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Short communication

Enantiomeric separation of tocainide and its analogues on an optically active crown ether-based stationary phase by liquid chromatography

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Abstract

Tocainide and its 14 analogues were resolved on a chiral stationary phase (CSP) based on (3,3′-diphenyl-1,1′-binaphthyl)-20-crown-6 covalently bonded to silica gel. The resolution was quite good, the separation (α) and resolution factors (R_S) being 1.84–15.32 and 1.34–13.78, respectively. Especially, the result for the resolution of tocainide on the CSP turns out to be the best one among others reported so far. The chromatographic resolution behaviors were demonstrated to be dependent on the content and the type of organic and acidic modifiers and the ammonium acetate concentration in aqueous mobile phase.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Crown ether-based stationary phase; Tocainide

1. Introduction

Two enantiomers consisting of racemic drugs have been known to often show different pharmacological activity. In consequence, the enantiomeric composition of chiral drugs is an important issue in the drug developments and in the clinical use of chiral drugs [1]. Now various methods are available for the determination of enantiomeric composition of chiral drugs. However, the liquid chromatographic separation of enantiomers on chiral stationary phases (CSPs) has been known to be one of the most accurate and convenient techniques of determining the enantiomeric composition of chiral drugs [2,3].

Tocainide is an antiarrhythmic agent. The (*R*)-enantiomer of tocainide has been reported to be three times more potent than the (*S*)-enantiomer [4]. In this instance, the exact determination of the enantiomeric composition of tocainide is important. Previously, several liquid chromatographic CSPs were used in the resolution of tocainide. For example, α_1-acid glycoprotein silica (AGP) [5] and modified diallyl-tartardiamide crosslinked in network and immobilized on silica [6] were used in the liquid chromatographic resolution of tocainide. We have also been interested in the resolution of tocainide and intended to utilize crown ether-based CSPs. Crown ether-based CSPs are known to be useful for the resolution of racemic compounds containing a pri-

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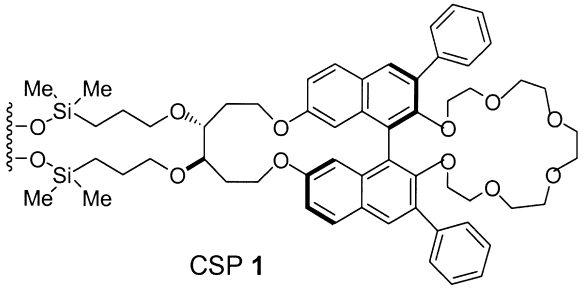
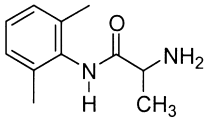
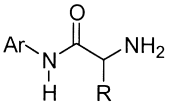
mary amino group [7]. Tocainide contains one primary amino group and consequently is expected to be resolved on crown ether-based CSPs.

Optically active (3,3′-diphenyl-1,1′-binaphthyl)-20-crown-6 dynamically coated on octadecyl silica gel (Crownpak CR, Daicel Chemical Industries) was actually utilized in the resolution of tocainide [8]. However, the baseline resolution was not successful (R_S=0.97) even though the separation factor was reasonable (α=1.44). Recently, we developed a very effective CSP based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid for the resolution of α-amino acids [9], primary amines [10], primary amino alcohols [10] and fluoroquinolone compounds containing a primary amino group [11] and we also applied it in the resolution of tocainide [9]. In the resolution of tocainide on the CSP based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, the baseline separation of the two enantiomers was observed (R_S=1.31), but the separation factor was only marginal (α=1.16). More recently, we developed another crown ether-based CSP (CSP **1**, Fig. 1) by covalently bonding (3,3′-diphenyl-1,1′-binaphthyl)-20-crown-6 to silica gel [12]. CSP **1** was also very effective in the resolution α-amino acids [12], primary amines [13] and primary amino alcohols [13]. However, CSP **1** has not been utilized in the resolution of tocainide. In this study, we wish to extend the use of CSP **1** to the resolution of tocainide (**2a**) and its analogues (**2b**–**2o**) shown in Fig. 1.





2a



2b-2o

Fig. 1. The structures of CSP **1**, tocainide (**2a**) and its analogues (**2b**–**2o**).

2. Experimental

Chromatography was performed with an HPLC system consisting of a Waters model 515 HPLC pump, a Rheodyne model 7725i injector with a 20 μl sample loop, a YoungLin M720 Absorbance detector (variable wavelength) and a YoungLin Autochro Data Module (Software: YoungLin Autochro-WIN 2.0 plus). The column temperature was controlled by using a Julabo F30 Ultratemp 2000 cooling circulator. The enantioselective column packed with CSP **1** (250 mm×4.6 mm I.D.) was available from previous study [13].

