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Improved crown ether-based chiral stationary phase

Toshio Shinbo, Tomohiko Yamaguchi, Hiroshi Yanagishita, Dai Kitamoto, Keiji Sakaki and Masaaki Sugiura

National Chemical Laboratory for Industry, 1–1 Higashi, Tsukuba, Ibaraki 305 (Japan)

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ABSTRACT

An improved crown ether-based chiral stationary phase (CSP) was prepared by dynamic coating of a reversed-phase silica gel with a new chiral crown ether which was designed to have more lipophilicity than the previously used one, while preserving the basic structure responsible for chiral recognition. The CSP showed not only excellent enantioselectivity for amino acids, but also higher stability against organic solvents in the mobile phase. No desorption of the crown ether from the support was observed in a mobile phase containing up to 40% of methanol. An increase in methanol concentration in the mobile phase gave rise to a decrease in the retention of amino acids and an increase in the "apparent" enantioselectivity, *i.e.*, the separation coefficient and the resolution factor. A possible retention mechanism is proposed to explain these behaviours.

INTRODUCTION

Recently, much attention has been paid to chiral stationary phases (CSPs) based on host–guest molecular recognition [1–6]. Cram and co-workers [1,2] developed chiral packings by binding chiral crown ethers covalently to polystyrene and silica gel, and showed that a chiral crown ether-based packing is a powerful tool for the optical resolution of amino acids.

In an earlier paper [3], we also reported another crown ether-based CSP prepared by dynamic coating of the chiral crown ether **1** [(*R*)-**1** and (*S*)-**1**], on reversed-phase (RP) silica gels. The CSP showed excellent enantioselectivity toward a wide variety of amino acids and amines. However, it had a disadvantage that only aqueous solutions of inorganic species could be used as mobile phase. As the crown ether was embedded in the alkyl chain of the RP support by hydrophobic interaction, passage of methanol-containing solutions through the CSP easily desorbed the coated crown ether from the support.

In this paper, we report an improved crown ether-based CSP that can be used in methanol-containing mobile phases. The crown ether (*R*)-**2**, a derivative of (*R*)-**1** having higher lipophilicity and preserving the basic structure responsible for chiral recognition, was prepared by attaching an octyl chain to the dinaphthyl ring, and was immobilized dynamically on RP silica gel in a similar manner to that described previously. The elution behaviour of racemic amino acids on this CSP was examined, and the characteristics of the CSP is discussed in comparison with those of the previous one.

EXPERIMENTAL

Reagents and materials

*Preparation of (R)-2,3:4,5-bis[1,2-(3-phenyl-6-octylnaphtho)]-1,6,9,12,15,18-hexaoxacycloeicosa-2,4-diene [(R)-**2**].* The *R*-enantiomer of **2** [(*R*)-**2**] was prepared according to Fig. 1.

*(R)-6,6'-Dioctyl-2,2'-dimethoxy-1,1'-dinaphthyl [(R)-**6**].* Bromine (4 ml) was added over a 20-min period to a solution of 10 g of (*R*)-2,2'-dimeth-

Correspondence to: Dr. Toshio Shinbo, National Chemical Laboratory for Industry, 1–1 Higashi, Tsukuba, Ibaraki 305, Japan.

