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MOLECULAR INTERACTIONS IN A UNIQUE SOLVENT–SOLUTE SYSTEM

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SUMMARY

A systematic study has been undertaken of the variables in gas chromatographic separation of enantiomeric substances using an optically active dipeptide stationary phase (N-trifluoroacetyl-L-α-amino-*n*-butyryl-L-α-amino-*n*-butyric acid cyclohexyl ester). Although this dipeptide phase showed excellent separation for derivatized D,L-α-amino acids, it was observed that other optically active solutes (*e.g.*, β-amino acids, β-amino alcohols, and derivatives of α-amino acids other than trifluoroacetyl-α-amino acid esters) did not meet the steric and structural requirements, and hence were not resolved.

Interaction between the optically active solvent (dipeptide phase) and optically active solute seems to be so unique that only α-amino acids and proline are capable of forming the hydrogen-bonded diastereoisomeric association complex, which has been shown to be requisite for the chromatographic resolution.

INTRODUCTION

The separation of D- and L-amino acid derivatives by gas–liquid partition chromatography (GLC) on optically active dipeptide stationary phases can now be easily accomplished[1–12]. The fact that separation is observed, has been attributed to the formation of a hydrogen-bonded diastereoisomeric association complex between the dipeptide solvent and amino acid solute (Fig. 1)[9,13].

However, it has only recently been demonstrated that the most probable type of complex formation is depicted by the structures in (A). CORBIN *et al.*[14] showed the lack of dependence upon the esterified amino acid portion of the dipeptide solvent and GROHMANN AND PARR[15] revealed that the steric requirements of the solvent are the simple amide or peptide bond and the amino group of the trifluoroacetylated (TFA) molecule. Hence, the actual portion of the dipeptide phase which is responsible for the separation of D- and L-amino acid derivatives is shown in Fig. 2.

These previous studies were made using TFA-α-amino acid derivatives, and the atomic distances within the solute are such that the atoms which are hydrogen bonded have an almost direct spatial correlation with those atoms on the solvent (Fig. 3) undergoing mutual bonding.

From the preceding discussion, the need for a more complete interpretation of the earlier findings becomes immediately evident. Since only the solvent was manipulated by previous investigators, there is a lack of description for the steric

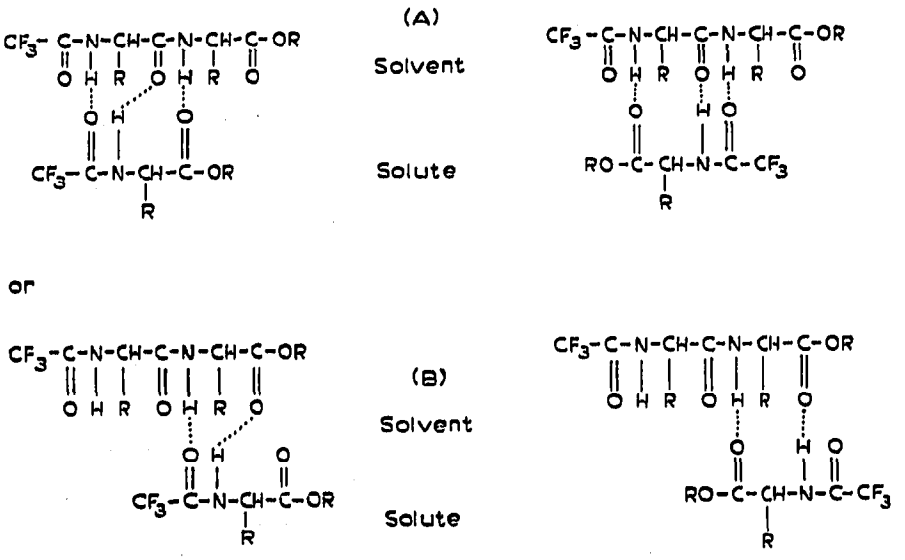


Fig. 1. Diastereoisomeric association complex.

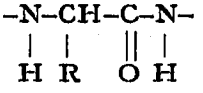


Fig. 2. Configurational requirements of the dipeptide solvent

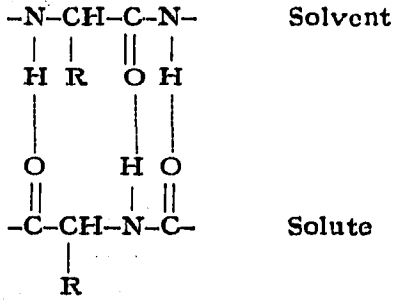


Fig. 3. Spatial correlation between solute and solvent.

properties of the solute as related to its role in formation of the association complex.

