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GAS CHROMATOGRAPHIC RESOLUTION OF ENANTIOMERIC AMPHETAMINES AND RELATED AMINES

I. STRUCTURAL EFFECTS ON SOME DIASTEREOMER SEPARATIONS

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SUMMARY

Eight structurally related enantiomeric primary amines including several amphetamines were resolved as diastereomers by gas chromatography. Optically active acylated amino acid chlorides were reacted with the amine racemates to yield volatile diastereomers which were chromatographed on polar and on nonpolar columns. Selectivity of diastereomeric pair separations was found to be dependent on substitution at the chiral centers of both the amino acids and the amines.

Baseline resolution was achieved on a 6-ft. diethylene glycol succinate column at 200° for seven of the eight amines. The polar stationary phases yielded higher relative separation factors than the nonpolar phase in all cases for the same chromatographic conditions. The effect of varying the perfluoroacyl group of the amino acid resolving agent from trifluoroacetyl to pentafluoropropionyl to heptafluorobutyryl was to significantly decrease the retention times without significantly decreasing these separation factors.

INTRODUCTION

The efficient separation of enantiomers is an intriguing and often difficult problem. Normal separation techniques are ineffective because the antipodes possess exactly the same physical properties and differ only in chirality. The potential utility of methods to resolve optical isomers is, however, very great, especially in pharmaceutical and biological applications, since it is known that the antipodes may have different pharmacological effects[1].

Classically, resolution was achieved by formation and repeated fractional crystallization of diastereomers which were then decomposed and purified[2]. Gas chromatography (GC) is a process that has the potential to give both high optical and chemical purities. GC has been demonstrated to be a rapid, sensitive, and efficient method for separating enantiomers, both by use of asymmetric stationary phases[3–15]

for direct resolution of enantiometric derivatives and by use of achiral phases for separation of diastereomers[16–22].

The chiral stationary phases studied have been of two types, the dipeptide amido esters[3,4,9,10] and the carbonyl bis-(amino acid esters)[11,12,14,15]. Asymmetric phases are particularly attractive as they offer separations of enantiomers directly (although derivatization is normally required to make the samples volatile), but their use is sometimes limited by two serious drawbacks. The differential interactions of the solutes with the stationary phase may be so small as to require the use of capillary or open-tubular columns. These must be carefully prepared, and their use precludes preparative-scale work. Also, the working temperature range of the chiral stationary phase may not be very great, and the maximum operating temperature may not be high enough for an analysis. Recently, certain carbonyl bis-(amino acid esters) have demonstrated large-scale (packed column) suitability for various separations and reasonably wide working temperature ranges[15].

Several workers examined resolution of a few amine enantiomers as diastereomers[19–22], and some have studied the mechanism(s) of such separations[18,21]. The uses of several derivatizing reagents for diastereomer formation have been reported[23,24], but the most widely used (and now commercially available) reagent has been N-trifluoroacetyl (N-TFA) L-prolyl chloride[25]. The latter reagent has proven especially useful for resolution of racemic amines.

Relatively few enantiomeric amine separations have been studied for drug compounds, and no reports exist of attempts to use other amino acid chlorides as resolving agents for amines. This paper presents results of separations of a variety of structurally related primary amine drug substances. Some new amino acid resolving reagents are compared in effectiveness to N-TFA L-prolyl chloride, and the effect of varying the perfluoroacyl group is examined. Resolution is also discussed with respect to the applicability to analytical methods.

EXPERIMENTAL

Reagents

L-Valine and L-alanine were obtained from Nutritional Biochemicals (Cleveland, Ohio, U.S.A.), L-leucine was from Sigma (St. Louis, Mo., U.S.A.), and L-proline was purchased from Merck (Rahway, N.J., U.S.A.). The perfluoroanhydrides were supplied by Pierce (Rockford, Ill., U.S.A.).

Free amines and amine salts were obtained from various in-house sources. Structures had been verified previously by spectroscopic methods and the samples were supplied in high purity.

N-Acyl amino acid chlorides were prepared in the following manner, which is a variation of the method by Wells[26] for N-TFA L-prolyl chloride. One gram of the resolved amino acid was weighed into a clean, dry 125-ml conical glass-stoppered flask and the flask was cooled in an ice-bath. Ten milliliters of fresh perfluorinated anhydride were added, and the flask was occasionally shaken until the acid had dissolved. The excess anhydride was evaporated under nitrogen and 5 ml of fresh thionyl chloride were added to the chilled flask. After having stood for 15 min, the excess thionyl chloride was evaporated under dry nitrogen and the residue was dissolved in 100 ml of methylene chloride. The flasks were tightly stoppered and were

stored refrigerated. The acylated amino acid chloride solutions were found to be stable for at least four months.

