Journal of Chromatography, 217 (1981) 71–79
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,938

NEW RESULTS IN THE GAS CHROMATOGRAPHIC SEPARATION OF ENANTIOMERS OF HYDROXY ACIDS AND CARBOHYDRATES

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SUMMARY

New chiral stationary phases for the gas chromatographic separation of the enantiomers of amines, amino alcohols, hydroxy acids and carbohydrates are described. Hydroxy acids can be separated on stationary phases prepared from *S*-2-hydroxyisopentanoic acid and *S*-2-hydroxyoctanoic acid by coupling with *S*-α-phenylethylamine. For the first time the separation of carbohydrate enantiomers could be achieved on a stationary phase obtained by connecting L-valine-*S*-α-phenylethylamide to the functionalized cyanoethyl side-chains of the polysiloxane XE-60.

INTRODUCTION

Chiral recognition in a gas chromatographic system was first utilized for configurational analysis in 1966 by Gil-Av *et al.*[1], who were able to separate amino acid enantiomers on optically active stationary phases in capillary columns. Subsequently this method was modified by the synthesis of new chiral stationary phases by several groups[2–5] and it was possible to extend the range of application to chiral amines, amino alcohols and carboxylic acid amides[6–8]. For the mechanism of molecular interaction hydrogen bonding between a chiral stationary phase and a chiral substrate was believed to be of major importance[8–11]. However, we were able to demonstrate that chiral recognition is not limited to systems in which hydrogen bonding interaction is possible, but that enantiomer separation may occur as a result of diastereomeric dipole–dipole interaction in a general way[12,13]. This was further demonstrated by the recent separation of hydroxy acid enantiomers on several stationary phases also derived from hydroxy acids[14,15]. The separation of isopropyl O-trifluoroacetylmandelate enantiomers on cyclohexyl O-benzyloxycarbonylmandelate (stationary phase) clearly shows that enantioselectivity is not restricted to nitrogen-containing systems[15]. These results encouraged us in our intention to separate other chiral compound classes on a variety of chiral stationary phases, thus leading to further improvements in the separation of hydroxy acids and for the first time to the separation of carbohydrate enantiomers.

As shown by Bayer and co-workers[16,17], the thermal stability of polymeric chiral stationary phases is superior to that of monomeric stationary phases. Polysiloxane with chiral constituents such as L-valine-*tert.*-butylamide show high enan-

0021-9673/81/0000–0000/$02.50

tioselectivity for amino acids and some amino alcohols. Similarly modified polysiloxane stationary phases can be obtained by hydrolysis of the cyanoalkyl sidechains of stationary phases such as OV-225 or Silar-10C, as demonstrated by Verzele and co-workers[18,19]. We have adopted this method for synthesizing optically active polymers. The cyano groups may be functionalized either by hydrolysis to carboxylic groups or by reduction to amino methyl groups[20]. This opens up the possibility of connecting a wide range of chiral constituents to the polymer.

EXPERIMENTAL

Synthesis of stationary phases I, II and III

The O-benzyloxycarbonyl derivative of *S*-3-phenyllactic acid was prepared according to Thamm[22] and coupled to *tert.*-butylamine after formation of the mixed anhydride with ethyl chlorocarbonate. Compound I was obtained as a colourless oil in 80% yield and had an optical rotation of $[\alpha]_D^{20} = -8.3°$ ($c = 1.4$, $CHCl_3$).

The O-benzyloxycarbonyl (*Z*) derivatives of racemic 2-hydroxyisopentanoic acid and 2-hydroxyoctanoic acid were prepared according to Thamm[21] and coupled to *S*-α-phenylethylamine as described above. The diastereoisomers obtained could be separated by fractional crystallization from chloroform–light petroleum and diisopropyl ether, respectively. The *Z*-derivative of II had m.p. = 122°C ($[\alpha]_D^{20} = -39.6°$; $c = 0.79$, $CHCl_3$). The *Z*-derivative of III as recrystallized from diisopropyl ether was a solid compound with m.p. = 109–110°C ($[\alpha]_D^{20} = -27.8°$; $c = 0.64$, $CHCl_3$).

