Liquid Chromatographic Resolution of 2-Hydroxy Acids on Chiral Stationary Phases: A Mechanistic Consideration

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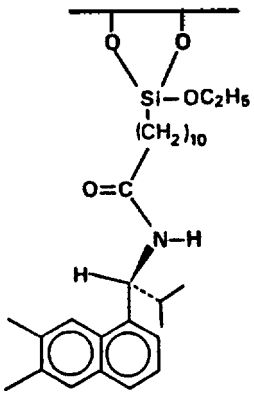
Two enantiomers of various 2-hydroxy acid esters have been resolved as the 3,5-dinitrophenyl carbamates on chiral stationary phases (CSPs) derived from α-arylalkylamines. Two CSPs, each of which contains the same type of chiral moiety, but shows different mode of connection to a silica support, have been found to show the contrasting resolution behaviors. From the contrasting resolution behaviours of two CSPs used in this study, two competing chiral recognition mechanisms are proposed.

Introduction

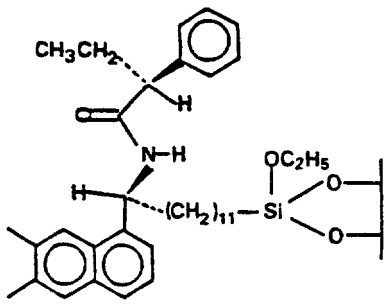
Optically pure 2-hydroxy acids are important as starting materials, chiral building blocks or intermediates for the synthesis of biologically active natural products.[1] Efforts to obtain single enantiomer of 2-hydroxy acids have been focused on the chemical or enzymatic asymmetric synthesis and, as results, various synthetic methods are currently available.[2,3] However, the methods which make possible the rapid and accurate assesment of enantiomeric composition of 2-hydroxy acids prepared asymmetrically are limited. NMR chemical shift reagent methods,[3] polarimetric methods and/or methods of chromatographic separation of diastereomers[4,5] have usually been employed for the determination of enantiomeric purity of 2-hydroxy acids even though all of these methods have shortcomings in convenience and accuracy.

Recently chromatographic direct separation of enantiomers on CSPs has beeen considered to be an effective analytical tool for the rapid and accurate determination of enantiomeric purity of chiral products. Gas chromatographic separation of racemic 2-hydroxy acids as their volatile derivatives on CSPs has been used for the evaluation of enantiomeric purities.[5] Enantiomeric 2-hydroxy acids have also been resolved by liquid chromatography including ligand exchange chromatography on CSPs.[6,7] In this area, our effort has also been described in a short communication dealing with the liquid chromatographic resolution of 2-hydroxy acid esters as their 3,5-dinitrophenyl carbamates on CSP **1** derived from (S)-1-[1-(6,7-dimethylnaphthyl)]isobutylamine.[8] However the chiral recognition mechanisms involved have not been systematically studied yet. An understanding of the chiral recognition mechanisms involved allows one to predict the separation of given analytes and to assign the absolute configuration of the enantiomers of 2-hydroxy acids from the elution order without recourse to configurationally known samples.

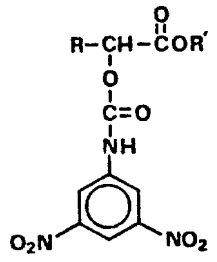
In this study, we wish to report that CSPs **1** and **2** which are derived from α-arylalkylamines can separate two enantiomers of various 2-hydroxy acid derivatives. CSP **1** has been known to separate a variety of racemates.[9-13] CSP **2** which has second stereogenic center was recently prepared by connecting (S)-N-[(S)-1-phenylbutanoyl]-1-[1-(6,7-dimethylnaphthyl)]alkylamine to silica gel.[14] The direction of connecting arm of CSP **2** is altered from that of CSP **1** as shown in their structure. Thus, the comparison of chiral resolution behaviours on these CSPs may provide a reasonable chiral recognition mechanism.



CSP 1



CSP 2



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Experimental

The chromatographic system used in this study consists of Waters Model 510 pump, Waters Model U6K Universal Chromatograph Injector, Waters Model 441 Absorbance Detector with 254 nm UV filter and Waters Model 740 Data Module Recorder.

All data for the chromatographic resolution were obtained by using a 250 mm × 4.6 mm I.D. stainless-steel column packed with CSP **1** or CSP **2** by conventional methanol slurry method. Mobile phase was 10% isopropyl alcohol in *n*-hexane on CSP **1** or 20% isopropyl alcohol in *n*-hexane on CSP **2**. The flow rate of mobile phase was 2 ml/min. Column temperature was maintained constant (20 °C) during the chromatographing period by using a Julabo F 30 cooling circulator.

