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Approaches to Drug Sample Differentiation. III: A Comparative Study of the Use of Chiral and Achiral Capillary Column Gas Chromatography/Mass Spectrometry for the Determination of Methamphetamine Enantiomers and Possible Impurities

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ABSTRACT: A gas chromatograph/mass spectrometer (GC/MS) system equipped with a chiral and an achiral capillary column is used to analyze control methamphetamine, an illicit methamphetamine preparation ("White Cross"), and a simulated illicit methamphetamine synthesis product. Samples are derivatized with *N*-trifluoroacetyl-*l*-prolyl chloride (*l*-TPC) before the GC/MS analysis. The results obtained lead to the conclusion that the resolution on an achiral column is adequate for the determination of methamphetamine enantiomers and impurities, providing the enantiomeric impurity of the *l*-TPC is known. Four new possible by-products of methamphetamine preparations were identified in the simulated illicit methamphetamine synthesis product.

KEYWORDS: toxicology, drug identification, chemical analysis, enantiomer, chiral stationary phase, capillary column, amphetamine, methamphetamine, *N*-trifluoracetyl-*l*-prolyl chloride, gas chromatography/mass spectrometry

Because of differences in pharmacological effects and government regulatory measures, forensic science analyses of optically active drug samples face unique challenges and controversies [*1*]. The development of analytical methods suitable for the differentiation and determination of optically active drugs is more than an academic exercise or useful only for clinical or pharmacokinetic studies; it fills apparent forensic analytical needs.

Our laboratory is interested in various approaches in drug sample differentiation methods [*2*] and recently has developed specific procedures for the determination of methamphetamine [*3*] and amphetamine[3] enantiomers by using chiral shift reagents in the nuclear magnetic resonance spectrometric method of analysis. The purpose of this study is to explore the use of capillary column gas chromatography/mass spectrometry for the determination of

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[3]Liu, J. H. and Tsay, J. T., "Comparison of Chiral Lanthanide Shift Reagents for the Nuclear Magnetic Resonance Spectrometric Determination of Amphetamine Enantiomers," *The Analyst*, in press.

methamphetamine enantiomers. Specifically, chiral and achiral stationary phase wall-coated capillary columns are used to determine *N*-trifluoroacetyl-*l*-prolyl chloride (*l*-TPC) derivatized *d*- and *l*-methamphetamine samples. Procedures developed are then applied to the analyses of a field sample and a simulated illicit synthesis product.

Materials and Procedure

d- and *l*-Amphetamine were obtained from Aldrich Chemical Company (Milwaukee, WI). *d,l*-Methamphetamine hydrochloride and *d*-methamphetamine hydrochloride were purchased from Sigma Chemical Company (St. Louis, MO). *d*-Methamphetamine and *d,l*-methamphetamine were obtained by dissolving the appropriate salt in water and extracting with ether under basic conditions.

An illicit methamphetamine ("White Cross") preparation was provided by the Criminalistics Division of the Chicago Police Department. A simulated illicit methamphetamine preparation was prepared in this laboratory by the commonly used Leukart reaction [*4*].

The chiral derivatizing reagent, 0.1*M* *l*-TPC in chloroform, was purchased from Regis Chemical Company (Morton Grove, IL). The reagent was found [*5*] to contain 5.19% of *d*-TPC. All chemicals were kept dry and were used without further purification.

The standard TPC derivatization procedure recommended by the supplier was adopted to form the diastereoismeric pair of *N*-(trifluoroacetyl-*l*-prolyl)-*d,l*-amphetamine and *N*-(trifluoroacetyl-*l*-prolyl)-*d,l*-methamphetamine. A typical derivatization experiment started with the addition of 15 μL of *d,l*-methamphetamine to 0.50 mL chloroform in a pressure acylation tube, followed by the addition of 1.0 mL of the *l*-TPC reagent. The mixture was allowed to stand for 5 min before the addition of 20 μL of triethylamine to take up excess unreacted *l*-TPC. After 15 min of intermittent shaking, 1.0 mL of 6*N* HCl was used to remove the ammonium salt. The mixture was finally washed with 1 mL of distilled water and then dried over anhydrous magnesium sulfate before dilution and analysis. Control *d*-methamphetamine and *d*- and *l*-amphetamine were converted to their *N*-trifluoroacetyl-*l*-prolyl derivatives in the same manner.

