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Investigation on the chiral discrimination mechanism using an axially asymmetric binaphthalene-based stationary phase for high-performance liquid chromatography

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ABSTRACT

The chiral discrimination mechanism of 3,5-dinitrophenyl-derivatized enantiomeric alcohols, amines and carboxylic acids using a chiral stationary phase (CSP) prepared by bonding (*aS*)-1,1′-binaphthyl-2,2′-dicarboxylic acid to 3-aminopropylsilanized silica gel was investigated. Studies of the elution behaviour of a series of structurally related analytes on the CSP and ^{1}H NMR measurements of a solubilized model compound of the CSP and analytes indicated that a π-donor–acceptor interaction between one of the naphthalene planes of the CSP and the 3,5-dinitrophenyl ring of the analyte cooperates with the dipole stacking interaction between two sets of amide linkages of the CSP and the analytes to determine the stability of the diastereomeric adsorbates.

INTRODUCTION

The direct separation of enantiomers by high-performance liquid chromatography (HPLC) on chiral stationary phases (CSPs) has been the subject of intense investigations and a wide variety of CSPs have been developed [1–4]. Although many of the exact mechanisms of the chiral recognition by such CSPs still remain to be elucidated, so-called "brush-type" CSPs, which are based on chiral molecules bonded to silica gel, are known to be most amenable to rationalization by the use of chiral recognition models [3] and hence theoretical treatments [5,6]. Among such models, those based on the π-donor–acceptor interactions between CSPs and analytes, as proposed by Pirkle and co-workers [7–9], have been the most successful, giving a good guide for the design of novel CSPs of predictable performance.

In a previous paper, we described the preparation and performance of CSPs derived from axially asymmetric 2′-substituted-1,1′-binaphthyl-2-carboxylic acids bonded to aminoalkylsilanized silica gels through an amide linkage by use of the 2-carboxylic function [10]. The 2′-substituents tested included –CN, –COOH, $-CONH_2$, –CONHEt, $-CONEt_2$ and $-OCH_3$. At first, we suspected that incorporation of highly polar substituents such as –COOH and $-CONH_2$ might be disadvantageous owing to non-stereoselective, excessive selector–analyte interactions via strong hydrogen bonding causing

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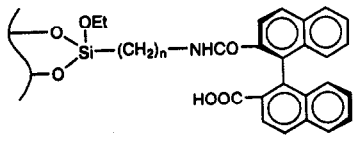

Fig. 1. Structure of CSP 1.

too much retention and peak tailing [11,12]. On the contrary, however, it has been found that a CSP with a 2'-carboxy substituent (CSP 1) is the best among those tested (Fig. 1); CSP 1 discriminates a wide range of enantiomeric amino acids, amines and alcohols as their 3,5-dinitrophenyl derivatives and biaryls bearing 2,2'-polar substituents in normal-phase HPLC [10]. Effective chiral discrimination of enantiomeric alcohols as the 3,5-dinitrophenylcarbamates is the most characteristic feature of CSP 1, and a tentative chiral discrimination model of such analytes has also been presented, in which simultaneous π-donor–acceptor interactions and dipole stacking interactions between the selector and the analyte play a critical role (Fig. 2). This paper presents the results of related investigations performed to shed more light on the mechanism by studying the HPLC behaviour of a series of structurally related analytes on CSP 1 and ^{1}H NMR measurements of a model compound of the CSP and analytes.

EXPERIMENTAL

General

Liquid chromatography was performed using a Shimadzu LC-6A apparatus equipped with a Shimadzu SPD-6A ultraviolet detector set at 254 nm.

IR spectra were measured on a Shimadzu IR-460 grating spectrophotometer. ^{1}H NMR spectra were recorded on a JEOL JNM-FX 60 instrument at 60 MHz or a Bruker AC-250T instrument at 250 MHz in the ^{2}H lock mode with tetramethylsilane as an internal standard.

Optical rotations were recorded on a Union PM-101 automatic digital polarimeter in a 1-cm cell.

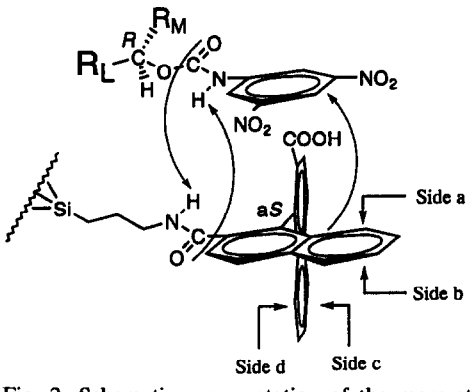

Fig. 2. Schematic representation of the more stable adsorbates formed from CSP 1 and derivatized (*R*)-alcohols.

Materials

Commercial materials were used as received unless stated otherwise. Solvents used for HPLC were distilled before use. Diethyl ether, tetrahydrofuran (THF) and dioxane were distilled from sodium diphenylketyl. Dimethylformanide (DMF), hexamethylphosphoric triamide (HMPA) and octylamine were distilled from calcium hydride under reduced pressure. Other amines and dichloromethane were distilled from calcium hydride. These materials were stored under nitrogen. Water-sensitive reactions were routinely carried out in a nitrogen atmosphere. Merck silica gel $60GF_{254}$ was used for analytical and preparative thin-layer chromatography (TLC). Column chromatography was performed using Nacalai Tesque silica gel 60.

Acyl azides used for the derivatization of 1-phenylethanol were prepared as described [13].

Chiral stationary phase

CSP 1 was as used in a previous study [10], prepared by bonding (*aS*)-1,1'-binaphthyl-2,2'-dicarboxylic acid (**5**) to 3-aminopropylsilanized silica gel (spherical 5-μm particles, microsphere diameter 100 Å); 0.47 mmol binaphthyl unit/g gel.

