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Differentiation of Side Chain Isomers of Ring-Substituted Amphetamines Using Gas Chromatography/Infrared/Mass Spectrometry (GC/IR/MS)

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ABSTRACT: Common analytical methods used for identifying samples obtained from clandestine laboratories were evaluated for their ability to differentiate between possible amphetamine isomers and homologs. A series of ring-substituted (4-methyl, 4-methoxy, and 3,4-methylenedioxy) amphetamine and *N*-methylphenethylamine isomers was analyzed using color tests, thin-layer chromatography, gas chromatography/mass spectrometry (GC/MS) and GC/infrared (GC/IR). The *N*-acetyl derivatives of the isomers were analyzed using GC/IR/MS. GC/IR/MS readily differentiated the 4-methylphenylalkylamine isomers. MS and IR spectra were also obtained for each pair of the 4-methoxyphenylalkylamine isomers and the 3,4-methylenedioxyphenylalkylamine isomers, but differentiation via GC/IR/MS was difficult. The *N*-acetyl derivatives of each pair of isomers could be readily differentiated using GC/IR/MS. Good library researchable spectra for *N*-acetylamphetamine could be obtained for IR identification with 10 ng (on-column) and MS identification with 2 ng. The spectrometrically independent IR and MS data obtained for the *N*-acetyl derivatives indicated that the combination of GC/IR/MS can add a significant level of confidence in the analysis of ring-substituted arylalkylamines.

KEYWORDS: toxicology, amphetamine, chromatographic analysis, chemical analysis, isomers

Because of the occurrence in the illicit drug market of clandestinely synthesized analogs and homologs of therapeutically useful drugs (often referred to as "designer drugs"), there is need for improved methods for differentiating these scheduled compounds from unregulated substances. In addition, clandestinely prepared drugs often contain impurities of manufacture that are structurally similar to those of the desired drug. Identification of these impurities often aids in understanding the manufacturing process as well as some of the pharmacological effects associated with clandestinely prepared substances [*1*,*2*].

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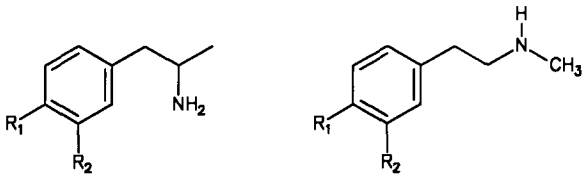
Although a variety of tests is used for characterization of compounds submitted for forensic identification, gas chromatography coupled with detection by mass spectrometry (GC/MS) is often considered the definitive method for identification of unknown samples submitted for analysis. The electron impact mass spectra have been useful in differentiating closely related analogs of the arylcycloalkylamines of phencyclidine [*3*], the arylalkylamine analogs of amphetamine [*4–7*] and methamphetamine [*8,9*], and the quinazolone analogs of methaqualone [*10—12*]. Although the mass spectra of most compounds are unique, when certain fragmentation pathways predominate, comparable mass spectra are obtained. Examples of similar mass spectra have been observed for ring-positional isomers of amphetamine [*4*] amphetamine/*N*-methylphenethylamine [*7*] and methamphetamine/phentermine [*13*]. The primary fragmentation pathway of the arylalkylamines is through fission beta to the amino function, which is also beta to the aromatic ring. This gives the same base peak and it accounts for a large percentage of the total ion abundance (that is, usually greater than 50%). Due to the low relative abundance of the remaining ions, it is often difficult to unambiguously differentiate these isomeric pairs using only the mass spectra unless synthetic standards or prior spectra of both isomers are available for direct comparison [*4*].

As methodology has improved for coupling gas chromatography with infrared detection (GC/IR), this analytical method has developed into a valuable adjunct with GC/MS to complement the differentiation and identification of closely related amphetamine isomers. Using spectrometrically independent IR and MS data, it was shown that the combined technique of GC/IR/MS could add an increased level of confidence in the differentiation of the C_3H_8N side chain isomers of amphetamine [*14*].

To further develop the application of GC/IR/MS for differentiation of positional isomers for which similar mass spectra would be anticipated, a series of ring-substituted amphetamine analogs and their *N*-methylphenethylamine isomers was studied. These compounds were characterized using GC/IR/MS as well as other common analytical tests used in the forensic analysis of arylalkylamines. It was anticipated that some structure specific trends would be observed that would allow differentiation of these side-chain isomers. This approach would then permit unambiguous differentiation of the arylisopropylamine side chain from the *N*-methylarylethylamine side chain for controlled substances in which synthetic standards may not be readily available. Unexpectedly, two of the arylalkylamine isomers were strikingly similar using GC/IR/MS and improved differentiation of these isomers required the preparation of their *N*-acetyl derivatives.

Experimental Procedure

Amphetamine (*I*), 4-methylamphetamine (*II*), 4-methoxyamphetamine (*III*) and 3,4-methylenedioxyamphetamine (*IV*) were provided by Dr. Richard Glennon of Virginia Commonwealth University. *N*-methylphenethylamine (*V*) was obtained from Aldrich Chemical Co. (Milwaukee, Wisconsin). The 4-methyl-*N*-methylphenethylamine (*VI*), 4-methoxy-*N*-methylphenethylamine (*VII*) and 3,4-methylenedioxy-*N*-methylphenethylamine (*VIII*) were prepared from their respective arylacetic acids which were commercially available. The acids were converted to their respective acid chlorides with thionyl chloride followed by the addition of methylamine. The resulting amides were reduced to the amine using lithium aluminum hydride ($LiAlH_4$) [*15*]. The amines were distilled and converted to the hydrochloric acid (HCl) salts with gaseous HCl. The melting points were 191° to 193°C for *VI*·HCl (literature, 191°C [*16*]); 180° to 182°C for *VII*·HCl (literature, 181° to 182°C [*17*]); and 181° to 183°C for *VIII*·HCl (literature, 183° to 185°C [*18*]). In addition to the analytical tests described in this paper, proton NMRs of the *N*-methylphenethylamine analogs were consistent with the proposed structures. The structures of the compounds evaluated are shown in Fig. 1.



