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ANALYSIS OF N-MONO-TRIFLUOROACETYL DERIVATIVES OF AMPHETAMINE ANALOGUES BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

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SUMMARY

N-Mono-trifluoroacetylated (TFA) derivatives of amphetamine, methamphetamine and some analogues were prepared by on-column derivatization with N-methyl-bis (trifluoroacetamide) and analyzed by gas chromatography (GC) and mass spectrometry (MS). Several of the commonly abused phenethylamines were readily separated on a 10% Carbowax 20M–2% KOH on 80–100 mesh Chromosorb W AW packed column as their N-TFA derivatives. The direct probe chemical ionization mass spectra and GC–MS chemical ionization mass spectra are presented in addition to the GC–MS electron impact spectra. Most mass spectral fragmentation pathways were predictable and these are compared to previously reported spectra.

INTRODUCTION

Because of the wide use and abuse of amphetamine and its derivatives, great importance has been placed on the development of methods for the rapid separation, identification, and quantitation of these drugs. Recently, the use of derivatizing reagents has played a major role in the development of such methods[1–13]. Several different derivatizing techniques have been employed for the separation of these compounds[1–3] and one of the more common derivatizing functional groups has been the trifluoroacetyl (TFA) derivative. Several groups have reported the use of this particular derivative for improving the chromatographic properties of this class of compounds[4–8]. More recently this derivative has also been used in conjunction with gas chromatography–mass spectrometry (GC–MS) studies for qualitative and quantitative analysis since the N-TFA derivative yields a much more complex and unique mass spectra[9–11].

Although previous chromatographic procedures have been excellent for their applications, complications often arise from other commonly abused amines when illicit samples must be analyzed in the forensic laboratory. Considering this, our laboratory employed the TFA derivative in analyzing illicit samples by GC–MS. We report an improved separation of the commonly abused amines by combining the TFA derivative with a common GC column, 10% Carbowax 20M–2% KOH. The

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derivatives were all confirmed by GC–MS and their spectra are presented, along with the direct probe chemical ionization mass spectra of the free bases.

Most of the TFA derivatives reported to this date have been formed by reaction with trifluoroacetic anhydride (TFAA)[6–11]. Foltz's group, however, chose N-methyl-bis(trifluoroacetamide) (MBTFA), in their quantitative determination for this class of compounds[13]. They reported complete reaction and a neutral by-product, which is most desirable. For these reasons and the ease of formation of the N-TFA derivative we chose to use MBTFA.

EXPERIMENTAL

Chemicals

MBTFA (Pierce), methamphetamine · HCl, phentermine · HCl, *p*-methylamphetamine · HCl, β-phenethylamine · HCl, ephedrine · HCl, (K&K Labs.), amphetamine sulfate, mephentermine (Wyeth Labs.), *p*-chlorophentermine · HCl (Warner-Lambert Research Institute), *o*-chlorophentermine · HCl (USV Pharmaceutical), norephedrine (Aldrich), 1-methylamino-2-phenylpropane · HCl, and *p*-hydroxy-amphetamine · HBr (SKF Labs.) were obtained from commercial sources.

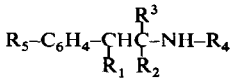
Preparation of samples for GC

A 1-μl volume of a chloroform solution of the phenethylamines in the free base form was drawn up in a 10-μl syringe along with 1 μl of MBTFA and was injected directly onto the column for gas chromatographic analysis.

The chemical ionization (CI) mass spectra were obtained by transferring 1 μl of a solution of free base phenethylamines in a 1-cm closed-end capillary tube and taking this solution to dryness. The tube was then inserted into the source of the mass spectrometer via the direct probe. The instrument was then scanned over the mass

TABLE I

STRUCTURES AND MOLECULAR WEIGHTS OF COMPOUNDS STUDIED



No.	*Compound*	R_1	R_2	R_3	R_4	R_5	*Molecular weight*	
							Free base	*TFA derivative*
1	β-Phenethylamine	H	H	H	H	H	121	217
2	Amphetamine	H	CH_3	H	H	H	135	231
3	Phentermine	H	CH_3	CH_3	H	H	149	245
4	Methamphetamine	H	CH_3	H	CH_3	H	149	245
5	N,β-Dimethylphenethylamine	CH_3	H	H	CH_3	H	149	245
6	*p*-Methylamphetamine	H	CH_3	H	H	CH_3	149	245
7	Mephentermine	H	CH_3	CH_3	CH_3	H	163	259
8	*p*-Chlorophentermine	H	CH_3	CH_3	H	Cl(-*p*)	188	279
9	*o*-Chlorophentermine	H	CH_3	CH_3	H	Cl(-*o*)	183	279
10	*p*-Hydroxyamphetamine	H	CH_3	H	H	OH	151	247
11	Ephedrine	OH	CH_3	H	CH_3	H	165	261
12	Norephedrine	OH	CH_3	H	H	H	151	247

region of interest as the direct probe was heated and the sample volatilized into the source.