Optically pure *S*-2-hydroxyisopentanoic acid-*S*-α-phenylethylamide (II) and *S*-2-hydroxyoctanoic acid-*S*-α-phenylethylamide (III) were obtained after catalytic hydrogenation with palladium–charcoal as catalyst in ethyl acetate solution. The optical purity was determined by gas chromatography of the diastereoisomers. Compound II had m.p. = 66–67°C ($[\alpha]_D^{20} = -118.9°$; $c = 0.185$, $CHCl_3$) and compound III was obtained as a colourless oil with $[\alpha]_D^{20} = -99.0°$ ($c = 0.63$, $CHCl_3$).

Synthesis of stationary phases IV and V

Stationary phases IV and V were obtained by refluxing a solution of the polysiloxane XE-60 in methanol with sodium methylate at 120°C for 5 h. Coupling with L-valine-*S*(*R*)-α-phenylethylamide was performed with dicyclohexylcarbodiimide in chloroform solution. Completion of the reactions was monitored by infrared spectroscopy.

Formation of derivatives

The formation of 2-(O-trifluoroacetoxy) acid isopropyl esters has been described previously[15].

Carbohydrate derivatives were obtained by reaction of 0.5-mg samples with 200 μl of dichloromethane and 50 μl of trifluoroacetic anhydride at 100°C for 15 min in a screw-capped vial with a PTFE lining in the cap. The methylglycosides of aldoses were prepared by heating carbohydrate samples in 1 ml of methanolic hydrochloric acid (1.5 *N*) at 100°C for 15 min. After removal of excess of reagent the residues were submitted to trifluoroacetylation as described above. The products were checked by mass spectrometry[22]. Trifluoroacetyl derivatives of chiral amines and amino alcohols were prepared as described elsewhere[20].

Preparation of glass capillary columns

Glass capillaries were drawn from Pyrex glass tubes with a Hupe and Busch capillary drawing machine and coated as described earlier[13]. Gas chromatography was performed in Carlo Erba Model 2101 gas chromatographs with hydrogen as the carrier gas.

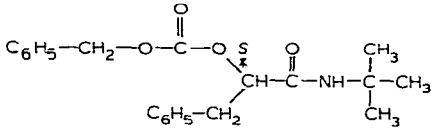
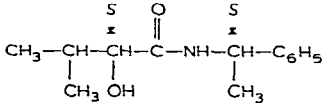
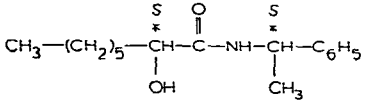
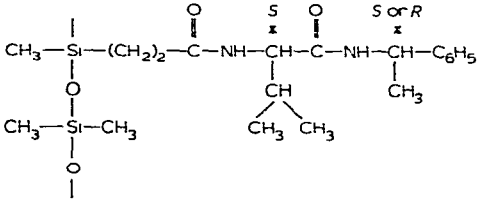
RESULTS AND DISCUSSION

Chiral hydroxy acids

In previous papers we have reported on the separation of α-hydroxy acid derivatives on a variety of chiral stationary phases derived from mandelic acid and 2-phenylbutyric acid[14,15]. From these results it was concluded that aromatic substituents and benzyloxycarbonyl substitution of the benzylic hydroxy group support enantioselectivity. Therefore, it was not unexpected to observe enantiomer separation of hydroxy acids on O-benzyloxycarbonyl-(*S*)-3-phenyllactic acid-*tert*.-butylamide (I). However, it was surprising that stationary phases II and III (Table I), derived from aliphatic α-hydroxy acids, show even better separation (Fig. 1). For the first time we observed partial separation of 3-hydroxybutyric acid enantiomers on stationary phase III. The α-values for the separation of hydroxy acids are given in

TABLE I

STRUCTURES OF NEW CHIRAL STATIONARY PHASES

No.	*Name*	*Formula*
I	O-Benzyloxycarbonyl-*S*-3-phenyllactic acid-*tert*.-butylamide	
II	*S*-2-Hydroxyisopentanoic acid-*S*-α-phenylethylamide	
III	*S*-2-Hydroxyoctanoic acid-*S*-α-phenylethylamide	
IV V	XE-60-*S*-valine-*S*-α-phenylethylamide XE-60-*S*-valine-*R*-α-phenylethylamide	