A slight variation in procedure was used for the derivatization of the illicit methamphetamine preparation. A 0.20-g pulverized sample was dissolved in 8.0 mL of 0.10*N* sodium hydroxide. The solution was centrifuged to remove insoluble adulterants. The supernatant was extracted with 30 mL of chloroform three times. The organic layers were combined, dried with magnesium sulfate, and then evaporated to 0.50 mL under dry nitrogen. The 0.50-mL aliquot was subsequently used for *l*-TPC derivatization as described previously for control samples. The simulated illicit methamphetamine preparation was derivatized by using 15 μL of the crude synthetic product and following the same procedure used for derivatizing control amphetamine and methamphetamine.

Samples were analyzed on a Hewlett-Packard (Palo Alto, CA) HP-5985 gas chromatograph/mass spectrometer/data system (GC/MS/DS). The mass spectrometer was operated in electron impact mode at 70 eV. The source temperature was maintained at 200°C. Mass unit and relative abundance were calibrated with perfluorotributylamine [*6*]. Spectra were collected in the range $m/e = 45$ to 450 and, in most cases, started at 10 min after injection. A 13-m and a 25-m fused silica glass (0.20 mm inside diameter) SP-2100 (Hewlett-Packard, Avondale, PA) and a 25-m glass (0.30 mm inside diameter) Chirasil-Val (Applied Science, State College, PA) capillary columns were used for this study. Helium was used as the carrier gas throughout all experiments. The inlet pressure was maintained at 275 kPa (40 psi).

Results and Discussion

Separation of Enantiomers

Figure 1 shows the chromatograms of representative samples. The four possible isomers resulting from the reaction of *d*- and *l*-amphetamine with *d*- and *l*-TPC are completely re-

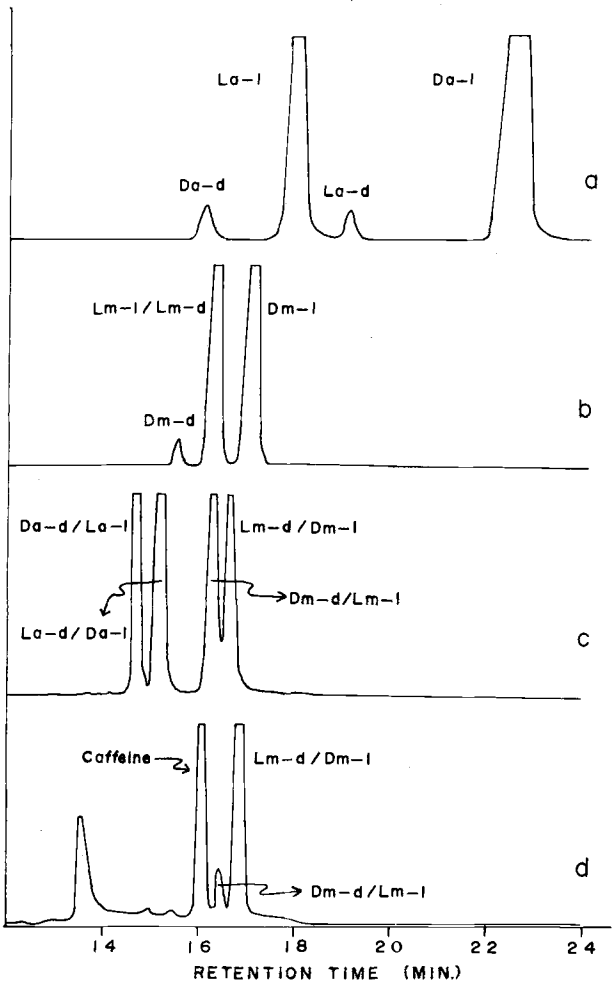

FIG. 1—*Total ion chromatograms of* (a) *authentic amphetamine and* (b) *authentic methamphetamine obtained from the Chirasil-Val column, and* (c) *authentic amphetamine and methamphetamine mixture and* (d) *illicit methamphetamine ("White Cross") obtained from the 25-m SP-2100 column. The GC conditions for (a) and (b) were starting temperature, 150°C; time at starting temperature, 1 min; final temperature, 200°C; temperature programming rate, 5°C/min; and carrier gas linear velocity, 44 cm/s. The corresponding conditions for (c) and (d) were 100°C; 1 min; 240°C; 10°C/min, and 28 cm/s.*