Compound	R_1	R_2
I	H	H
II	CH_3	H
III	OCH_3	H
IV	OCH_2O	

Compound	R_1	R_2
V	H	H
VI	CH_3	H
VII	OCH_3	H
VIII	OCH_2O	

FIG. 1—*Arylisopropylamines and* N*-methylarylethylamines used in study.*

All of the amines were tested as the free base. Color tests and spray reagents were of standard composition [*19*]. Fluram® was obtained from Roche Diagnostics (Nutley, New Jersey). Thin-layer chromatography (TLC) analyses were performed using 250-μm-thick layers of Silica GF on 10-cm glass plates precoated by Analtech, Inc. (Newark, Delaware). The GC separations for Fig. 2 were obtained from a single injection using a Hewlett-Packard 5890A gas chromatograph and a 20 m 5% phenylmethylsilicone column (HP Ultra 2) with a 0.52-μm film thickness and 0.32 mm inside diameter. The initial oven temperature was 40°C for 2 min, then temperature programed to 220°C at 7°C/min. Helium was the carrier gas at a head pressure of 34.5 kPa (5 psi). The GC/IR/MS interface, data analysis, and library searching have been previously described [*14*], and the system is commercially available.

The on-column acetylation was achieved utilizing the following procedure. Using a 10-μL Hamilton glass syringe (Reno, Nevada), the sample was prepared by drawing up in the syringe 1 μL of chloroform, 1 μL air, 1 μL of sample dissolved in choroform, 1 μL of air, 1 μL of acetic anhydride, and 1 μL of air. The sample was injected manually (injection port 300°C) into the splitless mode of the capillary port.

Results

The analytical tests routinely used by the Drug Examination Section, Division of Forensic Science, Richmond, Virginia, for screening of drug samples include color tests, TLC followed by visualization with specific reagents, GC, and GC/MS. Each isomer was evaluated in all of the analytical systems.

The results of the color tests are reported in Table 1. The nitroprusside test gave a positive intense blue color or precipitate for the secondary amines (*V–VIII*) and gave no color for the primary amines except for *IV*. The cobalt thiocyanate test gave a positive intense blue color for the secondary amines (*V–VIII*) and no color for the primary amines (*I–IV*). An appropriate combination of color tests can differentiate each positional isomer and homolog provided a sufficiently pure sample is being analyzed.

The results of the analyses of the isomers using TLC in six different solvent systems are given in Table 2. The chloroform/methanol (9:1) and acetone solvent systems differentiated the arylisopropylamine isomers from their respective *N*-methylarylethylamine isomers. The primary amines (*I–IV*) could be visualized and differentiated from the secondary amines (*V–VIII*) using Fluram and 366 nm light or ninhydrin. All of the isomers could be detected after TLC analysis with 254 nm light, iodoplatinate, and

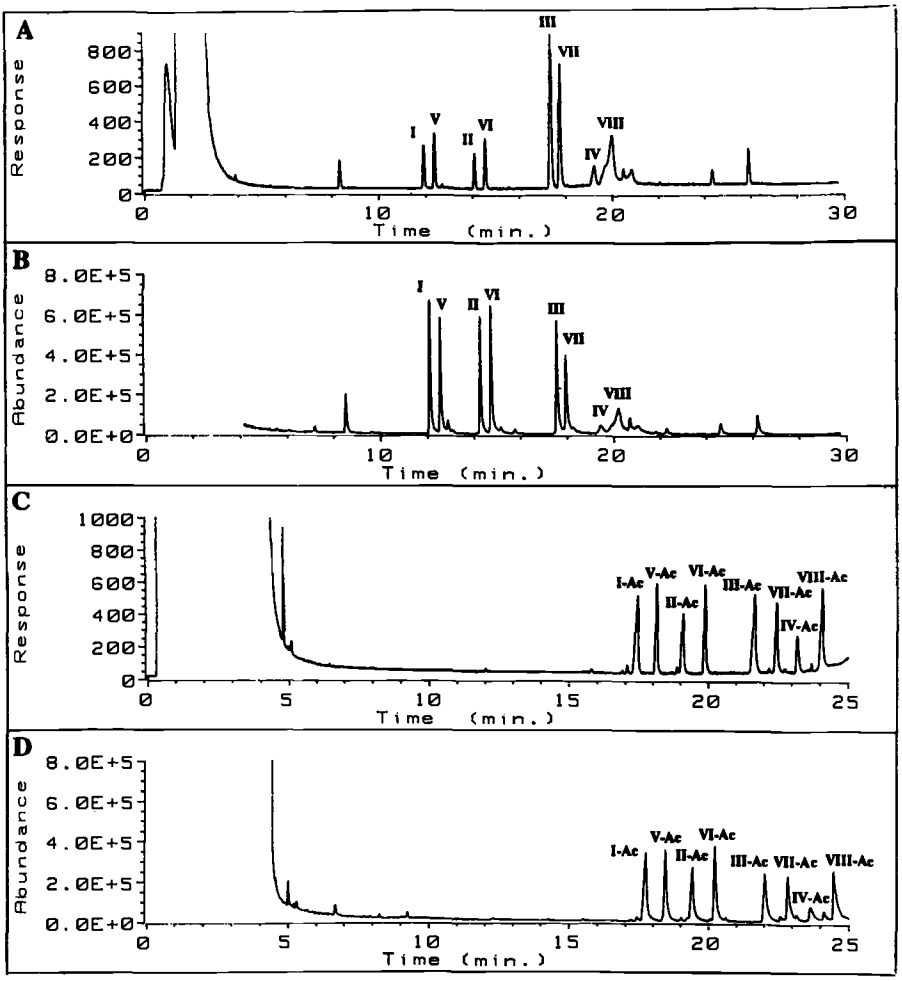


FIG. 2—*Chromatograms from a single injection of the arylalkylamine with detection using* (a) *total IR response and* (b) *total ion response. Chromatograms from a single injection of the* N-*acetyl derivatives of the arylalkylamines bases with detection using* (c) *total IR response and* (d) *total ion response.*

Dragendorff reagent. No single system was capable of differentiating all eight compounds. Several combinations of systems with appropriate visualization would enable differentiation of the arylalkylamines.