^{1}H NMR spectra for the characterization of the derivati-

Table 1. The Resolution of 3,5-Dinitrophenylcarbamates **3** of 2-Hydroxy Acid Esters on CSP 1 and CSP 2

Carbamates **3**		CSP 1			CSP 2		
R	R'	α^a	$k_1{}^b$	Conf.^{c}	α^a	$k_1{}^b$	Conf.^{c}
Methyl	Methyl	1.84	4.49	S	2.83	5.49	S
Methyl	Ethyl	1.89	3.24	S	2.90	4.38	S
Methyl	*n*-Propyl	1.93	2.65	S	2.81	3.88	S
Methyl	*n*-Butyl	1.93	2.38	S	2.75	3.50	S
Methyl	*n*-Octyl	1.93	1.75	S	2.27	2.69	S
Methyl	*n*-Dodecyl	1.94	1.39	S	2.00	2.19	S
Phenyl	Methyl	1.89	4.95	S	3.13	11.53	S
Phenyl	Ethyl	1.98	3.75	S	3.16	9.28	S
Phenyl	Isopropyl	2.00	2.93	S	3.29	7.06	S
Phenyl	*n*-Butyl	1.99	2.99	S	2.91	7.48	S
Phenyl	*n*-Octyl	2.08	2.28	S	2.35	5.75	S
Phenyl	*n*-Dodecyl	2.10	1.88	S	2.03	4.53	S
Methyl	Ethyl^d	1.89	3.24	S	2.90	4.38	S
Ethyl	Ethyl	2.03	2.88		3.71	4.50	
n-Butyl	Ethyl	1.94	2.25		4.19	3.90	
n-Hexyl	Ethyl	1.88	1.90		4.12	3.25	
n-Octyl	Ethyl	1.80	1.69		4.16	2.71	
n-Dodecyl	Ethyl	1.69	1.38		4.17	2.00	

^{a}Separation factor. For definition, see reference 19. ^{b}Capacity factor for the first eluted enantiomer. For definition, see reference 19. ^{c}Absolute configuration of the lately eluted enantiomer. For blanks, elution orders have not been established. ^{d}This data is duplicated for the purpose of comparison.

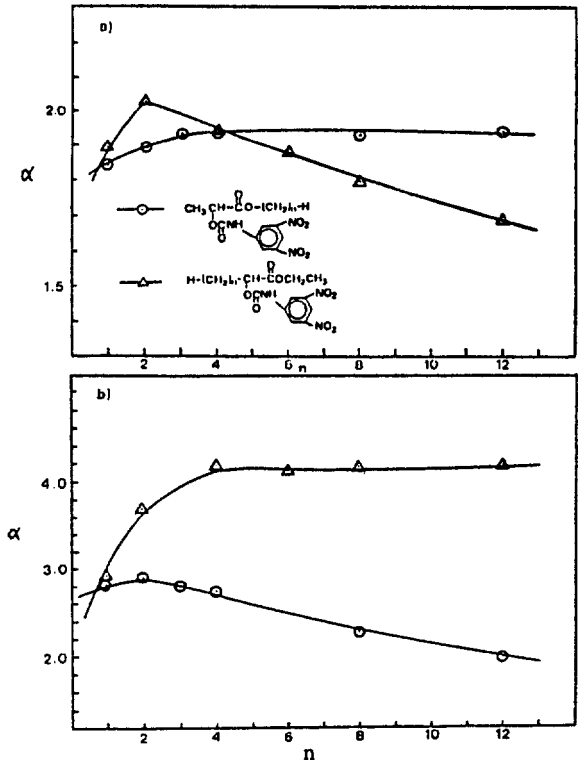

Figure 1. Resolution of a series of carbamates **3** on a) CSP **1** and b) CSP **2**. All chromatographic conditions are given in the experimental part.

ves of 2-hydroxy acids were obtained on a Varian EM-360A spectrometer. IR spectra were recorded on a Mattson Polaris FT-IR spectrometer. Melting point determination was performed by using a Rigaku Thermal Analyzer TAS 100.

2-Hydroxy acids used in this study were commercially available or were prepared by reducing with sodium borohydride α-keto esters obtained from the reaction of Grignard reagent with dialkyl oxalate.[2,15] Derivatization of 2-hydroxy acids were performed by using conventional esterification reaction and then, by using the method of the derivatization of alcohols or diols.[10,16] As an example, the spectroscopic and physical data of methyl 2-hydroxybutanoate and its 3,5-dinitrophenyl carbamate are presented in the following.

