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Dimethyl-, dichloro- and chloromethylphenylcarbamates of amylose as chiral stationary phases for high-performance liquid chromatography

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Abstract

Twelve dimethyl-, dichloro- and chloromethylphenylcarbamate derivatives of amylose were prepared and their chiral recognition abilities were evaluated as chiral stationary phases for high-performance liquid chromatography. *ortho*-Substituted phenylcarbamate derivatives of amylose showed high chiral recognition abilities, although cellulose phenylcarbamate derivatives with *ortho* substituents on the phenyl moiety showed low chiral recognition. The superiority of 5-chloro-2-methylphenylcarbamate over the corresponding dimethyl- and dichlorophenylcarbamate derivatives of amylose was demonstrated. The roles of the NH residue of the carbamate moieties and methyl and chloro groups in chiral recognition were elucidated using IR and ^{1}H NMR spectroscopic data. The tris(5-chloro-2-methylphenylcarbamate) of amylose exhibited the highest chiral recognition ability among the amylose derivatives synthesized, and can be used for the separation of some chiral drug enantiomers.

1. Introduction

Many derivatives (acetate, benzoates, carbamates, etc.) of polysaccharides such as cellulose [1–4], amylose [1,3,4], chitosan [1,5] and guaran [6] have been examined as chiral stationary phases (CSPs) for enantioseparation by HPLC. Among them, cellulose derivatives, especially phenylcarbamate derivatives, have been most extensively studied and many empirical correlations have been established between the physical or chemical properties and enantiomer recognition abilities of CSPs [7]. Amylose derivatives are also of potential use as CSPs, but they have not yet been studied in detail [1,3,4]. More extensive investigations will provide more practical applications of this promising chiral natural polymer for enantioseparation, and will contribute to the elucidation of the chiral recognition mechanism on polysaccharide CSPs, which still remains obscure.

The purposes of this study were to evaluate (i) the role of the nature and the position of methyl and chloro groups in the chiral recognition abilities of disubstituted phenylcarbamate derivatives of amylose; (ii) the effect of introducing both an electron-donating methyl group and an electron-withdrawing chloro group on to the phenyl moieties on the chiral recognition abilities; and (iii) the correlations between chiral

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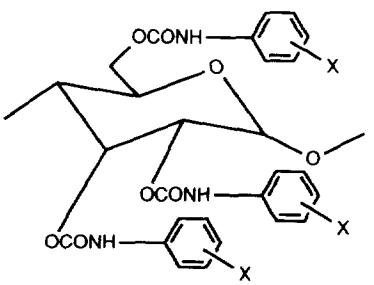

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X=	a: 3,4-$(CH_3)_2$	e: 5-Cl-2-CH_3	i: 3-Cl-2-CH_3
	b: 2,5-$(CH_3)_2$	f: 2-Cl-5-CH_3	j: 4-Cl-2-CH_3
	c: 3,4-Cl_2	g: 4-Cl-3-CH_3	k: 2-Cl-6-CH_3
	d: 2,5-Cl_2	h: 3-Cl-4-CH_3	l: 2-Cl-4-CH_3

Fig. 1. Structures of CSPs.

recognition abilities and the NH frequencies in IR spectra and the chemical shifts of the NH protons in ^{1}H NMR spectra of amylose phenylcarbamate derivatives. The potential use of above amylose derivatives for the enantioseparation of some practically important drugs is also demonstrated.

In this study, twelve dimethyl-, dichloro- and chloromethylphenylcarbamate derivatives of amylose (Fig. 1) were prepared and their chiral recognition abilities as CSPs for HPLC were evaluated.