The analogs were resolved using capillary gas chromatography (Fig. 2); however, relatively long retention times were necessary for *III*, *IV*, *VII*, and *VIII*. Within this series the arylisopropylamine isomer consistently eluted before its *N*-methylarylethylamine isomer.

The results of the mass spectral analyses are tabulated in Table 3 for the amines and their respective *N*-acetyl derivative. The electron impact (EI) spectra are dominated by beta cleavage of the molecular ion to give the dimethyl amine ion at *m/z* 44 (base peak), as shown in Fig. 3. In small abundance are fragment ions due to formation of the appropriately substituted tropilium ion for Compounds *I*, *II*, *V*, and *VI* (M-44). As the ring substitution increases (*III*, *IV*, *VII*, and *VIII*), the fragment ion associated with

TABLE 1—*Color test data.*[a]

Compound	Marquis	Nitroprusside	Mecke	Nitric Acid	Froehde	Cobalt Thiocyanate	Sulfuric Acid	Mandelin
I	OR	. . .	. . .	. . .	. . .	. . .	. . .	GR
V	OR/BRN	BL-pt	. . .	. . .	. . .	PUR-pt	. . .	GR/GY
II	RED	. . .	YL	. . .	. . .	. . .	. . .	BRN
VI	RED	BL-pt	GY	. . .	. . .	PUR	. . .	BRN
III	GY	. . .	GR	YL-wk	BL/BLK	. . .	. . .	PINK
VII	OR	BL-pt	BLK	YL	PUR-wk	PUR-pt	. . .	PUR
IV	PUR	PUR	GR	YL	GR-PUR	. . .	PUR	BRN
VIII	BRN	BL-pt	GR	BRN	BRN	PUR-pt	YL	BRN

[a]Key to abbreviations: OR = orange; BRN = brown, GR = green; BL = blue; BLK = black; YL = yellow; PUR = purple; GY = gray; pt = precipitate; wk = weak.

TABLE 2—*Thin-layer chromatography on silica (rf).*[a]

Solvent system	A	B	C	D	E	F	G	H
I	0.49	0.71	0.52	0.39	0.66	0.65	+	pink
V	0.39	0.64	0.26	0.39	0.55	0.59	–	purple
II	0.27	0.66	0.13	0.29	0.83	0.58	+	pink
VI	0.43	0.65	0.24	0.40	0.54	0.56	–	purple
III	0.22	0.64	0.29	0.32	0.29	0.53	+	pink
VII	0.15	0.54	0.07	0.24	0.19	0.35	–	purple
IV	0.43	0.68	0.45	0.26	0.55	0.60	+	pink
VIII	0.23	0.57	0.14	0.25	0.33	0.42	–	purple

[a]Key to abbreviations: A = chloroform/methanol (9/1, *v/v*); B = chloroform saturated with ammonia/methanol (18/1, *v/v*); C = acetone; D = cyclohexane/toluene/diethylamine (75/15/10, *v/v*); E = chloroform/methanol (1:1, *v/v*); F = ammonium hydroxide/methanol (1.5/100, *v/v*); G = Fluram, + indicates positive visualization; H = ninhydrin.

benzylic cleavage and hydrogen transfer (M-43) increases in relative abundance (see Fig. 3). Following injection of *IV*, a mass spectral library search of the Wiley/NBS file (Registry of Mass Spectral Data, Electronic Data Division, Wiley, 605 Third Ave., New York, New York 10158) gave a match of 78 for methylenedioxyamphetamine when using "quality"-based matching [*20*]. Upon injection of *VIII* it was also identified as methylenedioxyamphetamine with a "quality" match of 78.

The base peak for the *N*-acetyl derivatives remains at *m/z* 44. As shown in Fig. 4, significant ions are observed for the M-59 ion in the arylisopropylamine series and an M-73 ion in the *N*-methylarylethylamine series of isomers. These ions were not present or were minor ions in the spectrum of the underivatized amines.

The results of the infrared analyses are tabulated in Table 4 for the amines and their respective *N*-acetyl derivatives. The *N*-methyl symmetric stretch (2803 cm^{-1}) [*21*] was a weak to moderate absorbance for compounds *V–VIII*. The absorbances associated with the *C-N* aliphatic stretch (1121 to 1126 cm^{-1} and 1117 cm^{-1}) and the methylene asymmetric stretch (3000 to 2850 cm^{-1}) became less intense as substitution on the aromatic ring increased [*21*].

The *N*-acetyl derivatives exhibited a strong Amide I band for the secondary amides (1712 cm^{-1}) formed by *I–IV* and the tertiary amides (1684 cm^{-1}) formed by *V–VIII*. The *C-N* bending for the secondary amide (1371–1368 cm^{-1}) as opposed to that of the tertiary amide (1398 cm^{-1}) is characteristic within each series. The *N-H* bending and *C-N* stretching (1245 cm^{-1}) is a weak absorbance that is difficult to detect for compounds *III*, *IV*, *VII*, and *VIII*. The methyl and methylene stretching for all of the *N*-acetyl derivatives were weak and broad.