solved by the Chirasil-Val column (Fig. 1a). This is important because commercial TPC contains a small amount of *d*-TPC. The elution order of these four isomers in increasing retention time is *d*-amphetamine-*d*-TPC (Da-d), *l*-amphetamine-*l*-TPC (La-l), *l*-amphetamine-*d*-TPC (La-d) and *d*-amphetamine-*l*-TPC (Da-l). The assignments of these four peaks in a chromatogram were based on relative peak sizes. Since the purity [5] of the TPC reagent and the relative concentration of *d*- and *l*-amphetamine in control samples are known, the relative intensities of Da-d, La-l, La-d, and Da-l are predictable and their corresponding peaks are assigned accordingly.

Contrarily, the four isomers resulting from the reaction of *d*- and *l*-methamphetamine with *d*- and *l*-TPC are resolved into three peaks (Fig. 1b) only. Based on the relative intensities and the known quantity injected, these three peaks, in order of increasing retention

time, are *d*-methamphetamine-*d*-TPC (Dm-d), *l*-methamphetamine-*l*-TPC/*l*-methamphetamine-*d*-TPC (Lm-l/Lm-d), and *d*-methamphetamine-*l*-TPC (Dm-l). The inability of the Chirasil-Val column to resolve the four resulting isomers is attributed to the replacement of the active hydrogen atom attached to the nitrogen atom by a methyl group. This replacement reduces the efficiency in forming a transient diastereoisomeric association complex between the substrate and the chiral phase [7]. Further discussion on the separation of enantiomers is presented elsewhere [8].

Quantitative Determination of Enantiomers

Since no strictly *l*-methamphetamine sample was available, a series of solutions containing different ratios of *d*- and *l*-methamphetamine was prepared by mixing different ratios of *d,l*-methamphetamine and *d*-methamphetamine. These solutions were analyzed on the Chirasil-Val column and results are presented in Table 1. The exact enantiomeric concentrations can be calculated only if the enantiomeric purities of *d,l*-methamphetamine and *d*-methamphetamine are known. These purities are calculated based on the reasoning described below.

Since excess *l*-TPC was present during the derivatizing process, both the *d*- and the *l*-methamphetamine present will be derivatized. The limited amount of *d*-TPC present will be competed for by *d*- and *l*-methamphetamine. Furthermore, since the first sample in Table 1 is a "racemic" mixture, the amounts of *d*- and *l*-methamphetamine are approximately the same and, therefore, equal amounts of these two enantiomers will form derivatives with *d*-TPC. The concentration ratio of these two enantiomers in the first sample is obtained by dividing the areas of the Lm-l/Lm-d peak by the sum of the areas of Dm-l and Dm-d peaks. The ratio thus calculated is L/D = 51.5:48.5. The chromatogram of the last sample in Table 1 does not show the Lm-l/Lm-d peak indicating the high optical purity of the *d*-methamphetamine used in this study.

With the volume ratio and compositions of *d,l*-methamphetamine and *d*-methamphetamine available, the absolute quantity of each enantiomer injected in each analysis is easily calculated and is listed in the last column of Table 1. The quantities of *d*-methamphetamine in these samples represent a reasonable spread and are plotted in Fig. 2. The peak areas are based on total ion and single ions (166 and 251; see Fig. 3 for the fragments of these ions).

