Optical Resolving Ability of 3,5-Dimethylphenylcarbamates of Oligosaccharides and Cyclodextrins

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3,5-Dimethylphenylcarbamates of oligosaccharides and cyclodextrins were synthesized and their chiral recognition abilities were evaluated as chiral stationary phases for high-performance liquid chromatography. In case of the carbamates of linear maltooligosaccharides, the optical resolution abilities of 4—7-mer were similar to each other and were not so different from the ability of amylose tris(3,5-dimethylphenylcarbamate). The chiral recognition abilities of the carbamates of cellooligosaccharides were lower than that of the cellulose tris(3,5-dimethylphenylcarbamate). The intensities of the CD spectra of the cellooligosaccharide derivatives were smaller than that of the cellulose derivative. These results suggest that the less ordered structure of the cellooligosaccharide derivatives may be correlated to the lower chiral recognition. On the other hand, 3,5-dimethylphenylcarbamates of cyclodextrins showed quite different optical resolution abilities from that of amylose tris(3,5-dimethylphenylcarbamate), probably because of a quite different high-order structure.

We reported that the phenylcarbamates of polysaccharides, such as cellulose and amylose, showed a high chiral recognition ability when used as chiral stationary phases (CSP) for high-performance liquid chromatography (HPLC).[1] The optical resolution ability of the derivatives carrying various substituents on the phenyl groups is greatly dependent on the kinds of substituents.[2,3] The inductive effect of the substituents affects the polarity of the urethane bond, which is the most important adsorbing site of a solute. The chiral recognition ability also depends on the high-order structure of the polymers. Therefore, the mechanism of optical resolution on these CSPs can not be simply understood, since the structures, including the high-order structure of the polysaccharide derivatives, delicately vary according to the substituents. In many phenylcarbamates, both the 3,5-dimethylphenylcarbamates of cellulose and amylose show high chiral recognition and can separate broad racemic compounds with high probability.[4]

In this study, 3,5-dimethylphenylcarbamates of linear oligomers of cellulose (cellooligosaccharide) and amylose (maltooligosaccharide), and cyclodextrins were synthesized; their chiral recognition ability was then compared with that of the corresponding polysaccharide derivatives in order to obtain information about the influence of the high-order structure of the polysaccharide derivatives. In addition, the circular dichlorism (CD) spectra of oligomer and polysaccharide derivatives were measured and the results correlated with the chiral recognition ability.

Experimental

Maltooligosaccharides (n=2: nacalai tesque; n=3—7: Nihon Shokuhin Kako), cellooligosaccharides (n=2, 4: NFI

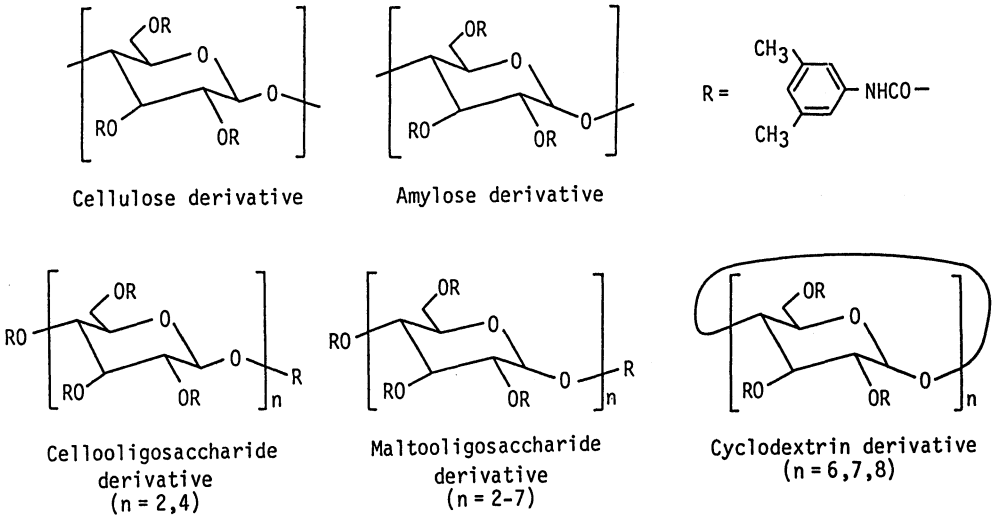

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Table 1. Elemental Analyses of 3,5-Dimethylphenylcarbamates of Oligosaccharides and Cyclodextrins[a)]