The IR spectrum of each isomer and its *N*-acetyl derivative was entered into a user-constructed drug library (utilizing the default parameters of the HP 5965B IRD software package). A search of the user-constructed library for an unknown provides a ranking of possible compounds as reflected by a "hit quality" index. The hit quality for each isomer injected and analyzed, along with the relative ranking of its positional isomer for that library search (the number is given in parentheses, if reported), is as follows: *I*, 940 (902); *II*, 979 (912); *III*, 990 (958); *IV*, 943 (921); *V*, 951 (925); *VI*, 982 (914); *VII*, 979 (955), *VIII*, 965 (940); *I-Ac*, 983; *II-Ac*, 982; *III-Ac*, 969 (894); *IV-Ac*, 963 (895); *V-Ac*, 988; *VI-Ac*, 989; *VII-Ac*, 971; *VIII-Ac*, 989 (914). Following a second injection and analysis of each isomer as both the free amine and *N*-acetyl derivative, the quality of the

TABLE 3—*Mass spectral data for the isomers.*[a]

I		*V*		*II*		*VI*		*III*		*VII*		*IV*		*VIII*	
44	(100)	44	(100)	44	(100)	44	(100)	44	(100)	44	(100)	44	(100)	44	(100)
51	(3.4)	51	(15.6)	77	(3.0)	77	(2.4)	65	(1.4)	65	(4.9)	77	(6.2)	77	(7.8)
63	(2.7)	63	(8.7)	78	(1.4)	78	(1.3)	77	(3.9)	77	(8.9)	78	(2.5)	78	(2.8)
65	(7.7)	65	(20.5)	91	(3.6)	91	(2.7)	78	(4.5)	78	(11.5)	89	(0.5)	89	(1.7)
77	(2.3)	77	(9.4)	105	(3.5)	105	(2.4)	89	(1.1)	89	(2.9)	91	(0.5)	91	(2.1)
78	(0.7)	78	(4.4)	106	(1.7)	106	(1.2)	91	(2.6)	91	(6.9)	105	(1.8)	105	(2.0)
91	(13.3)	91	(21.5)	117	(0.8)	117	(1.0)	106	(1.1)	106	(1.3)	106	(1.8)	106	(1.3)
103	(1.5)	103	(3.6)	133	(0.2)	133	(0.5)	107	(2.0)	107	(1.9)	135	(9.5)	135	(10.1)
105	(. . .)	105	(7.5)	134	(0.7)	134	(0.1)	121	(8.7)	121	(13.2)	136	(21.3)	136	(33.4)
115	(2.2)	115	(0.3)	148	(0.3)	148	(0.5)	122	(20.0)	122	(34.5)	147	(0.2)	147	(1.1)
120	(4.5)	120	(. . .)	149	(0.4)	149	(1.4)	134	(0.8)	134	(1.1)	148	(0.2)	148	(1.0)
134	(1.2)	134	(. . .)					135	(0.3)	135	(1.4)	179	(1.6)	179	(2.4)
135	(0.3)	135	(4.0)					165	(1.2)	165	(2.4)				
I-Ac		V-Ac		II-Ac		VI-Ac		III-Ac		VII-Ac		IV-Ac		VIII-Ac	
44	(100)	44	(100)	44	(100)	44	(100)	44	(100)	44	(100)	44	(100)	44	(100)
51	(3.8)	51	(3.5)	86	(13.6)	86	(13.1)	77	(5.9)	77	(5.3)	51	(8.6)	51	(8.2)
65	(10.0)	65	(6.1)	77	(5.7)	77	(5.2)	78	(6.3)	78	(5.9)	77	(9.0)	77	(7.4)
77	(2.1)	77	(3.2)	78	(2.4)	78	(2.3)	86	(8.9)	86	(6.2)	86	(7.0)	86	(4.8)
78	(1.1)	78	(1.8)	91	(3.6)	91	(3.5)	91	(3.8)	91	(4.8)	135	(11.6)	135	(10.4)
86	(31.4)	86	(23.4)	105	(8.7)	105	(6.7)	117	(2.5)	117	(0.3)	162	(27.9)	162	(. . .)
91	(17.2)	91	(7.7)	117	(7.7)	117	(5.9)	121	(17.8)	121	(17.7)	148	(. . .)	148	(47.4)
104	(0.5)	104	(6.6)	118	(1.4)	118	(28.5)	122	(5.5)	122	(2.3)	221	(1.6)	221	(1.6)
118	(25.5)	118	(0.5)	132	(32.0)	132	(0.4)	134	(0.9)	134	(55.3)				
177	(1.5)	177	(9.5)	191	(0.8)	191	(6.2)	148	(36.5)	148	(. . .)				
								207	(0.5)	207	(1.6)				

[a]Fragment ion, *m/z* (percent relative abundance to the base ion).

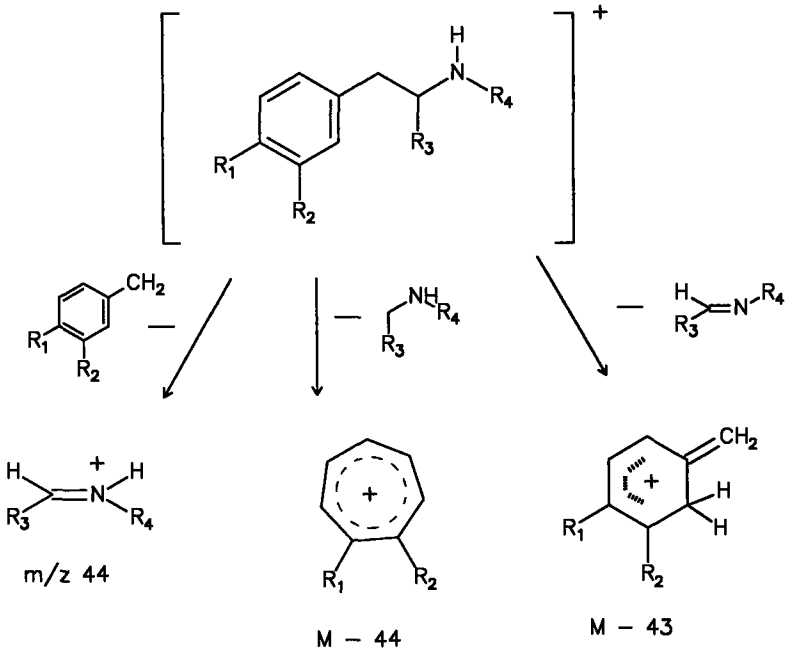


FIG. 3—*Proposed fragmentation pathway for the arylisopropylamines and* N-*methylarylethylamines.*