2. Experimental

2.1. Chemicals

Amlyose B ($M_r \approx 16\,000$) was purchased from Nacalai Tesque (Kyoto, Japan). (3-Aminopropyl)triethoxysilane, 2,5-dimethylaniline, 3,4-dimethylaniline, 2,5-dichlorophenyl isocyanate, 3,4-dichlorophenyl isocyanate, 3-chloro-2-methylaniline and 3-chloro-4-methylaniline were of guaranteed reagent grade from Tokyo Kasei (Tokyo, Japan). 2-Chloro-6-methylaniline was obtained from Jansen Chimica (Beerse, Belgium) and triphosgene, pyridine-d_5, 2-chloro-4-methylaniline, 4-chloro-2-methylaniline, 5-chloro-2-methylaniline and 2-chloro-5-methylaniline from Aldrich (Milwaukee, WI, USA). Isocyanates were prepared from the corresponding anilines by the conventional method using triphosgene. Wide-pore silica gel (Daiso gel SP-1000, pore size 100 nm, particle size 7 μm) was kindly supplied by Daiso (Osaka, Japan) and was silanized using (3-aminopropyl)triethoxysilane in benzene at 80°C before use. Hexane and 2-propanol, used as components of the eluents, were of analytical-reagent grade. Racemic compounds (**6**, **13**, **14** and **15**) were gifts from Professor Suda and Dr. Kanoh of Kanazawa University, and other compounds were commercially available or were prepared by the usual procedures [8].

2.2. Preparation of tris(disubstituted phenylcarbamate) derivatives of amylose

Amylose tris(disubstituted phenylcarbamate) derivatives (**1a–l**) (Fig. 1) were prepared by the reaction of amylose with an excess of the corresponding isocyanates in dry pyridine at ca. 100°C using the same procedures described previously [2] and isolated as methanol-insoluble fractions. Elemental analyses (Table 1) and IR and ^{1}H NMR spectra were used to determine the degree of conversion of the hydroxy groups of amylose into the carbamate moieties. The reactivity of amylose with the isocyanates seemed to be lower than that of cellulose. The conversion of the hydroxy groups into the carbamates was more than 90% after 24 h in most instances, but sometimes small amounts of partially or completely unreacted amylose were found in the reaction flask even after 200 h at ca. 100°C. This fraction could be easily removed by decantation or by selective dissociation of the desired carbamate derivatives in tetrahydrofuran (THF), where the unreacted amylose is insoluble.

2.3. Preparation of stationary phase

Column packing materials were prepared as described previously [2] using macroporous silica gel (Daiso gel SP-1000) and packed into 25 cm × 0.46 cm I.D. stainless-steel tubes by the conven-

Table 1
Elemental analyses and NH chemical shifts of **1a–l**

Amylose derivative	C (%)	H (%)	N (%)	Cl (%)	NH proton, δ (ppm)		
					I	II	III
1a	61.80	6.02	6.14	–	9.56	9.28	
1b	57.24	6.04	4.92	–	8.80	8.35	
1c	43.86	3.32	4.94	–	10.84	10.12	10.06
1d	44.57	2.72	5.64	28.71	9.27	9.21	8.99
1e	52.66	4.41	5.75	14.64	9.55	8.95	8.21
1f	52.72	4.59	5.53	14.89	8.80	8.53	8.10
1g	51.69	4.66	5.08	12.63	10.33	9.71	9.33
1h	52.57	4.28	6.02	15.65	10.55	9.82	
1i	48.41	4.46	4.60	12.60	9.76	9.20	8.94
1j	53.67	4.31	6.17	14.82	9.26	8.94	8.42
1k	52.70	4.29	5.92	15.04	9.06[a]		
1l	53.39	4.46	5.95	14.98	9.50	8.92	

Calculated values: C 65.89, H 5.82, N 6.99% (**1a** and **b**); C 44.75, H 2.35, N 5.80, Cl 29.42% (**1c** and **d**); C 54.33, H 3.92, N 6.34, C 16.08% (**1e–l**).
[a] Very broad peak ranging from 8.2 to 10.5 ppm.

tional high-pressure slurry packing technique using a model CCP-085 Econo packer pump (Chemco, Osaka, Japan). The plate numbers of the columns were 3000–4000 for benzene with hexane–2-propanol (90:10) as the eluent at a flow-rate of 0.5 ml/min at 20°C. The dead time (t_0) of the columns was determined using 1,3,5-tri-*tert.*-butylbenzene as a non-retained compound.

