Journal of Chromatography, 195 (1980) 317–327
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,803

N-DOCOSANOYL-L-VALINE-2-(2-METHYL)-*n*-HEPTADECYLAMIDE AS A STATIONARY PHASE FOR THE RESOLUTION OF OPTICAL ISOMERS IN GAS–LIQUID CHROMATOGRAPHY

R. CHARLES and E. GIL-AV*
Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot (Israel)
(Received March 5th, 1980)

SUMMARY

The synthesis and chromatographic properties of N-docosanoyl-L-valine-2-(2-methyl)-*n*-heptadecylamide are described. It has been employed as a stationary phase at column temperatures up to 200°C and it shows excellent stereoselectivity for various classes of compounds, including, in particular, N-trifluoroacetyl (N-TFA) esters of α- and γ-amino acids, aromatic N-TFA amines, N-TFA-O-acyl amino alcohols and N-TFA-α-methylvaline isopropyl ester. The influence of lengthening the chains of R′ and R‴ on the properties of the diamides R′CONHCH(R″)CONHR‴ is discussed.

INTRODUCTION

It is well established that diamides of the type R′CONHCH(R″)CONHR‴, when used as stationary phases in gas chromatography (GC), show high stereoselectivity for enantiomeric amides derived from amino acids, amines, amino alcohols[1–3], α-hydroxy acids[4] and esters of aromatic diols[4]. Studies in our laboratory were concerned with the influence on chromatographic properties and chiral recognition of modifications of R′ and R‴[2,3] and, more recently, of R″[5]. Other workers have examined the use of diamide phases where R′ is a polymeric matrix[4,6]. At present these various phases are being employed routinely for the GC analysis of enantiomeric mixtures, particularly of α-amino acids, using capillary and packed columns[7–10]. The increasing application of these solvents lends special interest to the pursuit of the above systematic investigations, which might elucidate the mechanism of chiral recognition observed and solve remaining analytical problems. Also, the further development of simple preparative procedures for these phases and control of the optical purity during synthesis is important.

In this paper we report the synthesis and properties of a diamide phase derived from valine (R″ = *i*-Pr), with R′ = *n*-$C_{21}H_{43}$ and R‴ = $-\overset{CH_3}{\underset{CH_3}{C}}-(CH_2)_{14}-CH_3$.

0021-9673/80/0000–0000/$02.25

EXPERIMENTAL

Materials

The N-trifluoroacetyl (N-TFA) isopropyl esters of the amino acids and the TFA derivatives of the other compounds resolved were prepared by esterification with 1.25 *N* hydrochloric acid–isopropanol in a sealed tube at 105–110°C for 4 h, followed by trifluoroacetylation, as described previously[11].

Synthesis of the stationary phase

N-Carbobenzoxy-L-valine-2-(2-methyl)-n-heptadecylamide (1). To a stirred solution of N-carbobenzoxy-L-valine-N-hydroxysuccinimide ester[3] (0.001 mole) in dry chloroform (60 ml) kept at −5 to −10°C, 2-methyl-2-heptadecylammonium chloride[3] (0.001 mole) and 2 equivalents of N-methylmorpholine in chloroform (40 ml) were added dropwise. Reaction was continued for 48 h and the mixture was then left to reach room temperature. The crude product was washed successively with water, 2% hydrochloric acid, water and 5% sodium hydrogen carbonate solution. After drying over magnesium sulphate, the solvent was removed rapidly.

L-Valine-2-(2-methyl)-n-heptadecylamide (2). The above crude product was dissolved in 50 ml of absolute methanol and reduced with hydrogen in the presence of 10% palladium–charcoal for 4 h. Filtration of the catalyst and evaporation of the solvent left the expected compound, which was identified by nuclear magnetic resonance (NMR) spectroscopy.

