J. H. Liu,[1] *Ph.D.; S. Ramesh,*[2] *Ph.D.; J. T. Tsay,*[1] *LL.B.; W. W. Ku,*[1] *B.S.; M. P. Fitzgerald,*[1] *B.S.; S. A. Angelos,*[3] *M.S.; and C. L. K. Lins,*[4] *Ph.D.*

Approaches to Drug Sample Differentiation. II: Nuclear Magnetic Resonance Spectrometric Determination of Methamphetamine Enantiomers

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ABSTRACT: Synthesized tris[*d,d*-dicampholylmethanato]europium(III), Eu(dcm)$_3$, and three other commercially available chiral lanthanide shift reagents are used to resolve the nuclear magnetic resonance (NMR) spectra of the enantiomeric N-CH_3 and C-CH_3 protons in *d*- and *l*-methamphetamine. The chemical shift difference between the corresponding enantiomeric protons induced by these four shift reagents are compared and evaluated in their prospective use for enantiomeric identification and determination. It is concluded that while the chemical shift difference between the two enantiomeric C-CH_3 protons induced by Eu(dcm)$_3$ is most suitable for qualitative identification of these enantiomers, the NMR spectra of the N-CH_3 protons are best resolved by Eu(dcm)$_3$ and most suitable for quantitative determinations.

KEYWORDS: toxicology, drug identification, chemical analysis, enantiomer, chiral shift reagent, amphetamine, methamphetamine

Mainly because of the widespread clandestine manufacture of methamphetamine and amphetamine [*1*], numerous papers [*2-7*] concerning these drugs have appeared in the forensic science literature. However, all of these studies address only the presence of various impurities that may be used to identify samples of common origin and to distinguish between samples of legitimate and illicit manufacture. Because *d*- and *l*-isomers have different potency of stimulating effect [*8*], the differentiation of these two forms of isomer is an issue of medical and regulatory significance.

The use of a derivatizing agent [*9*]; chiral solvent, alone or with an achiral lanthanide shift reagent [*10*]; and chiral lanthanide shift reagents [*11-13*] has rendered the differentiation of enantiomers by nuclear magnetic resonance (NMR) spectrometry possible. These approaches have also been applied to the determination of cocaine isomers [*14,15*]. The pur-

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[1] Assistant professor, graduate student, graduate student, and graduate student, respectively, Department of Criminal Justice, University of Illinois at Chicago Circle, Chicago, Ill.

[2] Research associate, Department of Chemistry, University of Illinois at Chicago Circle, Chicago, Ill.

[3] Forensic chemist, North Central Regional Laboratory, Drug Enforcement Administration, Chicago, Ill.

[4] Research associate, Department of Medicinal Chemistry, University of Illinois at the Medical Center, Chicago, Ill.

pose of this study is to explore the use of more effective chiral shift reagents for the determination of methamphetamine enantiomers. Specifically, four chiral shift reagents, one synthesized and three commercially obtained, were used in this study.

Materials and Methods

Four shift reagents used for this study are tris(*d,d*-dicampholylmethanato)europium(III) [Eu(dcm)$_3$]; tris(3-trifluoroacetyl-*d*-camphorato)europium(III) [Eu(tfac)$_3$ or Eu(facam)$_3$], also called tris(3-trifluoromethylhydroxymethylene-*d*-camphorato)europium(III) [Eu(tfc)$_3$]; tris(3-heptafluorobutyryl-*d*-camphorato)europium(III) [Eu(hfbc)$_3$], also called tris(3-heptafluoropropyl-hydroxymethylene-*d*-camphorato)europium(III) [Eu(hfc)$_3$]; and tris(3-heptafluorobutyryl-*d*-camphorato)praseodymium(III) [Pr(hfbc)$_3$].

Eu(hfbc)$_3$ and Pr(hfbc)$_3$ were obtained from Stohler Isotope Chemicals (Waltham, Mass.). Eu(tfac)$_3$ was purchased from Norell, Inc. (Landisville, N.J.). Eu(dcm)$_3$ was synthesized as described in the literature [*12*] by reacting *d,d*-dicampholylmethane with sodium methoxide and then with europium(III) chloride hexahydrate. *d,d*-Dicampholylmethane was prepared by reacting *d*-campholylmethane with lithium diisopropylamide and then with *d*-campholyl chloride. Both *d*-campholylmethane and *d*-campholyl chloride were derived from *d*-campholic acid, which was prepared by fusion of *d*-camphor and potassium hydroxide in a rocking steel bomb. All special reagents except methyllithium, which was obtained from Alfa Products Div., Ventron Corp. (Danvers, Mass.), were purchased from Aldrich Chemical Co. (Milwaukee, Wis.).

l-Amphetamine was obtained from Aldrich. *d*-Amphetamine sulfate, *d,l*-methamphetamine hydrochloride, and *d*-methamphetamine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, Mo.). *d*-Methamphetamine, *d,l*-methamphetamine, and *d*-amphetamine were obtained by dissolving the appropriate salt in water and extracted with ether under basic conditions.

