Detection of Phenethylamine, Amphetamine, and Tryptamine Imine By-Products from an Acetone Extraction

Mary A. Yohannan* and Arthur Berrier
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166
[email: mary.a.yohannan -at- usdoj.gov]

ABSTRACT: The formation of imine by-products from phenethylamines, amphetamines, and tryptamines upon an acetone extraction is presented. These imine by-products were characterized using GC/MSD and exhibited preferential cleavage at the α-carbon of the alkyl chain. Further characterization of the imine by-products of phenethylamine and tryptamine was done using IR and NMR.

KEYWORDS: phenethylamine, tryptamine, imine, acetone, schiff base, drug chemistry, forensic chemistry

In most forensic laboratories, the solvents used to extract drugs are chosen based upon their solubility properties and their ability to not interact with the drug. In fact, there are very few publications where a solvent used to extract a drug reacts with the drug and forms by-products [1-3].

This laboratory recently discovered that an additional component was formed when acetone was used to extract a sample containing a known tryptamine. Analysis by gas chromatography/mass spectroscopy (GC/MS) of the acetone extract yielded an extra peak in the total ion chromatogram that was approximately half the abundance of the known tryptamine peak. The known tryptamine peak was identified from the fragmentation pattern and retention time. The unknown peak's fragmentation pattern exhibited a molecular ion that was 40 mass units higher than that of the known tryptamine molecular ion and was subsequently identified as the imine formed from the reaction with acetone. Primary aliphatic amines are known to react with aldehydes and ketones, typically in the presence of an acid catalyst, to produce an imine, or a carbon-nitrogen double bond, Figure 1 [4, 5].

This study reports that the following phenethylamines, amphetamines, and tryptamines form imines with acetone under mild conditions: phenethylamine; 2,5-dimethoxy-4-iodo-phenethylamine (2C-I); 2,5-dimethoxy-4-ethylthio-phenethylamine (2C-T-2); 2,5-dimethoxy-4-ethyl-phenethylamine (2C-E); 2,5-dimethoxy-4-n-propylthio-β-phenethylamine (2C-T-7); 2,5-dimethoxy-4-chloro-phenethylamine (2C-C); 2,5-dimethoxy-4-bromo-phenethylamine (2C-B); 2,5-dimethoxyamphetamine; 4-methoxyamphetamine, 3,4-methylenedioxyamphetamine (MDA); amphetamine; tryptamine; α-methyl-tryptamine; and 5-methoxy-α-ethyl-tryptamine. This study also reports that the GC/MSD of all imine compounds showed preferential cleavage at the α-carbon on the alkyl chain. In addition to GC/MS, the imines formed from phenethylamine base and tryptamine base were characterized by Fourier transform-infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectroscopy.

Experimental

Solvents, Chemicals, and Materials

Acetone was ACS/HPLC grade from Burdick and Jackson Laboratories (Muskegon, MI). Phenethylamine base and tryptamine base were obtained from Sigma-Aldrich Chemicals (Milwaukee, WI). All other compounds were obtained from the authentic reference collection of the DEA Special Testing and Research Laboratory.

Gas Chromatography/Mass Spectrometry (GC/MS)

GC/MS analyses were performed using an Agilent Model 5975C inert XL mass-selective detector (MSD) interfaced with an Agilent Model 7890A gas chromatograph. The GC system was fitted with a 30 m x 0.250 mm ID fused-silica capillary column coated with HP-5 (0.25 μm) supplied by J & W Scientific. The injection port temperature was maintained at 280ºC and was operated in the split mode (25:1). The oven temperature was programmed as follows: initial temperature, 90ºC; initial hold, 2 minutes; program rate 14ºC/minute; final temperature, 300ºC; final hold, 10 minutes. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-550 mass units, and at 2.83 scans/second. The MS source and MSD were maintained at 230ºC and 150ºC, respectively.

Fourier Transform-Infrared Spectroscopy (FTIR)

FTIR analyses were performed using a Thermo Scientific Model Smart Golden Gate attenuated total reflectance (ATR)

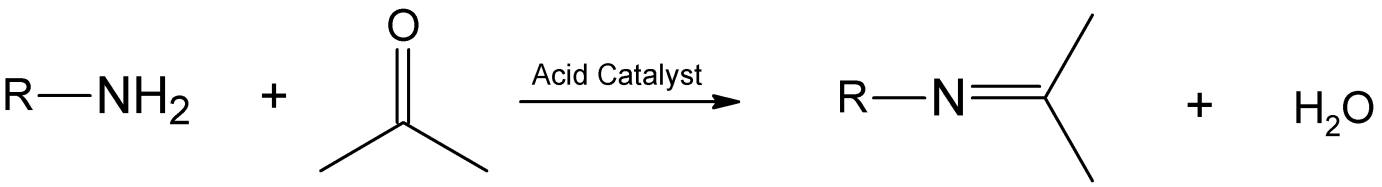

Figure 1 - Simple reaction of a primary amine with acetone to produce an imine.

accessory with KRS-5 focusing elements, single bounce, attached to a Thermo Scientific Model Nicolet IZ10 spectrophotometer. The FTIR parameters were as follows: number of background scans, 32; number of scans, 32; resolution, 4.000; and sample gain, 8.0.