N-Docosanoyl-L-valine-2-(2-methyl)-n-heptadecylamide (3). The diamide was obtained by coupling compound 2 (0.001 mole) with the docosanoate of N-hydroxysuccinimide (0.001 mole) in dry chloroform in the presence of 1 equivalent of N-methylmorpholine at −5 to −10°C for 48 h. The reaction mixture was treated with active charcoal, filtered and then washed as for compound 1. After evaporation of the solvent, the crude product was coated on 30–60-mesh Chromosorb W AW and placed on top of a column containing silica gel deactivated with 6% of water. The fraction eluting with 10% ethyl acetate in *n*-hexane was the desired compound, as demonstrated by NMR spectroscopy (m.p. 54–57°C; yield 46%). Elemental analysis: found, C 78.10, H, 13.18%; calculated for $C_{45}H_{90}N_2O_2$, C 78.19, H 13.13%; $[\alpha]_D^{25}$ −15.2 (*c* 1.85 in chloroform). The optical purity was 95%, as determined by GC of the N-TFA-valine isopropyl ester, obtained after hydrolysis of the phase at 110°C for 4 h with a 1:1 mixture of 6 and 1.25 *N* hydrochloric acid in isopropanol*, followed by trifluoroacetylation[11].

Chromatographic conditions

A stainless-steel capillary column (50 m × 0.5 mm I.D.) was mounted in a Varian Series 2700 gas chromatograph, provided with a stream splitter and a flame-ionization detector (FID). The temperatures were: injector 240°C and detector 240°C; the column temperatures used are indicated in the tables. The carrier gas (helium) flow-rate was 3 ml/min. The resolution factors ($r_{II/I}$, see tables) were not corrected for the optical purity of the stationary phase[2]. Thermogravimetric analysis was performed with a Perkin-Elmer TG-S-1 instrument provided with a thermo-

* It was shown that under these conditions the diamides are not racemized[12].

balance. The amount of the sample was 2.8 mg; between 50 and 300°C the temperature was raised at a rate of 8°/min. Loss of weight was observed to start above 200°C.

RESULTS AND DISCUSSION

As mentioned in the Introduction, the use of diamide stationary phases in the enantiomeric analysis of amino acids has become routine in many laboratories. There remain, however, some aspects of the method that could still be improved, for instance the resolution of aspartic acid and of proline, the degree of peak overlap of some protein amino acids and the ease of synthesis of the stationary phases and their thermal stability.

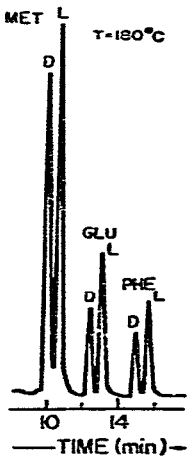
The separation problems can be solved, at least in part, by the use of highly efficient capillary columns. However, it would be advantageous to develop stationary phases or combinations of them and/or to employ solute derivatives that would permit the complete enantiomeric analysis of mixtures of protein amino acids with less peak overlap, and preferably also on packed columns[3].

As to thermal stability, incorporation of the diamide moiety into a polymeric matrix increases its thermal stability[4,6]. It has been shown[3], however, that modification of the substituents R′ and R‴ equally permits bleeding of "low-molecular-weight" diamides to be reduced. Thus, N-docosanoyl-L-valine-*tert.*-butylamide (4) and N-lauroyl-L-valine-2-(2-methyl)-*n*-heptadecylamide (5) could be used at column temperatures of 180–190°C[3].

The synthesis of stationary phase 3 has been carried out as part of a study on the influence of the chain lengthening of R′, R″ and R‴ on the thermal stability and chromatographic behaviour of the diamides[3].