Preparation of phenyl-substituted homologous alcohols and carboxylic acids

Enantiomeric (*R*)-(+)-1-phenylethanol (**2a**), (*R*)-(−)-2-phenylpropionic acid (**4a**) and (*R*)-(−)-3-phenylbutanoic acid (**4b**) were commercially available, and were used for the preparation of the phenyl-substituted homologous alcohols and carboxylic acids of known enantio-

meric composition. Typical preparations are as follows.

3-Phenyl-1-butanol (2c). To a stirred suspension of $LiAlH_4$ (1.26 g, 33.2 mmol) in dry diethyl ether (10 ml) was added a solution of 3-phenylbutanoic acid (**4b**) (1.86 g, 11.3 mmol) in diethyl ether (40 ml) under a nitrogen atmosphere and the mixture was refluxed for 1.5 h. After the reaction had cooled to 0°C, 2 *M* HCl (50 ml) was added slowly to the mixture. The organic layer was separated and the aqueous layer was extracted with diethyl ether (2 × 20 ml). The combined organic layer was washed with 2 *M* Na_2CO_3 (30 ml) and saturated aqueous NaCl solution (2 × 30 ml) and dried over $MgSO_4$. After filtration, the solvent was removed *in vacuo* to give 1.60 g of **2c** (94% yield). ^1H NMR (C^2HCl_3), δ (ppm) 1.28 (3H, d, CH_3), 1.34 (1H, s, OH), 1.82 (2H, m, CH_2), 2.86 (1H, m, CH), 3.60 (2H, t, CH_2), 7.20 (5H, s, Ar-H); IR (liquid film) (cm^{-1}), 3330, 3055, 2950, 1601, 1493, 1450, 1374, 1047, 762, 700.

1-Bromo-3-phenylbutane. To a mixture of **2c** (1.40 g, 9.32 mmol) and pyridine (0.81 g, 10.2 mmol) was added tribromophosphine (3.28 g, 12.1 mmol) slowly at −10°C. The mixture was then heated to 100°C and kept at this temperature for 15 h. After 2 *M* HCl (30 ml) and diethyl ether (30 ml) had been added to the mixture, it was stirred for 15 min and the precipitate was filtered off, then the solids were rinsed with diethyl ether. The organic layer was separated and the aqueous layer was extracted with diethyl ether (2 × 20 ml). The combined organic layer was washed with 1 *M* Na_2SO_3 (30 ml), 2 *M* Na_2CO_3 (2 × 30 ml) and with saturated aqueous NaCl solution (2 × 30 ml) and dried over $MgSO_4$. After filtration, the solvent was evaporated *in vacuo* and the residue was distilled by the Kugelrohr method under reduced pressure to give 1.70 g of 1-bromo-3-phenylbutane (86% yield). ^1H NMR (C^2CHl_3), δ (ppm) 1.27 (3H, d, CH_3), 2.08 (2H, m, CH_2), 2.65–3.51 (3H, m, CH_2, CH), 7.20 (5H, s, Ar-H); IR (liquid film) (cm^{-1}), 3055, 2950, 1601, 1491, 1453, 1375, 1258, 762, 700.

4-Phenylpentanoic acid (4c). To magnesium turnings (1.56 g, 64.1 mmol) in dry diethyl ether (25 ml) was added 1,2-dibromoethane (1.20 g, 6.41 mmol) under a nitrogen atmosphere and the mixture was irradiated with ultrasound (35 W, 41 kHz) for 30 min. To the activated magnesium was added dropwise a solution of 1-bromo-3-phenylbutane (1.37 g, 6.41 mmol) in diethyl ether (30 ml) over 1 h under ultrasound irradiation and the mixture was irradiated under reflux for 1 h to give a solution of 3-phenylbutylmagnesium bromide. The solution of the Grignard reagent was then added to crushed dry-ice (50 g) and the mixture was allowed to warm to room temperature. The mixture was extracted with 2 *M* NaOH (3 × 50 ml) and combined aqueous layer was washed with diethyl ether (2 × 30 ml). After the aqueous layer had been acidified with concentrated HCl and saturated with NaCl, the liberated carboxylic acid was extracted with diethyl ether (5 × 50 ml) and the combined organic layer was washed with saturated aqueous NaCl solution (2 × 50 ml) and dried over $MgSO_4$. After filtration, the solvent was removed *in vacuo* to give 0.89 g of **4c** (78% yield). ^1H NMR (C^2HCl_3), δ (ppm) 1.27 (3H, d, CH_3), 1.92 (2H, m, CH_2), 2.23 (2H, t, CH_2), 2.73 (1H, m, CH), 7.16–7.32 (5H, m, Ar-H), 11.0 (1H, br, COOH); IR (liquid film) (cm^{-1}), 3500–2500, 3025, 2950, 1710, 1450, 1283, 1221, 938, 762, 700.

Other compounds. 2-Phenyl-1-propanol (**2b**), 4-phenyl-1-pentanol (**2d**) and 5-phenylhexanoic acid (**4d**) were prepared similarly as above.

2b: ^1H NMR (C^2HCl_3), δ (ppm) 1.23 (3H, d, CH_3), 1.36 (1H, s, OH), 2.90 (1H, m, CH), 3.66 (2H, d, CH_2), 7.20 (5H, s, Ar-H); IR (liquid film) (cm^{-1}), 3350, 3030, 2960, 1605, 1500, 1455, 1040, 1020, 760, 700.

2d: ^1H NMR (C^2HCl_3), δ (ppm) 1.26 (3H, d, CH_3), 1.34–1.68 (5H, m, CH_2 and OH), 2.69 (1H, m, CH), 3.56 (2H, t, CH_2), 7.14–7.31 (5H, m, Ar-H); IR (liquid film) (cm^{-1}), 3330, 3025, 2950, 1600, 1491, 1448, 1058, 760, 700.