Nuclear Magnetic Resonance Spectroscopy (NMR)

One and two dimensional NMR analyses were performed on a Varian VNMRS 600 MHz NMR using a 3 mm triple resonance Varian indirect detection probe. The samples were prepared in deuterated chloroform containing tetramethylsilane ($CDCl_3$ with TMS, Sigma-Aldrich Chemicals (Milwaukee, WI)). Gradient versions of the two dimensional NMR experiments, HSQC (one bond correlation of hydrogens directly bonded to carbon) and HMBC (correlation of hydrogens 2, 3, or 4 bonds from a carbon) were performed to make the assignments listed in Tables 1-2.

Synthesis

The syntheses of all the imines was performed by dissolving approximately 20 mg of the HCl salt or free base form of the phenethylamine, amphetamine, or tryptamine into 5 mL of acetone in a 20 mL centrifuge tube. Each sample was capped and vortexed for 5 to 10 sec. GC/MS analysis was performed on 1 mL aliquots.

Approximately 4 drops of phenethylamine base and 50 mg of tryptamine base were added to 2 mL GC vials and diluted with acetone to the 1.5 mL mark on the vial. The reactions were allowed to come to room temperature for 8 hours prior to performing GC/MS analysis. The acetone was evaporated with air at room temperature to obtain an oil subsequently used for the FTIR and NMR analyses.

Results and Discussion

Primary amines are known to react readily with aldehydes and ketones to form imines. In this work, a series of primary amine containing drugs, in either the free base or HCl salt forms, were dissolved in acetone and allowed to react prior to GC/MSD analysis. In all cases, an additional peak was observed in the total ion chromatograph after a short period of time, which was identified as the imine product by analysis of its mass spectrum. To further confirm the structure of the reaction products of phenethylamine base and tryptamine base with acetone, analyses by FTIR and NMR spectroscopy were performed.

The mass spectra of the imine products from the phenethylamine–type compounds are shown in Figures 2-5. All the phenethylamine-based imine products show a base peak of *m/z* 70, indicating predominant α-cleavage on the alkyl chain, Figure 6. The imine products with methoxy groups in the two and five positions of the aromatic ring also exhibited a smaller peak that was 31 mass units less than the molecular ion. This is due to the loss of one of the methoxy groups.

The mass spectra of the imine products formed from the

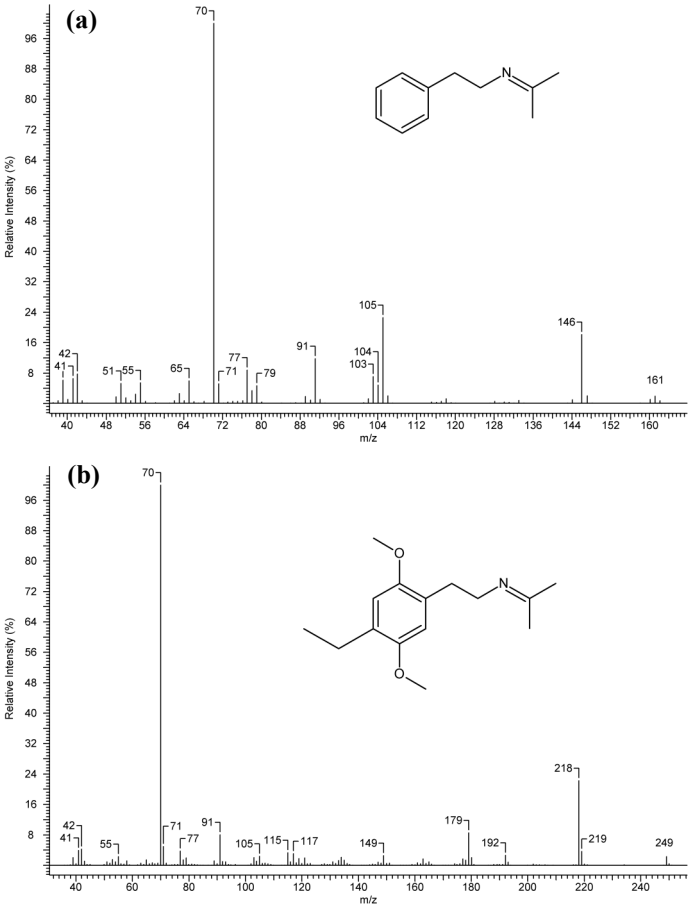

Figure 2 - Mass spectra of (a) N-isopropylidenephenethyl-amine and (b) 2,5-dimethoxy-4-ethyl-N-isopropylidene-phenethylamine.