These solutions were also analyzed on a 25-m achiral SP-2100 column (Fig. 1c). Since Dm-l and Lm-d and Dm-d and Lm-l are enantiomers to each other and not resolved by the achiral column, only two peaks are observed. By observing the relative intensity of these two peaks, it is concluded that the Lm-l/Lm-d pair elute first. The peak areas contained in Table 2 have been corrected for the small amount of the other enantiomer co-eluted by using the procedure described elsewhere [5].

TABLE 1—*Observed peak areas of* d- *and* l-*methamphetamine-*l-*TPC and quantity versus response correlation of* d-*methamphetamine-*l-*TPC obtained from the analysis on the Chirasil-Val column.*

Volume Ratio *d,l/d*	Area Ratio (Dm-d + Dm-l)/(Lm-l + Lm-d)			Quantity Injected, ng (*d/l*)
	Total Ion	251	166	
100:0	4 092:4410	239:300	1173:1222	4.72:5.10
90:10	8 538:480	373:22	2111:189	8.80:1.02
84:17	1 559:212	144:22	495:94	2.20:0.461
75:25	961:218	84:11	304:84	1.45:0.510
0:100	16 076:0	895:0	3735:0	18.7:0
Intercept	−94.6	17.6	143.3	...
Slope	883.7	45.9	198.0	...
Correlation	0.997	0.996	0.994	...

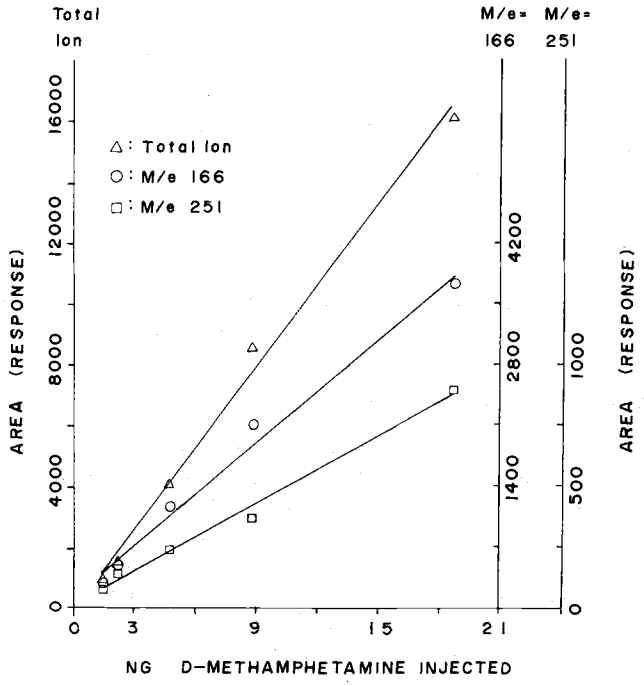

FIG. 2—*Plot of peak area versus quantity for* d-*methamphetamine obtained from the Chirasil-Val column. The GC conditions were identical to those of Figs. 1c and 1d.*

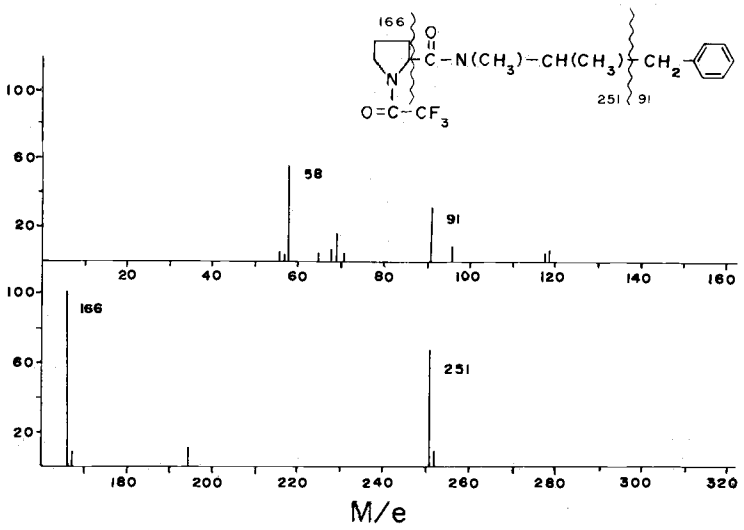

FIG. 3—*Mass spectrum of TPC-derivatized methamphetamine.*

TABLE 2—*Quantity ratios of* d- *and* l-*methamphetamine*-l-*TPC obtained from the analysis on the SP-2100 column.*