2.4. Apparatus

All chromatographic experiments were performed on a Jasco Trirotar-II liquid chromatograph equipped with UV (Jasco 875-UV) and polarimetric (Jasco 181-C) detectors at ambient temperature. An injector with a 100-μl loop (Model 7125, Rheodyne, Cotati, CA, USA) was used for injection of samples. IR spectra were measured using a Jasco Fourier transform IR spectrometer with a Jasco PTL-396 data processor. UV spectra of amylose phenylcarbamate derivatives were measured in THF solutions using a Jasco Ubest-55 spectrophotometer. Circular dichroism (CD) spectra were measured in THF solutions in a 0.01-cm cell using a Jasco J-720 L spectropolarimeter. ^{1}H NMR spectra were measured in pyridine-d_5 solutions at 80°C with a Varian VXR-500S NMR spectrometer (500 MHz). Tetramethylsilane (TMS) was used as the internal standard.

3. Results and discussion

The results of enantioseparation of fourteen racemic compounds (**2-15**) (Fig. 2) are given in Table 2. On the basis of separation factors (α), the chiral recognition abilities of 2,5-dimethyl- (**1b**) and 2,5-dichlorophenylcarbamates (**1d**) of amylose were not substantially low compared

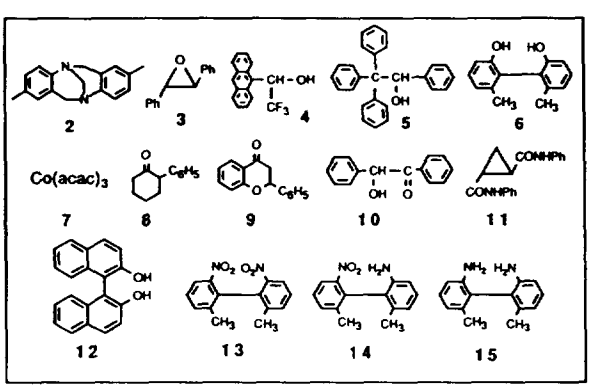

Fig. 2. Structures of racemic solutes.

