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Short communication

Chiral separation of 2,4-dinitrophenyl amino acids using 3-*O*-methyl-β-cyclodextrin-bonded stationary phase in reversed-phase liquid chromatography

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Abstract

Reversed-phase liquid chromatography (RPLC) separation factors for racemic 2,4-dinitrophenyl (DNP) amino acids (Phe and Trp) having various substituents at different positions were measured on native β-CD and heptakis(3-*O*-methyl)-β-CD as the chiral stationary phase in order to compare enantioselectivity of these phases. Both native β-CD and heptakis(3-*O*-methyl)-β-CD showed good enantioselectivity for the DNP–amino acids investigated. Enantioselectivities of the two CD phases for the DNP–amino acids vary with the type and position of the substituent on the DNP moiety of the amino acids. Heptakis(3-*O*-methyl)-β-CD, the cavity of which is more electron-rich than that of native β-CD, showed in general much better enantioselectivity for the amino acid derivatives studied. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Application of cyclodextrins (CDs) in high-performance liquid chromatography (HPLC) either as a chiral additive in the mobile phase or as a chiral stationary phase (CSP) for the separation of optical isomers has received increasing attention [1–8]. One of the most interesting developments in CD-bonded stationary phases is their derivatization [8–10]. The 2-OH and/or 3-OH positions of CDs can be functionalized with designed groups [11,12]. Presence of these hydroxyl groups on the CD rim changes the size of the CD cavity and hence inclusion of guest molecules in the CD [3]. Previous studies have focused on the CD derivatives with both 2-OH and 3-OH positions substituted with other functional groups. Chromatographic resolution by 2,3-methylated CD derivatives are known to occur mainly by inclusion process in the CD cavity [9,10]. Tanaka et al. [10] reported separation of various positional isomers on a 2,3-methylated CD derivative. Kuroda et al. [9] reported the separation of racemic 3,5-dinitrobenzoylated amino acids on the methylated CDs. These CDs were found to show only limited enantioselectivity. Optimization of separation conditions such as the solute concentration,

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the organic composition and pH of the aqueous mobile phase and the concentration of buffer in enantioseparation of racemic amino acids have been extensively studied on various CDs as CSPs [6,8,13,14].

Recently we reported that enantioselectivities of native, 3-*O*-methylated and 2,3-*O*-methylated β-CDs in the separation of some racemic 2,4-dinitrophenyl (DNP)–amino acids (Val, Leu, Met, Ile, His, Phe, Tyr) [13]. Native β-CD and heptakis(3-*O*-methyl)-β-CD showed good enantioselectivity for the DNP–amino acids while heptakis(2,3-di-*O*-methyl)-β-CD showed very poor enantioselectivity. This indicated that the presence of at least one OH group on the CD rim is essential for chiral recognition. Heptakis(3-*O*-methyl)-β-CD has recently been utilized as the chiral selector in capillary electrophoresis for separation of dansyl amino acids and basic drugs [3,15]. While enantioselectivities of native β-CD and heptakis(3-*O*-methyl)-β-CD are very good for the amino acid derivatives studied it was difficult to see any systematic variation of the enantioselectivity for the two CD phases with the type of the amino acid residues. The effect of derivatization of amino acid residues of the DNP–amino acids was not investigated.

Although inclusion into the CD cavity is not the sole mechanism for retention and enantioselectivity on the CD stationary phases, inclusion is considered to be a main factor for determining the retention and enantioselectivity [9,10]. For DNP–amino acids inclusion of the DNP moiety into the CD cavity is likely to be the predominant factor. However the side chain on the chiral carbon is also thought to be concerned. A chiral retention of amino acid derivatives is based on the competitive inclusion complexation between the substituent group and the side chain or aromatic moiety of the amino acid derivatives with CDs [6]. In view that the size and geometry of the guest molecule are important factors affecting inclusion, it would be more useful to compare separation of a single type of amino acid derivatives having various substituents on different positions than those for different types of amino acids. It is thought that different type and position of various substituents will render different size and shape for the amino acids and have them better sense subtle differences in the cavities of native and methylated CDs.

