Liquid Chromatographic Resolution of Mexiletine and Its Analogs on Crown Ether-Based Chiral Stationary Phases

KAB BONG JIN, HEE EUN KIM, AND MYUNG HO HYUN*

Department of Chemistry and Chemistry Institute for Functional Materials, Pusan National University, Busan, Republic of Korea

ABSTRACT Mexiletine, an effective class IB antiarrhythmic agent, and its analogs were resolved on three different crown ether-based chiral stationary phases (CSPs), one (CSP **1**) of which is based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid and the other two (CSP **2** and CSP **3**) are based on (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6. Mexiletine was resolved with a resolution (R_S) of greater than 1.00 on CSP **1** and CSP **3** containing residual silanol group-protecting n-octyl groups on the silica surface, but with a resolution (R_S) of less than 1.00 on CSP **2**. The chromatographic behaviors for the resolution of mexiletine analogs containing a substituted phenyl group at the chiral center on the three CSPs were quite dependent on the phenoxy group of analytes. Namely, mexiletine analogs containing 2,6-dimethylphenoxy, 3,4-dimethylphenoxy, 3-methylphenoxy, 4-methylphenoxy, and a simple phenoxy group were resolved very well on the three CSPs even though the chiral recognition efficiencies vary with the CSPs. However, mexiletine analogs containing 2-methylphenoxy group were not resolved at all or only slightly resolved. Among the three CSPs, CSP **3** was found to show the highest chiral recognition efficiencies for the resolution of mexiletine and its analogs, especially in terms of resolution (R_S). *Chirality 26:272–278, 2014.*

KEY WORDS: chiral stationary phase; enantiomer separation; liquid chromatography; mexiletine; mexiletine analogs

INTRODUCTION

Mexiletine, 1-(2,6-dimethylphenoxy)propan-2-amine, (Fig. 1) has been known to be an effective class IB antiarrhythmic agent and approved as a racemic form for the treatment of ventricular arrhythmias.[1,2] Between the two enantiomers of mexiletine, (−)-(*R*)-enantiomer was reported to posses greater antiarrhythmic properties than the opposite enantiomer.[3] In addition, the two enantiomers of mexiletine analogs were reported to show different activity in producing a tonic block or as blockers of voltage-gated Na^+ channels and, consequently, the enantioselective synthesis of mexiletine analogs has attracted much attention.[4,5] In this instance, the exact determination of the enantiomeric composition or enantiomeric purity of mexiletine and its analogs are quite important.

For the determination of the enantiomeric composition or purity of mexiletine and its analogs, the liquid chromatographic method utilizing chiral stationary phases (CSPs) has been applied. For example, Chiralcel OD, OD-R, and OD-H (cellulose tris-3,5-dimethylphenylcarbamate) columns from Daicel Chemical Industries (Tokyo, Japan) were successfully used for the liquid chromatographic determination of the enantiomeric composition or purity of mexiletine and its analogs.[4,5] Chiralcel OD-H and OJ-H (cellulose tris-4-methyl-benzoate) columns were also used for the resolution of *N*-acyl derivatives of mexiletine.[6,7]

Mexiletine and its analogs contain a primary amino group at their chiral center. Racemic compounds containing a primary amino group have been successfully resolved on crown ether-based CSPs.[8,9] In this instance, mexiletine and its analogs are expected to be resolved on crown ether-based CSPs. Among various crown ether-based CSPs, CSP **1** (Fig. 1) based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid was very successful in the resolution of α-, β-, and γ-amino acids, fluoroquinolone compounds, amino alcohols and various primary amino compounds.[10–15] CSP **1** was also very successful in the resolution of secondary amines.[16–20] In addition, CSP **2** and CSP **3** (Fig. 1) based on (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6 were very successful in the resolution of α- and β-amino acids, fluoroquinolone compounds, amino alcohols, and various primary amino compounds.[21–27] However, study of the liquid chromatographic resolution of mexiletine and its analogs on crown ether-based CSPs is quite rare. Only CSP **2** was used for the resolution of racemic mexiletine during the evaluation of the CSP for the resolution of various primary amino compounds, but the baseline separation was not obtained.[22] In this study, we prepared various mexiletine analogs by changing the methyl group at the chiral center of mexiletine to the substituted phenyl group and by varying the 2,6-dimethylphenoxy group of mexiletine to another substituted phenoxy group. Racemic mexiletine and its analogs thus prepared were resolved on CSP **1**, CSP **2,** and CSP **3** and the chromatographic behaviors for the resolution of racemic mexiletine and its analogs on the three CSPs were compared.

