Journal of Chromatography, 298 (1984) 253–261
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,797

GAS CHROMATOGRAPHIC RESOLUTION OF OPTICAL ISOMERS ON TWO NEW DIAMIDE STATIONARY PHASES, N-LAUROYL-L-VALINE *tert.*-OCTYLAMIDE AND N-DOCOSANOYL-L-LEUCINE *tert.*-OCTYLAMIDE

ROSITA CHARLES* and KATSUNORI WATABE
Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot (Israel)
(Received April 3rd, 1984)

SUMMARY

Two novel diamide chiral stationary phases are described: N-lauroyl-L-valine *tert.*-octylamide (I) and N-docosanoyl-L-leucine *tert.*-octylamide (II). Their chromatographic behaviour in stainless-steel capillary columns and on whisker-walled glass capillary columns is reported. The phases have been employed at a maximum column temperature of 180°C for I and 220°C for II, showing good stereoselectivity for N-trifluoroacetyl (N-TFA) esters of α- and γ-amino acids, a N-TFA-dipeptide ester and N-TFA-O-pivaloyl aminoalcohols.

INTRODUCTION

Studies carried out in our laboratory on diamide phases of the type R'CONHCH(R'')CONHR''', their chromatographic behaviour and the influence of modification of R', R'' and R''' have already been reported[1–4]. We have found that by increasing the length of R' from $C_{11}H_{23}$ to $C_{21}H_{43}$ while R'' and R''' are unchanged, the thermal stability of the phase is increased by 50°C[2]. Also, increasing R'' and R''' permits operation at higher temperatures. In the present paper we report two new stationary phases I and II, where R''' = *tert.*-octyl, *i.e.*, $(CH_3)_3CCH_2C(CH_3)_2$–.

Stainless-steel and whisker-walled glass capillary columns were used to study the stereoselectivity of these phases and their comparative behaviour will be discussed in detail.

EXPERIMENTAL

Stationary phases

The synthetic method used has already been reported[4].

N-Lauroyl-L-valine *tert.*-octylamide (I) was obtained in 55% yield, m.p. 71–73°C, optical purity (o.p.) 92%; the NMR spectrum was as expected. N-Docosanoyl-L-leucine *tert.*-octylamide (II) was obtained in 50% yield, m.p. 38–40.5°C, o.p. 99.8%; the NMR spectrum was in agreement with the chemical structure.

0021-9673/84/$03.00

Chromatographic conditions

Stainless-steel capillary columns (50 m × 0.5 mm I.D.) were coated by the plug method with 5% solutions of the above stationary phases in chloroform. Each column was mounted in a Varian Series 2700 gas chromatograph, provided with a stream splitter and a flame ionization detector. The temperatures were: injector, 240°C; detector, 240°C. The column temperatures used are indicated in the tables. The carrier gas (helium) flow-rate was 3 ml/min, and the splitting ratio was 1:10.

Whisker-walled glass capillary columns. Two whisker-walled glass capillaries (9 m × 0.35 mm I.D.) were prepared by a slight modification[5] of the method described by Schieke *et al.*[6]. They were washed with chloroform and dichloromethane, and then dried with nitrogen gas. Each capillary was coated by use of a 10% dichloromethane solution of the diamide phase. Before coating phase II, the inner surface of the capillary was deactivated with benzyltriphosphonium chloride at 350°C for 3 h. The columns (9 m × 0.35 mm I.D.) coated with phases I and II were preconditioned for 1 day at 170°C and at 200°C, respectively, before sample injection.

A Varian Aerograph Series 1200 gas chromatograph, equipped with a flame ionization detector, was modified for connection to the glass capillary columns. The gas chromatographic (GC) conditions were as follows: injection temperature, 200°C; detector temperature, 230°C; carrier gas (helium) flow-rate, 0.5–1.5 ml/min; splitting ratio, 1:25.

RESULTS AND DISCUSSION

We studied the resolution of N-trifluoroacetyl (TFA)-isopropyl esters of α- and γ-amino acids, N-TFA-O-acyl-2-aminoalkan-1-ols and the N-TFA-isopropyl ester of a dipeptide.