Nuclear magnetic resonance spectra were obtained at 60 MHz with two Varian (Walnut Creek, Calif.) Model T-60 spectrometers. One of these spectrometers was operated on the "FT" mode by interfacing the spectrometer to a Nicolet (Madison, Wis.) Model TT-7 Fourier transform accessory. The pulse width and relaxation delay used in the FT determinations were 10 μs and 1 s, respectively. Data presented in Figs. 1 and 2 on Eu(dcm)$_3$/methamphetamine in Table 1 were obtained at FT mode.

d-Methamphetamine, *l*-methamphetamine, and shift reagents were dissolved in deuterochloroform (1*M*, 1*M*, and 0.05*M*, respectively). A typical series of experiments, intended for the measurement of chemical shift difference ($\Delta\Delta\delta$) as a function of the molar ratio *MR* of shift reagent to amphetamine, started with 20 μL of *d,l*-methamphetamine stock solution and one drop of tetramethylsilane (TMS) in 0.5 mL of deuterochloroform. Five-microlitre aliquots of shift reagent solution were successively added until the *MR* was about 0.5 or until the NMR spectrum became too broad. A NMR spectrum was taken at ambient temperature before the addition of each aliquot of shift reagent solution. A typical set of experiments designed for the quantitative determination of individual enantiomers started with 20 μL of *d,l*-methamphetamine stock solution and one drop of TMS in 0.5 mL of deuterochloroform. Eu(dcm)$_3$ (160 μL) was added to produce an optimum *MR* (see Results and Discussion). Fifty-microlitre aliquots of deuterochloroform were successively added to provide different substrate concentrations. An NMR spectrum and an integration were obtained before the addition of each aliquot of solvent.

Results and Discussion

Of the four possible ways of generating $\Delta\Delta\delta$ between the corresponding groups of enantiomers previously discussed, the use of a proper chiral shift reagent is usually the most effective [*12*]. Figure 1 shows the variation of N-CH$_3$ and C-CH$_3$ proton NMR spectra of *d*- and