match for each isomer in the library generated was excellent and the correct isomer was always identified. However, as heteroatom substitution increased on the aromatic ring, the hit-quality values for the isopropylamine and *N*-methylethylamine isomer pairs were high and comparable in value. This makes unambiguous identification of each isomer more difficult and indicates that standards of each isomer would be helpful in unambiguously differentiating these compounds. In contrast, when identifying the *N*-acetyl derivatives using library searching, the equivalent positional isomer is usually not included in the hit-quality list. When present on the list, differentiation of the isomers is readily apparent upon visual inspection of the spectra. When analyzing the *N*-acetyl derivative of *II* (hit quality 982), the second compound listed was the *N*-acetyl derivative of *I* (its alkyl homolog) with a hit quality of 966. Comparable results were obtained when analyzing the *N*-acetyl derivative of *I*, *V*, and *VI*, but not for the *N*-acetyl derivatives of *III*, *IV*, *VII*, and *VIII*.

Conversion of the free amine to the amide enhanced the lower limit of detection for these compounds. Library searchable spectra for MS and IR were obtained for 20 ng and 92 ng of *I* on-column, respectively. Library searchable IR spectra for *N*-acetylamphetamine were obtained with 9.2 ng on-column (Fig. 5) and library searchable MS spectra at 2 ng on-column.

Discussion

Over ten arylisopropylamines in addition to their ring-positional isomers are regulated by the Drug Control Act, Code of Virginia. Most of these compounds are rarely observed and standards of the controlled substance, let alone possible positional isomers, are often not readily available. Due to their limited availability, the identification of these sub-

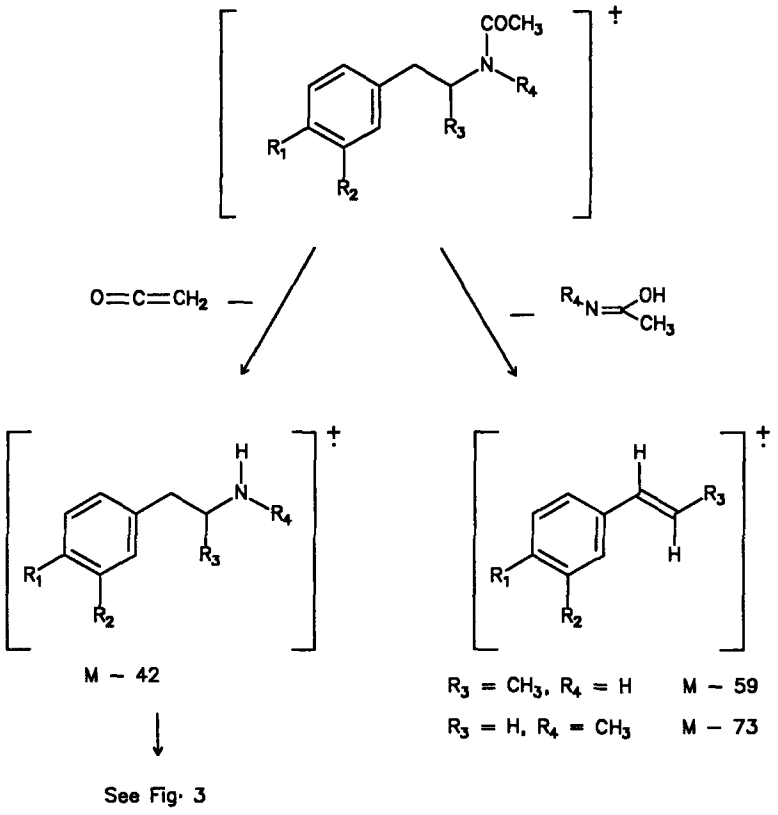


FIG. 4—*Proposed fragmentation pathway for the* N-*acetyl derivatives of the arylisopropylamines and* N-*methylarylethylamines.*

stances is based on analytical information provided in the literature. The studies in the literature have concentrated on differentiation of the substitution pattern on the aromatic ring or on homologs of the arylisopropylamine side chain (that is, *N*-methyl or *N*-ethyl derivatives). Prior work has rarely investigated whether the analytical method or methods used in the study would differentiate possible side-chain positional isomers, even though the analytical methods commonly used are based primarily on functional group differences or molecular weight differences or both. This is probably because of limited availability of the *N*-methylphenethylamine isomers, since minimal pharmacological activity would be anticipated for these compounds. When synthetic standards are available for comparison purposes it has been shown that ring-positional isomers or side-chain isomers can be differentiated. For example, even when the mass spectrum is similar, careful examination of the mass spectra of amphetamine and *N*-methylphenethylamine indicate minor differences. It is important to emphasize that when standards are available, other analytical tests, that is, the color tests, in conjunction with TLC and GC, also allow differentiation of positional isomers [*22*]. When a pure compound is being identified, the color tests associated with TLC can be directly correlated with more structure-specific information, that is, GC/MS. However, when analyzing samples submitted for analysis, the material being identified is frequently impure. The sample may contain synthetic by-products and other structurally related and unrelated compounds for which complete resolution by TLC may not always be achieved without use of special TLC techniques.