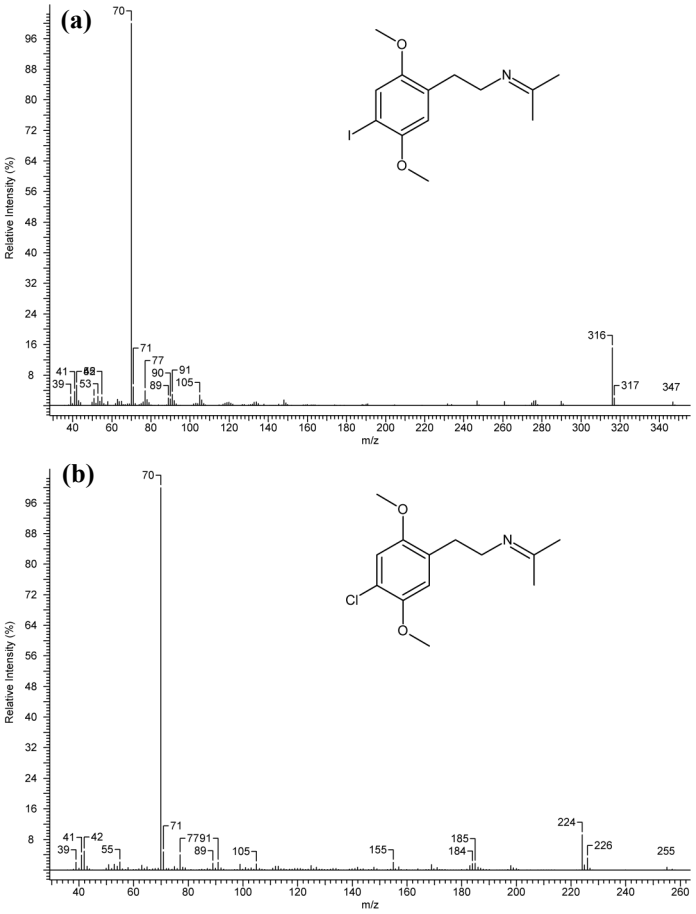

Figure 3 - Mass spectra of (a) 2,5-dimethoxy-4-iodo-N-isopropylidenephenethylamine and (b) 2,5-dimethoxy-4-chloro-N-isopropylidenephenethylamine.

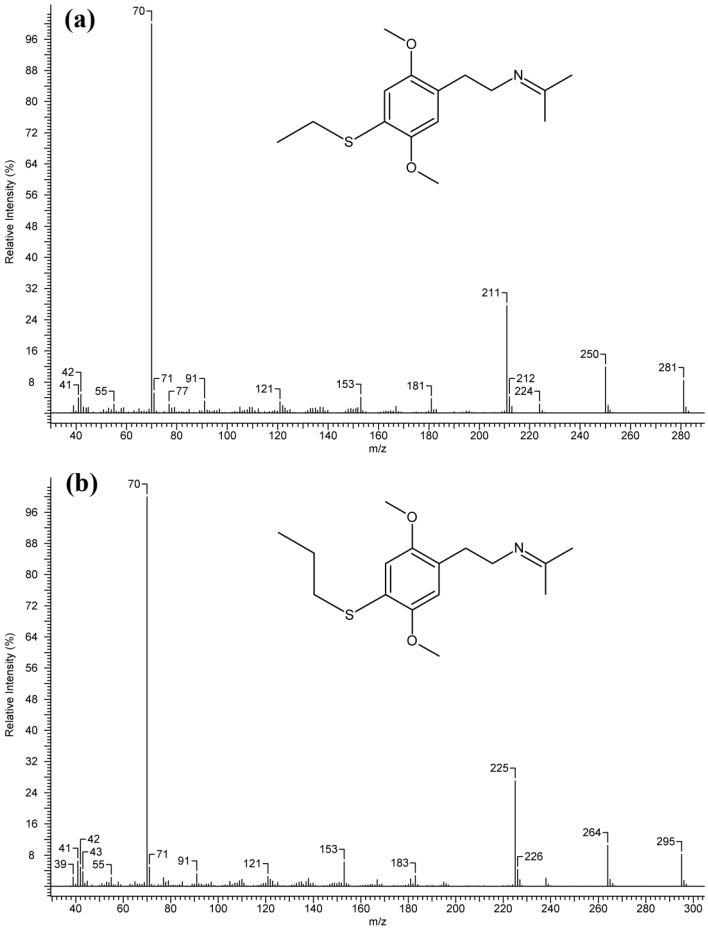

Figure 4 - Mass spectra of (a) 2,5-dimethoxy-4-ethylthio-N-isopropylidenephenethylamine and (b) 2,5-dimethoxy-4-propylthio-N-isopropylidenephenethylamine.