Polysaccharide	n	C %	H %	N %
Amylose	2	67.67	6.24	7.68
		(66.39	6.23	7.37)
	3	65.95	6.22	7.36
		(66.18	6.22	7.26)
	4	65.70	6.20	7.17
		(66.07	6.21	7.19)
	5	65.48	6.14	7.16
		(65.99	6.20	7.15)
	6	66.40	6.22	7.41
		(65.94	6.20	7.12)
	7	66.14	6.29	7.41
		(65.90	6.20	7.10)
Cellulose	2	66.73	6.30	7.50
		(66.39	6.23	7.37)
	4	66.85	6.28	7.49
		(66.07	6.21	7.19)
Cyclodextrin	6	65.13	6.18	6.93
		(65.66	6.18	6.96)
	7	65.45	6.11	6.80
		(65.66	6.18	6.96)
	8	65.14	6.15	6.99
		(65.66	6.18	6.96)

a) Calculated values are shown in parentheses.

Laboratories), and cyclodextrins (n=6—8: nacalai tesque) were reacted with 3,5-dimethylphenyl isocyanate in pyridine for 10—24 h at 80 °C. Pyridine was evaporated under reduced pressure and the residue washed with hexane. After isolated products were dissolved in *N,N*-dimethylacetamide or tetrahydrofuran (THF), the insoluble parts, characterized as a urea derivative from the infrared spectra, were removed by centrifugation. The soluble parts were repricipitated in a methanol-water (4:1) mixture, filtrated, and dried in vacuo at 60 °C. Elemental analyses (Table 1) showed that hydroxyl groups were almost completely converted to carbamate moieties.

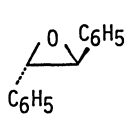
The carbamates of oligosaccharides (0.75 g) were dissolved in a solvent (ca. 10 ml), THF for cellulose oligomer and cyclodextrin derivatives and *N,N*-dimethylacetamide for amylose oligomer derivatives, and adsorbed on 3-aminopropylsilylated silica gel (NUCLEOSIL 4000-7, particle size: 7 μm, pore size: 400 nm, 3.0 g). These packing materials were packed in a stainless-steel tube (25×0.46 (id)cm) by a slurry method. Optical resolution was carried out with a JASCO TRIROTAR-II chromatograph equipped with JASCO UVIDEC-100-III UV and DIP-181C polarimetric detectors. Chromatographic analyses were performed at a flow rate of 0.5 ml min^{-1} using hexane or a hexane-2-propanol mixture as an eluent. An elution time of 1,3,5-tri-*t*-butylbenzene was used as the dead time (t_0) of the chromatography.

CD spectra were measured on a JASCO J-500 apparatus with a 0.1 mm cell in THF. Gel permeation chromatographic (GPC) analysis was carried out with Shodex A80M and KF803 GPC columns connected in series with THF as an eluent.

Results and Discussion

The results for the optical resolution of racemic compounds **1**—**5** on 3,5-dimethylphenylcarbamates of maltooligosaccharides (2—7-mer) are summarized in Table 2. For a comparison, the optical resolving ability of amylose tris(3,5-dimethylphenylcarbamate)[5)] (ADMPC) is also shown. A nonpolar eluent, hexane or hexane-2-propanol (99:1), was used for the oligomeric CSPs, since the oligomer derivatives were soluble in polar solvents. No simple correlation was observed between the number (n) of glucose units and the separation factors (α). Although the maltose derivative (n=2) showed no optical resolving ability, other derivatives showed the characteristic optical resolving ability. ADMPC showed the highest optical resolving ability for **1** and **2**. However, other racemic compounds were best resolved on 4-mer or 5-mer. In most cases, the elution order of enantiomers was the same on amylose and oligomer derivatives, except for that for **5**.

