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GAS CHROMATOGRAPHIC RESOLUTION OF OPTICAL ISOMERS BY DIAMIDE STATIONARY PHASES, R'CONHCH(R'')CONHR'''

EFFECT OF NON-POLAR SUBSTITUENTS (R'') AT THE α-CARBON ATOM

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SUMMARY

Four chiral diamide stationary phases of formula $C_{11}H_{23}CONHCH(R'')CONH$-*tert.*-$C_4H_9$ derived from L-alanine (I, R'' = CH_3), L-leucine [II, R'' = $CH_2CH(CH_3)_2$], D-phenylglycine (III, R'' = C_6H_5) and L-phenylalanine (IV, R'' = $CH_2C_6H_5$) were synthesized in order to study the influence of R'' on stereoselectivity in gas chromatography. N-TFA-isopropyl (TFA = trifluoroacetyl) esters of α-, β- and γ-amino acids, N-TFA-O-acyl-2-aminoalkan-1-ols and N-TFA-amines were the solutes with which resolution was studied.

An increase in the size of R'' from methyl (I) to isobutyl (II) leads throughout to larger resolution factors. Aromatic R'' substituents (III, IV) can effect stereoselectivity both by their size and through specific interactions of the benzene ring with polar functions of solutes, compared with R'' = isobutyl (II), either an increase or a decrease in resolution factors may result. Methyl substitution in N-TFA esters of neutral α-amino acids in the β- position leads to a decrease, and in the γ-position to an increase, respectively, in the stereoselectivity of the solutes on the stationary phases studied. The most useful are stationary phases II, which is suitable for all the above compounds except the β-amino acids, and IV, which is appropriate for the enantiomeric analysis of both α- and β-amino acids.

INTRODUCTION

Diamide stationary phases of structure R'CONHCH(R'')CONHR''', derived from optically active α-amino acids, have been investigated in our laboratory for a number of years[1,2]. All compounds of this type prepared until recently were derived from valine (R'' = iso-C_3H_7). The influence of variation of the R' and R''' substituents has been reported in several papers[3–5]. A recent modification of this approach is the incorporation of the valyl-*tert.*-butylamide moiety into a polymeric backbone[6,7]. All of these valine-derived stationary phases have been shown to have high stereoselectivity for various classes of optical isomers, and their use is becoming routine, particularly for the enantiomeric analysis of α-amino acids.

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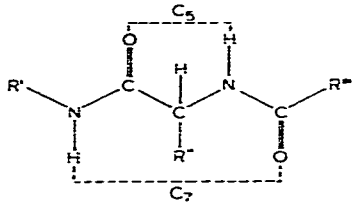
We have proposed[3–5] that chiral recognition by the diamides involves diastereomeric association complexes in which the solute is hydrogen-bonded through the "C_5" or "C_7" side:



It was felt that the substituent at the asymmetric centre of the solvent molecule must, on the one hand, be sufficiently bulky in order to show stereoselective effects, but, on the other hand, it should not be so large as to make association with the solute difficult. The isopropyl group of valine seemed to be a good compromise.

However, having demonstrated the usefulness of the valine type of stationary phases, we have recently started to explore systematically the effect of the nature of the R'' group. The work appeared of interest for gaining a better understanding of the process of chiral recognition, and for improving and extending the procedures for the resolution of optical isomers by gas chromatography.

In this paper we report on four new diamide stationary phases, I–IV, where R' and R''' remain unchanged, being $C_{11}H_{23}$ and *tert.*-butyl, respectively, and R'' is a non-polar aliphatic or aromatic substituent: I, (L-alanine phase)[8], R'' = CH_3; II, (L-leucine phase), R'' = iso-C_4H_9; III, (D-phenylglycine phase), R'' = C_6H_5; IV, (L-phenylalanine phase), R'' = $C_6H_5CH_2$.

EXPERIMENTAL

Derivatization of compounds chromatographed

The N-TFA*-isopropyl esters of the amino acids and the TFA derivatives of the other compounds were prepared as described previously[9].

Synthesis of stationary phases

The above-mentioned stationary phases were prepared by either Scheme A or Scheme B:

N-Carbobenzoxy-tert.-butylamides of α-amino acids (2)[4]. A solution of N-carbobenzoxy-α-amino acid** (0.01 mole) in dry ethyl acetate (70 ml) was cooled to −20 C and N-hydroxysuccinimide (0.011 mole) and dicyclohexylcarbodiimide (0.01 mole) were added, to obtain 1. The mixture was stirred overnight at 0°C, filtered and the filtrate cooled to −20°C. Then an ethyl acetate solution containing 0.01 mole each of *tert.*-butylamine and N-methylmorpholine was dropped in, and stirring at −20 to −5°C continued for 2 days. After filtration, the solvent was removed under reduced pressure, and the residue was dissolved in diethyl ether and washed succes-

* TFA = trifluoroacetyl.

** The derivatives of L-alanine and L-phenylalanine were kindly supplied by the Biophysics Dept. of the Weizmann Institute. The derivative of L-leucine was prepared by Dr. L. Wackerle in our laboratory.

$$C_6H_5CH_2OCONHCH(R'')COOH + SuOH^{*} \xrightarrow[CH_3COOC_2H_5 \text{ or } CHCl_3,\ -20°C]{DCC} C_6H_5CH_2OCONHCH(R'')COOSu \quad (1)$$

$$1 + H_2NC(CH_3)_3 \xrightarrow[-20 \text{ to } -5°C]{\text{N-CH}_3\text{-morpholine}} C_6H_5CH_2OCONHCH(R'')CONHC(CH_3)_3 \quad (2)$$

$$2 + H_2 \xrightarrow[C_2H_5OH]{10\%\ Pd/C} H_2NCH(R'')CONHC(CH_3)_3 \quad (3)$$

$$3 + CH_3(CH_2)_{10}COOSu \xrightarrow[-20 \text{ to } -5°C]{\text{N-CH}_3\text{-morpholine}} CH_3(CH_2)_{10}CONHCH(R'')CONHC(CH)_3 \quad (4)$$

I, R'' = CH_3
II, R'' = iso-C_4H_9
IV, R'' = $C_6H_5CH_2$

Scheme A.

$$H_2NCH(R'')COOH \xrightarrow{2\ N\ NaOH} \xrightarrow[2\ N\ NaOH]{CH_3(CH_2)_{10}COCl} \xrightarrow[pH\ 2]{H^+,\ H_2O}$$

$$CH_3(CH_2)_{10}CONHCH(R'')COOH \quad (5)$$

$$5 + SuOH \xrightarrow[CH_3COOC_2H_5,\ -20 \text{ to } -5°C]{DCC} CH_3(CH_2)_{10}CONHCH(R'')COOSu \quad (6)$$

$$6 + H_2NC(CH_3)_3 \xrightarrow[-20 \text{ to } -5°C]{\text{N-CH}_3\text{-morpholine}}$$

$$CH_3(CH_2)_{10}CONHCH(R'')CONHC(CH_3)_3 \quad (4)$$

Scheme B.