Table 2
Optical resolution of racemates **2–15** on amylose derivatives **1a–l**

Compound	**1a**: 3,4-$(CH_3)_2$			**1b**: 2,5-$(CH_3)_2$			**1c**: 3,4-Cl_2			**1d**: 2,5-Cl_2		
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s
2	1.00(+)	1.23	1.4	0.70(−)	ca. 1		0.82	1.22	0.8	1.33(−)	1.38	0.8
3	0.83(+)	1.36	1.8	0.55(+)	1.67	2.2	0.43(+)	1.23	0.6	1.33(+)	1.18	0.7
4	2.10	1.00		1.40(−)	1.14	1.2	1.53	1.00		1.40	1.00	
5	2.10(+)	1.67	3.5	1.40(+)	1.60	2.5	0.93(+)	1.50	0.6	0.47(−)	3.14	2.0
6	3.43(−)	1.58	3.0	2.67(−)	1.08		1.40(−)	ca. 1		2.20(+)	1.16	
7	0.87(−)	1.06		0.62(+)	ca. 1		1.60(−)	ca. 1		1.10(+)	ca. 1	
8	1.33(−)	1.06		1.07(−)	1.13	1.0	1.13(+)	ca. 1		2.00(−)	1.24	1.0
9	2.10(+)	1.38	3.0	1.87(+)	1.18	1.2	1.25(+)	1.08		2.37	1.00	
10	4.92(−)	1.04		3.22(+)	1.24	2.0	3.43(−)	1.31	1.3	0.38(+)	ca. 1	
11	2.70(−)	1.11	0.6	1.67	1.00		0.58(+)	1.14		0.83(+)	2.04	1.0
12[a]	6.87	1.00		7.80	1.10		2.73	1.00		6.67	1.15	
13	2.27(−)	ca. 1		2.20(−)	ca. 1		2.58(−)	1.26	1.0	6.13(−)	ca. 1	
14	2.22(−)	1.20	1.7	1.32(+)	1.25	1.3	1.25(−)	1.52	2.0	3.03(−)	1.38	1.4
15	2.43(+)	1.12	1.4	1.72(+)	1.14	1.2	0.70(−)	1.19	0.7	1.60(−)	ca. 1	
	1e: 5-Cl-2-CH_3			**1f**: 2-Cl-5-CH_3			**1g**: 4-Cl-3-CH_3			**1h**: 3-Cl-4-CH_3		
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s
2	0.83(−)	1.13		0.93(−)	1.39	1.1	0.77(+)	1.43	1.0	0.78(+)	1.28	1.4
3	0.67(+)	1.70	2.3	0.77(+)	1.43	0.8	0.60(+)	1.66	2.0	0.57(+)	1.29	1.3
4	0.90(−)	1.14	0.6	2.30(−)	1.09		0.73	1.00		0.83	1.00	
5	1.20(+)	1.67	1.5	1.53(+)	2.11	1.7	1.30(+)	1.88	2.3	1.67(+)	1.77	3.5
6	1.27(+)	1.28	0.8	2.53(+)	1.07		2.07(−)	1.32	0.8	1.43(−)	1.09	
7	1.10(+)	1.18	1.0	0.55	1.00		1.07(−)	ca. 1		0.68(−)	ca. 1	
8	1.37(−)	1.24	1.0	0.90(−)	1.19		0.93(−)	ca. 1		1.03(−)	ca. 1	
9	2.00(+)	1.18	1.1	2.13	1.00		1.13(+)	1.11		1.57(+)	1.49	3.5
10	4.30(+)	1.16	0.8	4.13	1.00		2.87(−)	1.05		3.83(+)	ca. 1	
11	1.32(+)	3.09	2.1	1.03(+)	2.09	1.2	0.97(+)	1.34	0.9	1.30(+)	1.08	
12[a]	3.07(−)	1.35	1.2	8.27	1.07		5.73	1.00		4.07	1.00	
13	3.67(−)	1.13	1.3	3.80(−)	1.10		2.03(−)	1.07		2.07(−)	1.06	
14	2.20(−)	1.06		2.80(−)	1.15	0.7	1.20(−)	1.39	1.3	1.33(−)	2.00	4.4
15	0.43(+)	2.38	1.7	3.80(+)	1.10		1.00(−)	ca. 1		1.10(−)	1.18	1.2
	1i: 3-Cl-2-CH_3			**1j**: 4-Cl-2-CH_3			**1k**: 2-Cl-6-CH_3			**1l**: 2-Cl-4-CH_3		
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s
2	0.93(+)	1.11		1.37(+)	1.11		0.87(+)	2.62	2.0	1.50	1.00	
3	0.50(+)	2.00	2.0	1.05(+)	1.54	2.1	0.60	1.00		0.82(+)	1.22	0.7
4	0.77	1.00		1.28	1.00		2.53	1.00		2.67	1.00	
5	1.07(+)	2.44	3.1	1.27(+)	1.84	1.5	1.32(−)	1.14		1.67(+)	1.36	1.1
6	1.38(−)	ca. 1		2.67	1.00		2.10	1.00		1.93	1.00	
7	0.95(+)	ca. 1		1.50(+)	ca. 1		3.30(−)	1.04		0.60(+)	ca. 1	
8	0.52(−)	ca. 1		1.73(−)	ca. 1		1.70(+)	ca. 1		0.87(−)	ca. 1	
9	1.63(+)	1.15	0.5	2.57(+)	1.04		1.70	1.00		2.28	1.00	
10	3.83(+)	1.09	0.5	5.50(+)	1.39	1.9	3.13(+)	1.09		3.70(+)	1.07	
11	1.00(+)	1.50	0.9	1.50(+)	1.42	1.0	1.38(+)	1.36	0.9	1.10(+)	2.12	1.4
12[a]	3.33	1.00		5.13	1.00		5.20	1.00		10.33	1.06	
13	2.90(−)	ca. 1		4.83(+)	ca. 1		5.67(−)	1.03		3.77	1.00	
14	1.87(+)	ca. 1		3.00(−)	ca. 1		3.07(+)	ca. 1		2.53(−)	ca. 1	
15	1.20(+)	1.06		1.26(+)	ca. 1		1.20(+)	ca. 1		1.47(+)	ca. 1	

k'_1 = Capacity factor with optical rotation (in parentheses) of the first-eluted enantiomer. Separation factor (α) = k'_2/k'_1. Resolution factor (R_s) = $2(t_2 - t_1)/(w_1 + w_2)$, where t_1 and w_1 are the retention time and band width of the first-eluted enantiomer and t_2 and w_2 are the retention time and band width of the second-eluted enantiomer, respectively. Eluent, hexane–2-propanol (90:10); flow-rate, 0.5 ml/min.
[a] Flow-rate, 1.0 ml/min.