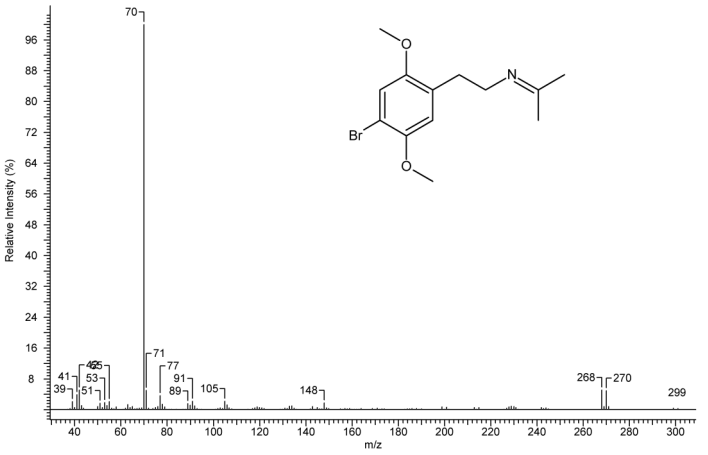

Figure 5 - Mass spectrum of 2,5-dimethoxy-4-bromo-N-isopropylidenephenethylamine.

reaction of amphetamine and amphetamine-type compounds with acetone are shown in Figures 7-8. The mass spectra of the imine products of amphetamine and amphetamine-type compounds all exhibited a base peak of *m/z* 84, indicating predominant α-cleavage on the alkyl chain, Figure 9. The imine product of 2,5-dimethoxyamphetamine exhibited a smaller peak that was 31 mass units less than the molecular ion, similar to the behavior seen with the dimethoxy-substituted phenethylamines, and is due to the loss of a methoxy group. However, 4-methoxyamphetamine did not exhibit loss of its methoxy group.

The mass spectra of the imine products formed from the reaction of tryptamine, α-methyltryptamine, and 5-methoxy-α-ethyl-tryptamine with acetone are show in Figures 10-11. All three compounds exhibited base peaks indicating predominant α-cleavage on the alkyl chain, Figure 12. In addition, a less intense peak is seen at *m/z* 130 due to initial ionization of the indole ring followed by α-cleavage or a less intense peak at *m/z* 160 due to initial ionization of the methoxy-substituted indole ring followed by α-cleavage, Figure 13.

In all of the cases, additional imine products were observed with longer reaction time. These peaks were either 40 mass units higher or 80 mass units higher than the initial imine product, and were subsequently identified as the imine products

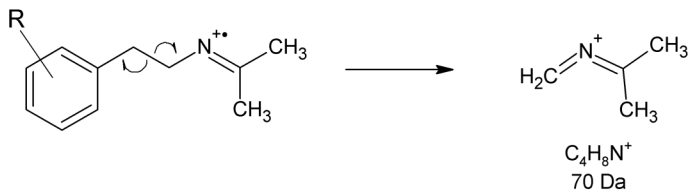

Figure 6 - Fragmentation Mechanism of N-isopropylidene-phenethylamines.

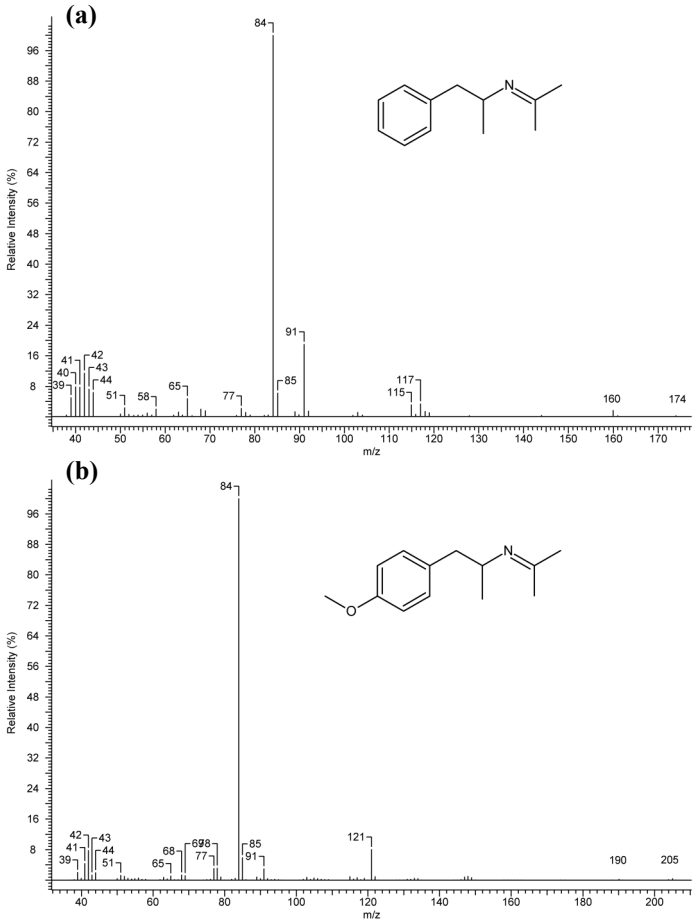

Figure 7 - Mass spectra of (a) N-isopropylideneamphetamine and (b) 4-methoxy-N-isopropylideneamphetamine.

