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Chiral identification and determination of ephedrine, pseudoephedrine, methamphetamine and metecathinone by gas chromatography and nuclear magnetic resonance

M.J. LeBelle*[a], C. Savard[a], B.A. Dawson[a], D.B. Black[a], L.K. Katyal[b], F. Zrcek[c], A.W. By[a]

[a]*Pharmaceutical Chemistry Division, Bureau of Drug Research Banting Building, Tunney's Pasture, Ottawa, Ontario, Canada K1A OL2*
[b]*Forensic Science Laboratory, Haryana Madhybam (Karnal), Karnal, India*
[c]*Institute of Criminalistics, Criminal Police Centre, Prague, Czech Republic*

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Abstract

The enantiomers of the related substances methamphetamine, ephedrine, pseudoephedrine and methcathinone were determined by both gas chromatography after derivatization and by nuclear magnetic resonance using a chiral solvating agent. For GC the substances were derivatized with (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) to give diasteromeric derivatives. Resolution (baseline) of at least 1.6 was obtained between all derivatives. NMR determination of the enantiomers was conducted in a chiral environment by the addition of the chiral solvating agent, (*R*)-(+)-1,1′-bi-2-naphthol, to NMR solutions of the substances. Racemization of methcathinone was demonstrated to be facile by exposure to alkaline solutions for varying periods of time. Enantiomeric ratios of some products derived from the oxidation of ephedrine were determined.

Keywords: Methamphetamine; Methcathinone; Ephedrine; Chiral derivatization; Resolution; Chiral solvating reagent; Gas chromatography; Nuclear magnetic resonance

1. Introduction

Recently we reported the chiral derivatization and identification of the phenylalkylamine alkaloids of Khat, *Catha edulis* Forsk [1]. This involved the derivatiza-

* Corresponding author.

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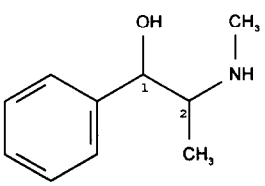
tion and gas chromatographic (GC) separation of the diastereomeric derivatives of norpseudoephedrine, norephedrine and cathinone. We subsequently compared the results obtained using GC with those obtained using NMR with a chiral solvating reagent [2].

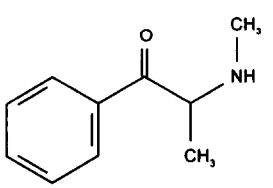
Ephedrine and pseudoephedrine are also of forensic importance. (*R*,*S*)-(−)-ephedrine and (*S*,*S*)-(+)-pseudoephedrine are both components of a number of accepted pharmaceutical preparations. Ephedrine, in particular, has become a valuable precursor as it serves as a starting material for two powerful and popular stimulants, methamphetamine and methcathinone (Fig. 1).

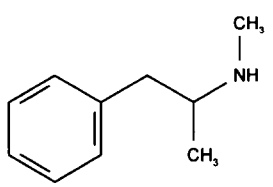
Methamphetamine is known by many names — speed, ice, crack, meth, and czecho — depending on the country. Despite the many schemes available for its synthesis, the red phosphorous-hydriodic acid procedure [3] for the reduction of ephedrine has often been identified as the most popular. In the Czech Republic where hydriodic acid is difficult to obtain, the reduction has been carried out by in situ generation of hydriodic acid from iodine and red phosphorous in concentrated phosphoric acid. With loss of the benzylic chiral centre, reduction of either (*R*,*S*)-ephedrine, **1RS**, or (*S*,*S*)-pseudoephedrine, **2SS**, yields (*S*)-methamphetamine, **4S**.

