the di-acetylated derivative (Figure 4, molecular ion at *m/z* 307), while the minor compound was the mono-acetylated compound (molecular ion at *m/z* 281; spectrum not shown). Comparison of the mass spectrum of MDHOET with that of its amphetamine analogue, N-(2-hydroxyethyl)amphetamine as described by Cry *et al*. and Carpenter *et al*. [4,5], further corroborates this interpretation.

Fourier Transform Infrared Spectrometry

The FTIR-ATR spectrum of the synthesized MDHOET HCl is shown in Figure 5. The principal wavebands are at 1031 and 1249 cm^{-1}, with additional characteristic bands at 3369, 2949 and 1578 cm^{-1}.

NMR Spectroscopy

The ^{1}H- and ^{13}C-NMR spectra of the synthesized MDHOET in D_2O are shown in Figures 6 and 7. The two active hydrogens are not visible due to deuterium exchange with the solvent (when dissolved in $CDCl_3$, both resonances are visible as broad singlets between 9.2 - 9.3 ppm). The ^{1}H-NMR spectrum of the original sample dissolved in D_2O is shown in Figure 8. In addition to MDHOET, MDMA, sorbitol, and caffeine are observed. The assignment of both the ^{1}H- and ^{13}C- resonances of MDHOET are given in Table 3.

Conclusions

Although MDHOET is quite unlikely to ever become a significant drug of abuse, future encounters are probable, both in Europe and elsewhere. The analytical data presented in this article should enable facile analyses of this unusual "designer drug," in accordance with SWGDRUG protocols [6].

Acknowledgements

The Sixth Framework Program of the European Commission is gratefully acknowledged for funding this project. The authors would like to thank the following persons for their contributions: Laura Aalberg (Vantaa, Finland), Fabrice Besacier (Ecully, France), Jiri Bolehovsky (Prague, Czech Republic), Rainer Dahlenburg (Wiesbaden, Germany), Susanne Dieckmann (Wiesbaden, Germany), Laurence Dujourdy (Ecully, France), Pierre Esseiva (Lausanne-Dorigny, Switzerland), Céline Delaporte (Lausanne-Dorigny, Switzerland), Henk Huizer (The Hague, The Netherlands), Libuse Kawulokova (Prague, Czech Republic), Eric Lock (The Hague, The Netherlands), Anneke Poortman (The Hague, The Netherlands), Milan Prazak (Prague, Czech Republic), Erkki Sippola (Vantaa, Finland), and Pavel Tomicek (Prague, Czech Republic) [See address header for respective addresses.]

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Table 1. Seizures of MDHOET in Europe During the Time Frame December 2004 - March 2006.

Country	Date	Logo	Wt., Diam., Width	Contents	Amount
France	12/04	Euro	282 mg, 9.1 mm, 3.6 mm	MDHOET, MDMA, caffeine	1000 tablets
Netherlands	02/05	-	-	MDHOET, MDMA	10 g powder
	06/05	-	-	MDHOET, MDMA	20 g powder
	03/06	-	-	MDHOET, MDMA	1.8 g powder
Austria	05/05	Love	190 mg, 6.7 mm, 4.1 mm	MDHOET	50 tablets
Switzerland	05/05	Love	278 mg, 7.1 mm, 4.5 mm	MDHOET, MDMA	Not Reported
U.K.	04/05	Prob. Unmarked	-	MDHOET	Tablet Fragments

Table 2. Overview of the Techniques Used to Identify MDHOET.

LAB	GC/FID	HPLC	GC/MS	GC/MS Deriv.	LC-MS/MS	DLC	CE-MS/MS	FTIR	NMR	Synthesis
1	X		Quad	X	X			X		
2	X		Quad	X		X		X		X
3			Quad	X						
4	X	X	Quad	X						
5	X		Quad/Ion Trap					X		
6			Ion Trap				X	X	X	
7	X	X	Quad	X				X	X	X

- -

Table 3. Assignment of ^{1}H- and ^{13}C-Resonances of MDHOET

Position	Proton (ppm, Peak Muliplicity, Coupling Constant)	Carbon (ppm)
CH_3	1.28 (d, *J* = 6.6 Hz)	18.0
CH_3-CH	3.6 (ddq, *J* = 5.8, 8.7, 6.6 (x3))	58.4
CH_3-CH-CH_2	3.07 (dd, *J* = 13.8, 5.8 Hz), 2.80 (dd, *J* = 13.8, 8.7 Hz)	41.2
N-CH_2-CH_2-OH	3.28 (td, *J* = 13.3, 5.3 Hz), 3.21 (td, *J* = 13.1, 5.2 Hz)	49.1
N-CH_2-CH_2-OH	3.85 (t, *J* = 5.2 Hz)	59.7
O-CH_2-O	5.96 (s)	104.1
phenyl #1	n/a	132.5
phenyl #2	6.85 (d, *J* = 1.47 Hz)	112.6
phenyl #3	n/a	150.4
phenyl #4	n/a	149.2
phenyl #5	6.88 (d, *J* = 7.92 Hz)	111.6
phenyl #6	6.80 (dd, *J* = 7.92, 1.47 Hz)	125.7
NH, OH	Exchanged with Solvent	N/A

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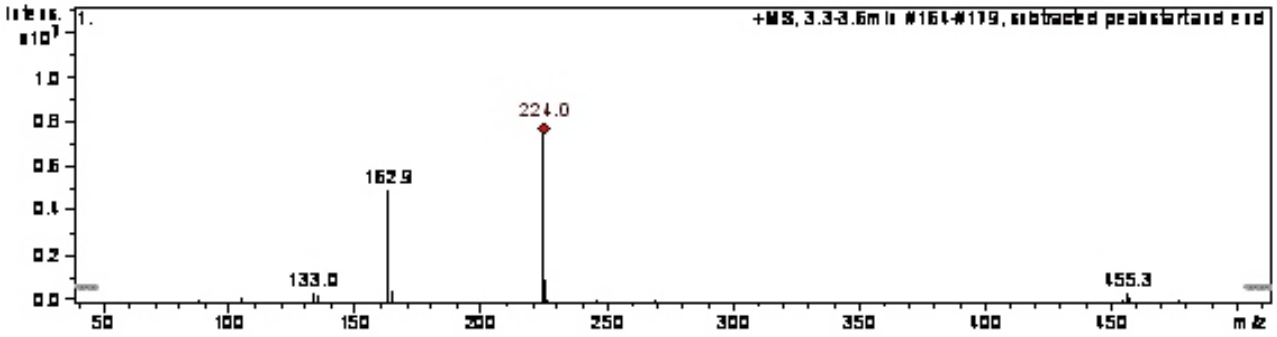

Figure 2. LC-MS/MS Mass Spectrum of MDHOET HCl.

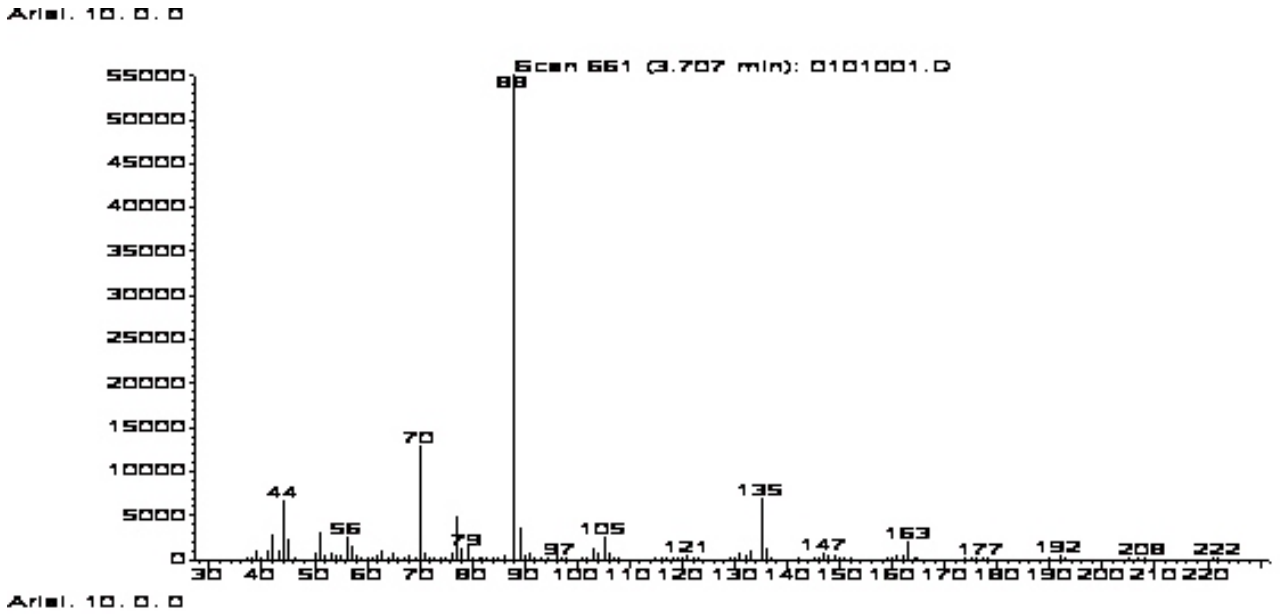

Figure 3. Mass Spectrum of MDHOET HCl.

