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Note

High-performance liquid chromatographic separation of amino acid enantiomers on urea derivatives of L-valine bonded to silica gel

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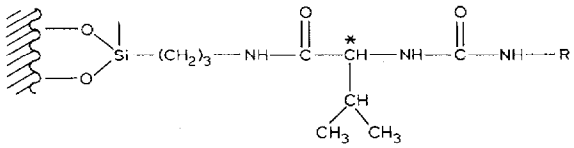
It is well known diastereomeric hydrogen bonding association, as well as host–guest, metal–chelate and charge-transfer complexations, can contribute to the separation of enantiomers by high-performance liquid chromatography. Hara and co-workers[1–4] reported that N-acetyl-DL-amino acid esters were resolved into their antipodes with a chrial amide-bonded stationary phase [(N-acetyl-L-valylamino)propyl silica gel]. This technique depends entirely on hydrogen bonding association, and involves no other strong complexations.

Recently we obtained very effective separation factors for separations of N-acetyl-DL-amino acid methyl esters on N-(*tert.*-butyl aminocarbonyl)-L-valylaminopropyl silica gel as a novel stationary phase[5].

In this paper we describe the detail of separations with novel chiral stationary phases I–III, consisting of urea derivatives of L-valine chemically bonded to γ-aminopropyl silanized silica.



R = (I) isopropyl, (II) *tert.*-butyl, (III) phenyl

EXPERIMENTAL

Preparation of chiral stationary phase

Phase I. To a solution of 4.69 g (0.04 mol) of L-valine in 40 ml of 1 *N* sodium hydroxide were added 3.4 g (0.04 mol) of isopropyl isocyanate and 5 ml of tetrahydrofuran and the mixture was stirred at room temperature overnight. The reaction mixture was acidified with 5 ml of 1 *N* hydrochloric acid. The solvent was removed by filtration to afford 2.5 g of crude N-isopropylaminocarbonyl-L-valine. This crude crystalline material was recrystallized from ethyl acetate–*n*-hexane. Analysis: calculated for $C_9H_{18}N_2O_3$, C 53.45, H 8.97, N 13.85%; found, C 52.73, H 8.73, N 13.41%.

To a solution of 1.19 g of N-isopropylaminocarbonyl-L-valine in 14 ml dimethylformamide were added 2.3 g of LiChrosorb-NH_2 (10 μm) (E. Merck, Darm-

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stadt, F.R.G.) and 1.02 g of 1-hydroxybenzotriazole, and 1.5 g of N,N'-dicyclohexylcarbodiimide (DCC) was added with stirring at 0°C for 1 h, then the mixture was stirred at room temperature overnight. Modified silica (I) was collected by centrifugation and washed exhaustively with chloroform, methanol, acetone and diethyl ether and dried under reduced pressure. It contained 0.73 mmol of N-isopropylaminocarbonyl-L-valine per gram of support (based on C) or 0.79 mmol/g (based on N).

Phase II. N-*tert.*-Butylaminocarbonyl-L-valine was synthesized as for N-isopropylaminocarbonyl-L-valine but using *tert.*-butyl isocyanate instead of isopropyl isocyanate. This compound was colourless cyrstals. Analysis: calculated for $C_{10}H_{20}N_2O_3$, C 55.53, H 9.32, N 12.95%; found, C 55.52, H 9.53, N 12.84%. Phase II was prepared as for phase I but using N-*tert.*-butylaminocarbonyl-L-valine instead of N-isopropylaminocarbonyl-L-valine, and this modified silica contained 0.57 mmol of N-*tert.*-butylamonocarbonyl-L-valine per gram of support (based on C) or 0.57 mmol/g (based on N).

Phase III. N-Phenylaminocarbonyl-L-valine was synthesized as for N-isopropylaminocarbonyl-L-valine but using phenylisocyanate instead of isopropyl isocyanate. This compound was colourless crystals. Analysis: calculated for $C_{12}H_{16}N_2O_3$, C 61.00, H 6.83, N 11.86%; found, C 61.17, H 6.91, N 11.72%.