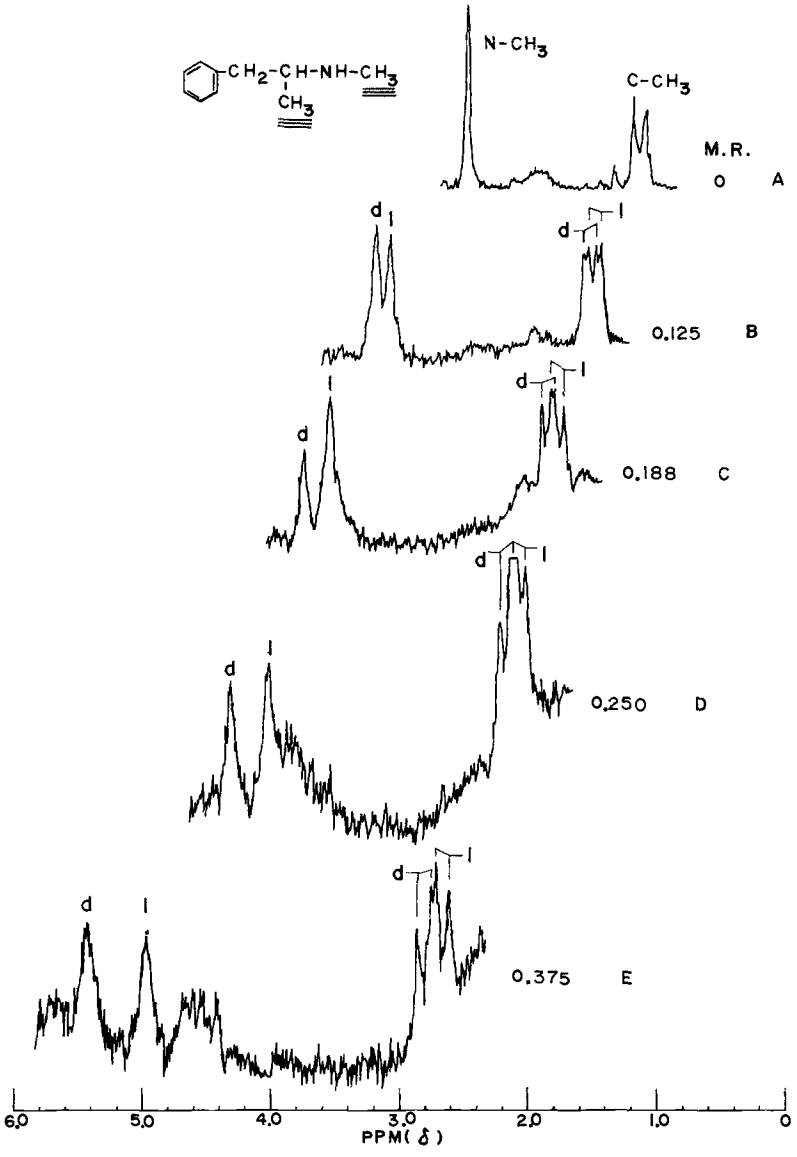

FIG. 1—*N-CH_3 and C-CH_3 proton NMR spectra of* d-*methamphetamine* (d) *and* l-*methamphetamine* (l) *mixture in the absence* (A) *and presence* (B *to* E) *of various amounts of Eu(dcm)$_3$.*

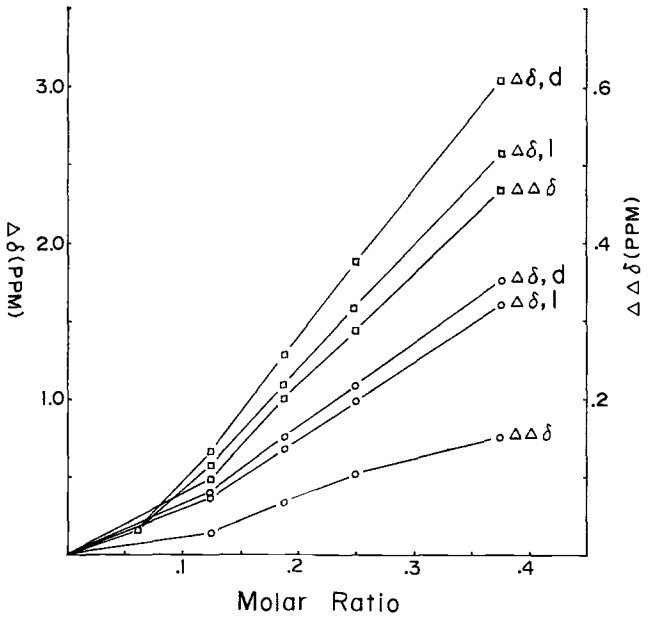

FIG. 2—*Shift in chemical shift* ($\Delta\delta$, left axis) *of* d-*methamphetamine* (d) *and* l-*methamphetamine* (l) *and chemical shift difference* ($\Delta\Delta\delta$, right axis) *between* d-*methamphetamine and* l-*methamphetamine* (*N-CH*$_3$ (□) *and C-CH*$_3$ (○) *protons*).

l-methamphetamine with various amounts of Eu(dcm)$_3$ added. The variation in chemical shifts ($\Delta\delta$) of both enantiomers and $\Delta\Delta\delta$ between these two enantiomers for both N-CH$_3$ and C-CH$_3$ protons are plotted in Fig. 2, where the $\Delta\delta$ values are on the left and the $\Delta\Delta\delta$ values are on the right. Considering the magnitude of $\Delta\Delta\delta$ and peak broadening, the optimum *MR* for resolving C-CH$_3$ proton spectra falls in the range of 0.02 to 0.15.

The use in trace analysis of the single proton attached to the chiral center is limited by its low intensity and multiplicity. Although C-CH$_3$ proton spectra can be used for qualitative differentiation between these two enantiomers, the resolution of the N-CH$_3$ proton is more suitable for quantitative determination. It should be noted that the methine group may interfere with the N-CH$_3$ spectra if the *MR* is not carefully chosen.