EXPERIMENTAL

A high-performance liquid chromatography (HPLC) system consisting of a Waters model 515 HPLC pump (Milford, MA), a Rheodyne model 7725i injector (Rohnert Park, CA) with a 20-μl sample loop, a Waters 2487 detector, and a YoungLin Autochro data module (Software: YoungLin Autochro-WIN 2.0 plus) was used for the liquid chromatography. The chiral column

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*Correspondence to: Myung Ho Hyun, Department of Chemistry, Pusan National University, Busan 609-735, Republic of Korea. E-mail: mhhyun@pusan.ac.kr
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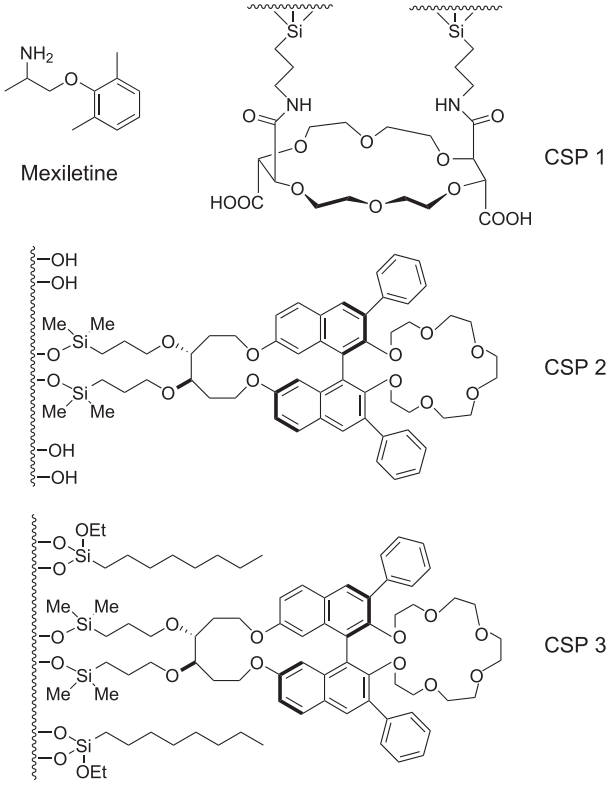


Fig. 1. Structures of mexiletine, CSP **1**, CSP **2,** and CSP **3**.

temperature was maintained at 20°C by using a JEIO TECH VTRC-620 cooling circulator (Daejeon, Korea). Chiral columns packed with CSP **1**, CSP **2,** and CSP **3** were available from prior studies.[10,21,23]

Mexiletine was available as its HCl salt from Sigma-Aldrich Korea (Seoul, Korea). All mexiletine analogs (Fig. 2) used in this study were prepared as shown in Scheme 1. As a representative example, the detailed synthetic procedure for the preparation of mexiletine analog, 2-(2,6-dimethylphenoxy)-1-phenylethanamine (**4a**), is described. 2-Bromo-1-phenylethanone (1 g, 5 mmole), 2,6-dimethylphenol (0.92 g, 7.5 mmole) and potassium carbonate (1.04 g, 7.5 mmole) were dissolved in 10 mL of dimethylformamide (DMF). After stirring the mixture for 24 h at room temperature under an argon atmosphere, water (30 mL) was added and the whole aqueous solution was extracted with diethyl ether three times. The organic solution was dried over anhydrous $MgSO_4$ and then the solution was concentrated using a rotary evaporator. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate, 5/1, v/v) to afford intermediate A (Scheme 1, X = H, $R_1 = R_2 = CH_3$, $R_3 = R_4 = R_5 = H$) (0.80 g, 66%). ^{1}H NMR ($CDCl_3$, ppm) δ: 7.98-7.95 (m, 2H), 7.64-7.58 (m, 1H), 7.52-7.46 (m, 2H), 7.06-6.94 (m, 3H), 5.12 (s, 2H), 2.31 (s, 6H). A solution of the intermediate A (Scheme 1, X = H, $R_1 = R_2 = CH_3$, $R_3 = R_4 = R_5 = H$) thus prepared (0.2 g, 0.83 mmole), ammonium acetate (CH_3COONH_4, 0.8 g, 10.4 mmole) and sodium cyanoborohydride ($NaBH_3CN$, 0.2 g, 3.1 mmol) in 25 mL of methanol was heated to reflux for 48 h. After cooling, the reaction mixture was acidified by adding concentrated HCl slowly until the pH became lower than 2. Methanol was removed using a rotary evaporator and then water was added to the residue. The resulting solution was extracted with diethyl ether twice. The aqueous layer was made basic by adding KOH pellets until the pH became higher than 10. The basic solution was extracted with diethyl ether (20 mL) twice. The organic solution was dried over anhydrous $MgSO_4$ and then evaporated to afford the mexiletine analog, 2-(2,6-dimethylphenoxy)-1-phenylethanamine (**4a**) (0.16 g, 80%).