Injected, ng (*d*/*l*)	Corrected Peak Area (*d*/*l*)		
	Total Ion	251	166
5.39:5.83	88 420:98 910	9 537:11 240	24 291:27 948
7.75:0.896	152 909:22 044	16 917: 2 417	43 853: 6 265
2.66:0.558	84 901:16 051	9 197: 1 775	22 629: 4 375
1.38:0.486	30 726: 8 818	3 028: 1 137	7 583: 3 053
19.6:0	379 719: 0	37 693: 0	108 014: 0

The *d*- and *l*-methamphetamine ratios measured from peak areas are compared to those calculated and found to be in good agreement as shown in the second half of Table 3.

Analysis of Methamphetamine Preparations

The SP-2100 columns were further used to analyze an illicit methamphetamine ("White Cross") sample (25 m) and a simulated illicit methamphetamine synthesis product (13 m). The chromatogram of the illicit sample is shown in Fig. 1d. The peak areas indicated a composition of 95.2% *d*-methamphetamine and 4.8% *l*-methamphetamine. The peak preceding the *l*-methamphetamine peak is caffeine, which is commonly found [*9*] in illicit methamphetamine preparations. Assuming equal extraction efficiency and response factor, the concentration of caffeine is about 48.3% of *d*-methamphetamine.

The nuclear magnetic resonance (NMR) spectra (taken before derivatization) and the chromatogram (taken after derivatization) of the simulated illicit methamphetamine synthesis product are shown in Figs. 4 and 5, respectively. Major components are identified in Table 4, which also includes major mass fragments and chemical shifts of identified compounds. As expected, identification of all compounds in a single NMR spectrum is difficult, and only some of these compounds are identified with their chemical shifts. Most of the NMR assignments are crude approximations. These spectra might have been contributed by similar protons of other compounds listed in the table.

Of the eleven compounds identified in Table 4 and Fig. 5, seven have been previously reported [*10*] as impurities in various illicit methamphetamine preparations. They are presented here together with the four newly identified compounds to demonstrate that the approach used here possesses a high quality of separation that has never been achieved before. Chromatogram peaks 6 and 7 are a diastereoisomeric pair of *N*, α,α'-trimethyldiphenethylamine. Barron et al [*11*] observed a pair of NMR spectra closely related to *N*, α,α'-trimethyldiphenethylamine but failed to identify them as diastereoisomers. To the authors' knowledge, the three compounds identified for chromatogram peaks 5, 8, and 9 have not been

TABLE 3—*Peak area ratios of* d- *and* l-*methamphetamine*-l-*TPC obtained from the analysis on the SP-2100 column.*

Calculated *d*/*l* Ratio	Measured *d*/*l* Ratio				
	Total Ion	251	166	Average	Standard Deviation
0.925	0.894	0.848	0.869	0.870	0.023
8.63	6.94	7.00	7.00	6.98	0.035
4.77	5.29	5.17	5.17	5.21	0.069
2.84	3.48	2.66	2.48	2.87	0.533

