Journal of Chromatography, 209 (1981) 91–95
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,591

Note

Gas chromatographic separation of enantiomers of amines and amino alcohols on chiral stationary phases

WILFRIED A. KÖNIG* and INGRID BENECKE
Institut für Organische Chemie und Biochemie der Universität, D-2000 Hamburg 13 (G.F.R.)
(Received December 17th, 1980)

Capillary gas chromatography is a fast and reliable method for the assignment of the configurations of optically active compounds. Both the formation of diastereomeric derivatives and the direct separation of enantiomers on chiral stationary phases have been applied in many versions, especially for amino acids, amines, amino alcohols and α-hydroxy acids.

Most chiral stationary phases have poor thermal stability. This problem was overcome by Bayer and co-workers[1,2] by synthesizing polysiloxane stationary phases with chemically bound chiral constituents. A simpler approach was demonstrated by Saeed *et al.*[3,4], who succeeded in modifying commercially available silicone polymers with cyano groups in their side-chains such as OV-225 and Silar-10C. The cyano groups were converted by acid hydrolysis into carboxylic groups and acid chlorides and coupled with L-valine-*tert.*-butylamide. With these stationary phases they were able to separate amino acids and some amino alcohols such as norephedrine.

In this work we introduced chiral constituents into silicone OV-225 after reduction of the cyano groups to amino groups with lithium aluminium hydride ($LiAlH_4$) and by coupling benzyloxycarbonyl-L-valine and -L-leucine to the amino groups.

EXPERIMENTAL

Reduction of OV-225 with $LiAlH_4$

To a suspension of 50 mg of $LiAlH_4$ in 5 ml of dry diethyl ether a solution of 100 mg of OV-225 (Applied Science Labs., College Station, PA, U.S.A.) in 3 ml of diethyl ether was slowly added and the mixture was refluxed for 5 h. Excess of $LiAlH_4$ was then decomposed with water. After addition of concentrated sodium hydroxide solution the phases separated. The organic phase was removed, the aqueous phase was extracted three times with diethyl ether and the combined extracts were dried over sodium sulphate. After removal of the solvent, completion of the reaction was proved by infrared spectroscopy.

Coupling of Z-L-Val and Z-L-Leu to OV-225 amine

Z-Amino acids were prepared according to the procedure described by Grassmann and Wünsch[5] and added in equimolar ratio to the OV-225 amine in chloroform solution. After the addition of a small excess of dicyclohexyl carbodiimide (DCC)

0021-9673/81/0000–0000/$02.50

the reaction mixture was stirred at room temperature for 12 h. After completion of the reaction the solution was washed with 1 *N* hydrochloric acid, saturated sodium hydrogen carbonate solution and water and dried over sodium sulphate. To remove dicyclohexylurea the product was chromatographed on a silica column with ethyl acetate as eluent.

Preparation of glass capillary columns

Pyrex glass capillary columns were drawn on a Hupe and Busch drawing machine and coated as described previously[6]. Gas chromatography with hydrogen as the carrier gas was performed on a Carlo Erba Model 2101 gas chromatograph.

Formation of derivatives

Samples of 1 mg or less of amines or amino alcohols were dissolved in 200 μl of dichloromethane and trifluoroacetylated by addition of 50 μl of trifluoroacetic anhydride. After 30 min at room temperature the excess of reagent was removed with a gentle stream of dry nitrogen and the residue was dissolved in 100 μl of dichloromethane and used for gas chromatographic investigation. Amino acids were derivatized as reported earlier[7].

RESULTS AND DISCUSSION

Various intermolecular forces participate in the formation of diastereomeric complexes between a chiral solvent and chiral solutes in a gas chromatographic system. Amino acid derivatives in many instances have proved to be adequate constituents of chiral stationary phases for effective separations of enantiomers. It has been shown[8–10] that the structure of an acyl, ester or amide moiety of a stationary phase derived from amino acids or dipeptides strongly influences enantioselectivity. From our recent work[7,11] on the separation of α-hydroxy acids on mandelic acid derivatives as stationary phases we concluded that benzyloxycarbonyl residues may support enantiomer separation. It also seemed interesting to compare the properties of these new stationary phases with those prepared by Saeed *et al.*[3].