Thermal stability

Thermogravimetric analysis showed that bleeding should start only at about 200°C. As can be seen in Figs. 1 and 2, a stable baseline was obtained when operating



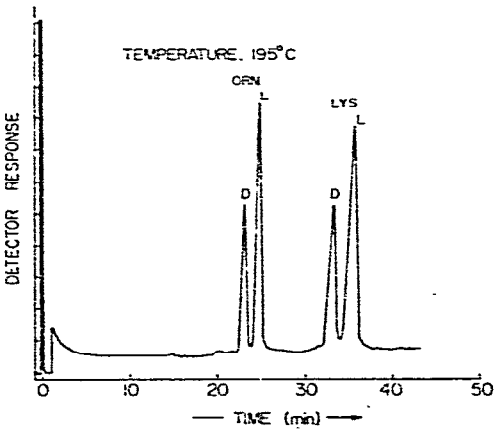


Fig. 1. Chromatogram of N-TFA-isopropyl esters of methionine, glutamic acid and phenylalanine.

Fig. 2. Chromatogram of N-TFA-isopropyl esters of ornithine and lysine.

at 180 and 195°C with the instrument set at $4 \cdot 10^{-11}$ full-scale deflection. The stationary phase was also used for the resolution of some less volatile protein amino acids at 200°C (Table I) and, in general, performed well when exposed for long periods to the above relatively high temperatures. It should be emphasized that efficient purification of 3 and other similar stationary phases, including a final clean-up by chromatography on silica gel, is considered essential for good thermal performance[§].

Chromatographic properties

Data on the resolution of amino acids, amino alcohols and amines are given in Tables I, II and III, respectively.

TABLE I

RESOLUTION OF N-TFA-AMINO ACID ISOPROPYL ESTERS

For chromatographic conditions, see Experimental.

Protein α-amino acids	*α-Amino acids*			*T (°C)*
	Enantiomer	r^*	r_{LID}**	
ALA	D-	4.76	1.155	120
	L-	5.50		
THRE	D-	6.74	1.098	120
	L-	7.40		
VAL	D-	7.70	1.104	120
	L-	8.50		
GLY	—	9.12	—	120
allo-ILE	D-	11.78	1.108	120
	L-	13.06		
ILE	D-	13.06	1.105	120
	L-	14.44		
SER	D-	12.30	1.100	120
	L-	13.56		
LEU	D-	16.08	1.230	120
	L-	19.80		
PRO	D-	19.60	1.025	120
	L-	20.08		
ASP	D-	37.12	1.042	120
	L-	38.68		
CYS	D-	18.98	1.178	140
	L-	22.36		
MET	D-	10.54	1.066	180
	L-	11.24		
GLU	D-	12.90	1.060	180
	L-	13.68		
PHE	D-	16.07	1.049	180
	L-	16.85		
ORN	D-	20.08	1.072	200
	L-	21.54		
LYS	D-	28.60	1.064	200
	L-	30.40		
TRP	D-	62.52	1.041	200
	L-	65.08		

[§] Some workers[4] have claimed that N-docosanoyl-L-valine-*tert.*-butylamide (4), when coated on glass capillaries, could not be used above 140°C. However, to make a meaningful statement on the thermal behaviour of these stationary phases one has to ascertain their purity.

TABLE I *(continued)*

Non-protein α-amino acids	*Enantiomer*	*r**	*$r_{L/D}$***	*T (°C)*
α-Aminobutyric	D-	6.34	1.148	120
	L-	7.28		
α-Aminopentanoic	D-	11.70	1.174	120
	L-	13.74		
α-Aminohexanoic	D-	6.48	1.126	150
	L-	7.30		
α-Aminoheptanoic	D-	10.75	1.102	150
	L-	11.85		
α-Aminooctanoic	D-	12.30	1.081	160
	L-	13.30		
tert.-Leucine	D-	8.06	1.052	120
	L-	8.48		
Phenylglycine	D-	19.90	1.043	150
	L-	20.76		
γ-Amino acids	*Enantiomer*	*r**	*$r_{D/L}$***	*T (°C)*
γ-Aminovaleric	L-	31.80	1.046	120
	D-	33.26		
γ-Amino-δ-methyl-hexanoic	L-	61.78	1.076	120
	D-	66.50		
γ-Amino-ε-methyl-heptanoic	L-	110.50	1.070	120
	D-	118.20		
α-Methyl-α-amino acids	*Enantiomer*	*r**	*$r_{II/I}$***	*T (°C)*
α-Methylvaline	I	10.88	1.048	110
	II	11.40		
α-Methylnorvaline	I	9.26	1.000	110
	II	9.26		
α-Methylleucine	I	12.36	1.000	110
	II	12.36		
α-Methylnor-leucine	I	16.04	1.000	110
	I	16.04		