TABLE 4—*Wave numbers and relative intensity*[a] *for amine isomers.*

I	*V*	*II*	*VI*	*III*	*VII*	*IV*	*VIII*
3071(m)	3034(m)	3024(m)	3026(m)	2963(w)	2943(s)	2963(w)	2941(m)
3034(m)	2942(s)	2967(m)	2936(s)	2924(w)	2847(w)	2924(w)	2803(m)
2969(s)	2859(w)	2930(s)	2803(m)	2848(w)	2803(m)		
2928(s)	2803(s)						
1612(m)	1603(w)	1622(w)	1515(m)	1613(m)	1612(w)	1619(w)	1491(m)
1496(m)	1455(m)	1515(m)	1454(m)	1513(s)	1513(s)	1491(s)	1445(m)
1454(m)	1360(w)	1455(w)	1359(w)	1378(w)	1454(w)	1455(m)	1355(w)
1377(m)	1126(s)	1378(w)	1124(m)	1249(s)	1359(w)	1351(w)	1246(s)
1114(w)		1119(w)	1020(w)	1176(m)	1249(s)	1247(s)	1192(w)
				1116(w)	1175(w)	1192(w)	1121(w)
				1042(m)	1126(w)	1117(w)	1050(m)
					1042(w)	1050(m)	
789(m)	743(s)	791(s)	803(m)	799(m)	823(w)	946(m)	946(m)
736(s)	698(s)					798(m)	806(m)
699(m)							
I-Ac	*V-Ac*	*II-Ac*	*VI-Ac*	*III-Ac*	*VII-Ac*	*IV-Ac*	*VIII-Ac*
3034(w)	3033(w)	3025(w)	2935(m)	2942(w)	2942(m)	2933(w)	2934(m)
2976(w)	2938(w)	2935(w)					
2934(w)							
1712(s)	1685(s)	1712(s)	1684(s)	1712(s)	1684(s)	1712(s)	1684(s)
1493(s)	1398(m)	1493(s)	1398(m)	1612(w)	1513(m)	1490(s)	1491(s)
1371(w)		1371(w)		1510(s)	1398(m)	1368(w)	1443(m)
1245(m)		1245(m)		1495(m)	1250(s)	1248(s)	1398(m)
1148(w)		1147(w)		1368(w)	1178(w)	1049(m)	1247(s)
				1248(s)	1038(w)		1048(m)
				1049(m)			
		801(w)	805(w)	946(w)	825(w)	946(w)	945(w)
				808(w)		808(w)	807(w)

[a]Relative intensity: s = strong, m = moderate, w = weak.

Therefore, when a compound is being identified from a mixture, much of the structural information obtained from color tests and TLC may no longer directly correlate with spectroscopic information obtained from a GC/MS analysis.

Before the routine use of GC/MS, IR was recognized as the preferred method for differentiation and identification of isomers. IR spectra are often considered to be characteristic and unique for a compound, especially when comparing the "fingerprint" region (900 to 1400 cm^{-1}). In addition, with the development of methodology for obtaining vapor phase IR, the relative intensities of the absorbances, including relative weak absorbances, become more important and reproducible. This is because the matrix effects are absent in the vapor state. However, until the commercial availability of GC/IR, which can purify the sample prior to analysis, vapor phase IR did not have a broad application.

With the advent of the combination of GC/IR/MS, this methodology has potential for rapid and unambiguous identification of compounds with a significant savings of laboratory time. Since some forensic scientists have additional responsibilities, in addition to drug analysis, it is important that this combination of techniques be robust in its ability to differentiate compounds and that limitations of the methods are recognized. Currently, the analytical information on positional isomers is limited and there is a tendency to extrapolate information obtained from a series of simple compounds to more complex

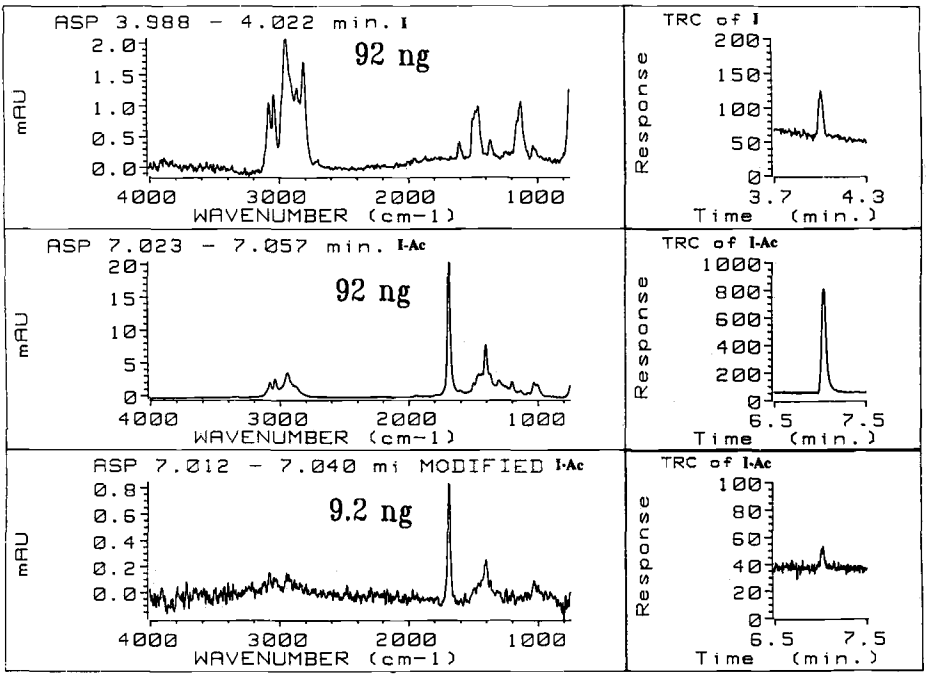


FIG. 5—*IR spectra of* (a) *amphetamine (92 ng),* (b) N-*acetylamphetamine (92 ng) and* (c) N-*acetylamphetamine (9.2 ng).*

homologs. For this study it was hypothesized that GC/IR/MS would be an unambiguous method for identification of any isomer or homolog of a regulated amphetamine even if analytical information specific to possible positional isomers was not available.