Results obtained by using the other three shift reagents are much less satisfactory. Figure 3 includes methamphetamine spectra in the absence (A) and presence (B to E) of optimum *MR* of shift reagents. Eu(dcm)$_3$ is the only shift reagent that is effective in resolving these corresponding enantiomeric protons. Whatever $\Delta\Delta\delta$ may have been induced by other shift reagents is unresolved as a result of peak broadening. Table 1 compares the $\Delta\delta$ and $\Delta\Delta\delta$ (C-CH$_3$ group protons) of methamphetamine with those of amphetamine obtained with optimum *MR*. Although only Eu(dcm)$_3$ is effective in resolving these corresponding protons in methamphetamine, Eu(dcm)$_3$, Eu(tfac)$_3$, and Eu(hfbc)$_3$ are all capable of resolving these protons in amphetamine. However, Eu(dcm)$_3$ is again the best choice in the analysis of amphetamine enantiomers. Numerical values in Table 1 also indicate the better resolution of the C-CH$_3$ protons in amphetamine.

A series of experiments were performed to test the applicability of using the integration of the N-CH$_3$ spectra for quantitative determination of methamphetamine enantiomers. Spectra of a solution containing *d*- and *l*-methamphetamine with *MR* = 0.42 was repeatedly

TABLE 1—*Comparison of the resolution power of shift reagents on the C-CH$_3$ proton (of amphetamine and methamphetamine) nuclear magetic resonance spectra.*

Amphetamine				Methamphetamine			
Molar Ratio[a]	Δδ, ppm *d-*	Δδ, ppm *l-*	ΔΔδ, ppm	Molar Ratio[a]	Δδ, ppm *d-*	Δδ, ppm *l-*	ΔΔδ, ppm
			Eu(dcm)$_3$				
0.0625	0.317	0.259	0.058	0.125	0.386	0.360	0.026
0.125	0.784	0.634	0.150	0.188	0.749	0.681	0.068
0.150	0.967	0.784	0.183	0.250	1.087	0.985	0.102
0.200	1.350	1.100	0.250	0.375	1.766	1.616	0.150
0.250	1.734	1.426	0.308	...	...	...	...
0.300	2.125	1.750	0.375	...	...	...	...
0.350	2.621	2.177	0.454	...	...	...	...
0.400	3.031	2.533	0.498	...	...	...	...
0.500	3.760	3.180	0.580	...	...	...	...
			Eu(tfac)$_3$				
0.0625	0.484	0.484	...	0.0833	0.300	0.300	...[b]
0.125	0.934	0.934	...	0.167	0.633	0.633	...
0.188	1.279	1.300	0.021	0.333	1.17	1.17	...
0.250	1.614	1.642	0.028	...	...	...	...
0.313	1.909	1.942	0.033	...	...	...	...
0.375	2.163	2.200	0.037	...	...	...	...
0.438	2.417	2.459	0.042	...	...	...	...
0.500	2.621	2.667	0.046	...	...	...	...
0.563	2.842	2.892	0.050	...	...	...	...
0.625	3.038	3.090	0.052	...	...	...	...
0.688	3.267	3.324	0.057	...	...	...	...
			Eu(hfbc)$_3$				
0.025	0.067	0.067	...	0.167	0.216	0.216	...[b]
0.050	0.134	0.134	...	0.333	0.433	0.433	...
0.125	0.367	0.367	...	...	...	...	...
0.188	0.571	0.546	0.025	...	...	...	...
0.214	0.625	0.596	0.029	...	...	...	...
0.250	0.684	0.650	0.034	...	...	...	...
0.300	0.767	0.725	0.042	...	...	...	...
0.375	0.934	0.875	0.059	...	...	...	...
0.500	1.591	1.508	0.083	...	...	...	...

[a] Shift reagent/substrate.
[b] Peaks are too broad to reveal any resolution.

taken at different dilutions by adding increasing amounts of solvent. The percentage of an enantiomer is calculated by dividing the integration area of the corresponding enantiomer by the total area. Results are presented in Table 2. The integrated area is plotted against concentration in Fig. 4. The comparability of calculated and measured composition and the linearity of the plot of area versus concentration indicate that quantitative determination is possible even with the relatively small amount of sample used. The fact that the plots of area versus concentration do not pass through zero is probably due to an error in the concentration axis. The assumption that the *d-*,*l-*methamphetamine sample used is truly racemic is probably not valid.