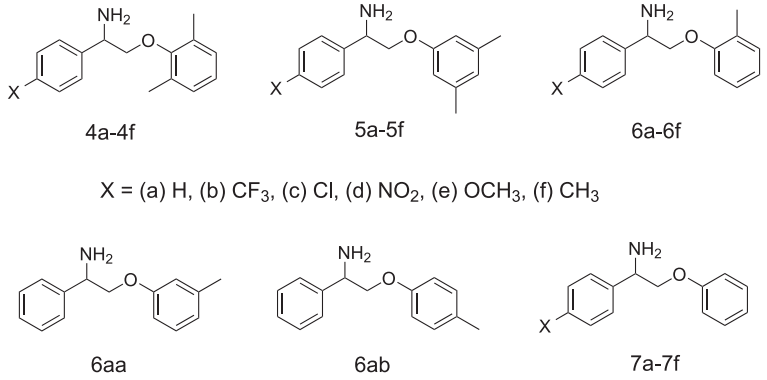
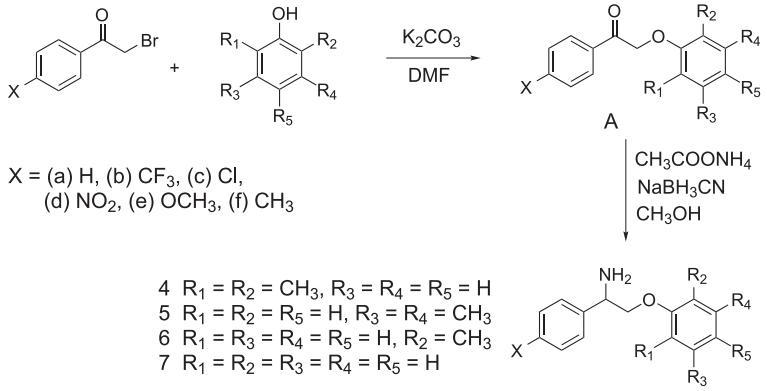


Fig. 2. Structures of mexiletine analogs.



Scheme 1. Synthetic route for the preparation of mexiletine analogs.

^{1}H NMR ($CDCl_3$, ppm) δ: 7.47-7.42(d, 2H), 7.39-7.27(m, 4H), 7.01-6.98 (d, 1H), 6.94-6.88(q, 1H), 4.48-4.43(q, 1H), 3.87-3.78(m, 2H), 2.26(s, 6H), 1.90(s, 2H). The structures of all other analytes (**4b-f**, **5a-f**, **6a-f**, **6aa**, **6ab,** and **7a-f**) prepared as shown in Scheme 1 were identified by ^{1}H NMR spectra. Injection samples were prepared by dissolving each analyte in methanol at a concentration of 1.0 mg/mL and an injection size was typically 2.0 μl.

RESULTS AND DISCUSSION

The two enantiomers of mexiletine and its analogs have been known to show different activity for blocking sodium currents of skeletal muscle fibers as mentioned above and the difference in their activity was found to be dependent on the structures.[4] Especially, mexiletine analogs containing a substituted phenyl group instead of a methyl group at the chiral center and/or a substituted phenoxy group instead of 2,6-dimethylphenoxy group were found to show different activity in their two enantiomers.[4,5] In this instance, in order to see the structural characteristics for the resolution of mexiletine and its analogs on CSP **1**, CSP **2,** and CSP **3**, various mexiletine analogs were prepared according to the procedure shown in Scheme 1. By treating 2-bromo-1-(substituted phenyl)ethanones with various substituted phenols in the presence of potassium carbonate in DMF, 2-(substituted phenoxy)-1-(substituted phenyl)ethanones (intermediate A in Scheme 1) were prepared. The resulting 2-(substituted phenoxy)-1-(substituted phenyl)ethanones were treated with ammonium acetate and sodium cyanoborohydride in methanol to afford 26 analogs of mexiletine.

