Optical Resolution by Supercritical Fluid Chromatography Using Polysaccharide Derivatives as Chiral Stationary Phases

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(Received March 27, 1992)

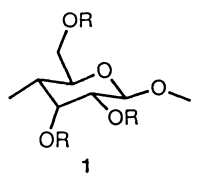
Synopsis. The optical resolution of ten racemates by supercritical fluid chromatography (SFC) was investigated using phenylcarbamates of cellulose and amylose and 4-methylbenzoate of cellulose as chiral stationary phases (CSPs) and carbon dioxide–alcohol as a mobile phase. The optical resolving abilities of phenylcarbamates of both cellulose and amylose in SFC were low compared with those in high-performance liquid chromatography (HPLC). However, 4-methylbenzoate of cellulose showed a slightly better optical resolving ability in SFC than in HPLC.

The development of supercritical fluid chromatography (SFC) is remarkable; it has been becoming one of the practically useful separation methods. However, the optical resolution by SFC using chiral-packed columns is still not very familiar, although some data are available.[1] Recently, Macaudiere et al.[2] and Nitta et al.[3] have reported the optical resolution by SFC using chiral columns packed with cellulose derivatives coated on silica gel.

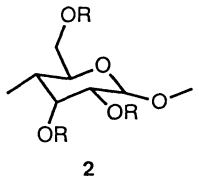
This paper describes the optical resolution on cellulose tris(3,5-dimethylphenylcarbamate) (**1a**),[4] cellulose tris(phenylcarbamate) (**1b**),[5] cellulose tris(3,5-dichlorophenylcarbamate) (**1c**),[4] cellulose tris(4-methylbenzoate) (**1d**),[6] and amylose tris(3,5-dimethylphenylcarbamate) (**2a**)[7] by SFC using carbon dioxide–alcohol as a mobile phase. The effect of alcohols as modifiers on optical resolution was also evaluated using **1a**. The obtained data were compared with those under HPLC.

Experimental

Racemic solutes, **3**,**4**,**5**,**7**,**8**,**10**, and **11** were commercially available; **6** and **9** were synthesized by conventional methods, and **12** was kindly provided by Professor Hiroshi Suda of Kanazawa University.



1
Cellulose derivatives



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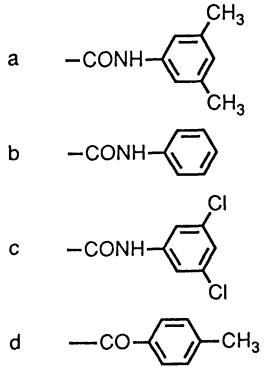
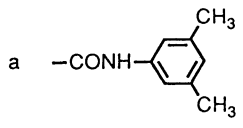
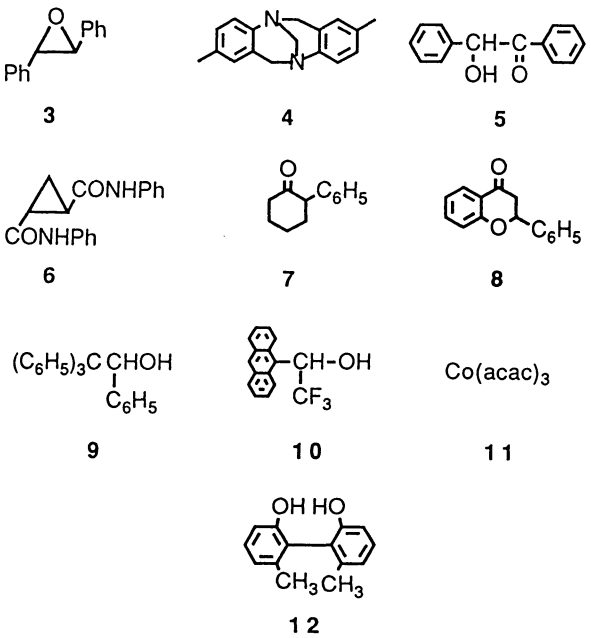
Amylose derivatives







Details concerning the preparation of CSPs were described previously.[4,6] Cellulose and amylose derivatives (25 wt% of silica gel) were coated on macroporous silica gel (Nucleosil 4000-7), which had been treated with (3-aminopropyl)-triethoxysilane.

