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Preparation and enantioselectivity of (*S*)-binaphthol-bonded phase for high-performance liquid chromatography

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Abstract

A novel chiral stationary phase (CSP) for high-performance liquid chromatography was prepared by covalently bonding (*S*)-2,2′-dihydroxy-1,1′-binaphthyl to silica gels. This CSP showed enantioselectivity mainly for secondary and tertiary amines. The experimental results suggested that the free hydroxyl groups of the binaphthol moiety play a very important role in the retention and the chiral recognition on this CSP. Addition of trifluoroacetic acid or diethylamine to a mobile phase affected the peak shape and the enantioselectivity. Trifluoroacetic acid improved the resolution between enantiomers for many analytes.

Keywords: Chiral stationary phases, LC; Amines; Promethazine; Propranolol; Crown ethers; Binaphthol

1. Introduction

Chiral crown ethers derived from 2,2′-dihydroxy-1,1′-binaphthyl (binaphthol) have excellent enantioselectivity for amino acids [1]. It was also demonstrated that the chiral crown ethers are available as chiral stationary phases (CSPs) for direct chiral resolution of amino acids and primary amines by high-performance liquid chromatography (HPLC) [2–5].

Since the binaphthol is the chiral source of the crown ethers and is also capable of forming complexes with aromatic amines by hydrogen bonding and $\pi-\pi$ interaction, it is expected to have enantioselectivity for aromatic amines. Toda and co-workers [6] reported that binaphthol forms complexes with compounds such as amines, alcohols and sulfoxides and that it has enantioselectivity for these compounds. Tamai et al. [7] reported that 3,5-dinitrophenylcarbamoyl derivatives of chiral alcohols were resolved by HPLC, using silica gel coated with polymethacrylates bearing binaphthol and silica gel to which binaphthol was covalently bonded. In their preparations, one hydroxyl group of binaphthol was used to bond itself to the stationary supports and the other was masked by methylation. This treatment apparently eliminates the acidic characteristic of the neighboring phenolic OH groups, which may take an important role in chiral recognition, from the chiral binaphthol moiety. The packing coated with the chiral polymethacrylate exhibited enantioselectivity but low efficiency of column, resulting in insufficient

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resolution between the enantiomers, whereas the enantioselectivity of the binaphthol-bonded packing was much lower than that of the chiral polymer-coated packing. Furthermore, some samples required π–acid derivatization, such as 3,5-dinitrophenylcarbamoylation, for their chiral resolution.

We report here, the preparation and characterization of a novel CSP possessing a binaphthol moiety (CSP–BIN). (*S*)-6-(4-carboxybutyl)-binaphthol is chemically bonded to aminopropylsilylated silica gel. It is designed to maintain two hydroxyl groups of the chiral selector, binaphthol, in an unmodified form and to make better use of their nature of hydrogen bonding formation for the chiral resolution of amines.

2. Experimental

2.1. Reagents and materials

(*S*)-2,2′-Dihydroxy-1,1′-binaphthol (100% e.e.) was provided by Mitsubishi Gas Chemicals (Tokyo, Japan). The silica gel used was M.S.GEL SIL (EP-DF grade, particle size 5 μm, pore size 120 Å and surface area 350 m^2/g) from Dohkai Chemicals (Fukuoka, Japan). Other reagents were obtained commercially and used without further purification.

Fig. 1 shows the structures of the compounds used as HPLC analytes. Salts of racemates such as (*R*)- and (*S*)-α-methyl-4-nitrobenzylamine hydrochloride, propranolol hydrochloride, butethamate citrate, chlorpheniramine maleate, meclizine hydrochloride, promethazine hydrochloride, tolperizone hydrochloride, trihexyphenidyl hydrochloride and verapamil hydrochloride were shaken with aqueous ammonia and chloroform, and the chloroform layers were used as HPLC analytes.

2.2. Preparation of CSP–BIN

Fig. 2 shows the preparation procedure of CSP–BIN.

2.3. (S)-2,2′-Dimethoxy-1,1′-binaphthyl [(S)-3]

To a solution containing 20 g (70 mmol) of (*S*)-2,2′-dihydroxy-1,1′-binaphthyl [(*S*)-2] in 700 ml of acetone, 40 g (290 mmol) of K_2CO_3 and 28 ml (450 mmol) of CH_3I were added, with stirring under N_2, and the mixture was refluxed for 24 h. An additional 14 ml (225 mmol) of CH_3I was added and the mixture was refluxed for an additional 15 h. The solvent was evaporated to a volume of 100 ml, then cooled to 25°C and 600 ml of water was added to the suspension. After the mixture was stirred for 8 h, the solid was collected, washed with water and dried under vacuum, first at 25°C and then at 95°C for 24 h, to yield 22 g (100%) of the desired product.

