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CHIRAL PHASES DERIVED FROM XE-60 FOR GLASS CAPILLARY GAS CHROMATOGRAPHY OF AMINO ACID ENANTIOMERS

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SUMMARY

The silicone GE XE-60 has been modified by hydrolysis and coupling with L-valine-*tert.*-butylamide to introduce a chiral property and by subsequent chain-opening polymerization with octamethylcyclotetrasiloxane or decamethylcyclopentasiloxane to obtain thermal stability and resolution efficiency. The resulting chiral phases can be used for the separation of amino acid enantiomeric mixtures by glass capillary gas chromatography at operating temperatures up to 200°C. The thermal stabilities, resolution factors and peak-to-peak separations of amino acids obtained with the different phases are compared.

INTRODUCTION

The analysis of amino acid enantiomer is important in the determination of any D-amino acid in synthetic or natural polypeptides, the distribution of D-amino acids in nature[1] and in the dating of geological materials from biological origin[2]. The outstanding method of analysis employed is glass capillary gas chromatography.

Since the direct resolution of amino acid enantiomers on chiral phases was first introduced by Gil-Av *et al.*[3] a large volume of work has been reported[4–6]. Chiral polysiloxane phases are thought to be very important for rapid analysis[7]. Bayer and co-workers[8] first introduced this type of phase, having the low volatility and high thermal stability Chirasil-Val, by coupling the L-valine-*tert.*-butylamide moiety to a copolymer of dimethylsiloxane and carboxyalkylmethylsiloxane units. The principal advantages of these phases depend on the low polarity and high thermal stability which greatly reduce solute retention time. Chiral phases of this type can also prepared from commercially available OV-225, Silar-10C, XE-60, etc., with nitrile groups in the side chain of the polysiloxane, after suitable modification as first proposed by Verzele and co-workers[9].

We have investigated two novel chiral phases derived from XE-60 and L-valine-*tert.*-butylamide by chain-opening polymerization with octamethylcyclotetrasiloxane or decamethylcyclopentasiloxane, respectively. They were found to be more efficient in resolving amino acid enantiomeric mixtures and more thermally stable than modified OV-225[9] (190°C), Silar-10C[10] (160°C) or XE-60[11] (180°C) reported elsewhere.

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EXPERIMENTAL

Synthesis of the chiral phases

L-Valine-tert.-butylamide trifluoroacetate. *tert.*-Butoxycarbonyl (BOC)-L-valine (5 g) and 1-hydroxybenzotriazole (3.1 g) were dissolved in 120 ml of dry tetrahydrofuran and cooled in an ice–water bath. N,N′-dicyclohexylcarbodiimide (5.2 g) and *tert.*-butylamine (1.7 g) were added successively to this stirred solution which was kept at 0–2°C for 1 h and then at room temperature for about 20 h. After filtration of the precipitates and evaporation of the solvent, the residue was dissolved in ethyl acetate. This solution was washed successively with 2 *N* citric acid, water, 5% $NaHCO_3$ and water and then dried over $MgSO_4$. After filtration and evaporation of the solvent, trifluoroacetic acid (30 g) was added and left at room temperature for 1 h to split off the BOC group. After evaporation of the trifluoroacetic acid, a brown oil remained which was crystallized from diethyl ether. Yield: 45%. M.p.: 195–197°C.

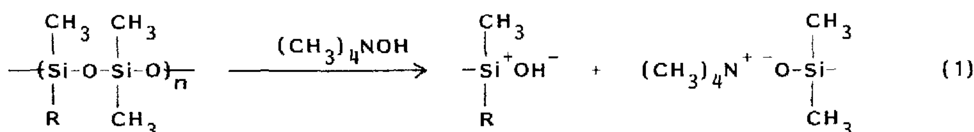
2-(Chlorocarbonyl)-1-ethylmethyldimethylsiloxane. XE-60 (1 g) was dissolved in 25 ml of dioxane and 25 ml of conc. hydrochloric acid were added. The solution was refluxed until the nitrile groups were almost completely hydrolyzed into carboxyl groups, as confirmed by the disappearance of the nitrile infrared spectrum. After evaporation of the solvent, the residue was dissolved in chloroform, filtered and the solvent evaporated. The acid thus obtained was dissolved in 5 ml of dry benzene and refluxed with 4 g of oxalyl chloride for 4 h. The excess of reagents was carefully evaporated.