Methcathinone appears to have had its genesis in what was, at that time, the USSR [4]. It is also known by many names including cat, ephedrone, and methylpropion. Its amphetamine-like effect [5] has led to its wide popularity, particularly in the Midwest of the USA [6]. One could expect both permanganate [3] and



NAME	REFERENCE
(1R,2S)-(-)-EPHEDRINE	**1RS**
(1S,2R)-(+)-EPHEDRINE	**1SR**
(1S,2S)-(+)-PSEUDOEPHEDRINE	**2SS**
(1R,2R)-(-)-PSEUDOEPHEDRINE	**2RR**





NAME	REFERENCE
(R)-(+)-METHCATHINONE	**3R**
(S)-(-)-METHCATHINONE	**3S**

NAME	REFERENCE
(R)-(-)-METHAMPHETAMINE	**4R**
(S)-(+)-METHAMPHETAMINE	**4S**

Fig. 1. Structures and stereochemical notation for ephedrine, pseudoephedrine, methcathinone and methamphetamine.

dichromate [7] to be used for the oxidation of ephedrine and pseudoephedrine depending on availability. The presumably more active (*S*)-enantiomer, **3S**, is derived from oxidation of (*R,S*)-ephedrine, **1RS**, and (*S,S*)-pseudoephedrine, **2SS**.

The need for specific methods to determine the isomeric composition of methamphetamine and methcathinone in order to help establish synthetic procedures and to provide intelligence information on samples (T.A. Dal Cason; F.T. Noggle, J. DeRuiter, A. Valaer and C.R. Clark; and F.T. Noggle, J. DeRuiter, L. Hayes and C.R. Clark, pers. commun.) has been described by several others [3,8–10].

Recently, a review [11] appeared describing the technical choices available for the chiral analysis of methamphetamine. Chiral derivatization for GC analysis was restricted to substituted prolyl chlorides. We found that the GC resolution of the trifluoroacetylprolyl chloride (TPC) derivatives of the khat related substances was inferior to that obtained with MTPA. The results reported by McKibben [9] indicate incomplete resolution of the four diastereomeric TPC derivatives of ephedrine and pseudoephedrine.

We report here the results of the determination of mixtures of the eight possible isomers of the titled-substances by both GC and NMR. GC analysis was performed after derivatization with (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid. NMR analysis was carried out using the chiral solvating agent, (*R*)-(+)-1,1′-bi-2-naphthol.

2. Experimental

2.1. Materials and supplies

The following reagents were obtained from Aldrich Chemical Co.: (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid; (+)-ephedrine hydrochloride, **1SR**; (*R*)-(+)-1,1′-bi-2-naphthol; and dicyclohexylcarbodiimide (DCC). The following were obtained from Sigma: (+,−)-methamphetamine hydrochloride, **4**; (*R,R*)-(−)-pseudoephedrine base, **2RR**, (+)-pseudoephedrine hydrochloride, **2SS**, and (+,−)-ephedrine hydrochloride, **1**. (+,−)-Methcathinone hydrochloride, **3**, was prepared from 2-bromopropiophenone and methylamine [12]. (*R*)-Methcathinone, **3R**, was prepared from **1SR** by permanganate oxidation [3]. The rest of the standard materials were available from the Bureau's collection of drug standards.

2.2. Instrumentation

Gas chromatograph-mass spectrometer. The GC was a Carlo Erba Vega Series 6000 equipped with a Grob-type split/splitless injector and a capillary column. The column, DB-5, 15 m × 0.25 mm, 0.25 μm film thickness, was obtained from J and W Scientific (Rancho Cordova, CA). The column was operated at a head pressure of 40 kPa of helium (linear velocity, 60 cm/s). The injector was maintained at 275°C. The column conditions were: 190°C, hold for 1 min, increase temperature at 2°/min to 220°C, hold for 1 min, then increase temperature at 20°/min to 275°C. Injections (1 μl) were made at a split ratio of approximately 25:1. The mass spectrometer was a Finnigan Mat 800 Ion Trap Detector. The gas chromatograph's column effluent was introduced to the spectrometer by direct attachment of the column to the Ion

Trap detector; no splitting occurred between the gas chromatograph and the detector. The Ion Trap was set to acquire data at 1 scan/s over a mass range of 40–500 amu. Chemical ionization spectra were acquired with methane using the standard operating procedures specified by the manufacturer.