2.4. (S)-6-(3-Carbomethoxybutyryl)-2,2′-dimethoxy-1,1′-binaphthyl [(S)-4]

To 60 ml of dichloromethane was added 6.4 g (48 mmol) of $AlCl_3$, under N_2, and cooled to 0°C. To this mixture, while being stirred under N_2, was added 6.6 ml (48 mmol) of methyl-4-(chloroformyl)butyrate and the mixture was stirred for 1 h to give a homogeneous solution. To this stirred solution was added 10 g (32 mmol) of (*S*)-3 and 10 ml of dichloromethane and the solution was stirred for 4 h. The solution was cooled to 0°C and 100 ml of 1.2 *M* HCl was carefully added to the stirred solution. The suspension was shaken with 30 ml of chloroform. The organic layer was dried, evaporated and chromatographed on 100 g of silica gel suspended in benzene. The product was eluted from the column with benzene and a benzene–ethyl acetate mixture (95:5, v/v) to give 6.9 g (49%) of (*S*)-4.

2.5. (S)-6-(4-Carboxybutyl)-2,2′-dimethoxy-1,1′-binaphthyl [(S)-6]

To a suspension containing 3.1 g (7.0 mmol) of (*S*)-4 in 40 ml of ethanol, 5 ml of water and 0.60 g (11 mmol) of KOH were added. The suspension was refluxed for 15 min, cooled to 25°C and shaken with a mixture of 50 ml of water, 1 ml of conc. HCl and chloroform. The organic layer was dried and evaporated to give (*S*)-6-(3-carboxybutyryl)-2,2′-dimethoxy-1,1′-binaphthyl [(*S*)-5].

A mixture containing 20 g of Zn, 2 g of $HgCl_2$, 30 ml of water and 1 ml of conc. HCl was stirred for 10 min. The water was poured off and the amalgamated Zn was rinsed once with water. To the amalgamated Zn were added 15 ml of water, 35 ml of conc. HCl

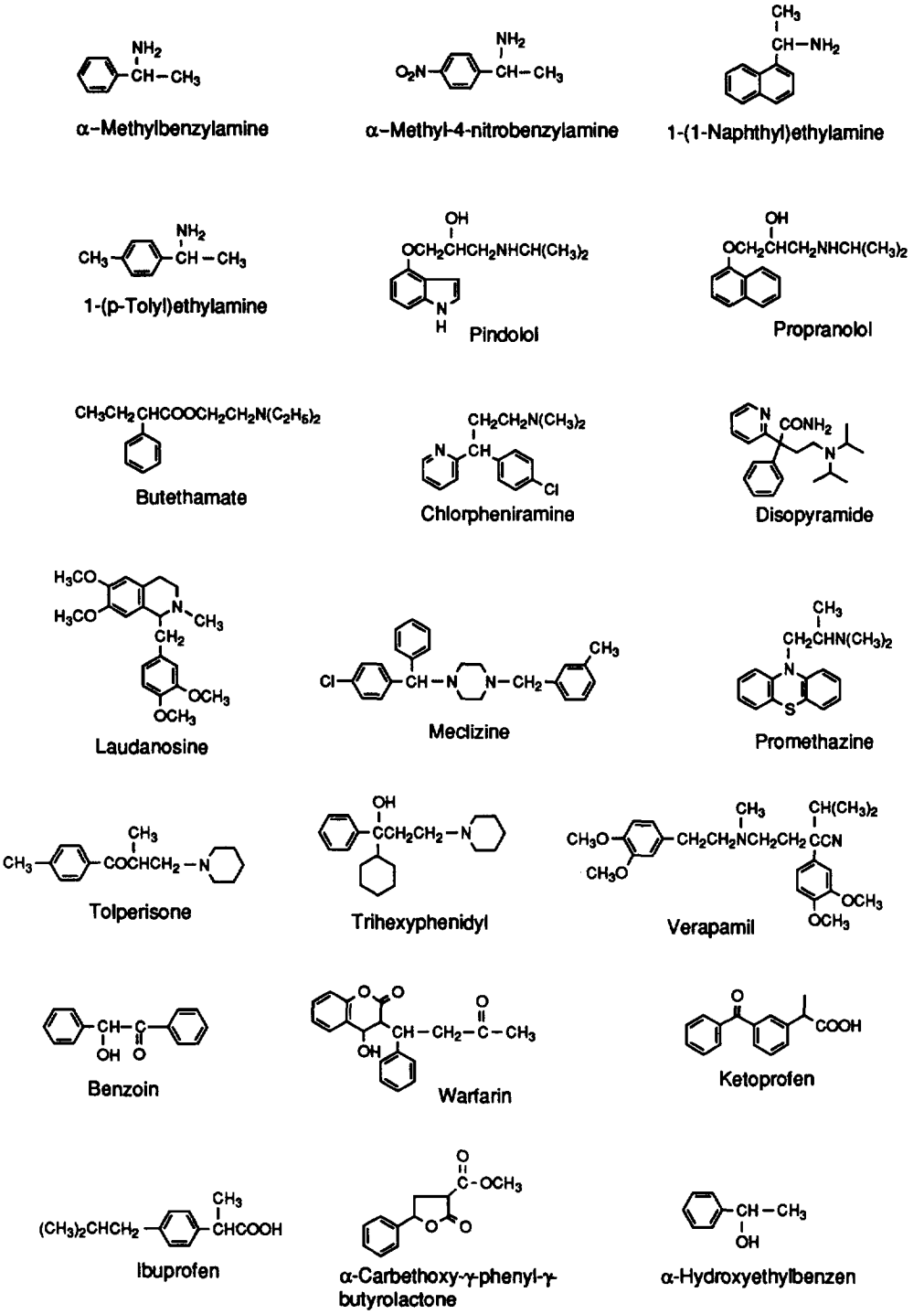

Fig. 1. Structure of analytes used to characterize CSP–BIN.

