ELSEVIER

Journal of Chromatography A, 694 (1995) 91–100

JOURNAL OF CHROMATOGRAPHY A

Review

Development of chiral stationary phases consisting of polysaccharide derivatives

Kazuma Oguni, Hirofumi Oda, Akito Ichida*

Daicel Chemical Industries Ltd., Research Center, Shinzaike, Aboshi-ku, Himeji, Hyogo 671-12, Japan

Abstract

Various chiral stationary phases (CSPs) consisting of polysaccharide derivatives were developed by adsorbing them on silica gel on the basis of information obtained from cellulose triacetate. The chiral recognition ability increases with an increase in conformational regularity. The regularity of cellulose triacetate depends greatly on the kind of solvents used in the coating procedure. This finding seems to be applicable to other coating-type CSPs based on polysaccharide derivatives. Some interactions were considered between a CSP and an enantiomer for chiral discrimination. By using ^{13}C NMR spectroscopy, 1-phenylethyl alcohol was chirally recognized on cellulose tris(4-methylbenzoate).

Contents

1. Introduction

The importance of the establishment of acquisition methods and analytical methods for chiral compounds has increased in various fields such as pharmaceuticals and agriculture and in asymmetric synthesis. High-performance liquid chromatographic (HPLC) resolution on chiral stationary phases (CSPs) is of interest as a simple and practical method applicable to the preparative separation and determination of optical isomers. CSPs are classified into two types according to the molecular mass of the chiral resolving agents. The first type is based on low-molecular-mass resolving agents bonded to silica gel, and the other on high-molecular-mass poly-

* Corresponding author.

SSDI 0021-9673(94)00789-6

mers. In the former type, the relationships between CSPs and resolved racemates has been substantially elucidated as it is possible to design CSPs based on the expected resolution mechanism. However, with high-molecular-mass CSPs, the explanation of the chiral discrimination mechanism is difficult from the structure of the constitutional monomer units. Many kinds of high-molecular-mass resolving agents are known, and various racemates from simple aliphatic carbonyl compounds to polyfunctional compounds have been resolved. In this paper, we describe our work on the development of CSPs consisting of polysaccharide derivatives.

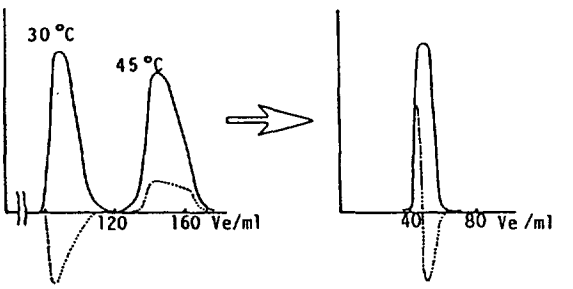


Fig. 1. Chromatograms of the separation of Tröger's base on MCTA (left) and on cellulose triacetate precipitated from acetone (right). Eluent, ethanol; column length, 16 cm; solid lines, UV detection; dotted lines, optical rotation; V_e, elution volume; column temperature, 30°C, increased to 45°C after the first component was eluted [3,4].

2. Development of CSPs

2.1. Factors controlling the appearance of chiral discrimination abilities of cellulose triacetates

A high chiral discrimination ability of optically active poly(triphenylmethyl methacrylate) coated on silica gel was already known [1,2] when we began the study on CSPs. Its ability comes from the one-handed helical structure of the polymer. We therefore focused on the higher order structure of optically active polymers to attain efficient chiral discrimination. Hesse and Hagel [3,4] succeeded in the complete resolution of Tröger's base on a column packed with microcrystalline cellulose triacetate (MCTA) synthesized by acetylation of microcrystalline cellulose under heterogeneous conditions. However, the type of compounds resolved was limited and the plate number of the column was low because ground MCTA was used as a CSP. Therefore expected that these defects could be overcome if various cellulosic derivatives could be prepared and coated on silica gel as in the case of poly(triphenylmethyl methacrylate). However, Hesse and Hagel also pointed out that dissolution and consequent reprecipitation of MCTA, which was accompanied by irreversible transformation of crystalline lattice from CTA-I to CTA-II, resulted in almost complete loss of chiral discrimination ability, which even showed reversed enantioselectivity recognized by a polarimeter (Fig. 1). Hence it was widely accepted that for efficient resolution on cellulose triacetate, it is very important to keep the crystalline structure as in natural cellulose. Therefore, procedures accompanying dissolution such as homogeneous acetylation and coating on silica gel had not been applied to CSPs consisting of cellulosic derivatives.

