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Investigation of the effect of acylation on the enantiomeric separation of amino acid isopropyl esters by gas chromatography

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ABSTRACT

The effects of acylation on the enantiomeric separation of isopropyl esters of alanine, valine, allo-isoleucine, isoleucine, leucine and aspartic acid were investigated with a cross-linked polycyanoethyl vinyl siloxane-L-Val-*tert.*-butylamide fused-silica capillary column. The gas chromatographic behaviour of N-formyl, N-acetyl, N-trifluoroacetyl and N-benzoyl derivatives of the amino acid isopropyl esters were studied. The chiral recognition mechanism of the solutes on diamide chiral stationary phases is discussed.

INTRODUCTION

Since Gil-Av *et al.* [1] described the first reproducible resolution of protein amino acids by gas chromatography (GC), various chiral stationary phases (CSPs) have been reported, such as Chirasil-Val [2] and cyclodextrin derivatives [3,4]. The enantiomeric separation of many different compounds has been reported on diamide CSPs [5,6], and all of the common protein amino acids can be separated into their antipodes in a single run by temperature programming [7]. The classical method of derivatization of amino acids is their conversion into N-perfluoroacyl alkyl esters [8]. It was assumed that the enatiomeric resolution was due to the hydrogen-bonded association of the "C_5–C_5" or "C_5–C_7" type between pertinent solute and solvent molecules [9]. The effect of the alkyl groups in the amino acid derivatives on enantiomeric separation have been systematically investigated by Liardon and Ledermann [10] and Beitler and Feibush [11]. However, except for perfluoroacyl, the effect of other N-acyl groups has not been fully studied yet.

Amino acids are elementary materials in biochemistry, and in biosynthesis the amino groups are usually protected with formyl (Fr), acetyl (Ac) or benzoyl (Bz) groups [12]. Therefore, the direct enantiomeric separation of amino acid esters acylated with Fr, Ac or Bz groups is certainly of interest in biochemistry.

In this work, the effect of acylation on the enantiomeric separation of amino acids and the chiral recognition mechanism on diamide CSPs were investigated.

EXPERIMENTAL

Derivatization

Esterification. Esterification was performed by heating the amino acid for 30 min at 110°C in acetyl chloride–isopropanol. The excess of reagent was removed by evaporation under a stream of nitrogen [13].

Amino acid isopropyl esters. The esterified samples were dissolved in dichloromethane, neutralized

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with dilute sodium hydroxide solution and washed with water.

N-TFAc-amino acid isopropyl esters. The N-TFAc-amino acid isopropyl esters were prepared according to McKenzie and Tenaschuk [13].

N-Fr-amino acid isopropyl esters. The esterified samples were dissolved in dichloromethane, neutralized with 2*M* sodium hydroxide solution, treated with formic acid–acetic anhydride (3:1, v/v), kept at room temperture for 1 h [14] and dried under a stream of nitrogen.

N-Ac-amino acid isopropyl esters. The esterified samples were treated with anhydrous sodium hydroxide and acetyl chloride, heated at 100°C for 30 min [15], dried under a stream of nitrogen, extracted with dichloromethane and washed with water to neutrality.

N-Bz-amino acid isopropyl esters. The esterified samples were dissolved in dichloromethane, treated with benzoyl chloride and anhydrous sodium hydroxide, heated at 100°C for 30 min [16], treated with sodium hydroxide to remove the excess of benzoyl chloride and washed with water to neutrality.

Chromatographic conditions

The cross-linked fused-silica chiral capillary column was prepared as described previously [17]. The chromatographic experiments were carried out with a GC R1A gas chromatograph equipped with a split injector and a flame ionization detector.

RESULTS AND DISCUSSION

The separation factors (α), capacity factors (k') and thermodynamic parameters of the N-TFAc, N-Fr, N-Ac and N-Bz amino acid isopropyl esters are given in Table I. The thermodynamic parameters were calculated from the following equations:

$$\Delta\Delta G = -RT \ln \alpha \quad (1)$$

$$\ln \alpha = -(\Delta\Delta H/RT) + (\Delta\Delta S/R) \quad (2)$$

Figs. 1–4 show plots of ln α *versus* $1/T$ for the solutes tested.