Methyl 2-Hydroxybutanoate. ^{1}H NMR (CCl_4) δ 0.80 (t, 3H), 1.35-1.95 (m, 2H), 3.35-3.50 (broad, 1H), 3.70 (s, 3H), 3.87-4.25 (m, 1H); IR (KBr) 3200-3650 (broad), 2970, 2880, 1740 cm^{-1}

3,5-Dinitrophenyl Carbamate of Methyl 2-Hydroxybutanoate. m.p. 114-115 °C; ^{1}H NMR ($CDCl_3$) δ 1.05 (t, 3H), 1.75-2.10 (m, 2H), 3.93 (s, 3H), 5.10 (t, 1H), 8.50-8.68 (m, 4H); IR (KBr) 3320, 3100, 2950, 1760, 1730 cm^{-1}.

Results and Discussion

For the enantioselective separation, CSPs should interact with analytes through a minimum of three simultaneous interactions, at least one of these interactions being stereochemically dependent.[17] Among others, π-π interaction between CSP **1** and analytes has been known to be of prime importance to achieve chiral recognition.[9-13] Both of CSPs **1** and **2** contain a strong π-basic site such as 6,7-dimethylnaphthyl group. However, 2-hydroxy acids do not have any π-acidic site needed for the π-π interaction with CSPs. Thus, to be resolvable on CSPs **1** and **2**, 2-hydroxy acids should be converted into the π-acidic derivatives.

Previously, racemic alcohols and racemic diols have been resolved as the 3,5-dinitrophenyl carbamates on CSP **1**[10,16] As a logical extension of the prior studies, 2-hydroxy acid esters were treated with 3,5-dinitrophenyl isocyanate obtained *in sutu* from 3,5-dinitrobenzoylazide to afford 3,5-dinitrophenyl carbamates **3**. In order to see the general chromatographic resolution trend depending on the structure of analytes, various 3,5-dinitrophenyl carbamates **3** with varying length of the ester alkyl tail(R') and the alkyl substituent(R) at the stereogenic center were prepared and identified by their satisfactory spectroscopic data.

Data for the chromatographic resolution of carbamates **3** on CSPs **1** and **2** are listed in Table 1. The elution orders noted in Table 1 were determined by chromatographing configurationally known samples. In every case, it is noted that (S)-enantiomers elute later than (R)-enantiomers and CSP **2** shows greater chiral recognition than CSP **1**. From the data, one sees an important trend of resolution behaviours. The magnitude of the separation factors(α) on CSP **1** remains almost constant after the initial increase as the ester alkyl tail (R') of carbamate **3** increases in length, but on the other hand the magnitude of separation factors decreases after the maximization of α at $n = 2$ as the alkyl substituent(R) increases in length. On the contrary, on CSP **2**, the magnitude of separation factors decreases with the maximization of α at $n = 2$ as the ester alkyl tail of carbamate **3** increases in length and re-

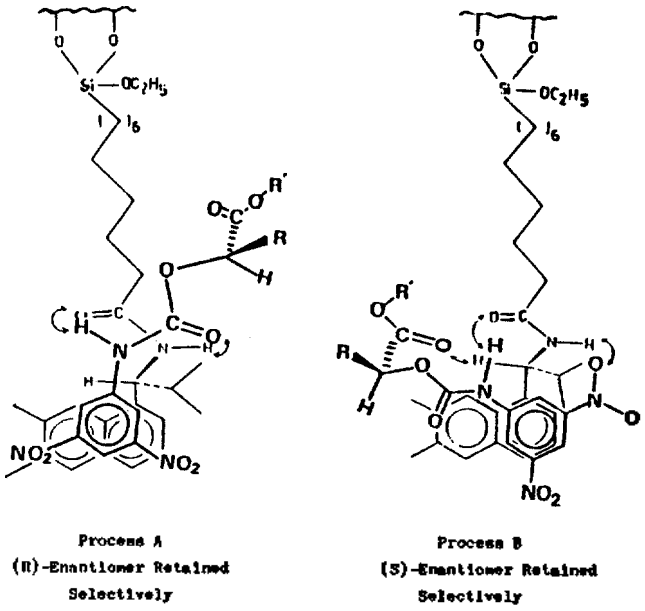

Figure 2. Two competing chiral recognition processes for the resolution of carbamate **3** on CSP **1**.

mains almost independent on the length of the alkyl substituent of carbamate **3** after the length of the alkyl substituent reaches *n*-butyl. To visualize those resolution trends given in Table 1, some of those are graphically shown in Figure 1.