Gas chromatography. The gas chromatograph was a model HP 5890 equipped with an HP 7673A autosampler, a flame ionization detector, a split/splitless injector and an HP 3365 ChemStation data system/system controller. The column and temperature program were identical to those used in the GC-MS. The instrument was operated at a head pressure of 10 kPa with a flow of 1.5 ml/min of helium. The detector was operated with a mixture of air at 400 ml/min and hydrogen 30 ml/min. Nitrogen was used as make-up gas at a flow of 30–40 ml/min. Injections (1 μl) were made in split mode.

Nuclear magnetic resonance. Spectra were recorded on a Bruker AM-400 spectrometer equipped with an Aspect 3000 computer and process controller. Standard microprograms from the Bruker Software Library were employed. Samples were recorded in a 5-mm NMR tube at 298 K using 32 transients and a standard 5-mm Bruker ^1H probe, a 30° flip angle and acquisition time of 2.92 s. A relaxation delay of 3 s was specified. Data from a sweep width of 5618 Hz was stored in 32 k data points. Spectra were processed using a line broadening of 0.343.

2.3. Sample preparation

Extraction and derivatization for GC analysis. The MTPA anhydride was prepared at a concentration of 0.025 M in dichloromethane [1] by mixing equal volumes of a 0.05 M solution of the acid and a 0.05 M solution of DCC prepared in the same solvent. The derivatization procedure for aqueous solutions of the alkaloids at concentrations up to 3.5 mg/ml in 0.05 N hydrochloric acid was the following. A 1-ml aliquot was made basic with saturated sodium bicarbonate and extracted immediately with 500 μl of methylene chloride. To a 200-μl aliquot of the organic extract was added 200 μl of the anhydride solution. The solution was then heated at 35°C for 1 h. The solution was cooled and injected into the GC.

Extraction and NMR analysis. Approximately 1 mg of the base form of the standard was dissolved in 0.45 ml of $CDCl_3$. The proton spectrum was recorded. The solvating agent, (*R*)-(+)-1,1′-bi-2-naphthol, 11 mg, was then added directly to the tube and the tube agitated slightly to dissolve the solid before recording the spectrum again. Where the standard was available as the hydrochloride salt, the base was extracted from aqueous solution with $CHCl_3$ after having been rendered basic with potassium carbonate. The resulting $CHCl_3$ solution was dried over sodium sulphate and evaporated with a stream of nitrogen. The residue was redissolved immediately in 0.45 ml of $CDCl_3$. The spectra were recorded as described above.

A 1-ml aliquot of aqueous solutions of known composition was made basic by the addition of potassium carbonate and extracted twice with 3-ml aliquots of $CDCl_3$. The combined organic extracts were then evaporated to dryness with a stream of nitrogen and the residue redissolved in 0.45 μl $CDCl_3$. This solution was used for the acquisition of the proton spectrum. The solvating agent, (*R*)-(+)-1,1′-bi-2-naphthol, 11 mg, was then added directly to the tube and the spectrum recorded again.

3. Results and discussion

The derivatization procedure was similar to that used for the derivatization of the phenylalkylamine alkaloids of khat [1]. However, as might be expected with the substances here, all secondary amines, the derivatization proceeded at a slower rate. The reaction mixtures were therefore heated at 35°C for 1 h.