For N-TFAc-amino acid akyl esters on diamide CSPs, the enatiomeric separation is explained by the "C_5–C_5" or "C_5–C_7" interaction model [9]. In this mechanism, the effect of the acylation group on enantioselectivity was not considered. The isopropyl esters of Ala, Val, Leu, and Pro also contain a

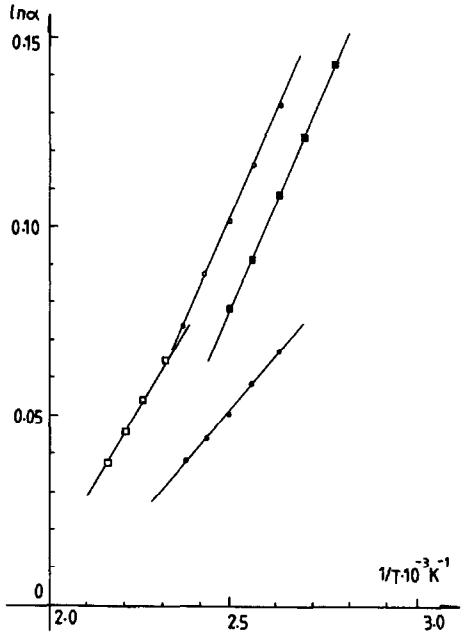

Fig. 1. Plots of ln α *versus* $1/T$ for N-acyl isopropyl esters of Ala. ○ = N-Ac; ■ = N-TFAc; ● = N-Fr; □ = N-Bz.

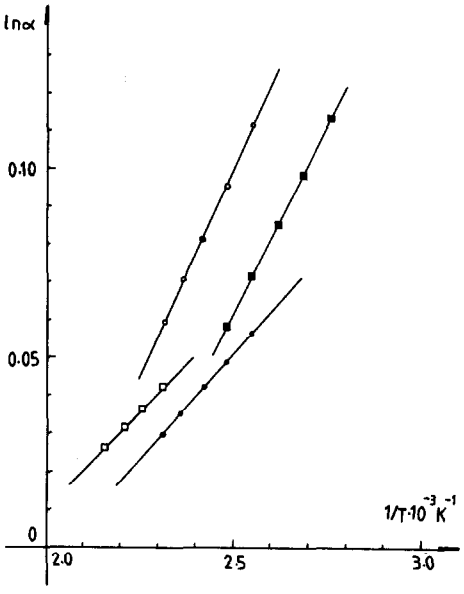

Fig. 2. Plots of ln α *versus* $1/T$ for N-acyl isopropyl esters of Val. Symbols as in Fig. 1.