III, R'' = C_6H_5

* SuOH = N-hydroxysuccinimide.

sively with water, 2% hydrochloric acid, water, 5% sodium hydrogen carbonate solution, water and saturated sodium chloride solution, and dried over anhydrous magnesium sulphate. The diethyl ether was removed under reduced pressure and the residue dried under high vacuum. The following N-carbobenzoxy-*tert.*-butylamides of α-amino acids 2 were prepared. L-Leucine: m.p. 58.5–60°C; yield 98%. Elemental analysis: found: C, 68.18; H, 8.90; N, 8.80%; calculated for $C_{18}H_{28}N_2O_3$: C, 67.47; H, 8.81; N, 8.74%. $[\alpha]_D^{24}$ − 29.1 (*c* 5 in chloroform); optical purity, *ca.* 100%. L-Phenylalanine: m.p. 98–99°C; yield 98%. Elemental analysis: found: C, 71.35; H, 7.52; N, 8.02%; calculated for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.39; N, 7.90%. $[\alpha]_D^{24}$ + 10.4 (*c* 5 in chloroform); optical purity, *ca.* 100%.

The L-alanine derivative was synthesized in the same way, but worked up to the final product 4 (I, R'' = CH_3) without isolating the intermediate 2.

tert.-Butylamides of α-amino acids (3). Hydrogen was bubbled for 4 h at a low flow-rate through an alcoholic solution of 2 (0.01 mole) keeping in suspension 0.1 g of 10% palladium/charcoal. After the catalyst had been filtered off and the alcohol removed under reduced pressure, the *tert.*-butylamide of α-amino acid 3 was obtained in quantitative yield.

N-Lauroyl-tert.-butylamides of α-amino acids (4). A solution of 3 (0.01 mole) in dry ethyl acetate (70 ml) was cooled to −20°C, stirred and the laurate of N-hydroxysuccinimide[3] (0.01 mole) added. N-Methylmorpholine (0.01 mole) was then dropped in and treatment continued as for 2.

The crude product was further purified by chromatography on silica gel containing 6% of water, starting with *n*-hexane as eluent and increasing the polarity with ethyl acetate. Pure N-lauroyl-L-alanine-*tert.*-butylamide (I), N-lauroyl-L-leucine-*tert.*-butylamide (II) and N-lauroyl-L-phenylalanine-*tert.*-butylamide (IV) were thus obtained in yields of 43%, 60% and 54%, respectively. The properties of these stationary phases are summarized in Table I.

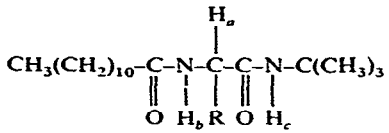
N-Lauroyl-α-amino acids (5). Condensation of lauroyl chloride with an α-amino acid to give N-lauroyl-α-amino acid was carried out by the usual Schotten–Baumann procedure. The α-amino acid (12.8 mmole) was dissolved in an ice-cold 2 *N* sodium hydroxide solution (7.1 ml), and 2 *N* sodium hydroxide solution (9.6 ml) and lauroyl chloride (15.4 mmole) were dropped in separately with vigorous stirring and cooling in an ice-bath. After addition of the reagents was complete, the mixture was stirred for a further 2 h at room temperature. The mixture was washed twice with diethyl ether, acidified to pH 2 with 6 *N* hydrochloric acid and extracted three times with ethyl acetate. The ethyl acetate extract was dried over anhydrous magnesium sulphate, the solvent removed under reduced pressure and the residue recrystallized from ethyl acetate. N-Lauroyl-D-phenylglycine was prepared by this method (m.p. 79.5–80.5 C; yield 43%). Elemental analysis: found: C, 72.01; H, 9.39; N, 4.11%; calculated for $C_{20}H_{31}NO_3$: C, 72.03; H, 9.37; N, 4.20%. $[\alpha]_D^{24}$ − 14.6 (*c* 1 in chloroform); optical purity, 93%.

N-Lauroyl-D-phenylglycine-tert.-butylamide (III)

For carrying out the reaction step 6 → 4, N-lauroyl-D-phenylglycine (5 mmole) in dry ethyl acetate (50 ml) was cooled to −20 C, N-hydroxysuccinimide (5.5 mmole) and dicyclohexyl carbodiimide (5 mmole) were added and the mixture was stirred overnight. After filtration to remove the dicyclohexylurea formed, the clear solution

TABLE I

PROPERTIES OF THE DIAMIDE STATIONARY PHASES



Property	*Stationary phase*			
	*I**, $R = CH_3$ (L-*alanine*)	*II**, $R = i\text{-}Bu$ (L-*leucine*)	*III***, $R = C_6H_5$ (D-*phenylglycine*)	*IV**, $R = C_6H_5CH_2$ (L-*phenylalanine*)
Formula	$C_{19}H_{38}N_2O_2$	$C_{22}H_{44}N_2O_2$	$C_{24}H_{40}N_2O_2$	$C_{25}H_{42}N_2O_2$
%C Calcd.	69.99	71.68	74.18	74.58
Found	69.89	71.75	74.29	74.75
%H Calcd.	11.85	12.03	10.38	10.52
Found	11.73	12.07	10.43	10.52
%N Calcd.	8.58	7.60	7.22	6.96
Found	8.65	8.03	7.10	7.25
M.p. (°C)	87–88.5	Sticky, viscous oil	73–74	75–76
$[\alpha]_D^{24}$ (c 5% $CHCl_3$)	−41.95	−49.6	−71.72	−0.3
Optical purity (%)***	98	≈100 §	81	≈100 §
^1H NMR shift (ppm):				
H_a (m)	4.40	4.39	5.54	4.50
H_b (d)	6.26	6.69	7.08	6.63
H_c (s)	6.22	6.45	6.31	5.39

* Prepared by Scheme A.
** Prepared by Scheme B.
*** See Experimental for details and accuracy of purity determination.
§ Only one peak observed (see Experimental).

was cooled to −20°C and dry ethyl acetate, containing 5 mmole of each *tert.*-butylamine and N-methylmorpholine, was added dropwise. The reaction and the work-up were then continued as above for 4 (scheme A). N-Lauroyl-D-phenylglycine-*tert.*-butylamide (III) was prepared by this method in 54% yield; for the properties of the stationary phase, see Table I.

Structures

NMR spectroscopy was used to check the structures of all products and intermediate compounds.

Determination of optical purity of compounds

Hydrolysis and esterification were carried out in one step by refluxing 5 mg of

sample with a mixture of 2 ml of 6 *N* hydrochloric acid and 2 ml of 1.25 *N* hydrochloric acid in isopropanol in a sealed tube at 110°C for 4 h. The hydrochloric acid and isopropanol were removed under reduced pressure and the residue was converted into N-TFA-isopropyl esters.

The derivatives were gas chromatographed on a chiral diamide stationary phase and the optical purity was calculated from the areas of the peaks corresponding to the D- and L-enantiomers.

The accuracy of determination was, in general, ± 1–2%. Where only one peak was found, the optical purity was considered to be 99–100% as the limit of detection under the conditions used was ≤0.5%.

Chromatographic conditions

Stainless-steel capillary columns (100 ft. × 0.02 in. I.D.) were coated by the plug method with I and II (mounted in a Varian Series 2700 chromatograph) and 150 ft. × 0.02 in. I.D. columns were coated with III and IV (mounted in a Varian Series 1200 chromatograph). Both instruments were provided with a splitter and a flame-ionization detector. The injector temperature was 240°C and the detector temperature 240°C; the column temperatures used are indicated in the Tables. The helium flow-rate was 2.8–3 ml/min for all columns.