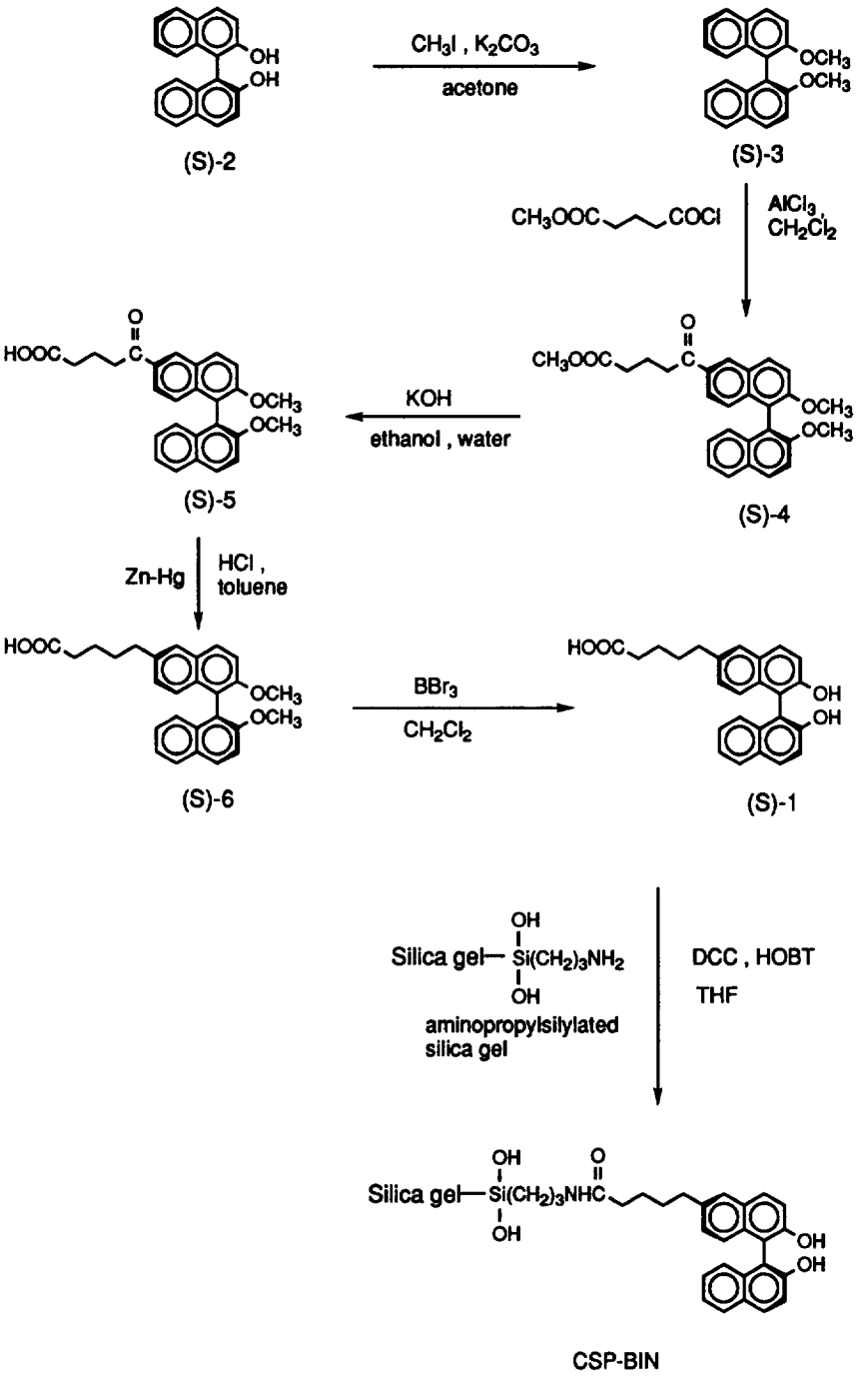

Fig. 2. Preparation procedure of CSP–BIN.

and the total amount of (*S*)-5. The mixture was vigorously refluxed for 24 h and 17 ml of conc. HCl was added twice during this period. The mixture was cooled to 25°C and shaken with 50 ml of water and 100 ml of chloroform. The organic layer was dried, evaporated and then chromatographed on 50 g of silica gel suspended in benzene. The product was eluted from the column with benzene and a benzene–ethyl acetate mixture (95:5 and 80:20, v/v) to gave 2.3 g (79%) of (*S*)-6.