Each CSP obtained was packed in a stainless-steel tube (25 cm×0.46 (i.d.) cm) by a slurry method. An SFC analysis was performed on a JASCO Super-200 system equipped with two pumps (JASCO 880-PU) and a UV detector (JASCO 875-UV). The line pressure was regulated by means of a JASCO 880-81 regulator. The optical resolution was performed with a carbon dioxide mixed alcohol modifier used as a mobile phase. The dead time (t_0) was estimated with 1,3,5-tri-*t*-butylbenzene. The temperature was controlled at 60 °C by a column oven (JASCO 888-CO). The conditions of the HPLC analysis were described previously.[4,6]

Results and Discussion

Figure 1 shows a chromatogram of the resolution of **3** on cellulose tris(3,5-dimethylphenylcarbamate) (**1a**). The capacity factors, $k'_1=(t_1-t_0)/t_0$ and $k'_2=(t_2-t_0)/t_0$, for the first and second eluted isomers were 1.47 and 2.09, respectively, and the separation factor $\alpha=k'_2/k'_1$, which represents the chiral recognition ability of CSPs, was 1.42. The resolution factor, $R_s=2(t_2-t_1)/(W_1+W_2)$, was estimated to be 4.13.

The results of optical resolution of ten racemates (**3**—**12**) by SFC on three cellulose phenylcarbamates (**1a**—**c**) and benzoate (**1d**) are summarized in Table 1, together

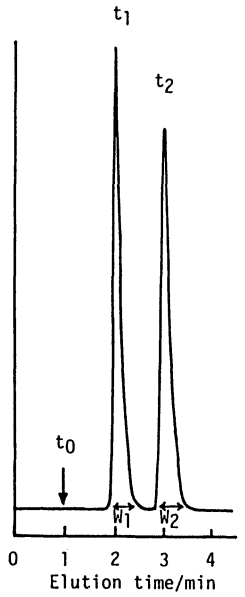


Fig. 1. Optical resolution of *trans*-stilbene oxide (**3**) on cellulose tris(3,5-dimethylphenylcarbamate) (**1a**) by SFC. (Mobil phase: CO_2–2-propanol (96 : 4), 3.2 ml mim,$^{-1}$ 60 °C)

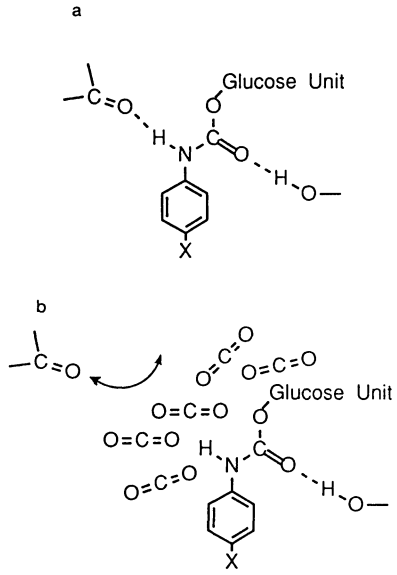


Fig. 2. Schematic interaction of phenylcarbamate of a polysaccharide; (a) with racemates; (b) with CO_2.

with the data obtained by HPLC. Most racemates were completely resolved with high α values by HPLC on **1a**—**c**. However, in SFC, the chiral recognition abilities of the three CSPs became lower. Particularly, the lowering was remarkable for **1c** and only three racemates (**7**—**9**) could be resolved.

These results may be understood by considering the interaction of a mobile phase CO_2 with CSPs. The most important adsorbing site of these CSPs for chiral recognition is the carbamate moieties of the CSPs (Fig. 2a).[4] Racemic compounds may interact with the carbamate moieties mainly through the hydrogen bond. Since CO_2 is used as a mobile phase, the racemates may not interact with the -NH groups of the carbamate moieties, because -NH may be surrounded by CO_2 (Fig. 2b). It has been shown that the polarity of the carbamate moieties depends greatly on the substituents introduced on the phenyl groups.[4] An electron-donating substituent, such as CH_3, enhances the electron density of C=O and an electron-withdrawing substituent, such as Cl, increases the acidity of -NH. The acidic -NH group of **1c** may be surrounded more preferentially by CO_2 than those of other derivatives. This appears to be associated with the more remarkable lowering of the chiral recognition of **1c**.

