Chiral Gas Chromatography as a Tool for Investigations into Illicitly Manufactured Methylamphetamine

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ABSTRACT The aim of this study was to develop a chiral gas chromatographic method for the separation of compounds likely to be found in the EMDE synthesis of methylamphetamine, a heavily abused stimulant drug. Here we describe the separation of the enantiomers of ephedrine, pseudoephedrine, chlorinated intermediates and methylamphetamine using fluorinated acid anhydrides as chemical derivatization reagents prior to gas chromatographic analysis on a 2,3-di-*O*-methyl-6-*t*-butyl silyl-β-cyclodextrin stationary phase (CHIRALDEX™ B-DM). Separation of the enantiomers of pseudoephedrine, methylamphetamine and chloro-intermediates was achieved using PFPA derivatization, and enantiomers of ephedrine using TFAA derivatization, in run times of less than 40 minutes. The use of HFBA as a derivatization reagent for this set of analytes is also discussed. *Chirality 23:519–522, 2011.*

KEY WORDS: Methylamphetamine, EMDE synthesis, chiral GC, cyclodextrin

INTRODUCTION

Methylamphetamine is a stimulant-type drug based on the phenethylamine structure and was reclassified in January 2007 in the United Kingdom from Class B to A within the Misuse of Drugs Act 1971. Here, we refer to the *N*-methylamphetamine substance. The terminology associated with this substance can be confusing with methylamphetamine used in this legislation and in the literature, with other commonly used terms such as methamphetamine, *N*,α-dimethylphenylethylamine, (2*S*)-*N*-methyl-1-phenyl-propan-2-amine, and deoxyephedrine particularly in the case of the (−)-(*R*)-enantiomer. The Chemical Abstract Service (CAS) registry number for methylamphetamine is 537-46-2. The drug is a major problem throughout the world particularly in East and Southeast Asia and the United States, which accounts for 40 and 56%, respectively, of the seized material worldwide in 2007.[1] The problems associated with methylamphetamine use have been well documented in the media, and a general summary can be found in the US Drug Enforcement Administration webpages.[2] Recent concerns have been reported involving the exposure of children[3,4] and the general population[5] to methylamphetamine and chemicals found in clandestine laboratories.

In the United States, differences in penalty exist between the two enantiomers, with Federal Sentencing Guidelines imposing greater penalties on seizures containing >80% of the (+)-(*S*)-methylamphetamine. Therefore, in the United States, chiral analysis is required and usually carried out by the formation of a diastereoisomeric *N*-trifluoroacetyl-L-prolyl derivative followed by achiral gas chromatography.[6] The reason for differences in sentencing is due to the greater activity of the (+)-(*S*)-enantiomer as a central nervous system stimulant, whereas the (−)-(*R*)-enantiomer shows greater peripheral activity and indeed has been used in nonprescription decongestants.[7] A recent review covers the pharmacology of methylamphetamine, including some stereochemical considerations.[8]

There have been several reports of stereospecific separation of methylamphetamine using chiral stationary phases in liquid chromatography and gas chromatography[9,10] and mobile-phase additives in capillary electrophoresis (CE).[9,11] In addition, the formation of diastereoisomers and subsequent analysis using a nonchiral gas chromatographic column has been attempted successfully.[12]

In terms of drug profiling studies applied to methylamphetamine, these studies have recently focused on isotope ratio mass spectrometry,[13] organic impurity characterization,[14] chiral analysis using reversed-phase CE with highly sulfated γ-cyclodextrin as chiral selector,[15] achiral gas chromatography–mass spectrometry with electron impact and chemical ionization,[16] and achiral gas chromatography–mass spectrometry using α-methoxy-α-(trifluoromethyl)phenylacetyl chloride for the formation of diastereoisomeric derivatives.[17]

Many less common synthetic routes for the illicit manufacture of methylamphetamine exist,[15] but the three most commonly encountered routes are Nagai (nonmetal reduction using hydriodic acid/red phosphorus), Birch reduction (dissolving metal reduction with liquid ammonia and lithium or sodium), and Emde (chlorination with thionyl chloride followed by hydrogenation over palladium).[18] These routes are illustrated in Figure 1.