2.6. (S)-6-(4-Carboxybutyl)-2,2'-dihydroxy-1,1'-binaphthyl [(S)-1]

To a solution containing 2.0 g (4.8 mmol) of (*S*)-6 in 150 ml of methylene chloride was added 1.8 ml (19 mmol) of BBr_3 at 0°C. The solution was stirred for 10 min at 0°C and the excess BBr_3 was decomposed, by dropwise addition of water. The mixture was shaken with 100 ml of water. The organic layer was dried, evaporated and chromatographed on 70 g of silica gel suspended in benzene. The product was eluted from the column with benzene and a benzene–ethyl acetate mixture (9:1, v/v) to give 0.93 g (50%) of (*S*)-1. Mass spectrum: *m/z* 386 (M^+). ^1H NMR (200 MHz): δ 1.65 (m, CH_2, 4H), 2.30 (m, $O{=}CCH_2$, 2H), 2.70 (m, $ArCH_2$, 2H), 7.4(m, ArH, 11H). The enantiomeric purity of (*S*)-1 was determined to be 100% e.e. by HPLC under the following conditions: column, Ceramospher Chiral RU-1 (150 × 4.6 mm I.D.) (Shiseido, Tokyo, Japan); mobile phase, acetic acid–methanol (0.5:100, v/v); flow-rate, 1 ml/min; temperature, 25°C; UV detection, 280 nm.

2.7. Preparation of aminopropylsilylated silica gel

Silica gel (10 g), in a vial, was dried under vacuum at 140°C for 8 h. After adding 38 ml of water, the vial was sealed, shaken and allowed to stand for 24 h. Then, 34 ml of toluene, 80 ml of triethylamine and 5 ml (29 mmol) of 3-aminopropyltrimethoxysilane were added, and the mixture was refluxed under N_2 for 16 h, cooled and filtered. The silica gel was washed sequentially with toluene, chloroform and methanol. The gel was stirred in a mixture containing 30 ml of acetonitrile and 20 ml of water for 1 h to hydrolyze the residual methoxy groups. The gel was filtered, washed with methanol and dried under vacuum at 120°C for 4 h.

To the silica gel were added 38 ml of toluene and 4.8 ml (23 mmol) of hexamethyldisilazane, to end-cap the residual silanols. The mixture was refluxed under N_2 for 16 h, cooled and filtered. The gel was washed sequentially with toluene, chloroform and methanol. To the silica gel were added 30 ml of acetonitrile and 20 ml of water, and the mixture was stirred for 30 min to hydrolyze the methylsilylated amino groups. The gel was filtered, washed with methanol and dried under vacuum at 120°C for 4 h to give aminopropylsilylated silica gel.

2.8. Preparation of binaphthol-bonded silica gel

Tetrahydrofuran (15 ml) was added to a mixture containing 3.5 g of aminopropylsilylated silica gel, 0.67 g (1.7 mmol) of (*S*)-1, 0.54 g (2.6 mmol) of 1,3-dicyclohexylcarbodiimide and 0.35 g (2.6 mmol) of 1-hydroxybenzotriazole [8], and the mixture was stirred for 24 h. To the mixture 0.54 g (2.6 mmol) of 1,3-dicyclohexylcarbodiimide, 0.35 g (2.6 mmol) of 1-hydroxybenzotriazole, 0.27 ml (4.8 mmol) of acetic acid and 5 ml of tetrahydrofuran were added. The mixture was stirred for an additional 24 h to acetylate the residual amino groups. The particles were filtered, washed sequentially with tetrahydrofuran, ethanol and hot ethanol and were then dried at 60°C, under vacuum, for 4 h to give the chiral stationary phase (CSP–BIN). The enantiomeric purity of CSP–BIN was estimated to be 100% e.e. because that of the condensed compound prepared from (*S*)-1 and butylamine by the same method as the preparation of CSP–BIN was determined to be 100% e.e. by HPLC under the same conditions as those used to determination of the enantiomeric purity of (*S*)-1 except that the mobile phase used was methanol.

2.9. Chromatographic measurements

The prepared CSP–BIN was packed into a stainless steel tube (150 × 4.6 mm I.D.) by conventional high-pressure slurry-packing procedures.

The HPLC system consisted of a pump (LC-10AD, Shimadzu, Kyoto, Japan), a UV detector

(SPD-10A, Shimadzu), a thermostated chamber (TCO-10AC, Shimadzu), a data processor (C-R4A, Shimadzu) and an injector (Model 7125, Rheodyne, USA). The dead time (t_0) of the column was determined by the retention time of 1,3,5-tri-*tert.*-butylbenzene [9]. The separation factor (α) between enantiomers was defined as

$$\alpha = k_2'/k_1'$$

where k_1' and k_2' are the capacity factors of the first and the second eluted enantiomers, respectively. The value of α is a measure of enantioselectivity.

