CHROM. 19 202

CHROMATOGRAPHIC CHIRAL RESOLUTION

XIV*. CELLULOSE TRIBENZOATE DERIVATIVES AS CHIRAL STATIONARY PHASES FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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(Received November 3rd, 1986)

SUMMARY

   Cellulose tribenzoate and its ten derivatives substituted on phenyl groups were adsorbed on silica gel and their chiral recognition abilities as stationary phases for high-performance liquid chromatography were investigated, using a hexane–2-propanol mixture as eluent. Alkyl, halogen, trifluoromethyl, and methoxy groups were selected as substituents. Inductive effect of the substituents seemed to affect greatly the optical resolution ability. 2-Substituted derivatives showed a low degree of resolution. Among 4-substituted tribenzoates, the derivatives having electron-donating substituents showed better chiral recognition ability than those having electron-withdrawing substituents. However, the most electron-donating methoxy group was not a suitable substituent because of the high polarity of the substituent itself. 3-Methyl-, 4-methyl-, and 3,4-dimethylbenzoates showed better chiral recognition for several enantiomers than the other benzoates. Most stationary phases possessed high durability and many racemic compounds were resolved on these methyl-substituted benzoates.

INTRODUCTION

   Recently, we prepared chiral stationary phases for high-performance liquid chromatography (HPLC) with cellulose triphenylcarbamate derivatives and macroporous silica gel[1–3]. The optical resolution abilities of the derivatives having various substituents on the phenyl groups were greatly influenced by the inductive effect of the substituents. The introduction of both electron-donating and electron-withdrawing substituents enhanced the chiral recognition power of the stationary phases. Thus, 3,5-dimethyl and 3,5-dichloro derivatives were found to be useful chiral stationary phases for the resolution of many racemic compounds.
   On the other hand, many chiral stationary phases have also been prepared with cellulose triesters including aliphatic and aromatic esters[4–6]. However, no sys-

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   * For Part XIII, see ref. 3.

tematic study has been done from a viewpoint of inductive effects of substituents on the phenyl groups of cellulose tribenzoate (CTB).

In the present study, we synthesized twelve cellulose tribenzoate derivatives (1–12), and examined their chiral recognition abilities as stationary phases for HPLC.
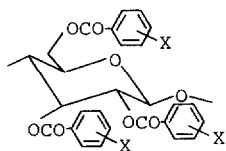
X =

1	$3,5-(CH_3O)_2$	7	$4-CF_3$
2	$4-CH_3O$	8	$3,5-Cl_2$
3	$4-(CH_3)_3C$	9	$3-CH_3$
4	$4-CH_3$	10	$2-CH_3$
5	H	11	$3,4-(CH_3)_2$
6	$4-F$	12	$3,5-(CH_3)_2$

EXPERIMENTAL

CTB derivatives were synthesized by the reaction of corresponding benzoyl chloride with cellulose (Avicel, Merck) dissolved in N,N-dimethylacetamide–lithium chloride–pyridine (15:1.5:7) at about 100°C, and isolated as the fraction insoluble in methanol; yields were 75–100%. Elemental analysis (Table I) and IR spectra showed that hydroxy groups of cellulose were nearly completely converted to ester groups.

Macroporous silica gel (Merck, LiChrospher SI 4000) was treated with a large excess of 3-aminopropyltriethoxysilane in benzene. CTB derivatives (0.75 g) except for 7 and 12 were dissolved in chloroform (15 ml). CTB derivative 7 was dissolved in tetrahydrofuran, and 12 was insoluble in common organic solvents. The above

TABLE I

ELEMENTAL ANALYSES OF CELLULOSE TRIBENZOATE DERIVATIVES

Calculated values are shown in parentheses.

Com-pound	C (%)	H (%)	Com-pound	C (%)	H (%)	Com-pound	C (%)	H (%)
1	60.53 (59.10	5.21 5.11)	5	67.56 (68.35	4.62 4.67)	9	69.32 (69.75	5.39 5.46)
2	63.12 (63.82	4.92 5.00)	6	61.16 (61.37	3.59 3.62)	10	68.90 (69.75	5.38 5.46)
3	72.35 (72.87	7.16 7.21)	7	52.85 (52.27	2.65 2.87)	11	69.40 (70.95	6.10 6.14)
4	69.53 (69.75	5.43 5.46)	8	47.49 (47.61	2.27 2.37)	12	70.07 (70.95	6.02 6.14)

solution (*ca.* 5 ml) was added to the silanized macroporous silica gel (3 g) and the wet silica gel was dried under vacuum. This coating process was repeated. Then the solution (*ca.* 5 ml) was again added to the silica gel, and the wet silica gel was added to hexane. The packing materials thus obtained were packed in a stainless-steel tube [25 × 0.46 I.D. cm] at 300 kg/cm² by a slurry method. The plate numbers of these columns were 2000–5000 for benzene using hexane–2-propanol (90:10, 0.5 ml/min) as eluent at 25°C. The dead time (t_0) of the columns was estimated to be 6.0 min with 1,3,5-tri-*tert.*-butylbenzene as a non-retained compound[7].