The C_3H_7N side chain was chosen for this initial study since it was the smallest side chain for which positional isomers were possible and for which comparable MS analytical data were anticipated. The choice of the *N*-methylphenethylamine side chain was based on prior reports in which this substitution pattern had been shown to give comparable mass spectra but different infrared spectra. In the earlier study using simple phenylalkylamines [*14*], the GC/IR data had indicated that the *C-H* and *C-N* stretching vibrations should be useful in differentiating positional isomers of homologs of amphetamine. The series of ring-modified homologs of the arylisopropylamines was chosen to increase the structural complexity of the analytical data. By keeping the structural changes simple and systematic, it was hoped that the spectral differences could be related back to structure. Information from this study can then be used to direct investigations with the C_4H_9N side chains found in the methamphetamine series, since comparable analytical data would be anticipated. Although correct identifications were provided, it was unexpected that for two of the isomers tested (*III* versus *VII* and *IV* versus *VIII*) the combination of MS and IR did not give as effective a differentiation of these isomers as previously observed for *I* versus *V* [*14*] or *II* versus *VI*.

The color tests, TLC, and GC analysis indicated that each pair of isomers can be differentiated provided a clean sample is being analyzed and proper conditions are used. The mass spectral study showed that the EI mass spectra of the respective arylisopropylamine and *N*-methylarylethylamine analogs are very similar (Fig. 6 for *III* and *VII*). The spectra are simple and are dominated by the dimethyl amine ion. The mass spectral library search was not capable of differentiating *IV* from *VIII* and gave an identical

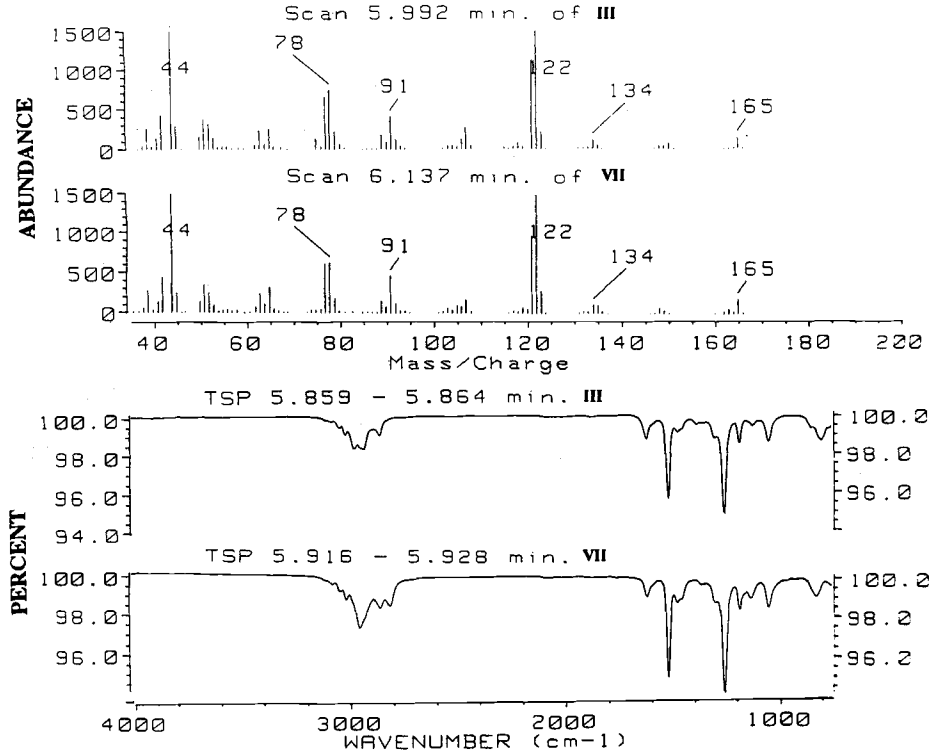


FIG. 6—*Spectrometric comparison of* III *and* VII *by* (a) *IR and* (b) *EI mass spectrometry.*

"quality" match regardless which isomer was being analyzed. Without standards of both or even one of the isomers for comparison, unambiguous differentiation of these isomers using GC/MS exclusively would be extremely difficult. The differences between the arylisopropylamines and the *N*-methylarylethylamines using IR for compounds *I* versus *V* and *II* versus *VI* were readily apparent. The IR spectra of *III* versus *VII* (Fig. 6) and *IV* versus *VIII* were very similar. Differentiation was dependent on the absence or presence of the weak to moderate *N*-methyl symmetric stretch (2803 cm^{-1}). In the earlier study the absorbances associated with the *C-N* aliphatic stretch and the methyl and methylene asymmetric stretch were characteristic for differentiating the isomers. In this series these absorbances decreased in relative intensity as substitution on the aromatic ring increased. This is probably due to the intense *C-O* stretch of the ether groups on the aromatic ring. Therefore, careful evaluation of the absorbances from 3000 to 2700 cm^{-1} is required for differentiating isomers with heteroatom substituents on the aromatic ring. These results indicate that both GC/MS and GC/IR will exhibit only minor differences that are useful in differentiating the more complex ring-substituted isomers when analyzed as the free base. The requirement for standards for comparison is still relatively important.