* Corrected retention time (minutes).

** $r_{L/D}$ = resolution factor = ratio of the corrected retention time of the L- over that of the D-enantiomer, calculated with r values expressed to the second decimal place.

α-Amino acids. Comparison with data for homologous low-molecular-weight diamides, *e.g.*, N-lauroyl-L-valine-2-(2-methyl)-*n*-heptadecylamide (5)[3], shows lower resolution factors [Ala: $r_{L/D} = 1.166$ on 5 (130°C), 1.155 on 3 (120°C). Glu (180°C): $r_{L/D} = 1.069$ on 5, 1.060 on 3. Met (180°C): $r_{L/D} = 1.080$ on 5, 1.066 on 3]. This can be readily interpreted as being due to the lengthening of the chains of R′ and R‴, which "dilutes" the effect of the central diamide group responsible for chiral recognition. However, the r values are higher throughout than for the polymeric chiral phases and are still amply sufficient for resolution on packed columns of 14 of the 16 optically active protein amino acids examined, and of aspartic acid and proline on capillaries. Using readily available starting materials, it is possible to synthesize higher homologues of 3, which might further increase the permissible operating temperature.

TABLE II

RESOLUTION FACTORS OF N-TFA-O-ACYL DERIVATIVES OF AMINOALKANOLS

For chromatographic conditions, see Experimental.

Aminoalkanol	*Enantiomer*	*Acyl group*								
		Propionyl			*Isobutyryl*			*Pivaloyl*		
		r^*	$r_{(D/L)}$**	T(°C)***	r^*	$r_{(D/L)}$**	T(°C)***	r^*	$r_{(D/L)}$**	T(°C)***
2-Aminopropan-1-ol	L-	14.58	1.040	120	9.40	1.051	140	12.00	1.075	140
	D-	15.16			9.88			12.90		
2-Aminobutan-1-ol	L-	25.00	1.076	120	14.56	1.089	140	15.86	1.112	140
	D-	26.90			15.86			17.64		
2-Aminopentan-1-ol	L-	42.28	1.089	120	10.94	1.075	160	11.48	1.097	160
	D-	46.00			11.76			12.60		
2-Aminohexan-1-ol	L-	69.88	1.087	120	16.86	1.071	160	18.14	1.096	160
	D-	76.00			18.06			19.88		
2-Aminoheptan-1-ol	L-	51.88	1.071	140	19.86	1.062	170	20.88	1.082	170
	D-	55.58			21.10			22.60		
2-Aminooctan-1-ol	L-	88.80	1.071	140	31.98	1.067	170	33.14	1.083	170
	D-	95.10			34.14			35.90		
2-Amino-3-methyl-butan-1-ol	L-	28.44	1.094	120	16.90	1.105	140	18.66	1.125	140
	D-	31.12			18.68			21.00		
2-Amino-4-methyl-pentan-1-ol	L-	24.10	1.057	140	29.84	1.085	140	30.04	1.130	140
	D-	25.48			32.38			33.94		

* See Table I.

** See Table I

*** Temperature at which good peak resolution and relatively short retention were observed.

TABLE III

RESOLUTION OF CHIRAL N-TFA-AMINES

For chromatographic conditions, see Experimental.