For these reasons we introduced chiral constituents into the silicone polymer OV-225 by reduction of the cyano groups to aminomethylene groups and by coupling Z-L-Val and Z-L-Leu to the newly formed amino groups with DCC as condensing agent (Fig. 1). As can be seen from the α-values in Table I, both the Z-L-Val and the Z-L-Leu phases give only poor separations of amino acid enantiomers. However, the separation factors for amino alcohols and amines are surprisingly high. Amino alcohols have repeatedly been identified as constituents of peptide antibiotics[12,13] and the determination of their configuration on a microgram scale has been difficult. The separation of trifuoroacetylated aliphatic amino alcohols and amines is not possible or very poor on such widely used chiral stationary phases as N-dodecanoyl-L-Val-*tert.*-butylamide[14], N-trifluoroacetyl-L-Val-L-Val-O-cyclohexyl ester[15] and Chirasil-Val[1]. Aliphatic, cyclic and aromatic amines were separated by Gil-Av and co-workers[16,17] on stationary phases derived from chiral aromatic amines and on carbonylbis(N-L-valine isopropyl ester)[18]. Good separation of the N,O-bis-trifluoroacetyl derivatives of DL-alaninol and DL-valinol was obtained on (O-benzyloxycarbonyl)-S-mandeloyl-L-valine-*tert.*-butylamide[7].

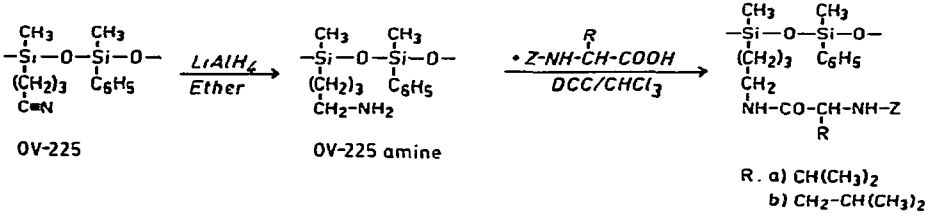

Fig. 1. Synthetic pathway for the preparation of chiral stationary phases.

TABLE I
α VALUES FOR CHIRAL STATIONARY PHASES

Racemate	*Z-L-Val-OV-225*		*Z-L-Leu-OV-225*	
	α value	*Column temperature (°C)*	*α value*	*Column temperature (°C)*
D,L-Alanine	1.00	70	1.013	80
D,L-Valine	1.026	80	1.015	80
D,L-*allo*-Isoleucine	1.011	80	1.00	80
D,L-Isoleucine	1.022	80	1.009	80
D,L-Leucine	1.017	80	1.00	80
R,S-2-Aminopentane	1.009	80	1.00	80
R,S-2-Amino-3-methylpentane	1.007	80	1.00	80
	1.010	80	1.005	80
R,S-2-Aminohexane	1.015	80	1.010	80
R,S-2-Amino-5-methylhexane	1.019	80	1.011	80
R,S-2-Aminoheptane	1.017	80	1.012	80
R,S-2-Amino-6-methylheptane	1.017	80	1.012	80
R,S-2-Aminooctane	1.020	80	1.015	80
R,S-Phenylethylamine	1.028	100	1.023	100
D,L-Alaninol	1.00	80	1.00	100
D,L-Aminobutanol	–	–	1.011	100
D,L-Valinol	1.008	80	1.018	100
D,L-Norvalinol	1.018	80	1.017	100
D,L-Leucinol	1.025	80	1.022	100
D,L-Norleucinol	1.023	80	1.019	100
D,L-Norephedrine	–	–	1.013	130
D,L-Mandelic acid	1.006	90	1.007	90

Chiral amino alcohols could also be separated as N-trifluoroacetyl-O-acyl derivatives[19,20] with propionyl, isobutyryl or pivaloyl as acyl residues. However, the procedure reported for the formation of derivatives does not seem to be easily applicable on a microgram scale.