4d: ^1H NMR (C^2HCl_3), δ (ppm) 1.24 (3H, d, CH_3), 1.47–1.63 (4H, m, CH_2), 2.30 (2H, t, CH_2), 2.69 (1H, m, CH), 7.15–7.32 (5H, m, Ar-H), 10.9 (1H, br, COOH); IR (liquid film) (cm^{-1}), 3500–2500, 3025, 2950, 1709, 1453, 1287, 1221, 938, 762, 700.

Preparation of derivatized enantiomeric analytes [14]

Derivatization of alcohols. Carbamates of 1-

phenylethanol were prepared by treating an excess amount of isocyanates or acyl azides (the azides were thermally converted *in situ* into the isocyanates [14]) with 1-phenylethanol (**2a**) in dioxane in the presence of triethylamine as described before for the preparation of 1′-phenylethyl 3,5-dinitrophenylcarbamate (**2α**) [10]. 3,5-Dinitrophenylcarbamates (**2β–δ**) of phenylalkanols (**2b–d**) were similarly prepared.

Derivatization of amines. The preparation of N′-(1′-phenylethyl) - N - (3,5-dinitrophenyl)urea (**3a**) is representative. 1-Phenylethylamine (20 mg, 0.17 mmol) was added to a solution of 3,5-dinitrophenyl isocyanate (50 mg, 0.24 mmol) in dioxane (1 ml) (the azide could not be used because it reacted with amines to give the corresponding amides) and stirred at room temperature for 30 min. After 3-dimethylaminopropylamine (20 μl) had been added to the mixture to remove excess isocyanate, it was subjected to TLC to give a sample of **3a**.

Derivatization of carboxylic acids. The preparation of 2′-phenylpropion-3,5-dinitroanilide (**4α**) is representative. A mixture of 2-phenylpropionic acid (**4a**) (20 mg, 0.13 mmol), 3,5-dinitroaniline (24 mg, 0.13 mmol), 1,3-dicyclohexylcarbodiimide (DCC) (54 mg, 0.26 mmol) and pyridine (10 μl) in dichloromethane (1 ml) was stirred at room temperature for 24 h and then subjected to TLC to give a sample of **4α**.

Although the reaction was much accelerated by the use of 4-dimethylaminopyridine in place of pyridine as the base, the derivatization of enantiomerically pure carboxylic acids resulted in the formation of the racemic anilides.

Preparation of model compounds for NMR analysis

The preparation of atropisomerically pure 1,1′-binaphthyl-2,2′-dicarboxylic acid (**5**) has been previously reported [15–17].

(aS)-2′-Octylcarbamoyl-1, 1′-binaphthyl-2-carboxylic acid [(aS)-1]. To a stirred solution of (*aS*)-**5** (1.00 g, 2.92 mmol) in THF (30 ml) was added a solution of DCC (0.602 g, 2.92 mmol) in THF (20 ml) at room temperature for 1 h under a nitrogen atmosphere. The mixture was stirred for another 2 h at that temperature and then heated at reflux for 4 h. After cooling to room temperature, triethylamine (0.5 ml) and octylamine (0.453 g, 3.50 mmol) were added, and the mixture was heated at reflux for 3 h. The mixture was allowed to cool to room temperature, precipitated N,N′-dicyclohexylurea was filtered off and the solids were rinsed with small portions of THF. The solvent was distilled from the filtrate under reduced pressure and the residue was dissolved in chloroform (50 ml). The solution was washed with concentrated HCl (2 × 50 ml) and then with water (4 × 50 ml) and dried over $MgSO_4$. After filtration, the solvent was evaporated *in vacuo* to give 1.29 g of a mixture of unchanged (*aS*)-**5**, (*aS*)-**1** and (*aS*)-2,2′-bis(octylcarbamoyl)-1,1′-binaphthyl.

As the separation of the mixture as such into each component was difficult, the desired (*aS*)-**1** was purified via the methyl ester as follows. The mixture was dissolved in HMPA (10 ml) and then a 25% (w/w) aqueous solution of NaOH (1.1 ml) was added. After stirring for 1 h at room temperature, methyl iodide (1.2 ml) was added to the solution and stirring was continued for another 1 h. Then, was added 2 *M* HCl (20 ml) to the mixture, which was then extracted with diethyl ether (3 × 20 ml). The combined organic layer was washed with 2 *M* HCl (2 × 20 ml) and water (2 × 20 ml) and dried over $MgSO_4$. After filtration, the solvent was evaporated *in vacuo* to give 1.14 g of the residue, which was chromatographed on a silica gel column (100 g) with hexane–ethyl acetate (2.5:1) as eluent to give 0.66 g of the methyl ester of (*aS*)-**1**. This was then dissolved in ethanol (10 ml) with warming, and a solution of KOH (1.0 g) in water (3 ml) was added. After the mixture had been heated at reflux for 3 h, volatiles were removed under reduced pressure. The residue was dissolved in water (40 ml) and washed with diethyl ether (2 × 10 ml) to remove non-acidic compounds. The aqueous layer was acidified with concentrated HCl and the resulting precipitate was extracted with ethyl acetate (3 × 20 ml). The combined organic layer was washed with water (3 × 20 ml) and dried over $MgSO_4$. After filtration, the solvent was removed *in vacuo* to give 0.53 g of (*aS*)-**1** as a colourless glass [40% yield based on the starting (*aS*)-**5**].

$[\alpha]_D^{20}$ −106° (*c* 1.00, acetone); ^{1}H NMR (C^2HCl_3), δ (ppm) 0.75–1.29 (15H, m, CH_2, CH_3), 3.00 (2H, m, CH_2), 6.62 (1H, m, NH), 6.90–8.01 (12H, m, Ar-H); IR (KBr) (cm^{-1}), 3750–2600, 3390, 2915, 1696, 1591, 1552, 822, 760.