We investigated the relationships between the chiral discrimination ability and the crystalline structure of cellulose triacetate in detail, and obtained some important results:

(i) the discrimination ability of MCTA decreased with an increase in the degree of crystallinity (Fig. 2) [5];

(ii) cellulose triacetate had chiral discrimination ability, even if it was prepared under homogeneous conditions (Fig. 3);

(iii) enantioselectivity was reversed between MCTA (CTA-I) and cellulose triacetate (CTA-II) (Fig. 4) [6,7].

(iv) high performance of the column was realized by coating on silica gel;

(v) for cellulose triacetate coated on silica gel, its chiral discrimination ability was optimized by changing the solvents used for coating (Fig. 5) [8].

The chiral discrimination ability was increased in the order of the coating solvent(s) CH_2Cl_2 only, CH_2Cl_2–CF_3COOH and CH_2Cl_2–phenol (Fig. 5). The conformation of cellulose triacetate seems to be different in the above solutions and

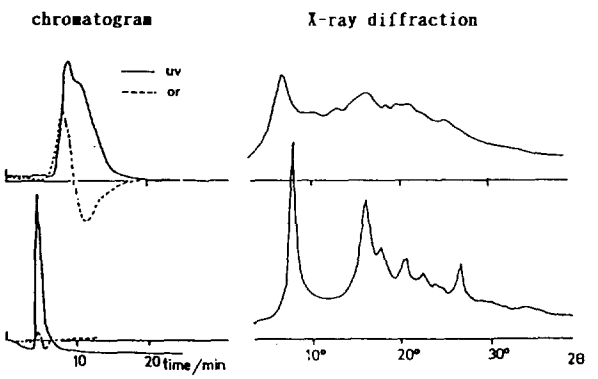


Fig. 2. Chromatograms and X-ray diffractograms of MCTA (top) and MCTA crystallized at 230°C for 1 h (bottom). Sample, mandelamide; eluent, 95% aqueous ethanol; flow-rate, 0.5 ml/min. The broken lines in the chromatograms show optical rotation curves.

the difference may be retained on the surface of silica gel after the solvents have been removed. The conformational difference was investigated by cross polarization magic angle spinning (CP/MAS)^{13}C NMR spectroscopy (Fig. 6) [9]. The chemical shifts for each carbon did not change at all. However, the half-widths of the signals became sharper for the C-1 carbon of anhydroglucose in the order CH_2Cl_2 (315 Hz), CH_2Cl_2–CF_3COOH (243 Hz) and CH_2Cl_2–phenol (218 Hz). In general, the line width of the NMR signal depends on the regularity of molecular conformation and mobility, but change in spin–lattice relaxation time (T_1), which reflects the latter, was not detected among the polymers recovered from the solutions. This result seems to show that the narrowing of the

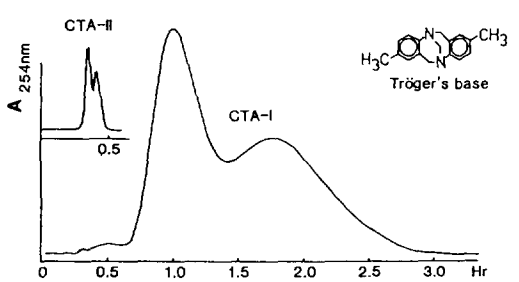


Fig. 3. Chiral discrimination of Tröger's base on CTA-II and CTA-I (MCTA). Eluent, ethanol; column, 25 cm × 0.46 cm I.D.; flow-rate, 0.2 ml/min.