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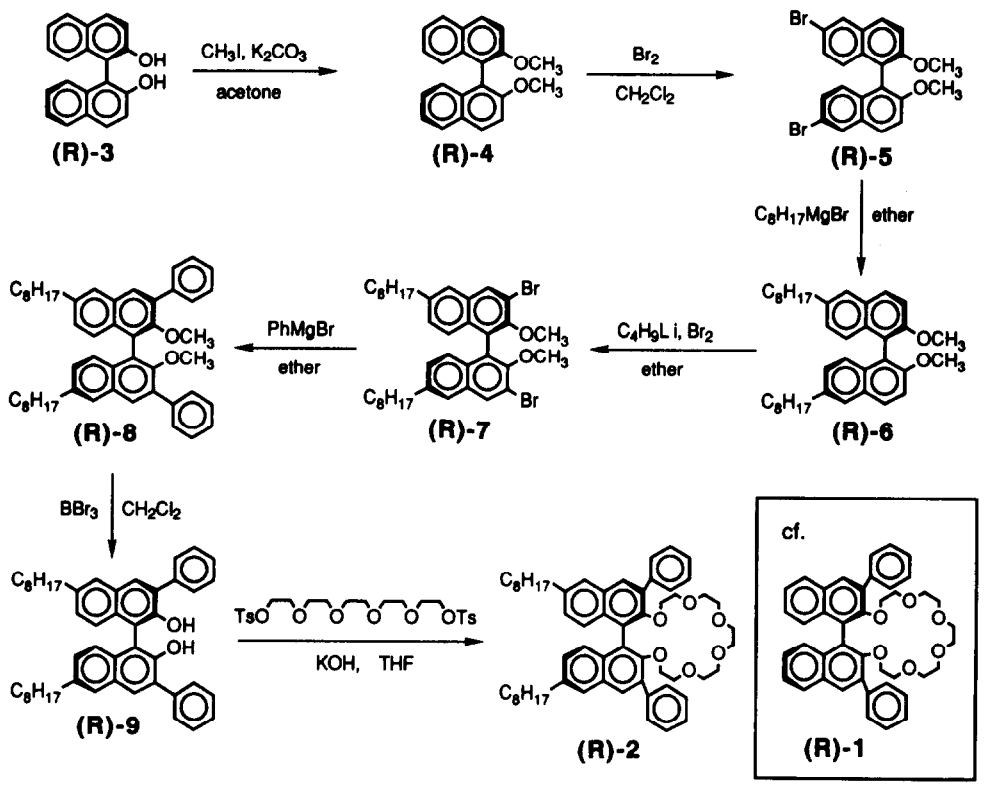

Fig. 1. Reaction scheme for the preparation of (*R*)-**2**.

oxy-1,1′-dinaphthyl [(*R*)-**4**] in 100 ml of dichloromethane stirred under nitrogen at −75°C. The mixture was stirred for 2.5 h, then gradually warmed to 25°C. After 30 min, 300 ml of 10% Na_2SO_3 were added to decompose unreacted bromine. The cake-like material that formed and the residue resulting from evaporation of the dichloromethane phase were combined and purified by column chromatography (silica gel, benzene–cyclohexane) to give 14 g (93%) of (*R*)-6,6′-dibromo-2,2′-dimethoxy-1,1′-dinaphthyl [(*R*)-**5**].

To a mixture of 10 g of (*R*)-**5** and 1.25 g of Ni[$(C_6H_5)_2P(CH_2)_3P(C_6H_5)_2$]$Cl_2$ [7] in 150 ml of diethyl ether under nitrogen were added 100 ml of a diethyl ether solution of octylmagnesium bromide (63 mmol) and the mixture was refluxed for 20 h. The reaction mixture was shaken with 800 ml each of 1 *M* HCl and dichloromethane. The organic phase was washed, dried and evaporated. The residue was chromatographed on silica gel (light petroleum–diethyl ether) and 8.1 g (72%) of (*R*)-6,6′-dioctyl-2,2′-dimethoxy-1,1′-dinaphthyl-[(*R*)-**6**] were obtained.

The following syntheses were carried out in a similar manner to those described in the literature [8].

*(R)-6,6′-Dioctyl-3,3′-diphenyl-2,2′-dihydroxy-1,1′-dinaphthyl [(R)-**8**].* To a solution of 5.2 g of tetramethylethylenediamine in 400 ml of diethyl ether were added under nitrogen 32 ml of 1.6 *M* butyllithium in hexane. After the mixture had been stirred for 15 min at 25°C, 6.8 g of (*R*)-**6** were added and the suspension was stirred for 3 h. The mixture was cooled to −75°C and 10 ml of bromine in 30 ml of pentane were added over a 10-min period. The mixture was warmed to 25°C, stirred for 1 h, then 300 ml of a saturated solution of Na_2SO_3 were added and the mixture was stirred for an additional 4 h. The mixture was then shaken with dichloromethane and the organic layer was dried and evaporated. The residue was purified by column chromatography (silica gel, cyclohexane–benzene) to give 4.8 g

(55%) of (*R*)-6,6′-dioctyl-3,3′-dibromo-2,2′-dimethoxy-1,1′-dinaphthyl [(*R*)-**7**].