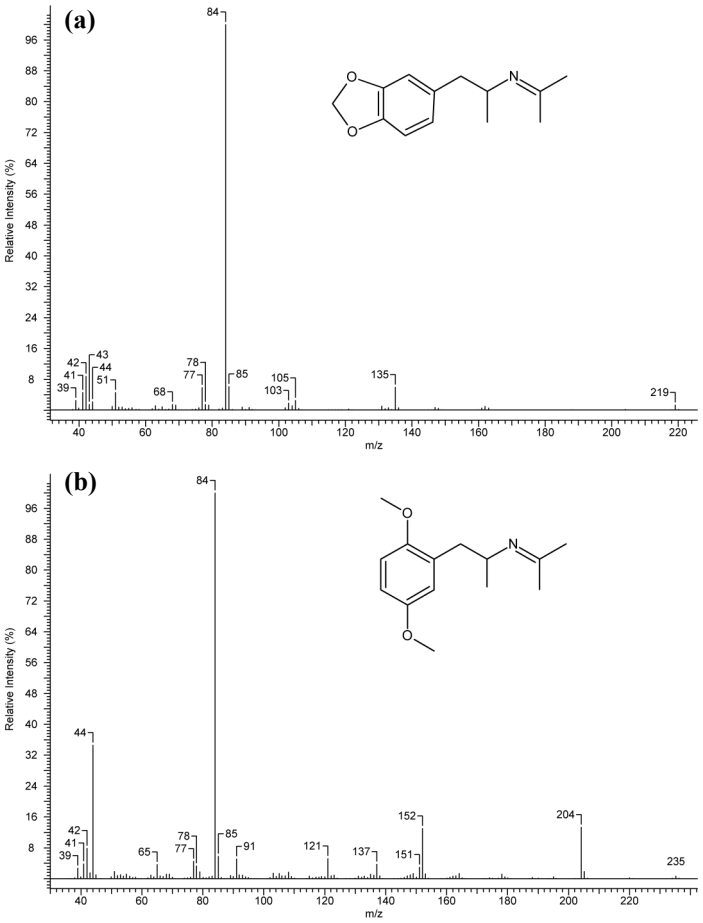

Figure 8 - Mass spectra of (a) 3,4-methylenedioxy-N-isopropylideneamphetamine and (b) 2,5-dimethoxy-N-isopropylideneamphetamine.

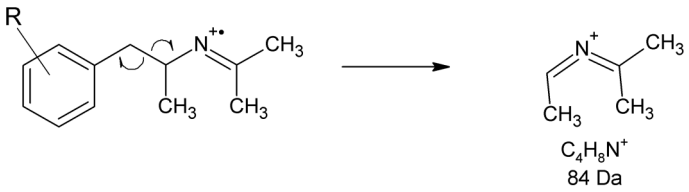

Figure 9 - Fragmentation mechanism of N-isopropylidene-amphetamines.

of the starting amine with either mesityl oxide, Figure 14, or isophorone, Figure 15. Both mesityl oxide and isophorone are condensation products formed by acetone in the presence of a base, such as amines. In the case of phenethylamine and tryptamine, the identity of the mesityl oxide and isophorone imine products were confirmed by reacting these primary amines with the appropriate ketone at 70°C for one hour. Analysis by GC/MS of these products matched the retention times and mass spectrums of the products formed from the reaction with acetone.

FTIR-ATR

FTIR-ATR was performed on the imine products of phenethylamine and tryptamine. An imine in the IR region