Racemic and optically active tocainide (**2a**) and its derivatives (**2b**–**2o**) were prepared from corresponding α-amino acids as follows. Racemic or optically active α-amino acids were treated with di-*tert*.-butyldicarbonate in the presence of triethylamine to afford *N*-*t*-BOC-α-amino acids. *N*-*t*-BOC-α-Amino acids thus prepared were treated with aniline, 2,6-dimethylaniline or benzylamine in the presence of coupling agent EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline) in methylene chloride to afford *N*-*t*-BOC-α-amino amides. Finally, treatment of *N*-*t*-BOC-α-amino amides in methylene chloride with HCl gas evolved from the reaction of H_2SO_4 and NaCl produced racemic and optically active tocainide and its analogues as their HCl salts. The structures of tocainide and its analogues thus prepared were consistent with ^{1}H NMR spectral data.

Each of racemic and optically active tocainide and its derivatives thus prepared was dissolved in water (usually 2.5 mg/ml) and then used for the resolution on CSP **1**. The usual injection volume was 0.1 μl.

3. Results and discussion

Resolution of tocainide (**2a**) and its analogues (**2b**–**2o**) on CSP **1** is summarized in Table 1. The mobile phase was 80% acetonitrile in water containing sulfuric acid (10 m*M*) and ammonium acetate (1 m*M*). Previously, it was demonstrated that complexation of ammonium ion ($R\text{-}NH_3^+$) inside the

Table 1
Resolution of tocainide (**2a**) and its analogues (**2b-o**) on CSP **1**[a]

	Analytes		k_1	k_2	α	R_S
	R	Ar				
2a	Methyl	2,6-Dimethylphenyl (Tocainide)	0.54 (*S*)	1.34 (*R*)	2.48	5.40
2b		Phenyl	0.45 (*S*)	1.63 (*R*)	3.62	7.73
2c		Benzyl	0.52 (*S*)	1.53 (*R*)	2.94	6.09
2d	Isopropyl	2,6-Dimethylphenyl	0.15 (*S*)	0.28 (*R*)	1.87	1.34
2e		Phenyl	0.17 (*S*)	0.34 (*R*)	1.94	1.62
2f		Benzyl	0.19 (*S*)	0.35 (*R*)	1.84	1.63
2g	Isobutyl	2,6-Dimethylphenyl	0.25 (*S*)	1.07 (*R*)	4.28	6.27
2h		Phenyl	0.18 (*S*)	0.79 (*R*)	4.39	5.05
2i		Benzyl	0.21 (*S*)	0.94 (*R*)	4.48	6.02
2j	Phenyl	2,6-Dimethylphenyl	0.25 (*S*)	3.83 (*R*)	15.32	13.78
2k		Phenyl	0.29 (*S*)	2.57 (*R*)	8.86	12.24
2l		Benzyl	0.29 (*S*)	2.24 (*R*)	7.72	11.19
2m	Benzyl	2,6-Dimethylphenyl	0.26 (*S*)	0.73 (*R*)	2.81	4.08
2n		Phenyl	0.23 (*S*)	0.92 (*R*)	4.00	5.43
2o		Benzyl	0.30 (*S*)	1.03 (*R*)	3.43	5.61

[a] Mobile phase: 80% CH_3CN in water+H_2SO_4 (10 m*M*)+CH_3COONH_4 (1 m*M*). Flow rate, 0.5 ml/min; detection, 210 nm UV; column temperature, 20 °C. k_1, retention factor of the first eluted enantiomer; k_2, retention factor of the second eluted enantiomer. Absolute configuration of the first and the second eluted enantiomer is indicated in the parenthesis; α, separation factor; R_S, resolution factor.

cavity of the crown ether ring is essential for the chiral recognition [14]. In this instance, sulfuric acid added to the mobile phase is believed to play a role in protonating the primary amino group of analytes and enhancing the diastereomeric complex formation of an analyte inside the cavity of the chiral crown ether ring of the CSP. Ammonium acetate added to the mobile phase was used to reduce the retention time of analytes on the chiral column by competing with the ammonium ion ($R\text{-}NH_3^+$) of analytes for complexation by the crown ether of the CSP [12,13]. The elution orders shown in Table 1 were determined by injecting configurationally known samples.