(S)-1'-Phenylethyl 3,5-dinitrophenylcarbamate [(S)-2α]. A solution of (*S*)-1-phenylethanol (**2a**) {0.28 g, 2.3 mmol; $[\alpha]_D^{23} = -41.3°$ (neat)}, 3,5-dinitrobenzoylazide (0.82 g, 3.5 mmol) and one drop of triethylamine in dioxane (10 ml) was stirred at 100°C for 1 h; the azide was converted into the isocyanate *in situ* and allowed to react with the alcohol. To the solution was added 3-dimethylaminopropylamine (0.5 ml) to remove excess isocyanate and the solvent was removed *in vacuo*. The residue was purified by silica gel column chromatography (100 g) with hexane–ethyl acetate (6:1) as eluent to give 0.76 g of (*S*)-**2α** as a pale yellow solid (94% yield). ^{1}H NMR (C^2HCl_3), δ (ppm) 1.65 (3H, d, CH_3), 5.94 (1H, q, CH), 7.28 (1H, br, NH), 7.26–7.41 (5H, m, Ar-H), 8.62 [2H, d, *o*-H of 3,5-$(NO_2)_2C_6H_3$], 8.68 [1H, t, *p*-H of 3,5-$(NO_2)_2C_6H_3$].

By using (*R*)-1-phenylethanol {$[\alpha]_D^{23} = +42°$ (neat)}, (*R*)-**2α** was similarly prepared as above.

^{1}H NMR chemical shift measurements

Samples for ^{1}H NMR measurements were prepared by diluting measured amounts of (*aS*)-**1** and (*S*)- or (*R*)-**2α** to 0.1 mol dm^{-3} with C^2HCl_3. All measurements were carried out at 250 MHz at 20°C.

RESULTS AND DISCUSSION

As is generally recognized, enantiomer separation on a CSP requires that the analyte should contain properly arranged functionalities, which may be either steric or electronic in nature, for interaction with complementary sites in the CSP [3]. Fig. 2 shows the proposed model for the more stable adsorbates of enantiomeric alcohols as the 3,5-dinitrophenylcarbamates on CSP 1 [10]. The plane which contains the CSP amide linkage, –CO–NH–CH–, is twisted *ca.* 50° downward from the connected naphthalene plane, and hence the lower side of the amide plane is shielded by the lower half of the vertical naphthalene (side d). This in turn means that side d of the said naphthalene plane is blocked by the amide hydrogen and the connecting arm of the CSP, allowing the dipole stacking interaction between the two sets of the amide linkages of the selector and analyte only from upper side of the horizontal naphthalene plane (side a). Thus, only the overlap of the 3,5-dinitrophenyl ring with the horizontal naphthalene plane on side a can cooperate with the dipole stacking interaction between two sets of the properly arranged amide linkages of the selector and analyte, resulting in the *R* enantiomer of the carbamate analyte being more retained by the chiral selector bearing the (*aS*)-binaphthyl axis. The conformation of the analyte in Fig. 2 is different from that postulated by Pirkle and House [18,19] to be the most heavily populated in solution, in that the carbinyl hydrogen is not eclipsed with the carbonyl oxygen. The latter conformational arrangement, however, has frequently been precedented. For example, Lipkowitz *et al.* [5] showed an example where a model carbamate has torsion angle defined by the carbonyl C=O and carbinyl C–H bonds is 60°, as suggested by MM2C and MM2D force-field calculations and MNDO semi-empirical molecular orbital methods (a similar *gauche* disposition of the carbonyl oxygen and the carbinyl hydrogen was also postulated by Uccelo-Barretta *et al.* [20]). Further, it is known that the energy barriers separating minima of conformational isomers are usually between 2 and 5 kcal mol^{-1} (1 kcal = 4.184 kJ), ensuring rapid interconversion between the conformational states. Therefore, it may not be unreasonable to assume that the balance between the steric repulsion and lipophilic interaction on approach of the analyte to the CSP allows a slight conformational change of the analyte to be approximated as suggested [10]. It has also been suggested that the π-donor–acceptor interactions may be possible between the CSP and analyte on both sides b and c, but seemingly they are non-stereoselective as space-filling Corey–Pauling–Koltun (CPK) model inspections indicate [10]. The following data support the soundness of the proposed

chiral discrimination mechanism of the 3,5-dinitrophenyl-derivatized analytes by CSP 1.

Importance of π-accepting site in the analyte

In order to assess the contribution of π-acidic sites of the analyte for chiral separation [18], ten carbamates were prepared from 1-phenylethanol (**2a**) and analysed on CSP 1. The data in Table I exhibit a large, although not monotonous, decrease in retention (k') and selectivity (α) with decrease in the electron-withdrawing ability of the aryl carbamate moiety. As expected, the retention and selectivity decrease considerably with the phenylcarbamates bearing an *ortho*-substituent, indicating that the vicinity of the amide nitrogen is vital for chiral recognition and the presence of a superfluous substituent in this region imposes severe steric repulsion between the selector and analyte. These results show that cooperation of the π-donor–acceptor interaction with the dipole stacking interaction between the selector and analyte plays the dominant role in the retention and chiral recognition by CSP 1, the highest retention and separation being obtained with the 3,5-dinitrophenylcarbamate. The longer retention of the 4-methoxyphenylcarbamate compared with the 4-methylphenyl counterpart, which is inconsistent with the order of the electronic effects of the substituents, may be indicative of the contribution of non-stereoselective hydrogen bonding interactions between the methoxyl oxygen and the 2′-carboxylic proton of the CSP. The isopropylcarbamate results in no separation with little retention, showing the critical importance of a π-accepting site in the analyte.

Table I also contains the results of the separation of enantiomeric 2-phenylpropionic acid (**4a**) as the various amides and will be discussed later.