Amino acid derivatives

Data for protein and non-protein α-amino acids and γ-amino acids are listed in Table I. For the α-amino acids the order of elution throughout is the L- after the D-isomer on both L-phases; this order is reversed for the γ-amino acids. Proline is not resolved on phase II, but shows a shoulder on I. Threonine overlaps with valine on I and L-threonine overlaps with D-valine on II. L-*allo*-Isoleucine overlaps with D-isoleucine on both phases. For the less volatile amino acids, *e.g.*, Met, Glu, Phe, Orn and Lys, no overlap is observed, as can be seen in Figs. 1 and 2.

In Table II are listed data obtained on phase I, at the same temperature (115°C) in both stainless-steel (50 m) and glass (9 m) capillary columns. The resolution factors measured on both columns were essentially the same.

The γ-amino acid derivatives are resolved with a reversed elution order, as is usually found on this type of phases[7]. β-Amino acid derivatives are not resolved.

2-Aminoalkan-1-ols

The N-TFA-O-pivaloyl derivatives of 2-aminoalkan-1-ols are well resolved on both phases, as can be seen from Table III and Fig. 3. Their elution order is reversed as compared with the N-TFA-α-amino acid esters. As discussed before[7], this behaviour can be ascribed to an interaction with the chiral solvent similar to that of the γ-amino acid derivatives.

TABLE I

RESOLUTION OF N-TFA-ISOPROPYL ESTERS OF α- AND γ-AMINO ACIDS ON DIAMIDE STATIONARY PHASES I AND II

See text for chromatographic conditions. r = Corrected retention time (min). $r_{L/D}$ = Resolution coefficient = ratio of the corrected retention volumes of the separated enantiomers. Subscripts, *e.g.*, $r_{L/D}$ or $r_{D/L}$ designate the configuration or the elution order of the enantiomers for which the ratio was determined.

N-TFA-isopropyl ester of	*Enantiomer*	*N-Lauroyl-L-valine tert.-octylamide (I)*						*N-Docosanoyl-L-leucine tert.-octylamide (II)*					
		Stainless-steel capillary column			*Whisker-walled glass capillary column*			*Stainless-steel capillary column*			*Whisker-walled glass capillary column*		
		r	$r_{L/D}$	T(°C)	r	$r_{L/D}$	T(°C)	r	$r_{L/D}$	T(°C)	r	$r_{L/D}$	T(°C)
Protein α-amino acids													
Ala	D	5.50	1.127	130	6.96	1.147	115	4.30	1.186	120	3.18	1.141	130
	L	6.20			7.98			5.10			3.63		
Thr	D	8.70	1.092	130	11.34	1.111	115	4.84	1.115	120	4.20	1.097	130
	L	9.50			12.60			5.40			4.52		
Val	D	8.68	1.080	130	11.43	1.092	115	5.40	1.118	120	5.19	1.099	130
	L	9.38			12.48			6.04			5.70		
Gly	—	10.80	—	130	14.22	—	115	7.30	—	120	5.97	—	130
allo-Ile	D	12.50	1.104	130	16.59	1.114	115	8.20	1.110	120	7.68	1.102	130
	L	13.80			18.48			9.10			8.46		
Ile	D	13.80	1.090	130	18.48	1.107	115	9.10	1.109	120	8.46	1.085	130
	L	15.04			20.46			10.10			9.18		
Ser	D	18.28	1.090	130	23.94	1.098	115	9.50	1.084	120	8.78	1.061	130
	L	19.92			26.31			10.30			9.31		
Leu	D	18.04	1.186	130	25.68	1.231	115	11.30	1.212	120	10.32	1.174	130
	L	21.40			31.62			13.70			12.12		
Pro	D	19.30	1.034	130	26.13	1.025	115	14.00	1.000	120	13.29	1.000	130
	L	20.00	(shoulder)		26.73			14.00			13.29		
Asp	D	55.70	1.030	130	84.15	1.032	115	36.84	1.038	120	46.14	1.044	130
	L	57.36			86.85			38.24			48.18		
Met	D	21.18	1.088	170	31.26	1.100	150	11.60	1.086	170	22.32	1.083	160
	L	23.06			34.38			12.60			24.18		
Glu	D	28.08	1.072	170	44.28	1.078	150	16.06	1.068	170	30.00	1.074	160
	L	30.10			47.73			17.16			32.22		