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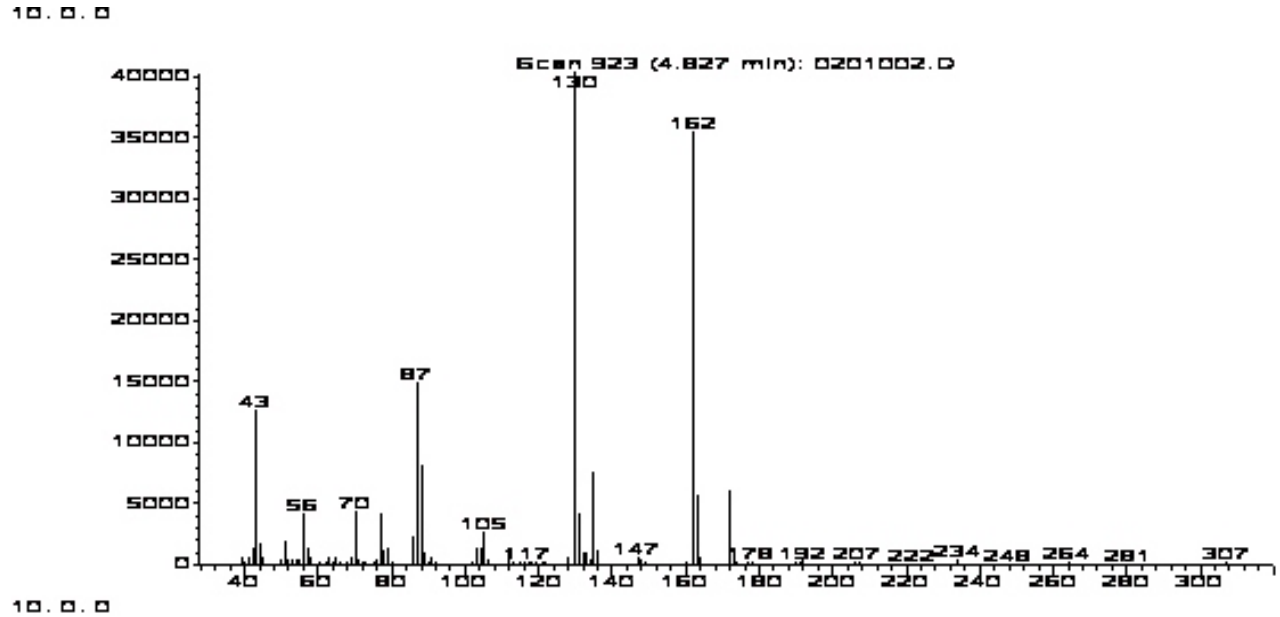

Figure 4. Mass Spectrum of the Double-Acetylated Derivative of MDHOET HCl.

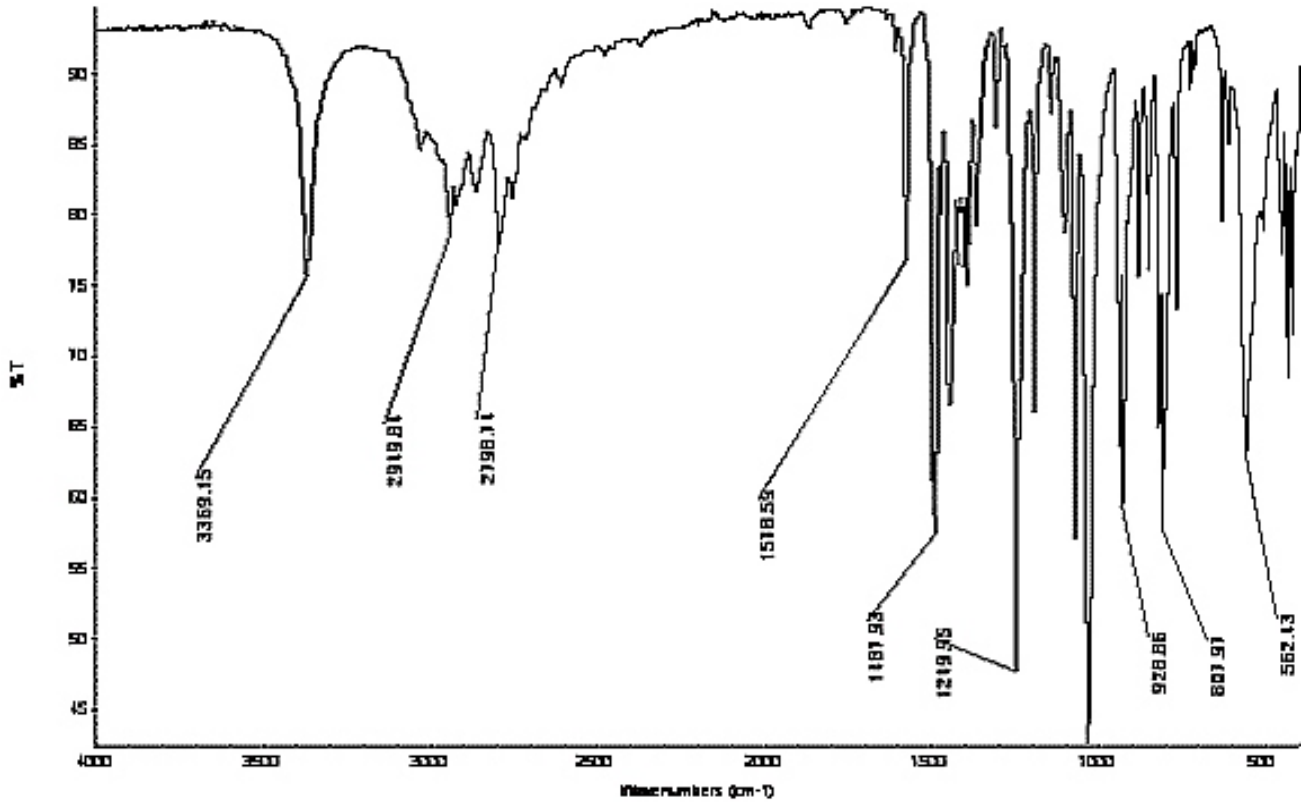

Figure 5. Uncorrected FTIR-ATR Spectrum of Synthesized Reference MDHOET HCl.

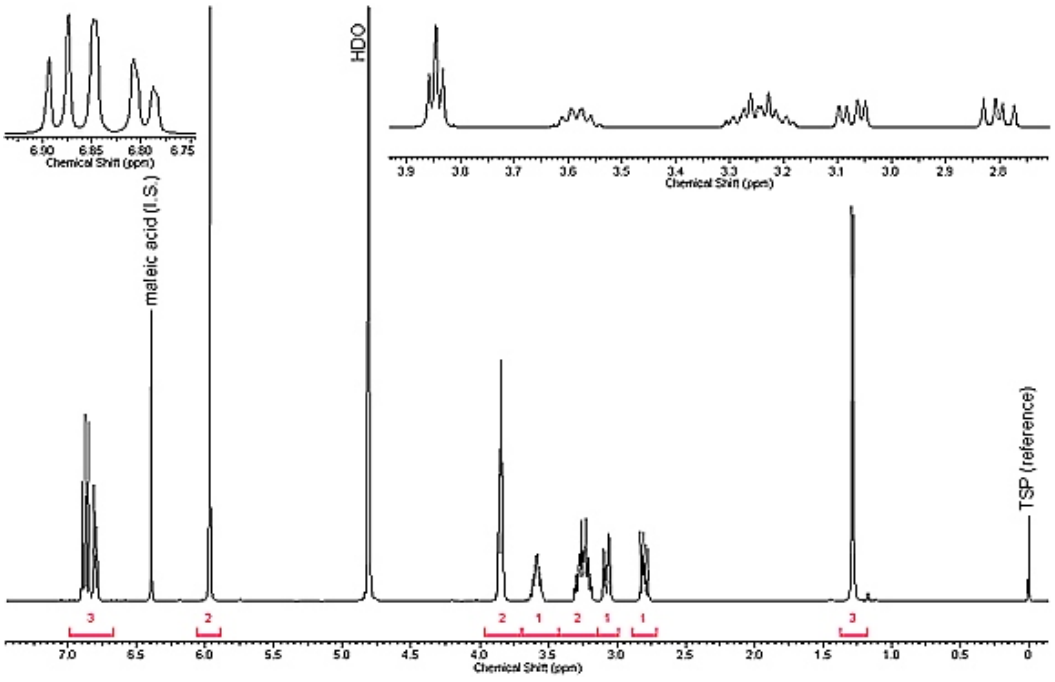

Figure 6. Proton NMR Spectrum of Synthesized Reference MDHOET HCl in D_2O.