TABLE I

α AND k'_L VALUES OF THE SOLUTES TESTED

Temperature (°C)	N-TFAc							
	Ala		Val		Leu		Asp	
	α	k'_L	α	k'_L	α	k'_L	α	k'_L
90	1.154	5.57	1.120	7.62				
100	1.131	3.28	1.103	4.41	1.201	11.48		
110	1.114	2.02	1.089	2.67	1.167	6.27		
120	1.095	1.28	1.074	1.67	1.141	3.75	1.043	12.47
130	1.081	0.83	1.060	1.06	1.116	2.26	1.036	7.35
140					1.098	1.47	1.030	4.46
150							1.026	2.75
160							1.021	1.84
$\Delta\Delta H$ (cal/mol)	−475		−398		−687		−177	
$\Delta\Delta S$ (cal/mol)	−1.03		−0.87		−1.48		−0.37	
	N-Ac							
	Ala		Val		Leu		Asp	
	α	k'_L	α	k'_L	α	k'_L	α	k'_L
110	1.141	9.99						
120	1.123	6.11	1.118	10.67				
130	1.106	3.79	1.100	6.52	1.133	10.93		
140	1.091	2.47	1.085	4.17	1.113	6.64		
150	1.067	1.63	1.073	2.61	1.096	4.05	1.041	12.04
160			1.061	1.76	1.081	2.62	1.034	7.38
170					1.065	1.71	1.029	4.71
180							1.024	3.12
$\Delta\Delta H$ (cal/mol)	−471		−439		−543		−207	
$\Delta\Delta S$ (cal/mol)	−0.97		−0.90		−1.10		−0.41	
	N-Fr							
	Ala		Val		Leu		Asp	
	α	k'_L	α	k'_L	α	k'_L	α	k'_L
110	1.069	8.82						
120	1.060	5.52	1.058	12.09	1.070	19.33		
130	1.051	3.44	1.050	7.48	1.061	11.01		
140	1.045	2.30	1.043	4.83	1.052	6.74		
150	1.039	1.56	1.035	3.05	1.045	4.33	1.028	13.98
160			1.030	2.10	1.038	2.83	1.024	8.42
170							1.020	5.48
180							1.016	3.54
$\Delta\Delta H$ (cal/mol)	−230		−230		−257		−149	
$\Delta\Delta S$ (cal/mol)	−0.47		−0.47		−0.52		−0.30	

(Continued on p. 234)

TABLE I *(continued)*

Temperature (°C)	N-Bz					
	Ala		Val		Leu	
	α	k'_L	α	k'_L	α	k'_L
160	1.066	13.53	1.043	17.20		
170	1.056	8.86	1.037	11.29	1.067	17.11
180	1.047	5.53	1.032	6.94	1.056	10.44
190	1.038	3.35	1.027	4.16	1.046	5.98
$\Delta\Delta H$ (cal/mol)	−352		−204		−405	
$\Delta\Delta S$ (cal/mol)	−0.69		−0.39		−0.79	

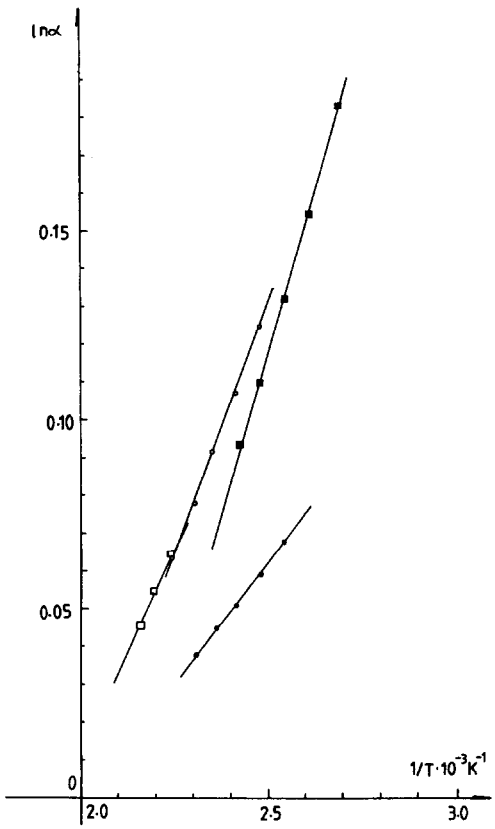

Fig. 3. Plots of ln α *versus* $1/T$ for N-acyl isopropyl esters of Leu. Symbols as in Fig. 1.

C_5 conformation (Fig. 5), and could undergo "C_5–C_5" or "C_5–C_7" association with the diamide CSPs, but no chiral resolution was observed in our experiment (Fig. 6). By comparison with the results obtained for N-acyl isopropyl esters of the amino acids (Table I), it can reasonably be concluded that in this instance the acylation group plays an important role in the chiral recognition. In fact, all reported chiral resolutions of amines or amino acids were carried out by derivatizing them into N-acyl amines or N-acyl amino acid akyl esters (or amides).