The optical resolving ability of the benzoate **1d** was slightly better in SFC than in HPLC. In case of **1d**, the interaction with CO_2 seems to be ignored because of the absence of NH groups; therefore, a significant decrease of the chiral recognition ability can not be expected.

Table 2. Optical Resolution of **3**—**12** by SFC and HPLC on **2a**[a]

	SFC			HPLC[b]		
	k'_1	α	R_s	k'_1	α	R_s
3	1.81	ca.1		0.42	3.04	6.67
4	8.15	1.13	0.77	0.53	1.58	2.30
5	5.10	1.13	1.37	3.14	1.21	2.07
6	20.0	ca. 1		3.25	2.01	3.59
7	6.30	ca. 1		0.61	ca. 1	
8	2.82	1.29	1.45	0.93	1.12	0.77
9	18.4	ca. 1		2.65	1.98	5.48
10	10.4	1.24	2.72	1.30	1.15	0.75
11	0.73	ca. 1		0.25	ca. 1	
12	5.29	2.40	5.46	2.46	2.11	6.38

a) CO_2–2-propanol (96 : 4), 3.2 ml min^{-1}, 60 °C.
b) Hexane–2-propanol (90 : 10), 0.5 ml min^{-1}, 25 °C.

Table 1. Optical Resolution of **3**—**12** by SFC and HPLC on **1a**—**d**[a]

	1a						**1b**						**1c**						**1d**					
	SFC			HPLC[b]			SFC			HPLC[b]			SFC			HPLC[c]			SFC			HPLC[b]		
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s
3	1.47	1.42	4.13	0.74	1.68	3.22	3.00	1.23	0.75	0.67	1.46	2.00	1.22	ca. 1		0.56	1.84	4.20	1.23	1.28	1.84	1.07	1.11	0.81
4	5.29	1.04		0.97	1.32	1.92	3.13	1.14	0.77	1.12	1.37	1.73	2.62	ca. 1		0.87	1.65	2.12	2.12	1.96	5.84	0.98	3.80	4.46
5	4.00	1.25	3.20	2.43	1.58	4.38	1.73	ca. 1		5.28	ca. 1		2.30	ca. 1		3.08	1.21	1.91	2.24	1.06		3.30	1.23	1.67
6	4.00	2.00	5.33	0.83	3.17	6.17	7.96	ca. 1		2.08	1.45	1.38	15.7	ca. 1		0.59	1.41	1.47	2.03	1.07				
7	12.4	ca. 1		1.17	1.15	0.90	8.52	ca. 1		1.88	1.17	0.98	7.21	ca. 1		2.65	1.26	1.95	1.72	1.23	1.52	1.22	ca. 1	
8	3.78	1.10	0.75	1.47	1.41	3.80	2.38	ca. 1		2.22	1.10	0.68	1.61	ca. 1		1.55	1.20	1.48	2.01	1.07		3.02	ca. 1	
9	17.7	ca. 1		2.36	1.83	4.39	9.14	1.31	2.55	2.58	1.22	0.77	2.49	1.10		0.40	1.29	0.84	11.5	1.43	0.97	3.22	1.44	0.84
10	1.75	2.01	4.43	2.13	2.59	6.40	3.50	1.42	2.11	1.56	1.45	1.38	1.29	1.42	1.90	0.28	1.38	0.87	12.8	1.38	0.77	3.73	1.17	0.95
11	1.98	1.08		0.42	ca. 1		1.13	1.36	1.36	2.57	1.24	0.75	1.32	1.63	2.20	0.76	1.82	4.06	0.68	ca. 1		0.57	ca. 1	
12	3.80	ca. 1		1.37	1.34	1.87	2.19	1.06		2.37	1.65	2.56	1.24	ca. 1		1.62	1.11	0.75	2.22	1.09		1.77	1.22	0.87

a) CO_2–2-propanol (96 : 4), 3.2 ml min^{-1}, 60 °C. b) Hexane–2-propanol (90 : 10), 0.5 ml min^{-1}, 25 °C. c) Hexane–2-propanol (95 : 5).