Instrumentation

GC was carried out on a Varian 1400 gas chromatograph with a flame-ionization detector. Nitrogen was used as the carrier gas at a flow-rate of 30 ml/min. The column used was a 10% Carbowax 20M–2% KOH on 80–100 mesh Chromosorb W AW (6 ft. × 2.0 mm I.D. glass column). Injector and detector temperatures were 210°C and 250°C, respectively. The column temperature was 165°C for all of the analyses. The GC column used in the GC–MS studies was connected by a glass transfer line to a DuPont-490 mass spectrometer. Isobutane was used as the reagent gas at a source pressure of approximately 0.5 to 1.0 Torr (66.6 to 133.3 Pa). The source temperature and transfer lines were set at 200°C. The ionizing voltage was 70 eV in both the electron impact (EI) and CI modes. All materials admitted into the source through the direct probe were recorded at probe temperatures of 150°C and 200°C. The jet separator was of the molecular jet type and was maintained at a temperature of 200°C.

RESULTS AND DISCUSSION

The compounds analyzed are listed in Table I along with the molecular weights corresponding to the free bases and N-TFA derivatives. Initial attempts to derivatize the salts of the phenethylamines with MBTFA yielded incomplete derivatization. This incomplete derivatization resulted in multiple peaks with poor peak shape and irreproducible results. No attempt was made to determine the composition of these multiple peaks. However, after converting the phenethylamines to free bases by dissolving the salts in 0.1 *N* sodium hydroxide solution and extracting into chloroform, complete derivatization was obtained which yielded single, sharp, symmetrical peaks.

The only phenethylamine which did not form the TFA derivative with MBTFA was mephentermine. All attempts failed in derivatizing this compound with MBTFA. Both the HCl salt and the free base were attempted by on-column derivatization and by reacting in a separate vessel for several hours at 60°C. Presumably, steric hindrance contributed to this result. Mephentermine, however, in the free base form did not interfere with the derivatization or the chromatography of the other phenethylamines when analyzed in a mixture. Mephentermine was able to be derivatized with TFAA however, and the data in the tables corresponding to N-TFA-mephentermine was collected by reacting mephentermine with TFAA in pyridine, concentrating over nitrogen, dissolving in chloroform, and injecting this onto the column.

The TFA derivatives, which were obtained by on-column injection, were analyzed on a 10% Carbowax 20M–2% KOH on 80–100 mesh Chromosorb W AW column. The derivatization was also attempted on a 6 ft. 10% Apiezon-L–2% KOH on 90–100 mesh Anakrom ABS glass column with irreproducible results. Table II shows the relative retention times of these derivatives on the Carbowax 20M column. The N-TFA derivatives were considerably less volatile than their parent amines and showed much better chromatographic properties. Fig. 1 shows the separation of five prominent phenethylamines as their N-TFA derivatives on this column. Most of the derivatives were better separated on this column as their N-TFA derivative than

TABLE II

RETENTION DATA OF N-MONOTRIFLUOROACETAMIDE PHENETHYLAMINES

GC conditions: 10% Carbowax 20M–2% KOH on 80–100 mesh Chromosorb W AW. Temperatures: column, 165°C; detector, 250°C; injector, 210°C. Nitrogen flow-rate, 30 ml/min.

Compound	*Relative retention times*
β-Phenethylamine-TFA	1.9
Amphetamine-TFA	1.0 (6.5 min)
Phentermine-TFA	0.6
Methamphetamine-TFA	0.8
N,β-Dimethylphenethylamine-TFA	0.7
p-Methylamphetamine-TFA	1.4
Mephentermine-TFA	0.7
p-Chlorophentermine-TFA	2.3
o-Chlorophentermine-TFA	1.4
p-Hydroxyamphetamine-TFA	9.0
Ephedrine-TFA	6.0
Norephedrine-TFA	4.0

when chromatographed as the free base. This is an important feature since our laboratory has encountered most of these compounds in illicit samples. Since it is often requested to quantitate the methamphetamine in a sample, the improved separation may be needed when one or more of these compounds are in a mixture with methamphetamine.