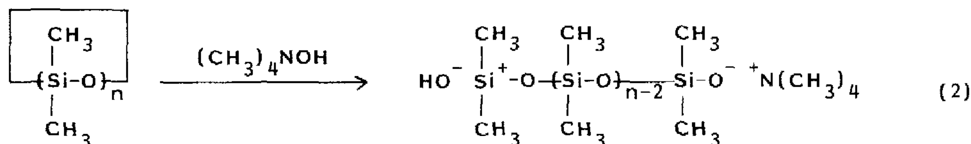
XE-60–L-valine-tert.-butylamide. The acid chloride of hydrolyzed XE-60 was dissolved in 5 ml of dry methylene chloride and cooled in an ice-bath. An equivalent amount of L-valine-*tert.*-butylamide trifluoroacetate and two equivalents of triethylamine in methylene chloride were added dropwise. After stirring overnight, the solvent was evaporated to dryness and the residue dissolved in diethyl ether and filtered. The filtrate was washed with 0.5 *M* HCl, twice with water and dried over $MgSO_4$. Further purification was not performed.

XE-60–L-valine-tert.-butylamide–D_4(D_5). XE-60-L-valine-*tert.*-butylamide (100 mg) and an equivalent amount of octamethylcyclotetrasiloxane (D_4) or decamethylcyclopentasiloxane (D_5) were dissolved in about 0.5 ml of *n*-butanol. A gentle stream of nitrogen was passed through this solution, and tetramethylammonium hydroxide (about 0.1% of the total volume of the solution) was added. The solution was heated slowly to 80°C, allowed to stand for 30 min and then heated to 130°C with continuous stirring. Evaporation of the solvent yielded a slightly yellow oily product which was dissolved in about 30 ml of toluene and then washed with an equivalent amount of 0.1 *M* hydrochloric acid and twice with water for complete removal of tetramethylammonium hydroxide.

Mechanism of the base-catalyzed chain-opening polymerization. The reaction of D_4 or D_5 with XE-60-L-valine-*tert.*-butylamide in the presence of tetramethylammonium hydroxide as a catalyst results in chain-opening polymerization[12]. The reaction mechanism may be written as shown in Fig. 1, and results in an increase in the number of dimethylsiloxane units between optically active groups and in the average molecular weight.

In the first step (1), XE-60-L-valine-*tert.*-butylamide is attacked by tetramethylammonium hydroxide with subsequent formation of the active intermediate. Reac-





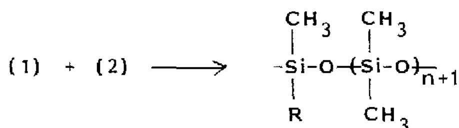


Fig. 1. Mechanism of the chain opening polymerization. R = N-Propionyl-L-valine-*tert.*-butylamide.

tion 2 represents the chain-opening of D_4 or D_5 to give the same type of intermediate is formed more readily in reaction 1 than in 2 because of the more powerful electron-inductive effect of propionic acid. Tetramethylammonium hydroxide was selected as basic catalyst for polymerization because it is convenient to remove. The catalyst is active in the siloxane solution at 80°C, but when heated to 130°C decomposes into trimethylamine and methanol which are volatile and easily removed by evaporation. For complete removal of the catalyst, a washing with extremely dilute hydrochloric acid is necessary, as described earlier.