^1H NMR studies of a model compound of CSP 1 and enantiomeric analytes

Although diastereomeric complexes formed from a working CSP and enantiomeric analytes may be significantly different from those of a solubilized model CSP analogue and analytes, it has been well demonstrated that NMR studies on rationally designed CSP model compounds and analytes can be a great aid in the elucidation of the chiral discrimination mechanism by CSPs [20,21].

In a previous paper [10], we reported a good separation of the 3,5-dinitrophenylcarbamates derived from enantiomeric alcohols by CSP 1; a separation factor (α) of 1.59 was obtained from the carbamate (**2α**) of 1-phenylethanol (**2a**) using

TABLE I

SEPARATION OF CARBAMATES OF 1-PHENYLETHANOL (**2a**) AND AMIDES OF 2-PHENYLPROPIONIC ACID (**4a**)

Mobile phases: hexane–2-propanol, (A) 95:5 and (B) 90:10. Flow-rate: 1 ml/min. k' = Capacity factor for the first-eluted enantiomer. The configuration of the first-eluted enantiomer is indicated in parentheses. The separation factor, α, is the ratio of the capacity factors of the enantiomers.

R	PhCH(CH$_3$)OCONH-R			PhCH(CH$_3$)CONH-R		
	Eluent	k'_1	α	Eluent	k'_1	α
3,5-(NO$_2$)$_2$C$_6$H$_3$	A	18.20(*S*)	1.59	B	14.18(*R*)	1.59
2,4-(NO$_2$)$_2$C$_6$H$_3$	A	1.44	1.00	B	2.10(*R*)	1.07
p-NO$_2$C$_6$H$_4$	A	10.89(*S*)	1.20	B	10.73(*S*)	1.32
3,5-Cl$_2$C$_6$H$_3$	A	3.21(*S*)	1.23	B	3.28(*R*)	1.40
2,4-Cl$_2$C$_6$H$_3$	A	0.34	1.00	B	0.71(*R*)	1.05
p-ClC$_6$H$_4$	A	3.10(*S*)	1.12	B	5.45(*R*)	1.22
C$_6$H$_5$	A	2.37(*S*)	1.06	B	3.77(*R*)	1.14
p-CH$_3$C$_6$H$_4$	A	1.98(*S*)	1.05	B	3.33(*R*)	1.14
p-CH$_3$OC$_6$H$_4$	A	4.04(*S*)	1.03	B	6.37(*R*)	1.11
i-C$_3$H$_7$	A	0.86	1.00	B	1.74(*R*)	1.06

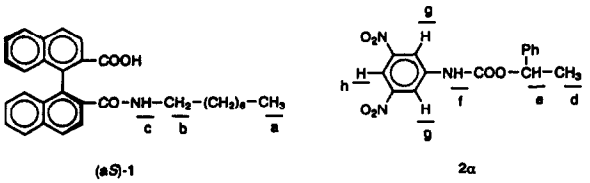

Fig. 3. Structures of model compounds of CSP 1 and analytes.

5% 2-propanol in hexane as the eluent (Table I). As chiral separation by the axially asymmetric binaphthalene-based CSPs seemed almost independent on the length of the arms connecting to the solid support [10], we chose (*aS*)-2′-octylcarbamoyl-1,1′-binaphthyl-2-carboxylic acid [(*aS*)-**1**] as the solubilized model compound for CSP 1 (Fig. 3). Taking into account of the results of Pirkle and Pochapsky [21] and of Uccelo-Barretta *et al.* [20], NMR studies in chloroform may be reasonably used to interpret normal-phase chromatographic separations by CSP 1. Thus, deuterochloroform (C^2HCl_3) was used as a solvent of low polarity to amplify the induced shift differences caused by mixing of the two pertinent components. Table II summarizes the results of the ^1H NMR measurements, and shows the chemical shifts of the selected protons in (*aS*)-**1**, (*S*)- and (*R*)-**2α** in the free, *aS*, *S* and *aS*, *R* mixtures. Although the induced shifts on mixing are small, they are generally in accordance with the expected shielding effect by the naphthalene plane and the deshielding effect by dipole stacking interactions and/or hydrogen bonding.

A larger upfield shift of aromatic H_g protons ($\Delta\delta H_g = +0.053$) of the *aS*, *R* complex compared with that ($\Delta\delta H_g = +0.015$) of the *aS*, *S* complex should be noted. This indicates that H_g protons of the *aS*, *R* complex are more closely disposed over the naphthalene plane via π-donor–acceptor interactions than those of the *aS*, *S* complex. Similarly, a downfield shift of the amide H_c proton ($\Delta\delta H_c = -0.020$) of the *aS*, *R* complex compared with a very small upfield shift ($\Delta\delta H_c = +0.006$) of the *aS*, *S* complex indicates that the H_c proton of the former complex is more deshielded than the latter, presumably via dipole stacking interaction with the urethane amide bond of **2α**. Induced shifts of the other protons are similar in both the *aS*, *S* and *aS*, *R* complexes, which may be the result of the non-stereoselective interactions of (*aS*)-**1** and the enantiomeric **2α** on mixing. These ^1H NMR observations may indicate that the attractive interaction of (*aS*)-**1** is stronger with (*R*)-**2α** than with (*S*)-**2α**, which is consistent with the chromatographic behaviour of enantiomeric **2α** in that (*R*)-**2α** is more retained than (*S*)-**2α** by CSP 1 bearing an (*aS*)-binaphthyl axis.

Here again, from the stereospecificity of the induced shifts of the H_g and H_c protons, it may be said that a π-donor–acceptor interaction between the 3,5-dinitrophenyl ring of **2α** and the naphthalene ring of CSP 1 and a dipole stacking

TABLE II

^1H NMR CHEMICAL SHIFTS OF SELECTED PROTONS IN (*aS*)-**1** AND (*S*)- OR (*R*)-**2α** IN THE FREE, *aS*, *R* AND *aS*, *S* MIXTURES

All shifts measured at 250 MHz relative to tetramethylsilane in C^2HCl_3 at 20°C. Concentration 0.1 *M* for components. Shifts are reported for the centre of multiplets.