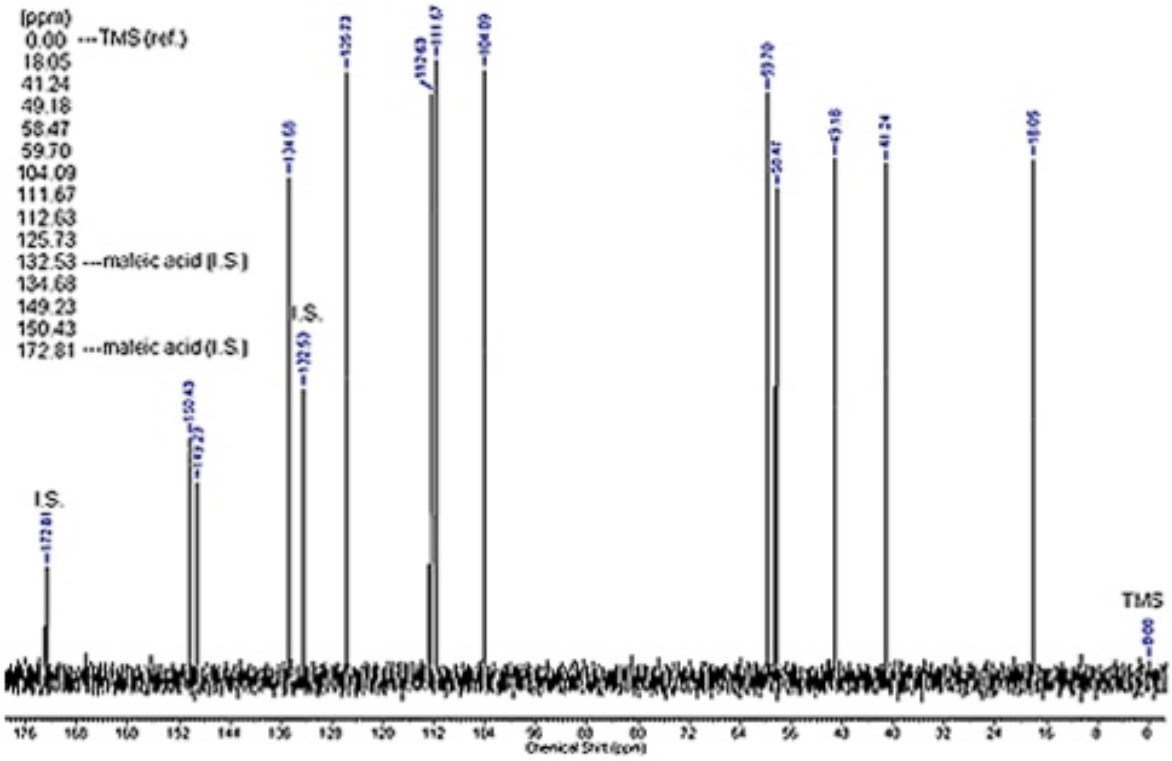

Figure 7. Carbon NMR Spectrum of Synthesized Reference MDHOET HCl in D_2O.

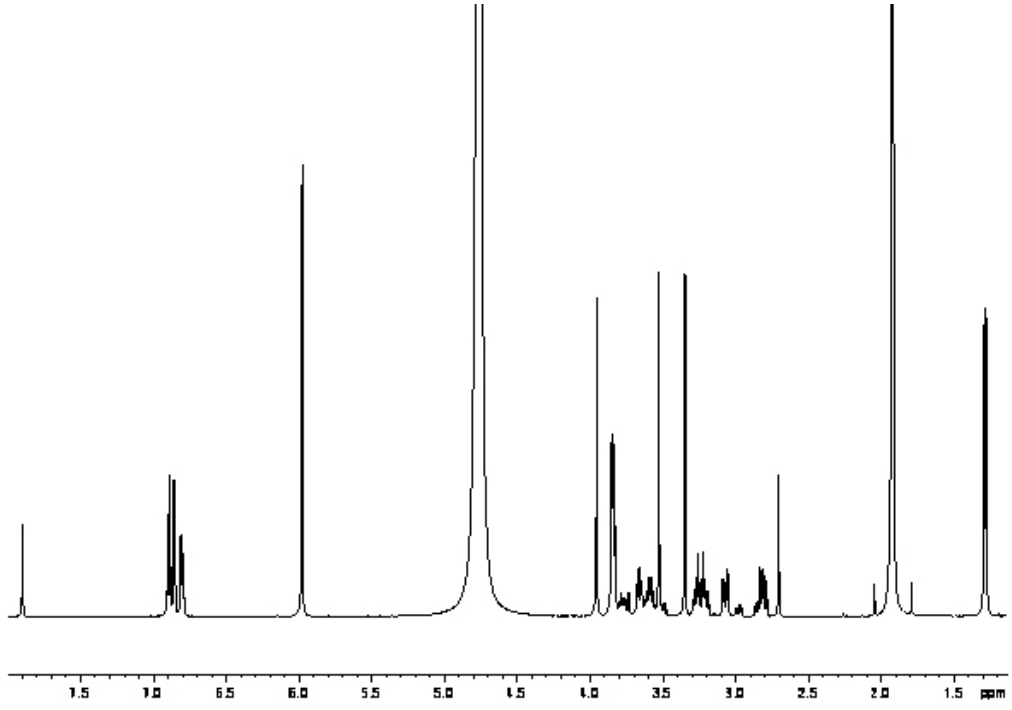

Figure 8. Proton NMR Spectrum of one of the French "Euro" Tablet in D_2O (Containing MDHOET HCl, MDMA HCl, Caffeine, and Sorbitol).

Technical Note

Analysis and Characterization of Psilocybin and Psilocin Using Liquid Chromatography - Electrospray Ionization Mass Spectrometry (LC-ESI-MS) with Collision-Induced-Dissociation (CID) and Source-Induced-Dissociation (SID)

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ABSTRACT: The rapid analysis of psilocybin and psilocin using liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS) is presented. Full-scan MS experiments provide molecular weight information, but little fragmentation. Similarly, collision-induced-dissociation (CID) experiments generate only a limited number of fragments. However, source-induced-dissociation (SID) experiments result in more extensive fragmentation. The combined results from these complementary techniques allows for the more complete characterization of psilocin and the thermally-labile psilocybin.

KEYWORDS: Psilocybin, Psilocin, Thermally-Labile, Liquid Chromatography-Mass Spectrometry (LC/MS), Tandem Mass Spectrometry, Collision-Induced-Dissociation, Source-Induced-Dissociation.

Introduction

Direct analysis of thermally-labile compounds using gas chromatography - mass spectrometry (GC/MS) is limited or impossible due to degradation caused by the high injector and column temperatures. Although derivatization is useful in many cases, direct analysis of the compounds of interest is always preferable. The development of the electrospray ionization (ESI) technique has enabled the transfer of thermally-labile compounds from solution into the gas phase without significant degradation [1]. The use of ESI in combination with liquid chromatography - mass spectrometry (LC/MS) techniques therefore provides a powerful analytical tool for the analysis of heat sensitive compounds.

A "classic" example of such a thermally labile compound is psilocybin, a powerful hallucinogen found in over 100 species of mushrooms, including *Psilocybe azurescens*, *Strophoria cubensis*, and *Psilocybe mexicana* [2-4]. Psilocybin is the phosphorylated ester of psilocin (Figure 1). The phosphate ester in psilocybin is delicate, and analysis of psilocybin-containing substrates by standard analytical techniques is therefore problematic. Because both psilocybin and psilocin are classified under Schedule I of the United States Controlled Substances Act, their analyses are important for forensic/law enforcement purposes.

Previous reports on the analysis of hallucinogenic mushrooms include descriptions of various extractions of the material, followed by instrumental analysis using liquid chromatography, gas chromatography, and mass spectrometry techniques [5-11]. In actuality, most of these analyses allow the detection of psilocin only, since the psilocybin did not survive the extraction and/or analysis. In addition, both psilocin and psilocybin have been indirectly analyzed following derivatization [12], and more recently, directly analyzed with the use of LC/MS

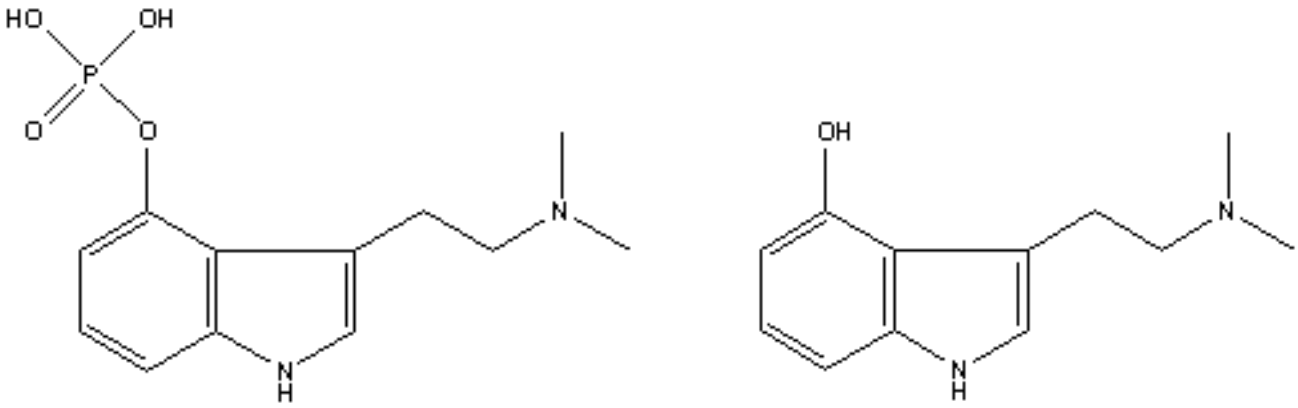

Figure 1. Chemical Structures of Psilocybin (Left) and Psilocin (Right).

and tandem mass spectrometry (MS/MS) techniques [13]. Unfortunately, even these advanced techniques give limited information beyond molecular weights.

However, when combined with multiple fragmentation techniques, LC-ESI-MS enables more complete characterization of thermally labile compounds. Collision-induced-dissociation (CID; MS/MS) can generate some fragment ions. For small compounds like tryptamines, a greater amount of dissociation and information can usually be obtained by performing source-induced-dissociation (SID) experiments, where ions are fragmented within the electrospray interface before they reach the mass analyzer.