(Continued on p. 256)

TABLE I (*continued*)

N-TFA-isopropyl ester of	*Enantiomer*	*N-Lauroyl-L-valine tert.-octylamide (I)*						*N-Docosanoyl-L-leucine tert.-octylamide (II)*					
		Stainless-steel capillary column			*Whisker-walled glass capillary column*			*Stainless-steel capillary column*			*Whisker-walled glass capillary column*		
		r	$r_{L/D}$	*T*(°C)	*r*	$r_{L/D}$	*T*(°C)	*r*	$r_{L/D}$	*T*(°C)	*r*	$r_{L/D}$	*T*(°C)
Phe	D	29.80	1.067	170	44.70	1.083	150	18.24	1.058	170	32.88	1.070	160
	L	31.80			48.37			19.30			35.19		
Tyr	D	–	–		–	–		24.20	1.074	170	57.00	1.096	160
	L							26.00			62.46		
Orn	D	104.80	1.088	180	–	–		22.44	1.061	200	32.49	1.042	195
	L	114.80						23.80			33.87		
Lys	D	150.00	1.076	180	–	–		31.26	1.045	200	47.55	1.026	195
	L	161.50						32.68			48.81		
Non-protein α-amino acids													
α-Amino butanoic acid	D	7.90	1.130	130	9.90	1.140	115	5.02	1.143	120	4.68	1.122	130
	L	8.74			11.28			5.74			5.25		
α-Amino-pentanoic acid	D	12.70	1.134	130	17.01	1.168	115	8.10	1.185	120	7.62	1.150	130
	L	14.40			1986			9.60			8.76		
α-Amino-hexanoic acid	D	21.28	1.142	130	28.56	1.180	115	13.10	1.198	120	12.45	1.147	130
	L	24.30			33.69			15.70			14.28		
α-Amino-heptanoic acid	D	27.10	1.122	140	51.36	1.175	115	15.00	1.153	130	21.69	1.147	130
	L	30.40			60.36			17.30			24.87		
α-Amino-octanoic acid	D	45.90	1.118	140	93.24	1.177	115	25.90	1.153	130	37.80	1.149	130
	L	51.32			109.71			29.86			43.44		
tert.-Leucine**	D	22.00	1.038	100	–	–		9.30	1.082	100	–	–	
	L	22.84						10.06					
Phenyl-glycine	D	33.90	1.047	150	23.40	1.038	150	18.26	1.049	150	17.70	1.037	160
	L	35.50			24.30			19.16			18.36		

*γ-Amino acids**													
γ-Amino-pentanoic acid	L	30.64	1.041	130	40.86	1.039	115	12.60	1.024 (shoulder)	130	18.00	1.030	130
	D	31.90			42.45			12.90			18.54		
γ-Amino-δ-methyl-hexanoic acid	L	62.86	1.053	130	28.02	1.064	142	24.30	1.074	130	36.45	1.082	130
	D	66.20			29.82			26.10			39.45		
γ-Amino-ε-methyl-heptanoic acid	L	108.50	1.068	130	46.80	1.060	142	41.40	1.075	130	61.83	1.071	130
	D	115.88			49.59			44.50			66.24		

* $r_{D/L}$ refers to γ-amino acids where the elution order is reversed.
** *tert.*-Leucine = α-amino-β,β-dimethylbutanoic acid.