The resolution trends influenced by the direction of the connecting arm of CSPs and the length of the ester alkyl tail and the alkyl substituent of carbamate **2** indicate the possibility of the operation of competing chiral recognition processes of opposite enantioselectivity. Utilizing two competing chiral recognition processes of opposite enantioselectivity has been well documented for the resolution of 3,5-dinitrobenzoyl derivatives of α-amino acid esters and α-arylalkylamines.[9] Similarily, possible two competing chiral recognition processes advanced from the model study to account for the dependence of the magnitude of separation factors upon the length of the ester alkyl tail and the alkyl substituent of carbamate **3** are shown in Figure 2.

In Figure 2, the analyte enantiomer is shown in its most favorable conformation. The carbonyl oxygen of the 3,5-dinitrophenyl carbamate group of analyte is essentially eclipsed with the hydrogen at the stereogenic center. This type of conformation has been rationalized by X-ray crystallography even though the conformation in solution may be different from that in solid state.[18] The proposed chiral recognition processes given in Figure 2 show that the (S)-enantiomer approaches the most accesible face of CSP **1** through the $\pi-\pi$ interaction between the 6,7-dimethylnaphthyl group of CSP and the 3,5-dinitrophenyl group of analyte and through the hydrogen-bondings indicated by arrow lines (Process B). On the other hand, the (R)-enantiomer approaches the most accessible face of CSP **1** through the $\pi-\pi$ interaction and the hydrogen-bondings indicated by arrow lines between the two amide groups of CSP and analyte (Process A). In those two processes, it is assumed that the carboalkoxy group (COOR') of analyte is sterically smaller than the alkyl substituent(R) of analyte. Hence, in both of the two processes, the smaller alkoxy group of analyte is directing toward the CSP. This assumption has previously been satisfied in explaining the chiral recognitions on CSPs derived from α-arylalkylamines.[9]

Competing of the two processes, then, essentially determines the degree of chiral recognition. Between the two processes, "process B" is thought to retain (S)-enantiomers more strongly than "process A" retains (R)-enantiomers because (S)-enantiomers always elute later as shown in Table 1. The alkyl substituent of analyte, in "process A", is not directing to the silica support or not being intercalated between the strands of connecting arm of CSP **1**. In "process B", however, the alkyl substituent of analyte is more or less parallel to the connecting arm of CSP **1** and being essentially intercalated between the strands of bonded phase. Therefore, as the length of the alkyl substituent of analyte increases, the initial dominance of "process B" over "process A" is diminished and, consequently, the magnitude of α should decrease progressively. On the other hand, the ester alkyl tail of analyte is not being intercalated between the strands of connecting arm of CSP **1** or not directing to the silica support in both of "processes A" and "B". Thus, the length of the ester alkyl tail of analyte does not influence the two processes and, consequently, the magnitude of α remains almost constant without being dependent on the length of the ester alkyl tail of analyte.

CSP **2** was essentially prepared by connecting the chiral moiety, α-arylalkylamine, to silica gel through the alkyl group of chiral moiety instead of the amide bond by which α-arylalkylamine is connected to silica gel to afford CSP **1**. Therefore, the direction of connecting arms of CSPs **1** and **2** are exactly opposite. As a consequence, "process B" intercalates the ester alkyl tail of analyte, but does not intercalate the alkyl substituent when carbamates **3** are resolved on CSP **2**. "Process A" does not intercalate both of the ester alkyl tail and the alkyl substituent of analyte. Hence, the long ester alkyl tail of analyte suppresses the initially dominant "process B" and the magnitude of α on CSP **2** should decrease progressively as the length of the ester alkyl tail of analyte increases. The alkyl substituent of analyte is not being intercalated between the strands of connecting arm of CSP **2** or not directing to the silica support in both of the two processes. Therefore, the magnitude of α on CSP **2** is not affected by the length of the alkyl substituent of analyte.

At this point, it should be noted that "process A" which is quite similar to the "dipole-stacking process" which was proposed to retain one of the two enantiomers selectively during the resolution of N-3,5-dinitrobenzoylamino acid esters on CSPs derived from α-arylalkylamines[9] is not the only process. Any process which does not intercalate both of the ester alkyl tail and the alkyl substituent of analyte can be used together with "process B" to explain the observed resolution behaviors of carbamates **3**.