Support for the effects of acylation on chiral recognition can also be found by comparing the α val-

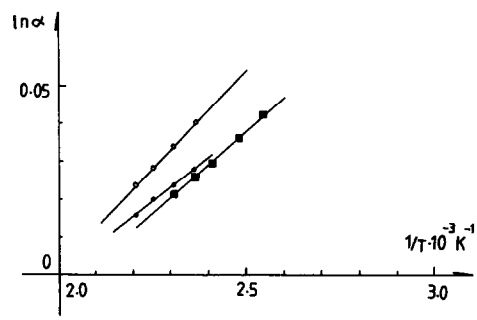

Fig. 4. Plots of ln α *versus* $1/T$ for N-acyl isopropyl esters of Asp. Symbols as in Fig. 1.

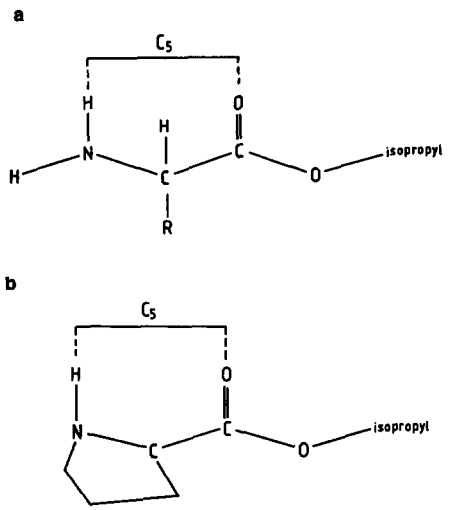

Fig. 5. Conformations of the amino acid isopropyl esters. (a) Ala, Val or Leu isopropyl ester; (b) Pro isopropyl ester.

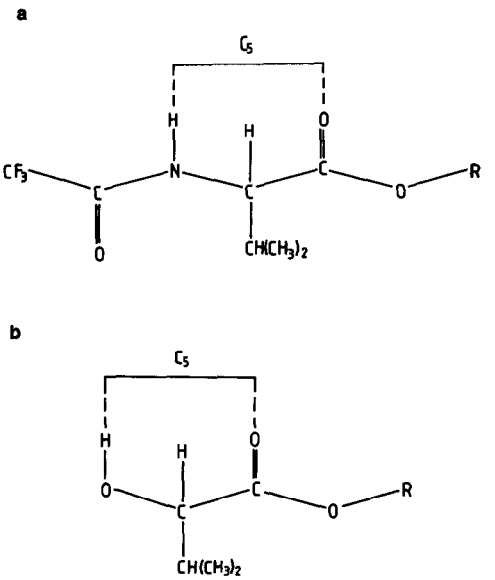

Fig. 7. Conformations of N-TFAc-Val and 2-hydroxy-3-methyl butyric acid alkyl esters. (a) N-TFAc-Val alkyl ester; (b) 2-hydroxy-3-methylbutyric acid alkyl ester.

ues of N-TFAc-isopropyl esters of Val and alkyl esters of 2-hydroxy-3-methylbutyric acid on Chirasil-Val (N-TFAc-isopropyl esters of Val, α = 1.129, 100°; 2-hydroxy-3-methylbutyric acid alkyl ester, α = 1.081, 70°C) [18,19]. Both of the solutes contain a similar C_5 conformation (Fig. 7), and can undergo "C_5–C_5" or "C_5–C_7" association with the solvent. It is well known that O–H · · · O hydrogen bonding is much stronger than N–H · · · O. However, the α value for the N-TFAc-isopropyl ester of Val is much higher than that of the alkyl ester of 2-hydroxy-3-methylbutyric acid. This might be explained by the assumption that the carbonyl moiety of the acylation group takes part in the chiral recognition and the ability of a hydrogen atom in the amide group to form hydrogen bonds was enhanced because of the strong electron-attracting property of the acyl group.