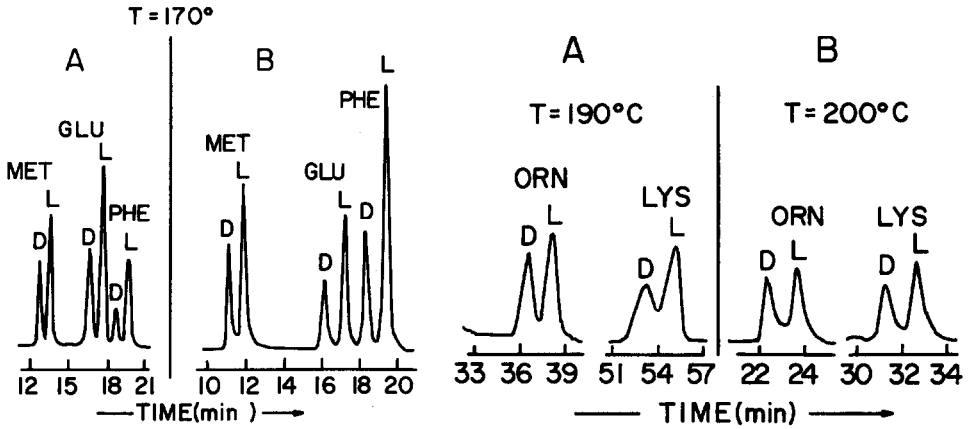

Fig. 1. Chromatograms of N-TFA-isopropyl esters of methionine, glutamic acid and phenylalanine on phase II. A, Whisker-walled glass capillary column; B, stainless-steel capillary column.

Fig. 2. Chromatograms of N-TFA-isopropyl esters of ornithine and lysine on phase II. A and B as in Fig. 1.

TABLE II

RESOLUTION OF N-TFA-ISOPROPYL ESTERS OF SOME α-AMINO ACIDS ON N-LAUROYL-L-VALINE *tert.*-OCTYLAMIDE AT 115°C

See Table I for definition of r and $r_{L/D}$, and text for chromatographic conditions.

N-TFA-isopropyl ester	*Enantiomer*	*Stainless-steel capillary column*		*Whisker-walled glass capillary column*	
		r	$r_{L/D}$	r	$r_{L/D}$
Ala	D	8.70	1.161	6.96	1.147
	L	10.10		7.98	
Val	D	14.40	1.097	11.43	1.092
	L	15.80		12.48	
allo-Ile	D	21.20	1.113	16.59	1.114
	L	23.60		18.48	
Ile	D	23.60	1.113	18.48	1.107
	L	26.26		20.46	
Leu	D	32.40	1.240	25.68	1.231
	L	40.20		31.62	
Pro	D	31.00	1.045	26.13	1.025
	L	32.40	(shoulder)	26.73	

N-TFA-isopropyl ester of a dipeptide

As phase II can be used up to 220°C, we tried to resolve on it a dipeptide, namely the N-trifluoroacetyl-(±)alanylglycine isopropyl ester. The elution order is reversed as can be seen in Fig. 4 and the resolution factor, $r_{D/L}$, was 1.045 at 200°C and 1.051 at 190°C. Other chromatographic results for derivatives of dipeptides and the mechanism of resolution will be published elsewhere.

TABLE III

RESOLUTION OF N-TFA-O-PIVALOYL DERIVATIVES OF 2-AMINOALKAN-1-OLS ON DIAMIDE STATIONARY PHASES I AND II

See text for chromatographic conditions and Table I for definitions of r and $r_{D/L}$.

Aminoalkanol	*Enantiomer*	*N-Lauroyl-L-valine tert.-octylamide*						*N-Docosanoyl-L-tert.-octylamide*					
		Stainless-steel capillary column			*Whisker-walled glass capillary column*			*Stainless-steel capillary column*			*Whisker-walled glass capillary column*		
		r	$r_{D/L}$	T(°C)	r	$r_{D/L}$	T(°C)	r	$r_{D/L}$	T(°C)	r	$r_{D/L}$	T(°C)
2-Amino-propan-1-ol	L	18.20	1.080	140	12.60	1.093	142	8.00	1.075	130	13.35	1.085	130
	D	19.66			13.77			8.60			14.49		
2-Amino-butan-1-ol	L	26.10	1.145	140	18.45	1.137	142	12.10	1.129	130	20.46	1.119	130
	D	29.90			20.97			13.66			22.89		
2-Amino-pentan-1-ol	L	39.60	1.159	140	28.41	1.148	142	19.00	1.131	130	32.25	1.138	130
	D	45.92			32.61			21.50			36.69		
2-Amino-hexan-1-ol	L	31.80	1.119	160	45.48	1.152	142	15.40	1.097	150	53.37	1.134	130
	D	35.60			52.38			16.90			60.51		
2-Amino-heptan-1-ol	L	50.44	1.124	160	75.09	1.151	142	25.20	1.109	150	91.20	1.134	130
	D	56.70			86.43			27.96			103.38		
2-Amino-octan-1-ol	L	80.60	1.124	160	125.82	1.153	142	42.10	1.109	150	159.39	1.138	130
	D	90.60			145.05			46.70			181.44		
2-Amino-3-methylbutan-1-ol	L	30.00	1.170	140	20.46	1.173	142	13.36	1.157	130	22.67	1.118	130
	D	35.10			24.00			15.46			25.36		
2-Amino-4-methylpentan-1-ol	L	49.10	1.165	140	34.35	1.150	142	23.60	1.127	130	39.22	1.107	130
	D	57.20			39.51			26.60			43.41		