The maximization of α at $n \simeq 2$ and the initial increase of α which are observed when methyl (R or R') of analyte is changed to ethyl as shown in Table 1 and Figure 1 is presumed to come from the increased conformational rigidity of analyte. Ethyl is significantly larger than methyl and, consequently, exerts a greater control of conformation favorable for chiral recognition. However, the alkyl substituents or the ester alkyls longer than ethyl are not effectively larger and do not exert additional conformational control. The longer alkyl

substituents or the longer alkyl tails of carbamates **3** are expected to show only the effect exerted by interfering with the connecting arm of CSP.

For the present study, the second stereogenic group of CSP **2** seems to work only as a steric group. The big chiral acyl group of CSP **2** may suppress "process A" more strongly than CSP **1** and hence CSP **2** is expected to show better chiral recognitions than CSP **1**. The larger capacity factors, k_1, on CSP **2** than on CSP **1** shown in Table **1**, however, is not properly explained by using the chiral recognition mechanisms proposed. The additional $\pi-\pi$ interaction between the phenyl group at the second stereogenic center of CSP **2** and the 3,5-dinitrophenyl group of carbamate **3** may provide the larger capacity factors on CSP **2**. However, we do not have any evidence for the additional $\pi-\pi$ interaction.

In conclusion, CSP **1** and CSP **2** which are derived from α-arylalkylamines have been shown to be quite effective for the resolution of 3,5-dinitrophenyl carbamates **3** of various 2-hydroxy acids. From the resolution behaviors influenced by the direction of connecting arm of CSPs and the length of the ester alkyl tail(R') and the alkyl substituent(R) of carbamates **3**, the two competing chiral recognition mechanisms are proposed to be operative. The magnitude of separation factors observed for every entry in Table 1 is greater than 1.60. Therefore, we expect that the methodology described herein allows for accurate and simple analytical assesment of enantiomeric purity of 2-hydroxy acids.

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References

1. J. W. Scott, in "Asymmetric Synthesis", J. D. Morrison and J. W. Scott, Eds., Vol. 4, Chap. 1, Academic Press, New York, 1984.
2. H. C. Brown, B. T. Cho and W. S. Park, *J. Org. Chem.*, **51**, 3398 (1986); and references cited therein.
3. M.-J. Kim and G. M. Whitesides, *J. Am. Chem. Soc.*, **110**, 2959 (1988); and references cited therein.
4. R. W. Souter, "Chromatographic Separations of Stereoisomers", CRC Press, Inc. Boca Raton, Florida, 1985.
5. P. Newman, Ed., "Optical Resolution Procedures for Chemical Compounds", Vol. 2, Optical Resolution Information Center, Riverdale, New York, 1984.
6. G. Gubitz and S. Mihellyes, *Chromatographia*, **19**, 257 (1984); and references cited therein.
7. N. Oi and H. Kitahara, *Bunseki Kagaku*, **33**, 387 (1984).
8. M. H. Hyun and C.-S. Min, *Bull. Korea Chem. Soc.*, **10**, 328 (1989).
9. W. H. Pirkle, M. H. Hyun and B. Bank, *J. Chromatogr.*, **316**, 585 (1984).
10. W. H. Pirkle, G. S. Mahler and M. H. Hyun, *J. Liq. Chromatogr.*, **9**, 443 (1986).
11. W. H. Pirkle, A. Tsipouras, M. H. Hyun, D. J. Hart and C.-S. Lee, *J. Chromatogr.*, **358**, 377 (1986).
12. M. H. Hyun and W. H. Pirkle, *Bull. Korean Chem. Soc.*, **8**, 45 (1987).
13. M. H. Hyun, Y.-W. Park and I.-K. Baik, *Tetra. Lett.*, **29**, 4735 (1988).
14. Manuscript in Preparation
15. L. M. Weinstock, R. B. Currie and A. V. Lovell, *Syn. Comm.*, **11**, 943 (1981).
16. W. H. Pirkle, G. S. Mahler, T. C. Pochapsky and M. H. Hyun, *J. Chromatogr.*, **388**, 307 (1987).
17. C. Dalgleish, *J. Chem. Soc.*, **75**, 1072 (1953).
18. A. M. Mathieson, *Tetra. Lett.*, 4137 (1965).
19. L. R. Snyder and J. J. Kirkland, "Introduction to Modern Liquid Chromatography" 2nd ed.; John Wiley and Sons, Inc.; New York, 1979.