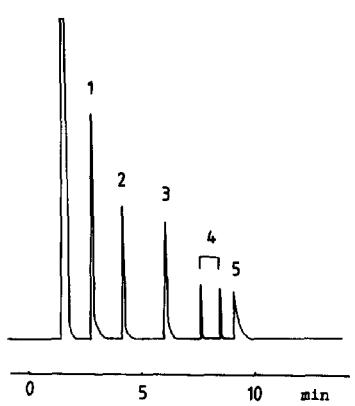

Fig. 6. Chromatogram of amino acid isopropyl ester and N-TFAc-Ala-isopropyl ester enantiomers. Column, cross-linked polycyanoethyl vinyl siloxane-L-Val-*tert.*-butylamide, fused silica, 20 m × 0.25 mm I.D.; temperature, 100 °C; carrier gas, nitrogen. 1 = D,L-Ala-isopropyl ester; 2 = D,L-Val-isopropyl ester; 3 = D,L-Leu-isopropyl ester; 4 = D,L-TFAc-Ala-isopropyl ester; 5 = D,L-Pro-isopropyl ester.

From Table I, it can be seen clearly that among the samples tested the N-Ac derivatives show the highest α values whereas the N-TFAc derivatives are the most volatile. The only difference between them is the acylation group. For TFAc, all the hydrogen atoms in Ac were replaced with fluorine. The variation of the α values might be explained by the difference in ability to form hydrogen bonds with the solvent. CF_3 is a strong electron-attracting group and the electron cloud density around the oxygen in TFAc is reduced. Therefore, the ability of

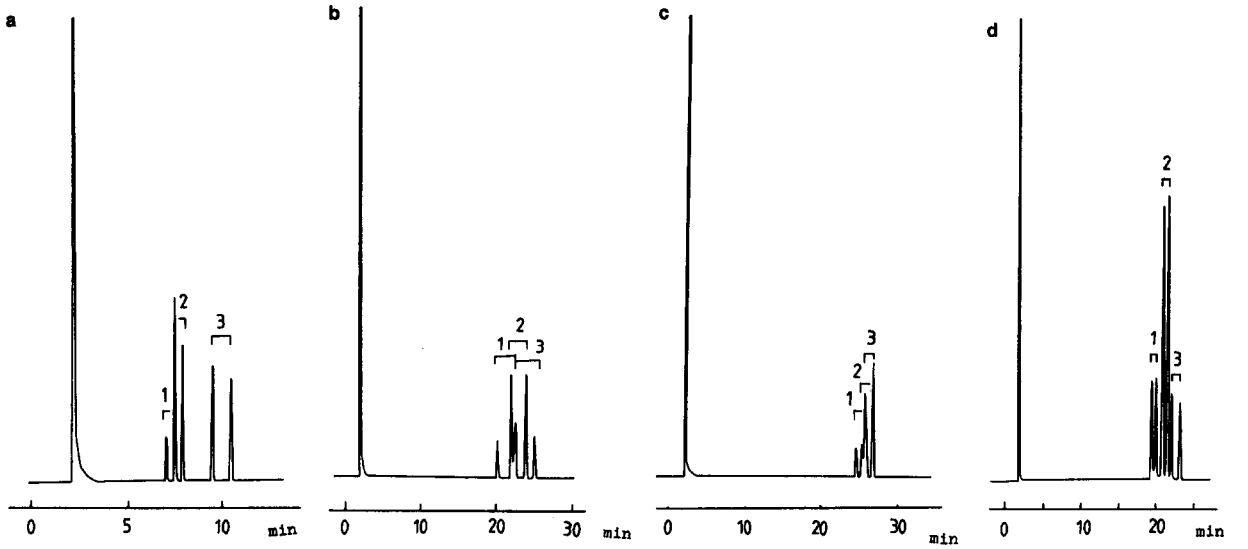

Fig. 8. Chromatograms of N-acyl isopropyl esters of a-Ile, Ile and Leu. Column, cross-linked polycyanoethyl vinyl siloxane-L-Val-*tert.*-butylamide, fused silica, 20 m × 0.25 mm I.D.; carrier gas, nitrogen. (a) N-TFAc, 120°C; (b) N-Ac, 130°C; (c) N-Fr, 130°C; (d) N-Bz, 180°C. Peaks: 1 = a-Ile; 2 = Ile; 3 = Leu (D-enantiomers eluted first).