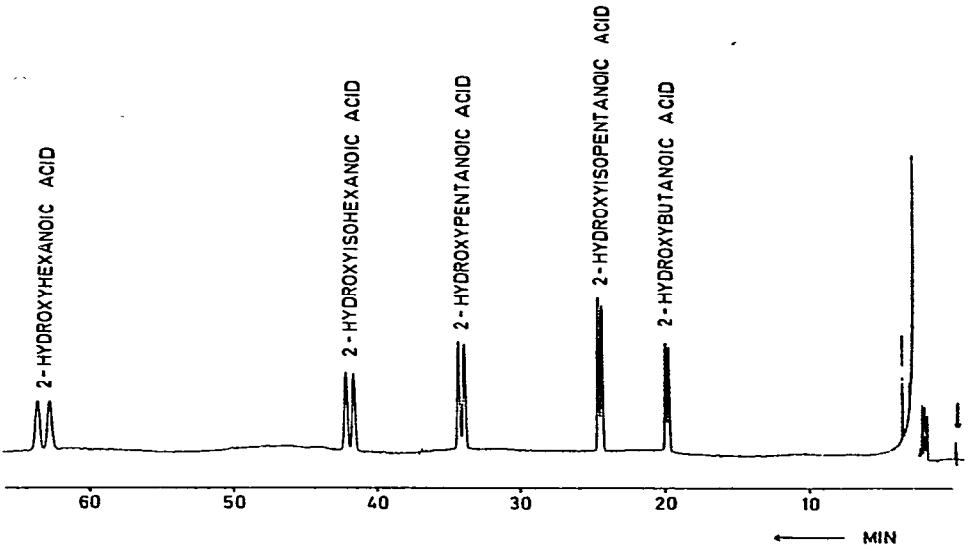


Fig. 1. Separation of 2-hydroxy acid enantiomers (O-TFA isopropyl esters) on a 37-m Pyrex glass capillary coated with stationary phase III. Column temperature, 45°C (isothermal). D-Enantiomers are eluted first.

Table II. It may be concluded that the chiral phenylethylamide residue in stationary phases II and III makes a major contribution to the enantioselectivity observed for hydroxy acids. Stationary phases I–III also show very good enantioselectivity for chiral amines. The best separation was obtained on stationary phase I (Fig. 2).

Carbohydrates

Carbohydrates, similarly to amino acids, occur in both enantiomeric forms in

TABLE II

SEPARATION FACTORS (α) AND OPERATING TEMPERATURES FOR ENANTIOMER SEPARATION OF CHIRAL HYDROXY ACIDS (O-TFA/ISOPROPYL ESTERS) ON STATIONARY PHASES I, II AND III

Racemate	*Phase I*		*Phase II*		*Phase III*	
	α	*Column temperature (°C)*	α	*Column temperature (°C)*	α	*Column temperature (°C)*
2-Hydroxybutyric acid	1.006	62	1.008	71	1.011	45
3-Hydroxybutyric acid	–	–	–	–	1.007	55
2-Hydroxyisopentanoic acid	1.007	62	1.010	71	1.011	45
2-Hydroxypentanoic acid	1.008	67	1.009	71	1.012	45
2-Hydroxyisohexanoic acid	1.011	62	1.012	71	1.013	45
2-Hydroxyhexanoic acid	1.011	62	1.012	71	1.014	45
2-Hydroxyoctanoic acid	1.006	81	1.015	71	1.011	92
Mandelic acid	1.008	100	1.026	81	1.019	92
Malic acid	–	–	1.011	91	1.012	92
3-Phenyllactic acid	–	–	1.013	81	1.009	100