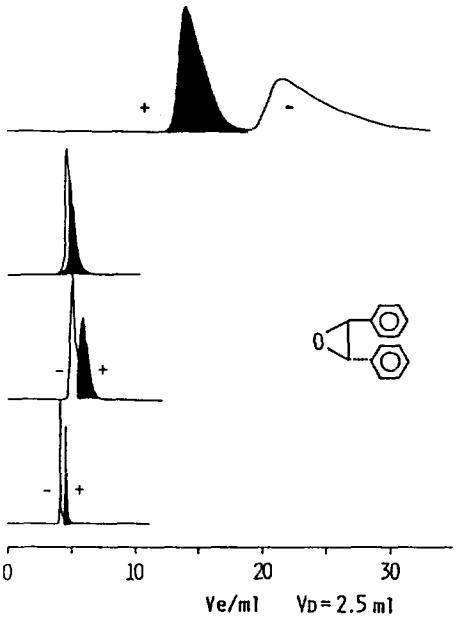


Fig. 4. Chiral discrimination of *trans*-1,2-diphenyloxirane racemate. From top to bottom: (1) on MCTA; eluent, ethanol; (2) on CTA crystallized in formamide at 180°C; eluent, ethanol; (3) on moderate crystalline CTA; eluent, ethanol; (4) on CTA coated on silica gel; eluent, *n*-hexane–2-propanol (9:1, v/v). Column, 25 cm × 0.46 cm I.D.; flow-rate, 0.5 ml/min; V_e, elution volume; V_D, dead volume.

C-1 signal is responsible for the higher regularity of the conformation of cellulose triacetate about the β-1,4-glycosidic linkage, not for enhanced molecular mobility. This phenomenon does not seem to be restricted to cellulose triacetate. For instance, cellulosic carbamate derivatives with liquid crystallinity, which relates to the regularity of the polymer conformation, have high chiral discrimination abilities [10]. From these results, it was concluded that a microcrystalline structure of cellulose triacetate was not always necessary for high chiral discrimination, and the formation of an adequate morphology of cellulose triacetate on silica gel allowed discrimination abilities to be achieved.

2.2. Other cellulosic derivatives

The procedure for coating cellulose triacetate on silica gel was readily applied to other derivatives of cellulose (Fig. 7). Cellulose nitrate

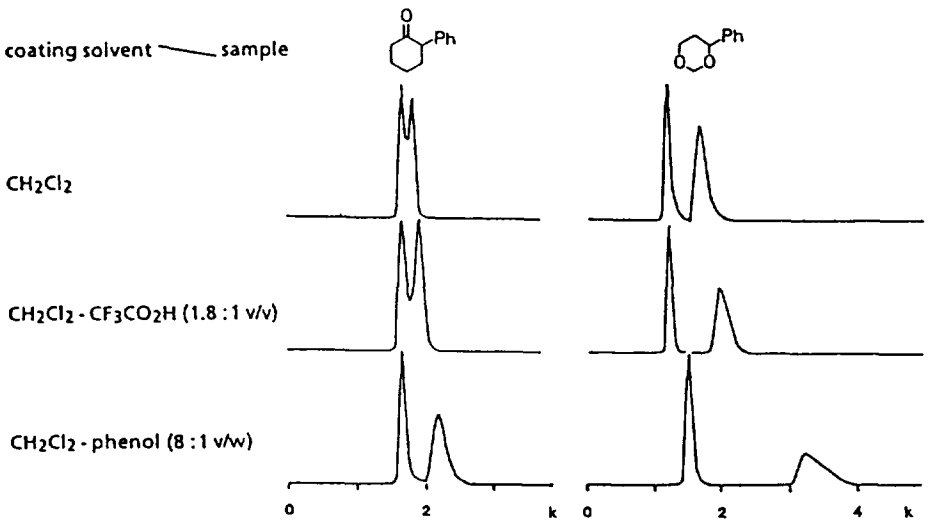


Fig. 5. Effect of coating solvents on chiral discrimination of cellulose triacetate. k = Capacity factor.

[5,11], aromatic esters such as benzoate [5,12] and cinnamate [6,7] and phenylcarbamate [6,7,13] have been prepared and coated on silica gel. These CSPs have characteristic chiral discrimination abilities, which are enhanced by the introduction of substituents on the phenyl groups of the derivatives [10,12]. Commercially available cellulosic CSPs coated on silica gel are shown in Fig. 8. Various racemates can be resolved on these CSPs by using *n*-hexane–2-propanol or ethanol mixtures as the mobile phase. Among the derivatives, 3,5-dimethylphenylcarbamate and 4-methylbenzoate particularly have high chiral discrimination abilities for aryloxypropanolamines and arylpropionic acids, respectively. Although the only difference between cellulose tribenzoate and cellulose tris(4-methylbenzoate) is whether they bear a methyl on the phenyl group or not, their chiral discrimination abilities are very different (Table 1). Compounds with a larger molecular size are often better resolved on the 4-methylbenzoate, e.g., Tröger's base can be well resolved on cellulose tris(4-methylbenzoate), but cannot be resolved on the benzoate. The shape and size of the chiral adsorbing sites of the two benzoates seem dramatically different. Although the difference and the chiral discrimination mechanisms have not been clarified, such chiral sites seem to exist regularly along the polymer chain because of the ordered arrangement of ester groups.