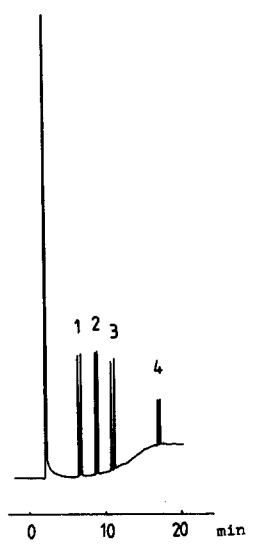

Fig. 9. Chromatogram of N-Ac amino acid isopropyl esters. Column, cross-linked polycyanoethyl vinyl siloxane-L-Val-*tert.*-butylamide, fused silica, 20 m × 0.25 mm I.D.; carrrier gas, nitrogen; temperature, 140°C (5 min), increased at 4°C/min to 180°C. Peaks: 1 = Ala; 2 = Val; 3 = Leu; 4 = Asp (D-enantiomers eluted first).

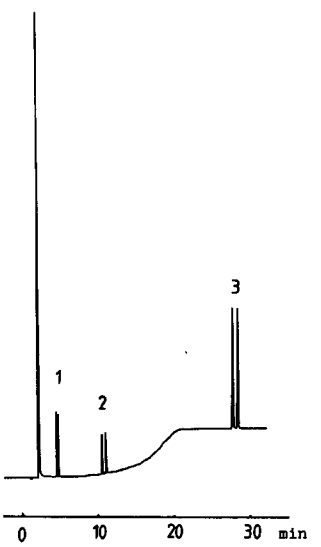

Fig. 10. Chromatogram of N-acyl isopropyl esters of Ala. Column, cross-linked polycyanoethyl vinyl siloxane-L-Val-*tert.*-butylamide, fused silica, 20 m × 0.25 mm I.D.; carrier gas, nitrogen; temperature, 120°C (5 min), increased at 4°C/min to 180°C. Peaks: 1 = N-TFAc; 2 = N-Ac; 3 = N-Bz (D-enantiomers eluted first).

the oxygen atom to form hydrogen bonds with the solvent might be decreased. CH_3 is an electron-donating group, and the ability of the oxygen atom in Ac to form hydrogen bonds with the solvent might be increased. On replacing the acylation group from Ac or TAFc to Fr, the α values for all the solutes tested are considerably decreased, that is, the skeleton of the acylation group has some effect on enantioselectivity.

All the $\Delta\Delta H$ and $\Delta\Delta S$ values shown in Table I are negative. The $-\Delta\Delta H$ values for Fr and Bz amino acid derivatives are much lower and the $\Delta\Delta S$ values are higher than those for the corresponding Ac and TFAc derivatives.

From the discussions above, the effect of the carbonyl moiety in the N-acyl group on enantioselectivity can be considered to be follows: (1) by introducing acyl groups on the nitrogen atom of the amino acid, the ability for hydrogen bonding of the hydrogen atom connected to the nitrogen atom with the solvents is increased owing to the electron-attracting property of the acyl group; (2) the carbonyl groups introduced into the solutes take part in the chiral recognition; and (3) the skeleton of the acyl group has some effect on chiral recognition.

Except for Ala, the N-Fr-amino acid isopropyl esters eluted after their corresponding N-Ac-amino acid isopropyl esters, in spite of the smaller carbon number. This behaviour might be caused by Fr being a more polar group than Ac. On replacing the acyl groups with Bz, the volatilities of all the solutes tested were greatly reduced but no higher α values were obtained.

In most diamide CSPs, N-TFAc isopropyl esters of allo-Ile (a-Ile) and Ile are seriously overplapped. The chromatogram of N-TFAc, N-Fr, N-Ac and N-Bz isopropyl esters of a-Ile, Ile and Leu are shown in Fig. 8. Among these, only when derivatized as N-Bz isopropyl esters can a-Ile, Ile and Leu be eluted without overlapping.