To a mixture of 4.4 g of (*R*)-**7** and 0.5 g of $Ni[P(C_6H_5)_3]_2Cl_2$ in 50 ml of diethyl ether stirred under nitrogen were added 20 mmol of phenylmagnesium bromide in 30 ml of diethyl ether and the mixture was refluxed for 20 h. After cooling, the reaction mixture was shaken with 500 ml each of 1 *M* HCl and dichloromethane. The organic layer was dried and evaporated, and the residue was purified by column chromatography (silica gel, cyclohexane–benzene) to obtain 1.66 g (38%) of (*R*)-6,6′-dioctyl-3,3′-diphenyl-2,2′-dimethoxy-1,1′-dinaphthyl [(*R*)-**8**].

To a solution of 1.5 g of (*R*)-**8** in 150 ml of dichloromethane at 0°C were added 7.5 g of BBr_3. The mixture was stirred for 24 h at 25°C, then cooled to 0°C. After the excess of BBr_3 had been decomposed with water, the organic layer was washed with water, dried, evaporated, and chromatographed on silica gel (cyclohexane–benzene) to obtain 1.55 g (80%) of (*R*)-6,6′-dioctyl-3,3′-diphenyl-2,2′-dihydroxy-1,1′-dinaphthyl [(*R*)-**9**].

(R)-2,3:4,5-Bis[1,2-(3-phenyl-6-octylnaphtho)]-1,6,9,12,15,18-hexaoxacycloeicosa-2,4-diene [(R)-2]. To a solution of 0.74 g of (*R*)-**9** and 0.68 *g* of pentaethylene glycol ditosylate in 100 ml of tetrahydrofuran stirred under nitrogen was added 0.24 g of KOH and the mixture was refluxed for 72 h. The mixture was shaken with 250 ml each of dichloromethane and water and the organic layer was dried and evaporated. The residue was purified by column chromatography (silica gel, light petroleum–diethyl ether) and gel permeation chromatography (polystyrene, chloroform) to obtain 0.44 g (46%) of desired compound [(*R*)-**2**]. Specific rotation, $[\alpha]_{589}^{22}$ + 7.7° (*c* 8.68, THF). Mass spectrum, *m/z* 864 (M^+). ^1H NMR (400 MHz), d, 0.87 (t, CH_3, 6H), 1.29 (m, other CH_2, 20H), 1.70 (m, β-CH_2, 4H), 2.71 (t, α-CH_2, 4H), 3.41 (m, OCH_2, 20H), 7.47 (m, ArH, 18H).

Preparation of (R)-2-coated CSP

The preparation of (*R*)-**2**-coated CSP was performed in a similar manner to that described in a previous paper [3]. A solution of (*R*)-**2** in methanol–water (97:3) was circulated through an ODS column [LiChrospher RP-18(e) or LiChrosorb RP-18, d_p 5 μm, 125 x 4 mm I.D.] using a liquid chromatographic pump. To the solution, water was added stepwise to reduce the methanol–water ratio gradually to about 70:30. The immobilization of the crown ether on the support was monitored by the decrease in the UV absorption of the solution. The amount of crown ether coated was chosen to be 79 and 173 mg per column, because preliminary experiments revealed that, although an increase in the amount of crown ether coated gave a larger separation of enantiomers, as reported previously [3], an excessive amount (> 300 mg) of crown ether led to a severe decrease in the number of theoretical plates.

Chromatographic procedures

Chromatography was performed using a liquid chromatographic pump (DIP-1; Jasco), an injector (Model 7125; Rheodyne), a UV detector (S-310A; Soma) and an integrator (Chromatocorder11; System Instrumenz). The column temperature was controlled by placing the column in a thermostated water bath. The eluent was 0.01 *M* perchloric acid.

The resolved enantiomers were detected using UV spectrophotometry at 200 or 254 nm. The configurations of the enantiomers in the eluates were identified by comparison of their retention times with those of authentic enantiomers.

Column parameters such as capacity factors (k_L, k_D), separation coefficient (α) and resolution factor (R_s) were calculated in the usual manner.