3. Results and discussion

Table 1 shows the results of HPLC analysis of 21 racemic compounds using the CSP–BIN. The CSP–BIN exhibited strong retention and high enantioselectivity for secondary and tertiary amines. On the other hand, it exhibited weak retention and low enantioselectivity for compounds without an amino group. These results indicate that the hydroxyl groups of the binaphthyl moiety, that are designed to be left without modification in the present work, play an important role in the chiral recognition on the CSP–BIN.

3.1. Effect of trifluoroacetic acid in a mobile phase

As shown in Table 1, a mobile phase without acidic or basic additives gave peak tailing during the chromatography of amine analytes on the CSP–BIN. The effects of trifluoroacetic acid (TFA) added to a mobile phase on the k' value, the α value and the peak shape are given in Table 1. TFA reduced peak tailing of the basic analytes and improved the resolution between enantiomers with a secondary or tertiary amino group (Fig. 3). The addition of organic acids to a mobile phase is known to reduce the peak tailing caused by the interaction between acidic sites on stationary phases and basic analytes in normal-phase HPLC; ion-pair formation with the acid additive prevents the basic analyte from being adsorbed at acidic sites on the stationary phase [10,11].

Interestingly, TFA remarkably increased the k' values of tertiary amine analytes, but did not change those of primary and secondary amine analytes so much. TFA seems to strengthen specifically the hydrogen bonding between tertiary amines and the binaphthol moiety of CSP–BIN. TFA improved enantioselectivity for pindolol, butethamate, benzoin and α-carbetoxy-γ-phenyl-γ-butyrolactone. It also improved the resolution between enantiomers of propranolol, promethazine, tolperisone and trihexyphenidyl, due to sharpening their peaks, although it slightly decreased α values for these analytes.

3.2. Effects of alcohols as a component of the mobile phase

As shown in Table 1, the values of α and k' were larger, in general, on the CSP–BIN as the polarity of alcohols in a mobile phase containing TFA was lower. A similar tendency was reported on a N-(3,5-dinitrobenzoyl)leucine-derived stationary phase [12]; enantioselectivity decreased with increasing polarity of the mobile phase. Among the mobile phases examined, a 2-propanol–hexane mixture gave the largest α value for many analytes, but also gave rise to strong peak-tailing. An ethanol–hexane mixture tended to give the best shape of peaks and a little smaller α value. The mobile phase containing methanol gave better shape of peaks but a much smaller α value than did the 2-propanol–hexane mixture. Fig. 4 shows the chromatograms of promethazine, as an example, obtained with the mobile phases containing each alcohol. Although 2-propanol is widely used as a polar component in a mobile phase in normal-phase HPLC for chiral separation, the best resolution between enantiomers on the CSP–BIN was achieved mostly using ethanol.

The value of k' increased as the ethanol content in the mobile phase was decreased. Fig. 5 shows the relation between the values of k' and α, for secondary and tertiary amines. The increase in k' value, which is associated with the decrease of the polarity (or ethanol content) in the mobile phase, led to an increase in the α value for many analytes. This result is well understood by considering the effect of hydrogen bonding between analytes and the hydroxyl

Table 1
Separation of racemates on (*S*)-binaphthol CSP

Number	Compound	Mobile phase		k_1'[b]	α	Peak shape[c]
		additive[a]	solvent			
Primary amines						
1	α-Methylbenzylamine	no	Ethanol–Hexane (2:98, v/v)	25.98	1	T
		TFA	Ethanol–Hexane (2:98, v/v)	27.75	1	S
		DEA	Ethanol–Hexane (2:98, v/v)	4.247	1	S
2	α-Methyl-4-nitrobenzylamine	no	Ethanol–Hexane (5:95, v/v)	23.00	1	S
		TFA	Ethanol–Hexane (5:95, v/v)	34.03	1	S
		DEA	Ethanol–Hexane (5:95, v/v)	10.00	1	L
3	1-(1-Naphthyl)ethylamine	no	Ethanol–Hexane (5:95, v/v)	13.09	1	T
		TFA	Ethanol–Hexane (5:95, v/v)	13.57	1	S
		DEA	Ethanol–Hexane (5:95, v/v)	4.721	1	L
4	1-(*p*-Tolyl)ethylamine	no	Ethanol–Hexane (2:98, v/v)	23.19	1	T
		TFA	Ethanol–Hexane (2:98, v/v)	24.46	1	T
		DEA	Ethanol–Hexane (2:98, v/v)	4.052	1	S
Secondary amines						
5	Pindolol	no	Ethanol–Hexane (20:80, v/v)	12.35	1.029	T
		TFA	Ethanol–Hexane (20:80, v/v)	11.24	1.036	L
		TFA	IPA–Hexane (20:80, v/v)	22.87	1.065	T
		DEA	Ethanol–Hexane (10:90, v/v)	12.16	1.037	T
		DEA	IPA–Hexane (10:90, v/v)	22.00	1	T
6	Propranolol	no	Ethanol–Hexane (20:80, v/v)	5.99	1.042	T
		TFA	Ethanol–Hexane (20:80, v/v)	5.44	1.033	S
		TFA	IPA–Hexane (20:80, v/v)	8.36	1.070	T
		DEA	Ethanol–Hexane (1:99, v/v)	10.25	1.079	T
		DEA	IPA–Hexane (1:99, v/v)	12.20	1.096	T
Tertiary amines						
7	Butethamate	no	Ethanol–Hexane (20:80, v/v)	2.876	1	S
		TFA	Ethanol–Hexane (20:80, v/v)	13.18	1.031	S
		TFA	IPA–Hexane (20:80, v/v)	25.00	1.048	S
		DEA	Ethanol–Hexane (1:99, v/v)	0.706	1	S
8	Chlorpheniramine	no	Ethanol–Hexane (30:70, v/v)	13.90	1	L
		TFA	Ethanol–Hexane (30:70, v/v)	20.88	1	L
		DEA	Ethanol–Hexane (1:99, v/v)	5.27	1	L
9	Disopyramide	no	Ethanol–Hexane (20:80, v/v)	47.32	1	L
		TFA	Ethanol–Hexane (20:80, v/v)	53.24	1	L
		DEA	Ethanol–Hexane (5:95, v/v)	8.839	1.058	S
		DEA	IPA–Hexane (5:95, v/v)	8.308	1.060	T
		DEA	Methanol–Hexane (5:95, v/v)	11.44	1	S
10	Laudanosine	no	Ethanol–Hexane (20:80, v/v)	28.00	1	T
		TFA	Ethanol–Hexane (20:80, v/v)	52.55	1.036	S
		DEA	Ethanol–Hexane (1:99, v/v)	13.72	1	S
11	Meclizine	no	Ethanol–Hexane (10:90, v/v)	1.388	1	T
		TFA	Ethanol–Hexane (10:90, v/v)	31.45	1	S
		DEA	Ethanol–Hexane (1:99, v/v)	0.974	1	S