RESULTS AND DISCUSSION

In this paper we deal with the resolution of N-TFA-isopropyl esters of α-, β- and γ-amino acids, N-TFA-O-acyl-2-aminoalkan-1-ols and N-TFA-amines. The resolution of diamide solutes, $CF_3CONHCH(R)CONH$-*tert.*-C_4H_9, has been published previously[10]; work on the behaviour of other types of optically active compounds on stationary phases I–IV will be reported in a subsequent publication.

Amino acid derivatives

The data for the various types of amino acids resolved are listed in Table II.

α-Amino acids. The stereoselectivity for the α-amino acid derivatives studied can be readily discussed with reference to the plots of the resolution factors on stationary phases I–IV (Fig. 1).

The order of emergence throughout is the L- after the D-isomer on the L-stationary phases. Further, both proline and aspartic acid have very low resolution coefficients*. These results, already observed for the valine analogues[2–5], show that the changes made thus far in the structure of the diamide solvents do not essentially affect the nature of the resolution mechanisms involved.

Of the four stationary phases synthesized, II and IV give the highest stereoselectivity, with only small differences in the *r* values on these two solvents for a given individual α-amino acid. The data for II and IV are also close to those obtained on the N-lauroyl-*tert.*-butylamide of valine (V), as can be seen in Table III. For the D-phenylglycine phase (III), it should be pointed out that its optical purity was not more

* Proline and aspartic acid not only have very low *r* values, but also their resolution involves mechanisms different to that of the other α-amino acids. Unless specifically mentioned, the following discussion does not relate to these two compounds.

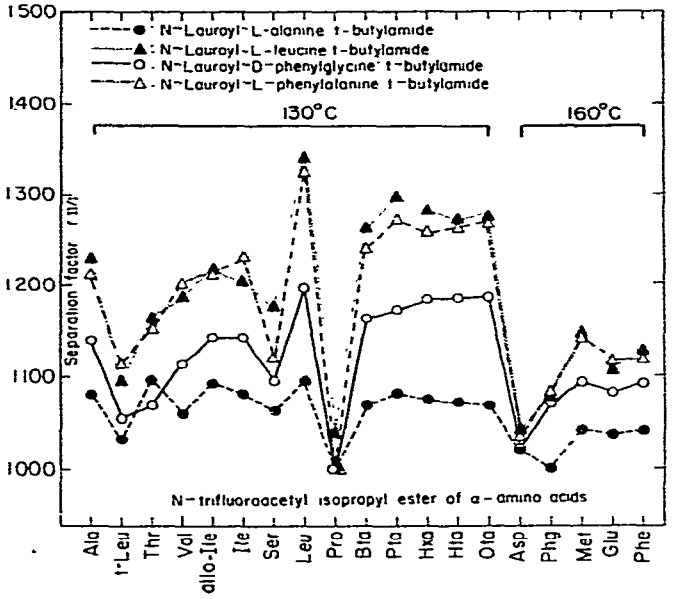


Fig. 1. Resolution factors of proteinic and non-proteinic amino acids on stationary phases I–IV. t = *tert.*; for Bta, Pta, Hxa, Hta and Ota, see Table II.

than 81 % and that the *r* values must be corrected[4] for proper comparison. However, even if the resolution factors are calculated for 100 % purity (see figures in parentheses in Table II), the stereoselectivity in most instances is found to be lower than for II and IV.

A feature in which the aromatic stationary phases III and IV deviate from the aliphatic stationary phases I and II is the complete lack of chiral differentiation for proline; also, compared with II, they have a relatively lower resolution factor for serine.

The alanine stationary phase (I) has the lowest *r* values throughout, except for threonine on III. Further, the stereoselectivity is only slightly affected by variations of the nature of the substituent in the α-position of the solutes; the spread of the data is relatively restricted (see the corresponding plot in Fig. 1). This pattern of behaviour can readily be rationalized with reference to the solvent–solute model proposed for explaining the chiral recognition, already discussed in previous papers for diamides and related optically active stationary phases[2–4] (Fig. 2).

If the R″ group in the solvent is methyl, its radius of interaction is limited, and consequently the system is not greatly affected by the length of the chain and branching of the substituent at the asymmetric carbon of the solute, and essentially only the change in configuration is detected (Fig. 1, data for I at 130°C, except proline); on the other hand, sensitivity to the nature of the α-substituent is observed when the R″ group is larger, as in II–IV (and also in V)[4,5].

It is of particular interest to examine the effect of branching in the aliphatic α-amino acids. If α-aminobutyric acid is taken as the reference solute, it is seen (Fig. 3) that the methyl substituents in the γ-position increase the resolution coefficient (leucine), whereas substitution in the β-position reduces the *r* values (alloisoleucine,

TABLE II

RESOLUTION OF α-AMINO ACIDS ON DIAMIDE STATIONARY PHASES

For chromatographic conditions see Experimental.

Type of amino acid	*N-TFA-isopropyl ester of*	*Stationary phase*							
		N-Lauroyl-L-alanine-tert.-butylamide (I)				*N-Lauroyl-L-leucine-tert.-butylamide (II)*			
		Enantiomer	r^*	r^{**}_{LD}	*T(C)*	*Enantiomer*	r^*	r^{**}_{LD}	*T(°C)*
Protein amino acids	Ala	D	5.94	1.091	120	D	–	–	–
		L	6.48			L			
		D	5.00	1.080	130	D	12.80	1.231	130
		L	5.40			L	15.76		
		–	–	–	–	D	8.46	1.163	150
						L	9.84		
	Thr	D	10.00	1.120	120	–	–	–	–
		L	11.20						
		D	6.90	1.096	130	D	20.12	1.175	130
		L	7.56			L	23.64		
		–	–	–	–	D	11.64	1.120	150
						L	13.04		
	Val	D	8.06	1.089	120	–	–	–	–
		L	8.76						
		D	6.60	1.061	130	D	20.50	1.187	130
		L	7.00			L	24.34		
		–	–	–	–	D	12.50	1.138	150
						L	14.22		
	Gly	–	13.40	–	120	–	–	–	–
		–	10.70	–	130	–	24.36	–	130
	Allo-Ile	D	11.86	1.104	120	–	–	–	–
		L	13.10						
		D	9.24	1.093	130	D	27.50	1.216	130
		L	10.10			L	33.44		
		–	–	–	–	D	17.58	1.142	150
						L	20.08		
	Ile	D	13.10	1.081	120	–	–	–	–
		L	14.16						
		D	9.86	1.081	130	D	30.24	1.204	130
		L	10.66			L	36.40		
		–	–	–	–	D	19.14	1.136	150
						L	21.74		
	Ser	D	15.80	1.063	130	D	38.64	1.175	130
		L	16.80			L	45.40		
	Leu	D	19.60	1.110	120	–	–	–	–
		L	21.76						
		D	15.70	1.095	130	D	38.80	1.342	130
		L	17.20			L	52.06		
		–	–	–	–	D	24.30	1.216	150
						L	29.54		
		–	–	–	–	–	–	–	–
		–	–	–	–	D	10.70	1.150	170
						L	12.30		