RESULTS AND DISCUSSION

Retention and resolution of various amino acids

Fig. 2 shows some examples of the elution profiles of racemic amino acids at 25°C on the CSP coated with 173 mg of (*R*)-**2**. Racemic amino acids were clearly separated into their enantiomers on this (*R*)-**2**-coated CSP, as was observed on (*R*)-**1**-coated CSP [3]. L-Enantiomers of all the amino acids were eluted prior to D-enantiomers. As D-enantiomers of amino acids form more stable complexes with the *R*-enantiomer of the crown ether [8], this observation reflects that the enantiomer forming a more stable complex with the crown ether was eluted after the enantiomer forming a less stable complex.

Table I shows the chromatographic data obtained at 25 and 4°C using the CSP coated with 79

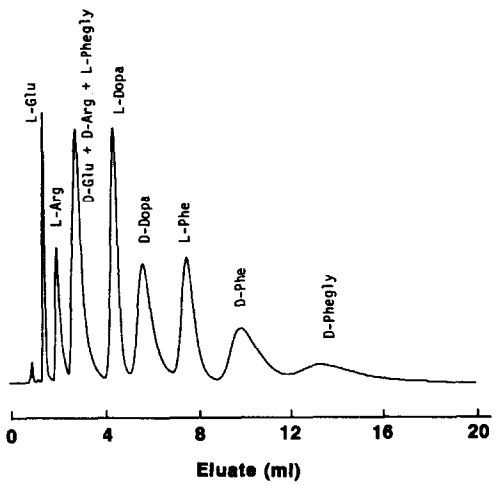

Fig. 2. Separation of five racemic amino acids on an (*R*)-**2**-coated CSP. Column, 125 mm x 4 mm I.D., LiChrospher RP-18(e) coated with 173 mg of (*R*)-**2**; eluent, 0.01 *M* perchloric acid; flow-rate, 1 ml/min; column temperature, 18°C; detection, UV (203 nm).

mg of (*R*)-**2**. Polar amino acids gave lower and hydrophobic amino acids gave higher retentions. Amino acids having one bulky substituent group at the β-carbon atom showed a large enantioselectivity, and amino acids having more than one substituent group at the β-carbon atom showed a smaller enantioselectivity (compare Leu with Ile and NVal with Val). Lower column temperatures gave higher retention and enantioselectivity.

The mechanism of the enantioselective retention of racemic amino acids on the crown ether-coated CSP can be described as follows. The injected amino acid (A^+) is distributed between the mobile and the stationary phases mainly by the following two reactions:

$$A^+ + X^- \rightleftharpoons \overline{A^+X^-} \quad (1)$$

$$A^+ + \bar{C} + X^- \rightleftharpoons \overline{(AC)^+X^-} \quad (2)$$

where X^- is the anion (perchlorate) in the mobile phase and C and AC^+ are the crown ether and the complex between the crown ether and amino acid, respectively. The bars above the letters represent the stationary phase. The crown ether-involved reaction (reaction 2) determines the enantioselectivity. The difference in the complex formation constants give rise to the difference in retention. As reaction 1 is not enantioselective, its contribution reduces the separation coefficient (*i.e.*, the apparent enantioselectivity).

This mechanism can explain the above results. The difference in retention reflects the nature of the RP stationary phase. Hydrophobic amino acids are retained strongly on RP stationary phases owing to the hydrophobic interaction and are eluted later.

The reason why the enantioselectivity depends on the number of substituent groups at the β-carbon atom is as follows. Although the bulkiness of a substituent at the α-carbon atom of an amino acid is important for chiral recognition, the presence of more than one substituent group at the β-carbon atom gives rise to extensive steric hindrance and decreases the complex formation constant of the favourable enantiomer. The small complex formation constant reduces the relative contribution of reaction 2 to the retention and hence gives small separation coefficients and resolution factors.

The temperature dependence of enantioselective retention is explained as described previously [3]. A lower temperature increases the chiral recognition of the crown ether [9], and also increases the retention due to reaction 1 [10]. The combination of these two effects causes an increase in enantioselective retention.