There has been no investigation of solutes which do not have the specific moiety shown in Fig. 3, nor has it been conclusively demonstrated that all three hydrogen bonds must be formed between the dipeptide solvent and the solute in order to effect good resolution of enantiomers.

In order to clarify the action of the solute in dipeptide solvent systems we have studied the behavior of other optical isomers (β-amino acids, β-amino alkanes, β-amino alcohols) and different α-amino acid derivatives on dipeptide stationary phases with respect to the separation of individual enantiomeric pairs of each.

EXPERIMENTAL

Preparation of the dipeptide stationary phase

N-TFA-L-α-amino-*n*-butyryl-L-α-amino-*n*-butyric acid cyclohexyl ester has

been synthesized in excellent yield via the hydroxybenztriazole technique[11,16]. Mass spectrometry and the results of a microanalysis verified the identity and high purity of the peptide:

	C	H	N	F
Calculated	52.45%	6.88%	7.59%	15.56%
Found	52.44%	6.88%	7.65%	15.66%

Preparation of the derivatives

Esterification and trifluoroacetylation of D,L-*α- and* D,L-*β-amino acids.* 5 mg of amino acid (ratio D:L = 1:3) were placed in a Pyrex tube, and after addition of 30 ml of isopropanol (3 *N* with respect to hydrogen chloride), the tube was capped and the contents were esterified at 100° for 1 h. Excess isopropanol and hydrochloric acid were removed *in vacuo*. The residue was suspended in 10 ml of methylene chloride, cooled to −20° and 2 ml of trifluoroacetic acid anhydride (TFAA) were added. After standing 1 h at room temperature, solvent and excess TFAA were removed *in vacuo*, and the sample residue was dissolved in 1 ml of chloroform.

Trifluoroacetylation of D,L-β-amino alkanes. 5 mg of sample were dissolved in methylene chloride, 2 ml of TFAA were added, and the mixture was stirred for 1 h at 0°. Methylene chloride and excess TFAA were removed carefully under high vacuum and ice-bath temperature in order to prevent loss of the derivatives. The sample residue was then dissolved in 1 ml of chloroform.

N,O-alkylation of amino acids. The N,O-diisopropyl derivatives of D,L-valine and D,L-leucine were prepared according to PETTITT AND STOUFFER[17].

Trimethylsilyl derivatives. The trimethylsilyl derivatives of D,L-valine and D,L-leucine were prepared according to SWEELEY *et al.*[18] using ready mixed TRI-SIL (Pierce Chemical Company).

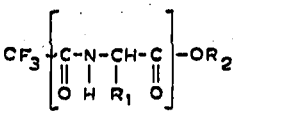
Apparatus

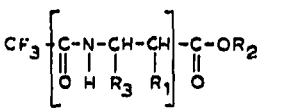
A Varian 1200-1 gas chromatograph, modified to minimize the dead volume in the system, was used in these investigations. The chromatograph was operated at a constant oven temperature of 110°, at a detector temperature of 280°, and at an injector temperature of 190°. An open tubular stainless-steel column (400 ft. × 0.02 in.) was cleaned as described earlier, and coated using a 10% w/v solution of N-TFA-L-*α*-amino-*n*-butyryl-L-*α*-amino-*n*-butyric acid cyclohexyl ester in anhydrous methylene chloride, at 10 p.s.i. (dry nitrogen). The column was conditioned at 120° for 24 h using helium as carrier gas at 5 p.s.i. and at 110° for 24 h.

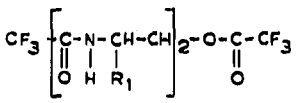
RESULTS AND DISCUSSION

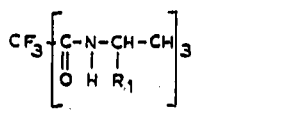
Because of its excellent separation properties, N-TFA-L-*α*-amino-*n*-butyryl-L-*α*-amino-*n*-butyric acid cyclohexyl ester was utilized as the stationary phase for investigating the separability of the compounds shown in Fig. 4.