N-Lauroyl-D-phenylglycine-tert.-butylamide (III)				*N-Lauroyl-L-phenyl-alanine-tert.-butylamide (IV)*			
Enantiomer	*r**	$r^{**}_{D/L}$	*T(°C)*	*Enantiomer*	*r**	$r^{**}_{L/D}$	*T(°C)*
L D	12.60 14.60	1.159	120	D L	—	—	—
L D	8.60 9.80	1.140 (1.175)	130	D L	4.82 5.84	1.212	130
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
L D	11.60 12.40	1.069 (1.085)	130	D L	7.16 8.26	1.154	130
—	—	—	—	—	—	—	—
L D	17.80 20.60	1.157	120	—	—	—	—
L D	12.00 13.40	1.117 (1.146)	130	D L	7.42 8.96	1.207	130
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	19.20	—	130	—	9.94	—	130
L D	25.80 30.20	1.171	120	—	—	—	—
L D	17.20 19.68	1.144 (1.181)	130	D L	10.70 13.00	1.215	130
—	—	—	—	—	—	—	—
L D	28.40 33.00	1.162	120	—	—	—	—
L D	18.88 21.60	1.144 (1.181)	130	D L	11.72 14.42	1.230	130
—	—	—	—	—	—	—	—
L D	22.08 24.20	1.096 (1.120)	130	D L	14.24 16.04	1.126	130
L D	38.68 47.92	1.235	120	D L	17.20 23.24	1.351	120
L D	25.20 30.20	1.198 (1.250)	130	D L	15.64 20.74	1.326	130
L D	11.00 12.60	1.145	150	D L	6.50 7.90	1.215	150
—	—	—	—	D L	5.00 5.88	1.176	160
—	—	—	—	—	—	—	—

(Continued on p. 96)

TABLE II *(continued)*

Type of amino acid	*N-TFA-isopropyl ester of*	*Stationary phase*							
		N-Lauroyl-L-alanine-tert.-butylamide (I)				*N-Lauroyl-L-leucine-tert.-butylamide (II)*			
		Enantiomer	*r**	$r^{**}_{L,D}$	*T(°C)*	*Enantiomer*	*r**	$r^{**}_{L,D}$	*T(°C)*
Protein amino acids	Pro	D	20.46	1.036	120	–	–	–	–
		L	21.20						
		D	17.70	1.000	130	D	46.04	1.041	130
		L	17.70			L	47.92		
		–	–	–	–	D	29.02	1.036	150
						L	30.06		
	Asp	D	29.64	1.022	140	–	–	–	–
		L	30.30						
		–	–	–	–	–	–	–	–
		D	15.10	1.020	160	D	52.28	1.042	160
		L	15.30			L	54.46		
		–	–	–	–	D	41.08	1.035	170
						L	42.50		
	Met	D	51.12	1.063	140	–	–	–	–
		L	54.32						
		–	–	–	–	–	–	–	–
		D	24.90	1.044	160	D	81.76	1.145	160
		L	26.00			L	93.63		
		–	–	–	–	D	63.14	1.118	170
						L	70.56		
		–	–	–	–	D	30.84	1.065	200
						L	32.86		
	Glu	D	71.34	1.058	140	–	–	–	–
		L	75.48						
		–	–	–	–	–	–	–	–
		D	34.80	1.040	160	D	114.14	1.110	160
		L	36.20			L	126.64		
		–	–	–	–	D	87.70	1.090	170
						L	95.50		
		–	–	–	–	D	61.14	1.089	180
						L	66.64		
		–	–	–	–	D	40.28	1.072	200
						L	43.20		
	Phe	D	76.64	1.069	140	–	–	–	–
		L	81.94						
		–	–	–	–	–	–	–	–
		D	39.00	1.044	160	D	118.06	1.131	160
		L	40.70			L	133.52		
		–	–	–	–	D	90.06	1.105	170
						L	99.44		

N-Lauroyl-D-phenylglycine-tert.-butylamide (III)				*N-Lauroyl-L-phenyl-alanine-tert.-butylamide (IV)*			
Enantiomer	*r**	$r^{*}_{D/L}$	*T(°C)*	*Enantiomer*	*r**	$r^{*}_{L/D}$	*T(°C)*
—	—	—	—	—	—	—	—
L D	40.40 40.40	1.000	130	D L	22.42 22.42	1.000	130
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
L D	36.80 38.00	1.033	150	—	—	—	—
L D	26.00 26.60	1.023 (1.031)	160	D L	26.86 27.88	1.038	160
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
L D	62.80 69.60	1.108	150	—	—	—	—
L D	43.20 47.20	1.093 (1.116)	160	D L	42.96 49.06	1.142	160
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
L D	87.60 96.00	1.096	150	—	—	—	—
L D	59.80 63.60	1.082 (1.102)	160	D L	58.14 64.98	1.118	160
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
L D	90.40 95.20	1.097	150	—	—	—	—
L D	60.80 66.40	1.092 (1.115)	160	D L	64.34 72.06	1.120	160
—	—	—	—	—	—	—	—

(Continued on p. 98)

TABLE II *(continued)*

Type of amino acid	*N-TFA-isopropyl ester of*	*Stationary phase*							
		N-Lauroyl-L-alanine-tert.-butylamide (I)				*N-Lauroyl-L-leucine-tert.-butylamide (II)*			
		Enantiomer	*r**	*$r^{**}_{L,D}$*	*T(°C)*	*Enantiomer*	*r**	*$r^{**}_{L,D}$*	*T (°C)*
Non-protein α-amino acids	α-Amino-butyric acid (Bta)	D	8.46	1.116	120	—	—	—	—
		L	9.44						
		D	8.60	1.070	130	D	11.00	1.264	130
		L	9.20			L	13.90		
	α-Amino-pentanoic acid (Pta)	D	14.56	1.118	120	—	—	—	—
		L	16.28						
		D	10.64	1.081	130	D	18.20	1.297	130
		L	11.50			L	23.60		
	α-Amino-hexanoic acid (Hxa)	D	24.64	1.102	120	—	—	—	—
		L	27.14						
		D	17.40	1.075	130	D	30.90	1.282	130
		L	18.70			L	39.60		
	α-Amino-heptanoic acid (Hta)	D	42.88	1.090	120	—	—	—	—
		L	46.74						
		D	29.70	1.071	130	D	53.60	1.272	130
		L	31.80			L	68.20		
	α-Amino-octanoic acid (Ota)	D	75.26	1.089	120	—	—	—	—
		L	82.02						
		D	51.50	1.070	130	D	93.20	1.277	130
		L	55.10			L	119.00		
	t-Leucine (t-Leu)	D	8.30	1.060	120	—	—	—	—
		L	8.80						
		D	5.90	1.034	130	D	12.70	1.095	130
		L	6.10			L	13.90		
	Phenylglycine (Phg)	D	37.08	1.018	140	—	—	—	—
		L	37.74						
		—	—	—	—	—	—	—	—
		D	19.70	1.000	160	D	64.96	1.076	160
		L	19.70			L	69.90		
		—	—	—	—	D	50.08	1.060	170
						L	53.08		
β-Amino acids	β-Amino butyric acid	No separation on I				D	21.88	1.028	130
						L	22.50		
	β-Amino-γ-methyl-pentanoic acid					D	44.84	1.047	130
						L	46.96		
	β-Amino-δ-methyl-hexanoic acid					D	63.90	1.051	130
						L	67.14		
γ-Amino acids	γ-Amino-valeric acid	L	28.20	1.000	130	L	56.60	1.009	130
		D	28.20			D	56.60		
	γ-Amino-δ-methyl-hexanoic acid	L	51.90	1.019	130	L	92.68	1.056	130
		D	52.90			D	97.90		
	γ-Amino-ε-methyl-heptanoic acid	L	94.70	1.037	130	L	159.20	1.047	130
		D	98.20			D	166.64		

* r = corrected retention time (min).