Comparison of (R)-2-coated CSP with (R)-1-coated CSP

In Table II, chromatographic data obtained using the CSP coated with 173 mg of (*R*)-**2** are compared with those obtained using the CSP coated with an equimolar amount (128 mg) of (*R*)-**1**. The retention and the enantioselectivity on the (*R*)-**2**-CSP were almost the same as those on the (*R*)-**1**-CSP, but there was a small difference between them. The capacity factors and separation coefficients of the (*R*)-**2**-CSP were slightly smaller than those of the (*R*)-**1**-CSP. As (*R*)-**2** was designed and synthesized to have the same crown ring and the same chiral barriers as (*R*)-**1**, their abilities for chiral recognition can be assumed to be the same. Therefore, the slight difference mentioned above is ascribed to the difference in their environment on the support. (*R*)-**1** is assumed to be present at the interface with the hydrophobic naphthyl group buried in the RP stationary phase and part of the relatively hydrophilic crown ring extruding toward the mobile

TABLE I

CAPACITY FACTORS AND ENANTIOSELECTIVITY FOR AMINO ACIDS ON (*R*)-**2**-COATED CSP

Column, 125 mm × 4 mm I.D. LiChrosorb RP-18 cooated with 79 mg of (*R*)-**2**; eluent, 0.01 *M* perchloric acid; flow-rate, 0.5 ml/min; detection, UV (200 nm); sample: 10^{-8} mol.

Amino acid[a]	18°C				2°C			
	k_L	k_D	α	R_s	k_L	k_D	α	R_s
Ala	0.31	0.51	1.64	0.97	0.48	0.98	2.04	2.44
AABA	0.60	0.76	1.27	0.71	0.64	1.01	1.57	1.79
Val	1.18	1.18	–	–	1.35	1.49	1.10	0.45
NVal	1.38	1.91	1.39	2.15	1.73	2.90	1.67	3.85
Leu	4.09	5.32	1.30	2.79	4.70	7.09	1.51	4.21
Ile	3.23	3.51	1.09	0.76	3.92	4.48	1.14	1.32
NLeu	4.50	5.93	1.32	2.64	5.62	8.54	1.52	4.40
Met	2.29	3.72	1.63	3.99	3.33	7.08	2.12	6.99
Phe	12.62	13.83	1.10	1.17	18.52	20.93	1.13	1.55
PGly	2.45	8.27	3.37	8.63	3.19	19.32	6.06	14.47
Ser	0.19	0.19	–	–	0.24	0.35	1.50	0.55
Thr	0.21	0.30	1.44	0.37	0.27	0.58	2.17	1.61
Cys	0.31	0.44	1.43	0.46	0.42	0.79	1.89	1.50
Tyr	9.42	10.24	1.09	1.04	20.74	22.97	1.11	1.22
Dopa	5.52	6.12	1.11	1.15	12.76	14.53	1.14	1.53
Asp	0.23	0.40	1.76	0.54	0.43	0.80	1.84	1.68
Asn	0.35	0.35	–	–	0.21	0.35	1.65	0.37
Glu	0.43	0.83	1.91	1.69	0.75	3.25	4.33	7.92
Gln	0.23	0.64	2.75	1.97	0.40	1.75	4.37	4.98
Lys	0.81	0.81	–	sh	2.00	2.36	1.18	0.97
Arg	0.47	0.81	1.73	1.61	0.92	2.40	2.60	4.11
His	0.28	0.28	–	–	0.64	0.64	–	–
PEA	17.37	19.70	1.13	0.77	22.85	27.66	1.21	1.67
AECL	1.31	1.86	1.42	2.13	1.81	3.15	1.74	3.88

[a]AABA = 2-Aminobutyric acid; NVal = norvaline; NLeu = norleucine; PGly = phenylglycine; PEA = 1-phenylethylamine; AECL = 3-amino-ε-caprolactam. Other abbreviations have their usual meanings.