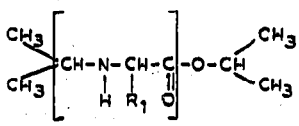
Compounds having the structure assigned to Type I were N-TFA-isopropyl esters of racemic *α*-amino acids, bearing different groups R_1; those assigned to

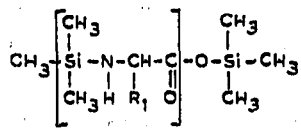

N-TFA-α-amino acid esters (I)


N-TFA-β-amino acid esters (II)


N,O-di-TFA-β-amino alcohols (III)


N-TFA-β-amino alkanes (IV)


N,O-diisopropyl-α-amino acids (V)


N,O-ditrimethylsilyl-α-amino acids (VI)

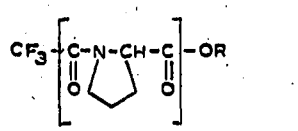

N-TFA-proline ester (cyclic derivative) (VII)

Fig. 4. Derivatized solutes used for TFA-L-L-dipeptide solvent system.

Group II belong to the N-TFA-D,L-β-amino acid isopropyl esters: D,L-β-amino-*n*-butyric acid ($R_1 = H$; $R_2 = CH(CH_3)_2$; $R_3 = CH_3$) and D,L-β-aminoisobutyric acid ($R_1 = CH_3$; $R_2 = CH(CH_3)_2$; $R_3 = H$); β-amino alcohol derivatives from Group III were N,O-di-TFA derivatives of D,L-alaninol ($R_1 = CH_3$) and D,L-leucinol ($R_1 = CH_2–CH(CH_3)_2$); Group IV was represented by N-TFA-D,L-β-amino pentane ($R_1 = (CH_2)_2CH_3$) and N-TFA-D,L-β-amino heptane ($R_1 = (CH_2)_4CH_3$); and in Group V, N,O-diisopropyl derivatives of α-amino acids are considered; these compounds are very similar in structure to the N,O-ditrimethylsilyl derivatives of α-amino acids represented by N,O-ditrimethylsilyl-D,L-valine and -D,L-leucine, which will be considered in Group VI; finally, the cyclic derivative is represented by an α-imino acid, N-TFA-D-L-proline isopropyl ester.

Table I and Fig. 5 show the separation of enantiomers for six amino acid derivatives from Group I and D,L-proline from Group VII on a 400 ft. × 0.02 in. stainless-steel capillary column coated with the optically active phase. These α-amino acids apparently find no difficulties in forming the diastereoisomeric association complex. Complete resolution of derivatized mixtures of D,L-alanine, D,L-valine, D,L-threonine, D,L-isoleucine, D,L-leucine, and D,L-serine is achieved. This depeptide phase can separate the amino acids from each other as well as differentiate between enantiomeric pairs. Less volatile D,L-α-amino acid derivatives can be separated on shorter columns without difficulties and therefore are not discussed here[8].

Table II demonstrates that there is no enantiomeric resolution which can be

TABLE I

GAS CHROMATOGRAPHIC DATA FOR N-TFA-D,L-α-AMINO ACID ISOPROPYL ESTERS (GROUP I)

GLC conditions: 400 ft. × 0.02 in. stainless-steel capillary column coated with N-TFA-L-α-amino-*n*-butyryl-L-α-amino-*n*-butyric acid cyclohexyl ester, 110° isothermal, carrier gas He. 15 p.s.i.

Amino acid	t_R (*min*)[a]	t_R (*L*)–t_R (*D*)[b] (*min*)	$\frac{t_R\,(L)}{t_R\,(D)}$[c]
D-Alanine	48.0		
		3.8	1.079
L-Alanine	51.8		
D-Valine	70.0		
		5.5	1.078
L-Valine	75.5		
D-Threonine	87.3		
		7.3	1.083
L-Threonine	94.6		
D-Isoleucine	100.0		
		9.3	1.093
L-Isoleucine	109.3		
D-Leucine	141.3		
		12.4	1.088
L-Leucine	153.7		
D-Serine	164.2		
		12.6	1.077
L Serine	176.8		
D-Proline	222.0		
		7.5	1.033
L-Proline	229.5		

[a] Retention time in minutes.
[b] Time difference in minutes between the isomers.
[c] Separation factor.

attributed to compounds having the moiety of Group II; derivatives of D,L-β-amino-*n*-butyric acid and D,L-α-aminoisobutyric acid emerge from the column as single peaks, completely unresolved.

β-Amino alcohols, such as N,O-di-TFA-D,L-alaninol and N,O-di-TFA-D, L-leucinol reveal that compounds having the Group III configuration show no detectable enantiomeric separation (Table II). The relative retention times of these compounds are slightly larger than that of derivatized D,L-alanine but there is no visible interaction with the stationary phase resulting in separation.