Table III shows the Cl mass spectra of these compounds both as the free bases

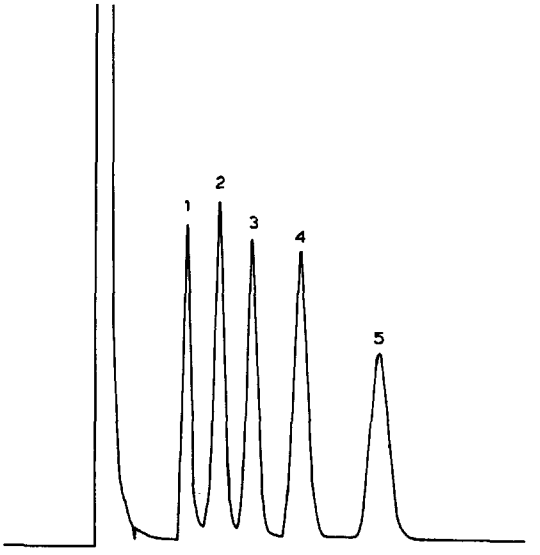


Fig. 1. Gas chromatographic separation of N-mono-trifluoroacetylated mixture of phenethylamines on 10% Carbowax 20M–2% KOH on 80–100 mesh Chromosorb W AW. Peaks: 1 = phentermine, 2 = methamphetamine, 3 = amphetamine, 4 = *p*-methylamphetamine, 5 = β-phenethylamine.

TABLE III

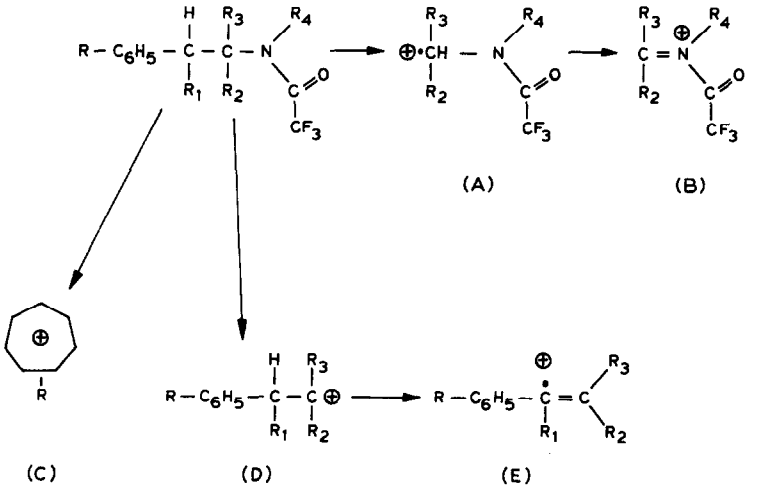
CHEMICAL IONIZATION MASS SPECTRAL DATA

Based on peaks > 10% of base peak.

Compound	*Direct probe, free base*	*GC–MS, TFA derivatives*
1	122*, 121(35%), 105(15%)	218*, 219(14%)
2	136*	232*, 233(13%)
3	150*	246*, 133(21%), 247(13%), 154(10%)
4	150*	246*, 247(17%)
5	150*	246*, 247(17%)
6	150* 149(49%)	246*, 247(12%), 132(10%), 133(10%)
7	164*	260*, 133(76%), 168(29%), 261(11%)
8	184*, 186(35%), 167(12%)	280*, 282(33%), 167(20%), 154(20%)
9	184*, 186(33%), 185(12%)	280*, 282(33%), 167(30%), 154(30%)
10	152*, 135(92%)	248*, 249(22%), 135(20%), 134(15%)
11	166*, 148(50%)	244*, 262(50%), 107(40%), 148(35%), 155(25%), 154(25%)
12	152*, 134(50%), 107(25%)	230*, 107(14%), 231(12%), 248(10%)

* Base peak.

and the N-TFA derivatives. All of the phenethylamines gave the M + H_1^+ molecular ion as the base peak when analyzed as free bases in the CI direct probe mode. *β*-Phenethylamine and *p*-methylamphetamine also gave a significant amount of the M^+. Why these two compounds were the only ones which behaved in this manner is not known. Ephedrine and norephedrine both gave fragmentation patterns charac-



Fragment	*Ions (m/e)*
(A)	127, 141, 155, 169
(B)	126, 140, 154, 168
(C)	91, 105, 107, 125, 127
(D)	105, 119, 133, 135
(E)	104, 118, 132, 134, 166, 168

Fig. 2. Mass spectral fragmentation pathways and fragment ions (ref. 9).

teristic of the loss of H_2O from the M + H^+ molecular ion. The *p*-hydroxyamphetamine did not show this characteristic loss. This is expected since the loss of the hydroxyl from the phenyl ring would not be a favored process.