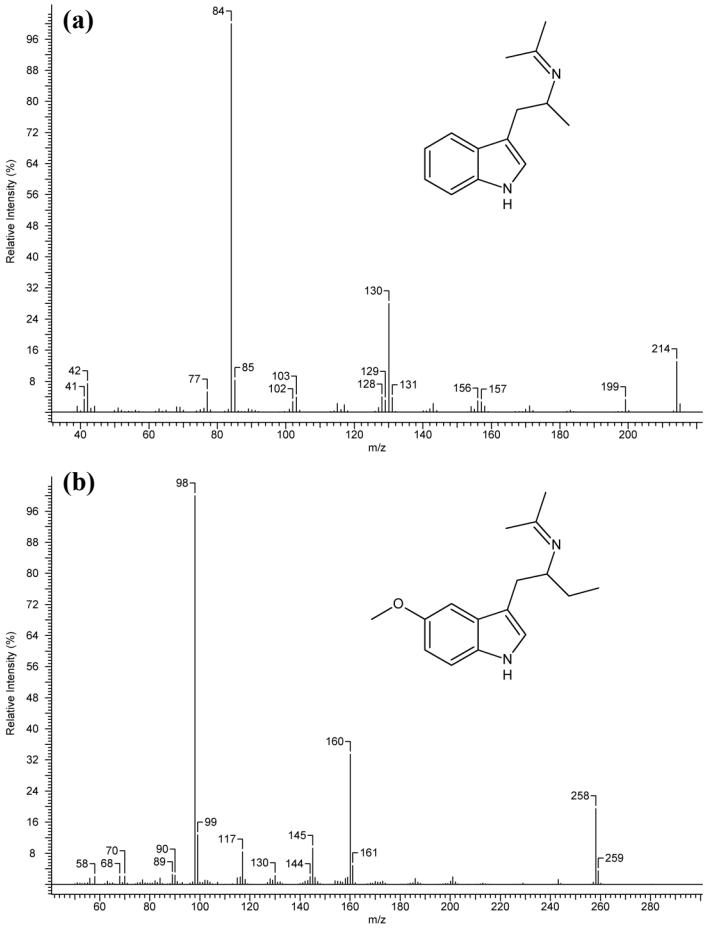

Figure 10 - Mass spectra of (a) α-methyl-N-isopropylidenetryptamine and (b) 5-methoxy-α-ethyl-N-isopropylidenetryptamine.

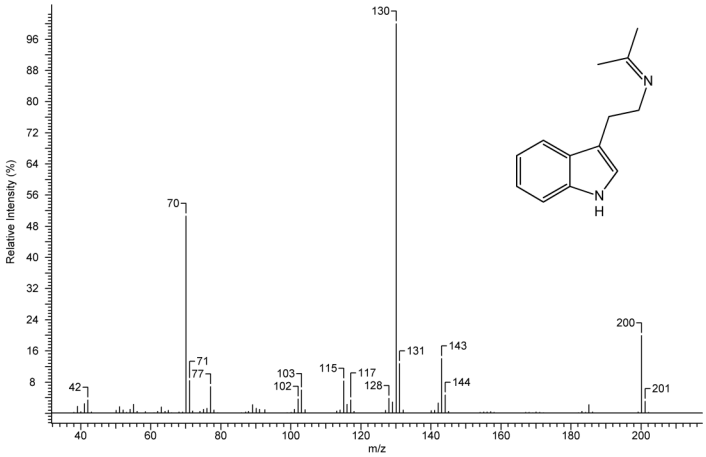

Figure 11 - Mass spectrum of N-isopropylidenetryptamine.

exhibits an absorption band in the region 1690-1640 cm^{-1}. The imine products of phenethylamine and tryptamine exhibited imine absorption bands at 1662 cm^{-1} and 1665 cm^{-1}, respectively, Figures 16-17. For comparison, the IR spectra of phenethylamine base and tryptamine base standards were also run. Comparison of all the spectra shows that the imine products of phenethylamine and tryptamine were formed. Also, there was no indication that any acetone was left in the samples

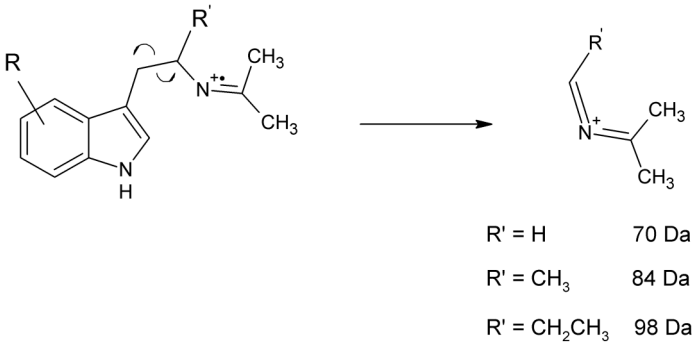

Figure 12 - Fragmentation mechanism of N-isopropylidene-tryptamines.

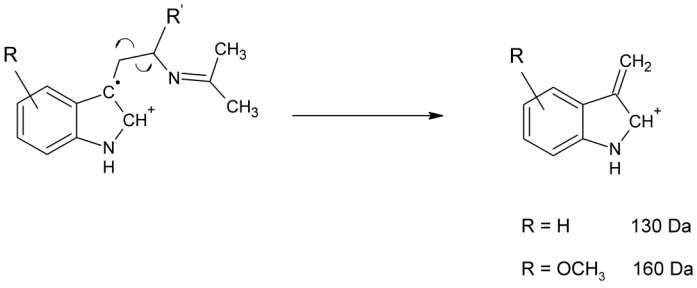

Figure 13 - Secondary fragmentation mechanism of N-isopropylidenetryptamines.