Herein, a method is presented for the separation and characterization of psilocybin and psilocin using LC-ESI-MS in combination with CID (MS/MS) and SID experiments.

Experimental

Experiments were performed using a ThermoFinnigan LCQ Advantage MAX quadrupole ion-trap mass spectrometer equipped with an electrospray ionization source and interfaced to a Surveyor HPLC system (solvent pump, autosampler/column, and photodiode array detector).

Liquid chromatography conditions were investigated in order to provide for the best separation possible during the shortest analysis time. Separations were performed using a Phenomenex Prodigy column (150 x 4.6 mm; 5 μm), and an isocratic flow of 89 % Solvent A and 11 % Solvent B. Solvent A is H_2O with 0.1 % (v/v) formic acid, while Solvent B is acetonitrile with 0.1 % (v/v) formic acid. The eluent flow rate was 400 μL/minute. Standard solutions of psilocin (Sigma Chemical) and psilocybin (Alltech) were prepared at concentrations of 20 μg/mL in Solvent A.

Sample injections of 10 μL were loaded into the isocratic flow and introduced into the mass spectrometer using the ESI interface. The transfer capillary was maintained at a temperature of 250 °C, while the capillary and tube lens were kept at 20 and 15 V, respectively. Nitrogen (99 %; 100 ± 20 psi) was used as both the sheath and auxiliary gas, and operated at 50 and 20 units, respectively.

Mass spectrometry data were collected in the positive ion mode using the full-scan and tandem (MS/MS) modes in order to provide both molecular weight and structural information. MS/MS experiments were performed using

a standard collision energy of 35 eV. Source-induced-dissociation (SID) experiments were performed using variable energies between 25 and 40 eV. Helium (99.999 %; 40 ± 10 psi) was used as both the trapping and collision gas.

Instrument control, data collection and analysis were performed using the Xcalibur software (version 1.4) provided by the instrument manufacturer.

Results and Discussion

Figure 2 shows the total ion chromatogram (TIC), UV-based chromatogram, and full-scan ESI mass spectral data obtained during a 10 minute isocratic separation (11 % Solvent B). Psilocybin elutes at 5.5 minutes, while psilocin elutes at 7.4 minutes. Clear separation is obtained and the full-scan spectra show the pseudo-molecular $(M+H^+)^+$ ions for psilocybin and psilocin at *m/z* 285 and 205, respectively. The full-scan ESI spectrum for psilocybin also shows a peak at *m/z* 307, corresponding to the $(M+Na^+)^+$ ion. The ESI data for psilocin also shows a small fragment ion at *m/z* 160. This experiment allows for the separation, detection, and determination of the molecular weights for these two compounds.

Figure 3 shows the tandem (MS/MS) fragmentation data obtained during the chromatographic separation of psilocybin and psilocin. During standard collision-induced dissociation (CID) experiments at 35 eV, psilocybin dissociates into two main fragments. The fragment observed at *m/z* 205 corresponds to loss of a neutral phosphate moiety (HPO_3; 80 Da), while the fragment at *m/z* 240 results from the loss of neutral dimethylamine ($HN(CH_3)_2$; 45 Da). Dissociation of psilocin is dominated by the loss of dimethylamine, producing a fragment at *m/z* 160. The dissociation patterns observed for psilocybin and psilocin are typical of tryptamine-type fragmentations previously reported [14,15], and are also in agreement with recent tandem MS experiments using a triple-quadrupole mass analyzer [13].

Source-induced-dissociation (SID) experiments provide an alternative fragmentation technique for compounds that show a limited number of fragments under MS/MS conditions. During SID, electrospray-generated ions are subjected to high-energy collisions with the background gas within the relatively high-pressure capillary-skimmer region of the ionization interface. As a result, characteristic fragments are generated and mass analysis provides additional structural information. Figures 4 and 5 show SID data obtained for psilocybin and psilocin, respectively, using dissociation energies of 25, 30, 35, and 40 eV.

For psilocybin, SID experiments result in the formation of multiple fragments. In addition to the fragments at *m/z* 240 and 205 observed with CID, other characteristic fragments are observed at *m/z* 222, 160, 142, and 115. The former three fragments correspond to loss of H_2O, HPO_3, and H_3PO_4 from the *m/z* 240 species, while the peak at *m/z* 115 is characteristic of the indole moiety. The fragment at *m/z* 160 can also be generated from the loss of $HN(CH_3)_2$ from *m/z* 205. As observed in the SID spectra, the sodiated psilocybin ion at *m/z* 307 does not undergo significant dissociation under these conditions. This is probably reflective of the greater stability of this species, due to the higher affinity of the phosphate group for sodium.

Increased fragmentation is also observed from SID experiments on psilocin. After production of *m/z* 160, subsequent dissociation reactions result in the appearance of fragments at *m/z* 142 and 132, due to loss of H_2O and $CH_2{=}CH_2$, respectively. The fragments observed at *m/z* 115 and 117 are again characteristics of the indole group, with and without the loss of H_2.

Conclusions

The presented LC-ESI-MS techniques allow for the direct, facile separation and identification of psilocybin and psilocin. The LC conditions used separated the two compounds in less than 8 minutes. Mass spectrometry

experiments in the full-scan and MS/MS mode provided molecular weight and partial structural information. Source-induced-dissociation experiments provided complementary fragment information, allowing for a much more complete structural characterization of the two species. The presented techniques illustrate the utility of LC-ESI-MS, CID, and SID experiments for the analysis and characterization of thermally-labile compounds.

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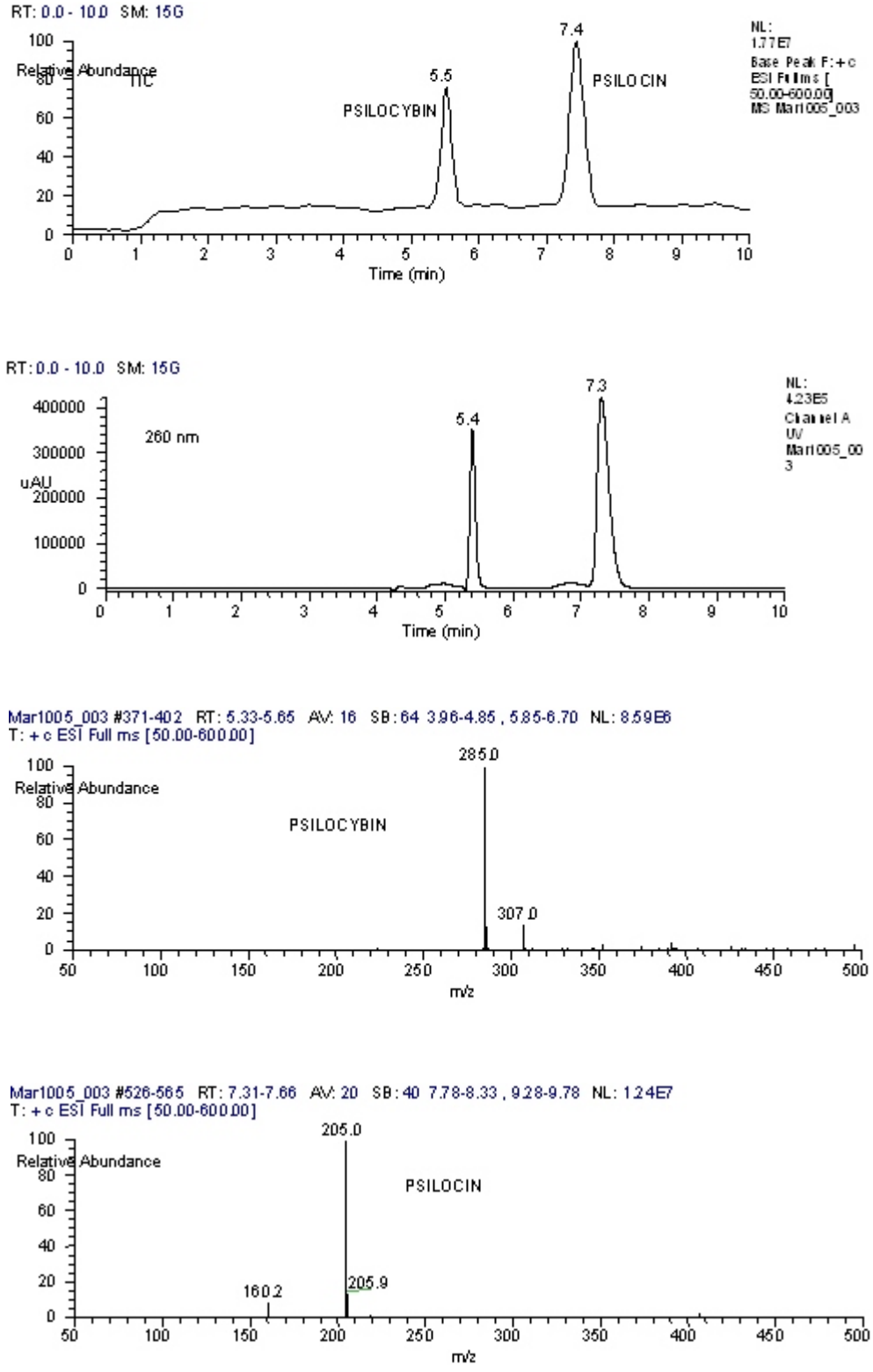

Figure 2. Total Ion Chromatogram, UV Chromatogram, and Full-Scan ESI Spectra Showing the Separation and Detection of Psilocybin (MW = 284) and Psilocin (MW = 204).