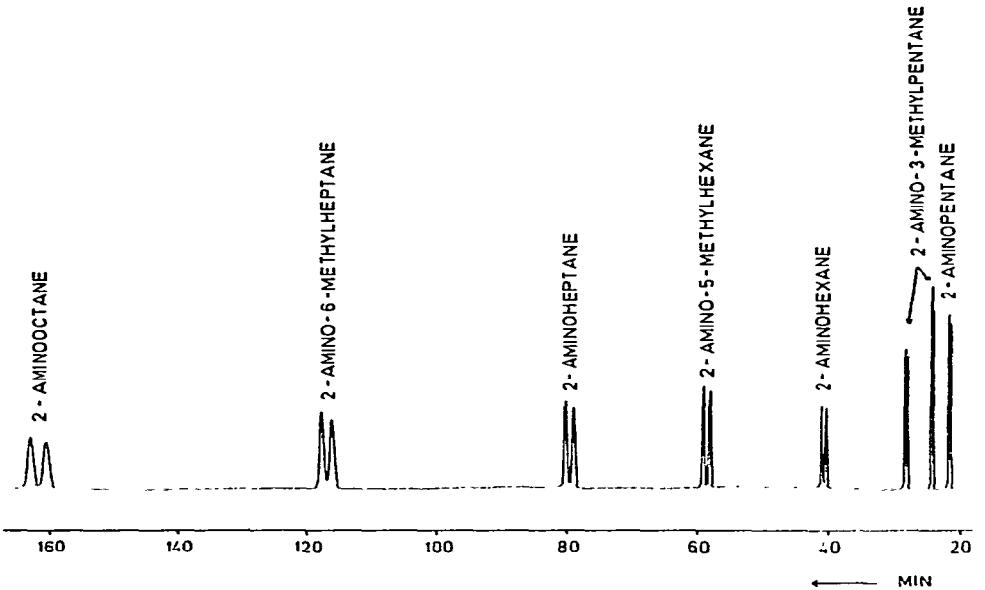


Fig. 2. Separation of 2-aminoalkane enantiomers (N-TFA derivatives) on a 36-m Pyrex glass capillary coated with stationary phase I. Column temperature, 84°C (isothermal). *R*-Enantiomers are eluted first.

nature. Determination of their configuration may be time consuming, as measurement of the optical rotation is of sufficient accuracy only for single components. This would imply the isolation of a sugar component from a mixture before its optical rotation can be determined. A simple gas chromatographic procedure, as already available for amino acids, would be very useful. Pollock and Jermany[23] in 1968 suggested oxidation of aldoses to aldonic acids and esterification with a chiral alcohol. A partial separation of these diastereomeric derivatives was obtained after acetylation. In a similar approach, the formation of diastereoisomers was achieved by glycosidation with (−)-2-butanol and separation of the trimethylsilyl derivatives[24]. In addition to difficulties with derivatization, these methods are affected with a systematic error due to the optical impurity of the chiral reagents.

By modification of the methylcyanoethyl-polysiloxane XE-60 we succeeded in preparing a chiral polymeric stationary phase that separates the enantiomers of carbohydrates. XE-60 was hydrolysed with sodium methylate to the corresponding acid and coupled with L-valine-*S*-α-phenylethylamide (IV) (Fig. 3).

Either the O-trifluoroacetylated D- and L-aldoses (Table III) or their methylglycosides were prepared on a 100-μg scale and chromatographed on a 40-m glass capillary coated with stationary phase IV. Fig. 4 shows the separation of the enantiomers of α- and β-methylglucopyranosides on stationary phase IV. The chromatogram in Fig. 5 shows a mixture of the enantiomers of glucose, galactose and mannose as their trifluoroacetylated methylglycosides. The separation factors for some other aldopentoses and aldohexoses are given in Table III.

As shown earlier[22], trifluoroacetylation of carbohydrates results in a mixture of α- and β-pyranosides and in most instances also in α- and β-furanosides. The same

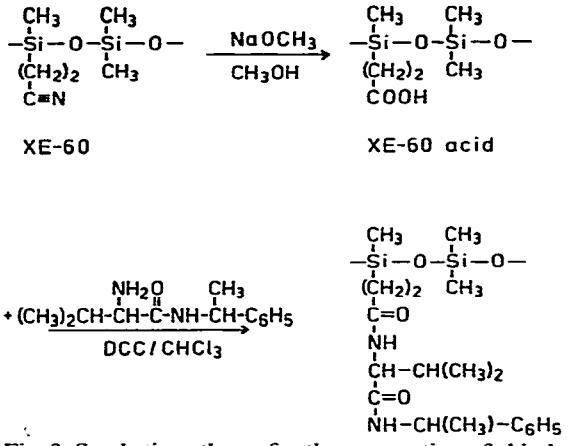


Fig. 3. Synthetic pathway for the preparation of chiral stationary phases IV and V.

TABLE III

SEPARATION FACTORS (α) AND OPERATING TEMPERATURES FOR ENANTIOMER SEPARATION OF CARBOHYDRATES ON A 40-m PYREX GLASS CAPILLARY COLUMN COATED WITH XE-60-L-VALINE-*S*-α-PHENYLETHYLAMIDE