Published investigations into the chlorination of the ephedrine enantiomers in the Emde route showed complete reversal of configuration at C-1 forming chloropseudoephedrine enantiomers, implying an S_N2 mechanism. Therefore, the predicted product for chlorination of (−)-(1*R*,2*S*)-ephedrine is the (+)-(1*S*,2*S*)-chloropseudoephedrine intermediate and for (+)-(1*S*,2*R*)-ephedrine is the (−)-(1*R*,2*R*)-chloropseudoephedrine. However, the chlorination of pseudoephedrine enantiomers does not follow S_N2 completely, and in fact, a mixture of 40% chloroephedrine: 60% chloropseudoephedrine enan-

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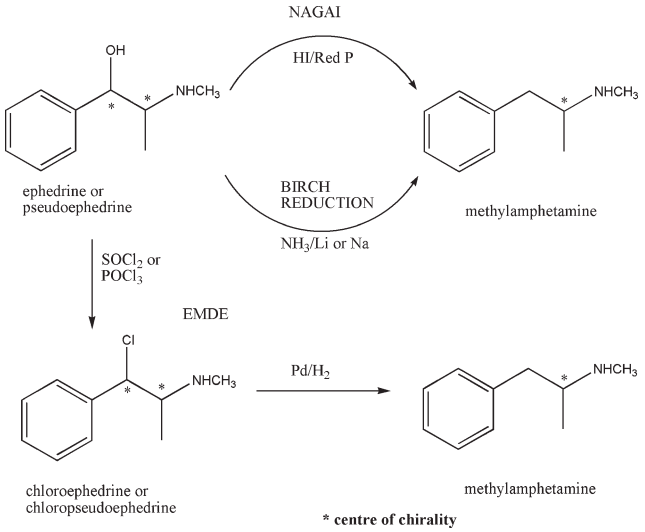

Fig. 1. Common synthetic routes to methylamphetamine.

tiomers is suggested, implying a mixture of S_N2 and S_Ni (retention of configuration).[19,20]

A chiral profile may therefore be used to determine the source of starting material and provide links to street batches. For example, (−)-(1*R*,2*S*)-ephedrine and (+)-(1*S*,2*S*)-pseudoephedrine have been identified as important components derived from the Ephedra plant (Ephedraceae family).[21]

The identification of the chlorinated intermediates would indicate the use of the Emde synthesis, rather than other routes such as the Birch reduction and Nagai methods.

MATERIALS AND METHODS

Chemicals

Methanol, ethyl acetate, chloroform, diethyl ether, and thionyl chloride were supplied by Fisher Scientific (Loughborough, UK). Trifluoroacetic anhydride (TFAA), pentafluoropropanoic anhydride (PFPA), and heptafluorobutyric anhydride (HFBA) were purchased from Sigma-Aldrich (Poole, UK).

(+)-(1*S*,2*R*)-Ephedrine hydrochloride [24221-86-1], (−)-(1*R*,2*S*)-ephedrine hydrochloride [50-98-6], (+)-(1*S*,2*S*)-pseudoephedrine [90-82-4], (−)-(1*R*,2*R*)-pseudoephedrine [321-97-1], (+)-(*S*)-methamphetamine hydrochloride [51-57-0], and (−)-(*R*)-deoxyephedrine [33817-09-3] were purchased from Sigma-Aldrich.

Chromatography

Enantiomeric separations were performed on a HP5890 gas chromatograph with FID detector connected to an Agilent 3396 series 3 integrator. The chromatographic column used was an Astec CHIRALDEX™ B-DM capillary column (30 m × 0.25 mm × 0.12 μm film thickness) supplied by Supelco (Bellefonte, PA). Helium (CP Grade) was used as a carrier gas and was supplied by BOC Gases Europe (Guildford, UK). The flow of carrier gas was 1 ml/min, with constant flow conditions being observed throughout. The temperature programs were as follows:

- **PFP derivatives:** Initial temperature of 115°C for 28 min followed by temperature gradient of 5°C/min for 5 min to a final temperature of 150°C, which was held for a further 5 min, giving a total run time of 40 min.
- **TFA derivatives of ephedrine:** Isothermal temperature at 110°C.
- The injector and detector temperatures were set at 200°C.

Derivatization Procedure

Stock solutions of single enantiomers were prepared in methanol at concentrations of approximately 1 mg/ml. The derivatization procedure was identical for all derivatizing reagents, with minor modifications for HFBA (described in Results and Discussion section):

1. Addition of 50 μl of methanol solution to vial.
2. Evaporation to dryness at 50°C under a stream of nitrogen, removing from heat at point of dryness.
3. Addition of 50 μl derivatizing reagent followed by 50 μl of ethyl acetate and heated for 15 min at 50°C.
4. Evaporation to dryness at 50°C under a stream of nitrogen.
5. Addition of 50 μl of ethyl acetate prior to chromatographic analysis.

Nitrogen was supplied by a generator (model UHPN 3001; Domnick Hunter, Gateshead, UK).

Summary of Preparation of Chloro Intermediates

The chlorination step of the Emde synthesis was carried out with minor modifications to the method outlined in the clandestine guide authored by Uncle Fester.[22] The ^{1}H NMR spectral data were compared with literature values and indicative with chloroephedrine/chloropseudoephedrine having been formed.[19,20] Additionally, infrared analysis of the intermediates showed the absence of the OH band and the presence of C—Cl signal, indicating chlorination had taken place. Furthermore, ^{1}H NMR investigation is required to differentiate chloroephedrine and chloropseudoephedrine in the synthesized samples.