** $r_{L,D}$ (for the L- stationary phases I, II and IV) and $r_{D,L}$ (for the D- stationary phase III) = resolution factor = ratio of the corrected retention time of the second over that of the first emerging enantiomer, calculated with r values expressed to the second decimal place. For III (see Table I), figures in parentheses are corrected[4] for 100% optical purity.

N-Lauroyl-D-phenylglycine-tert.-butylamide (III)				*N-Lauroyl-L-phenyl-alanine-tert.-butylamide (IV)*			
Enantiomer	*r**	$r^{**}_{D/L}$	*T (°C)*	*Enantiomer*	*r**	$r^{**}_{L/D}$	*T (°C)*
–	–	–	–	D L	7.92 10.00	1.263	120
L D	11.00 12.80	1.164 (1.206)	130	D L	7.36 9.14	1.242	130
–	–	–	–	D L	13.84 18.64	1.347	120
L D	18.60 21.80	1.172 (1.216)	130	D L	12.04 15.34	1.274	130
–	–	–	–	D L	25.08 32.32	1.289	120
L D	29.00 34.40	1.186 (1.234)	130	D L	20.70 26.02	1.257	130
–	–	–	–	D L	43.92 56.48	1.286	120
L D	49.60 58.80	1.187 (1.234)	130	D L	34.68 43.92	1.266	130
–	–	–	–	D L	75.76 96.52	1.274	120
L D	85.20 101.20	1.188 (1.235)	130	D L	59.58 75.76	1.272	130
–	–	–	–	–	–	–	–
L D	11.00 11.60	1.055 (1.068)	130	D L	7.20 8.02	1.114	130
–	–	–	–	–	–	–	–
L D	47.20 51.40	1.089	150	–	–	–	–
L D	33.00 35.40	1.073 (1.090)	160	D L	33.16 35.96	1.084	160
–	–	–	–	–	–	–	–
L D	21.80 22.80	1.046	130	D L	13.52 14.60	1.050	120
L D	40.00 43.00	1.075	130	D L	29.00 33.40	1.152	120
L D	56.80 60.40	1.063	130	D L	41.40 46.40	1.120	120
	No separation on III				No separation on IV		

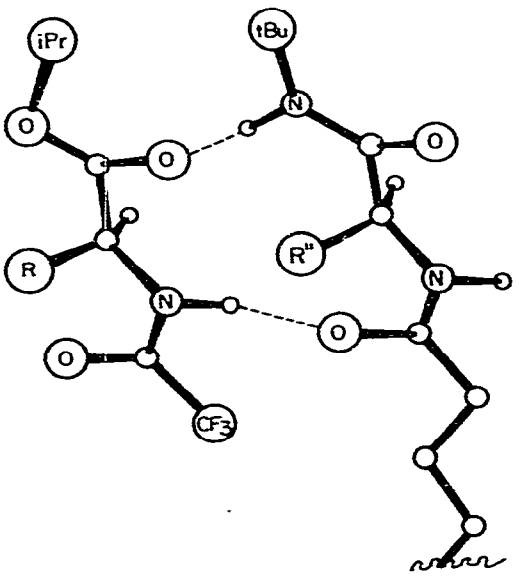
TABLE III

COMPARISON OF RESOLUTION FACTORS ON THE N-LAUROYL-*tert.*-BUTYLAMIDE STATIONARY PHASES DERIVED FROM D-VALINE (V), L-LEUCINE (II) AND L-PHENYLALANINE (IV) AT 130°C

For experimental conditions and definition of $r_{L/D}$ see Table II.

α-Amino acid	*Stationary phase*		
	V:* $r_{D\,L}$	*II:* $r_{L/D}$	*IV:* $r_{L/D}$
Ala	1.224	1.231	1.212
Val	1.172	1.187	1.207
Allo-Ile	1.188	1.216	1.215
Ile	1.175	1.204	1.230
Leu	1.288	1.342	1.326
α-Aminopentanoic acid	1.244	1.297	1.274
t-Leucine	1.094	1.095	1.114

* The purest sample of V available (97.8% optical purity) was derived from D-valine. For this reason $r_{D\,L}$ and not $r_{L\,D}$ values are given; the data were remeasured under the same conditions and the same instrument as those employed for II and IV (Table II). The figures are corrected for 100% optical purity[4]. It should be mentioned that the resolution factors for V given in ref. 4 are too high, as those values were corrected using too low optical purities. It has been found since (see Experimental) that under the normal conditions of peptide hydrolysis the diamides are readily racemized.



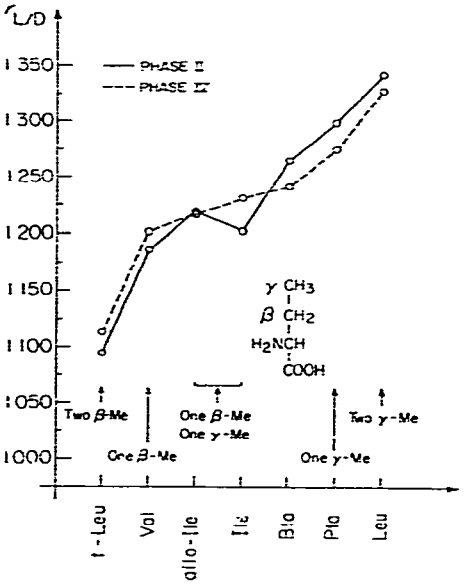


Fig. 2. Schematic representation of an L-solute–L-solvent interaction model (N-TFA-L-amino acid isopropyl ester–diamide stationary phase). iPr = Isopropyl; tBu = *tert.*-butyl.

Fig. 3. Influence of β- and γ-methyl substitution on the resolution factor of some neutral α-amino acids on diamides II and IV. Me = Methyl; Bta = α-aminobutyric acid; Pta = α-aminopentanoic acid.

isoleucine, valine). When two methyl groups are placed in the β-position, the resolution is very low indeed (*t*-leucine) with respect to the unsubstituted α-aminobutyric acid.

This behaviour has already been observed and discussed previously[11] for the resolution of aliphatic amino acids on N-TFA-L-phenylalanine-L-leucine cyclohexyl ester. As it is assumed[12] that the mechanism of the resolution on the dipeptide stationary phases is similar to that on the diamides, the phenomena would appear to be related. It should be mentioned that examination of CPK* models of L-solvent–L-solute pairs indicates that a β-substituent tends to interfere with a good fit of R″ with the corresponding alkyl group of the solute, whereas this is not the case for a γ-substituent. The resulting weakening or strengthening of the L–L solvent–solute associate should decrease (β-effect) or increase (γ-effect) the r_L values.

Possibly a better understanding of these effects might be achieved in the future through computation[13] of the minimal energies of the diastereomeric solvent–solute association complexes considered to be responsible for the chiral differentation.