Proton	Free (δ, ppm)	*aS*, *R* (δ, ppm)	Δδ (ppm)	*aS*, *S* (δ, ppm)	Δδ (ppm)	$\Delta\lvert\delta(aS, S) - \delta(aS, R)\rvert$
H_a	0.873	0.870	+0.003	0.870	+0.003	0.000
H_b	3.024	3.062	−0.038	3.051	−0.027	0.011
H_c	6.627	6.647	−0.020	6.621	+0.006	0.026
H_d	1.648	1.574	+0.074	1.560	+0.088	0.014
H_e	5.933	5.875	+0.058	5.876	+0.059	0.001
H_f	7.279	8.180	−0.901	8.193	−0.909	0.008
H_g	8.621	8.568	+0.053	8.607	+0.015	0.038
H_h	8.681	8.602	+0.079	8.607	+0.073	0.006

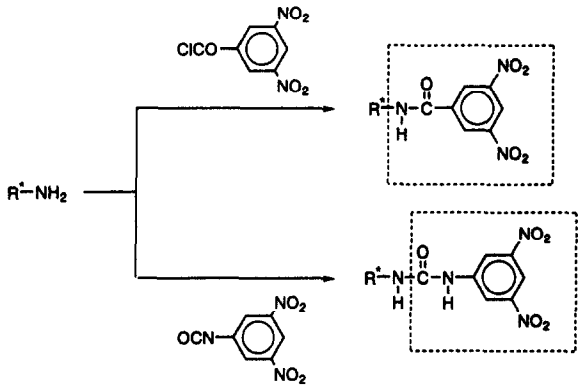

Fig. 4. Derivatization of enantiomeric amines.

interaction between the urethane bond of **2**α and the amide bond of CSP 1 act cooperatively in chiral recognition by CSP 1.

Improvement of the chiral separation of amines by conversion into 3,5-dinitrophenylureas

The chiral discrimination model for enantiomeric alcohols as the 3,5-dinitrophenylcarbamates (Fig. 2) emphasizes that the direction of the –CO–NH– linkage connecting to the 3,5-dinitrophenyl nucleus, *i.e.*, –CO–NH–Ar (Ar = 3,5-dinitrophenyl), also plays an important role in attractive interactions with the CSP by dipole stacking interactions. Previously [10], however, chiral separation of amines was carried out as their 3,5-dinitrobenzoyl derivatives. This seemingly resulted in an inferior separation of the derivatized amines by CSP 1 because of the mismatched direction of the amide linkage, *i.e.*, –NH–CO–Ar, for the dipole stacking interaction to cooperate effectively with the π-donor–acceptor interaction. This reasoning led us to adjust the mode of the attachment of the π-accepting moiety by converting amines into the 3,5-dinitrophenylurea derivatives. This could be accomplished by using 3,5-dinitrophenyl isocyanate (Fig. 4). The chromatographic data in Table III and Fig. 5 clearly show the increased retention as judged from the composition of the eluent used and the significant improvement in the chiral separation.

Chiral separation of enantiomeric carboxylic acids as the 3,5-dinitroanilides

Derivatization of carboxylic acids into the 3,5-dinitroanilides builds up a structure that is closely related to the 3,5-dinitrophenylcarbamates from the corresponding alcohols in that both have the –CO–NH–Ar linkage as indicated in Fig. 6. Further, the chiral centre of the acid derivative is α to the carbonyl centre, whereas that of the alcohol derivative is β, separated by the intervening ester oxygen. This situation strongly suggests that carboxylic acids as the 3,5-dinitroanilides should be better separated by CSP 1 than the corresponding alcohols as the 3,5-dinitrophenylcarbamates based on the proposed chiral discrimination model (Fig. 2). Table IV gives several examples of such separations of

TABLE III

COMPARISON OF THE SEPARATION OF AMINES AS THE 3,5-DINITROPHENYLUREAS (**3a–c**) WITH THAT AS THE 3,5-DINITROBENZAMIDES (**3'a–c**)

Mobile phases: hexane–2-propanol, (B) 90:10 and (D) 80:20, and hexane–ethanol, (E) 90:10 and (F) 80:20. See Table I for HPLC conditions.

R^1	R^2	$R^1CHR^2NHCONH$-3,5-$(NO_2)_2C_6H_3$ (**3**)				R^1CHR^2NHCO-3,5-$(NO_2)_2C_6H_3$ (**3'**)			
		3	Eluent	k'_1	α	**3'**	Eluent	k'_1	α
CH_3	Ph	**3a**	F	4.48(*S*)	1.30	**3'a**	D	6.55(*S*)	1.17[a]
CH_3	1-Naphth	**3b**	F	5.47(*S*)	1.24	**3'b**	D	7.90	1.07[a]
CH_3	n-C_6H_{13}	**3c**	E	4.45	1.16	**3'c**	B	5.34	1.00[a]

[a] Data from ref. 10.

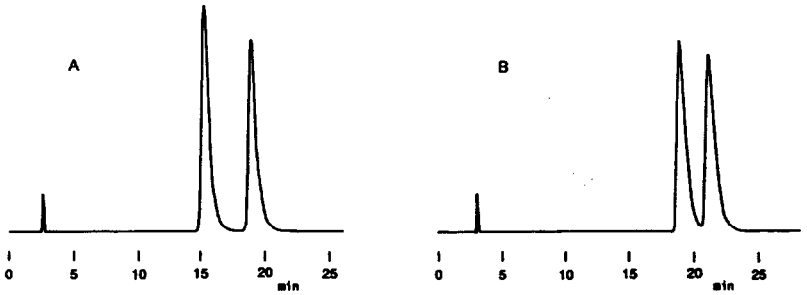

Fig. 5. Chromatographic separation of enantiomers on CSP 1. (A) N'-(1'-Phenylethyl)-N-(3,5-dinitrophenyl)urea (**3a**); (B) N-(3',5'-dinitrobenzoyl)-1-phenylethylamine (**3'a**). Chromatographic conditions as in Table III.