Fig. 2 is a GC chromatogram showing the separation of the eight diastereomeric MTPA derivatives of racemic methamphetamine, methcathinone and ephedrine and (+)- and (−)-pseudoephedrine. The identity of the derivatives was confirmed by mass spectrometry. Fig. 3 is the electron impact (EI) mass spectrum of the methcathinone derivative. None of the derivatives gave molecular ions in EI mode; the major ions were analogous to those derived from the *N*-demethyl series [1]. All of the derivatives gave M+1 ions in chemical ionization (methane) mode. However, whereas methamphetamine and methcathinone gave strong M+1 ions, loss of water in the derivatives of ephedrine and pseudoephedrine was predominant with a resulting ion at $m/z = 364$.

Measurement of the relative response factors by GC-FID of each of the diastereomeric pairs of derivatives indicated significant differences for some. The relative responses of each of the diastereomeric isomers is presented in Table 1. For each pair of enantiomers, the more responsive derivative was assigned a value of 100. The

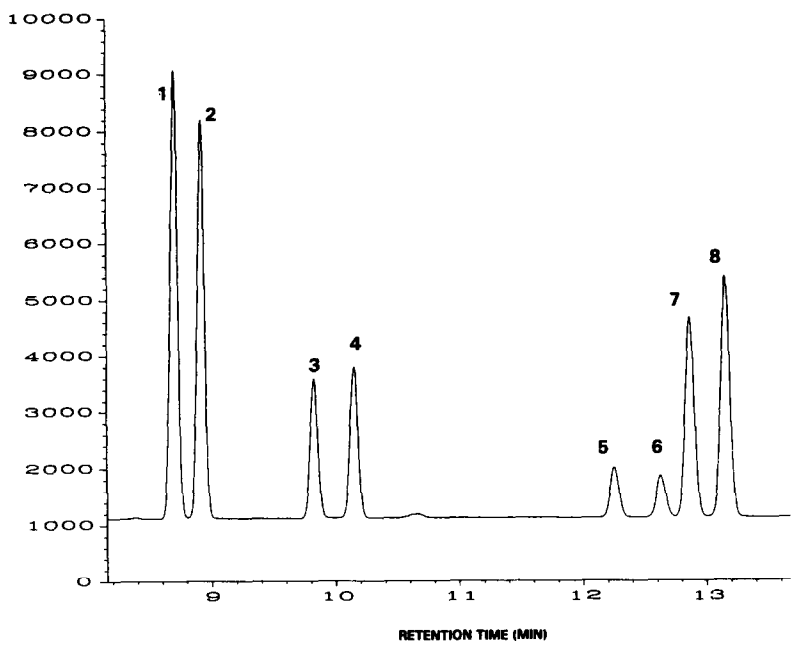


Fig. 2. GC chromatogram of the (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid derivatives. Peak 1, (*S*)-methamphetamine derivative; Peak 2, (*R*)-methamphetamine derivative; Peak 3, (*S*)-methcathinone derivative; Peak 4, (*R*)-methcathinone derivative; Peak 5, (*R,S*)-ephedrine derivative; Peak 6, (*S,R*)-ephedrine derivative; Peak 7, (*S,S*)-pseudoephedrine derivative; Peak 8, (*R,R*)-pseudoephedrine derivative.