2-Amino-n-alkanes (R-CH-CH_3 with NH_2)	*Enantiomer*	r^*	$r_{D/L}$**	R_S***	T (°C)
2-Aminoheptane	L-	11.72	Shoulder		110
	D-	12.06			
2-Aminooctane	L-	23.82	1.021	~0.2	110
	D-	24.32			
2-Aminononane	L-	42.86	1.026	~0.6	110
	D-	44.60			
2-Aminodecane	L-	88.10	1.028	~0.5	110
	D-	90.56			
3-Aminocyclenes	*Enantiomer*	r^*	$r_{II/I}$**	R_S***	T (°C)
3-Aminocyclohexene	I	7.96	1.000		120
	II	7.96			
	I	35.50	1.020	~0.3	80
	II	36.20			
	I	70.10	1.021	~0.4	65
	II	71.60			
Aromatic N-TFA-amines	*Enantiomer*	r^*	$r_{S/R}$**	T (°C)	
α-Phenylethylamine	*R*	16.68	1.035	130	
	S	17.26			
α-(1-Naphthyl)ethylamine	*R*	42.80	1.033	180	
	S	44.20			

* See Table I.

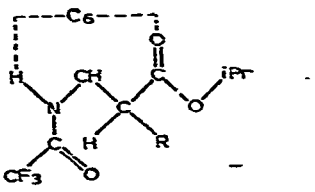
** See Table I.

*** R_S defined by the expression $2d/(w_1 + w_2)$, where d is the distance between the peak maxima and w_1 and w_2 are the half widths (at the baseline) of the first and second peak, respectively.

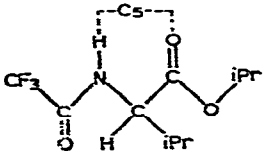
Increasing the chain length, *e.g.*, of R′ in 3, shifts the relative retentions of some amino acids, *e.g.*, Phe, as compared with Glu, is more retained on 3 than on 5 (at 180°C). Another result of chain lengthening is a decrease in the melting point, *e.g.*, 54–57° *vs.* 85°C for 4. Low-melting stationary phases permit the use of a wider temperature programming range and advantage to be taken of the higher resolution factors at the lower temperatures [*e.g.*, $r_{L/D}$ at 80°C (120°C): Ala, 1.306 (1.155); Pro, 1.040 (1.025); Leu, 1.470 (1.230)]. These effects could be combined to solve problems of peak overlap with protein amino acids on packed and, in special instances, on capillary columns. Two or more thermally stable homologous diamides, coupled with different temperature programming profiles, could be employed consecutively for this purpose. Alternative or additional possibilities for reducing peak overlap, such as coupling in series of a second column coated with an achiral phase or the injection of derivatives other than N-TFA isopropyl esters, have been discussed earlier[3].

β- and γ-amino acids. The β-amino acids examined (β-aminobutyric, β-amino-γ-methylpentanoic and β-amino-δ-methylhexanoic acid) showed either no resolu-

tion or, in the best instances, only a hint of a shoulder. As has been reported recently[13], the β-amino acids, which can form hydrogen bonds via a "C_6" conformation:



have considerably lower resolution factors than the corresponding α-amino acids, which can hydrogen bond via a "C_5" ring:



The above-mentioned diluting effect of chain lenghtening further decreases chiral recognition. It should also be recalled that there is a strong influence for this class of compounds of the nature of the R''' substituent on stereoselectivity, as manifested by the reversal of the order of emergence on N-lauroyl-L-valine-*tert.*-butylamide as compared with N-lauroyl-L-valine-6-undecylamide[13].