Continued on next page.

Table 1
Continued

Number	Compound	Mobile phase		k_1'[b]	α	Peak shape[c]
		additive[a]	solvent			
12	Promethazine	no	Ethanol–Hexane (20:80, v/v)	7.248	1.113	T
		TFA	Ethanol–Hexane (20:80, v/v)	14.76	1.107	S
		TFA	IPA–Hexane (20:80, v/v)	29.31	1.141	S
		TFA	Methanol–Ethanol–Hexane (10:10:80, v/v)	10.61	1.074	L
		DEA	Ethanol–Hexane (1:99, v/v)	2.189	1	S
13	Tolperisone	no	Ethanol–Hexane (20:80, v/v)	6.993	1.073	T
		TFA	Ethanol–Hexane (20:80, v/v)	14.80	1.059	S
		TFA	IPA–Hexane (20:80, v/v)	27.87	1.055	T
		TFA	Methanol–ethanol–hexane (10:10:80, v/v)	10.54	1.055	L
		DEA	Ethanol–Hexane (1:99, v/v)	0.990	1.079	S
14	Trihexyphenidyl	no	Ethanol–Hexane (10:90, v/v)	6.338	1.108	T
		TFA	Ethanol–Hexane (20:80, v/v)	8.189	1.082	S
		TFA	IPA–Hexane (20:80, v/v)	14.32	1.109	T
		TFA	Methanol–Ethanol–Hexane (10:10:80, v/v)	6.363	1.055	T
		DEA	Ethanol–Hexane (1:99, v/v)	0.679	1	S
15	Verapamil	no	Ethanol–Hexane (40:60, v/v)	16.44	1.063	L
		TFA	Ethanol–Hexane (40:60, v/v)	21.29	1.076	L
		TFA	IPA–Hexane (40:60, v/v)	77.04	1.065	T
		DEA	Ethanol–Hexane (10:90, v/v)	9.336	1.050	S
Compounds without amino groups						
16	Benzoin	no	Ethanol–Hexane (1:99, v/v)	4.845	1	S
		TFA	Ethanol–Hexane (1:99, v/v)	4.558	1.016	S
		DEA	Ethanol–Hexane (1:99, v/v)	4.272	1	S
17	Warfarin	no	Ethanol–Hexane (5:95, v/v)	27.59	1	T
		TFA	Ethanol–Hexane (5:95, v/v)	13.95	1	S
		DEA	Ethanol–Hexane (20:80, v/v)	38.64	1	T
18	Ketoprofen	no	Ethanol–Hexane (5:95, v/v)	51.69	1	T
		TFA	Ethanol–Hexane (1:99, v/v)	19.37	1	S
		DEA	Ethanol–Hexane (20:80, v/v)	33.80	1	T
19	Ibuprofen	no	Ethanol–Hexane (1:99, v/v)	11.48	1	T
		TFA	Ethanol–Hexane (1:99, v/v)	2.284	1	S
		DEA	Ethanol–Hexane (5:95, v/v)	39.62	1	T
20	α-Carbetoxy-γ-phenyl-γ-butyrolactone	no	Ethanol–Hexane (1:99, v/v)	7.527	1.036	L
		TFA	Ethanol–Hexane (1:99, v/v)	7.291	1.047	S
		DEA	Ethanol–Hexane (1:99, v/v)	5.686	1.036	S
21	α-Hydroxyethylbenzene	no	Ethanol–Hexane (1:99, v/v)	2.837	1	S
		TFA	Ethanol–Hexane (1:99, v/v)	2.613	1	S
		DEA	Ethanol–Hexane (1:99, v/v)	2.779	1	S