Free racemic amines were treated directly with the amino acid chlorides. Amine salts were treated with aqueous sodium hydroxide and the free amines were then extracted into chloroform. To form diastereomers, 30 mg of free racemic amine were dissolved in 30 ml of chloroform in a separatory funnel, and 4 ml of amino acid reagent in methylene chloride were added. The solution was briefly shaken and then allowed to stand 30 min. At the end of that time, 30 ml of 0.1 *N* NaOH were added and the mixture was shaken for 2 min. The chloroform layer was then filtered through anhydrous sodium sulfate and the aqueous layer was re-extracted with chloroform. The chloroform solution was reduced in volume with a rotary evaporator to about 3 ml, and a sample was injected into the chromatograph.

Gas chromatography

All experiments were performed using a Hewlett-Packard Model 402 high-efficiency dual-FID gas chromatograph. The carrier gas used was helium with a flow-rate of 60 ml/min. The carrier gas inlet system had essentially no dead volume. Columns were 6 ft. × 1/8-in.-I.D. glass U-tubes packed with one of the following materials: 5% SE-30 on 70–80 mesh Anakrom AB, 5% diethylene glycol succinate (DEGS) on the same support, 5% Carbowax 20M TPA on 80–100 mesh Chromosorb W AW DMCS. In the cases of the DEGS and SE-30 columns, the glass was silanized before use with a solution of 1% hexamethyldisilazane in toluene. All per cent loadings are by weight.

RESULTS

The utility of N-TFA L-prolyl chloride as a resolving agent for enantiomers was first demonstrated by Weygand *et al.* [16,25] and the reagent has been tested for resolution of racemic amino acids[17], of cyclic amines[21], of amphetamine[27,28] and of other aromatic amines[29]. Several amines closely related to amphetamine have been resolved with the new reagent N-pentafluorobenzoyl-S-(−)-prolyl 1-imidazolide[30]. This paper reports results of separations of amphetamines and related amines as diastereomers. The use of other amino acids in addition to proline as resolving agents for the amines is discussed.

Amino acids used for resolving agents included L-proline, L-valine, L-leucine, and L-alanine. The acyl groups attached to the amine acids were trifluoroacetyl (TFA), pentafluoropropionyl (PFP), and heptafluorobutyryl (HFB).

The wide acceptance of N-TFA L-prolyl chloride is due largely to its effectiveness in creating diastereomers exhibiting large differential interactions with a particular stationary phase. These large differences are at least partially a result of the conformational immobility that the proline ring system can lend to the amine structure to which it bonds. However, as this paper demonstrates, the proline reagent will not always be significantly more effective than other amino acid reagents for resolution of racemic amines, and will in fact be significantly less effective in some cases.

Bonner[31] has discussed the precautions necessary to avoid racemization during derivatization of leucine to form the N-TFA L-prolyl derivative. Although no evidence

of racemization was observed for a small selection of optically pure amine samples in this work, not all amine-resolving agent combinations were examined for such. N-TFA L-prolyl chloride has been observed to contain small amounts of antipodic impurities[30], and corrections for this are necessary should it be used for quantitative analysis[31].

Structures of the amines that were resolved as diastereomers are shown in Fig. 1. All compounds were primary amines and were either amphetamines or related compounds.