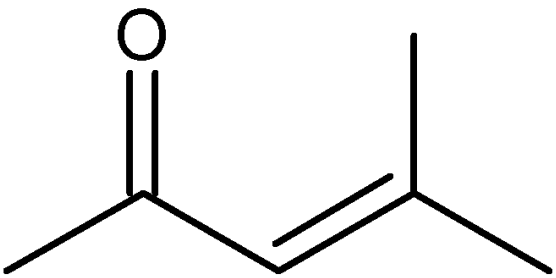

Figure 14 - Structure of mesityl oxide.

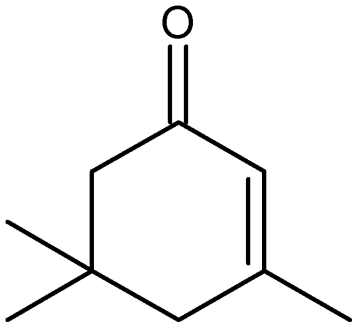

Figure 15 - Structure of isophorone.

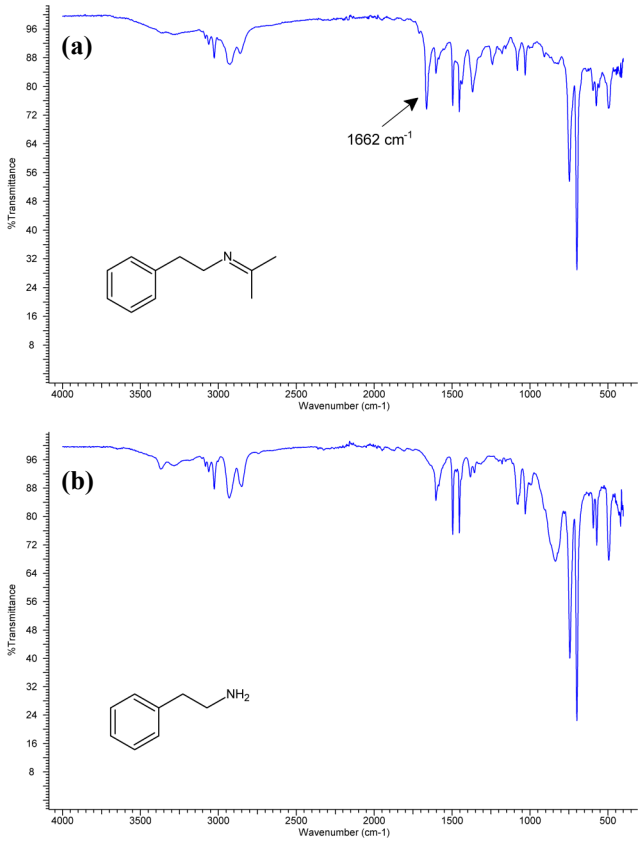

Figure 16 - FTIR spectrum of (a) N-isopropylidene-phenethylamine and (b) phenethylamine base.

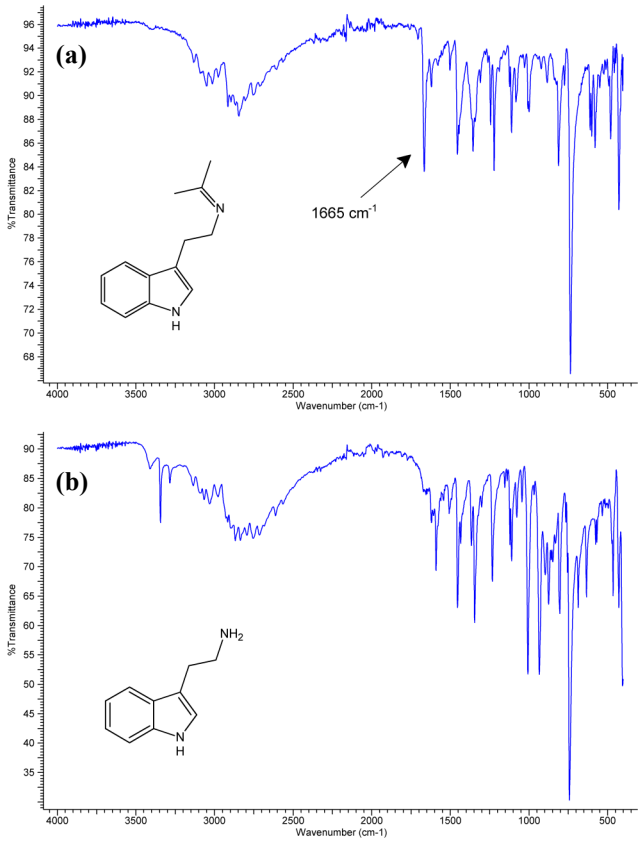

Figure 17 - FTIR spectrum of (a) N-isopropylidenetryptamine and (b) tryptamine base.