with those of 3,4-dimethyl- (**1a**) and 3,4-dichlorophenylcarbamates (**1c**) of amylose, respectively, although for cellulose phenylcarbamate derivatives, *ortho* substitution with either a methyl or a chloro group lowered the chiral resolving power [2]. Differences in the chiral recognition abilities between 2,5- and 3,4-disubstituted phenylcarbamates of amylose were observed, depending on the racemic compounds. For instance, the CSP **1a** separated or at least partially separated the racemic compounds **2**, **7** and **11**, which were not separated on **1b**. Similarly, the CSP **1b** separated the racemic compounds **4** and **12**, which were not separated on **1a**. The same tendency was observed for dichlorophenylcarbamate derivatives of amylose; the racemic compounds **9**, **10** and **15** were not resolved on **1d**, but were resolved on **1c**, and the racemic compounds **6**, **8** and **12** were separated on **1d**, but not on **1c**. The differences in chiral recognition abilities of the same dimethyl- and dichlorophenylcarbamates can also be found by comparing the chiral recognition abilities of the CSPs **1a** and **1c** or **1b** and **1d**. The chiral recognition abilities of 2,5- and 3,4-disubstituted phenylcarbamate derivatives of amylose for the racemic compounds used in this study depend on the position and the type of the substituents on the phenyl moieties.

We have already demonstrated that the main chiral adsorbing sites for chiral separation on phenylcarbamate derivatives of polysaccharides are considered to be the polar carbamate residues, which can interact with a solute via hydrogen bonding with NH and C=O groups [2–4,9–11]. The importance of the carbamate residues for chiral recognition has recently been supported by a ^{1}H NMR study on chiral recognition using cellulose tris(4-trimethylsilylphenylcarbamate) [12]. It has also been reported [2,4] that the introduction of either electron-donating or electron-withdrawing substituents at the *meta* or *para* position on to the phenyl moieties tends to improve the optical resolution abilities of phenylcarbamate derivatives of cellulose. For instance, cellulose tris(3,5-dimethylphenylcarbamate), having electron-donating substituents, could effectively resolve polar compounds which can interact with the carbonyl groups of the carbamate moieties, and the derivatives, for instance, cellulose tris(3,5-dichlorophenylcarbamate), having electron-withdrawing substituents could possess a higher resolving power for the compounds that can be adsorbed on the NH groups of the carbamate moieties [2]. The characteristic enantioseparation of many racemic compounds on the cellulose phenylcarbamate derivatives confirmed this explanation, but recently we have found that a better resolution of some racemic compounds was attained on some *meta*- and *para*-disubstituted chloromethylphenylcarbamates of cellulose than on the corresponding dimethyl- and dichlorophenylcarbamates [7,13]. This suggests that the above explanation needs some modification.

In order to examine the effect of the simultaneous introduction of both electron-donating and electron-withdrawing substituents on to the phenyl moieties on the chiral recognition abilities of amylose phenylcarbamate derivatives, two series of 2,5- and 3,4-substituted dimethyl-, dichloro- and chloromethylphenylcarbamate derivatives of amylose were prepared and their chiral recognition abilities were evaluated. For 3,4-disubstituted derivatives, the effect of the introduction of methyl and chloro groups was not marked and the CSPs **1a**, **1c**, **1g** and **1h** exhibited approximately the same chiral recognition abilities, whereas the chiral resolving power of 2,5-disubstituted phenylcarbamate derivatives of amylose was not similar, but was dependent on the type of the substituents. For example, the CSP **1b** could separate ten compounds among fourteen racemic compounds tested and **1d** separated eight of them, whereas **1f** separated eleven and, moreover, **1e** all fourteen racemic compounds. In some instances **1e** exhibited high separation factors (α), shorter retention times and better resolution factors (R_s).