The chromatograms of the N-TFAc, N-Ac, N-FR and N-Bz isopropyl esters of the amino acids are shown in Figs. 9–13.

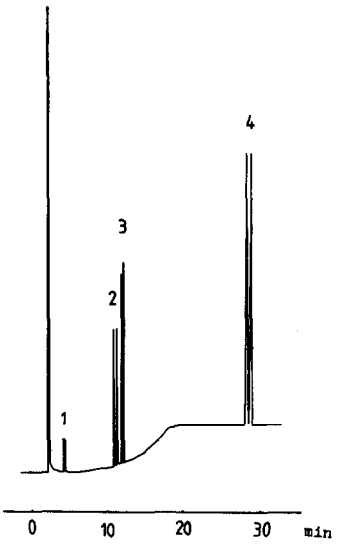

Fig. 11. Chromatogram of N-acyl isopropyl esters of Val. Column, cross-linked polycyanoethyl vinyl siloxane-L-Val-*tert.*-butylamide, fused silica, 20 m × 0.25 mm I.D.; carrier gas, nitrogen; temperature, 130° (5 min), increased at 4°C/min to 180°C. Peaks: 1 = N-TFAc; 2 = N-Ac; 3 = N-Fr; 4 = N-Bz (D-enantiomers eluted first).

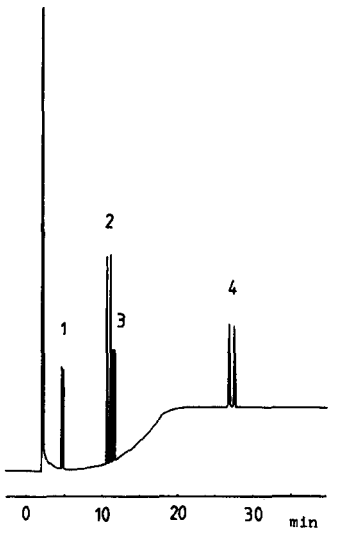

Fig. 12. Chromatogram of N-acyl isopropyl esters of Leu. Column, cross-linked polycyanoethyl vinyl siloxane-L-Val-*tert.*-butylamide, fused silica, 20 m × 0.25 mm I.D.; carrier gas, nitrogen; temperature, 140°C (5 min), increased at 4°C/min to 190°C. Peaks: 1 = N-TFAc; 2 = N-Ac; 3 = N-Fr; 4 = N-Bz (D-enantiomers eluted first).

CONCLUSIONS

For the enantiomeric separation of amino acids, the acylation process is important. The carbonyl moiety in the acylation group takes part in the chi-

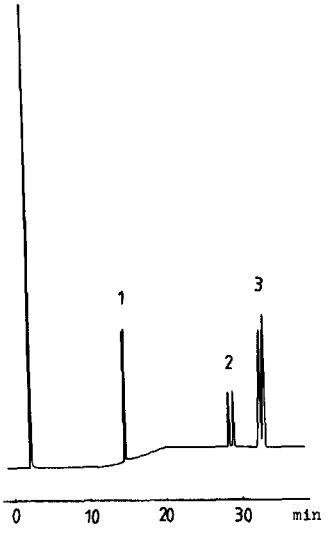

Fig. 13. Chromatogram of N-acyl isopropyl esters of Asp. Column, cross-linked polycyanoethyl vinyl siloxane-L-Val-*tert.*-butylamide, fused silica, 20 m × 0.25 mm I.D.; carrier gas, nitrogen; temperature, 130°C (10 min), increased at 4°C/min to 160°C. Peaks: 1 = N-TFAc; 2 = N-Ac; 3 = N-Fr; (D-enantiomers eluted first).

ral recognition interaction and enhances the ability of the hydrogen atom linked to the nitrogen to form hydrogen bonds with the diamide CSPs. The skeleton of the acylation group has a considerable effect on chiral recognition.

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