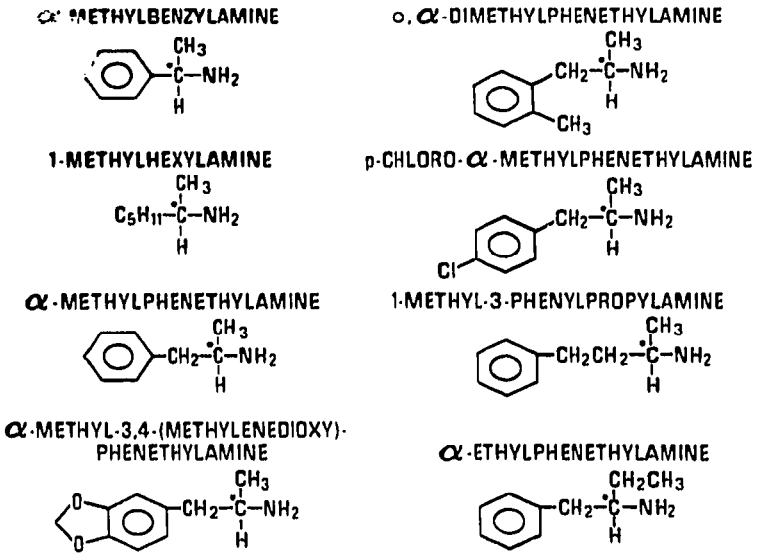

Fig. 1. Structures of the primary amines resolved as diastereomers by gas chromatography.

Extensive studies with the simple model compound α-methylbenzylamine were made by Lochmüller *et al.*[11,14,15] on optically active stationary phases, and results indicated that the presence of the bulky phenyl group at the asymmetric center in close proximity to hydrogen bonding involving the amino group (as an amide) gave excellent resolution of the antipodes. The chiral center is removed from the phenyl ring by one methylene group in α-methylphenethylamine (amphetamine) so its GC behavior and that of several homologs was examined. The compound α-ethylphenethylamine bears an ethyl group at the chiral center (instead of a methyl group as in amphetamine). The distance from the chiral center to the phenyl ring is increased by another methylene group (from amphetamine) in 1-methyl-3-phenylpropylamine, and 1-methylhexylamine bears an *n*-pentyl group at the asymmetric center rather than a phenyl group as in α-methylbenzylamine.

Results of GC measurements for diastereomers of the amines in Fig. 1 are given in Tables I and II. Measurements were made at three temperatures on the DEGS column to allow comparisons of data. The results are presented as the separation factor, α, where $\alpha = (t_{r2} - t_m)/(t_{r1} - t_m)$ and as differential standard free energy of solution (or interaction) $\Delta(\Delta G^\circ) = RT \ln \alpha$. t_{r2} and t_{r1} are the retention

times of the most and least retained species, respectively; t_m is the retention time of an "unretained" species (methane in this study); R is the gas constant 1.987 cal/mole °K; and T is the column temperature in °K.

All chromatographic measurements on the nonpolar SE-30 column were made at 170 °C. Some data at that temperature are also given for the polar DEGS column, so comparisons may be made. The α and $\Delta(\Delta G°)$ values were larger on the polar columns than on SE-30, and values of α were found to be slightly temperature dependent, as in the case of 1-methylhexylamine diastereomers on DEGS at 140 and 170 °C. The α and $\Delta(\Delta G°)$ values were found to decrease slightly with increasing temperature. Retention times on the polar columns were significantly longer at any particular temperature than on SE-30. Larger values of α and increased free energy differences on polar *versus* nonpolar columns were observed by Rose *et al.* for diastereomeric esters[18].

The values of α calculated for diastereomers on the Carbowax 20M TPA column at 200 °C showed that for perfluoroacyl L-prolyl diastereomers, α and $\Delta(\Delta G°)$ values decreased from α-methylbenzylamine to α-methylphenethylamine, increased slightly at α-ethylphenethylamine, and then dropped sharply at 1-methyl-3-phenylpropylamine. The α values for separations of L-prolyl diastereomers are only slightly affected by the perfluoroacyl group, and this is very important since the PFP and HFB analogs elute in much less time than the TFA compounds. Thus, analysis times may be sharply cut by use of PFP and HFB derivatives without significantly diminishing the separations. In the case of α-ethylphenethylamine, N-TFA L-leucyl and N-TFA L-alanyl derivatives gave lower α values than N-TFA L-prolyl derivatives. However, for 1-methyl-3-phenylpropylamine the α values for the N-TFA L-leucyl and N-TFA L-alanyl diastereomers are much larger than for N-TFA L-prolyl.