Column preparation

Surface pretreatment. Pyrex glass tubing was drawn into a capillary of 0.25 mm I.D. with a Shimadzu glass-drawing machine, Type GDM-1B (Shimadzu, Kyoto, Japan). The capillaries (20 m × 0.25 mm) were first rinsed with methylene chloride and dried with a stream of nitrogen. They were filled to one quarter of their length with a plug of 0.5% HF, and then rinsed with distilled water. Care must be taken to ensure that the contact time of the HF solution with the glass surface is 3–5 min. The capillary was then filled, to about 90% of its length, with 6 *M* hydrochloric acid, sealed at both ends and leached for 6 h at 180°C. The leaching acid was flushed with water, acetone and then dried with a stream of nitrogen. A plug of 0.3% PEG-20M in methylene chloride was passed through the capillary, which was then dried with a stream of nitrogen, sealed at both ends and heated at 280°C for 16 h. This procedure was repeated. After rinsing with 5 ml of methylene chloride, the capillary was dried with a stream of nitrogen.

Coating of the liquid phases. The pretreated glass capillary was coated statically with 0.3% methylene chloride–*n*-pentanol (1:1) solution. Finally, the capillary columns were conditioned at 200°C for at least 48 h.

Sample preparation

All amino acids were derivatized to their N(O)-trifluoroacetyl (TFA) isopropyl esters as described elsewhere[5].

RESULTS AND DISCUSSION

Table I shows the retention data and resolution factors of seventeen N(O)-TFA isopropyl esters of D,L-amino acids. The resolution factors and orders of emer-

TABLE I

RETENTION DATA OF N-TFA ISOPROPYL ESTERS OF PROTEIN AMINO ACIDS

Columns: I, XE-60–L-valine-*tert.*-butylamide; II, XE-60–L-valine-*tert.*-butylamide–D_4; III, XE-60–L-valine-*tert.*-butylamide–D_5. t_R = Retention time; $r_{L/D}$ = resolution factor (corrected).

Amino acid		*Column temp. (°C)*	*Column I*		*Column II*		*Column III*	
			t_R *(min)*	$r_{L/D}$	t_R *(min)*	$r_{L/D}$	t_R *(min)*	$r_{L/D}$
Alanine	D	90	3.94	1.202	5.01	1.239	6.75	1.187
	L		4.49		5.93		7.74	
Valine	D		4.63	1.173	6.89	1.185	8.52	1.151
	L		5.22		7.95		9.59	
Threonine	D		5.89	1.203	9.29	1.187		
	L		6.84		10.81			
allo-Isoleucine	D		6.20	1.212	9.90	1.224	11.97	1.187
	L		7.26		11.86		13.94	
Isoleucine	D		6.84	1.178	11.00	1.184	13.26	1.161
	L		7.84		12.81		15.16	
Glycine			8.82	—	10.50	—	15.16	—
Leucine	D		10.14	1.307	15.72	1.363	19.94	1.281
	L		12.88		21.00		25.14	
Proline	D		11.96	1.021	16.96	1.022	21.18	1.020
	L		12.18		17.30		21.58	
Serine	D		13.58	1.141	19.48	1.159		
	L		15.32		22.40			
Aspartic acid	D	120	8.04	1.061	11.35	1.059	14.36	1.054
	L		8.46		11.96		15.06	
Methionine	D		14.27	1.168	18.75	1.180	26.00	1.153
	L		16.48		21.93		29.76	
Phenylalanine	D		18.48	1.155	25.49	1.158	34.44	1.137
	L		21.16		29.35		38.96	
Glutamic acid	D		19.90	1.148	27.95	1.156	36.64	1.134
	L		22.68		32.15		41.36	
Tyrosine	D	170	4.79	1.078	6.48	1.073		
	L		5.08		6.88			
Ornithine	D		13.72	1.087	13.84	1.093	22.06	1.080
	L		14.82		15.03		23.72	
Lysine	D		19.28	1.082	19.69	1.081	31.12	1.074
	L		20.78		21.21		33.32	
Tryptophan	D		18.82	1.073	25.13	1.067	33.92	1.060
	L		20.12		26.75		35.88	