TABLE II

CAPACITY FACTORS AND ENANTIOSELECTIVITY FOR AMINO ACIDS OBTAINED USING (*R*)-**1**- AND (*R*)-**2**-COATED CSPs

Column, 125 mm × 4 mm I.D., LiChrospher RP-18(e) coated with an equimolar amount of crown ether, (*R*)-**1** 128 mg and (*R*)-**2** 173 mg; temperature, 18°C; eluent 0.01 *M* perchloric acid; flow-rate, 1.0 ml/min; detection, UV (200 nm); sample, 10^{-7} mol.

Amino acid	(*R*)-**2**				(*R*)-**1**			
	k_L	k_D	α	R_s	k_L	k_D	α	R_s
Ala	0.56	1.04	1.86	1.73	0.50	1.02	2.03	2.09
Val	0.88	1.02	1.16	sh	0.98	1.22	1.25	0.86
Met	2.16	4.61	2.14	3.03	2.26	5.90	2.61	4.68
Phe	7.59	9.49	1.25	1.11	8.73	13.22	1.51	2.33
PGly	2.70	12.76	4.73	6.56	2.81	17.54	6.25	7.39
Thr	0.32	0.59	1.86	1.26	0.31	0.53	1.69	1.08
Tyr	6.29	8.13	1.29	1.03	6.15	9.39	1.53	2.38
Glu	0.66	2.38	3.63	3.87	0.59	2.34	3.95	5.05
Arg	0.88	1.80	2.06	1.75	0.71	1.36	1.93	2.10

phase. As (*R*)-**2** is more hydrophobic than (*R*)-**1** owing to the additional octyl chain on the naphthyl group, it is reasonable to assume that the crown ring of (*R*)-**2** is buried deeper than (*R*)-**1** in the RP stationary phase. Accordingly, the interaction of an amino acid in the mobile phase is weaker with (*R*)-**2** than with (*R*)-**1**. This means that with (*R*)-**2**, the contribution of reaction 2 to the retention becomes smaller, and the "apparent enantioselectivity" is decreased.

Stability of the (R)-2-CSP towards methanol-containing mobile phases

As reported previously [3], the crown ether-coated CSP is stable when aqueous mobile phases are used. However, application of this CSP to highly lipophilic amino acids (or amines) requires the addition of an organic solvent to the mobile phase, because they are strongly retained in a merely aqueous mobile phase [11].

Table III shows the stability of the (*R*)-**2**-CSP towards methanol-containing mobile phases. The amounts of crown ether desorbed from the (*R*)-**2**-CSP using mobile phases containing various concentrations of methanol are compared with those desorbed from the (*R*)-**1**-CSP. As can be seen, the (*R*)-**2**-CSP showed excellent stability and no loss of the crown ether was observed even in 40% methanol solution, whereas the (*R*)-**1**-CSP lost the crown ether gradually even in the mobile phase containing only 10% of methanol. This result indicates that octyl chain gives satisfactory hydrophobicity for the crown ether to be held on the RP stationary phase in the ordinary methanol-containing mobile phase.

Effect of methanol in the mobile phase on retention and enantioselectivity

Table IV gives column parameters obtained using mobile phases containing up to 40% of methanol. The capacity factors, as expected, decreased with increase in methanol concentration, and for Trp it was reduced to be less than one sixth on changing the methanol concentration from 0 to 40%. On the other hand, the separation coefficients were unexpectedly increased with increase in methanol concentration. Examples of elution profiles are shown in Fig. 3.

The reason for the increase in separation coefficients is not clear, but a possible explanation is as follows. According to the aforementioned mechanism, the enantioselective retention of amino acids on this CSP arises from the two distribution reactions: simple distribution (reaction 1), and crown ether-involved complex formation (reaction 2). If it is assumed that part of the crown ring is extruded into the mobile phase, an increase in methanol concentration will bring about an increase in the retention due to reaction 2 because the complex formation constant in methanol is, generally, larger than that that in water [12]. On the other hand, the retention due to reaction 1 is decreased with increase in methanol concentration. Therefore, an increase in methanol concentration leads to an increase in the relative contribution of reaction 2 to the retention, and hence to an increase in the separation coefficient.

TABLE III

AMOUNT OF CROWN ETHER DESORBED FROM (*R*)-**1**- and (*R*)-**2**-COATED CSPs IN AQUEOUS MOBILE PHASES CONTAINING METHANOL

Temperature, 18°C; flow-rate, 0.5 ml/min.