As a peculiarity of the new chiral stationary phases the order of elution of amino acid enantiomers is not consistent. On the Z-L-Val phase the derivatives of D-Val, D-Ile and D-*allo*-Ile have longer retention times than the corresponding L-enantiomers.

With the Z-Leu-OV-225 stationary phase the derivatives of L-Ala but D-Val are retarded. Similar observations have been made with carbonylbis(N-L-valine isopropyl ester) as stationary phase[21]. With the amino alcohols (Fig. 2) the D-enan-

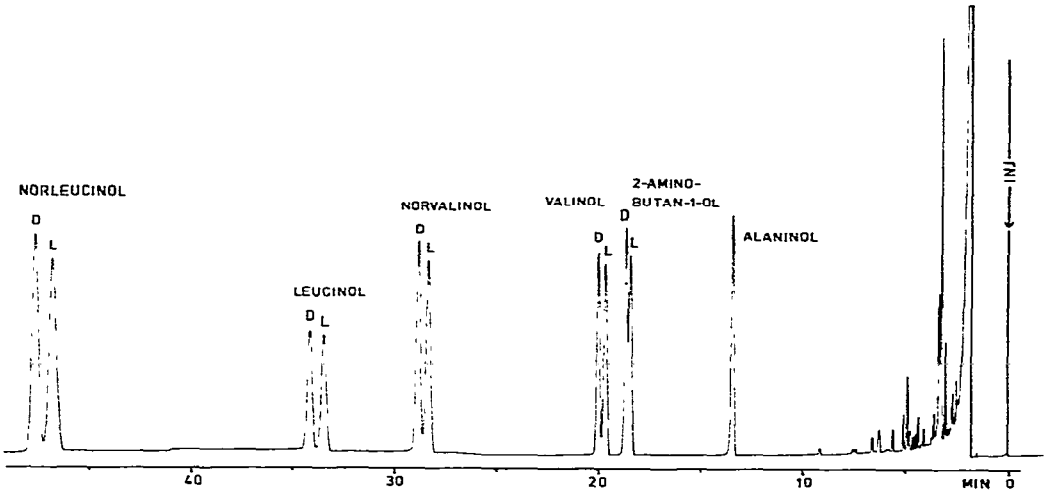

Fig. 2. Separation of N-trifluoroacetylamines on a Carlo Erba 2101 gas chromatograph with a 35 m × 0.2 mm I.D. Pyrex glass capillary coated with Z-L-Val-OV-225. Column temperature, 80°C; carrier gas, hydrogen (0.7 bar).