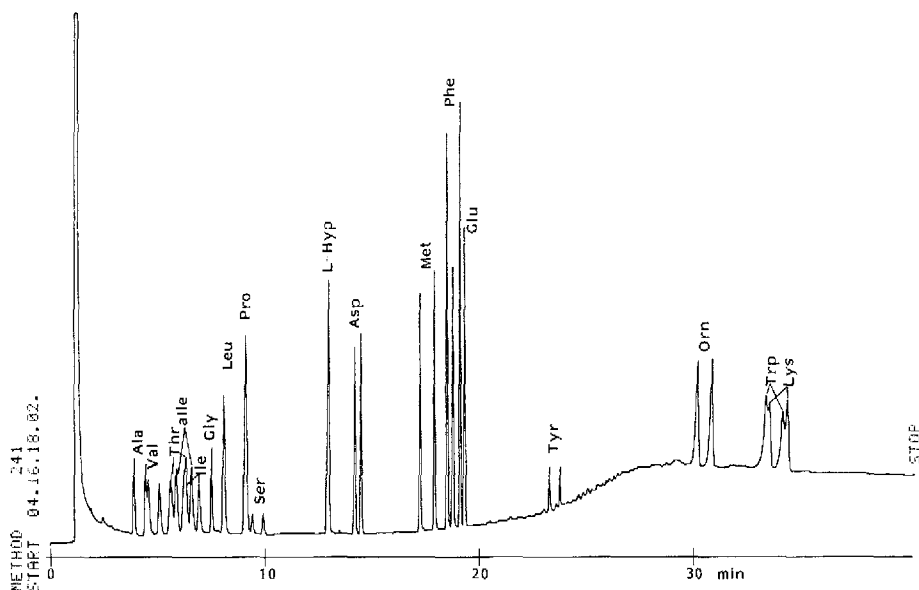


Fig. 2. Chromatogram of the N-TFA isopropyl esters of an amino acid enantiomeric mixture. Column: XE-60–L-valine-*tert.*-butylamide; 20 m × 0.25 mm I.D. glass capillary. Column temperature: 90°C, 4 min hold and then programmed at 4°C/min to 180°C. Injection temperature: 250°C. Carrier gas: helium. Splitting ratio: 1:40. For each pair of enantiomers the D-enantiomer emerges first.

gence are somewhat different from chemical properties of the phases. For example, glycine, of only one amino acid without α-alkyl side-chain, is said to have polar nature and to be effective for the formation of transient hydrogen bond with the phase, therefore, the elution order of glycine is more sensitive to the chemical properties of the phases and also to the inner surface of the glass capillary. The reverse is given here for proline lacked –NH group in the N-TFA part.

Column I (XE-60–L-valine-tert.-butylamide)

Column I, coated with XE-60–L-valine-*tert.*-butylamide which forms an alternate arrangement of methyl N-propionyl-L-valine-*tert.*-butylamide siloxane and dimethylsiloxane, resulted in considerable peak overlapping of relatively volatile amino acids, but gave complete resolution of less volatile amino acids.

As shown in Fig. 2, aspartic acid, methionine, phenylalanine, glutamic acid, tyrosine and ortnithine can be well resolved. However peak overlap is observed for L-alanine and D-valine, L-threonine and D-isoleucine, L-leucine and DL-proline, D-lysine and D-tryptohan and also L-lysine and L-tryptophan.

It may be concluded that column I is useful for resolving relatively less volatile amino acid, but a little bleeding was observed above 170°C.

Column II (XE-60–L-valine-tert.-butylamide- D_4)

Column II is coated with a phase modified by chain-opening polymerization of XE-60—L-valine-*tert.*-butylamide with octamethylcyclotetrasiloxane, resulting in N-propionyl-L-valine-*tert.*-butylamide units separated by less than five dimethylsiloxane units from each other.