The β-amino alkanes (IV) have been investigated by FEIBUSH AND GIL-AV[6], and their D,L-isomers have been well resolved on ureide stationary phases. However, when subjected to the dipeptide phase in this study, N-TFA-D,L-β-amino pentane and the corresponding heptane derivatives were not resolved and reflected no apparent interaction with the stationary phase (Table II).

The N,O-ditrimethylsilyl derivatives of D, L-amino acids, and N,O-diisopropyl-D,L-valine and -D,L-leucine which were prepared according to the method of PETTITT AND STOUFFER[7], are not resolved. Structures corresponding to Group VI do

TABLE II

GAS CHROMATOGRAPHIC DATA FOR DERIVATIZED OPTICALLY ACTIVE SOLUTES

GLC conditions: 400 ft. × 0.02 in. stainless-steel capillary column coated with N-TFA-L-α-amino-*n*-butyryl-L-α-amino-*n*-butyric acid cyclohexyl ester.

Group	*Optical isomers*	t_R (*min*)[a]	t_R (*L*)–t_R (*D*) (*min*)[b]	*Conditions*
II	N-TFA-D,L-β-amino-*n*-butyric acid isopropyl ester	138.3	—	110°, 25 p.s.i. He
	N-TFA-D,L-β-amino isobutyric acid isopropyl ester	124.8	—	
III	N,O-di-TFA-D,L-alaninol	73.1	—	110°, 25 p.s.i. He
	N,O-di-TFA-D,L-leucinol	123.5	—	
IV	N-TFA-D,L-β-amino-pentane	91.3	—	90°, 5 p.s.i. He
	N-TFA-D,L-β-amino-heptane	110.0	—	
V	N-isopropyl-D,L-valine isopropyl ester	78.1	—	100°, 12.5 p.s.i. He
	N-isopropyl-D,L-leucine isopropyl ester	103.5	—	
VI	N,O-ditrimethylsilyl-D,L-valine	118.3	—	110°, 15 p.s.i. He
	N,O-ditrimethylsilyl-D,L-leucine	138.4	—	
VII	N-TFA-D-proline isopropyl ester	222.0	7.5	110°, 25 p.s.i. He
	N-TFA-L-proline isopropyl ester	229.5		

[a] Retention time in minutes.
[b] Time difference in minutes between isomers.

not show any distinguishable solute–solvent interaction on the dipeptide phase, since no resolution of the respective enantiomers is observed.

All chromatograms were obtained under conditions which allowed sufficient sample retention time for resolution. Many of the Group-I compounds were easily resolved in less time than was allowed for other samples.

Comparison of the chromatograms for the seven types of compounds studied here yields some interesting facts concerning those bracketed groups in Fig. 4.

The α-amino acids from Group I are easily resolved since the arrangement of atoms fulfills the solute requirements postulated in Fig. 3.

The β-amino acids of Group II, however, are not resolved. Here the functional groups in the solute, necessary for complex formation, are present, but the insertion of a methylene group now gives a length of five atoms to the association-oriented segment. The added length apparently precludes facile solute–solvent association due to the inability of the larger chain to occupy the proper spatial coordinates for hydrogen-bond formation.

For similar reasons, the enantiomeric derivatives of β-amino alcohols are not resolved (Group III, Table II). In this case, the amide group and carbonyl group are again available for hydrogen bonding, but the length of the entire segment has now