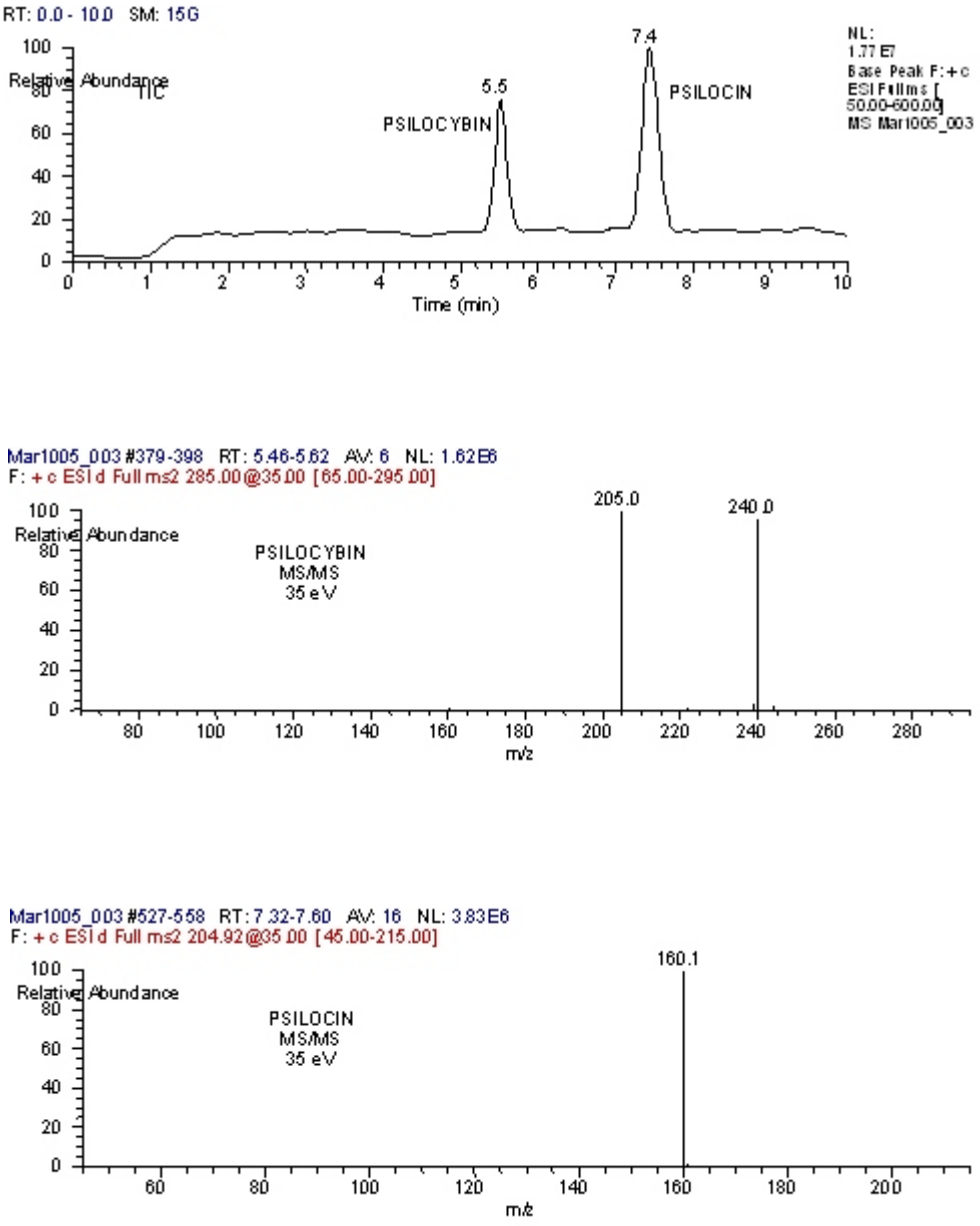

Figure 3. Total Ion Chromatogram and MS/MS Spectra Showing the Fragmentation of Psilocybin and Psilocin under Standard Collision-Induced-Dissociation Conditions at 35 eV.

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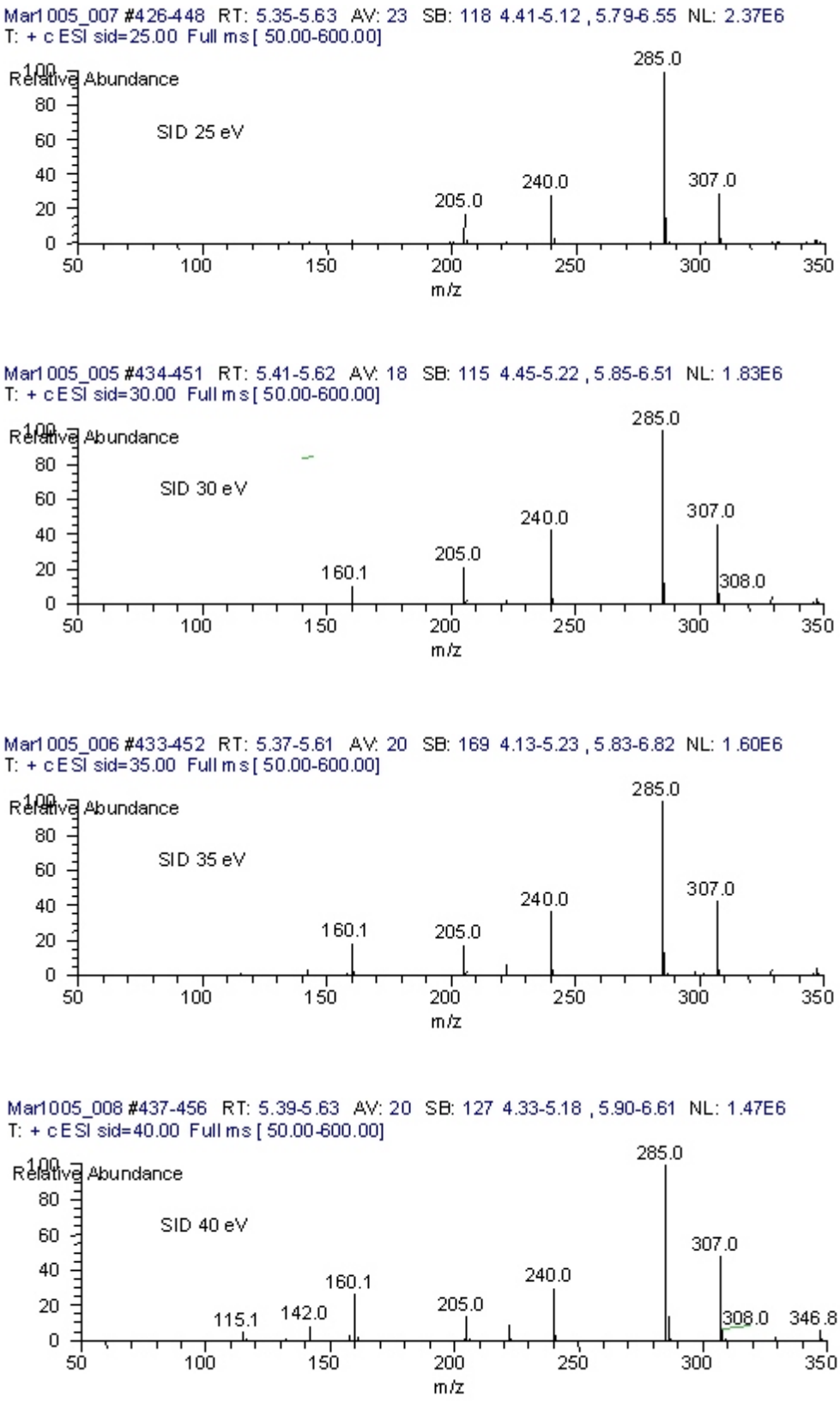

Figure 4. SID Spectra for Psilocybin Obtained Using Fragmentation Energies of 25, 30, 35, and 40 eV.