In total, 27 analytes including mexiletine and its analogs were resolved on CSP **1**, CSP **2,** and CSP **3** and the resolution results are summarized in Table 1. In order to find the appropriate mobile phase conditions, various mobile phases were tested and finally we found that methanol or acetonitrile in water containing sulfuric acid or perchloric acid as an acidic modifier are appropriate as mobile phases. For the resolution of mexiletine on CSP **1**, 80% methanol in water containing sulfuric acid (10 mM) was found to be the best mobile phase condition. The acidic modifier added to the mobile phase has been known to be used to protonate the primary amino group of analytes. The two enantiomers of resulting primary ammonium ion ($R\text{-}NH_3^+$) of analytes have been known to induce the enantioselective complexation inside the cavity of the crown ether ring of the CSP.[8,9] For the resolution of mexiletine analogs **4a-4f**, **5a-5f**, **6aa**, **6ab,** and **7a-7f** on CSP **1**, 80% methanol in water containing perchloric acid (10 mM) was found to be the most widely applicable as a mobile phase. However, mexiletine analogs **6a-6f** were not resolved at all with the use of 80% methanol in water containing perchloric acid (10 mM) as a mobile phase and even under other various mobile phase conditions.

TABLE 1. Resolution of racemic mexiletine and its analogs on CSP 1, CSP 2 and CSP 3

	CSP **1**			CSP **2**			CSP **3**		
Analytes	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
Mexiletine	1.50[a]	1.15	1.25	3.17[c]	1.20	0.67	8.65[e]	1.15	1.33
4a	4.72[b]	1.38	1.95	2.76[c]	1.66	1.43	13.32[e]	1.54	2.09
4b	4.35[b]	1.44	1.97	1.97[c]	1.73	1.57	14.81[e]	1.72	1.91
4c	5.00[b]	1.46	1.67	2.53[c]	1.86	1.64	15.85[e]	1.88	2.08
4d	8.13[b]	1.47	4.23	2.31[c]	1.70	2.51	10.98[e]	1.76	4.29
4e	5.03[b]	1.41	2.25	3.04[c]	1.79	1.89	15.23[e]	1.83	2.57
4f	3.99[b]	1.38	1.67	2.79[c]	1.61	1.38	15.92[e]	1.64	1.74
5a	3.80[b]	1.41	1.90	4.88[d]	3.49	4.43	9.99[d]	4.14	7.91
5b	4.10[b]	1.29	1.55	3.75[d]	2.87	3.82	8.31[d]	3.23	6.09
5c	4.81[b]	1.40	2.11	5.10[d]	3.13	4.21	10.51[d]	3.66	6.99
5d	7.60[b]	1.31	3.05	3.54[d]	2.92	4.90	7.43[d]	3.33	12.09
5e	3.87[b]	1.47	2.63	5.62[d]	3.79	4.99	10.93[d]	4.51	9.08
5f	3.50[b]	1.40	2.19	5.13[d]	3.62	4.76	10.51[d]	4.25	7.76
6a	4.77[b]	1.00		2.61[d]	1.00		6.06[d]	1.09	1.02
6b	5.34[b]	1.00		1.80[d]	1.00		5.35[d]	1.00	
6c	6.10[b]	1.00		3.76[d]	1.00		6.57[d]	1.00	
6d	9.80[b]	1.00		1.86[d]	1.00		17.13[c]	1.15	1.19
6e	5.03[b]	1.00		3.97[d]	1.00		6.56[d]	1.10	1.17
6f	4.47[b]	1.00		3.81[d]	1.00		6.36[d]	1.10	1.19
6aa	4.09[b]	1.43	3.86	3.29[d]	3.32	5.40	7.31[d]	3.29	12.50
6ab	4.15[b]	1.45	3.79	3.13[d]	3.81	4.56	7.22[d]	2.72	12.31
7a	4.23[b]	1.42	2.26	3.70[d]	2.30	2.94	7.87[d]	2.26	4.63
7b	4.41[b]	1.32	1.67	1.81[d]	3.07	2.98	4.86[d]	2.50	4.10
7c	5.43[b]	1.42	2.38	3.85[d]	2.09	2.64	8.23[d]	2.10	5.13
7d	8.39[b]	1.32	3.12	2.23[d]	2.31	3.21	5.31[d]	2.04	7.80
7e	3.79[b]	1.42	2.20	4.09[d]	2.58	4.24	8.58[d]	2.48	6.68
7f	4.92[b]	1.46	2.94	3.86[d]	2.36	3.12	8.32[d]	2.34	5.35

Mobile phase:
[a]80% Methanol in water + sulfuric acid (10 mM).
[b]80% Methanol in water + perchloric acid (10 mM).
[c]80% Acetonitrile in water + perchloric acid (10 mM).
[d]80% Acetonitrile in water + perchloric acid (10 mM) + ammonium acetate (1.0 mM).
[e]50% Acetonitrile in water + perchloric acid (10 mM) + ammonium acetate (1.0 mM). Flow rate: 0.5 ml/min. Detection: 254 nm UV. Temperature: 20°C. k_1: Retention factor of the first eluted enantiomer. α: Separation factor. R_S: Resolution.