In the present study we measured and compared retention and separation factors of two types of 2,4-DNP–amino acids – Phe and Trp – having various substituents at different positions on native β-CD and heptakis(3-*O*-methyl)-β-CD as CSPs in order to see the effect of derivatization of amino acid residues of the DNP–amino acids on enantioselectivity on the two CDs as CSPs.

2. Experimental

2.1. Apparatus and procedures

A Varian Model 5560 liquid chromatographic system consisting of a Model 7125 injector with a 10-μl sample loop (Rheodyne, Cotati, CA, USA) and a Model UV-200 wavelength UV detector set at 360 nm was used. A Varian (CA, USA) Model 4270 integrating recorder was used to record chromatograms. The columns (150×4.6 mm I.D., Phenomenex, CA, USA) were laboratory-packed by using an Alltech (Deerfield, IL, USA) Model 1666 slurry packer at ca. 7000 psi (1 p.s.i.=3894.76 Pa).

DNP–amino acids were derivatized according to the procedure described in the literature [16]. The mobile phases were mixtures of acetonitrile and ammonium acetate buffer (0.01 *M*). The ammonium acetate buffer was made up from ammonium acetate and acetic acid to the desired pH. The buffer was then filtered through a membrane filter of 0.5-μm pore size and degassed prior to use. The flow-rate was 1.0 ml min^{-1}. Methanol was used as the dead time marker. The chromatograms of the racemates of amino acids were obtained under ambient conditions and the peak identification was carried out by injecting standard solutions of each enantiomer.

2.2. Reagents and materials

L- and D,L-amino acids, 2,4-dinitrofluorobenzene and β-CD hydrate were obtained from Sigma (St. Louis, MO, USA) or Aldrich (Milwaukee, WI, USA). HPLC-grade acetonitrile was purchased from Burdick and Jackson (Muskegon, MI, USA). Lichrosorb Si 60 (Merck, Darmstadt, Germany), having a mean pore size of 6 nm and a mean particle diameter of 5 μm, was used as the support. Water was processed

with a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other reagents were used as received without any further purification.

2.3. Preparation of stationary phases

3-O-Me-β-CD was prepared according the procedure reported in the literature [11]. The structure of the CD derivative as identified by nuclear magnetic resonance (NMR) spectroscopy [^{1}H NMR (DMSO-d_6, 500 MHz): δ 4.99(s, 7H), 4.26(brs, 14H), 3.58–3.72(m, 28H), 3.49(s, 21H), 3.35(m, 7H), 3.15(d, 7H); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 99.7 (C-1), 82.1 (C-4), 81.9(C-3), 72.8, 71.6,(C-2,5), 59.9(C-6), 59.6(OCH_3) (Fig. 1). The preparation of the CD-bonded stationary phase has been described elsewhere [13]. The amount of bonded CD moiety was calculated from the results of the C, H and N elemental analysis. The values were 121 μmol g^{-1} for native and 148 μmol g^{-1} for heptakis(3-O-methyl)-β-CD, respectively.

3. Results and discussion

Structures of DNP–amino acids studied are shown in Scheme 1. Table 1 lists the retention factors (k') and separation factors (α) for DNP–Phe and DNP–

