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# GAS CHROMATOGRAPHIC RESOLUTION OF ENANTIOMERIC AMPHETAMINES AND RELATED AMINES

## II. EFFECTS OF CYCLIC STRUCTURES ON DIASTEREOMER AND ENANTIOMER RESOLUTION

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## SUMMARY

Proline and other cyclic amino acids were used as acylated acid chlorides for the resolution of enantiomers of amphetamine and related amines as diastereomers by gas chromatography. Variation of ring size or incorporation of a heteroatom into the ring of the cyclic resolving agent changed retention times and separation factors, depending on the racemic amine derivatized. Separations were achieved on a short, packed Carbowax 20M column.

Because N-trifluoroacetyl L-prolyl chloride has proved especially effective for derivatization of amine enantiomers to give diastereomers which often may be resolved by gas chromatography, the proline ring was incorporated into a new optically active stationary phase. For the enantiomeric derivatives examined, the new proline ester phase gave separations essentially equivalent to those obtained on the commercially available phase, carbonyl bis-(L-valine isopropyl ester). The fact that resolution was achieved on the new phase is evidence that, as has been previously suggested, the ester carbonyls of this type of phase are the significant sites of interaction with the antipodes undergoing separation.

## INTRODUCTION

Gas chromatography is now well established as a highly useful technique for resolution of amine enantiomers, either directly on optically active stationary phases[1–10] or as diastereomers on achiral phases[11–19]. Gas chromatography is a simple, relatively fast method to determine optical purity as well as chemical purity since impurities are normally well resolved from the enantiomer peaks.

Separations of optical isomers on achiral phases are based upon use of a chiral reagent to form diastereomers that differ in physical properties and thus can be resolved on the column[14,15]. The mechanism of separation on optically active stationary phases is through the formation of transient hydrogen-bonded association complexes between the active groups on the stationary phase and on the solute molecules[7–10].

Lochmüller and Souter[2,3] demonstrated that both steric and electronic effects were important in enantiomer separations on optically active carbonyl bis-(amino acid ester) stationary phases, and that the ester carbonyl groups of those phases were the only significant points of "attachment" in formation of the complexes with the amide solutes[1].

In the first part of this work[20] several new amino acid resolving agents and the commercially available N-trifluoroacetyl (N-TFA) L-prolyl chloride were compared in effectiveness for separating racemic amines. The purpose of this paper is to examine effects of changes of ring size and substitution of cyclic amino acid chloride resolving agents on the resolution of amphetamine and related amines. In addition, since the proline ring (from N-TFA L-prolyl chloride) often yields diastereomers having large free-energy differences, a new chiral stationary phase was synthesized incorporating L-proline.

The proline phase has no free amide N–H groups as in other carbonyl bis-(amino acid esters)[1–3], and its ability to separate enantiomers would indicate that the N–H groups play no part in the separation mechanism.

## EXPERIMENTAL

### *Reagents*

The 12.5% phosgene solution in benzene was purchased from Matheson, Coleman & Bell (East Rutherford, N.J., U.S.A.). L-2-Pyrrolidone 5-carboxylic acid, L-thiazolidine 4-carboxylic acid, and L-2-azetidine carboxylic acid were obtained from Aldrich (Milwaukee, Wisc., U.S.A.), and L-proline from N.B.C. (Cleveland, Ohio, U.S.A.). The perfluoroanhydrides were supplied by Pierce (Rockford, Ill., U.S.A.). Free amines or amine salts were obtained in-house, and the structures were verified by spectroscopic methods. Carbonyl bis-(L-valine isopropyl ester) was purchased from Sigma (St. Louis, Mo., U.S.A.).

The N-acyl amino acid chloride resolving agents were prepared as previously described[20] for naturally occurring amino acids. Acylated amines were prepared as described by Lochmüller *et al.*[1]. Diastereomers were formed from free amine samples using the resolving agents and the procedure outlined earlier[20].

Carbonyl bis-(L-proline isopropyl ester) was synthesized by a variation of the method of Lochmüller *et al.*[1]. Into a suspension of 20 g of L-proline in 300 ml of 2-propanol was bubbled dry hydrogen chloride gas for 6 h while the solution was refluxed. The amino acid dissolved in about 1 h. After cooling and standing for two days, excess of alcohol was removed under vacuum, leaving a thick syrup. This was dissolved in water, neutralized with sodium hydroxide and extracted with chloroform. The chloroform solution of the free amine was cooled to 0° in ice, and one equivalent of phosgene (as a benzene solution) was slowly added to each two equivalents of the stirred cold amine in chloroform. After phosgene addition the solution was refluxed 2 h, immediately followed by distillation of about half the solvent. Remaining solvent was removed under vacuum. The carbonyl bis-(L-proline isopropyl ester) was purified by crystallization from carbon tetrachloride and by subsequent washing with cyclohexane; melting point was 90°. The structure was verified by NMR and by mass spectrometry.