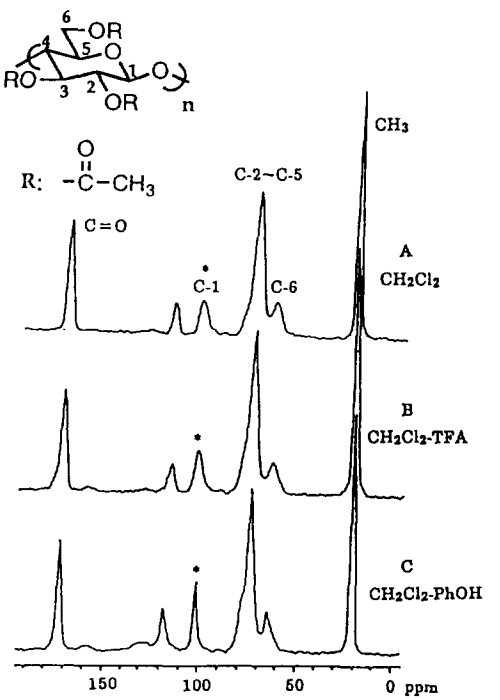


Fig. 6. 67.8 MHz CP/MAS ^{13}C NMR spectra of cellulose triacetates solidified from different solvents.

The CSP consisting of cellulose tris(3,5-di-

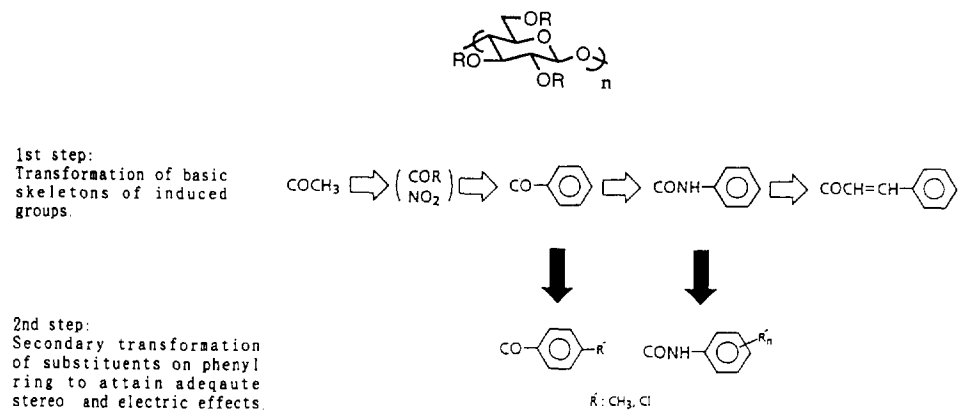


Fig. 7. Concepts of derivatization.

methylphenylcarbamate) also shows high chiral discrimination abilities when using aqueous mobile phases such as acetonitrile containing water or aqueous perchlorate buffer solution [14] (Fig. 9). Some racemates, e.g., verapamil and trimepramine, which were not resolved using *n*-hexane–2-propanol, could be resolved with acetonitrile containing water. The degree of resolution depends on the composition of the mobile phase, pH, kind of additive salt, etc. [15]. In general, the resolution of neutral compounds is not influenced by the acidity of a mobile phase and the kind of salt. In order to attain sufficient resolution of acidic compounds such as N-derivatized amino aids, a sufficient acidity of the mobile phase is necessary for the suppression of ionization of the carboxyl group. Perchloric acid and phosphoric acid are suitable for the separation of acids. For basic compounds with a primary, secondary and tertiary nitrogen such as propranolol and alimemazine, their retention and resolution depend on the kind of counter anion of the additive salt. The retention increases in the order $ClO_4^- > SCN^- > I^- > N_3^- > Br^- > Cl^- > AcO^-$. This order is in good agreement with that of chaotropicity [16] characterized by less localized electric charge, high polarizability and low degree of hydration of anions.