CSP	Amount of crown ether desorbed (mg/ml of eluate)				
	0% CH_3OH	10% CH_3OH	20% CH_3OH	30% CH_3OH	40% CH_3OH
(*R*)-**1**	–[a]	$3.5 \cdot 10^{-7}$	$6.7 \cdot 10^{-7}$	$9.6 \cdot 10^{-6}$	$1.5 \cdot 10^{-4}$
(*R*)-**2**	–	–	–	–	–

[a] –, Not detected (less than 10^{-7} mg/ml of eluate).

TABLE IV

EFFECT OF METHANOL CONCENTRATION IN THE MOBILE PHASE ON RETENTION AND ENANTIOSELECTIVITY FOR AMINO ACIDS

Column, 125 mm × 4 mm I.D., LiChrospher RP-18(e) coated with 173 mg of (*R*)-**2**; temperature, 18°C; eluent, 0.01 *M* perchloric acid + methanol; flow-rate, 1.0 ml/min; detection, UV (200 nm); sample, 10^{-7} mol.

Amino Acid	Enantiomer	0% CH_3OH			10% CH_3OH			20% CH_3OH			30% CH_3OH			40% CH_3OH		
		k'	α	R_s	k'	α	R_s	k'	α	R_s	k'	α	R_s	k'	α	R_s
Met	L	2.16	2.14	3.03	1.60	2.50	3.34	1.35	3.00	3.45	1.17	3.48	3.82	1.01	3.91	3.58
	D	4.61			4.01			4.06			4.08			3.96		
Phe	L	7.59	1.25	1.11	4.32	1.37	1.38	3.04	1.54	1.70	2.11	1.79	1.98	1.56	2.00	1.91
	D	9.49			5.90			4.68			3.77			3.12		
PGly	L	2.70	4.73	6.56	2.00	5.69	6.11	1.78	6.71	5.82	1.39	7.88	5.56	1.14	8.64	5.05
	D	12.76			11.39			11.92			10.97			9.82		
Trp	L	47.70	1.15	0.73	21.45	1.27	1.24	12.52	1.44	1.58	7.26	1.66	1.80	4.55	1.89	1.68
	D	54.89			27.25			17.98			12.07			8.59		
Tyr	L	6.29	1.29	1.03	3.17	1.49	1.57	2.23	1.75	1.92	1.50	2.05	2.27	1.12	2.28	2.19
	D	8.13			4.72			3.90			3.08			2.56		
Glu	L	0.61	3.63	3.87	0.58	4.17	3.65	0.54	4.78	3.61	0.53	5.15	4.12	0.50	5.41	4.02
	D	2.38			2.42			2.60			2.74			2.73		
Arg	L	0.88	2.06	1.75	0.63	2.27	1.75	0.47	2.39	1.83	0.39	2.50	1.79	0.33	2.53	1.56
	D	1.80			1.43			1.13			0.97			0.83		

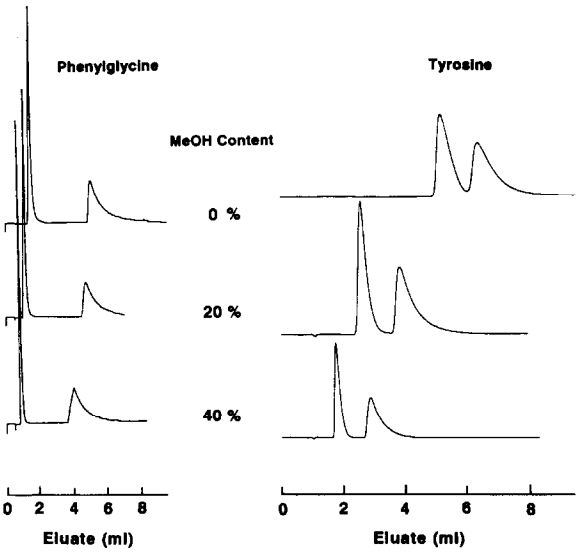

Fig. 3. Elution profiles of racemic phenylglycine and tyrosine in aqueous mobile phases containing various concentrations of methanol. Details as in Table IV.

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