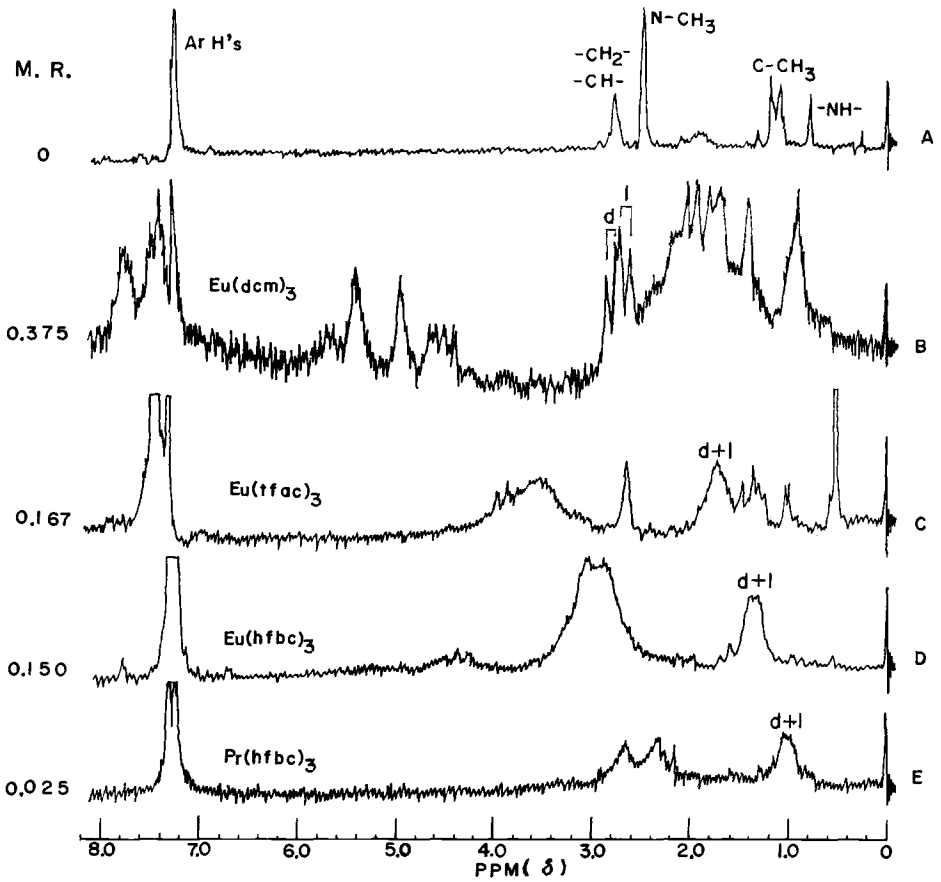

FIG. 3—*Comparison of the resolving power (with optimum ratio of shift reagent to methamphetamine) of the four chiral shift reagents on the N-CH_3 and C-CH_3 protons of* d- *and* l-*methamphetamine:* (A) *no shift reagent,* (B) *Eu(dcm)$_3$,* (C) *Eu(tfac)$_3$,* (D) *Eu(hfbc)$_3$, and* (E) *Pr(hfbc)$_3$.*

TABLE 2—*Use of integrated peak area (N-CH_3 proton) for the determination of* d- *and* l-*methamphetamine composition.*

Experiment	Concentration Used, m*M*	Integrated Area	Calculated,[a] %	Measured,[b] %
1	*d*- 75	18.0	50.0	51.4
1	*l*- 75	17.0	50.0	48.6
2	*d*- 68	16.5	50.0	52.4
2	*l*- 68	15.0	50.0	47.6
3	*d*- 63	15.0	50.0	51.7
3	*l*- 63	14.0	50.0	48.3
4	*d*- 58	14.0	50.0	52.8
4	*l*- 58	12.5	50.0	47.2
5	*d*- 54	13.0	50.0	53.1
5	*l*- 54	11.5	50.0	46.9

[a] Quantity of the corresponding enantiomer in the solution divided by the total methamphetamine present.

[b] Peak area of the corresponding enantiomer divided by the total peak area.

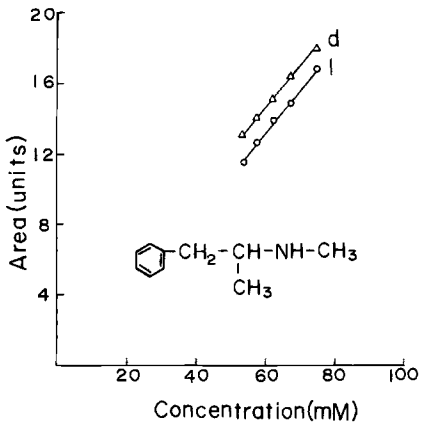

FIG. 4—*Integrated area of* d- *and* l-*methamphetamine versus concentration.*

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Address requests for reprints and additional information to
Juei H. Liu, Ph.D.
Department of Criminal Justice
University of Illinois at Chicago Circle
Box 4348
Chicago, Ill. 60680