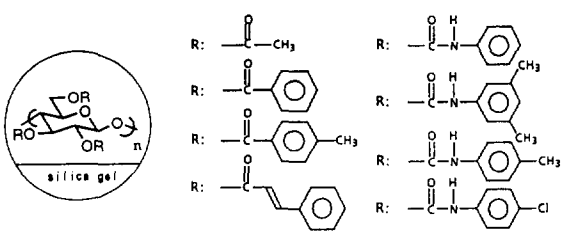
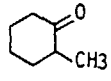
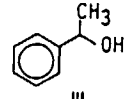
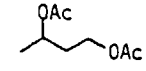
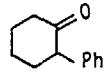
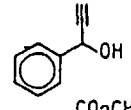
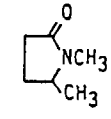
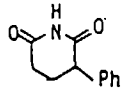
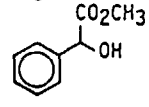
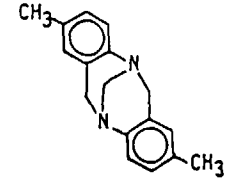
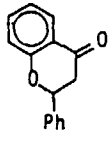
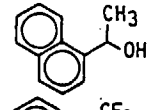
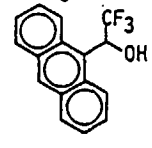


Fig. 8. Commercially available CSPs consisting of cellulose derivatives.

2.3. CSPs consisting of amylosic derivatives

Amylose is also a well known polysaccharide. The monomer unit of amylose is again D-glucose, which is the same as that of cellulose. However, amylose is said to have a helix structure as a higher order structure based on the α-linkage of D-glucose units. Hence, the chiral discrimination ability of amylose derivatives [17] is very different from that of cellulose. For example, arylpropanolamines such as propranolol and pindolol are well resolved on cellulose tris(3,5-dimethylphenylcarbamate) coated on silica gel, but are not well resolved on the corresponding amylose derivative. Some other differences are shown in Table 2. The tris(3,5-dimethylphenylcarbamate) derivatives of cellulose seem to be complementary from the viewpoint of resolved compounds. A large difference in chiral discrimination ability was observed on (*S*)-methylbenzylcarbamate derivatives. With the amylose, racemates with a β-lactam skeleton were well resolved, but they could not be resolved on the corresponding cellulosic derivative.

Table 1
Difference in enantioselectivity (α-value) between cellulose tris(4-methylbenzoate) (OJ) and cellulose tribenzoate (OB)

	OJ	OB		OJ	OB		OJ	OB
	1.0	1.15		1.17	1.57		1.0	1.73
	1.18	1.47		1.58	1.20		1.0	1.21
	2.15	1.19		1.21	1.0		6.05	ca. 1.0
	1.17	1.0		1.54	1.37			
				1.22	1.0			