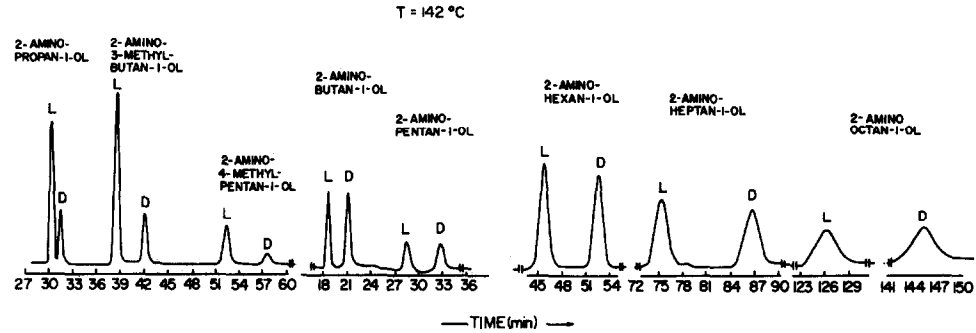

Fig. 3. Chromatogram of N-TFA-O-pivaloyl of 2-aminoalkan-1-ols on a whisker-walled glass capillary column coated with phase I. Chromatographic conditions as in the text.

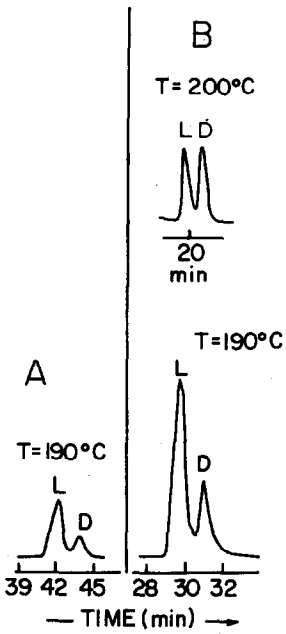

Fig. 4. Chromatogram of N-TFA-alanylglycine isopropyl ester on phase II: upper, racemate; lower, L/D enriched dipeptide. A and B as in Fig. 1.

TABLE IV

INFLUENCE ON THE THERMAL STABILITY OF CHANGING R''' FROM *tert.*-BUTYL TO *tert.*-OCTYL

Phase	*Maximum operating temperature (°C)*
N-Lauroyl-L-valine *tert.*-butylamide	140
N-Lauroyl-L-valine *tert.*-octylamide	180
N-Docosanoyl-L-valine *tert.*-butylamide	190
N-Docosanoyl-L-leucine *tert.*-octylamide	220

Thermal stability

By changing R''' from *tert.*-butyl to *tert.*-octyl, the thermal stability of the diamide phases was increased as expected. Comparative results are listed in Table IV.

ACKNOWLEDGEMENT

We are indebted to Professor E. Gil-Av for support and fruitful discussions.

REFERENCES

1 B. Feibush, *Chem. Commun.*, (1971) 554.
2 R. Charles, U. Beitler, A. Feibush and E. Gil-Av, *J. Chromatogr.*, 112 (1975) 121.
3 U. Beitler and B. Feibush, *J. Chromatogr.*, 123 (1976) 149.
4 S. C. Chang, R. Charles and E. Gil-Av, *J. Chromatogr.*, 235 (1982) 87.
5 K. Watabe, *Ph.D. Thesis*, Tokyo Metropolitan University, Tokyo, 1983.
6 J. D. Schieke, N. R. Comins and V. Pretorius, *J. Chromatogr.*, 112 (1975) 97.
7 B. Feibush, A. Balan, B. Altman and E. Gil-Av, *J. Chem. Soc., Perkin Trans. II*, (1979) 1230.