Phase III was prepared as for phase I but using N-phenylaminocarbonyl-L-valine instead of N-isopropylaminocarbonyl-L-valine, and this modified silica contained 0.59 mmol of N-phenylaminocarbonyl-L-valine per gram of support (based on C) or 0.61 mmol/g (based on N).

Liquid chromatography

The experiments were carried out with a Shimadzu LC-3A high-performance liquid chromatograph equipped with a UVD-2 ultraviolet detector (230 nm).

Steel columns (250 × 4 mm I.D.) were slurry packed with the modified silicas using a conventional technique.

RESULTS AND DISCUSSION

The chromatographic results are summarized in Table I. It was found that the three phases gave good chiral recognition for N-acetyl-DL-amino acid methyl esters. Typical chromatograms are shown in Figs. 1 and 2.

It is emphasized that the separation factors obtained are larger than those obtained by Hara and co-workers with N-(formyl-L-valylamino)propyl silica gel. Excellent separation factors were obtained on phase II.

As shown in Table II, the separation factors depended on changes involving the O-alkyl group of N-acetyl-DL-amino acid O-alkyl esters, and the most effective value was obtained for the O-*tert.*-butyl ester.

Some solvent systems tried on the mobile phases were found to have excellent separation factors and peak resolutions obtained with *n*-hexane–1,2-dichloroethane–ethanol, as shown in Table III.

In these separations the retention of L-isomers was always longer than that of D-isomers, showing that the hydrogen bond association between the stationary phase and L-isomers was the more stable.

These results show that the bonding of urea nitrogen to the asymmetric carbon

TABLE I

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF N-ACETYL-DL-AMINO ACID METHYL ESTERS ON CHIRAL STATIONARY PHASES

4% (v/v) of isopropanol in *n*-hexane was used as the mobile phase. A flow-rate of 1.0 ml/min was typically used for the 250 × 4 mm I.D. columns at room temperature.

Amino acid	*Phase I*		*Phase II*		*Phase III*	
	α^*	k'^{**}	α^*	k'^{**}	α^*	k'^{**}
Alanine	1.15	7.40	1.21	9.37	1.17	10.78
Valine	1.30	2.90	1.47	3.54	1.36	4.62
Leucine	1.34	3.04	1.68	3.86	1.36	4.10
Isoleucine	1.35	2.47	1.59	3.04	1.41	3.91
Phenylalanine	1.29	3.68	1.44	4.46	1.36	6.30
Phenylglycine	1.13	4.51	1.22	5.40	1.14	7.54

* The separation factor of the enantiomers (α) is the ratio of their capacity ratios.
** k' is the capacity ratio for the initially eluted enantiomer (D-enantiomer).

atom is the active site for diastereomeric hydrogen bond association in the separation of enantiomers by liquid chromatography.

In a study of the direct separation of enantiomers by gas chromatography, Feibush and Gil-Av[6] reported a ureide formed by the condensation of phosgene with L-valine isopropyl ester that gave good chiral recognition for some N-acyl primary amines. However, such phases have never been used in liquid chromatography.

It is interesting these novel phases, which contain an asymmetric carbon atom attached to the nitrogen atom of the urea group, are efficient for the separation of N-acetyl amino acid ester enantiomers by high-performance liquid chromatography.

