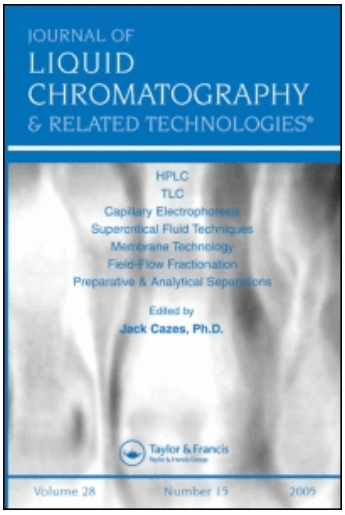

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Liquid Chromatographic Resolution of Enantiomers on Chiral Amide Bonded-Silica Gel Normal Phase Separation of Racemic α-Amino Acid Derivatives by N-Acetyl-L-valyl-aminopropyl-silanized Silica (AVA) Phase

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LIQUID CHROMATOGRAPHIC RESOLUTION OF ENANTIOMERS ON CHIRAL AMIDE BONDED-SILICA GEL NORMAL PHASE

SEPARATION OF RACEMIC α-AMINO ACID DERIVATIVES BY N-ACETYL-L-VALYL-AMINOPROPYL-SILANIZED SILICA (AVA) PHASE

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ABSTRACT

A new optically active stationary phase obtained by grafting N-acetyl-L-valine with 3-aminopropylsilanized silica was developed. The high-efficiency chiral column was prepared by slurry packing procedure. Direct resolution of the racemates of α-amino acid derivatives was accomplished by using normal phase liquid chromatography.

INTRODUCTION

Systematic procedures for the design of stationary and mobile phases employed in chromatography for direct resolution of enantiomers have recently been developed. Chiral recognition by liquid chromatography has been exploited by incorporating such highly selective interactions as host-guest complexation[1] and ionic ligand-exchange[2,3]. In both cases,

specific chiral phases such as biaryl crown macrocyclic ethers were constructed. The charge-transfer complex-forming stationary phase has been introduced, leading to the direct optical resolution of helicene enantiomers by Gil-Av and his co-workers by utilizing a properly designed stationary phase having π-electron acceptors[4,5]. They suggested that the main factors necessary for enantiomer resolution are the ability for strongly binding interactions and extensive spatial recognition by the physical configuration of the stationary surface[4]. Chiral differentiation employing weak diastereomeric association has not yet been achieved.

We wish to report a direct resolution of the optical isomers of α-amino acid derivatives on a silica gel surface grafted with an α-amino acid derivative involving lower configurational selectivity between the solute and the stationary phase and weaker hydrogen bonding than in the work mentioned above. This represents a new application of liquid chromatographic techniques to the resolution of enantiomers using chiral amide bonds as mild active sites grafted to the support.

MATERIALS AND METHODS

Preparation of N-Acetyl-L-Valyl-Aminopropylsilanized Silica (AVA)

3-Aminopropylsilanized silica (APS) was prepared from micro-particulate silica (Kusano Scientific, Tokyo; average particle size: 10 μm, average pore diameter: 95 A, surface area: 380 m^2/g) and 3-aminopropyltriethoxysilane in toluene.

To a suspension of APS (2.2 g) in 8 ml of dimethylformamide (DMF) degassed under vaccum for 5 min were added a solution of 1-hydroxybenztriazole 1.02 g (7.53 mmole) in 3 ml of DMF and a solution of N-acetyl-L-valine 799 mg (5.02 mmole) in 3 ml of DMF. The resulting mixture was treated with 3 ml of DMF containing 1.14 g of dicyclohexylcarbodiimide at 0° C for 1 hr under stirring, and then stirred at room temperature for 48 hr. The grafted silica was separated from dicyclohexylurea by centrifugation and washed successively with chloroform, acetone, methanol and diethyl ether and then dried over phosphorous pentoxide under vaccum for 6 hr. Elemental analysis of the grafted silica showed that it contained 2.04 % nitrogen. From this value, it was estimated that 28.30 % of the APS was grafted with N-acetyl-L-valine.