[a]TFA, containing 13 m*M* trifluoracetic acid; DEA, containing 9.7 m*M* diethylamine.
[b]Capacity factor of first eluted enantiomer.
[c]T = tailing; L = leading and S = symmetry.

groups of the binaphthol moiety on the CSP–BIN. The complexation via hydrogen bonds becomes stronger by decreasing the ethanol content, resulting in an increase of the k' value. This positive correlation between the values of α and k' suggests that the unmodified hydroxyl groups of the binaphthol moie-

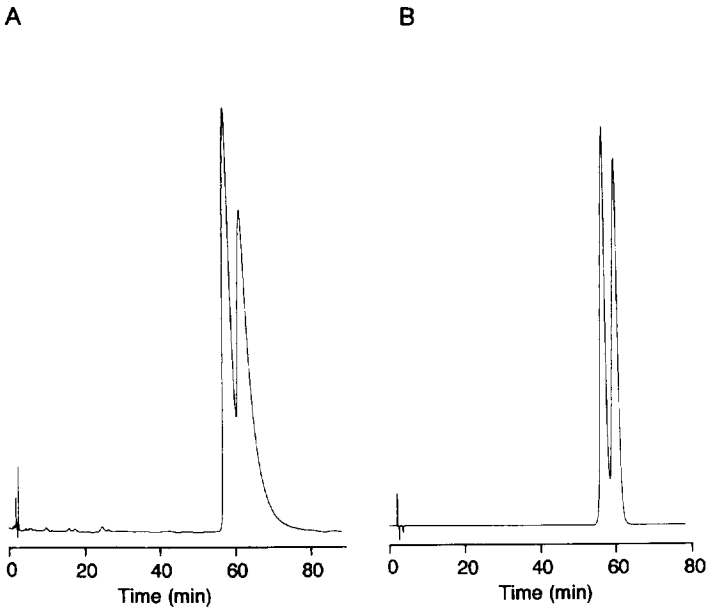

Fig 3. Chromatogram of propranolol. HPLC conditions: stationary phase, CSP–BIN; mobile phase, (A) ethanol–hexane (5:95, v/v); (B) ethanol–hexane (5:95, v/v) containing 12 m*M* TFA; flow-rate, 1 ml/min; UV detection, 254 nm; temperature, 25°C.

ty play a very important role in the chiral recognition on the CSP–BIN. For a few analytes, however, the increase in the k' value led to a decrease in the α value.

3.3. Effect of amines in the mobile phase

Amines are often added to a mobile phase in normal-phase HPLC in order to reduce peak tailing

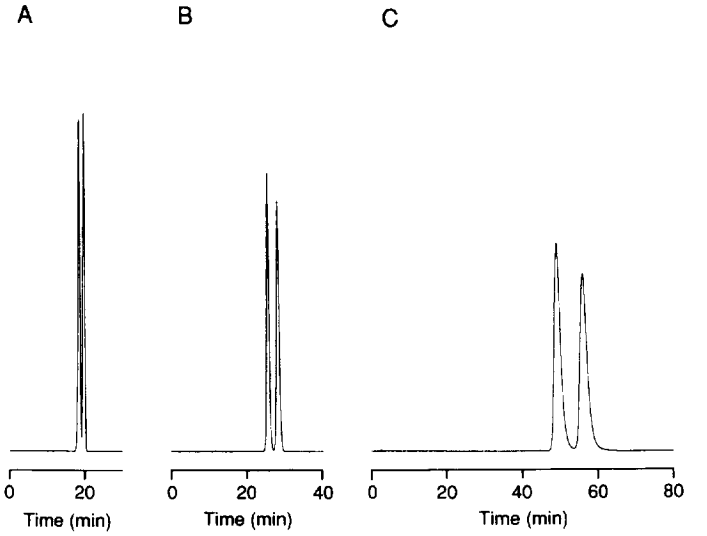

Fig. 4. Chromatogram of promethazine. The same HPLC conditions were used as for Fig. 3 except for the mobile phase. (A) methanol–ethanol–hexane (10:10:80, v/v) containing 12 m*M* TFA; (B) ethanol–hexane (20:80, v/v) containing 12 m*M* TFA; (C) 2-propanol–hexane (20:80, v/v) containing 12 m*M* TFA.