Results are presented in Table I for measurements on SE-30 at 170 °C. The α and $\Delta(\Delta G°)$ values are high for perfluoroacyl L-prolyl α-methylbenzylamine diastereomers. These values then drop significantly for the perfluoroacyl α-methylphenethylamines as the phenyl ring and asymmetric center are separated by a methylene group. Addition of a chlorine to the phenyl ring of α-methylphenethylamine again improves the separations. A slight increase in α and $\Delta(\Delta G°)$ values is observed for α-ethylphenethylamine diastereomer separations *versus* separations of α-methylphenethylamine analogs. The former amine bears an ethyl group on the asymmetric center as compared to a methyl group in the latter case. Perfluoroacyl L-prolyl diastereomers of 1-methyl-3-phenylpropylamine were not resolved, and this is probably not surprising if steric effects at the asymmetric centers are important, as this amine has the chiral center and phenyl ring separated by two methylenes. Use of N-TFA L-alanyl and N-TFA L-leucyl resolving agents for 1-methyl-3-phenylpropylamine give good separations.

Relatively little variation of α with amino acid structure was observed for α-methylbenzylamine on SE-30. The steric effect of the phenyl ring directly at the asymmetric center of the amine probably overshadows the steric effect of the amino acid structure. Much larger effects of the resolving agents on α and $\Delta(\Delta G°)$ values were observed for amines in which the phenyl ring was removed successively from the asymmetric center of the amine by methylene groups. Variation of acyl group from TFA to PFP to HFB with L-prolyl chloride showed a generally small effect on

TABLE I

GC RELATIVE RETENTION DATA FOR DIASTEREOMER SEPARATIONS ON A 6-ft. COLUMN OF 5% SE-30 ON 70–80 MESH ANAKROM AB AT 170°C WITH A HELIUM FLOW-RATE OF 60 ml/min

Compound	α	$\Delta(\Delta G^\circ)$ *(cal/mole)*
N-TFA L-valyl *dl*-α-methylbenzylamine	1.212	−169
N-TFA L-alanyl *dl*-α-methylbenzylamine	1.214	−171
N-TFA L-leucyl *dl*-α-methylbenzylamine	1.200	−161
N-TFA L-prolyl *dl*-α-methylbenzylamine	1.212	−169
N-PFP L-prolyl *dl*-α-methylbenzylamine	1.192	−155
N-HFB L-prolyl *dl*-α-methylbenzylamine	1.221	−176
N-TFA L-prolyl *dl*-α-methylphenethylamine	1.075	− 63.7
N-PFP L-prolyl *dl*-α-methylphenethylamine	1.068	− 57.9
N-HFB L-prolyl *dl*-α-methylphenethylamine	1.107	− 89.6
N-HFB L-leucyl *dl*-α-methylphenethylamine	NS*	—
N-HFB L-valyl *dl*-α-methylphenethylamine	1.123	−102
N-TFA L-prolyl *dl*-*p*-chloro-α-methylphenethylamine	1.176	−143
N-PFP L-prolyl *dl*-*p*-chloro-α-methylphenethylamine	1.175	−142
N-HFB L-prolyl *dl*-*p*-chloro-α-methylphenethylamine	1.188	−152
N-TFA L-prolyl *dl*-α-ethylphenethylamine	1.075	− 63.7
N-PFP L-prolyl *dl*-α-ethylphenethylamine	1.175	−142
N-HFB L-prolyl *dl*-α-ethylphenethylamine	1.117	− 97.4
N-TFA L-alanyl *dl*-α-ethylphenethylamine	1.051	− 43.8
N-TFA L-leucyl *dl*-α-ethylphenethylamine	NS	—
N-TFA L-prolyl *dl*-1-methyl-3-phenylpropylamine	NS	—
N-PFP L-prolyl *dl*-1-methyl-3-phenylpropylamine	NS	—
N-HFB L-prolyl *dl*-1-methyl-3-phenylpropylamine	NS	—
N-TFA L-alanyl *dl*-1-methyl-3-phenylpropylamine	1.073	− 62.1
N-TFA L-leucyl *dl*-1-methyl-3-phenylpropylamine	1.139	−115

* NS = No separation observed.

α values. Diastereomers of *p*-chloro-α-methylphenethylamine gave much larger α values than the unchlorinated analogs.

Table II presents results of diastereomer separations on the DEGS column at 140, 170 and 200 °C. Fig. 2 shows a comparison of gas chromatograms for separations of N-HFB L-prolyl α-methylbenzylamine on the polar DEGS column and on the nonpolar SE-30 column at 170 °C. Resolution and relative retentions were observed to be much greater on the polar columns than on SE-30, and α values show a small but definite temperature dependence.