The general synthetic method is as follows: (+)-(1*S*,2*S*)-pseudoephedrine (5.02 g, 0.030 mol) was dissolved in 10 ml chloroform and added to a mixture of 10 ml thionyl chloride and 10 ml chloroform. The reaction was heated in a water bath at a temperature of 70–75°C for a period of 5 h. The reaction mixture was then cooled in an ice bath followed by the addition of 100 ml diethyl ether. The crystals formed were filtered under vacuum, washed with acetone, weighed as a crude product, and recrystallized from methanol.

The method was applied to all other stereoisomers of ephedrine hydrochloride and pseudoephedrine using similar mole ratios between reactants.

RESULTS AND DISCUSSION

The analytical approach involved evaluation and development of various chromatographic conditions for enantiomer separations of ephedrine, pseudoephedrine, methylamphetamine, and the chlorinated intermediates, chloroephedrine and chloropseudoephedrine, from the Emde synthetic route. An important consideration was provision of a chromatographic system that can separate all analytes and therefore quickly provide additional useful information for analysts involved in drug profiling studies.

The chromatographic column used for the separations was a derivatized β-cyclodextrin (2,3-di-*O*-methyl-6-*t*-butyl silyl).

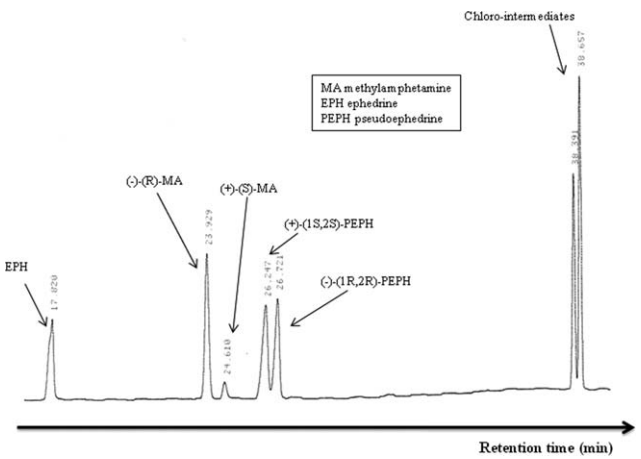

Fig. 2. Separation of enantiomers after PFPA derivatization.

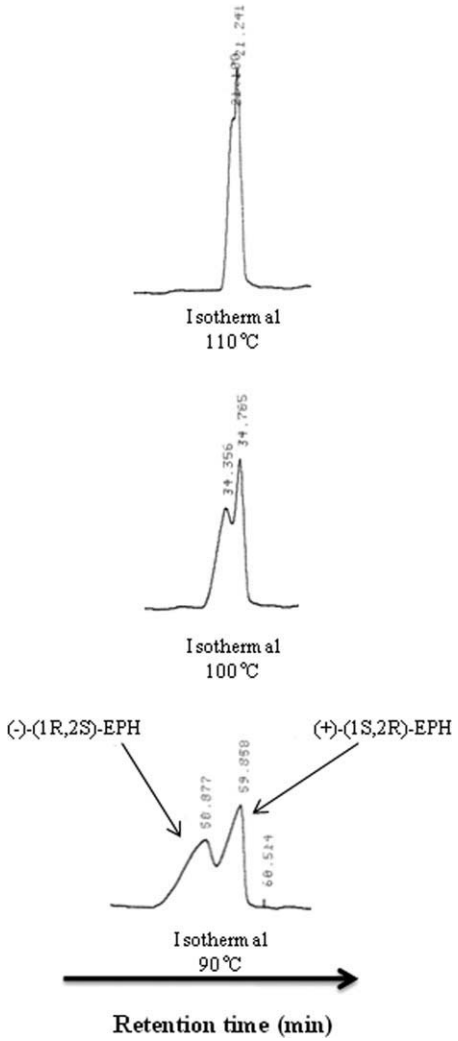

Fig. 3. Effect of temperature on the separation of ephedrine after PFPA derivatization.

Compound classes separated on this phase include aliphatic, olefinic, and aromatic enantiomers, with examples including selegiline (tertiary amine) and ibuprofen (carboxylic acid) enantiomers.[23] The desired blocking effect of surface silanols from the 6-*t*-butyl silyl groups on the cyclodextrin was not sufficient for the compounds of interest in this investigation, that is, secondary amine (methylamphetamine) and secondary amine/alcohol (ephedrine and pseudoephedrine), and therefore, a derivatization procedure using fluorinated acid anhydrides was used.