Analytical considerations. For the resolution of individual α-amino acids, stationary phases II and IV are equivalent or even superior to the commonly used diamide materials derived from valine. Further, the increase in R″ permits a higher operating temperature to be used than on the respective N-acyl-*tert.*-butylamide derivative of valine (160–170°C for II and IV, compared with 140°C for V).

Considering peak overlap of neighbouring α-amino acids, stationary phases II and IV (particularly II) give ready separations of all the isomers of alloisoleucine and isoleucine (Fig. 4), and stationary phases II and IV permit all components of coinjected N-TFA-isopropyl esters of (±)-phenylalanine and (±)-glutamic acid to be resolved completely (Fig. 5).

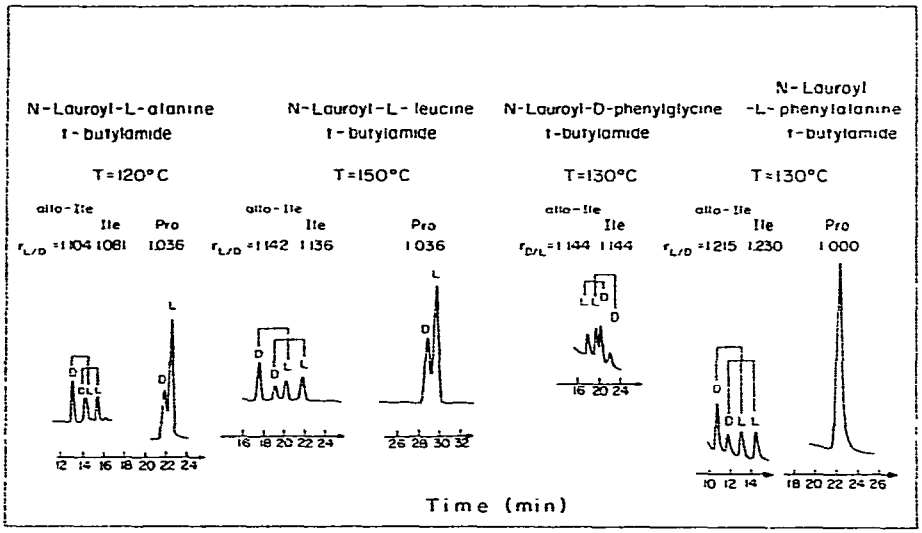


Fig. 4. Chromatogram of N-TFA-isopropyl esters of allo-Ile, Ile and Pro on capillary columns coated with I–IV.

* Corey–Pauling–Koltun.

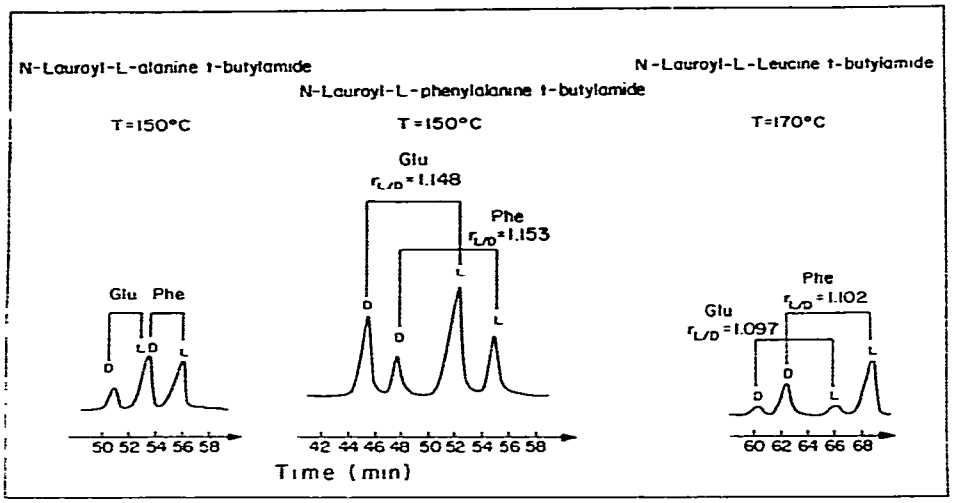


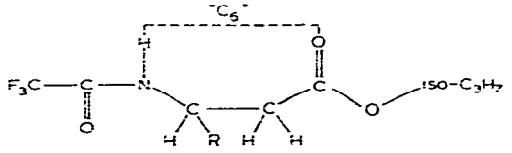
Fig. 5. Chromatogram of N-TFA-isopropyl esters of Glu and Phe on capillary columns coated with I, II and IV.

Stationary phases I and II (especially II) resolve proline reasonably well (Fig. 4) but, as already stated above, the aromatic stationary phases III and IV do not show chiral recognition for this secondary amino acid.

In a preceding paper[10], the stereoselectivity observed in the chromatography of N-TFA-*tert.*-butylamides of α-amino acids on stationary phases I–IV was reported, and the remarkable resolution of proline obtained through such derivatization was discussed.

In conclusion, II and IV are the most useful stationary phases for α-amino acid analysis of the four diamides studied here. The phenylglycine stationary phase (III) is not expected to be very stable optically, owing to the presence of a benzylic carbon atom in its molecule. The main advantage of the alanine stationary phase (I) lies in its application to the resolution of α,α-dialkylamino acids[8], which will be reported in a forthcoming publication.

β-Amino acids. Placing the amino group in the β-position with respect to the carboxylic group imparts a "C_6" moiety to these compounds, and strongly affects the nature of the stereoselective interaction with the diamides derived from α-amino acids.



In agreement with previous work[12], the β-amino acids listed in Table II gave considerably lower r values than those found for the α-amino acids. The alanine stationary phase (I) does not show any chiral recognition for the derivatives examined, and II gives relatively low resolution factors (Table II) of the same magnitude and same order of emergence as on V[12]. On the other hand, the aromatic

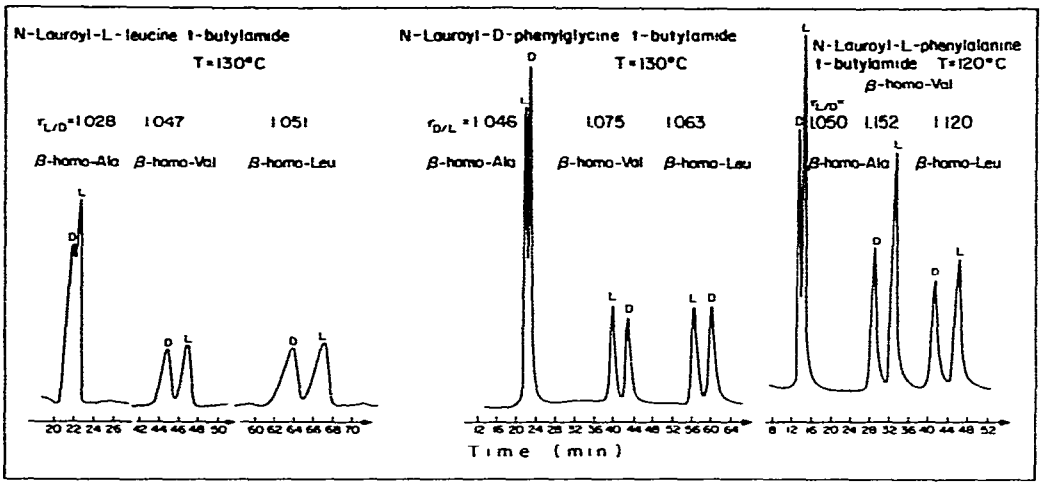
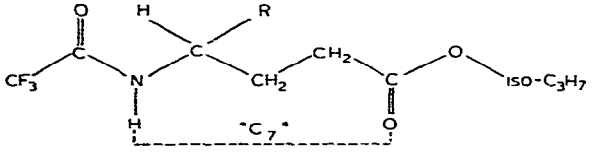


Fig. 6. Chromatogram of N-TFA-isopropyl esters of (±)-β-amino acids on capillary columns coated with II–IV. The term "homo" signifies that a methylene group is inserted between the carboxylic group and the asymmetric carbon of the reference substance.

diamides III and IV are more stereoselective in their interaction with this class of compounds. This is true in particular for the phenylalanine stationary phase (IV), which is recommended for analytical applications (Fig. 6).