*Gas chromatography*

All experiments were performed with a Hewlett-Packard Model 402 high-efficiency dual-FID gas chromatograph. 5% Carbowax 20M on 80–100 mesh Chromosorb G AW DMCS from Ohio Valley Specialty Chemical Company (Marietta, Ohio, U.S.A.) was used to pack a 3 ft. × 3 mm I.D. column for diastereomer separations. The optically active stationary phases were coated 5% (w/w) on 80–100 mesh Chromosorb G HP by the "pan" method[21]. Columns were acid-washed glass and were (except for the Carbowax) hexamethyldisilazane-treated.

## RESULTS AND DISCUSSION

A rapid simple technique adaptable to both preparative and quantitative analytical applications of enantiomer separations is of obvious importance in research and industry. Optical isomers of certain structural types may now be resolved either directly (on chiral stationary phases) or indirectly (as diastereomers on non-asymmetric phases) by gas chromatography. Existing reports have provided some basis for attempts at practical applications. However, much more work is necessary to elucidate asymmetric solute–solvent interactions to the extent that one may easily and successfully predict the structural and/or other requirements for optimum separations on chiral stationary phases.

TABLE I

SEPARATION FACTORS ($\alpha$) AND RESOLUTION ($R$) FOR DIASTEREOMER SEPARATIONS ON CARBOWAX 20M

Column: 5% (w/w) Carbowax 20M on 80–100 mesh Chromosorb G AW DMCS, 3 ft. × 3 mm I.D.; temperature, 210° (unless otherwise indicated); helium flow-rate, 60 ml/min.

| *Amine* | L-*Proline* | | L-*Azetidine* | | L-*Thioproline* | |
|---|---|---|---|---|---|---|
| | $\alpha$ | $R$ | $\alpha$ | $R$ | $\alpha$ | $R$ |
| $\alpha$-Methylbenzylamine | 1.31 | 1.7 | 1.25 | 1.5 | 1.25 | 1.8 |
| 1-Methylhexylamine | 1.12 | 0.44 | —** | — | 1.09 | 0.35 |
| $\alpha$-Methylphenethylamine | 1.15 | 1.3 | 1.18 | 1.2 | 1.18 | 1.3 |
| $o,\alpha$-Dimethylphenethylamine | 1.24 | 1.6 | — | — | 1.15 | 1.4 |
| $p$-Chloro-$\alpha$-methylphenethylamine* | 1.22 | 1.4 | 1.21 | 1.6 | 1.18 | 1.1 |
| 1-Methyl-3-phenylpropylamine | 1.06 | 0.28 | 1.05 | 0.31 | 1.21 | 0.86 |
| $\alpha$-Ethylphenethylamine | 1.21 | 1.2 | 1.21 | 1.4 | 1.20 | 1.5 |

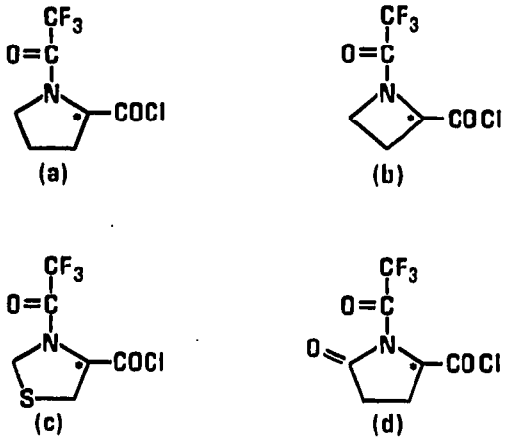
* At 230°.

** No measurements made.

The previous paper in this series[20] examined the structural effects of some new amino acid resolving agents on amphetamine and related amines. Karger *et al.*[14] showed that separations of racemic cyclic amines as N-TFA L-prolyl derivatives improved as conformational immobility at the amine chiral centers increased. No studies exist so far of effects on enantiomer separations via diastereomers of resolving agents with cyclic structures different from proline. In addition, no reports exist of attempts to use the proline ring in a chiral stationary phase.

Table I presents chromatographic data for separations of amphetamine and

related amines as diastereomers, prepared from the N-TFA acid chlorides of L-proline (a), L-2-azetidine (b) and L-thioproline (c).

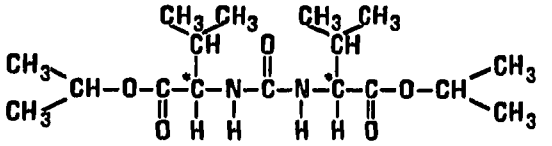



A change of the size of the ring in the resolving agent from 5-member (proline) to 4-member (azetidine) produced a small effect on both separation factor ($\alpha$) and resolution ($R$) for the diastereomers. The conformation of L-2-azetidine carboxylic acid is known to closely resemble that of proline[22] so the potential steric interactions are apparently retained although ring size is decreased by one methylene group. $R$ accounts for both peak broadness and for separation of peak maxima, and, if $R = 1$, the resolution of two equal-area peaks is about 98 % complete[21]. The only compounds not well resolved (Table I) by the proline or azetidine reagents were 1-methylhexylamine and 1-methyl-3-phenylpropylamine. Both of those amines have a relatively low steric bulk in proximity to the asymmetric center, so the separations might be expected to be poorer than in a case such as $\alpha$-methylbenzylamine.