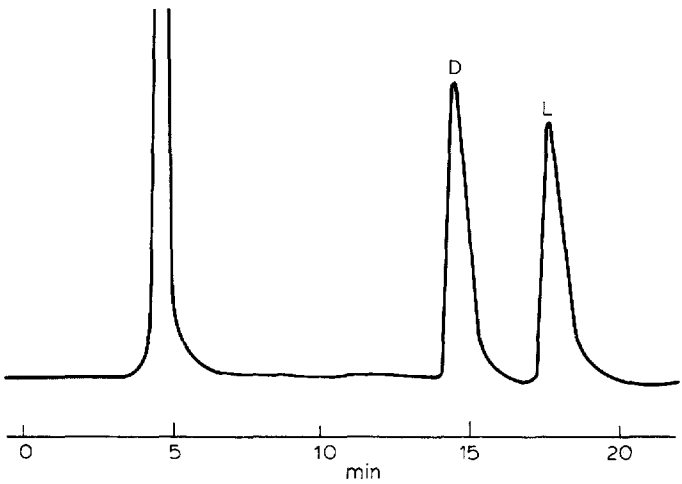

Fig. 1. Chromatographic separation of the enantiomers of N-acetyl-DL-leucine methyl ester on chiral stationary phase I. Chromatographic conditions as in Table I.

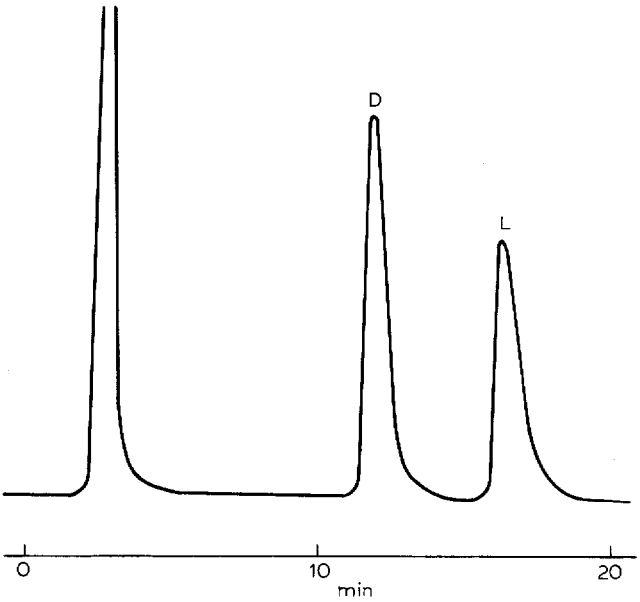

Fig. 2. Chromatographic separation of the enantiomers of N-acetyl-DL-valine methyl ester on chiral stationary phase II. Chromatographic conditions as in Table I.

TABLE II

SEPARATION OF N-ACETYL-DL-LEUCINE ESTERS ON CHIRAL STATIONARY PHASES

Chromatographic conditions, α and k' in Table I.

Compound	*Phase I*		*Phase II*		*Phase III*	
	α	k'	α	k'	α	k'
N-Acetyl-DL-leucine methyl ester	1.32	2.73	1.68	3.86	1.36	4.10
N-Acetyl-DL-leucine ethyl ester	1.42	1.83	1.79	2.34	1.45	2.83
N-Acetyl-DL-leucine isopropyl ester	1.52	1.33	1.93	1.66	1.54	2.04
N-Acetyl-DL-leucine *tert.*-butyl ester	1.73	0.97	2.29	1.20	1.73	1.50

TABLE III

SEPARATION OF N-ACETYL-DL-LEUCINE ESTERS IN DIFFERENT SOLVENT SYSTEMS

Solvent I = *n*-hexane–isopropanol (24:1); solvent II = *n*-hexane–isopropanol (49:1); solvent III = *n*-hexane–1,2-dichloroethane–ethanol (50:10:1). Chiral stationary phase II was used. α and k' as in Table I.

Compound	*Solvent I*			*Solvent II*			*Solvent III*		
	k'	α	R_s	k'	α	R_s	k'	α	R_s
N-Acetyl-DL-leucine methyl ester	3.86	1.68	3.61	3.92	1.46	4.50	3.31	1.70	5.94
N-Acetyl-DL-leucine ethyl ester	2.52	1.86	4.01	2.75	1.56	4.45	2.38	1.79	5.69
N-Acetyl-DL-leucine isopropyl ester	1.73	2.03	4.66	2.08	1.67	5.55	1.83	1.93	6.43
N-Acetyl-DL-leucine *tert.*-butyl ester	1.21	2.36	4.91	1.54	1.92	6.48	1.39	2.24	6.96

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