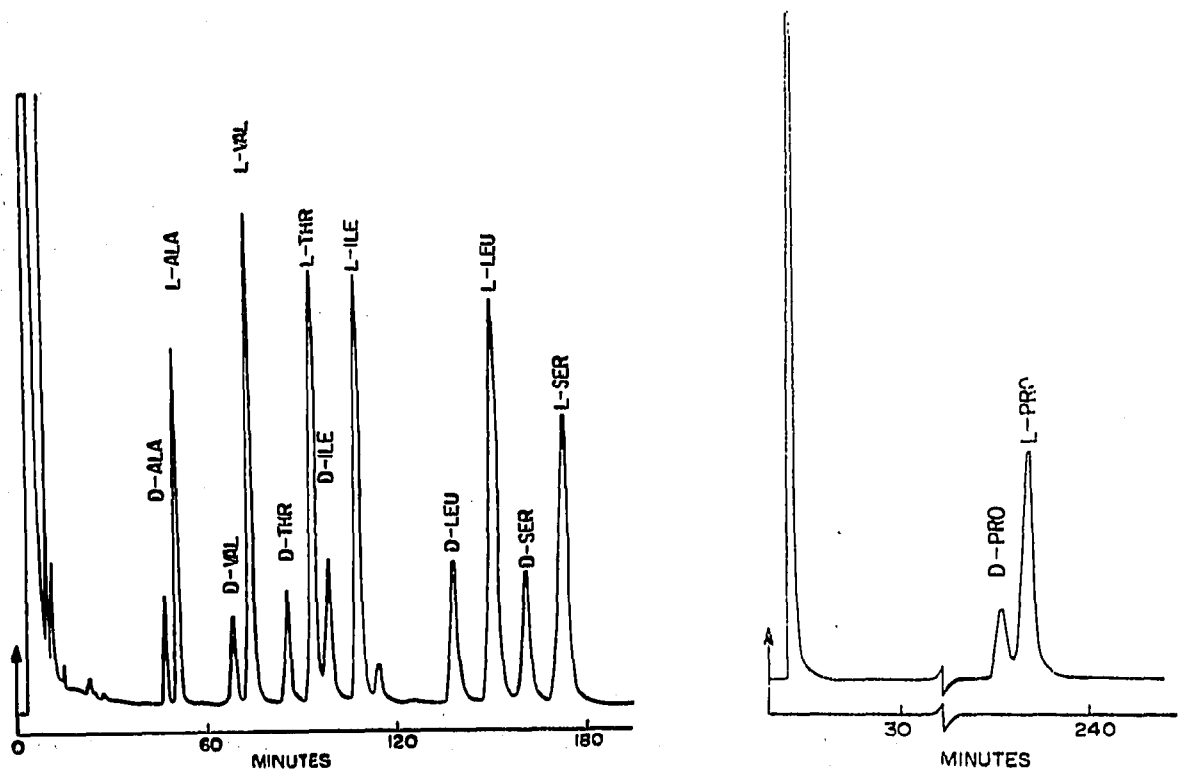


Fig. 5. Chromatogram of N-TFA-D,L-α-amino acid isopropyl esters on N-TFA-L-α-amino-n-butyryl-L-α-amino-n-butyric acid cyclohexyl ester stationary phase. Chromatographic conditions: 400 ft. × 0.02 in. stainless-steel capillary columns; 110° isothermal, injector temperature, 190°; carrier gas, He at 15 p.s.i.

Fig. 6. Chromatogram of N-TFA-D,L-proline isopropyl ester on N-TFA-L-α-amino-n-butyryl-L-α-amino-n-butyric acid cyclohexyl ester stationary phase. Chromatographic conditions: 400 ft. × 0.02 in. stainless-steel capillary columns; 110° isothermal, injector temperature 190°; carrier gas, He at 15 p.s.i.

grown to six atoms, and again, the correct arrangement between solute–solvent associated atoms within the solute itself is not realized.

β-Amino alkanes (Group IV) do not have the group postulated as being necessary for formation of the diastereoisomeric complex of Fig. 3. The amide group is present, but the second carbonyl group is not. The lack of resolution for these compounds by the dipeptide stationary phase indicates that both carbonyl groups must be present on the solute, with the proper distance between them, in order for formation of the association complex to take place.

The lack of resolution for N,O-diisopropyl amino acids and for N,O-ditrimethylsilyl-D,L-amino acids of Groups V and VI, respectively, corroborates the observations from Group-IV compounds (Table II). In this molecule, the amide carbonyl group is missing, and the association complex cannot be readily formed, due to the lack of available sites for hydrogen bonding on the solute; furthermore, the introduction of a bulky group could cause a certain steric hindrance.

D,L-Proline (Group VII) differs from the earlier groups, in that the length of the atom chain is correct, and both the amide and ester carbonyl groups are present with the proper atomic separation in the molecule. The amide nitrogen, however, has no hydrogen available for hydrogen bonding, yet N-TFA-D,L-proline is well separated by the dipeptide (Fig. 6). In this case, only two of the theoretically possible hydrogen bonds can be formed, resulting in a decrease in the separation factor (Tables I, and II), compared with the other α-amino acids.

In conclusion, it has been shown that the structure of the solute derivatives must be one containing two monocyclic carbonyl groups separated by two atoms, in order for the formation of the diastereoisomeric association complex to proceed readily. The protonated nitrogen atom on the amide portion of the solute does not appear to be necessary in order to insure complex formation, but the resolution of enantiomers by dipeptide stationary phases must be carried out on solutes having the correct arrangements of the hydrogen-bonded atoms before sterically suitable solute–solvent interaction can occur.

ACKNOWLEDGEMENT

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