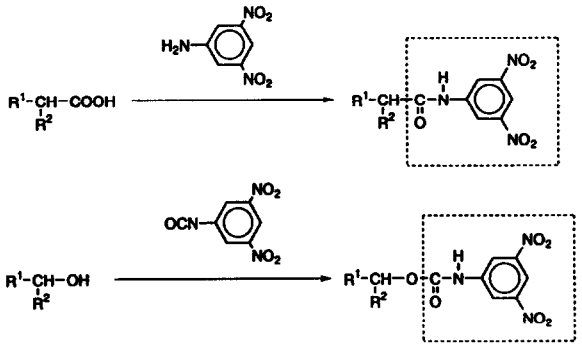

Fig. 6. Appropriate derivatization of enantiomeric carboxylic acids compared with that of alcohols.

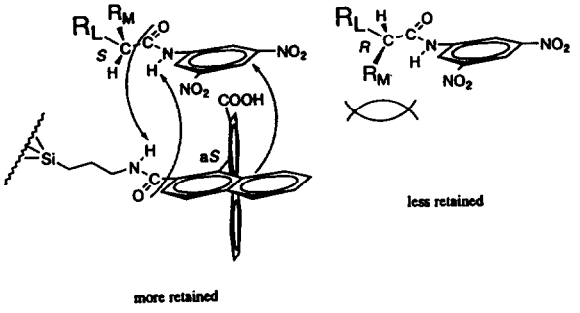

Fig. 7. Chiral discrimination model for enantiomeric carboxylic acids as the 3,5-dinitroanilides.

carboxylic acid derivatives compared with those of the alcohol counterparts. Interestingly, the anilides of (*R*)-carboxylic acids elute first on the (*aS*)-binaphthyl chiral selector. This is in good accord with the chiral discrimination model depicted in Fig. 7, which is a rational outcome of the model shown in Fig. 2.

The right-hand side of Table I clearly shows

TABLE IV

COMPARISON OF THE SEPARATION OF CARBOXYLIC ACIDS AS THE 3,5-DINITROANILIDES WITH THAT OF ALCOHOLS AS THE 3,5-DINITROPHENYLCARBAMATES

Mobile phases: hexane–2-propanol, (B) 90:10 and (C) 85:15. See Table I for HPLC conditions.

R^1	R^2	R^1CHR^2CONH-3,5-$(NO_2)_2C_6H_3$			R^1CHR^2OCONH-3,5-$(NO_2)_2C_6H_3$		
		Eluent	k'_1	α	Eluent	k'_1	α
CH_3	C_2H_5	B	12.33	1.08	B	5.36	1.00
CH_3	n-C_4H_9	B	8.97(*R*)	1.27	B	4.38	1.15
CH_3	n-C_6H_{13}	B	7.93	1.37	B	3.93(*S*)	1.21
CH_3	Ph	C	7.57(*R*)	1.63	C	3.86(*S*)	1.54[a]
C_2H_5	Ph	C	7.26(*R*)	1.46	C	3.44	1.53[a]
n-C_3H_7	Ph	C	7.02	1.47	C	3.33	1.43[a]

[a] Data from ref. 10.

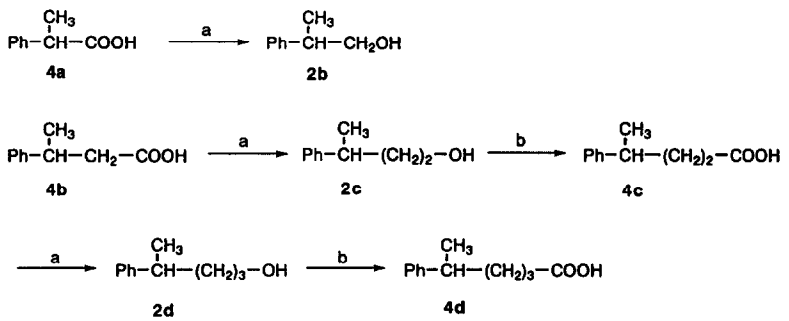

Fig. 8. Preparation of phenyl-substituted homologous alcohols and carboxylic acids. (a) $LiAlH_4$–Et_2O; (b) (1) PBr_3–pyridine, (2) Mg–Et_2O, (3) CO_2, (4) H^+–H_2O.

the importance of a π-acidic site for the derivatized carboxylic acid analytes. Table I also shows the better separation, in general, of the carboxylic acid derivatives than the corresponding alcohol counterparts.

Separation of homologous α- to δ-chiral alcohols and carboxylic acids as the 3,5-dinitrophenyl derivatives

Studies of the HPLC elution behaviour of suitable homologous series of compounds, in which stereochemistry and performance can be correlated, are of great help in elucidating the arrangement of the diastereomeric complexes formed from a chiral selector and analytes.

Enantiomerically pure 1-phenylethanol (**2a**), 2-phenylpropionic acid (**4a**) and 3-phenylbutyric acid (**4b**) are readily available, and were utilized for the preparation of a series of samples of phenyl-substituted homologous alcohols (**2a–d**) and carboxylic acids (**4a–d**) of known enantiomeric compositions (Fig. 8). Table V summarizes the separation of the derivatized homologous alcohols (**2α–δ**) and carboxylic acids (**4α–δ**) bearing a phenyl substituent on the α- to δ-carbon atom from the hydroxyl oxygen and carboxyl carbon, respectively.