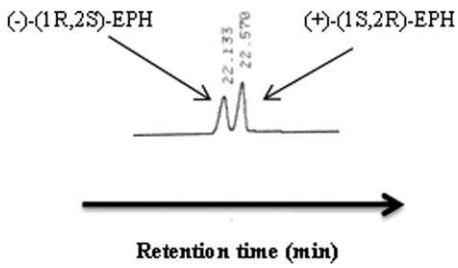

Fig. 4. Separation of ephedrine after TFAA derivatization.

TABLE 1. Retention time data from Figures 2 and 4

Enantiomer	Rt	Rt_0	k'
(−)-(*R*)-Methylamphetamine PFP	23.93	2.47	8.69
(+)-(*S*)-Methylamphetamine PFP	24.61	2.47	8.96
(+)-(1*S*,2*S*)-Pseudoephedrine PFP	26.25	2.47	9.63
(−)-(1*R*,2*R*)-Pseudoephedrine PFP	26.72	2.47	9.82
Chloro-intermediate PFP	38.39	2.47	14.54
Chloro-intermediate PFP	38.66	2.47	14.65
(−)-(1*R*,2*S*)-Ephedrine TFA	22.13	2.18	9.15
(+)-(1*S*,2*R*)-Ephedrine TFA	22.57	2.18	9.35

Rt and Rt_0 are retention times in minutes of analyte and nonretained compound. k' is the retention factor.

Chemical Derivatization

Chemical derivatization was investigated using fluorinated acetylating reagents, TFAA, PFPA, and HFBA, as a means of reducing polarity/basicity of analytes and improving enantiomeric separations. The derivatizing reagents were added prior to the addition of ethyl acetate to maximize reaction with the analytes.

Pentafluoropropionyl derivatives of pseudoephedrine and methylamphetamine separated well as shown in Figure 2. However, poor resolution was noted with ephedrine, even after decreases in column temperature, as illustrated in Figure 3. This is also the first eluting enantiomeric pair, and therefore, further decreases in temperature were not considered. The trifluoroacetyl derivatives of ephedrine showed good resolution at 110°C, as shown in Figure 4; however, peak coelution was noted for methylamphetamine and pseudoephedrine at this temperature. By reducing the temperature to 100°C, a separation of all six enantiomers was provided, but retention times were long and peak shapes poor. Therefore, for samples containing ephedrine and pseudoephedrine, two separate derivatization procedures are required, with TFAA derivatization recommended for ephedrine enantiomers only. Retention time data along with separation factors and resolutions are listed in Tables 1 and 2 for the pentafluoropropionyl (PFP) and trifluoroacetyl (TFA) derivatives.

The chloro intermediate of ephedrine/pseudoephedrine showed enantiomer resolution for TFA and PFP derivatives, but distinguishing chloroephedrine from chloropseudoephedrine has not been accomplished at present. Future investigations will focus on the NMR analysis of the samples for chloroephedrine and chloropseudoephedrine intermediate content and determine if both intermediates are present as predicted.[19,20]

When HFBA was used as a derivatizing reagent, partial resolution of pseudoephedrine and chloro intermediate could be achieved with single peaks observed for ephedrine and methylamphetamine under the same conditions (isothermal temperature 130°C). Additionally, oily residues of heptafluor-

TABLE 2. Separation factors and resolution of PFP and TFA derivatives

Analyte	α	Rs
Pseudoephedrine PFP	1.02	0.94
Methylamphetamine PFP	1.04	1.51
Chloro-intermediate PFP	1.01	0.90
Ephedrine TFA	1.02	1.23

obutyric acid remain after derivatization and evaporation. Further steps are therefore required before chromatography, for example, addition of larger volumes/repeated additions of ethyl acetate (approximately 200–300 μl) prior to evaporation under N_2. This reagent was not investigated further as an important aim of the research was simplification of the derivatization procedure.

Internal Standards

Internal standards were not used in this study as the focus here was on the ratios of enantiomers. However, should quantification of methylamphetamine be required, for example, when using mass spectrometric detectors,[24,25] deuterated analogs of methylamphetamine are available from commercial sources as mixtures of optical isomers.

CONCLUSION

The gas chromatographic analysis of the optical isomers of important compounds found within the Emde synthesis of methylamphetamine, including the starting materials and intermediates, can be achieved using readily available derivatizing reagents in combination with a chiral stationary phase. The derivatization procedure is quick and simple. In particular, the enantiomers of pseudoephedrine, methylamphetamine, and chlorinated intermediates can be separated in a run time of approximately 40 min, using PFPA as a derivatizing reagent. A separate derivatization procedure with trifluoroacetic anhydride is required for the separation of ephedrine enantiomers. HFBA requires a more labor-intensive derivatization procedure and is therefore not recommended for this type of analysis.

The identification of chlorinated intermediates within methylamphetamine samples is indicative of the Emde synthetic route rather than the Birch or Nagai routes.

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