Sugar	*TFA derivative*			*TFA–methylglycoside*		
	*Designation**	α	*Column temperature (°C)*	*Designation**	α	*Column temperature (°C)*
Glucose	α-p	1.071	140	α-p	1.032	120
	α-f	1.044	140	β-p	1.035	120
	β-f	1.031	140			
	β-p	1.140	140			
Mannose	α-p	1.036	140	α-p	1.053	120
	α-f	1.045	140	β-p	1.084	120
	β-p	1.247	140			
Galactose	α-p	1.019	140	β-f	1.010	120
	α-f	1.019	140	α-f	1.044	120
	β-p	1.045	140	α-p	1.049	120
	β-p	1.029	140	β-p	1.089	120
Xylose	β-p	1.030	100	β-p	Not separated	
Arabinose	α-p	1.028	100	α-f	1.019	100
	α-f	1.017	100	α-p	1.023	100
	β-f	1.048	100	β-p	1.046	100
	β-p	1.026	100			
Ribose	(c)	Not separated		(c)	Not separated	
	(c)	1.031	100	(c)	1.021	100
	(c)	1.041	100	(c)	Not separated	
	(c)	Not separated		(c)	Not separated	
Lyxose	(c)	Not separated		(c)	1.014	100
	(c)	1.019	100	(c)	Not separated	
	(c)	1.054	100	(c)	Not separated	
Fucose	(c)	Not separated		(c)	Not separated	
	(c)	Not separated		(c)	Not separated	
	(c)	1.035	100	(c)	1.014	100
				(c)	1.046	100

* p = pyranoside; f = furanoside; (c) = designation uncertain.

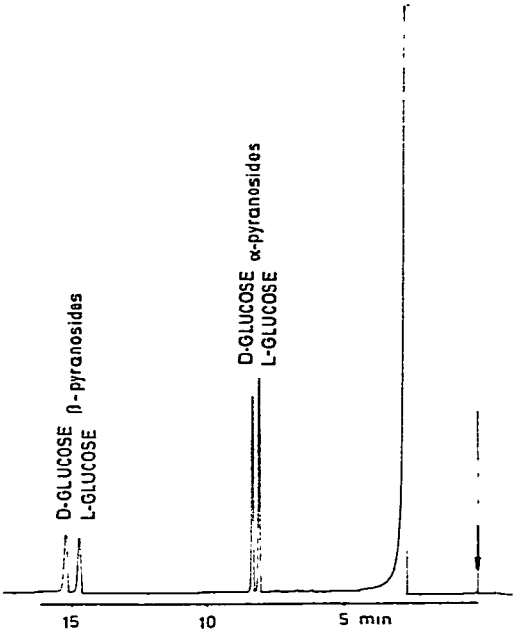


Fig. 4. Separation of the enantiomers of glucose isomers as O-trifluoroacetylated methylglycosides on a 40-m Pyrex glass capillary coated with stationary phase IV. Column temperature, 100°C (isothermal).

is true for the corresponding methylglycosides, although these derivatives are more stable toward hydrolysis than the fully acylated derivatives. Acyl residues seem to be essential for enantiomer separation of carbohydrates; the corresponding trimethylsilyl derivatives were not separated. It is conceivable that the polar trifluoroacetyl groups support the formation of diastereomeric association complexes with the stationary phase, whereas the large trimethylsilyl groups prevent the interacting molecules from coming into close contact, which is necessary for the formation of association complexes.

The order of elution is not consistent for the different stereoisomers. With glucose and mannose the D-enantiomers are retarded on stationary phase IV. A reverse order of elution was observed for galactose and fucose (6-deoxygalactose) and for one of the fully trifluoroacetylated arabinose isomers.

Stationary phase V, with a reverse configuration of the α-phenylethylamine moiety, also shows enantioselectivity for carbohydrate enantiomers. The separation factors are not as large as those with stationary phase IV. The order of elution of the enantiomers is the same for both stationary phases. Apparently only the configuration of the valine moiety determines the order of elution.

The new technique of configurational analysis has already been applied in the investigation of snail galactans[25]. These mucopolysaccharides contain varying amounts of L-galactose together with D-galactose. With less than 0.5 mg of the hydrolysate of a galactan of the snail *Helix pomatia* the portion of 14.4% L-galactose could be determined by electronic peak integration in the gas chromatogram. The amount of L-galactose and its presence in the outer region of the macromolecule may be of importance for the antigene–antibody interaction of galactans with some proteins[26].