Table 1 - NMR assignments and multiplicities of N-isopropylidenetryptamine.

Position	^{13}C (ppm)	^{1}H (ppm)	Multiplicity
NH	-	8.0	Broad singlet
2	121.9	7.05	Doublets
3	114.8	-	-
3a	127.6	-	-
4	119.2	7.64	Doublets
5	118.9	7.12	Doublet of triplets
6	121.6	7.19	Doublet of triplets
7	111.0	7.36	Doublet
7a	136.2	-	-
α	26.8	3.11	Broad triplet
β	52.4	3.57	Broad triplet
imino	167.5	-	-
methyl	18.4, 29.3	1.74, 2.03	Singlets

Table 2 - NMR assignments and multiplicities of N-isopropylidenephenethylamine.

Position	^{13}C (ppm)	^{1}H (ppm)	Multiplicity
1	140.5	-	-
2,6	128.8	7.18-7.24	Multiplet
3,5	128.3	7.27-7.32	Multiplet
4	126.0	7.18-7.24	Multiplet
α	37.4	2.94	Doublet of doublets
β	53.5	3.47	Broad Doublet of doublets
imino	167.7	-	-
methyl	18.3, 29.3	1.72, 2.01	Singlets

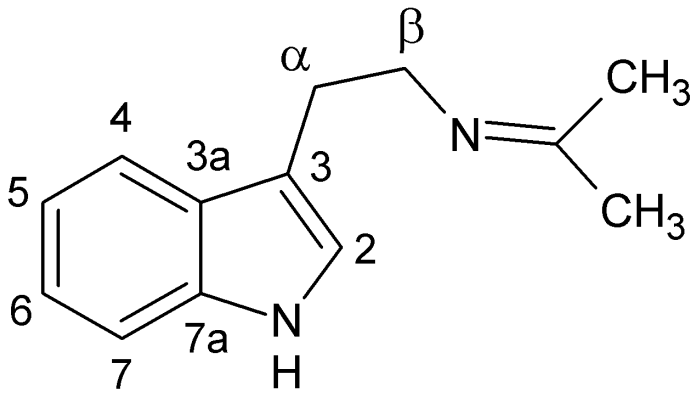

Figure 18 - Position of the carbons and protons for N-isopropylidenetryptamine

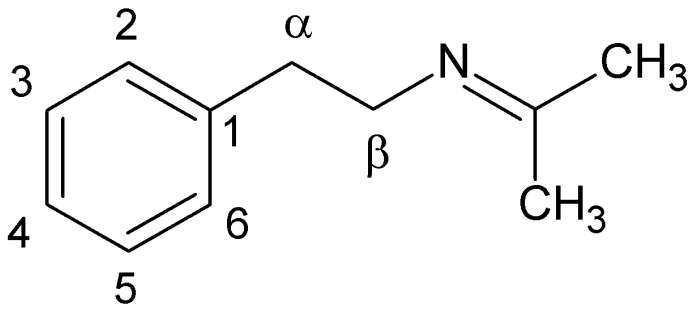

Figure 19 - Position of the carbons and protons for N-isopropylidenephenethylamine

as there was no absorption band at 1710 cm^{-1}. The imine absorption band for tryptamine, 1665 cm^{-1}, compares well with that reported in the literature [5].

NMR

The NMR chemical spectra are consistent with the imine structure for the condensation products of acetone with phenethylamine and tryptamine, Tables 1-2. The proton chemical shifts for the two methyl groups of the tryptamine imine product are in good agreement with those reported earlier [5].

References

1. Brandt SD, Martins CPB, Freeman F, Dempster N, Wainwright M, Riby PG, Alder JF. N,N-Dimethyltryptamine and Dichloromethane: Rearrangement of Quaternary Ammonium Salt Product During GC-EI and CI-MS-MS Analysis. J Pharm Biomed Anal 2008;47: 207-212.
2. Clark CR, DeRuiter J, Noggle FT. GC-MS Identification of Amine-Solvent Condensation Products Formed During Analysis of Drugs of Abuse. J Chromatogr Sci 1992;30:399-404.
3. Sparkman, OD, Curtis M, Jones PR. Interaction of Dichloromethane Solvent with n-Alkylamines Analyzed by Electron Ionization GC-MS. http://chromatographyonline.findanalytichem.com/lcgc/interaction-of-dichloromethane-solvent-with-n-alky/articlestandard/article/detail/659406 (cited 7/6/2010).
4. Jackson AH, Smith AE. The Melting Point and Structure of Tryptamine. J Chem Soc 1965; 3498-3500.
5. Hester JB. Cyclodehydration Reactions of Tryptamine Derivatives with Acetone. J Org Chem 1964;29: 2864-2867.