Column Packing Procedure

To 2.1 g of AVA was added a solution which contained 5 ml of tetrachloromethane, 10 ml of chloroform, and 10 ml of dioxan and which had been degassed in an ultrasonic bath for

3 min. Slurry Solvent B conc (Machery-Nagel, Düren, Germany) was added to stabilize the slurry. The slurry was transferred to a reservoir and pumped into a column (precision-bore stainless steel, length 20 cm, I.D. 4 mm) with constant pressure pump (Model DSTV-122G, Haskel Eng. and Supply, Burbank, Calif.) at 6000 psi of initial pressure. A solution consisting of chloroform and methanol (1:1 v/v, 200 ml) was used to pressurize the slurry. When half of the slurry solvent had been eluted, the pressure was at 8000 psi. After passing 200 ml of n-hexane, the pump was shut off and the column pressure relieved.

Apparatus and Chromatographic Procedure

The apparatus was a Spectra-Physics (Stenderway, Santa Clara, Calif.) Model SP 8000 Chromatograph equipped a Jasco UVIDEC-100-II (Japan Spectroscopic, Tokyo) variable wave length UV detector operated at 230 nm.

RESULTS AND DISCUSSION

It was previously thought that three active sites or steric barriers differentiating three particular groups in a chiral compound were absolutely necessary for recognition of asymmetric carbon compounds. An amide bond was selected as the active group, in this experiment, because the amide

bonding is chemically stable and also because the amide group has the ability to serve as either donor or acceptor in hydrogen bonding. Optically active N-acetyl-L-valine as a chiral element was activated and grafted onto the aminoalkylsilanized silica gel packing. In this process, another amide bond was introduced into the stationary phase. The chiral surface structure containing the two amide functions and alkyl group bound to the asymmetric carbon can differentiate chiral solute molecules.

A slurry of the new chiral materials was packed into stainless steel tubes, yielding highly efficient columns. Racemic N-acyl alkyl α-amino acid esters were selected as target compounds for examining the chiral recognition of the new columns. When the solvent strength was adjusted to afford medium capacity factors from 6 to 9, pairs of enantiomers were separated into corresponding antipodes. Separation factors for pairs of isomers were approximately 1.06 (Table 1). It was found that the D-isomers of the amino acid derivatives and the L-L-isomers of the dipeptide derivatives were eluted faster than their antipodes.

Since this selective-adsorption system employing an organic phase was constructed with the chiral α-amino acid derivatives as both the solute element and the grafted moiety

TABLE 1.

Resolution of DL-α-Amino Acid and Dipeptide Derivatives by HPLC Using AVA

Racemates	k'_1	k'_2	α	R_s	Mobile phase: 2-propanol in n-hexane v/v %
Ac-Leu-OMe	6.40	6.95	1.09	0.71	4
Ac-Norleu-OMe	6.35	6.87	1.08	0.63	4
Ac-Ile-OMe	5.65	6.06	1.07	0.54	4
Ac-Val-OMe	6.70	7.19	1.07	0.55	4
Ac-Phe-OMe	8.74	9.35	1.07	0.57	4
Ac-S-Bz-Cys-OMe	8.28	8.53	1.03	0.24	4
Ac-Met-OMe	6.37	6.64	1.04	0.31	8
Ac-Ala-OMe	7.42, shoulder				8
Z-Leu-Leu-OMe (L-L, D-D)	5.80	6.14	1.06	0.46	2
Z-Phe-Phe-OMe (L-L, D-D)	7.36, shoulder				3

(1) Resolution R_s was calculated according to the formula, $R_s = 1/4\,(\alpha - 1)\sqrt{N} \cdot k'/(1 + k')$. Capacity factor k' and separation factor α were calculated according to the formulae, $k' = (k'_1 + k'_2)/2$, $\alpha = k'_2/k'_1$.
(2) The column gave about 1300 theoretical plates per 20 cm by using N-Ac-L-Leu-OMe as the solute employing a linear velocity of 0.17 cm/sec, a temperature of 40° C and a mobile phase consisting of 8 v/v % 2-propanol in n-hexane (k' = 3.0).

on the support, it may be considered analogous to biological systems in which chiral recognition of α-amino acid derivatives is performed on the surface of proteins in the aqueous phase.

For exploration of the application of the new column, the chiral recognition of a variety of optical isomers was extensively examined. The results will be reported in future.

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