γ-Amino acids. Data on the γ-amino acids examined are given in Table I. They all show reversal of the order of emergence with respect to α-amino acids, as has also been noted on other diamide phases. This chromatographic behaviour has been tentatively correlated with the predominant formation of solvent–solute hydrogen-bonded association[2,14,15] of the "C_5–C_7" type, with "non-parallel" orientation of the alkyl groups at the respective asymmetric carbons for the less strongly retained L-enantiomer (Fig. 3). Frank *et al.*[4] have also used this type of structure to explain the stereoselectivity observed with their Chirasil-val phase. It is of interest that in the example given[4], where the solute was pentafluoropropionyl-L-lactic acid cyclohexylamide and the association was of the "C_5–C_7" type, the enantiomer with the higher retention ($r_{L/D} > 1$) showed, as expected, a parallel orientation of the pertinent alkyl groups.

α,α-Dialkyl-α-amino acids. N-TFA derivatives of this type of amino acid have been separated into their enantiomers on carbonylbis-(N-L-valine isopropyl ester)[16]. On "low-molecular-weight" diamides, however, resolution has been observed only in exceptional instances, *e.g.*, for α-methylvaline on 3 (Fig. 4); ($r_{II/I} = 1.085$ at 80°C). Isovaline, α-methylnorvaline, α-methylnorleucine and α-methylleucine gave only one peak. It seems that for this class of compounds the polymeric diamides have an advantage, as isovaline is reported to have been resolved on Chirasil-val[9].

2-Amino-1-alcohols. It has been already reported that aliphatic 2-aminoalkan-1-ols in the form of their N-TFA-O-acyl esters can be readily resolved[13]. The same is true for stationary phase 3, as can be seen in Table II and Fig. 5. The order of

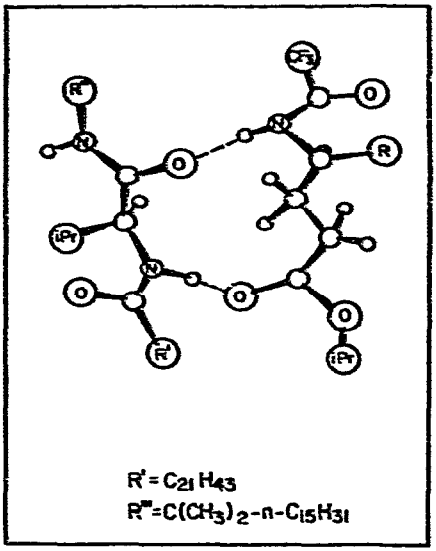


Fig. 3. Scheme of the hydrogen-bonded association for γ-amino acid derivatives with "non-parallel" orientation of the alkyl substituents at the asymmetric carbons for the less strongly retained L-enantiomer.

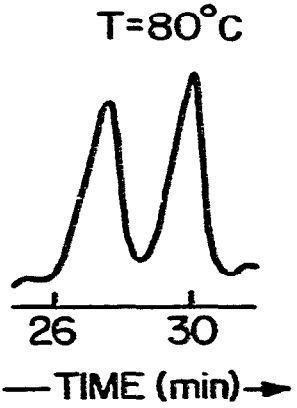


Fig. 4. Resolution of N-TFA-α-methylvaline isopropyl ester.

emergence is the reverse of that for the α-amino acids, *i.e.*, the D-enantiomers are more strongly retained on the L-phase. This behaviour, which is similar to that of the above γ-amino acid derivatives, has been discussed elsewhere[13].

Amines. N-Acyl derivatives of amines, which lack the additional carbonyl group present in amino acids and amino alcohols, are resolved by a different mechanism than the latter. It has been established that their resolution is, in general, best achieved with stationary phases such as carbonylbis-(N-L-valine isopropyl ester)[16] and N-lauroyl-S-α-(1-naphthyl)ethylamine[17]. In agreement with this, the