Table 3. Optical Resolution of **3**—**12** by SFC on **1a** Using Various Alcohols as Modifiers[a)]

	Methanol			Ethanol			2-Propanol		
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s
3	1.35	1.42	3.83	0.99	1.43	1.70	1.47	1.42	4.13
4	3.39	ca. 1		7.30	ca. 1		5.29	1.04	
5	3.34	1.22	2.88	2.63	1.11	1.50	4.00	1.25	3.20
6	20.8	1.39	3.27	4.20	1.56	5.33	4.00	2.00	5.33
7	3.89	ca. 1		8.25	ca. 1		12.4	ca. 1	
8	2.08	1.10	2.09	6.04	1.03		3.78	1.10	0.75
9	8.00	1.07		8.80	ca. 1		17.7	ca. 1	
10	9.10	ca. 1		3.15	2.17	4.95	1.75	2.01	4.43
11	0.83	ca. 1		1.04	ca. 1		1.98	1.08	
12	3.80	ca. 1		1.27	ca. 1		3.80	ca. 1	

a) CO_2–alcohol (96 : 4), 3.2 ml min^{-1}, 60 °C.

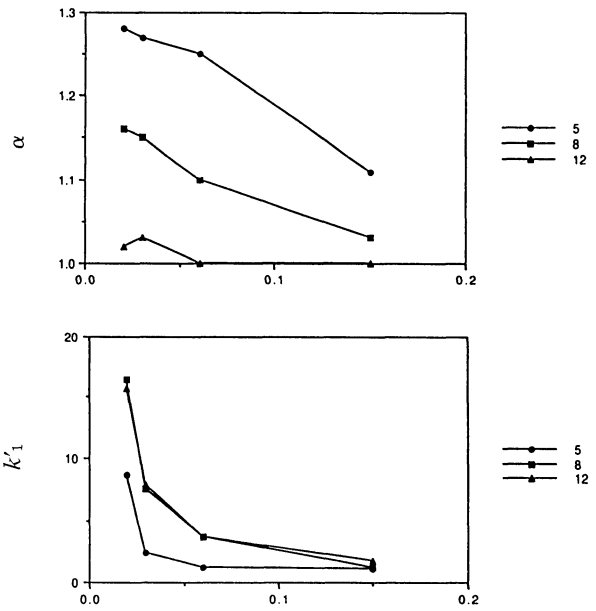


Fig. 3. Influences of the alcohol composition on the k'_1 and α, in the resolution of (●) benzoin (**5**), (■) flavanone (**8**), and (▲) 2,2′-dihydroxy-6, 6′-dimethylbiphenyl (**12**).

This column may be useful for optical resolution of many other racemates by SFC.

Table 2 shows the results of the optical resolution of ten racemates on **2a** by SFC. The optical resolving ability in SFC was also lower than that in HPLC, as observed for the cellulose derivatives.

Table 3 summarizes the results of the optical resolution on **1a** using various alcohols as modifiers. The α values depended on the kind of alcohols; for several racemates, 2-propanol was the most suitable modifier.

Figure 3 shows plots of α and k'_1 for three racemates (**5**, **8**, and **12**) against the 2-propanol composition in the mobile phases. Both the k'_1 and α values decrease with an increase in the modifier content.

Conclusion

Phenylcarbamates of cellulose and amylose showed lower optical resolving abilities in SFC using carbone dioxide modified with alcohols as a mobile phases than in HPLC using hexane and 2-propanol as an eluent. However, 4-methylbenzoate of cellulose in SFC showed a high optical resolving ability, comparable to that in HPLC. The optical resolving ability of cellulose tris(3,5-dimethylphenylcarbamate) depended on the kind and composition of the modifiers. The SFC using the cellulose benzoate may be useful not only for the analytical optical resolution of racemates, but also for preparative separation to obtain optically pure isomers.

This research was supported in part by a Grant-in-Aid for Scientific Research No. 02555184 from the Ministry of Education, Science and Culture.

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