Examples of the enantioseparation of the racemic compounds **7** and **12** characterized with different functionalities are given on Figs. 3 and 4. As can be seen, racemic cobalt(III) tris(acetylacetonate) (**7**) was not resolved on either **1b** or **1d**, but was effectively resolved on **1e**. More-

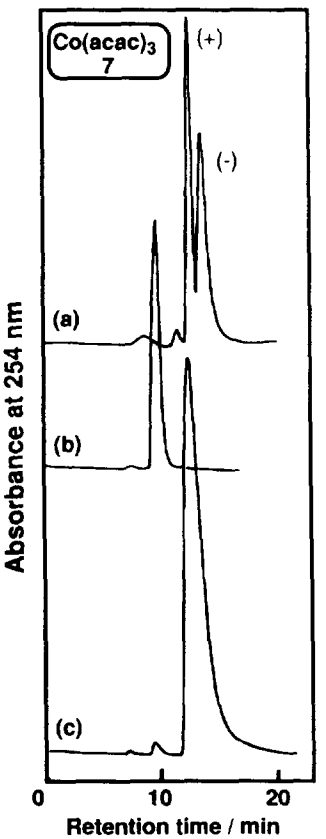

Fig. 3. Separation of enantiomers of cobalt(III) acetylacetonate (**7**) on (a) **1e**, (b) **1b** and (c) **1d**. Chromatographic conditions as in Table 2.

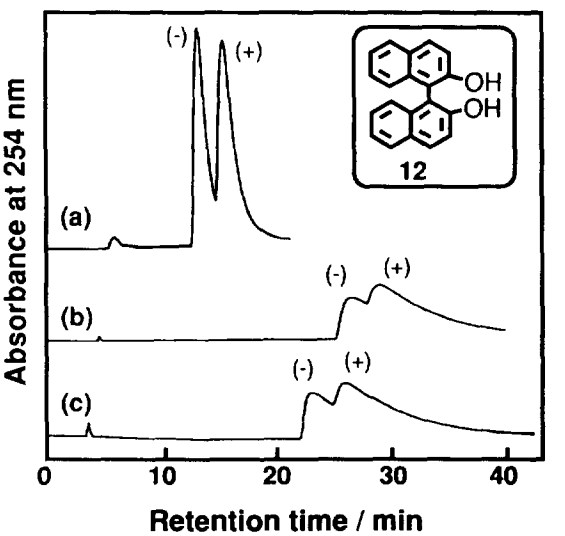

Fig. 4. Separation of enantiomers of 2,2′-dihydroxy-1,1′-binaphthyl (**12**) on (a) **1e**, (b) **1b** and (c) **1d**. Flow-rate, 1.0 ml/min.

over, racemic 2,2′-dihydroxy-1,1′-binaphthyl (**12**) was partially separated on **1b** and **1d**, whereas almost complete separation was achieved on **1e**, and the retention times are shorter and R_s higher on **1e**. Another example is the racemate **13**, which was not resolved on **1b** and **1d** but was resolved on **1e** and **1f**. The chiral recognition abilities exhibited by **1e** seem higher than those of the commercially available tris(3,5-dimethylphenylcarbamate) of amylose, which shows poor chiral recognition abilities for the racemic compounds **7**, **8**, **12** and **13** [3].

The ^{1}H NMR and IR spectral data for the amylose derivatives gave useful information to discuss the role of electron-donating and electron-withdrawing substituents in chiral recognition abilities of CSPs. As can be seen in the IR spectra of the amylose phenylcarbamate derivatives (Fig. 5), two peaks of the NH groups can be observed. The peak in the range 3400–3430 cm^{-1} may be assigned to a free N–H bond and the band in the range 3300–3350 cm^{-1} to an NH group involving intramolecular hydrogen bonding [7]. The high fraction of the latter band indirectly means a more ordered secondary structure of the CSPs. Both free NH and intramolecularly hydrogen-bonded NH groups were observed in the IR spectra of the amylose trisphenylcarbamates.