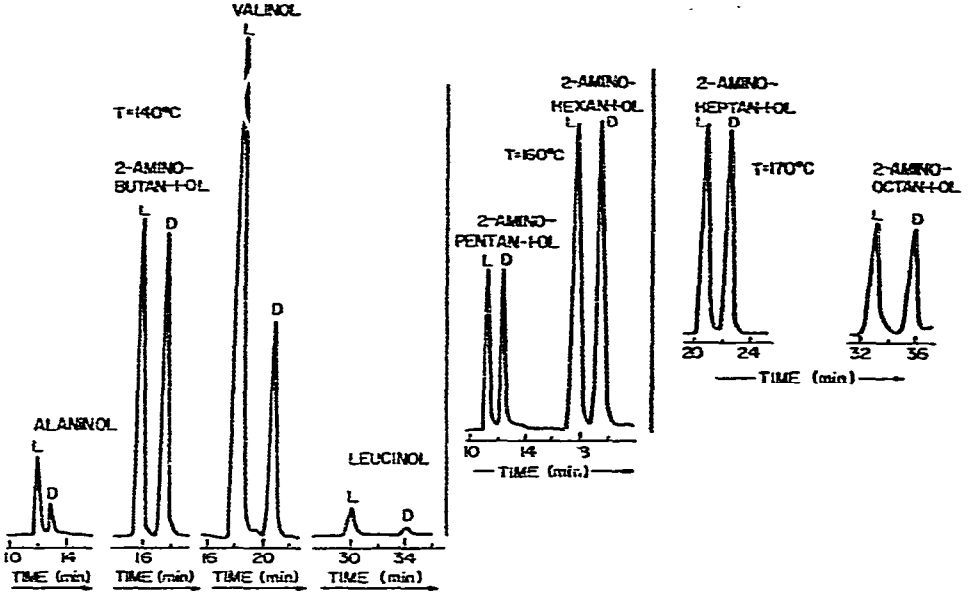


Fig. 5. Chromatogram of N-TFA-2-amino-alkane-1-ol pivaloyl esters.

separation of enantiomeric N-TFA aliphatic amines (Table III) on 3 is inefficient. The aromatic amines, on the other hand, although having far smaller r values than on N-lauroyl-S-α-(1-naphthyl)ethylamine, show good peak resolution (Table III, Fig. 6). It is also of interest that 3-aminocyclohexene could be relatively well resolved on 3 at 65°C, whereas conditions for the resolution of seven- and eight-membered homologues could not be found. On stationary phases showing typical amine stereoselectivity[16,17], the cyclohexene derivative is the one most difficult to separate.

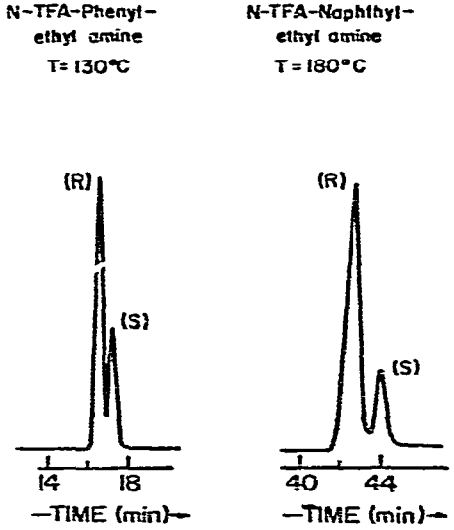


Fig. 6. Chromatogram of aromatic N-TFA amines.

CONCLUSIONS

Chain lengthening of the R substituents of the diamides R'CONHCH(R")-CONHR"', as in N-docosanoyl-L-valine 2-(2(methyl)-*n*-heptadecylamide (3) led to a stationary phase with considerable thermal stability and high stereoselectivity for a wide range of compounds. In particular, N-TFA-amino acid esters are resolved with relatively large resolution factors. The results indicate that further pursuit of systematic studies on the influence of the size and nature of the substituents should lead to optimization of analytical procedures and reveal many more significant details of the properties of these phases and the mechanism of their stereoselective action.

The conditions employed for the synthesis of 3 led to a product of high optical purity.

ACKNOWLEDGEMENT

The authors are indebted to the Stiftung Volkswagenwerk for support of the research.

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