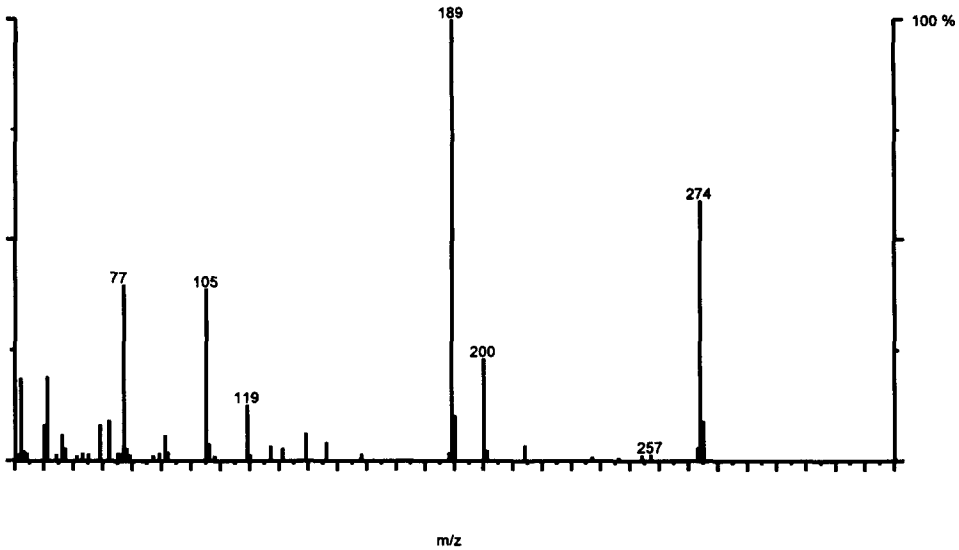


Fig. 3. Electron Impact mass spectrum of the (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid derivative of methcathinone.

response of the other derivative then was assigned as a percentage of the more responsive. However, the relative responses of other than enantiomeric pairs were not determined. The values of 100 for (*S*)-methamphetamine and (*R*)-methcathinone do not indicate equivalent responses.

Fig. 4 is a partial ^{1}H-NMR spectrum from the same sample analysed by GC and presented in Fig. 2. Two distinct regions are visible: the *C*-methyl region (0.6–1.2 p.p.m.) and the *N*-methyl region (1.9–2.4 p.p.m.). The *C*-methyl signals are doublets due to coupling to the *C*-2 methine proton. Signal assignments which were confirmed by recording the spectra of individual substances are labelled according to the reference codes assigned in Fig. 1.

Table 1
Relative responses of diastereomeric MTPA derivatives using flame ionization detection

Analyte	Isomer	Percentage relative response[a] of enantiomeric pairs
Methamphetamine	*R*	88
	S	100
Methcathinone	*R*	100
	S	89
Ephedrine	*RS*	100
	SR	86
Pseudoephedrine	*RR*	98
	SS	100

[a]Relative responses are expressed as a percentage of most responsive derivative within enantiomeric pairs only.

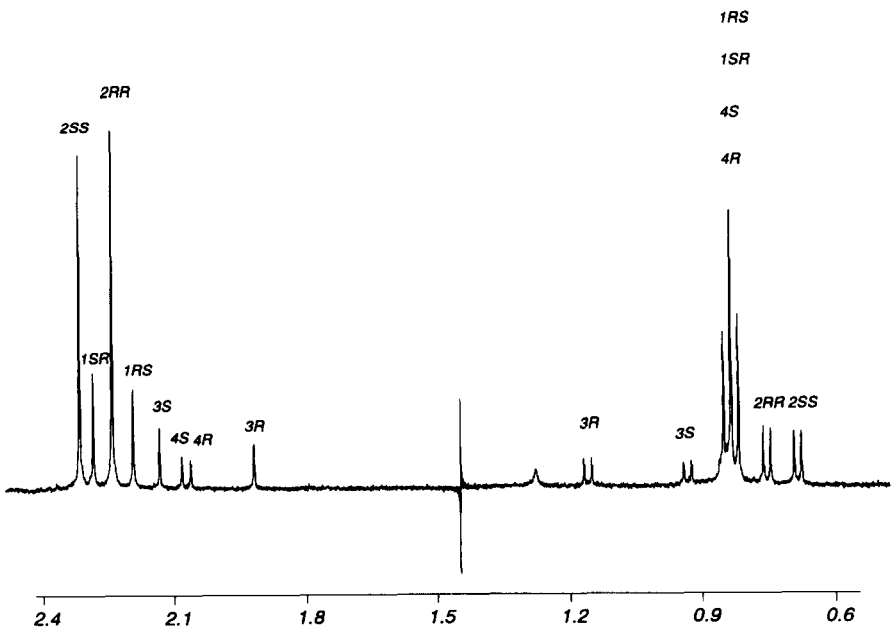


Fig. 4. NMR spectrum of $CDCl_3$ solution containing eight enantiomers. Signal assignments: *N*-methyl region; 1.9–2.4 p.p.m.; *C*-methyl region; 0.7–1.2 p.p.m. Singlet *N*-methyl signals and doublet *C*-methyl signals labelled according to reference codes assigned in Fig. 1.