The largest α and $\Delta(\Delta G^\circ)$ values in a particular set of amine diastereomers were generally observed for N-TFA L-propyl compounds. An exception was 1-methyl-3-phenylpropylamine, which gave greater values with the N-TFA L-leucyl and N-TFA L-alanyl reagents. Fig. 3 presents a comparison of separations of 1-methyl-3-phenylpropylamine and α-ethylphenethylamine using N-TFA L-propyl and N-TFA L-leucyl resolving agents. Nearly all other amines studied gave lowest α values with the N-TFA L-leucyl reagent.

The small effect of the perfluoroacyl group (of the amino acid reagent) on α seems to indicate that the central amide linkage of the diastereomers is the more important in determining solute–stationary phase interaction. Lochmüller *et al.*[32] showed that the electronic effect of various perfluoroacyl groups at the amide bond

TABLE II

GC RELATIVE RETENTION DATA FOR DIASTEREOMER SEPARATIONS ON A 6-ft. COLUMN OF 5% DEGS ON 70–80 MESH ANAKROM AB AT 140, 170, AND 200°C WITH A HELIUM FLOW-RATE OF 60 ml/min

Compound	α	$\Delta(\Delta G°)$ *(cal/mole)*
140°C		
N-TFA L-prolyl *dl*-1-methylhexylamine	1.190	−143
N-TFA L-valyl *dl*-1-methylhexylamine	1.135	−104
N-TFA L-leucyl *dl*-1-methylhexylamine	1.099	− 77.5
N-TFA L-alanyl *dl*-1-methylhexylamine	1.135	− 104
170°C		
N-TFA L-prolyl *dl*-1-methylhexylamine	1.188	−152
N-TFA L-valyl *dl*-1-methylhexylamine	1.122	−101
N-TFA L-leucyl *dl*-1-methylhexylamine	1.051	− 43.8
N-TFA L-alanyl *dl*-1-methylhexylamine	1.117	−138
N-TFA L-prolyl *dl*-α-methylbenzylamine	1.420	−309
N-PFP L-prolyl *dl*-α-methylbenzylamine	1.380	−284
N-HFB L-prolyl *dl*-α-methylbenzylamine	1.428	−314
N-TFA L-valyl *dl*-α-methylbenzylamine	1.397	−294
N-TFA L-leucyl *dl*-α-methylbenzylamine	1.389	−289
N-TFA L-alanyl *dl*-α-methylbenzylamine	1.461	−333
N-TFA L-prolyl *dl*-α-methylphenethylamine	1.340	−258
N-TFA L-valyl *dl*-α-methylphenethylamine	1.183	−148
N-TFA L-leucyl *dl*-α-methylphenethylamine	1.112	− 93.5
N-TFA L-alanyl *dl*-α-methylphenethylamine	1.187	−151
200°C		
N-TFA L-prolyl *dl*-α-methylbenzylamine	1.326	−265
N-PFP L-prolyl *dl*-α-methylbenzylamine	1.298	−245
N-HFB L-prolyl *dl*-α-methylbenzylamine	1.304	−250
N-TFA L-valyl *dl*-α-methylbenzylamine	1.327	−266
N-TFA L-leucyl *dl*-α-methylbenzylamine	1.315	−257
N-TFA L-alanyl *dl*-α-methylbenzylamine	1.322	−262
N-TFA L-prolyl *dl*-α-methylphenethylamine	1.259	−217
N-PFP L-prolyl *dl*-α-methylphenethylamine	1.214	−182
N-HFB L-prolyl *dl*-α-methylphenethylamine	1.206	−176
N-TFA L-valyl *dl*-α-methylphenethylamine	1.163	−142
N-TFA L-leucyl *dl*-α-methylphenethylamine	1.095	− 85.4
N-TFA L-alanyl *dl*-α-methylphenethylamine	1.138	− 122
N-TFA L-prolyl *dl*-α-methyl-3,4-(methylenedioxy)-phenethylamine	1.331	−268
N-TFA L-valyl *dl*-α-methyl-3,4-(methylenedioxy)-phenethylamine	1.154	−136
N-TFA L-leucyl *dl*-α-methyl-3,4-(methylenedioxy)-phenethylamine	1.076	− 68.9
N-TFA L-alanyl *dl*-α-methyl-3,4-(methylenedioxy)-phenethylamine	1.163	−142
N-TFA L-prolyl *dl*-*p*-chloro-α-methylphenethylamine	1.309	−253
N-TFA L-valyl *dl*-*p*-chloro-α-methylphenethylamine	1.102	− 91.3
N-TFA L-leucyl *dl*-*p*-chloro-α-methylphenethylamine	1.036	− 33.3
N-TFA L-alanyl *dl*-*p*-chloro-α-methylphenethylamine	1.136	−120
N-TFA L-prolyl *dl*-α-ethylphenethylamine	1.252	−211
N-TFA L-leucyl *dl*-α-ethylphenethylamine	1.078	− 70.6
N-TFA L-alanyl *dl*-α-ethylphenethylamine	1.120	−107
N-TFA L-prolyl *dl*-1-methyl-3-phenylpropylamine	1.096	− 86.2
N-TFA L-leucyl *dl*-1-methyl-3-phenylpropylamine	1.153	−134
N-TFA L-alanyl *dl*-1-methyl-3-phenylpropylamine	1.177	−153
N-TFA L-prolyl *dl*-*o*,α-dimethylphenethylamine	1.294	−242
N-TFA L-leucyl *dl*-*o*,α-dimethylphenethylamine	1.082	− 74.1
N-TFA L-alanyl *dl*-*o*,α-dimethylphenethylamine	1.152	−133