As shown in Table 1, resolution of tocainide (**2a**) and its analogues (**2b**–**2o**) on CSP **1** is quite good. Resolution of tocainide (**2a**) on CSP **1** was especially noteworthy because the result for the resolution of tocainide (**2a**) on CSP **1** turns out to be the best one among others reported so far for the resolution of tocainide on other CSPs [5,6,8,9,15–17]. Resolution of tocainide analogues (**2b**–**2o**) on CSP **1** is also interesting in that the effect of the structural variation of tocainide on the chiral recognition might be inferred. As shown in Table 1, when the 2,6-dimethylphenyl group (Ar group) of tocainide is changed to phenyl or to benzyl, the resolution was slightly improved (see the data for the resolution of analytes **2a**, **2b** and **2c**). However, when the R group is phenyl, the analyte containing 2,6-dimethylphenyl group as an Ar group is resolved best (see the data for the resolution of analytes **2j**, **2k** and **2l**). When the methyl group at the stereogenic center of tocainide is changed to a larger group, the resolution was generally improved except for the resolution of the analytes containing an isopropyl group at the stereogenic center (see the data for the resolution of analytes **2d**, **2e** and **2f**). Overall, resolution of tocainide (**2a**) and its analogues (**2b**–**2o**) on CSP **1** is very excellent without regard to the size or the type of the Ar and R group of the analyte.

In order to see the trends for the resolution of tocainide (**2a**) and its analogues (**2b**–**2o**) on CSP **1**, we selected three analytes including tocainide (**2a**, **2i** and **2j**) and resolved them with the variation of the content and the type of organic and acidic modifiers and the ammonium acetate concentration in aqueous mobile phase. The resolution results are summarized in Table 2. As shown in Table 2, the chromato-

Table 2
Resolution of tocainide (**2a**) and its analogues (**2i** and **2j**) on CSP **1** with the variation of the content and the type of organic and acidic modifiers and the ammonium acetate concentration in aqueous mobile phase[a]

	Mobile phase	**2a**			**2i**			**2j**		
		k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
a	30% CH_3CN+H_2SO_4 (10 m*M*)+$CH_3CO_2NH_4$ (1 m*M*)	0.96 (*S*)	2.17	5.13	0.80 (*S*)	4.18	9.32	1.50 (*S*)	12.37	13.90
b	50% CH_3CN+H_2SO_4 (10 m*M*)+$CH_3CO_2NH_4$ (1 m*M*)	0.70 (*S*)	2.24	5.33	0.40 (*S*)	4.39	8.13	0.67 (*S*)	12.27	14.46
c	80% CH_3CN+H_2SO_4 (10 m*M*)+$CH_3CO_2NH_4$ (1 m*M*)	0.54 (*S*)	2.48	5.40	0.21 (*S*)	4.48	6.02	0.25 (*S*)	15.32	13.78
d	50% CH_3OH+H_2SO_4 (10 m*M*)+$CH_3CO_2NH_4$ (1 m*M*)	0.61 (*S*)	2.02	3.32	0.50 (*S*)	3.31	5.72	0.68 (*S*)	9.01	8.78
e	80% CH_3OH+H_2SO_4 (10 m*M*)+$CH_3CO_2NH_4$ (1 m*M*)	0.32 (*S*)	2.32	3.59	0.11 (*S*)	4.80	4.47	0.14 (*S*)	11.50	9.39
f	80% CH_3CN+H_2SO_4 (1 m*M*)+$CH_3CO_2NH_4$ (1 m*M*)	1.08 (*S*)	1.82	4.30	0.59 (*S*)	2.56	5.83	0.70 (*S*)	7.04	12.45
g	80% CH_3CN+H_2SO_4 (2 m*M*)+$CH_3CO_2NH_4$ (1 m*M*)	0.78 (*S*)	1.99	4.35	0.36 (*S*)	3.18	5.02	0.44 (*S*)	8.70	12.24
h	80% CH_3CN+H_2SO4 (5 m*M*)+$CH_3CO_2NH_4$ (1 m*M*)	0.68 (*S*)	2.09	4.68	0.29 (*S*)	3.46	4.87	0.36 (*S*)	9.87	12.61
i	80% CH_3CN+H_2SO4 (10 m*M*)+$CH_3CO_2NH_4$ (0 m*M*)	1.44 (*S*)	2.19	6.70	0.55 (*S*)	4.42	9.11	0.80 (*S*)	12.15	14.38
j	80% CH_3CN+H_2SO4 (10 m*M*)+$CH_3CO_2NH_4$ (0.1 m*M*)	1.22 (*S*)	2.07	6.02	0.44 (*S*)	4.49	8.53	0.62 (*S*)	12.50	14.39
k	80% CH_3CN+H_2SO4 (10 m*M*)+$CH_3CO_2NH_4$ (0.5 m*M*)	0.86 (*S*)	2.15	5.18	0.33 (*S*)	4.03	6.56	0.45 (*S*)	11.31	13.83
l	80% CH_3CN+$HClO4$ (10 m*M*)+$CH_3CO_2NH_4$ (1 m*M*)	0.46 (*S*)	2.63	4.47	0.10 (*S*)	8.05	6.47	0.18 (*S*)	19.00	14.47
m	80% CH_3CN+CH_3CO_2H (10 m*M*)+$CH_3CO_2NH_4$ (1 m*M*)	2.10 (*S*)	1.71	4.88	1.24 (*S*)	2.23	6.44	1.19 (*S*)	5.91	13.36