The ion *m/e* 135 in the CI spectra of *p*-hydroxyamphetamine is most likely due to the loss of the amine to form the cation $HO\text{–}C_6H_4\text{–}\overset{|}{\underset{|}{C}}\text{–}C\oplus$ (CH_3). This ion also appears in the CI mass spectra of the N-TFA derivative of this compound. A similar mechanism also appears likely for β-phenethylamine which gives the ion *m/e* 105 in the CI mass spectra of the free base corresponding to $C_6H_5\text{–}\overset{|}{\underset{|}{C}}\text{–}C\oplus$. This is also a possible explanation for the formation of the ion *m/e* 167 in the CI-MS of the free base of *p*-chlorophentermine. Why this is not a major ion with *o*-chlorophentermine is not known since this ion appears in both of these compounds' GC–CI mass spectra when the N-TFA derivative is formed.

The ion *m/e* 107 in both of norephedrine's chemical ionization mass spectra is most likely due to the stable cation $C_6H_5\text{–}\overset{|}{C}\oplus$ (OH). This ion would also be expected in the ephedrine spectra but did not appear to be significant when analyzed as the free base but became an important contribution (40%) to the CI mass spectra of the N-TFA derivative of ephedrine.

In the CI mass spectra of the N-TFA derivatives, all of the compounds analyzed except ephedrine and norephedrine, gave the corresponding M + H^+ molecular ion as the base peak. The base peaks for both of these compounds most likely correspond to the stable cations that were similarly observed in the spectra of the free bases, $C_6H_5\text{–}\overset{|}{\underset{|}{C}}\text{–}\overset{|}{C}\text{–}\overset{R}{\overset{|}{N}}\text{–TFA}$ (CH_3).

A general observation of the GC–CI mass spectra of the N-TFA derivatives was the appearance in most of the spectra of M + $2H^+$. It was not known if this was a function of the source pressure, of the type of reagent gas, or of a phenomenon unique to the N-TFA derivative. It was reproducible however.

Other important ions were *m/e* 154 and *m/e* 133 which appeared in quite a few of the N-TFA derivatives spectra. The probable structures for these ions are shown in Fig. 2.

The N-Mono-TFA derivatives of the compounds listed in Table I required confirmation, and therefore, each derivative was subjected to EI mass spectral analysis. These mass spectral data are listed in Table IV. Most of the fragments either compared to or were readily predictable from previously reported spectra. The major ions are similar to the ions shown in the fragmentation pattern of Fig. 2 which was previously reported by Coutts *et al.*[9] for some ephedrine analogues. Some of the

TABLE IV

GC–EI-MS OF TFA DERIVATIVES

Based on eight largest peaks.

Compound	*GC–EI-MS*
1	104*, 91(44%), 105(14%), 126(10%), 217(6%), 92(5%), 65(5%), 103(4%)
2	104*, 118(84%), 91(21%), 119(12%), 92(6%), 117(6%), 141(4%), 65(4%)
3	154*, 132(16%), 91(8%), 59(6%), 155(6%), 114(6%), 140(5%), 118(5%)
4	154*, 118(36%), 110(22%), 91(8%), 155(6%), 119(6%), 117(3%)
5	105*, 118(76%), 140(31%), 154(14%), 119(12%), 106(10%), 77(9%), 79(7%)
6	132*, 105(69%), 140(47%), 106(15%), 133(15%), 154(12%), 117(9%), 91(8%)
7	168*, 110(52%), 69(14%), 56(11%), 91(10%), 169(10%), 132(6%), 117(6%)
8	154*, 59(39%), 166(12%), 155(8%), 114(8%), 125(7%), 127(5%), 168(5%)
9	154*, 59(45%), 125(16%), 155(14%), 166(14%), 114(10%), 126(8%), 131(7%)
10	107*, 134(54%), 140(16%), 135(12%), 77(11%), 108(9%), 70(9%), 133(8%)
11	155*, 154(85%), 110(85%) 105(71%), 77(59%), 56(55%), 51(41%), 116(38%)
12	107*, 141(86%), 79(43%), 77(34%), 123(32%), 105(25%), 72(16%), 108(10%)

* Base peak.

additional important ions were the $CH_3N^+ = CCF_3$, $m/e = 110$; $CH_3–C^+ = N–CH_3$, $m/e = 56$; CF_3^+, $m/e = 69$; and $C_6H_5^+$, $m/e = 77$. The fragment ions with m/e of 106 and 108 are most likely the protonated 105 and 107 ions respectively. The following major ions were not identified: 51, 59, 114, 116, and 123. Additional fragment ions were present in all of the spectra, but these were insignificant when compared to the base peak. Any differences from the spectra reported here and previously published spectra may be due to the differing conditions existing within the source of the mass spectrometer.

In conclusion, an improved separation of amphetamine type compounds can be achieved by derivatizing the free bases with MBTFA and separating on a 10% Carbowax 20M–2% KOH on 80–100 mesh Chromosorb W AW column. The mass spectral fragmentation pathways of these derivatives are predictable. All the derivatives analyzed except ephedrine and norephedrine gave the M + 1 ion as the base peak in the CI-MS mode.

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