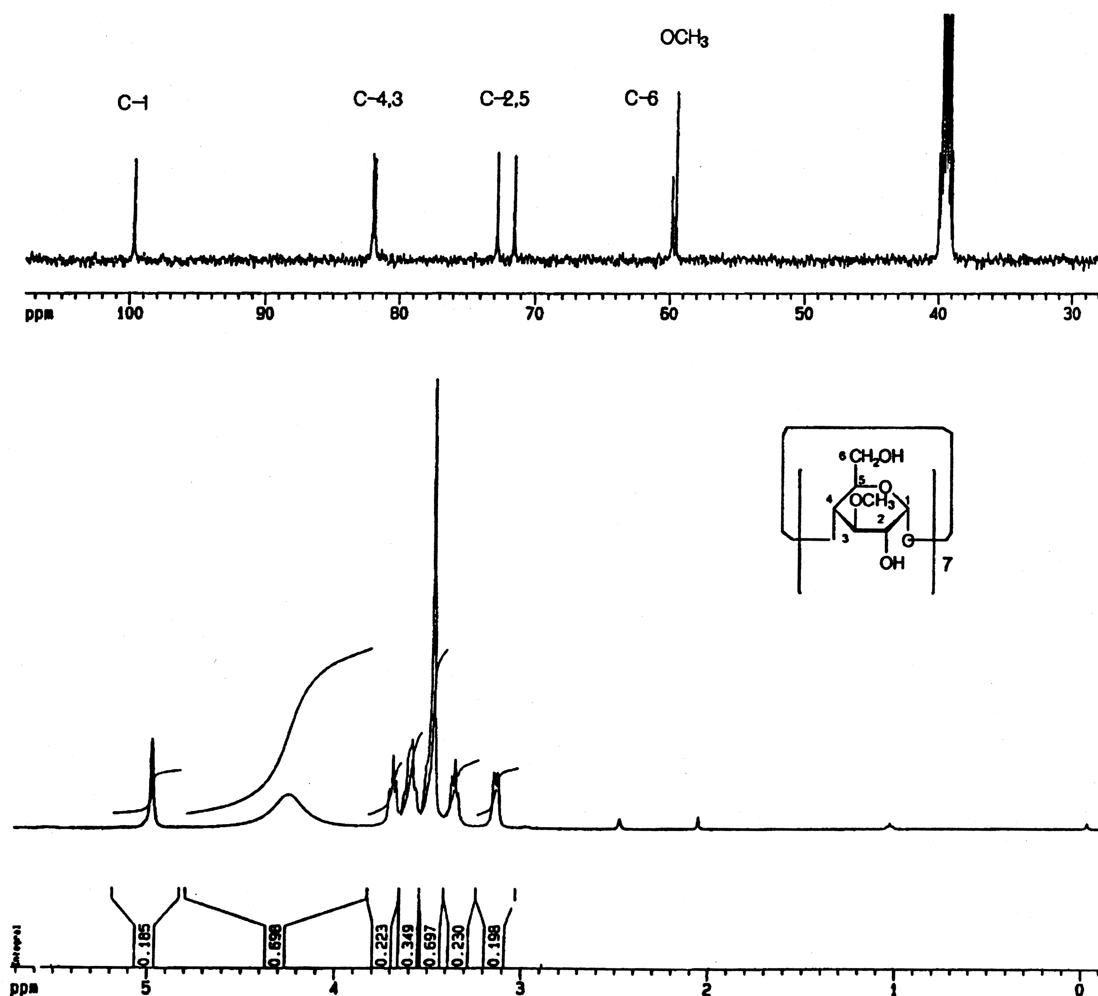
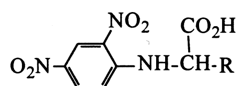


Fig. 1. ^{13}C and ^{1}H NMR spectra of 3-O-methyl-β-CD.



Compound	R=	Compound	R=
DNP-Phe		DNP-Trp	
DNP-2-F-Phe		DNP-4-F-Trp	
DNP-3-F-Phe		DNP-5-F-Trp	
DNP-4-F-Phe		DNP-6-F-Trp	
DNP-4-Cl-Phe		DNP-5-OCH3-Trp	

Scheme 1. Chemical structures of DNP–amino acids.

Trp on β-CD (phase I) and heptakis(3-*O*-methyl)-β-CD (phase II) in 10% acetonitrile–ammonium acetate buffer (0.01 *M*, pH 6.8).