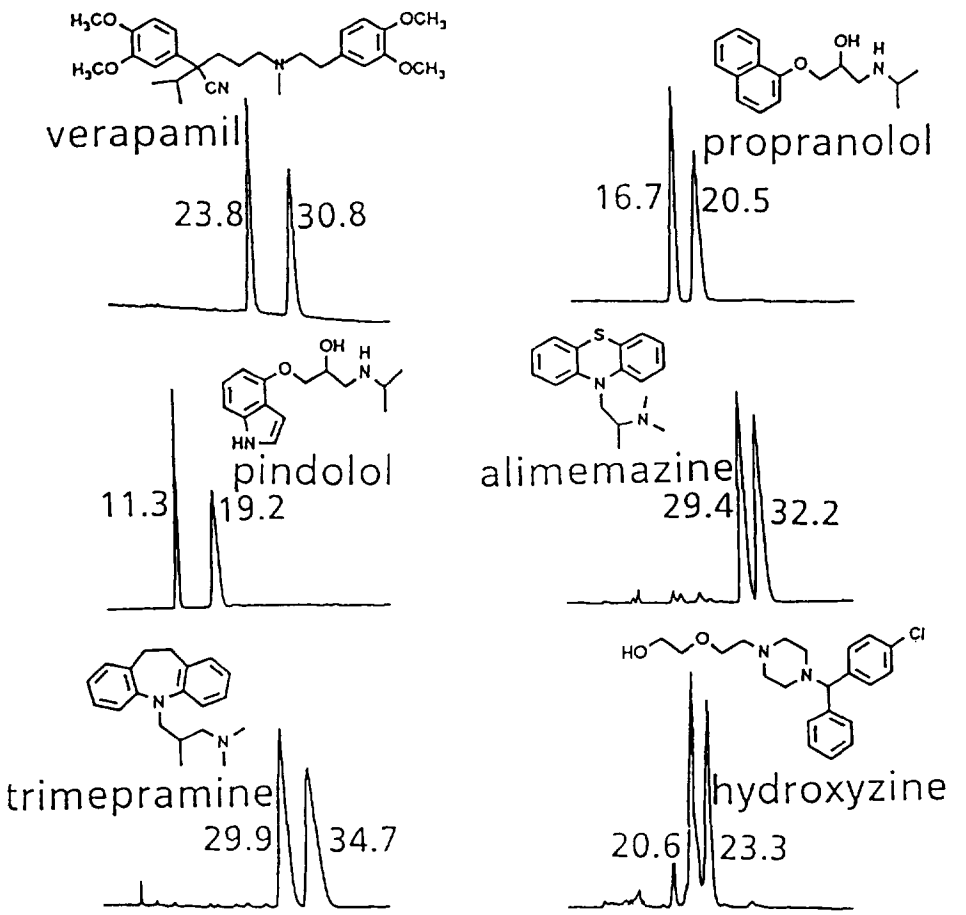


Fig. 9. Compounds resolved on cellulose tris(3,5-dimethylphenylcarbamate) (Chiralcel OD-R). The numbers on the peaks are retention times (min). Eluent, 0.1 *M* $NaPF_6$–CH_3CN (6:4, v/v); flow-rate, 0.5 ml/min; detection, UV at 245 nm.

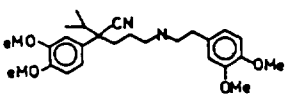
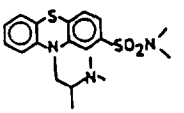
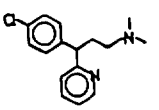
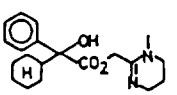
The chiral discrimination abilities of polysaccharide derivatives appear to depend on the conformation of the main chain and the structure of the substituents.

Other polysaccharide derivatives, e.g., benzoate derivatives of xylan and mannan and acetate derivatives of curdlan [10], are known. Curdlan triacetate has a considerable chiral discrimination ability. However, this ability decreases with time. Phenylcarbamate derivatives of xylan, curdlan, dextran and inuline were investigated and among these, xylan bis(3,5-dichlorophenylcarbamate) showed a high chiral discrimination ability for dihydropyridine derivatives.

2.4. Intermolecular interactions between CSPs and chiral molecules

The interactions between CSPs and enantiomers have been intensively discussed. The participation of hydrogen-bonding interactions was considered for the resolution on cellulose phenylcarbamate derivatives [10]. The carbonyl and amino groups of the carbamate function are considered to be a sort of Lewis base and acid, respectively. Introduction of an electron-withdrawing atom or group on the phenyl group can enhance the acidity of the hydrogen on the amino group, which can lead to a stronger hydrogen bonding interaction with Lewis basic

Table 2
Difference in enantioselectivity between 3,5-dimethylphenylcarbamate derivatives of amylose (AD) and cellulose (OD)

Name	Racemate	Mobile phase[a]	AD	OD
Verapamil		A	$k_1 = 2.36$ $\alpha = 1.27$ $R_s = 2.19$	$k_1 = 3.73$ $\alpha = 1.0$
Dimethotiazine		A	$k_1 = 3.74$ $\alpha = 1.19$ $R_s = 1.67$	$k_1 = 8.29$ $\alpha = 1.14$ $R_s = 0.65$
Chlorpheniramine		B	$k_1 = 3.25$ $\alpha = 1.29$ $R_s = 2.11$	$k_1 = 1.40$ $\alpha = 1.0$
Oxyphencyclimine		A	$k_1 = 0.75$ $\alpha = 1.85$ $R_s = 2.83$	$k_1 = 0.96$ $\alpha = 1.0$

[a] Mobile phases: A = *n*-hexane–2-propanol (9:1, v/v); flow-rate, 1.0 ml/min; B = *n*-hexane–2-propanol–diethylamine (98:2:0.1, v/v/v); flow-rate, 1.0 ml/min.

parts of analytes. In contrast, an electron-donating group can polarize the carbonyl group considerably, by increasing its basicity, and this can enhance the interaction with hydroxyl or amino groups of analytes. With cellulose ester derivatives, some aliphatic compounds, such as β-methylpropiolactone, 3-acetyl-4-cyclopentenone and 1,3-butanediol diacetate, without any aromatic or hydroxyl groups, could be separated. The ester groups of racemates may interact with the CSP through dipole–dipole interactions [5,11].