The amide derivatives, in contrast to the amines, are recognized for their improved chromatographic behavior [*23*]. When detecting the amides using either quadrupole or ion trap detectors, the amides exhibited improved mass spectral "character" by altering the fragmentation pathways [*23,24*]. On-column acetylation with acetic anhydride was chosen for this study due to its moderate reactivity, ready availability, and low cost. The derivatization reaction appeared to be quantitative and the acyl derivatives exhibited a

modified mass spectrum that enhanced the intensity of the other fragment ions relative to the base peak. Many of the same major ions observed for the free amine were also major ions for the amide. This probably results from cleavage of the carbonyl carbon-nitrogen bond followed by cleavage of the carbon-carbon bond beta to the nitrogen atom with rearrangement of a hydrogen atom to the nitrogen-containing fragment [*25,26*]. This fragmentation pathway indicates that other acyl derivatives (such as benzoyl and trifluoroacetyl) would fragment by a different pathway and provide different spectroscopic information [*27*]. Regardless, each acetylated isomer could be differentiated from each other, since the arylisopropylamine series gave a strong M-59 ion due to the *N*-acetyl fragment versus a strong M-73 ion for the *N*-methylacetyl fragment ion of the *N*-methylarylethylamines (see Fig. 7 for *III-Ac* and *VII-Ac*). The ratio of these other ions to the base peak has been enhanced, and differentiation of the isopropylamine side chain from the *N*-methylphenethylamine isomers is readily possible. The presence or absence of these ions can be used to differentiate these isomers.

More pronounced differences can also be observed in the IR of these derivatives (See Fig. 7 for *III-Ac* and *VII-Ac*). In particular, the strong carbonyl absorbance due to the Amide I band for the secondary amide (that is, the *N*-acetylarylisopropylamine) is easily differentiated from the Amide I band for the tertiary amide (*N*-acetyl-*N*-methylarylethylamine). The *C-N* bending for the secondary amide versus the tertiary amide is also useful in the differentiation of these isomers. The IR of the acetyl derivatives was unique for each pair of isomers. The infrared library search of the acetylated compounds of this series correctly identified each compound with good discrimination. These results indicate

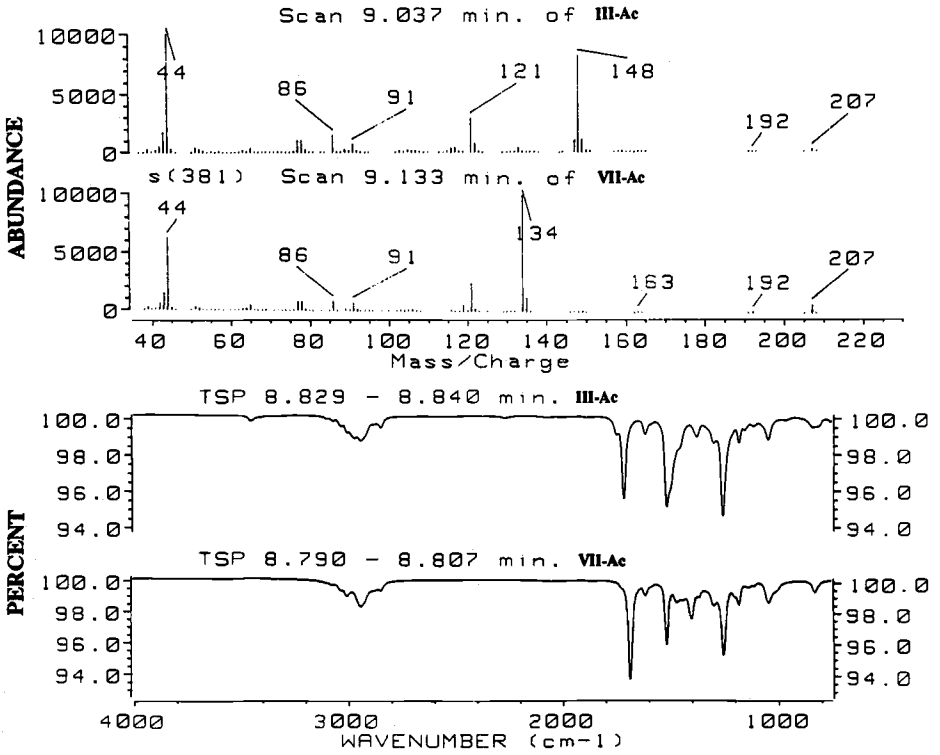


FIG. 7—*Spectrometric comparison of the* N-*acetyl derivative of* III *and* VII *by* (a) *IR and* (b) *EI mass spectrometry.*

that the derivatives are useful for the identification of amphetamine homologs and rule out possible side-chain positional isomers for which standards may not be readily available.

It should be mentioned that the carbonyl absorbance for the *N*-acetyl derivatives is much stronger than for the methyl and methylene asymmetric stretch, making these derivatives less effective in differentiating the amphetamine homologs in which the substituents on the ring are hydrogen and alkyl groups. For these compounds, the mass spectral data provide more definitive differentiation of the homologs.

Another advantage of the *N*-acetyl derivatives was the tenfold lower limit of detection for which library searchable IR or MS spectra could be obtained. The drug chemist is normally not limited in the quantity of material available for analysis; however, toxicological analysis of body fluids requires much lower limits of detection. The toxicologist finds it necessary to routinely derivatize arylalkylamines to enhance chromatography and quantification [*23,24*]. These results suggest that some derivatives may be useful for GC/IR detection and may provide additional confidence in their identification when used in conjunction with MS. Information is limited on the most effective derivatives for enhancing the sensitivity for GC/IR analysis.

Conclusion

Although the amphetamine and ring-modified analogs of amphetamine are not often observed in the illicit drug market, and although the *N*-methylarylethylamines have never been reported, when these compounds are encountered their unambiguous differentiation is important. Normally, IR complements the molecular weight and structural data from MS for identification of a compound. Although the GC/IR/MS analysis of the ring-substituted arylalkylamines was capable of differentiating the free-base form in this series, the differences were not striking. Techniques that are routinely used for GC or GC/MS (that is, on-column derivitization) were found to be directly applicable for GC/IR analysis. The spectrometrically independent IR and MS data of the derivatives show that the combined technique of GC/IR/MS can add a significant level of confidence to the analysis of arylisopropylamine compounds and can be extrapolated to compounds for which analytical standards may not be readily available.

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