Introduction of the methyl substituents at the 2- and 5-positions increased the ratio of hydrogen-bonded NH groups, which means the formation of a more ordered rigid structure of the polysaccharide CSP, whereas the introduction of chloro substituents at the 2- and 5- positions on the phenyl moieties leads to an increase in a free NH bond, which means an increase in the number of adsorptive sites accompanied by a decrease in the regularity of the chiral polymers.

Other information on the effect of the substituents can also be obtained from the ^{1}H NMR spectra. Three NH resonances in the ^{1}H NMR spectra corresponding to the NH protons of carbamate groups at the 2-, 3-, and 6-positions of

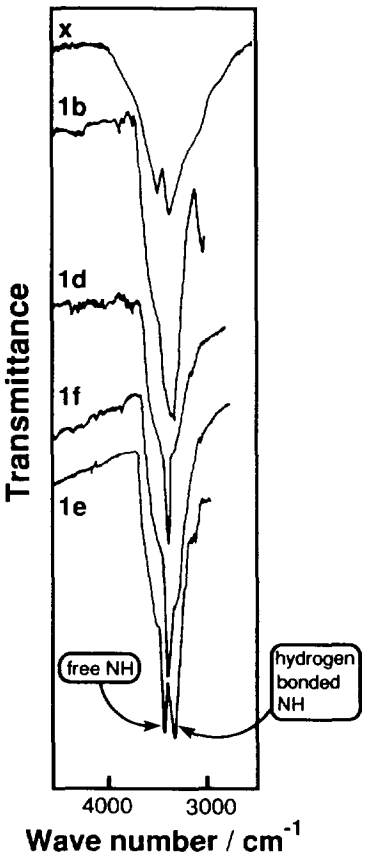

Fig. 5. IR spectra of the NH groups of 2,5-disubstituted chloromethylphenylcarbamates of amylose (**1b**, **1d**, **1e** and **1f**) and amylose trisphenylcarbamate (x).

the glucose units were observed. The resonances of the NH protons shifted upfield on introducing the methyl groups at the 2- and 5- positions, whereas the resonances of the NH protons shifted downfield on introduction of the chloro substituents at the 2- and 5-positions (Table 1). The NH chemical shifts reflect the acidity of the NH protons and the NH resonances will shift downfield with an increase in the acidity.

The effects of methyl and chloro substituents on the phenyl moieties on the properties of amylose phenylcarbamate derivatives can be summarized as follows. The introduction of the methyl groups contributes to maintaining a higher order structure of CSPs through intramolecular hydrogen bonding, but this results in decreases in the number and the acid strength of adsorptive sites, which leads to a decrease in adsorbing power to solutes capable of interacting with the NH groups of the carbamate moieties. In contrast, the introduction of the chloro groups on the phenyl moieties induces a disordered structure of CSPs, but it may cause increases in the number and the acid strength of adsorptive sites. In some instances this will lead to an increase in retention times and α for some racemates, but to a decrease in R_s because of peak broadening. The superiority of the 2,5-disubstituted chloromethylphenylcarbamates, especially **1e**, over the corresponding dimethyl- and dichlorophenylcarbamates must be the result of the balance of the above-mentioned effects. The same changes may be induced on the C=O sites.

The CSP **1b**, in which most of the NH groups are involved in intramolecular hydrogen bonding and the acidity of the NH is low, will not exhibit universal separation abilities, but some racemic compounds could be separated on **1b** with a very high R_s. In contrast, the CSP **1d** with a high proportion of free NH groups, and therefore the most disordered structure, will exhibit smaller R_s and large α for some racemates (**2**, **5**, **8**, **11** and **14**). The CSP **1f**, having a high proportion of free NH groups, also showed a small R_s. A balanced ratio of the free NH groups to hydrogen-bonded NH groups seems to be the reason for very high chiral recognition abilities of **1e**. The high acid strength of the NH groups of **1e** may also be important for high chiral recognition. This appears to improve both α and R_s. The high enantioselectivity of **1e** for most racemic compounds was not accompanied by an increase in retention times, indicating that the higher acidity of the NH groups is more important for intramolecular hydrogen bonding than for interaction with racemic solutes as adsorbing sites.