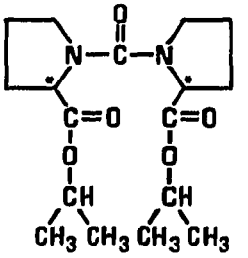
The use of thioproline as a resolving agent rather than proline had small effects on separations except for 1-methyl-3-phenylpropylamine. While resolution was still poor, both $R$ and $\alpha$ increased substantially over values obtained with proline or azetidine. Retention times were much longer for thioproline derivatives than for derivatives of the other reagents. The N-TFA acid chloride of L-2-pyrrolidone 5-carboxylic acid (d) was also examined as a resolving agent since the free acid is known to be of use for formation of diastereomeric amine salts which are then separable by fractional crystallization[23]. Peaks obtained by use of this reagent for a couple of amines were very broad and long-retained at the operating temperature, so no further experiments were attempted using it. Use of higher column temperatures might overcome this disadvantage.

One important conclusion from work by Lochmüller *et al.*[1] and Lochmüller and Souter[2,3,24] was that the ester carbonyls of those phases were the important sites of attachment of enantiomeric amide solutes in the formation of transient hydrogen-bonded association complexes.

In this work a new optically active proline stationary phase was tested. As may be seen from the structures below, the valine isopropyl ester phase contains free N–H groups not present in the proline phase.



Carbonyl-bis-(L-valine isopropyl ester)



Carbonyl-bis-(L-proline isopropyl ester)

As shown in Table II, the valine isopropyl ester phase and the proline isopropyl ester phase give essentially the same separations, indicating that the amide N–H groups in the solvent are not essential for achieving selectivity for the solutes studied.

TABLE II

SEPARATION FACTORS ($\alpha$) FOR ENANTIOMERS ON OPTICALLY ACTIVE STATIONARY PHASES

Column: 5% (w/w) stationary phase on 80–100 mesh Chromosorb G HP; temperature, 140°; helium flow-rate, 60 ml/min. PFP = pentafluoropropionyl; HFB = heptafluorobutyryl.

| *Solute* | *Carbonyl bis-(amino acid isopropyl ester) phase* | |
|---|---|---|
| | *Valine* | *Proline* |
| N-TFA *d,l*-$\alpha$-methylbenzylamine | 1.09 | 1.08 |
| N-PFP *d,l*-$\alpha$-methylbenzylamine | 1.09 | 1.09 |
| N-HFB *d,l*-$\alpha$-methylbenzylamine | 1.09 | 1.12 |
| N-TFA *d,l*-$\alpha$-methylphenethylamine | 1.00 | 1.00 |
| N-HFB *d,l*-1-methylhexylamine | 1.00 | 1.00 |
| N-TFA 1-methyl-3-phenylpropylamine | 1.03 | 1.03 |

The melting point of the proline isopropyl ester phase was 90° compared with 110° for the valine ester. Above 125°, both phases showed noticeable bleed, and for each amine chromatographed (as an N-acyl derivative) both phases gave essentially identical $\alpha$ values under identical operating conditions. Separations were best (but not complete) for $\alpha$-methylbenzylamine derivatives with typical retention times of 20–40 min. The N-TFA 1-methyl-3-phenylpropylamine showed only slight selectivity with the optically active phases after 1.5–2 h of retention and the amphetamine derivative gave no hint of separation in about 1 h. Retention times were typically longer on the proline isopropyl ester phase. Resolution of amides on the optically active phases

was, except for the α-methylbenzylamines, very poor. Retention times for other amines were impractically long with no observable resolution.

An attempt was made to resolve *d,l*-mandelic acid after trimethylsilylation (with trimethylsilylimidazole, Pierce) of the hydroxyl and carboxyl groups. No separation was observed even though mandelic acid has a benzene ring attached to the chiral center, and this may be due to the fact that derivatization leaves no active hydrogen as does acylation of primary amines.

## CONCLUSIONS

Derivatization of enantiomers with a chiral reagent gives diastereomers with permanent differences in overall chirality and boiling point. Formation of diastereomeric hydrogen-bonded association complexes between an asymmetric stationary phase and racemic solutes is a transient process. Differential interactions in the former case are often larger than in the latter allowing free choice of chromatographic operating conditions. Numerous separations on chiral phases have required capillary columns to supply enough theoretical plates so that differential interactions could be seen.

The proline isopropyl ester stationary phase gives essentially the same separations as the valine isopropyl ester compound. However, it has no central N–H groups as in the valine case, so it is apparent that these groups are not always required for achieving selective interaction with enantiomers (and probably indicates too that the ester carbonyls of the solvent are the significant points of attachment).

One major disadvantage of chiral gas chromatographic stationary phases presently in use is their high bleed rates when columns are operated above their melting points. This obviates their use for racemic derivatives with high boiling points, unless one does not mind waiting perhaps several hours for broad peaks to elute. Certainly this is of no value for practical analysis.

Highly selective, high-temperature chiral stationary phases are a critical need for fast direct gas chromatographic enantiomer separations. Work is in progress to try to develop such phases which will give practical resolution at high operating temperatures.

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