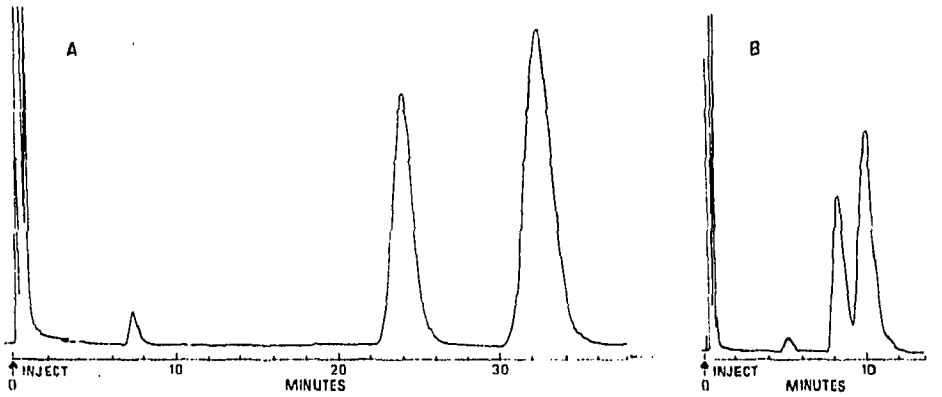

Fig. 2. Comparison of separations of racemic N-HFB L-prolyl *dl*-α-methylbenzylamine on 6-ft. glass columns packed with (A) 5% DEGS on 70–80 mesh Anakrom AB (polar) and (B) 5% SE-30 on 70–80 mesh Anakrom AB (nonpolar) at 170°C with a helium flow-rate of 60 ml/min. The diastereomers were prepared from amines enriched in one antipode.