The CD spectra of 3,5-dimethylphenylcarbamates of oligomers and amylose were measured in THF, and those of 2-mer, 3-mer, 7-mer, and amylose are shown in Fig. 1. The CD patterns of these carbamates, except for 2-mer, are quite similar. These results suggest that 3—7-mer and ADMPC derivatives may have similar high-order structures. This may be the reason for the similar optical resolving abilities of oligomer derivatives and ADMPC. The 2-mer



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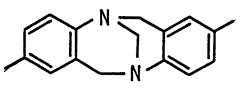

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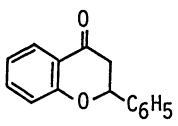

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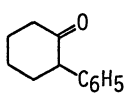

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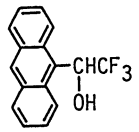

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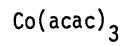

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Table 2. Optical Resolution of Racemates **1**—**5** on 3,5-Dimethylphenylcarbamates of Maltooligosaccharides and Amylose[a)]

Racemate	n=2		n=3[b)]		n=4		n=5		n=6		n=7		Amylose	
	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α
1	0.20(+)	ca. 1	1.65(+)	1.28	1.04(+)	1.11	0.73(+)	1.11	0.74(+)	ca. 1	0.74(+)	1.07	1.39(+)	4.29
2	0.59(−)	ca. 1	5.2	1.00	2.26(+)	1.34	1.48(+)	1.12	1.47(+)	1.11	1.58(+)	1.07	5.63(+)	1.42
3	0.94	1.00	7.65(+)	1.31	5.05(+)	1.60	2.53(+)	1.21	2.79(+)	1.36	2.65(+)	1.28	0.93(+)	1.12[c)]
4	0.49	ca. 1	3.87	1.00	1.98(−)	1.06	1.83(−)	1.14	1.78(−)	1.06	1.19(−)	1.12	2.67(−)	1.10
5	3.26(+)	ca. 1			18.5(−)	1.54	10.1(−)	1.59	7.68(−)	1.18			1.30(+)	1.15[c)]

Capacity factor: k_1'=(retention time of the first-eluted isomer−dead time)/(dead time).
Separation factor: α=(Capacity factor of the second-eluted isomer)/k_1'.
The sign of optical rotation of the first-eluted isomer is shown in parentheses.
a) Eluent: hexane-2-propanol (99 : 1), 0.5 ml min^{-1}. b) Eluent: hexane. c) Eluent: hexane-2-propanol (90 : 10).

appears to have a different conformation from others, which may be associated with the low chiral recognition ability, as shown in Table 2.

The influence of two terminal glucose units, each of which has four carbamate moieties, appears to be more pronounced on the oligomer derivatives than on the amylose derivative, and probably differs for each oligomer derivative. This influence may also be responsible for the delicate difference in chiral recognition by the 3,5-dimethylphenylcarbamates shown in Table 1. These conformational and structural influences on chiral recognition seem to depend on the structure of racemic compounds. In a chromatographic system with the phenylcarbamates of polysaccharides, a variety of racemic compounds including polar compounds, such as alcohol[6)] and acid,[7)] and nonpolar compounds, such as halides[8)] and aromatic hydrocarbons,[9)] have been resolved into optical isomers. This means that some interactions, for instance the hydrogen bond on the CO or NH group of the carbamate moiety, the dipole-dipole interaction on the CO group, and the π-π interaction on the phenyl group, are probably involved in the chiral discrimination by the CSP. The relative importance of these interactions may depend on the solutes. For a better understanding of the results shown in Table 2 by a diastereomeric interaction between the CSP and enantiomers, precise data concerning the sterical structures of CSPs and solutes are required. The clarification of these points is an attractive and challenging problem for the future.