Table 1
Comparison of retention factors (k') and separation factors (α) of DNP–amino acids on β-CD (phase I) and heptakis(3-*O*-methyl)-β-CD (phase II)

Compound	Phase I		Phase II	
	k'	α	k'	α
DNP–Phe	3.77 (D)	1.27	10.22 (D)	2.06
DNP–2-F-Phe	3.47	1.14	10.14	1.75
DNP–3-F-Phe	3.43	1.26	9.73	2.11
DNP–4-F-Phe	3.32	1.07	9.73	1.67
DNP–4-Cl-Phe	7.03	1.18	28.91	1.80
DNP–Trp	3.66 (L)	1.31	16.68 (D)	1.52
DNP–4-F-Trp	4.22	1.23	21.65	1.54
DNP–5-F-Trp	3.58	1.00	17.61	1.56
DNP–6-F-Trp	2.67	1.06	18.28	1.28
DNP–5-OCH_3-Trp	3.08	1.00	21.79	1.73

Retention factors are given for the first eluting enantiomers. Mobile phase; 10% acetonitrile containing 0.01 *M* ammonium acetate, pH 6.8.

Table 1 shows that retention for all the amino acid derivatives is much longer on the phase II than that on the phase I. The cavity of phase II is more hydrophobic due to the presence of a methyl group than that for native β-CD. More stable inclusion complexes of DNP–amino acids are likely to form with heptakis(3-*O*-methyl)-β-CD than native β-CD, rendering a greater retention on phase II than on phase I.

Retention behavior is interestingly different on the two CD phases for different types of amino acids. Introduction of a fluorine atom onto the amino acid residue of the DNP–Trp derivatives either increases or decreases retention of the amino acid derivatives, depending on the substitution position. In the case of DNP–Phe derivatives introduction of a fluorine atom always decreases retention regardless of the substitution position. The effect of the F substitution on the extent of the change in retention is greater for DNP–Trps than that for DNP–Phes. While the elution order of the F-substituted DNP–Phe derivatives is the same on both phases, the elution order of the

F-substituted DNP–Trp derivatives is different on the two phases. Different retention behavior on the native and 3-*O*-methylated CD phase indicates that inclusion capability of the CD cavity is considerably altered by the presence of 3-*O*-methyl groups on the CD rim.

Separation factors (α) in Table 1 indicate that enantioselectivity of phase II is much better than that of phase I for the amino acid derivatives studied. Enantioselectivities of the two CD phases for the DNP–amino acids are considerably different and vary with the type and position of the substituents on the amino acid residues. Introducing a substituent onto the amino acid residue of DNP–Phe always decreases enantioselectivity on phase I compared to nonsubstituted derivatives, regardless of the substituent type and the substitution position but on phase II enantioselectivity for 3-F-Phe is slightly increased.

In the case of DNP–Trps variation of enantioselectivities with the type and position of the substituents on the amino acid residue is quite different from that for DNP–Phe on the two CD phases. Introduction of a substituent always decreases enantioselectivity on phase I regardless of the substituent type and the substitution position while it increases enantioselectivity on phase II except for 6-F-Trp. It is noteworthy that enantiomers of 5-F-Trp and 5-OCH_3-Trp are not resolved at all on phase I while they are well resolved on phase II. In view of the fact that enantiomers of the dansyl derivative of Trp are not well separated on β-CD phases [6,14,17,18], it is interesting that DNP–Trp enantiomers are all well resolved on phase II.

Fig. 2 shows chromatograms for separation of a mixture of DNP–L-Trp and DNP–D-Trp (concentration ratio, 2:1) on phases I and II. It is interesting that elution order is reversed on the two CD phases. On phase I the L-form is eluted first while on phase II the D-form is eluted first. It is also interesting to note that DNP–5-OCH_3-Trp is better resolved than DNP–Trp on phase II but not resolved at all on phase I. It seems that the less polar indole ring is preferentially included into the CD cavity over the more polar DNP moiety. The cavity of heptakis(3-*O*-methyl)-β-CD is more hydrophobic due to the presence of a methyl group than that for native β-CD. More stable diastereomeric inclusion complexes of DNP–Trps are expected to form with heptakis(3-*O*-methyl)-β-CD than native β-CD, rendering a greater enantioselectivity to phase II than to phase I.