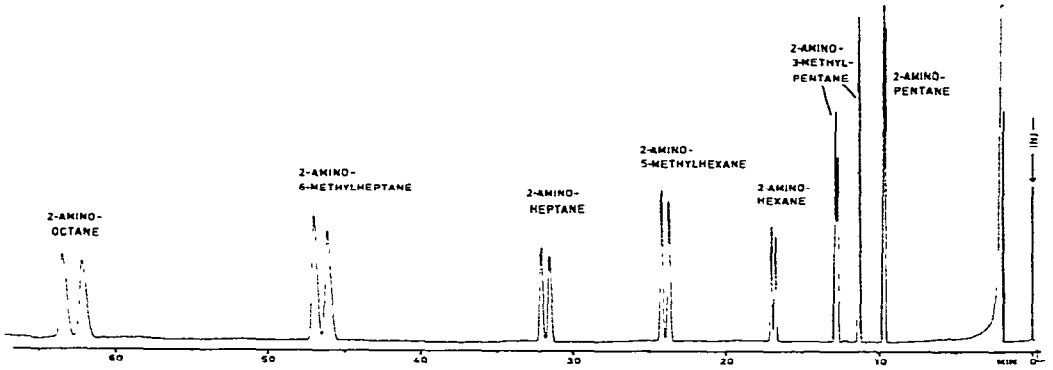

Fig. 3. Separation of N,O-bistrifluoroacetylamino alcohols on a Carlo Erba 2101 gas chromatograph with a 35 m × 0.2 mm I.D. Pyrex glass capillary coated with Z-L-Leu-OV-225. Column temperature, 100°C; carrier gas, hydrogen (0.7 bar).

tiomers consistently have longer retention times than the corresponding L-forms on both the Z-Val and Z-Leu phases. For the amines (Fig. 3) the S-enantiomers have longer retention times in the case of 2-aminopentane and phenylethylamine. The other 2-aminoalkanes were available only as racemates.

The major advantage of polymeric stationary phases over low-molecular-weight phases is their higher thermal stability. In this instance the limit of thermal stability has not yet been tested, but operation of the column at up to 130°C for several weeks did not decrease its separation efficiency. It is expected that further interesting results will be obtained by connecting other chiral compounds to functionalized silicone polymers.

REFERENCES

1 H. Frank, G. J. Nicholson and E. Bayer, *Angew. Chem.*, 90 (1978) 396.
2 H. Frank, G. J. Nicholson and E. Bayer, *J. Chromatogr.*, 146 (1978) 197.
3 T. Saeed, P. Sandra and M. Verzele, *J. Chromatogr.*, 186 (1979) 611.
4 T. Saeed, P. Sandra and M. Verzele, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 35.
5 W. Grassmann and E. Wünsch, *Chem. Ber.*, 91 (1958) 462.
6 W. A. König, K. Stölting and K. Kruse, *Chromatographia*, 10 (1977) 444.
7 W. A. König and S. Sievers, *J. Chromatogr.*, 200 (1980) 189.
8 W. Parr, C. Yang and E. Bayer, *J. Chromatogr. Sci.*, 8 (1970) 591.
9 K. Grohmann and W. Parr, *Chromatographia*, 5 (1972) 18.
10 W. Parr and P. Y. Howard, *Anal. Chem.*, 45 (1973) 711.
11 W. A. König, S. Sievers and U. Schulze, *Angew. Chem.*, 92 (1980) 935; *Angew. Chem. Int. Ed. Engl.*, 19 (1980) 910.
12 G. Jung, W. A. König, D. Leibfritz, T. Ooka, K. Janko and G. Boheim, *Biochim. Biophys. Acta*, 433 (1976) 164.
13 H. Brückner, W. A. König, M. Greiner and G. Jung, *Angew. Chem.*, 91 (1979) 508; *Angew. Chem., Int. Ed. Engl.*, 18 (1979) 476.
14 B. Feibush, *Chem. Commun.*, (1971) 544.
15 E. Gil-Av and B. Feibush, *Tetrahedron Lett.*, (1967) 3345.
16 S. Weinstein, B. Feibush and E. Gil-Av, *J. Chromatogr.*, 126 (1976) 97.
17 B. Feibush and E. Gil-Av, *J. Gas Chromatogr.*, 5 (1967) 257.
18 J. A. Corbin and L. B. Rogers, *Anal. Chem.*, 42 (1970) 974.
19 B. Feibush, A. Balan, B. Altman and E. Gil-Av, *J. Chem. Soc., Perkin Trans. II*, (1979) 1230.
20 R. Charles and E. Gil-Av, *J. Chromatogr.*, 195 (1980) 317.
21 B. Feibush, E. Gil-Av and T. Tamari, *J. Chem. Soc., Perkin Trans. II*, (1972) 1179.