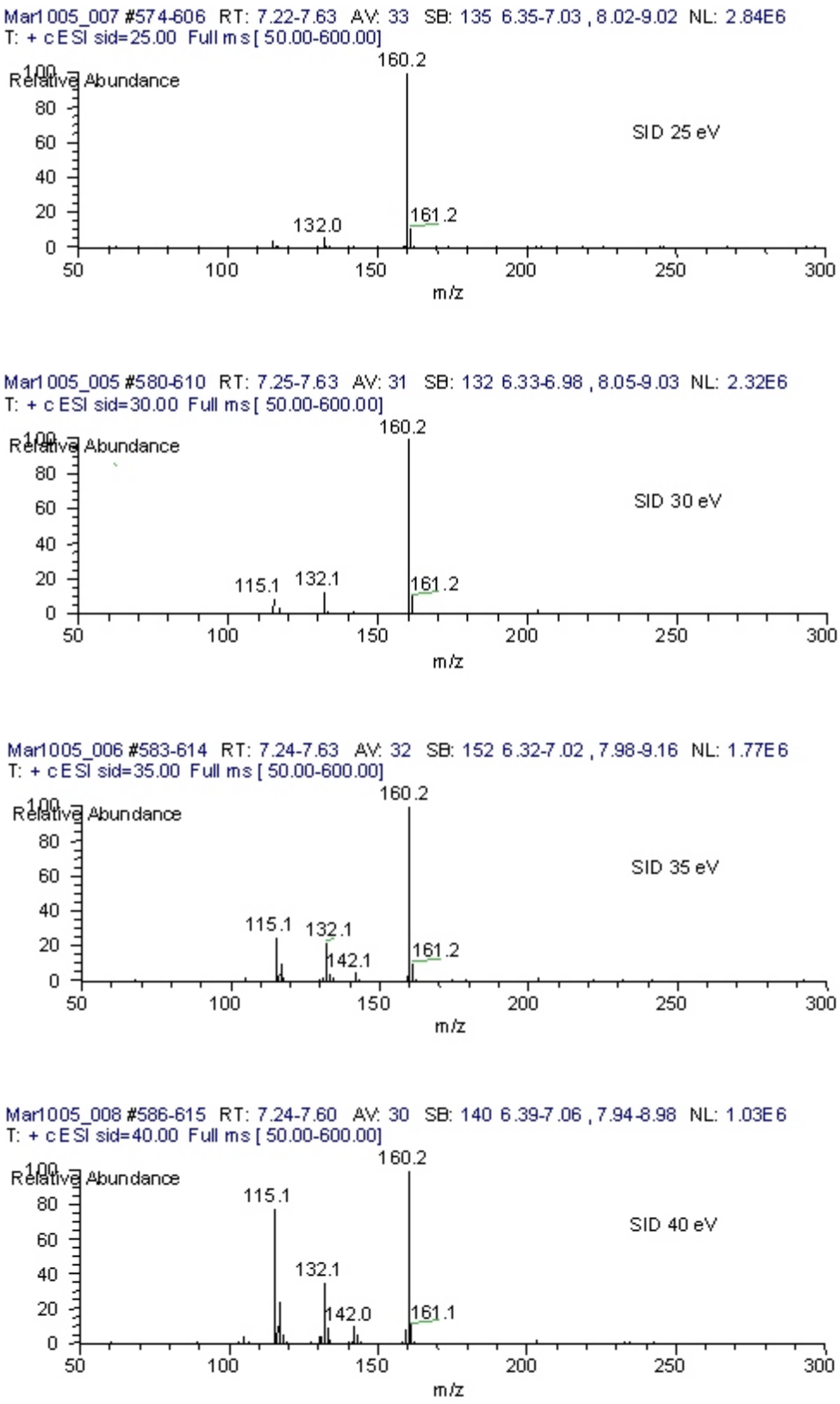

Figure 5. SID Spectra for Psilocin Obtained Using Fragmentation Energies of 25, 30, 35, and 40 eV.

Technical Note

Specificity of the Duquenois-Levine and Cobalt Thiocyanate Tests Substituting Methylene Chloride or Butyl Chloride for Chloroform

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ABSTRACT: The use of alternative solvents in the Duquenois-Levine and Cobalt Thiocyanate tests were explored due to substandard results with recently purchased lots of chloroform. Methylene chloride provided satisfactory results when substituted for chloroform in both tests. Butyl chloride provided satisfactory results in the Duquenois-Levine test.

KEYWORDS: Duquenois-Levine, Cobalt Thiocyanate, Marijuana, Cocaine, Chloroform, Methylene Chloride, n-Butyl Chloride

Introduction

The Rapid Modified Duquenois-Levine test and Cobalt Thiocyanate test (Scott test) are proven screening tests for the presence of marijuana and cocaine, respectively. The organic solvent traditionally used in these tests is chloroform. However, chloroform recently purchased by this laboratory produced little or no color change when performing the Duquenois-Levine and Cobalt Thiocyanate tests. Shortly after opening, this chloroform became yellow to green in color, at which point it was unsuitable to perform these tests. According to the manufacturer, this unusual decomposition of the chloroform was due to insufficient amounts of preservatives. This experience led to the investigation of using alternative organic solvents, specifically methylene chloride and n-butyl chloride, in the Duquenois-Levine and Cobalt Thiocyanate tests.

Experimental

Reagents and Solvents

Hydrochloric acid, methylene chloride, and n-butyl chloride were obtained from Fisher Scientific. Acceptable quality chloroform was obtained from OmniSolv. The Duquenois reagent was prepared by adding 10 grams of vanillin and 5 milliliters of acetaldehyde to 500 milliliters of ethanol. The vanillin, acetaldehyde, and ethanol were obtained from Kodak, EM Science, and Fisher Scientific, respectively. The cobalt thiocyanate reagent was prepared by dissolving ten grams of cobalt (II) thiocyanate in a mixture of 490 milliliters of distilled water and 500 milliliters of glycerin. The cobalt (II) thiocyanate and glycerin were obtained from Aldrich Chemical and Fisher Scientific, respectively.

Procedures

The Duquenois-Levine test was performed on 17 different substances using chloroform, methylene chloride, and butyl chloride as the organic solvent. The test was performed by placing approximately 10 to 20 milligrams of a target substance in a glass test tube, then 10 drops of the Duquenois reagent. After shaking, 10 drops of concentrated hydrochloric acid were added, and the tube was again shaken. Any color that resulted after the

hydrochloric acid step was recorded. Twenty drops of chloroform were then added, and the tube was vortexed, then allowed to settle and separate into two layers. Any color that transferred into the organic layer was recorded (Table 1). This procedure was repeated for each target substance by substituting methylene chloride or butyl chloride for chloroform.

The cobalt thiocyanate test was performed on 14 different substances using chloroform, methylene chloride, and butyl chloride. The test was performed by placing approximately 2 to 4 milligrams of a target substance in a glass test tube, then 5 drops of cobalt thiocyanate reagent. After shaking,1 or 2 drops of concentrated hydrochloric acid were added, and the tube was again shaken. Ten drops of chloroform were then added, and the tube was vortexed, then allowed to settle and separate into two layers. The final color of the chloroform (organic) layer was recorded (Table 2). This procedure was repeated for each target substance by substituting methylene chloride or butyl chloride for chloroform.

Results and Discussion

The results for the Duquenois-Levine test using either methylene chloride and butyl chloride were consistent with results obtained using chloroform. The marijuana became purple with the addition of the Duquenois reagent and hydrochloric acid. Upon addition of the organic solvent, the purple color transferred to the organic layer, indicating a positive test for cannabinoids. The color was consistent in all tests involving marijuana, regardless of the solvent used. None of the remaining 16 substances tested gave the characteristic purple color in the organic solvent layer.

Similarly, the results of the Cobalt Thiocyanate test were equivalent whether chloroform or methylene chloride was used. However, the results for the butyl chloride were mixed. Addition of the cobalt thiocyanate reagent to cocaine hydrochloride resulted in the surface of the particles turning a bright blue (faint blue for cocaine base). The solution changed back to pink upon adding one or two drops of hydrochloric acid and mixing. Addition of 10 drops of chloroform, vortexing, and allowing the solution to settle resulted in a blue organic layer for both cocaine hydrochloride and cocaine base. The test had similar results when methylene chloride was substituted for chloroform. In the case of butyl chloride, however, the organic layer stayed clear, giving an inconclusive test. Diphenhydramine and lidocaine also gave blue organic layers with either chloroform and methylene chloride. These compounds are known false positives for cocaine. However, in the case of butyl chloride, the organic layers were clear for diphenhydramine and white for lidocaine. The other ten materials had consistent negative test results for all three organic solvents.

Conclusions

Methylene chloride may be substituted for chloroform in both the Rapid Modified Duquenois-Levine test and Cobalt Thiocyanate test. Similarly, butyl chloride may be substituted for chloroform in the Duquenois-Levine test. However, butyl chloride was not a reliable substitute solvent for use in the Cobalt Thiocyanate test. Methylene chloride also works well as an extraction solvent in place of chloroform.

[Tables 1 and 2 Follow.]

Table 1. Duquenois-Levine Test Results

	Chloroform	Methylene Chloride	Butyl Chloride
Material	aqueous/organic	aqueous/organic	aqueous/organic
Allspice	brown/clear	brown/clear	brown/clear
Celery Flakes	yellow/clear	yellow/clear	yellow/clear
Chamomile	yellow/clear	yellow/clear	yellow/clear
Chamomile Tea	yellow/clear	green/clear	green/clear
Coffee	brown/clear	brown/clear	brown/clear
Dill Seed	yellow/clear	yellow/clear	yellow/clear
Hops	yellow/clear	yellow/clear	yellow/clear
Ginger	orange/orange	orange/orange	orange/clear
Ginseng	brown/brown	green/clear	green/clear
Marijuana	purple/purple	purple/purple	purple/purple
Marjoram	yellow/clear	green/clear	green/clear
Mint	green/clear	green/clear	green/clear
Sage	yellow/clear	yellow/clear	yellow/clear
Salvia Divinorum	green/clear	brown/clear	green/clear
Thyme	yellow/clear	green/clear	green/clear
Tobacco	brown/clear	brown/clear	brown/clear
White Pepper	orange/yellow	orange/yellow	orange/yellow

Table 2. Cobalt Thiocyanate Test Results

	Chloroform	Methylene Chloride	Butyl Chloride
Material	organic layer	organic layer	organic layer
Benzocaine	clear	clear	clear
Cocaine	blue	blue	clear
Cocaine Base	blue	blue	clear
Dextrose	clear	clear	clear
Diphenhydramine	blue	blue	clear
Heroin	clear	clear	clear
Inositol	clear	clear	clear
Lidocaine	blue	blue	white
Methamphetamine	clear	clear	clear
MDMA	clear	clear	clear
Morphine	clear	clear	clear
Procaine	clear	clear	clear
Soap	clear	clear	clear
Sodium Bicarbonate	clear	clear	clear

* * * * *

Technical Note

The Identification of 1-Dehydromethandrostenolone

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ABSTRACT: A recent steroid seizure was identified as 1-dehydromethandrostenolone, a positional isomer of methyltestosterone. GC/MS results are reported.