As can be seen from the CD spectra of these derivatives (Fig. 6), more intense peaks in the region of 210 nm (C=O region) were observed for **1e** than for **1b**, indicating that the conformation of **1e** is more regular than that of **1b**.

For 3,4-disubstituted phenylcarbamates of

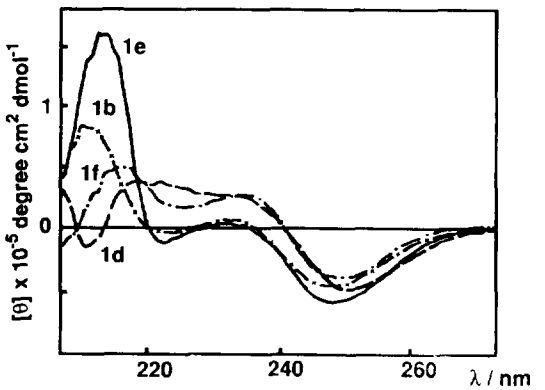

Fig. 6. CD spectra of 2,5-disubstituted chloromethylphenylcarbamates of amylose (**1b**, **1d**, **1e** and **1f**).

amylose (**1a**, **1c**, **1g** and **1h**), the ratios of free and intramolecular hydrogen-bonded NH groups were not changed substantially except for **1h**. This may be the reason for similar enantiomer resolving abilities of these derivatives. 2,4-Disubstituted derivative (**1j**) with similar NH chemical shifts to **1e** showed low chiral recognition; the geometry of the substituted phenyl group may be involved.

Besides the above-mentioned strong interactions through hydrogen bond, sometimes we must also take into account weak interactions ($\pi-\pi$, hydrophobic interactions, etc.) when discussing the mechanism of enantioseparation using polysaccharide CSPs.

Additional studies seem necessary to be able to discuss more precisely the effects of the electron-donating and electron-withdrawing substituents.

High chiral recognition abilities of amylose tris(2-methyl-5-chlorophenylcarbamate) were also shown in the separation of some other compounds (Fig. 7). These racemic drugs were completely separated on **1e**.

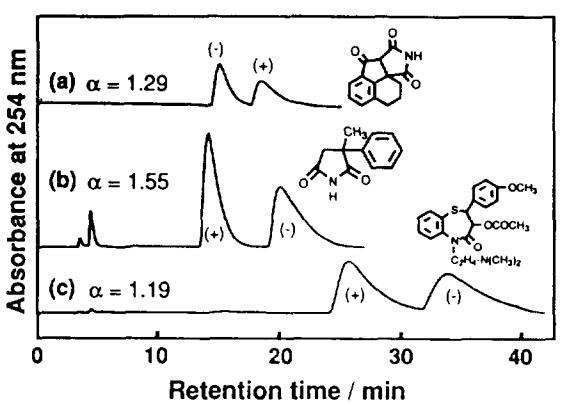

Fig. 7. Chromatographic separation of (a) 3a,4,5,6-tetrahydrosuccinimido[3,4-*b*]acenaphthen-10-one, (b) α-methyl-α-phenylsuccinimide and (c) *cis*-diltiazem on amylose tris(2-methyl-5-chlorophenylcarbamate) (**1e**). Eluent, (a) hexane–2-propanol (80:20) and (b,c) hexane–2-propanol (90:10); flow-rate, 1.0 ml/min.

4. Conclusions

The chiral recognition abilities of twelve dimethyl-, dichloro- and chloromethylphenylcarbamate derivatives of amylose were evaluated as CSPs for HPLC and it was established that chiral recognition ability of amylose tris(2-methyl-5-chlorophenylcarbamate) is superior to that of other 2,5-dimethyl- and 2,5-dichlorophenylcarbamates. An explanation is proposed for the role of the electron-donating and electron-withdrawing substituents on the phenyl moieties on the chiral recognition abilities of the polysaccharide phenylcarbamate derivatives.

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