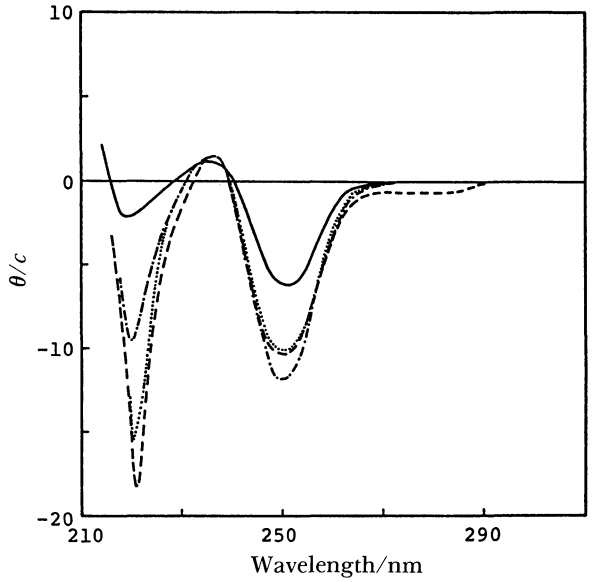

Fig. 1. CD spectra of 3,5-dimethylphenylcarbamates of maltooligosaccharide of (——: 2-mer; —·—: 3-mer; ·····: 7-mer) and amylose tris(3,5-dimethylphenylcarbamate) (----). (Solvent: THF, Concn: $c\approx$1 mg ml^{-1}).

The results of the optical resolution of **1**—**5** on 3,5-dimethylphenylcarbamates of cellobiose (n=2), cellotetraose (n=4), and cellulose[10)] are summarized in Table 3. Although, the dimer derivative separated **1** and **3**, the optical resolving ability of the cellulose derivative was generally higher than that of the oligomer derivatives. The α values, and sometimes the elution orders, of the enantiomers were fairly different between the cellulose and oligomer derivatives, and also between the two oligomers. These results suggest that the conformation of a glucose residue and the arrangement of glucose

Table 3. Optical Resolution on 3,5-Dimethylphenylcarbamates of Cellobiose, Cellotetraose, and Cellulose[a)]

Racemate	Cellobiose		Cellotetraose[b)]		Cellulose	
	k_1'	α	k_1'	α	k_1'	α
1	1.38(+)	1.37	9.38	1.00	2.84(−)	1.39
2	0.26(−)	ca. 1	1.54(+)	1.21	1.23(−)	2.61
3	0.29(+)	1.38	3.70(+)	ca. 1	2.10(+)	1.20
4	1.72	1.00	Not eluted		2.13(−)	2.59[c)]
5	0.25(−)	ca. 1	2.13(−)	1.13	2.21(−)	1.15

a) Eluent: hexane-2-propanol (98 : 2), 0.5 ml min^{-1}. b) Eluent: hexane. c) Eluent: hexan-2-propanol (90 : 10).

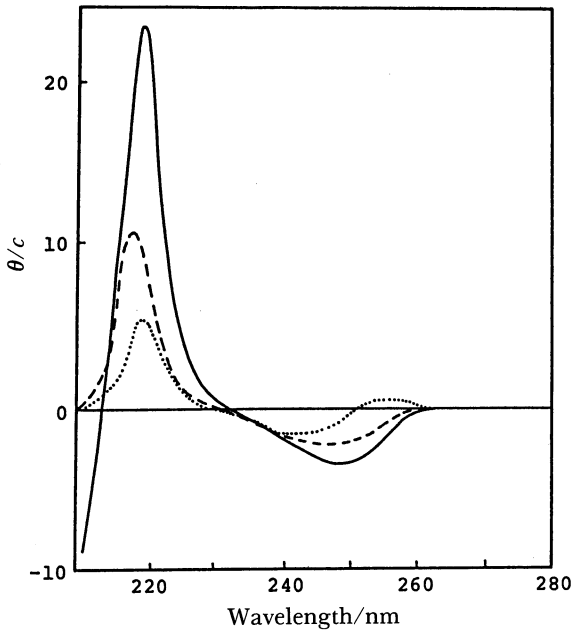

Fig. 2. CD spectra of 3,5-dimethylphenylcarbamates of cellulose (——), cellotetraose (----), and cellobiose (·····) in THF. (Concn: $c \approx 1$ mg ml^{-1}).