KEYWORDS: 1-Dehydromethandrostenolone, 1,(5α)-Androsten-17α-methyl-17ß-ol-3-one, Anabolic Steroid, GC/MS, Forensic Chemistry

Introduction

The Naval Criminal Investigative Service (NCIS) Regional Forensic Laboratory (San Diego, California) recently received 22 white capsules containing a dark granular material, a suspected steroid (see Photo 1). The exhibits were seized in San Diego by NCIS personnel from a member of the U.S. military who was believed to a member of a steroid trafficking group (details not available). Analysis of a methanolic extract by GC/MS indicated a mixture of niacinamide and an unknown steroid with the same molecular weight as methyltestosterone (302), but with a different retention time and fragmentation pattern. Library searches on the unknown spectrum were inconclusive.

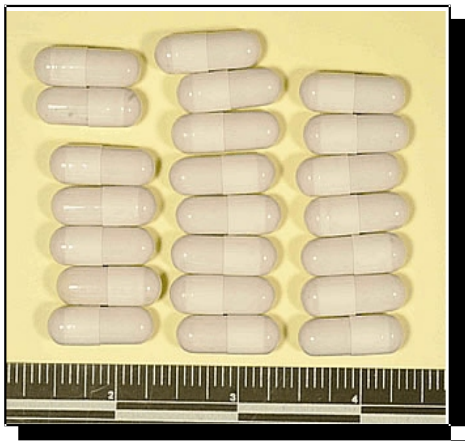

Photo 1

Experimental

1-Dehydromethandrostenolone standard was acquired from Steraloids (Code A4450-000; www.steraloids.com/pages/page028.html). GC/MS data were acquired using an HP 6890 GC interfaced with a HP 5972A MSD. The GC was equipped with a 30 m x 250 µm x 25.0 µm J&W DB-5MS capillary column, with an initial temperature of 70 ºC (2 minutes), then a ramp of 20 ºC/minute up to 300 ºC, then held for 15 minutes.

Results and Discussion

Figure 1a shows the Total Ion Chromatogram resulting from analysis of the methanolic extract. The first peak was identified as niacinamide (Figure 1b). The second peak (Figure 2a) was apparently isomeric with methyltestosterone (Figure 2b). After consultation with DEA laboratory personnel, the second peak was tentatively identified as 1-dehydromethandrostenolone (1,(5α)-androsten-17α-methyl-17ß-ol-3-one). Analysis of a commercial standard confirmed 1-dehydromethandrostenolone (Figure 2c; Note that the variations in relative mass fragment abundances between Figures 2a and 2c are due to acquisition on different mass spectrometers).

1-Dehydromethandrostenolone is controlled under Schedule III of the U.S. Controlled Substances Act. It is a positional isomer of methyltestosterone, with the only difference being the location of the double bond within the steroid "A" ring (see Figure 3). In 1-dehydromethandrostenolone the double bond is between the 1 and 2 carbons, whereas in methyltestosterone it is between the 4 and 5 carbons. Both 1-dehydromethandrostenolone and methyltestosterone are classified as anabolic steroids.

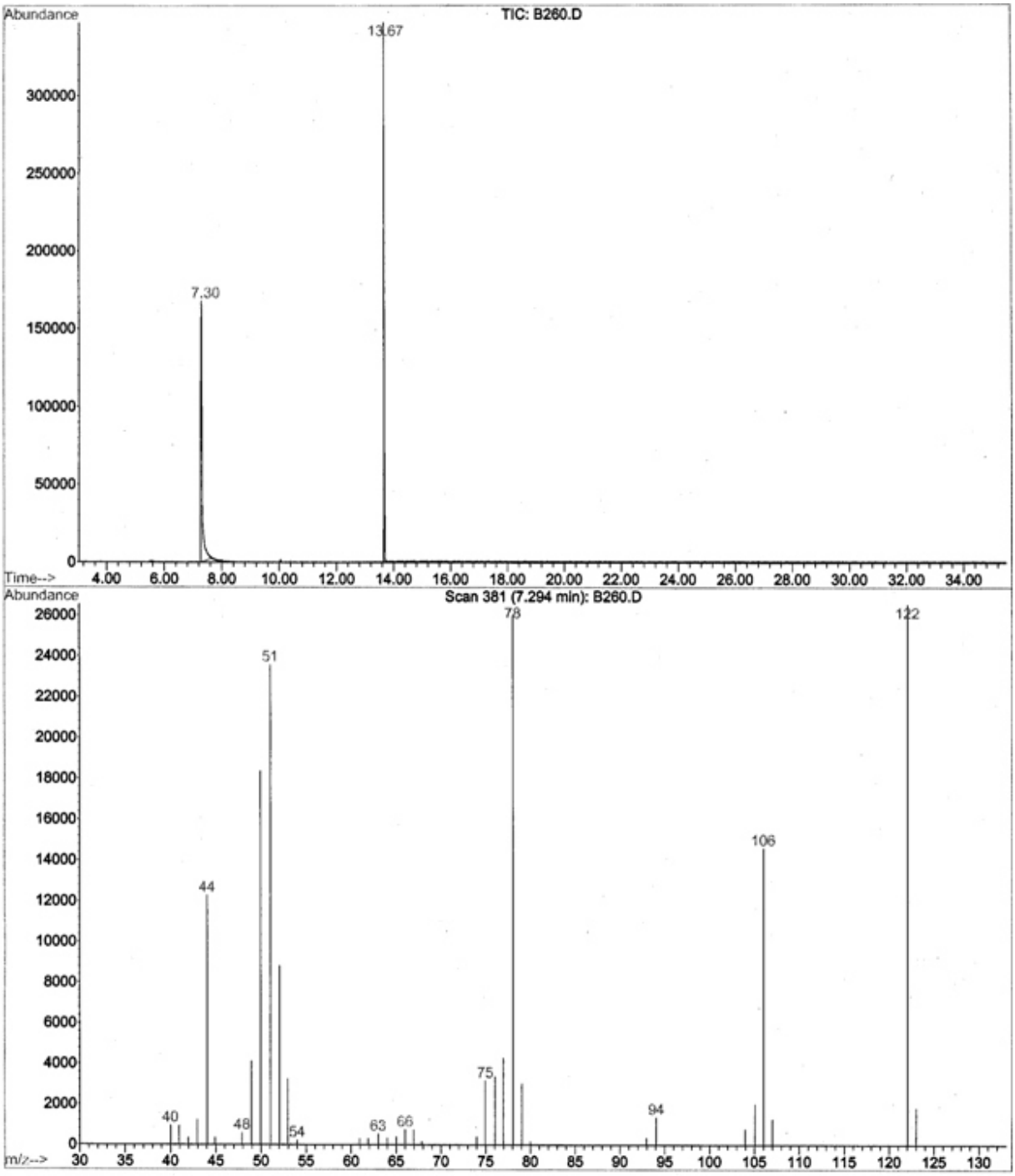

Figure 1. (a) Total Ion Chromatogram; (b) Mass Spectrum of Niacinamide (7.3 Minutes).

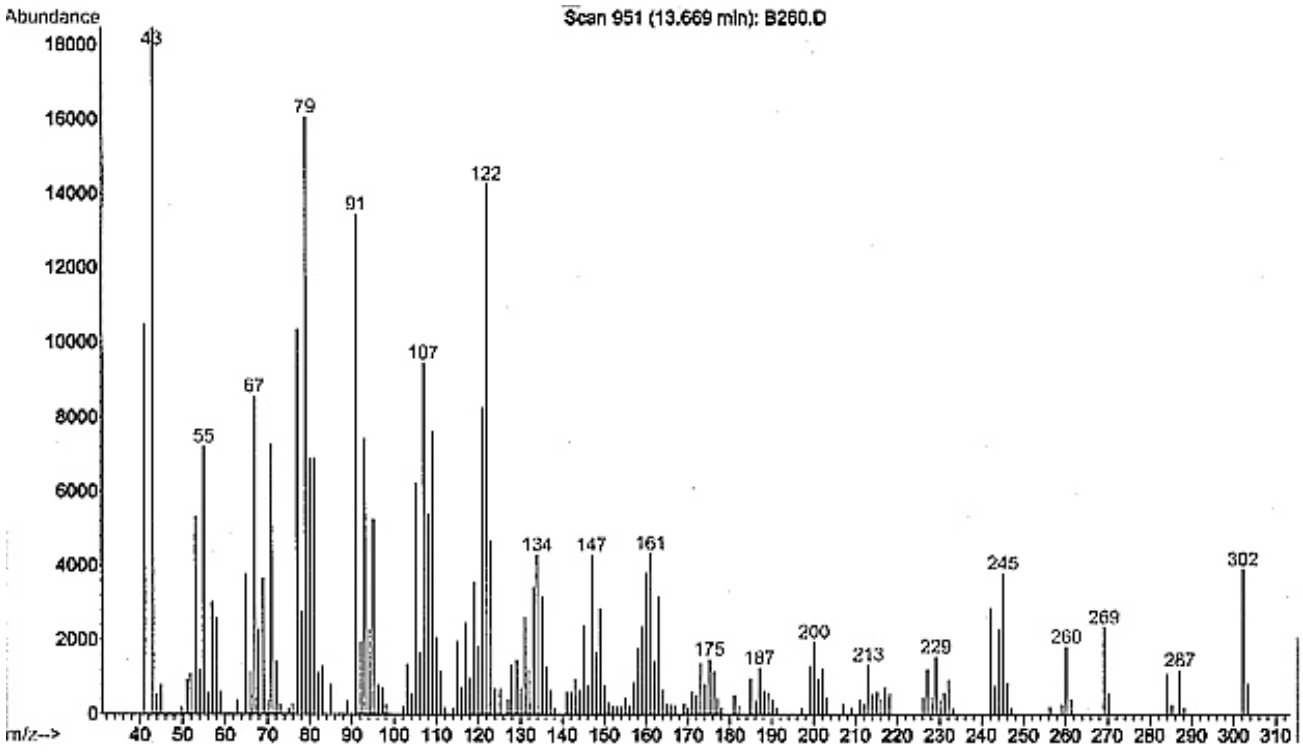

Figure 2a. Mass Spectrum of Unknown Steroid (13.67 Minutes in Figure 1a).