As shown in Fig. 3, almost all amino acids can be resolved and an extremely

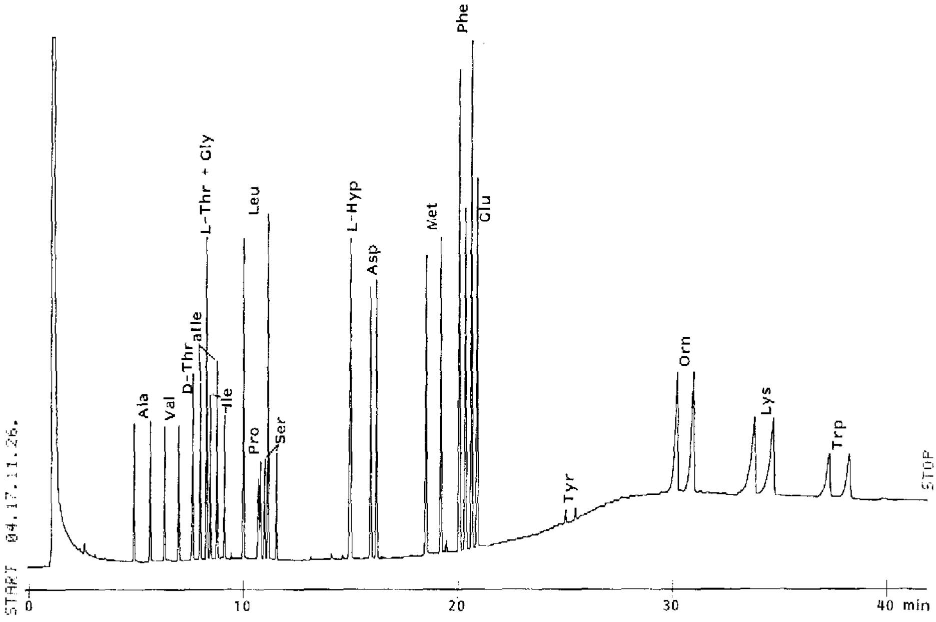


Fig. 3. Chromatogram of the N-TFA isopropyl esters of an amino acid enantiomeric mixture. Column: XE-60 -L-valine-*tert.*-butylamide-D_4. Conditions as in Fig. 2.

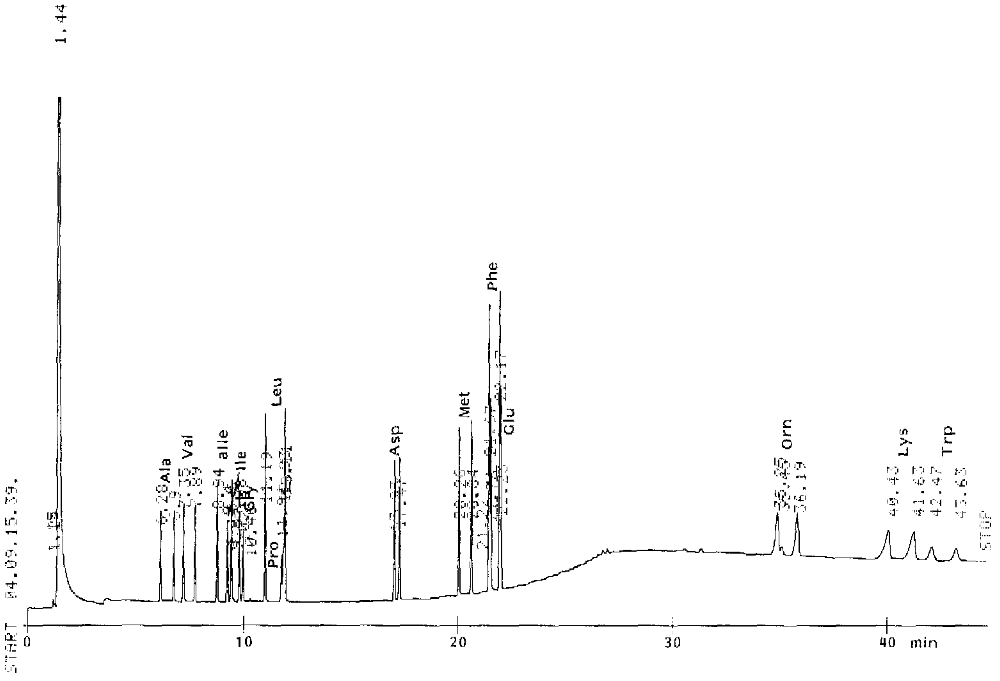


Fig. 4. Chromatogram of the N-TFA isopropyl esters of an amino acid enantiomeric mixture. Column: XE-60–L-valine-*tert.*-butylamide–D_5. Conditions as in Fig. 2.

large resolution factor was obtained for leucine enantiomers, see Table I. Also, threonine, *allo*-isoleucine, isoleucine, glycine, leucine, proline and serine are well resolved. Efficient resolution was obtained also of relatively less volatile amino acids. Bleeding is not high within the temperature limits of 90–180°C, and the baseline is stable.