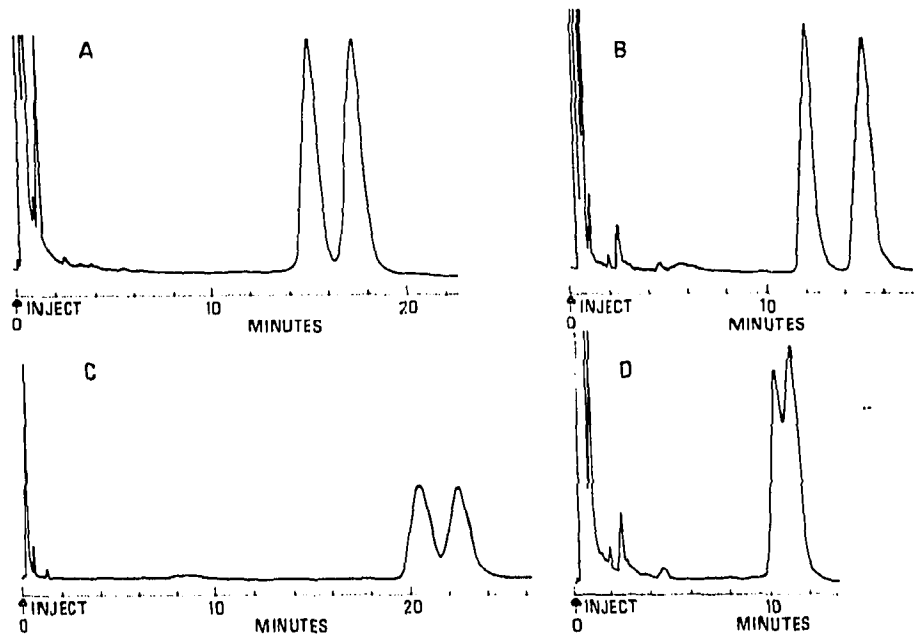

Fig. 3. Comparison of separations of N-TFA L-prolyl and N-TFA L-leucyl amine diastereomers at 200°C on a 6-ft. glass column packed with 5% DEGS on 70–80 mesh Anakrom AB with a helium flow-rate of 60 ml/min. (A) N-TFA L-leucyl *dl*-1-methyl-3-phenylpropylamine, $\alpha = 1.153$. (B) N-TFA L-prolyl *dl*-α-ethylphenethylamine, $\alpha = 1.243$. (C) N-TFA L-prolyl *dl*-1-methyl-3-phenylpropylamine, $\alpha = 1.096$. (D) N-TFA L-leucyl *dl*-α-ethylphenethylamine, $\alpha = 1.078$.

is not very different. In addition, Gil-Av and Nurok[33] found no change in relative retention for a series of α-alkanoyloxypropionates of 2-butanol, probably indicating that the ester linkage between the two asymmetric centers is the more important of the two in terms of differential interactions.

TABLE III

CALCULATED VALUES OF RESOLUTION (R_s) FOR DIASTEREOMER SEPARATIONS ON A 6-ft. COLUMN OF 5% DEGS ON 70–80 MESH ANAKROM AB AT 200°C WITH A HELIUM FLOW-RATE OF 60 ml/min

Compound	R_s
N-TFA L-prolyl *dl*-α-methylbenzylamine	2.4
N-TFA L-prolyl *dl*-α-methylphenethylamine	1.7
N-TFA L-prolyl *dl*-α-methyl-3,4-(methylenedioxy)-phenethylamine	3.3
N-TFA L-prolyl *dl*-*p*-chloro-α-methylphenethylamine	3.0
N-TFA L-prolyl *dl*-*o*,α-dimethylphenethylamine	2.6
N-TFA L-prolyl *dl*-α-ethylphenethylamine	2.0
N-TFA L-alanyl *dl*-1-methyl-3-phenylpropylamine	1.9

Retention order was examined on the DEGS column at 200 °C using diastereomers prepared from α-methylbenzylamine and from α-methylphenethylamine samples which were enriched with one enantiomeric form. It was found that the (+) amine eluted first regardless of the L-amino acid reagent used in the formation of the diastereomers.

Table III gives resolution factors (R_s) for diastereomer separations on the DEGS column at 200 °C. Resolution is a measure of the true separation of two consecutive peaks[34]. If $R_s = 1$, resolution of two equal-area peaks (as for diastereomers) is 98% complete, and if $R_s = 1.5$, the separation is baseline and 99.7% resolution is achieved. Results in the table show that complete baseline resolution is possible under the stated chromatographic conditions for each amine listed, by use of one of the amino acid reagents. In some cases, baseline resolution was achieved with a shorter analysis time by use of PFP or HFB (rather than TFA) amino acid reagents. A consideration of resolution is important in determining whether or not a particular separation is suitable for a practical assay method.

CONCLUSION

Four N-acyl amino acid chlorides were evaluated as resolving agents for a series of amphetamines and related amines. The proper choice of resolving agent gave baseline resolution for every amine chromatographed on a polar DEGS column at 200 °C, all with reasonable retention times (15–30 min). Significant reductions of retention time were possible without loss of baseline resolution by variation of the perfluoroacyl group on the amino acid reagent.

Separation factors generally dropped for a set of diastereomers through the series α-methylbenzylamine, α-methylphenethylamine, α-ethylphenethylamine and 1-methyl-3-phenylpropylamine. The distance between asymmetric centers was the same in each diastereomer compound, but the substituents at the amino acid or amine chiral centers were changed.

Since the amine–amino acid peptide bond is probably important in the interaction of the diastereomers with the polar stationary phases, substituent changes at the chiral centers (which both are very close to this linkage) will affect the interaction with the stationary phase, increasing or decreasing the differential interactions. The

effects of the chiral-center substituents are accentuated by the rigidity of the peptide bond[35].

Further experiments are in progress to evaluate the effects of other structural features in enantiomer and diasteromer separations.

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