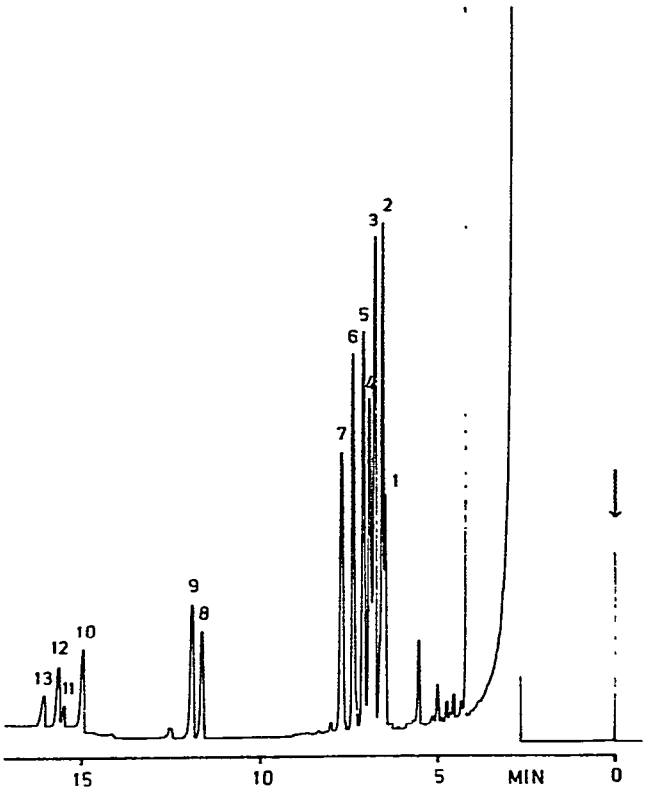


Fig. 5. Separation of a mixture of the enantiomers of glucose, galactose and mannose (O-TFA methylglycosides) on a 40-m Pyrex glass capillary coated with stationary phase IV. 1 = β-(D/L)-Galactofuranoside; 2 = α-L-mannopyranoside; 3 = α-D-mannopyranoside; 4 = α-L-glucopyranoside; 5 = α-D-glucopyranoside; 6 = α-D-galactopyranoside; 7 = α-L-galactopyranoside; 8 = β-L-glucopyranoside; 9 = β-D-glucopyranoside; 10 = β-D-galactopyranoside; 11 = β-L-mannopyranoside; 12 = β-L-galactopyranoside; 13 = β-D-mannopyranoside. Column temperature, 120°C programmed at 3°C/min to 180°C.

Enantioselectivity of stationary phases IV and V is not limited to carbohydrates. Stationary phase IV shows high resolution factors for amino acid enantiomers and stationary phase V shows high selectivity for aliphatic amino alcohols, as demonstrated in Fig. 6.

CONCLUSION

Micro-methods for configurational analysis of all kinds of chiral compounds are of increasing importance not only in structural investigations of natural compounds but also for the control of asymmetric syntheses. It may be expected that these new examples of chiral recognition will initiate further investigations with new types of chiral stationary phases and with chiral substrates not yet suitable for gas chromatographic separation.

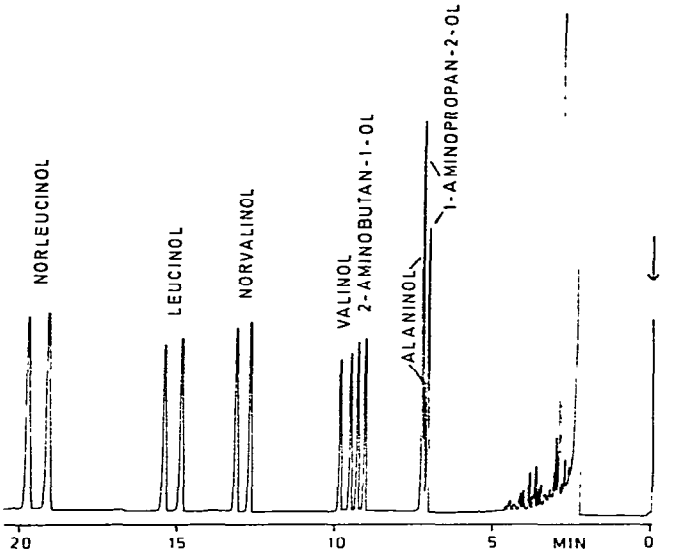


Fig. 6. Separation of the enantiomers of aliphatic α-amino alcohols (N,O-bis-TFA derivatives) on a 40-m Pyrex glass capillary coated with stationary phase V. Column temperature, 130°C (isothermal). L-Enantiomers are eluted first.

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