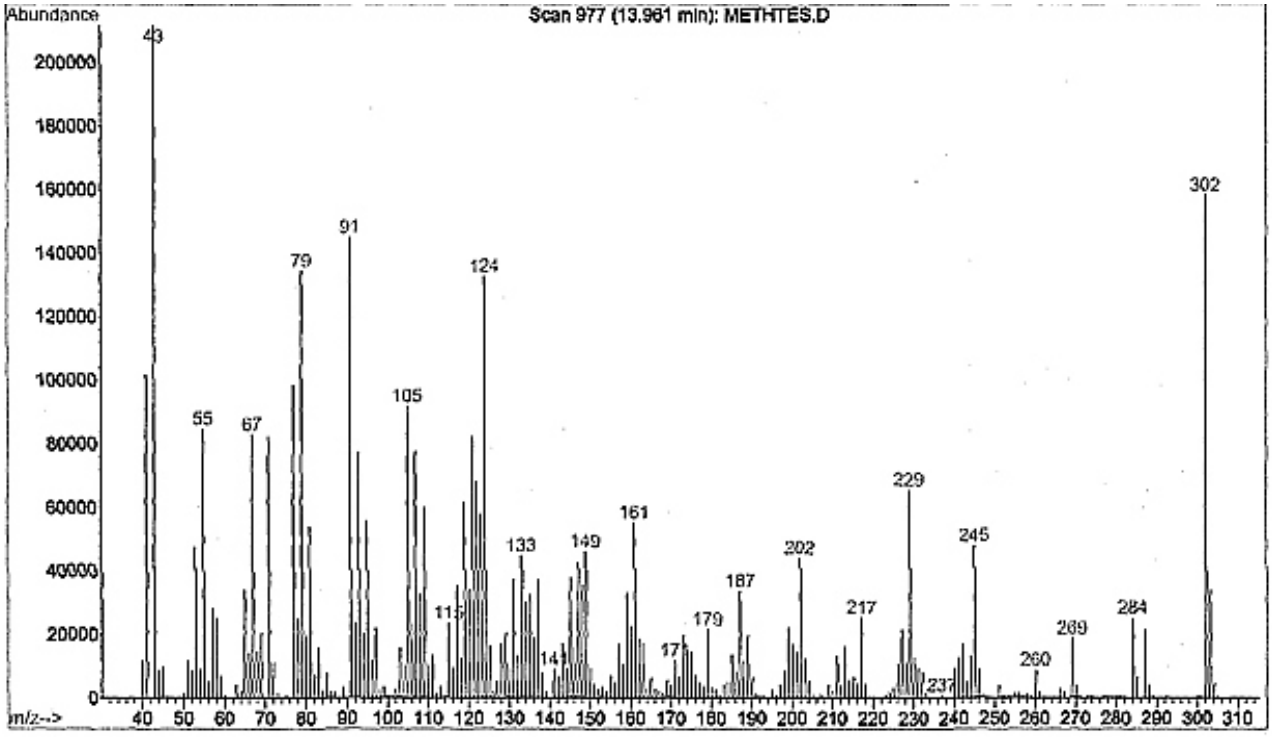

Figure 2b. Mass Spectrum of Methyltestosterone Standard. Contrast the Peaks at *m/z* 107 and 122 in Figure 2a Versus the Peaks at *m/z* 105 and 124 in this Spectrum.

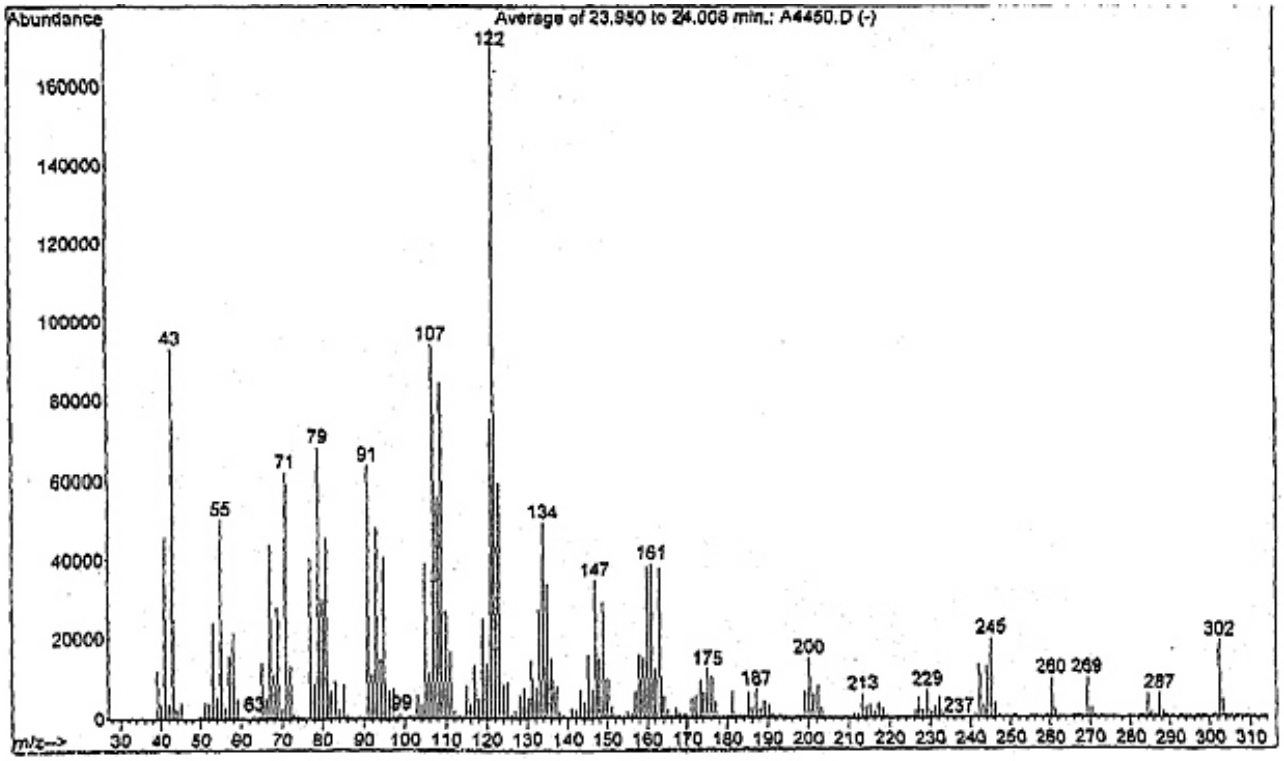

Figure 2c. Mass Spectrum of 1-Dehydromethandrostenolone Standard.

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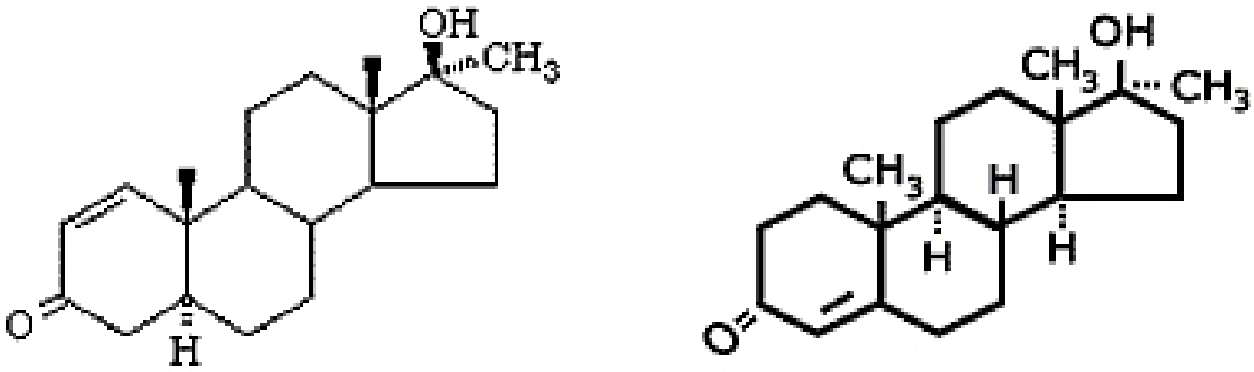

1-Dehydromethandrostenolone Methyltestosterone

Figure 3. Structural Formulae

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[1] - The NCIS San Diego Laboratory ceased operations in early 2006.