[a] Flow rate, 0.5 ml/min; detection, 210 nm UV; temperature, 20 °C. k_1, retention factor of the first eluted enantiomer. In the parentheses, the absolute configuration of the first eluted enantiomer is presented. α, separation factor; R_S, resolution factor.

graphic resolution behaviors are dependent on the content and the type of organic and acidic modifiers and the ammonium acetate concentration in aqueous mobile phase. The retention factors (k_1) for the first eluted enantiomers always decrease as the content of organic and acidic modifier and the ammonium acetate concentration in aqueous mobile phase increases and these trends are consistent with those for the resolution of amines and amino alcohols on CSP **1** [13]. The enantioselectivity (α) is generally improved by increasing the content of organic and acidic modifier in aqueous mobile phase. However, the effect of the ammonium acetate concentration on the enantioselectivity is not significant. The trends of the resolution factors (R_S) with the variation of the content and the type of organic and acidic modifiers and the ammonium acetate concentration are not so consistent.

The practical usefulness of CSP **1** in the determination of enantiomeric purity of tocainide (**2a**) was clear from the chromatograms shown in Fig. 2. Fig. 2a shows the chromatogram for the resolution of racemic tocainide (**2a**) on CSP **1**. The chromatogram for the resolution of (*R*)-tocainide prepared from (*R*)-alanine in this study on CSP **1** is shown in Fig. 2b and its expanded chromatogram is shown in Fig. 2c. Based on the computer-generated peak areas corresponding to the two enantiomers shown in Fig. 2b or c, the enantiomeric purity of (*R*)-tocainide was calculated to be 99.4% ee (*R*:*S*=99.7:0.3). However, enantiomerically more enriched (*R*)-tocainide is not available and the detection limit of the (*S*)-enantiomer present in (*R*)-tocainide was not able to be checked further.

In summary, CSP **1** was found to be quite useful in the resolution of tocainide and its analogues. Especially, the resolution of tocainide on CSP **1** turns out to be the best one among others reported so far. The chromatographic behaviors for the resolution of tocainide and its analogues on CSP **1** were also found to be dependent on the content and the type of organic and acidic modifiers and the ammonium acetate concentration in aqueous mobile phase. Based on the chromatographic resolution behaviors, the analytical time for the resolution of tocainide and its analogues on CSP **1** is concluded to be reducible quite substantially by increasing the content of organic and acidic modifiers and the ammonium acetate concentration in aqueous mobile phase without loss of enantioselectivity or resolution.

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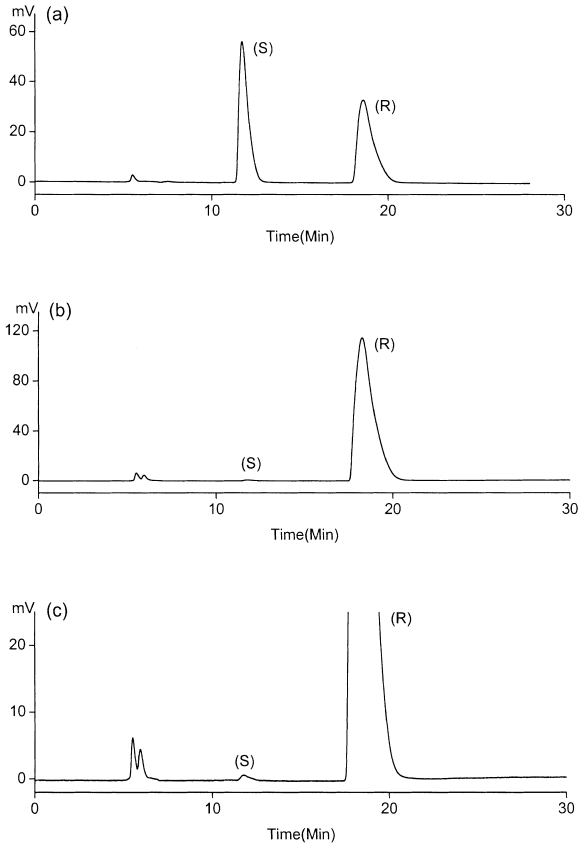

Fig. 2. (a) Chromatogram for the resolution of racemic tocainide (**2a**) on CSP **1**. (b) Chromatogram for the resolution of (*R*)-enriched tocainide on CSP **1**. (c) The expanded chromatogram of (b). Chromatograms were obtained with the mobile phase of 80% acetonitrile in water containing sulfuric acid (10 m*M*) and ammonium acetate (1 m*M*). Flow rate, 0.5 ml/min; detection, 210 nm UV; temperature, 20 °C.

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