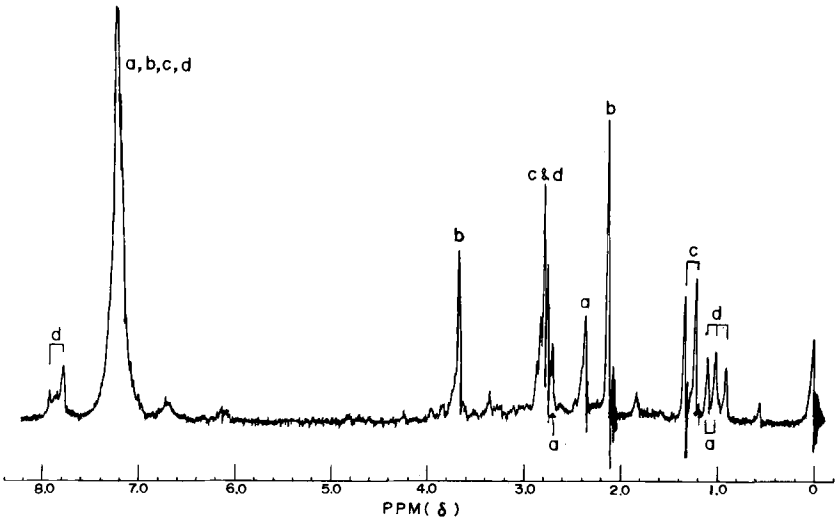

FIG. 4—*Nuclear magnetic resonance spectra of the simulated illicit methamphetamine synthesis crude product.*

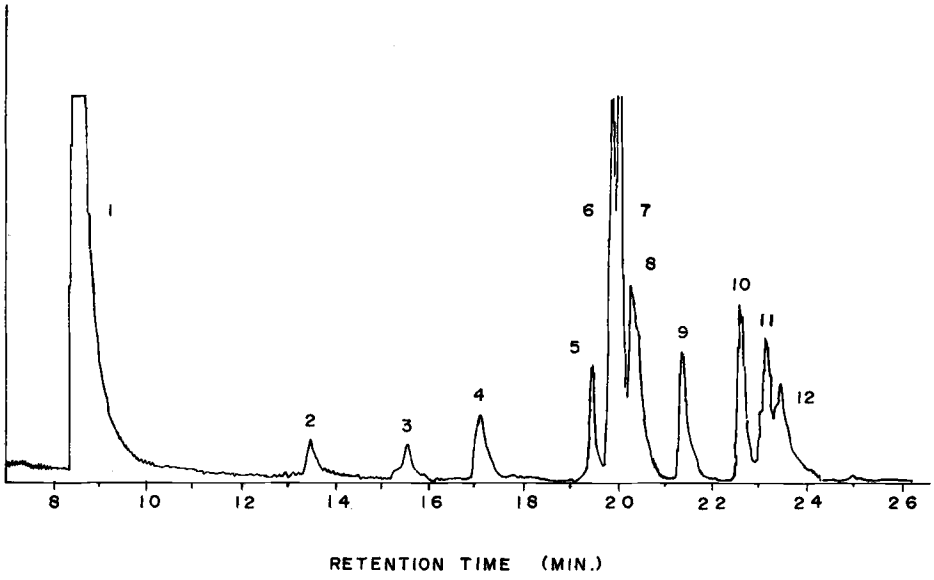

FIG. 5—*Total ion chromatogram of the* l-*TPC-derivatized simulated illicit methamphetamine synthesis crude product, obtained from the 13-m SP-2100 column. The GC conditions were starting temperature, 100°C; time at starting temperature, 1 min; final temperature, 180°C; temperature programming rate, 4°C/min; and carrier gas linear velocity, 33 cm/s.*

reported. These three compounds are identified here based on their mass spectra. The major fragments of these spectra are shown in Fig. 6. They are possible dehydration products of compounds derived from aldol condensation of methyl benzyl ketone. It should be noted, though, that these compounds might have been formed during the derivatization process, which was conducted under basic conditions.

TABLE 4—*Major mass fragments and chemical shifts for compounds found in the simulated illicit methamphetamine synthesis product.*