More data on the behaviour of β-amino acid derivatives will have to be collected before attempting to propose a mechanism for their chiral recognition.

γ-Amino acids. The aliphatic stationary phases I and II were found to manifest chiral differentiation (r = 1.026–1.056) for the N-TFA-isopropyl esters of the branched γ-amino acids, but not for γ-aminovaleric acid (Table II). When a comparison is made with the resolution factors obtained on V and on N-lauroyl-L-valine-6-undecylamide (VI)[12], taking into account the difference in column temperature (VI, 140°C) and optical purity (V, 75%; VI, 88%), it is found that I and II have lower stereoselectivity for the γ-amino acids resolved. The order of emergence is the same as for the valine stationary phase (V) ($r_{L/D} < 1.00$). This latter finding is consistent with a common mechanism of resolution which is considered to involve hydrogen bonding between the "C_7" side of the solutes and the "C_5" side of the diamide stationary phases[12].



On the other hand, in contrast to the results for the β-amino acids, the aromatic stationary phases III and IV did not show any stereoselectivity for the γ-amino acids examined (reduction of the temperature below 130°C did not change this result).

LUTION OF N-TFA-O-ACYL DERIVATIVES OF 2-AMINO-ALKAN-1-OLS [$RCH(NHCOCF_3)CH_2OCOR_1$] ON DIAMIDE STATIONARY PHASES

le II for chromatographic conditions and definition of t and $r_{D\,L}$.

		Stationary phase																					
		N-Lauroyl-L-alanine-tert-butylamide (I)								*N-Lauroyl-L-leucine-tert-butylamide (II)*								*N-Lauroyl-L-phenylalanine-tert-butylamide (IV)*						
		Acyl group (COR_1)						T (°C)		*Acyl group (COR_1)*						T (°C)		*Acyl group (COR_1)*						T (°C)
	Enantiomer	*Propionyl*		*Isobutyryl*		*Pivaloyl*			*Enantiomer*	*Propionyl*		*Isobutyryl*		*Pivaloyl*			*Enantiomer*	*Propionyl*		*Isobutyryl*		*Pivaloyl*		
		t	$r_{D\,L}$	t	$r_{D\,L}$	t	$r_{D\,L}$			t	$r_{D\,L}$	t	$r_{D\,L}$	t	$r_{D\,L}$			t	$r_{D\,L}$	t	$r_{D\,L}$	t	$r_{D\,L}$	
o-	L	10.40		13.50		15.00			L	13.14		15.64		17.28			L							
l-ol	D	11.60	1.018	13.90	1.030	15.50	1.033	140	D	13.44	1.023	16.12	1.031	17.90	1.036	170	D	8.02	1.000	9.70	1.000	11.40	1.000	130
o-	L	14.36		16.84		17.64			L	19.22		22.66		24.44			L							
-ol	D	14.84	1.033	17.62	1.046	18.78	1.065	140	D	20.04	1.043	23.88	1.054	26.14	1.070	170	D	15.34	1.000	19.12	1.000	19.66		
o-	L	21.68		25.34		26.36			L	28.56		33.26		35.30			L					20.34	1.035	130
l-ol	D	22.66	1.045	26.92	1.062	28.48	1.080	140	D	30.04	1.032	35.34	1.063	38.06	1.078	170	D	26.16	1.000	31.20	1.000	32.10		
o-	L	34.26		39.68		42.36			L	43.48		49.92		53.24			L					33.42	1.041	130
-ol	D	36.04	1.052	42.34	1.067	45.94	1.085	140	D	45.74	1.052	52.86	1.059	57.32	1.077	170	D	25.66	1.000	29.44	1.000	30.60		
o-	L	36.40		40.92		42.70			L	67.98		79.28		83.64			L					31.76	1.038	150
l-ol	D	38.04	1.045	43.28	1.058	45.96	1.076	150	D	71.54	1.052	84.44	1.065	90.92	1.085	170	D	44.60	1.000	49.60	1.014	60.24		
o-	L	56.62		66.34		69.66			L	109.96		127.18		134.06			L					50.30	1.052	150
-ol	D	59.12	1.044	70.48	1.062	75.06	1.078	150	D	115.70	1.053	136.52	1.073	145.30	1.084	170	D	73.16	1.000	81.60	1.014	92.20		
o-3-	L	15.36		18.46		19.78			L	21.00		24.70		26.74			L					82.74	1.040	150
utan-	D	15.94	1.038	19.50	1.056	21.14	1.069	140	D	22.30	1.062	26.54	1.075	29.02	1.085	170	D	15.70	1.009	19.24	1.041	20.54		
o-4-	L	27.00		33.02		32.32			L	33.90		40.30		41.28			L	15.84		20.02		21.72	1.058	130
entan-	D	28.24	1.046	34.92	1.058	34.92	1.081	140	D	35.60	1.050	42.64	1.058	44.70	1.083	170	D	30.40	1.000	36.28	1.000	34.56		
																						35.60	1.030	130

2-Aminoalkan-1-ols

The N-TFA-O-acyl-2-aminoalkan-1-ols are considered to have a resolution mechanism similar to that of the above N-TFA esters of γ-amino acids[12]. In fact, on stationary phases I–IV the two classes of compounds show similar behaviour (Table IV), including, in particular, the order of emergence and a marked reduction in stereoselectivity by an R″ aryl substituent.

No chiral recognition occurred on the phenylglycine stationary phase (III) under any conditions tried, and none of the O-propionyl derivatives could be resolved in IV. For isobutyryl as the O-acyl group, only three of the compounds examined showed weak chiral differentiation on IV. Even the introduction of the usually very effective O-pivaloyl group led to no separation for 2-aminopropan-1-ol and very small r values for the other aminoalkanols.

On the other hand, far more efficient separation of the enantiomeric amino alkanols is possible on aliphatic stationary phases, paralleling the findings for the γ-amino acids. Not unexpectedly, the leucine stationary phase (II), with the larger R″ group, is superior to the alanine stationary phase (I). A comparison of chiral differentiation of the aminoalkanol derivatives on II and on the previously studied VI[12] cannot be readily made, as the respective r values were measured at column temperatures differing by 40°C. Stationary phase II is excellent for the enantiomeric analysis of aminoalkanols (see Fig. 7) and can also be readily operated at a temperature as high as 170°C.