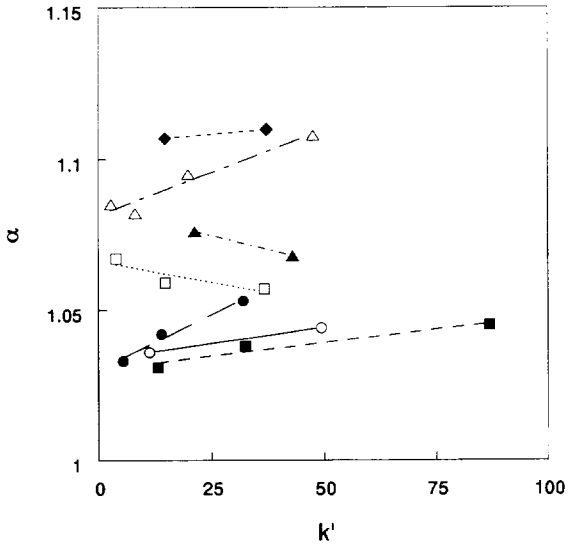

Fig. 5. Relationship between k' and α. The HPLC conditions used were the same as for Fig. 3, except for the mobile phase; the ethanol–hexane ratio containing 12 m*M* TFA was changed. Analytes: ○ = pindolol; ● = propranolol; ■ = butethamate; ◇ = promethazine; □ = tolperisone; △ = trihexyphenidyl and ▲ = verapamil.

caused by an interaction between acidic sites on stationary phases and basic analytes [10,13]. In HPLC using chiral stationary phases, amines are used also as anti-tailing agents for basic analytes, to improve resolution between enantiomers [14].

As shown in Table 1, a small amount of diethylamine (DEA) added to a mobile phase greatly reduced the k' value and peak-tailing for basic analytes on the CSP–BIN. These results indicate that DEA blocked the acidic sites on the stationary phase and prevented the analytes from forming hydrogen bonds. DEA remarkably deteriorated the enantioselectivity for promethazine and trihexyphenidyl. This is reasonable because amine additives block hydroxyl groups of the binaphthol moiety as well as residual silanols. On the other hand, a pronounced improvement of the α value was observed for disopyramide.

Fig. 6 shows the relationship between the values of k' and α for several basic analytes when primary, secondary and tertiary amines were used as additives to the mobile phase. The k' value tended to be larger with an increase in the carbon number of the amine additives. In the case of analytes with a tertiary amino group, the values of k' and α were correlated linearly, irrespective of the structure of the amine additives. Conversely, in the case of analytes with a secondary amino group (pindolol and propranolol), the correlation between the values of k' and α became non-linear, when primary amines indicated

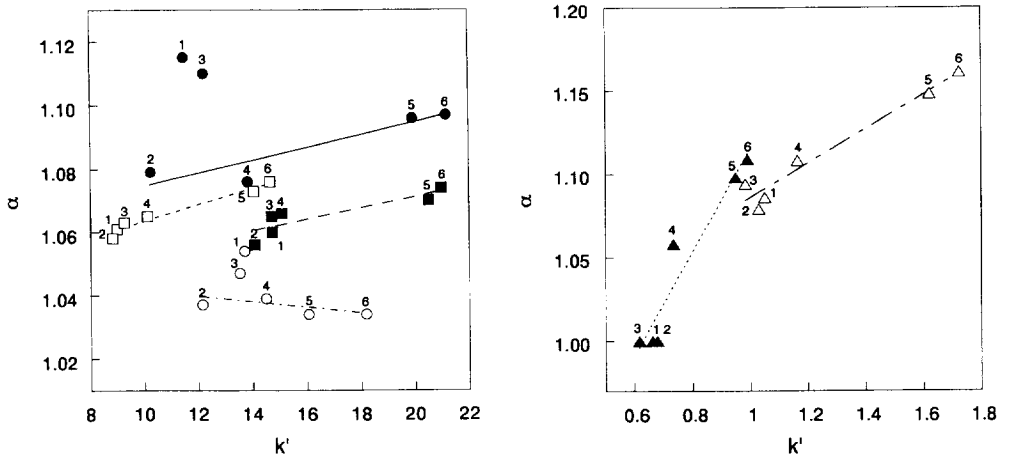

Fig. 6. Relationship between k' and α, when various amines were used as a modifier of the mobile phase. The amines added were (1) butylamine; (2) diethylamine; (3) hexylamine; (4) dipropylamine; (5) triethylamine and (6) dimethylbutylamine. Analytes: ● = propranolol; ○ = pindolol; □ = disopyramide; ■ = verapamil; △ = tolperisone and ▲ = trihexyphenidyl. The same HPLC conditions were used as for Fig. 3, except for the mobile phase: (●, △, ▲) ethanol–hexane (1:99, v/v) containing 9.7 m*M* amine; (□, ■) ethanol–hexane (5:95, v/v) containing 9.7 m*M* amine; (○) ethanol–hexane (10:90, v/v) containing 9.7 m*M* amine.

by 1 and 3 in Fig. 6 were added; the primary amine additives exhibited extraordinarily large α values. Although it was reported that bulky amine additives disimproved enantioselectivity when pindolol was separated on the CSP derived from glycyl-L-proline [15], bulky amines did not effect enantioselectivity on the CSP–BIN.

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