Compound	Peak No.[a]	Relative Retention Time[b]	Major Mass Fragments (*m/e*)[c]	Chemical Shifts, ppm[d]
Methyl benzyl ketone[e]	b	...	...	CCH_3(s): 2.13; CH_2(s): 3.68; ArH: ~7.23
N-formylmethamphetamine	1,d	0.371	86(B), 58(51), 91(14), 118(9)	CCH_3(t): 1.05; OCH(d): 7.85; NCH_3, CH_2(m): 2.75-3.35; ArH: ~7.23
Dibenzyl ketone	2	0.582	91(B), 65(23), 86(16), 49(15)	...
N-methyldiphenethylamine	3	0.672	91(B), 148(97)	...
α,α'-dimethyldiphenethylamine[f]	4	0.373	162(B), 91(99), 119(29), 70(17)	...
1,5-diphenyl-4-methyl-4-penten-2-one	5	0.836	91(B), 131(39), 115(15), 65(14)	...
N,α,α'-trimethyldiphenethylamine	6,c	0.862	176(B), 91(93), 58(43), 177(16)	CCH_3(d): 1.29; NCH_3, CH_2, CH(m): 2.75-3.35; ArH: ~7.23
N,α,α'-trimethyldiphenethylamine	7	0.866	91(B), 176(98), 58(46), 199(19)	CCH_3(d): 1.29; NCH_3, CH_2, CH(m): 2.75-3.35; ArH: ~7.23
3,5-diphenyl-4-methyl-3-penten-2-one	8	0.875	159(B), 91(43), 144(26), 141(20)	...
1,5-diphenyl-4-methyl-3-penten-2-one	9	0.922	91(B), 131(50), 159(22), 115(16)	...
l-methamphetamine-*l*-TPC/*d*-methamphetamine-*d*-TPC	10,a	0.974	166(B), 58(72), 251(71), 91(34)	CH_3(d): 1.09; NCH_3(s): 2.37; CH_2, CH(m): 2.70; ArH: ~7.23
d-methamphetamine-*l*-TPC/*l*-methamphetamine-*d*-TPC	11,a	1.00	166(B), 58(72), 251(71), 91(34)	...
SP-2100 column bleed	12	1.01	143(B), 71(70), 142(59), 99(50)	...

[a] 1 to 11 represent chromatogram peak numbers in Fig. 5 and a to d represent NMR spectrum peak numbers in Fig. 4.

[b] Retention times are listed in relation to *d*-methamphetamine (23.2 min).

[c] B = base peak.

[d] s = singlet; d = doublet; t = triplet; m = multiplet. The assignments of these spectra are crude approximations. These spectra might have been contributed by similar protons of other compounds in this table.

[e] This compound was eluted with solvent. Mass spectrum was not taken.

[f] This compound was not derivatized by *l*-TPC, presumably because of steric effect.

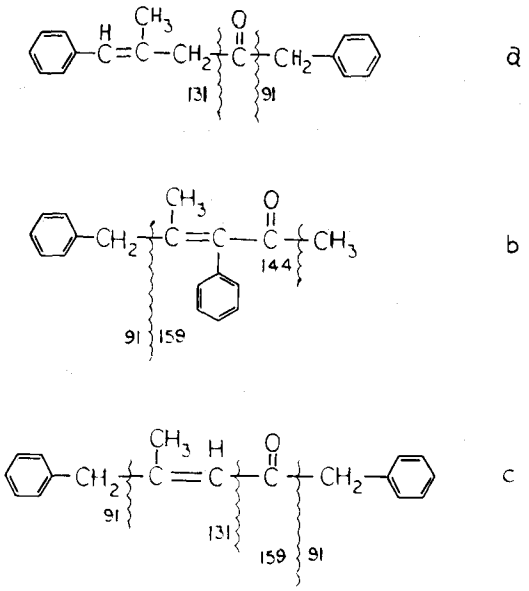

FIG. 6—*Major fragmentation patterns of* (a) *1,5-diphenyl-4-methyl-4-penten-2-one;* (b) *3,5-diphenyl-4-methyl-3-penten-2-one, and* (c) *1,5-diphenyl-4-methyl-3-penten-2-one.*

It should also be noted that although the derivatized product of control amphetamine and methamphetamine is very stable, the derivatized product from the simulated illicit methamphetamine sample shows signs of deterioration in less than one week. This suggests that the derivatization of unknown samples should not be conducted too long before chromatographic analysis.

The results reported here clearly demonstrate the merit of using capillary columns. It appears that the combination of an achiral column and the chiral *l*-TPC derivatizing reagent is adequate for analyzing *d*- and *l*-methamphetamine, providing the enantiomeric purity of *l*-TPC is known.

Acknowledgment

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