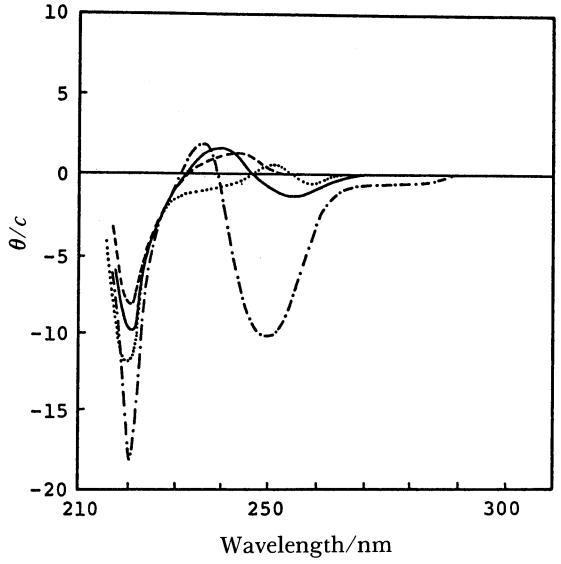

Fig. 3. CD spectra of 3,5-dimethylphenylcarbamates of α- (·····), β- (----), γ-cyclodextrins (——) and amylose (— · —) in THF. (Conc: $c \approx 1$ mg ml^{-1}).

residues may differ in each derivative. The CD spectra of these derivatives are shown in Fig. 2. The CD intensity greatly increased in the order 2-mer, 4-mer, and polymer. The regularity of the high-order structure of these derivatives may increase in this order.

Phenylcarbamate of β-cyclodextrin has been chemically bonded to silica gel and its optical resolving power was evaluated for alanyl-2-naphthylamide.[11] In the present study, we prepared 3,5-dimethylphenylcarbamates of α-, β-, and γ-cyclodextrins and used them as chiral stationary phases for HPLC (Table 4). For a comparison, the optical resolving ability of ADMPC is also shown. Although cyclodextrins and amylose have the same monomeric structure, the separation factor and elution order of the enantiomers were sometimes quite different between them. The linear amylose oligomer (n=4—7) derivatives showed a rather similar elution order and separation factor (Table 2). However, the cyclodextrin derivatives showed a different elution order and separation factor, depending on the number of monomeric units of the ring. Each of the cyclodextrin derivatives resolved at least one of racemates with the highest α value. This indicates that chiral recognition in these systems is very sensitive to even a small difference in structure.

The CD spectra of the cyclodextrin derivatives were compared with that of ADMPC (Fig. 3). The CD pattern of cyclodextrin derivatives differed slightly from each other in the aromatic region, suggesting that the conformation of these derivatives may be different. These CD patterns were quite different from that of ADMPC. The structures of cyclodextrin derivatives and ADMPC seem to be quite different. According to an X-ray analysis by Zugenmaier,[12] amylose tris(phenylcarbamate) probably exists as a four- or five-fold helix. Cyclodextrin derivatives must have a quite different cyclic structure from this helix. This structural difference may be correlated to different chiral recognition.

The oligosaccharide derivatives described here showed higher solubility in solvents compared to the

Table 4. Optical Resolution on 3,5-Dimethylphenylcarbamates of Cyclodextrins and Amylose[a]

Racemate	α-CD[b]		β-CD		γ-CD		Amylose	
	k_1'	α	k_1'	α	k_1'	α	k_1'	α
1	1.00(+)	1.36	0.87(+)	1.37	0.66(−)	1.16	1.39(+)	4.29
2	2.42(−)	ca. 1	1.49(−)	1.15	0.95(−)	1.52	5.63(+)	1.42
4	Not eluted		7.75	1.00	7.34(−)	1.10	1.30(+)	1.15[c]
5	2.49(−)	1.30	1.76(−)	1.22	1.36(+)	1.05	2.67(−)	1.10
6	Not eluted		2.50(−)	1.15	1.83(−)	1.11	2.98(−)	1.11

a) Eluent: hexane-2-propanol (99 : 1), 0.5 ml min^{-1}. b) Eluent: hexane. c) Eluent: hexane-2-propanol (90 : 10).

polysaccharide derivatives. Therefore, only nonpolar eluents like hexane and hexane containing less than 2% 2-propanol were used. Although some racemic compounds were better resolved on the oligosaccharide derivatives than on the polysaccharide derivatives, these oligomeric stationary phases can not be applied to practical use. The preparation of oligosaccharide derivatives chemically bonded to silica gel may improve this defect.

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5) Molecular weight of original amylose was 16000 which corresponds to degree of polymerization 99.

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