Recently, we found that enantiomers could be discriminated in the presence of a cellulose derivative by ^{13}C NMR spectroscopy in C^2HCl_3 [18]. In this study, cellulose tris(4-methylbenzoate) (CTMB) and 1-phenylethyl alcohol (1PE) enantiomers was chosen (Fig. 10). Both enantiomers of 1PE were well resolved by HPLC on a column packed with CTMB ($\alpha = 1.20$). The *R* and *S* enantiomers of 1PE showed different chemical shifts in the presence of CTMB (Table 3). The chemical shift difference was large at the

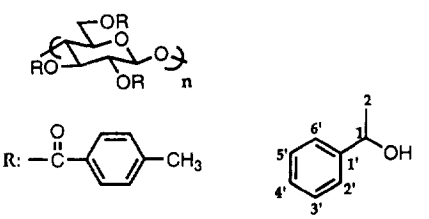


Fig. 10. Structures of compounds used for NMR study.

Table 3
Chemical shifts (ppm) of 1PE in the presence of CTMB in C^2HCl_3 at 35°C

Carbon	(*R*)-1PE	(*S*)-1PE	Δδ (ppm)[a]
1	70.214	70.224	−0.010
2	25.184	25.178	+0.006
1′	146.009	145.990	+0.019
2′, 6′	125.436	125.436	0
3′, 5′	128.441	128.444	−0.003
4′	127.337	127.348	−0.011

[a] Chemical shift difference between the enantiomers: δ[(*R*)-1PE] − δ[(*S*)-1PE].

Table 4
Spin–lattice relaxation times, T_1(s), of 1PE in the presence of an equimolar amount of CTMB in C^2HCl_3 at 35°C

Carbon	(*R*)-1PE	(*S*)-1PE
1	7.12	7.95
2	3.19	3.30
1′	11.1	12.9
2′, 6′	4.88	5.16
3′, 5′	4.71	4.97
4′	3.50	3.68

aromatic *ipso* carbon (C-1′) and asymmetric carbon (C-1) with a hydroxyl group. Spin–lattice relaxation times (T_1) were also measured for comparison with the mobility of each enantiomer in the presence of CTMB (Table 4). The T_1 values for each carbon of (*R*)-1PE were smaller than those for corresponding carbons of (*S*)-1PE; the presence of CTMB restricts the mobility of (*R*)-1PE more than that of (*S*)-1PE. The T_1 difference was large at C-1. These results indicate that 1-PE may be chirally recognized by CTMB at the C-1 site.

These data obtained in C^2HCl_3 solution may not reflect the real interactions in an HPLC packed column with *n*-hexane–2-propanol as the mobile phase. We are now investigating the interaction by solid-state NMR.

2.5. Application of the CSPs to large-scale separations

From the beginning of the development of CSPs, the centre of our interest was the use of the CSPs for large-scale separations. As for the CSPs for preparative separations, a low cost of preparation, a high loading capacity of samples, high durability, etc., are necessary. CSPs consisting of cellulosic derivatives meet such demands. In a large-scale separation, one must obtain a large amount of an anantiomer with high optical purity in a short time. Hence, it is important to optimize the separation conditions such as the amount of sample loaded and temperature. In Fig. 11, the column performances are shown for preparative and analytical columns. Some results obtained by using a preparative column are summarized in Table 5. The efficiency of separation is very different depending on the sample. For instance, for entry 1 each enantiomer was obtained in an amount of about 160 g per day, compared with less than 0.5 g for entry 5. However, the optical purities of the enantiomers obtained were over 99%. Productivity is affected

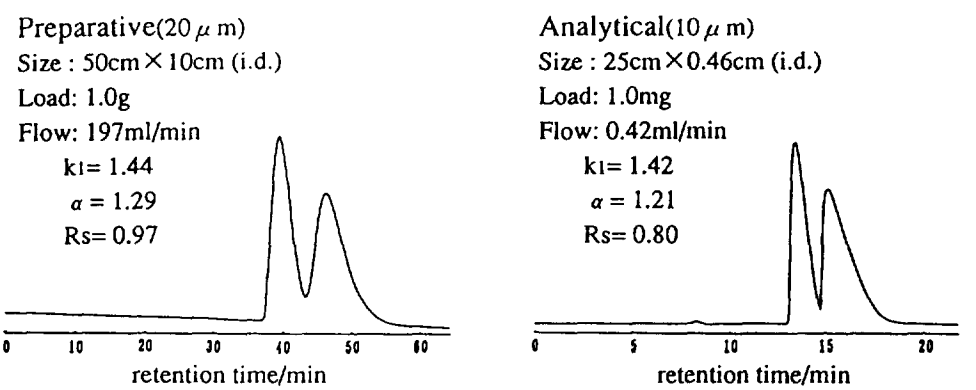


Fig. 11. Column performance of a preparative column vs. an analytical column. The values in parentheses (20 μm, 10 μm) are the silica gel particle size; sample, *trans*-1,2-diphenyloxirane racemate; k_1, capacity factor of first peak; α, separation factor; R_s, resolution.