For the resolution of mexiletine and its analogs **4a-4f** on CSP **2**, 80% acetonitrile in water containing perchloric acid (10 mM) was used as an appropriate mobile phase. However, for the resolution of mexiletine analogs **5a-5f**, **6a-6f**, **6aa**, **6ab,** and **7a-7f** on CSP **2**, the retention times were quite long when 80% acetonitrile in water containing perchloric acid (10 mM) was used as a mobile phase. For example, the resolution of **5e** on CSP **2** with the use of 80% acetonitrile in water containing perchloric acid (10 mM) was found to take more than 120 min, as shown in Figure 3. However, the addition of a small amount of ammonium acetate to the mobile phase was found to decrease the retention times quite a lot. As shown in Figure 3, when the content of ammonium acetate added to the mobile phase was increased from 0 to 0.1 to 0.5 and then to 1.0 mM, the retention times were decreased continuously and the resolution was completed within 40 min without hurting the separation factor (α) and resolution (R_S) noticeably. The ammonium ion (NH_4^+) added to the mobile phase is expected to compete with the primary ammonium ion (R-NH_3^+) of analyte for the complexation inside the cavity of the crown ether ring of the CSP and reduce the retention times of analytes.[8,9] Consequently, for the resolution of mexiletine analogs **5a-5f**, **6aa**, **6ab,** and **7a-7f** on CSP **2**, 80% acetonitrile in water containing perchloric acid (10 mM) and ammonium acetate (1.0 mM) was successfully used as a mobile phase. However, mexiletine analogs **6a-6f** were not resolved at all under various mobile phase conditions.

For the resolution of mexiletine and its analogs **4a-4f** on CSP **3**, 50% acetonitrile in water containing perchloric acid (10 mM) and ammonium acetate (1.0 mM) was found to be quite successful. However, for the resolution of **5a-5f**, **6a-6f**, **6aa**, **6ab,** and **7a-7f** on CSP **3**, the retention times were quite long when 50% acetonitrile in water containing perchloric acid (10 mM) and ammonium acetate (1.0 mM) was used as a mobile phase. For example, the resolution of **5d** on CSP **3** was found to take almost 4 h. When 80% acetonitrile in water containing perchloric acid (10 mM) and ammonium acetate (1.0 mM) was used as a mobile phase, the resolution of **5d** on CSP **3** was completed within 30 min, as shown in Figure 4. By increasing the acetonitrile content in the aqueous mobile phase from 50 to 80%, the mobile phase polarity is expected to be decreased and, consequently, the lipophilic interaction between the CSP and analytes is decreased. In this instance, the retention times should be decreased. The use of 80% acetonitrile in water containing perchloric acid (10 mM) and ammonium acetate (1.0 mM) as a mobile phase was quite successful for the resolution of **5a-5f**, **6aa**, **6ab,** and **7a-7f** on CSP **3**. Mexiletine analogs **6a**, **6e,** and **6f** were also resolved with the use of 80% acetonitrile in water containing perchloric acid (10 mM) and ammonium acetate (1.0 mM) as a mobile phase. For the resolution of **6d**, the use of 80% acetonitrile in water containing perchloric acid (10 mM) was found to afford the baseline resolution. However, mexiletine analogs **6b** and **6c** were not resolved at all under various mobile phase conditions.

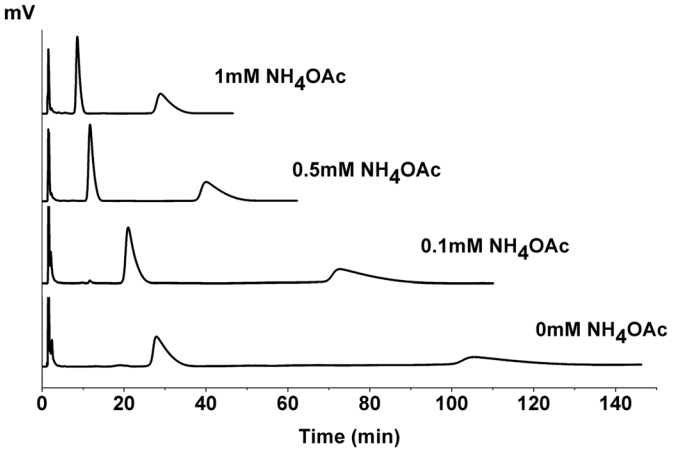


Fig. 3. Chromatograms for the resolution of **5e** on CSP **2** with the variation of the content of ammonium acetate in aqueous mobile phase consisting of 80% acetonitrile in water containing perchloric acid (10 mM). Flow rate: 0.5 ml/min. Detection: 254 nm UV. Temperature: 20°C.