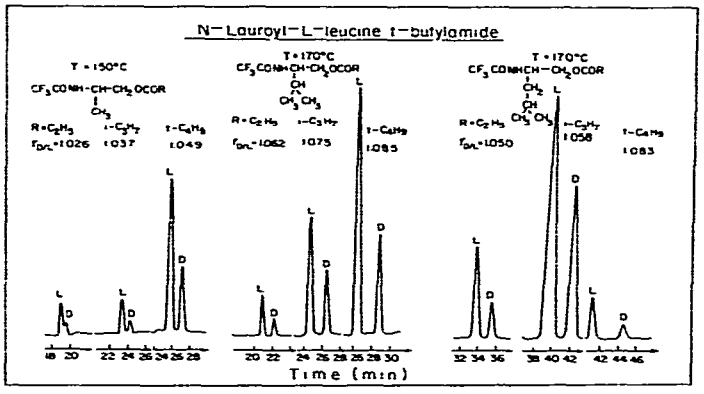


Fig. 7. Chromatogram of (±)-N-FTA-O-acyl-2-aminoalkan-1-ols on II.

Amines

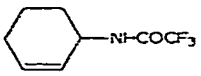
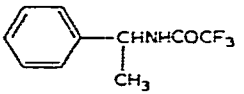
In a recent paper[5], the chromatography of N-TFA-amines on the diamide N-docosanoyl-L-valine-2-(2-methyl)-*n*-heptadecylamide (VII) was discussed. It was pointed out that the pattern of solute–solvent hydrogen bonding and, hence, the mechanism of resolution for this class of stationary phase differs from that of amino acids and amino alcohol derivatives.

In this work it was found that the aliphatic, but not the aromatic, stationary phases have stereoselectivity for the amine derivatives; further, the aliphatic amines studied were resolved only on II, and not on I. Wherever separation of enantiomers was achieved, the order of emergence on the L-stationary phases was *S* after the *R* isomer, as on all other stationary phases permitting resolution of amines[5,13–14].

TABLE V

RESOLUTION OF CHIRAL N-TFA-AMINES ON N-LAUROYL-L-ALANINE-*tert.*-BUTYLAMIDE (I) AND N-LAUROYL-L-LEUCINE-*tert.*-BUTYLAMIDE (II)

For chromatographic conditions see Table II.

Compound	*I*			*II*		
	*r** *(min)*	$r^{**}_{II,I}$	*T*(°C)	*r** *(min)*	$r^{**}_{II,I}$	*T*(°C)
n-$C_5H_{11}CHNHCOCF_3$ (CH$_3$)	No separation on I			*R* 14.04 *S* 14.40	Shoulder	140
n-$C_6H_{13}CHNHCOCF_3$ (CH$_3$)				*R* 23.50 *S* 24.14	1.027	140
n-$C_7H_{15}CHNHCOCF_3$ (CH$_3$)				I 40.64 II 41.64	1.025	140
n-$C_8H_{17}CHNHCOCF_3$ (CH$_3$)				I 71.74 II 73.36	1.023	140
$C_2H_5CHCH_2CHNHCOCF_3$ (CH$_3$, CH$_3$)				I 12.66 II 13.16	1.040	150
	I 32.60 II 33.60	1.023	80			
	*R* 54.60 *S* 57.36	1.050	110	*R* 33.90 *S* 35.56	1.049	150

* See Table II
** See Table II.

Compared with the above valine stationary phase (VII), II shows a higher selectivity[5]. For instance, the aliphatic amines listed in Table V have r values at 140°C similar to those on VII[5] at 110°C, and peak resolution is improved (Fig. 8).

Remarkably, N-TFA-3-aminocyclohexene, which is difficult to resolve, separated to some extent into two peaks on the alanine stationary phase, as was also observed on VII[5]. On I at 80°C, the retention times of the peaks were 32.60 and 33.30 min, respectively ($r_{II,I} = 1.023$; $R_s = 0.25$); resolution on II was not possible under any of the conditions tried.

As reported previously[5,13], the N-TFA derivative of α-phenylethylamine is much better resolved than aliphatic amines. The difference between the leucine and alanine is also apparent in this instance. Whereas for II the resolution factor is 1.05 at 150°C, a similar r value can be obtained on I only at 110°C.

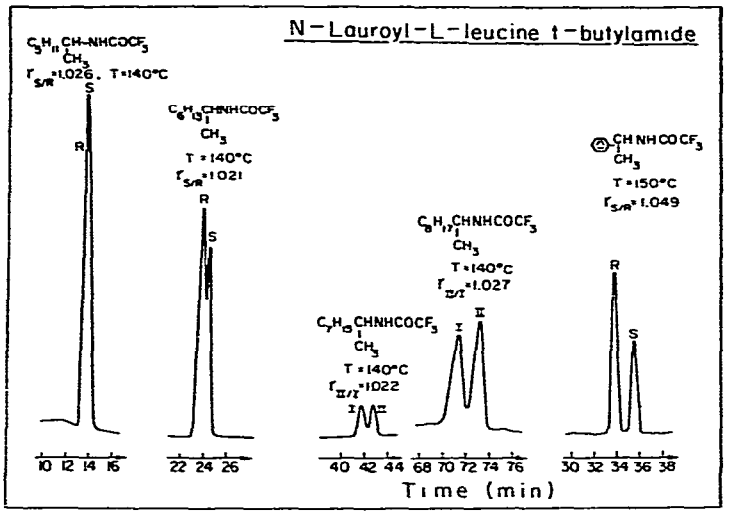


Fig. 8. Chromatogram of N-TFA-(±)amines on II.

On columns with a sufficient number of theoretical plates, II should be as useful for the enantiomeric analysis of amines (Fig. 8) as the stationary phases previously introduced for this purpose, *e.g.*, carbonylbis-(N-L-valine isopropyl ester)[14] and N-lauroyl-S-α-(1-naphthyl)ethylamine[13].

Aromatic R″ groups. Some comments can be made on the influence of the phenyl and the benzyl groups in III and IV, respectively, when substituted in the α-position of the solvent molecule. As far as the resolution of derivatives of the α-amino acids is concerned, the aromatic groups behave similarly to isobutyl (II), suggesting that their effect is essentially one of bulk. However, this parallelism with the isobutyl group breaks down for the other classes of solutes examined. Stationary phases III and IV are more selective with respect to derivatives of the β-amino acids. On the other hand, for γ-amino acids, aminoalkanols and amines, either no chiral recognition occurs or only very low resolution factors were observed (see N-TFA-O-pivaloylaminoalkanols, Table IV).

Stereoselective interactions between solutes and chiral solvents or supports, which seem to involve specifically an aromatic ring, have been observed in many instances. Thus, the interpretation of the resolution of aromatic amino acids on cellulose invokes absorption of the flat benzene residue as part of a "three-point" interaction model[15]. For various solutes it has been found that the gas chromatographic resolution of an aromatic compound is much more efficient than that of corresponding aliphatic or cycloaliphatic derivatives, as demonstrated, for example, by the amines listed in Table V. Also, the different stereoselective shifts of NMR signals of enantiomeric carbonyl-containing compounds in the presence of a chiral aromatic additive are assumed to be due to the formation of diastereomeric contact complexes with oriented donor–acceptor association between the carbonyl and benzene groups[16]. In the examples studied in this work, depending on the mechanism of resolution, additional associations with the benzene ring might have either a synergic effect or, on the contrary, interfere with the formation of the solute–solvent complex responsible for chiral recognition.

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