CSP 1 can discriminate the α- and β-chiral alcohols as the 3,5-dinitrophenylcarbamates (**2α** and **2β**), but not the derivatized γ-chiral alcohol (**2γ**). As will be discussed later, the stereochemistry of the first eluting enantiomer of **2α** is *S*, whereas that of **2β** alters to *R* by intervention of one methylene unit between the chiral centre and the hydroxyl oxygen.

In the series of the carboxylic acid derivatives, an increase in the distance of the chiral centre from the carbonyl group by intervention of methylene unit(s) also results in a significant decrease in selectivity (α). However, the derivat-

TABLE V

SEPARATION OF HOMOLOGOUS α- TO δ-CHIRAL ALCOHOLS AND CARBOXYLIC ACIDS AS THE 3,5-DINITROPHENYL DERIVATIVES

Mobile phase: hexane–2-propanol (85:15). See Table I for HPLC conditions.

n	$PhCH(CH_3)(CH_2)_nOCONH$-3,5-$(NO_2)_2C_6H_3$ (2)			$PhCH(CH_3)(CH_2)_nCONH$-3,5-$(NO_2)_2C_6H_3$ (4)		
	2	k'_1	α	4	k'_1	α
0	2α	3.86(*S*)	1.54[a]	4α	7.57(*R*)	1.63
1	2β	5.05(*R*)	1.09	4β	7.82(*R*)	1.50
2	2γ	5.23	1.00	4γ	8.14(*S*)	1.07
3	2δ	4.99	1.00	4δ	9.79	1.00

[a] Data from ref. 10.

ized γ-chiral carboxylic acid (**4γ**) can still be discriminated by CSP 1. Here again, alternation of the absolute configuration of the first-eluting enantiomers of the homologous acid derivatives, $PhCH(CH_3)(CH_2)_nCONHAr$, with the intervening methylene unit number ($n = 0$, 1 and 2) should be noted.

Table V indicates that the separability of **2α** is similar to that of **4β**. The chiral centre of these analytes is located β from the carbonyl carbon, and the absolute stereochemistry of the first-eluting enantiomers is the same as illustrated in Fig. 9B. A similar situation holds for the chromatographic behaviour of **2β** and **4γ**, except that the chiral centre is located γ from the carbonyl carbon and the stereochemistry of the first-eluting enantiomers is altered (Fig. 9C). Hence it may be concluded that CSP 1 discriminates the chiral centre of $PhCH(CH_3)(CH_2)_n$–X–CONHAr ($n = 0$ and 1) irrespective of whether the intervening X group is O or CH_2. On the other hand, shorter retentions of the derivatized alcohols (**2α** and **2β**) compared with those of the corresponding carboxylic acid derivatives (**4β** and **4γ**), respectively, may be ascribed to the electron-donating resonance effect of the ester oxygen lone pair in the amide system reducing the dipole stacking interaction. It should be noted that 1-phenylethylamine as the 3,5-dinitrophenylurea (**3a**) falls in the same category as **2α** and **4β** (Fig. 9B), although the presence of a superfluous, mismatched amide linkage seemingly reduces the separability to some extent.

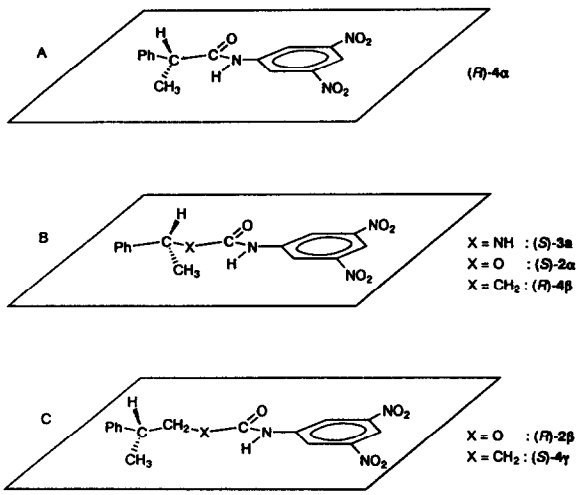

Fig. 9. Configuration of the first-eluting 3,5-dinitrophenyl-derivatized analytes.

For convenience, Fig. 9 schematically presents selected examples of the first-eluting analytes bearing a –CO–NH–Ar linkage when eluted on CSP 1. This kind of illustrative presentation is of great help in grasping the absolute stereochemistry of the chiral centre of these molecules, because the *R* and *S* designation (*e.g.*, Table V) varies with the substituent priority sequence. The molecular planes in Fig. 9 are defined by disposing pertinent atoms or groups of the analytes according to the proposed chiral discrimination model by CSP 1 (Fig. 2) [10]. It should be noted that the molecular arrangement is not inconsistent with Prelog's generalization to predict the stereochemistry of the addition of a nucleophile to chiral alkyl esters of benzoylformic acid [22]. It can be seen that those enantiomeric analytes which have the methyl substituent disposed on the underside of the molecular plane always elute faster than the enantiomeric counterparts which have the hydrogen disposed on the underside, irrespective of whether the intervening X functionality is O, CH_2 or NH.

CONCLUSIONS

The chiral discrimination mechanism of enantiomeric analytes by CSP 1 has been investigated. Generally, the model depicted in Fig. 2 explains well the HPLC behaviour of the 3,5-dinitrophenyl derivatized alcohols, and the model can be applied to the corresponding amine and carboxylic acid derivatives. The π-donor–acceptor interaction between one of the naphthalene planes of the CSP and the 3,5-dinitrophenyl ring of the analyte cooperates with the steric fit of the amide linkages for dipole stacking between the two species mainly to determine the magnitude of the resolution and retention. Because of this, the elution order of the enantiomers shows a high degree of regularity, which may permit the assignment of the absolute configurations of properly derivatized analytes based on the elution order with considerable confidence.

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