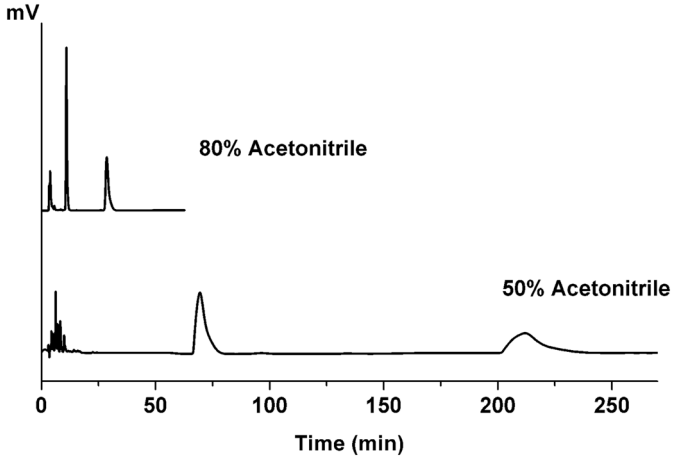


Fig. 4. Comparison of the representative chromatograms for the resolution of **5d** on CSP **3** with the use of 80% or 50% acetonitrile in water containing perchloric acid (10 mM) and ammonium acetate (1 mM) as a mobile phase. Flow rate: 0.5 ml/min. Detection: 254 nm UV. Temperature: 20°C.

Even though the resolution of mexiletine on CSP **1**, CSP **2,** and CSP **3** was not so significant, when the methyl group at the chiral center of mexiletine was changed to phenyl (**4a**) or 4-substituted phenyl (**4b**, **4c**, **4d**, **4e,** or **4f**), the resolution was improved quite a lot on each of the three CSPs. However, the effect of the type of the substituent (electron-donating or electron-accepting substituent) of the phenyl group at the chiral center of mexiletine analogs on the separation factors (α) was not so significant, the separation factors (α) for the resolution of **4a-4f** on CSP **1**, CSP **2,** and CSP **3** being in the relatively narrow range of 1.38–1.47, 1.61–1.86, and 1.64–1.88, respectively. On the contrary, the resolutions (R_S) for the resolution of **4a-4f** on CSP **1**, CSP **2,** and CSP **3** were found to be quite dependent on the type of the substituent on the phenyl group at the chiral center of the mexiletine analogs. The resolutions (R_S) for the resolution of **4a-4f** on CSP **1**, CSP **2,** and CSP **3** were in the range of 1.67–4.23, 1.38–2.57, and 1.74–4.29, respectively. On each of the three CSPs, **4d** containing 4-nitrophenyl group at the chiral center was found to give the highest resolution (R_S) value, but the reason is not clear yet.

Change of the 2,6-dimethylphenoxy group of analytes **4a-4f** to 3,5-dimethylphenoxy group (**5a-5f**) or to simple phenoxy group (**7a-7f**) does not affect the chiral recognition on CSP **1** significantly, as shown in Table 1. The separation factors and resolutions for the resolution of **4a-4f** on CSP **1** are in the range of 1.38–1.47 and 1.67–4.23, respectively, but those for the resolution of **5a-5f** and **7a-7f** on CSP **1** are in the range of 1.29–1.47 and 1.55–3.12, respectively. Change of the