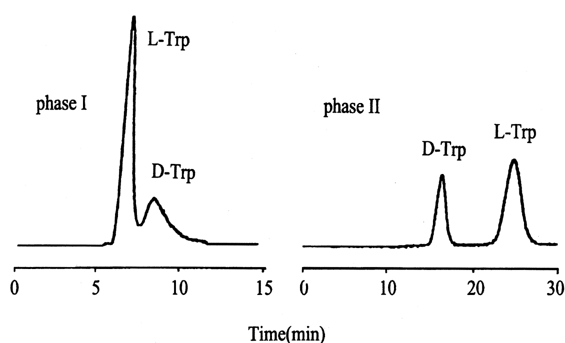


Fig. 2. Chromatograms of the separation of DNP–Trp on phases I and II. Experimental conditions as in Table 1.

4. Conclusions

Enantioselectivity of two β-CD bonded phases, native β-CD and heptakis(3-*O*-methyl)-β-CD, as CSPs were studied for reversed-phase liquid chromatography (RPLC) separation of DNP–Phe and DNP–Trp derivatives. Both native β-CD and heptakis(3-*O*-methyl)-β-CD showed good enantioselectivity for the DNP–amino acids. Heptakis(3-*O*-methyl)-β-CD, the cavity of which is more electron-rich than that of native β-CD, showed much better enantioselectivity for the amino acids derivatives studied.

References

[1] J. Szejtli, T. Osa, Comprehensive Supramolecular Chemistry, Vol. 3, Cyclodextrins, Pergamon, Oxford, 1996.
[2] A.M. Krutulovic, Chiral Separations by HPLC, Wiley, New York, 1989.
[3] M. Yoshinaga, M. Tanaka, J. Chromatogr. A 679 (1994) 359.
[4] B.J. Spencer, W.C. Purdy, Anal. Lett. 28 (1995) 1865.
[5] D.W. Armstrong, C.D. Chang, S.H. Lee, J. Chromatogr. 539 (1991) 83.
[6] K. Fujimura, S. Suzuki, K. Hayashi, S. Masuda, Anal. Chem. 62 (1990) 2198.
[7] T.J. Ward, D.W. Armstrong, J. Liq. Chromatogr. 9 (1986) 407.
[8] S. Li, W.C. Purdy, J. Chromatogr. 543 (1991) 105.

[9] Y. Kuroda, T. Kato, H. Ogoshi, Bull. Chem. Soc. Jpn. 66 (1993) 716.
[10] M. Tanaka, J. Okazaki, H. Ikeda, T. Shono, J. Chromatogr. 370 (1986) 293.
[11] K. Takeo, H. Mitoh, K. Uemura, Carbohydr. Res. 187 (1989) 203.
[12] P. Fugedi, Carbohydr. Res. 192 (1989) 366.
[13] J.W. Ryu, D.W. Kim, K.P. Lee, Anal. Sci. 13 (1997) 217.
[14] S.H. Lee, A. Berthod, D.W. Armstrong, J. Chromatogr. 603 (1992) 83.
[15] G. Weseloh, H. Bartsch, W.A. Konig, J. Microcol. Sep. 7 (1995) 355.
[16] J.F. Lawrence, R.W. Frei, Chemical Derivatization in Liquid Chromatography, Elsevier, New York, 1976.
[17] A.M. Rizzi, S.C. Runge, H. Jonsson, S. Osla, J. Chromatogr. A 710 (1995) 287.
[18] W.L. Hinze, T.E. Richl, D.W. Armstrong, W. Demond, A. Alak, T. Ward, Anal. Chem. 57 (1985) 237.