The accuracy of the determination of the enantiomeric ratios was determined by preparing a series of solutions that contained known concentrations of all the enantiomers. Table 2 shows the theoretical and experimentally determined values by both GC and NMR for the %*R* (of the total *R* + *S*) for each of the substances. Excellent agreement was obtained with the theoretical values by both methods.

Methcathinone can be expected to racemize under conditions similar to cathinone. The latter is known to be subject to racemization especially under alkaline conditions [13]. If the racemization of methcathinone is sufficiently facile, one might expect products prepared under uncontrolled conditions as may exist in a clandestine laboratory to contain measurable and characteristic amounts of the two enantiomers. The likelihood of this phenomenon providing the forensic chemist with information was determined by incubating methcathinone in an alkaline solution. A 0.5-mg/ml solution of *R*-methcathinone hydrochloride was made basic with saturated sodium bicarbonate and allowed to stand at room temperature. The enantiomeric ratios were determined immediately, and after 5, 60 and 120 min. The percentage *S*-enantiomer present as the total methcathinone was 1%, 4%, 19%, and 31%, respectively.

To mimic conditions that may prevail in a clandestine laboratory, the oxidation of **1SR** was carried out using both the permanganate method and the dichromate method. The permanganate method was carried out as described by Zhingel et al.

Table 2
GC and NMR results of the determination of synthetic mixtures

Substance	Solution no.	Enantiomer	Percentage of total		
			Theory	GC	NMR
Methamphetamine	1	*R*	50	50	53
	2		91	91	91
	3		75	75	75
	4		25	26	25
Methcathinone	1	*R*	50	50	55
	2		95	94	95
	3		83	84	83
	4		50	50	56
Ephedrine	1	*RS*	50	50	53
	2		9	11	10
	3		25	26	25
	4		75	75	72
Pseudoephedrine	1	*RR*	55	55	55
	2		91	91	91
	3		76	75	75
	4		26	26	24

[4]. At the extraction stage, the aqueous solution was adjusted to pH 12 with 5 N sodium hydroxide. Methylene chloride was added as extraction solvent and a portion withdrawn immediately. The aqueous organic mixture was allowed to stand with periodic agitation. Subsequent aliquots of the methylene chloride were withdrawn after 30 and 80 min. GC analysis of the three extracts indicated the percentage of (*S*)-methcathinone content increased from 5% (immediately) to 9% (30 min) and 34% (80 min). A similar approach was taken with the dichromate oxidation which was performed as described by L'Italien and Rebstock [7]. The reaction mixture was adjusted to pH 12 with 5 N NaOH and extracted with methylene chloride. Sequential aliquots of the organic fraction were withdrawn at the same time intervals as for the permanganate reaction. However, in contrast to the permanganate reaction the three extracts all contained equivalent (12%) amounts of the (*S*)-enantiomer of methcathinone.

It appears from these limited results that enantiomeric ratios may provide intelligence information on batches of illicitly produced methcathinone. In fact the variability of the chiral purity of methcathinone produced by the oxidation of ephedrine has been reflected in a number of reports (Ref. [5] and T.A. Dal Cason and C. Randall Clark, pers. commun.).

Both GC and NMR were shown to be capable of measuring the enantiomeric ratios of mixtures of ephedrine, pseudoephedrine methcathinone and methamphetamine. Clandestine laboratory conditions may be expected to lead to partially racemized products even when chiral starting materials are used which could be used to characterize batches of methcathinone.

Acknowledgement

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