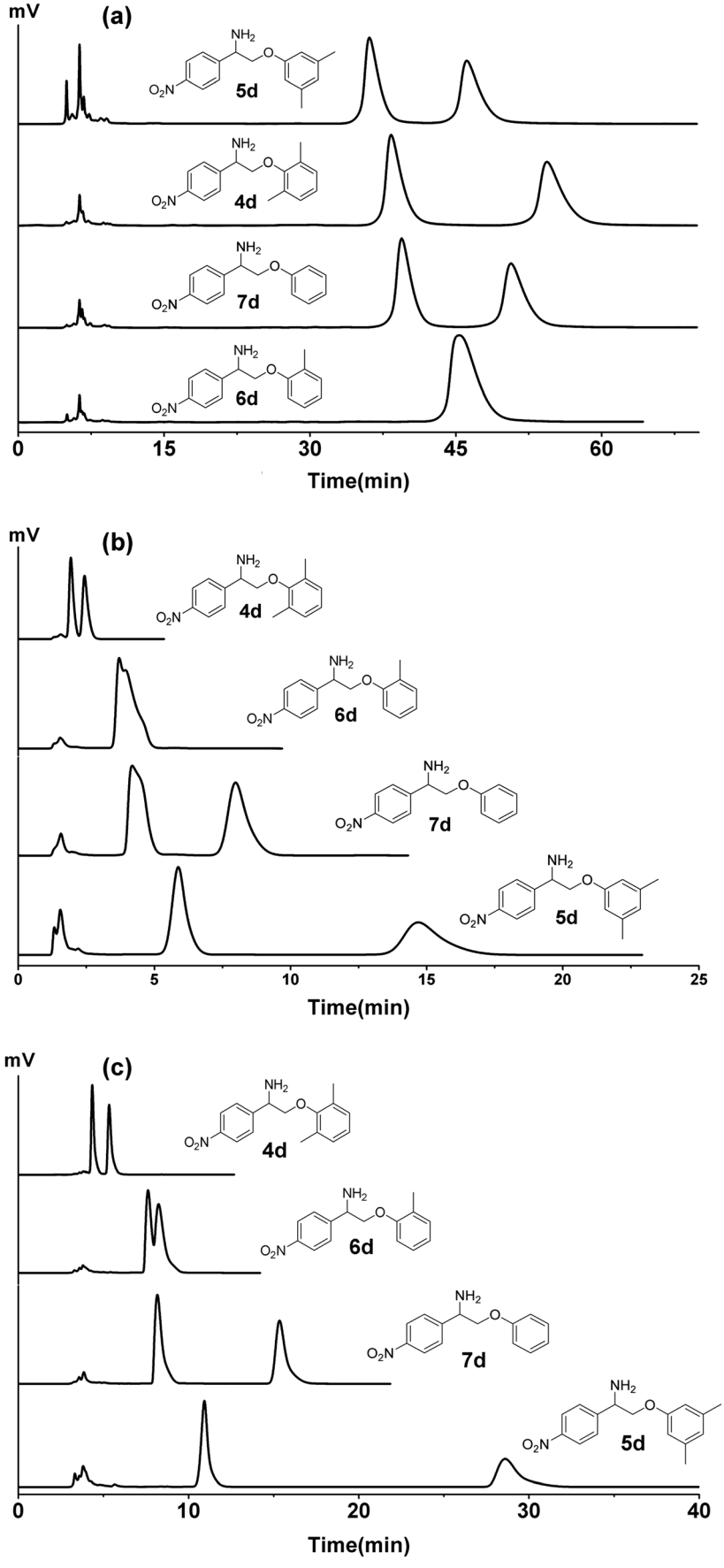


Fig. 5. Chromatograms for the resolution of **4d**, **5d**, **6d,** and **7d** on (**a**) CSP **1**, (**b**) CSP **2,** and (**c**) CSP **3**. Mobile phase: 80% methanol in water + perchloric acid (10 mM) for CSP **1**, 80% acetonitrile in water + perchloric acid (10 mM) + ammonium acetate (1.0 mM) for CSP **2,** and CSP **3**. Flow rate: 0.5 ml/min. Detection: 254 nm UV. Temperature: 20°C.

2,6-dimethylphenoxy group of analyte **4a** to 3-methylphenoxy (**6aa**) or to 4-methylphenoxy (**6ab**) also does not affect the chiral recognition on CSP **1** significantly. However, when the 2,6-dimethylphenoxy group of analytes **4a-4f** was changed to 2-methylphenoxy group (**6a-6f**), the chiral recognition was not observed at all on CSP **1**. From these results, the chiral recognition for mexiletine analogs on CSP **1** was concluded to be not affected significantly by the type of phenoxy group of analytes except for the 2-methylphenoxy group.

In contrast, the type of phenoxy group of mexiletine analogs was found to show a significant effect on the chiral recognition on CSP **2** and CSP **3**. As shown in Table 1, analytes **5a-5f** containing the 3,5-dimethylphenoxy group were resolved greater than analytes **4a-4f** containing the 2,6-dimethylphenoxy group on CSP **2** and CSP **3** in terms of the separation factors and resolutions. Between CSP **2** and CSP **3**, the latter was found to show a greater chiral recognition ability for analytes **5a-5f** than the former. Analytes **7a-7f** containing a simple phenoxy group were also resolved better than analytes **4a-4f** on CSP **2** and CSP **3**, but slightly worse than analytes **5a-5 f**. Analytes **6aa** and **6ab** containing a 3-methyl or 4-methylphenoxy group were also resolved better than analyte **4a** on CSP **2** and CSP **3**. However, analytes **6a-6f** containing the 2-methylphenoxy group were not resolved at all on CSP **2.** Analytes **6b** and **6c** were also not resolved at all on CSP **3** and analytes **6a**, **6d-6f** were only slightly resolved on CSP **3**. The reason for the nonresolution or poor resolution of analytes **6a-6f** on the three CSPs is not clear.