Table 5
Productivity of preparative chiral separation by HPLC on the 50 cm × 10 cm I.D. columns

Entry	Parameters on analytical column			Elution order	Productivity		Yield (%)	Optical purity (% e.e.)[c]
					g/d[a]	kg/yr[b]		
	k_1	α	R_s					
1	1.15	2.61	4.90	1	162	121	97	>99.6
				2	159	119	94	99.4
2	0.77	2.44	4.00	1	78	59	85	>99.8
				2	73	55	80	99.2
3	1.92	2.11	1.10	1	86	65	93	>99.8
				2	82	61	89	99.8
4	1.98	1.77	3.62	1	11	8.3	93	>99.6
				2	11	8.3	98	>97.6
5	6.94	1.24	2.89	1	0.4	0.3	83	>99.6
				2	0.2	0.1	57	>99.6

[a] 8 h/d.
[b] 24 h/d, 250 d/yr.
[c] e.e. = Enantiomeric excess.

by the solubility of the sample to mobile phase and the degree of separation.

3. Conclusions

The conformational regularity of the polysaccharide derivatives plays an important role in chiral discrimination. The regularity depends on the polysaccharides, the substituents introduced on the phenyl groups and the kind of coating solvent. The chiral recognition mechanism has not yet been clarified, but a certain "chiral field" which is constructed from a regular arrangement of ester or urethane groups of cellulosic derivatives appears to be responsible for efficient chiral recognition. A future project is to clarify the interactions between a CSP and an enantiomer at the molecular level, and NMR measurements are important in this respect.

References

[1] Y. Okamoto, K. Suzuki, K. Ohta, K. Hatada and H. Yuki, *J. Am. Chem. Soc.*, 101 (1979) 4763.
[2] Y. Okamoto, S. Honda, I. Okamoto, H. Yuki, S. Murata, R. Noyori and H. Takaya, *J. Am. Chem. Soc.*, 103 (1981) 6971.
[3] G. Hesse and R. Hagel, *Chromatographia*, 6 (1973) 277.
[4] G. Hesse and R. Hagel, *Liebigs Ann. Chem.* (1976) 996.
[5] T. Shibata, I. Okamoto and K. Ishii, *J. Liq. Chromatogr.*, 9 (1986) 313.
[6] A. Ichida, T. Shibata, I. Okamoto, Y. Yuki, H. Namikoshi and Y. Toga, *Chromatographia*, 19 (1984) 280.
[7] Y. Okamoto, M. Kawashima, K. Yamamoto and K. Hatada, *Chem. Lett.* (1984) 739.
[8] T. Shibata, T. Sei, H. Nishimura and K. Deguchi, *Chromatographia*, 24 (1987) 552.
[9] T. Sei, H. Matsui, T. Shibata and S. Abe, *Viscoelasticity of Biomaterials*, American Chemical Society, Washington, DC, 1992, Ch. 4, pp. 53–64.
[10] Y. Okamoto, M. Kawashima and K. Hatada, *J. Chromatogr.*, 363 (1986) 173.
[11] A. Ichida and T. Shibata, in M. Zief and L.J. Crane (Editors), *Chromatographic Chiral Separations*, Marcel Dekker, New York, 1988, p. 219.
[12] Y. Okamoto, R. Aburatani and K. Hatada, *J. Chromatogr.*, 389 (1987) 95.
[13] Y. Okamoto, M. Kawashima and K. Hatada, *J. Am. Chem. Soc.*, 106 (1984) 5357.
[14] K. Ikeda, T. Hamasaki, H. Kohno, T. Ogawa, T. Matsumoto and J. Sakai, *Chem. Lett.*, (1989) 1089.
[15] A. Ishikawa and T. Shibata, *J. Liq. Chromatogr.*, 16 (1993) 859.
[16] Y. Hatehi and W.G. Hanstein, *Proc. Natl. Acad. Sci. U.S.A.*, 62 (1969) 1129.
[17] Y. Okamoto, R. Aburatani, T. Fukumoto and K. Hatada, *Chem. Lett.*, (1987) 1857.
[18] K. Oguni, A. Matsumoto and A. Isokawa, *Polym. J.*, submitted for publication.