Column III (XE-60–L-valine-tert.-butylamide-D_5)

The molecular structure of this phase closely resembles that of column II but has one extra dimethylsiloxane unit between the N-propionyl-L-valine-*tert.*-butylamide units. Table I shows that the remarkably decreased resolution factors for column III compared with I or II.

Fig. 4 shows that L-leucine and L-proline, D-phenylalanine and D-glutamic acid, L-phenylalanine and L-glutamic acid are overlapped or incompletely resolved. The other amino acids, alanine, valine, *allo*-isoleucine, isoleucine, glycine, aspartic acid, methionine, ornithine and tryptophan, are well resolved.

Of the three types of chirally modified XE-60 phases prepared here, column III is the most stable and can be operated up to a temperature of 220°C.

Fig. 5 shows a representative chromatogram of N-pentafluoropropionyl (PFP) *n*-propyl ester derivatives of amino acids using XE-60–L-valine-*tert.*- butylamide-D_5 as stationary phase coated on a 28 m × 0.3 mm glass capillary as described. All amino acids are resolved into their enantiomers with reasonable separation between enantiomeric pairs. It is suggested that this phase will be applicable to the practical analysis of amino acid enantiomers.

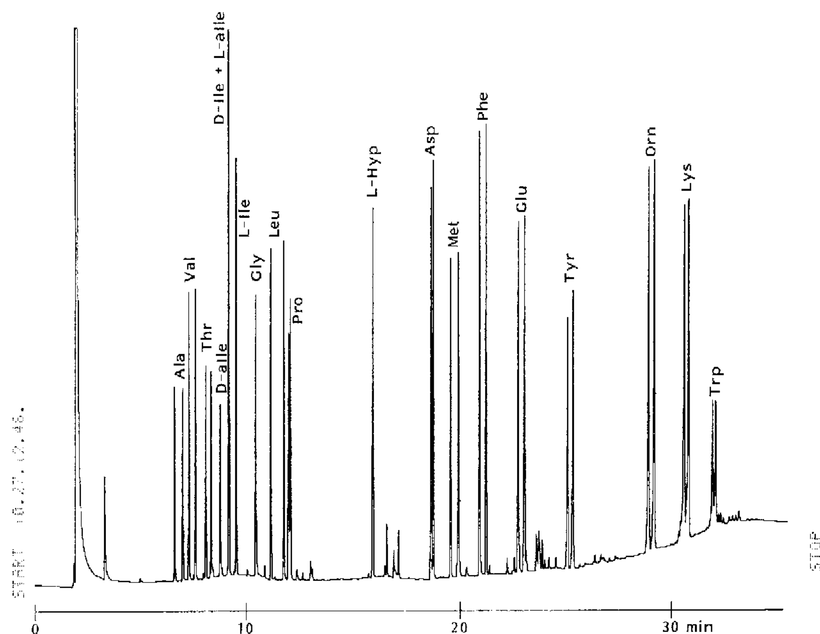


Fig. 5. Chromatogram of the N-PFP *n*-propyl esters of an amino acid enantiomeric mixture. Column: XE-60-L-valine-*tert.*-butylamide-D_5; 28 m × 0.3 mm I.D. glass capillary. Column temperature: 90°C, 4 min hold and then programmed at 4°/min to 200°C. Injection temperature: 200°C. Carrier gas: helium. Splitting ratio: 1:80. For each pair of peaks the D-enantiomer emerges first.

CONCLUSIONS

A chain-opening polymerization of XE-60–L-valine-*tert.*-butylamide with octamethylcyclotetrasiloxane or decamethylcyclopentasiloxane results in an improvement in thermal stability and enantioselectivity for amino acid analysis by gas chromatography.

For instance, leucine exhibits a large resolution factor, and proline and aspartic acid which are generally known difficult to resolve completely can be well resolved by using these phases.

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