All of these results indicate that the chiral recognition behaviors for the resolution of mexiletine and its analogs on CSP **1** are somewhat different from those on CSP **2** and CSP **3**. The different chromatographic behaviors on CSP **1**, CSP **2,** and CSP **3** are clearly demonstrated by the trends of the retention times for the resolution of **4d**, **5d**, **6d,** and **7d** on the three CSPs shown in Figure 5. The retention times of the first eluted enantiomers for the resolution of **4d**, **5d**, **6d,** and **7d** on CSP **1** with the use of 80% methanol in water containing perchloric acid (10 mM) as a mobile phase were only slightly different and were on the order of **5d** < **4d** < **7d** **6d**. However, the retention times for the resolution of **4d**, **5d**, **6d,** and **7d** on CSP **2** and CSP **3** under an identical mobile phase condition with the use of 80% acetonitrile in water containing perchloric acid (10 mM) and ammonium acetate (1 mM) as a mobile phase were very much different and were on the order of **4d** < **6d** < **7d** < **5d**. CSP **2** and CSP **3** should be quite lipophilic because of the lipophilic 3,3'-diphenyl-1,1'-binaphthyl group, while CSP **1** is relatively less lipophilic compared with CSP **2** and CSP **3**. In this instance, the lipophilic interaction of analytes with CSP **1** is expected to be not significant and does not seem to be dependent on the lipophilicity of analytes significantly. In this instance, the retention times of analytes **4d**, **5d**, **6d,** and **7d** on CSP **1** should be similar. However, the lipophilic interaction of analytes with CSP **2** or CSP **3** is expected to be quite significant because of the high lipophilicity of CSP **2** or CSP **3**, the retention times of the analytes being quite dependent on the lipophilicity of analytes. Analyte **5d** should be more lipophilic than analyte **6d** because of the additional 3,5-dimethyl group. In this instance, the lipophilic interaction of analyte **5d** with CSP **2** or CSP **3** should be greater than that of analyte **6d** with CSP **2** or CSP **3** and, consequently, analyte **5d** was retained longer than analyte **6d** on CSP **2** or CSP **3**. CSP **3** should be more lipophilic than CSP **2** because of the additional n-octyl groups on the silica surface of the stationary phase. In this instance, the analytes should be retained longer on CSP **3** than on CSP **2**. Analytes **4d** and **6d** might be more lipophilic than analyte **7d** because of the additional dimethyl or methyl group on the phenoxy ring. However, the retention times of analyte **4d** and **6d** are shorter than those of analyte **7d** on CSP **2** and CSP **3**. The 2,6-dimethyl group of analyte **4d** and the 2-methyl group of analyte **6d**, which are near the complexation site, might exert some steric hindrance for the complexation of the primary ammonium ions of the protonated analytes inside the cavity of the crown ether ring of the stationary phase and, consequently, reduce the retention times. The steric hindrance might originate from the steric interaction between the 3,3'-diphenyl-1,1'-binaphthyl of CSP **2** or CSP **3** and the nearby 2,6-dimethyl or 2-methyl group of analytes. In addition, the steric hindrance should be more significant with the 2,6-dimethylphenoxy group than with the 2-methylphenoxy group of the analyte. In this instance, the retention times for analyte **4d** should be shorter than for analyte **6d**. However, steric hindrance is not expected with CSP **1** because of the lack of sterically bulky 3,3'-diphenyl-1,1'-binaphthyl group.

In conclusion, three crown ether-based CSPs based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid or (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6 were quite successful for the resolution of mexiletine and its analogs. The chiral recognition efficiencies of the three CSPs for the resolution of mexiletine analogs were found to be quite dependent on the type of phenoxy group of analytes. Even though the CSP based on (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6 containing residual silanol group-protecting n-octyl groups on the silica surface was found to be most effective for the resolution of mexiletine and its analogsm especially in terms of resolutions (R_S), any one of the three CSPs is concluded to be useful for the determination of the enantiomeric composition of mexiletine analogs containing a primary amino group.

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