Chromatographic resolution was accomplished on a JASCO Trirotar-II chromatograph equipped with a JASCO UVIDEC-100-III UV and a JASCO DIP-181C polarimetric detector. Separation was carried out with a hexane–2-propanol (90:10) mixture at a flow rate of 0.5 ml/min at 25°C unless otherwise stated. The ¹³C NMR spectrum was measured with a JEOL JNM-FX100 (25 MHz) spectrometer using TMS as an internal standard. The infrared spectrum was taken on a JASCO IR-810 spectrophotometer in nujol.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatogram of resolution of Tröger base (13) on a column of benzoate 4. The enantiomers eluted at t_1 and t_2, and were completely separated. The capacity factors (k'_1 and k'_2) which are found from $(t_1 - t_0)/t_0$ and $(t_2 - t_0)/t_0$ were 0.98 and 3.73, respectively. The separation factor ($\alpha = k'_2/k'_1$) and the resolution ($R_s = 2(t_2 - t_1)/(W_1 + W_2)$) were determined to be 3.80 and 4.46, respectively.

Table II shows the results of optical resolution of 13, trans-2,3-diphenyloxirane (14), benzoin (15), 1-(2-naphthyl)ethanol (16), 2-phenylcyclohexanone (17), and methyl phenyl sulphoxide (18) on CTB derivatives 1–8. Retention times of acetone (t_a) on these stationary phases are also listed. The electron-withdrawing power of the substituents increases in the order of 1 to 8. As benzoate 3 was slightly soluble in hexane–2-propanol (90:10), hexane–2-propanol (98:2) was used as eluent for this column.

The resolution depended greatly on the substituents. Although a simple correlation was not observed between the α values and the nature of the substituents on the phenyl ring, compounds 13 and 16 seemed to be better resolved on the benzoates
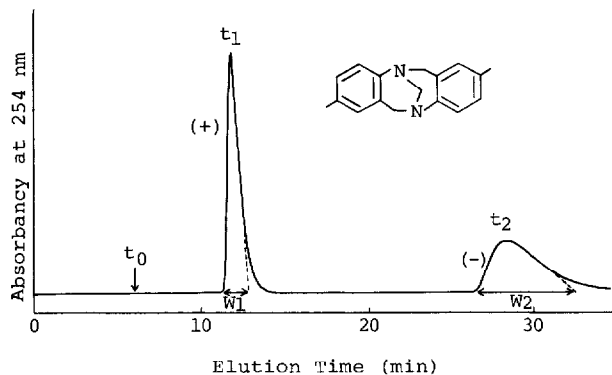

Fig. 1. Chromatographic resolution of Tröger base (13) on a cellulose tris(4-methylbenzoate) (4) column.

TABLE II

RESOLUTION OF ENANTIOMER AND ELUTION TIMES OF ACETONE (t_a) ON CELLULOSE TRIBENZOATE DERIVATIVES (1–8)

Eluent, hexane–2-propanol (90:10).

Column	13			14			15			16			17			18			t_a
	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	
1 3,5-(CH₃O)₂	0.76(+)	1.86	1.02	0.76(+)	1.21	0.72	3.90(−)	~1		1.14(−)	1.12	0.58	2.01(−)	1.34	0.91	2.07(−)	~1		9.53
2 4-CH₃O	1.15(+)	2.03	1.13	0.98(+)	1.25	0.81	4.56(−)	1.14	0.81	1.90(−)	1.11	0.56	1.85(−)	1.11		2.83(−)	~1		9.28
3 4-(CH₃)₃C*	0.55(+)	1.55	2.00	0.50(+)	~1					1.87(−)	1.18	1.11	0.92(−)	1.31	1.31	1.37(+)	~1		8.20
4 4-CH₃	0.98(+)	3.80	4.46	1.07(+)	1.17	0.81	3.30(−)	1.23	1.67	2.07(−)	1.23	1.45	1.22(−)	~1		1.92(−)	~1		8.90
5 H	1.53	1.00		0.87(+)	1.31	1.45	3.78(+)	1.08		1.60(−)	1.15	0.85	2.02(−)	1.32	0.85	2.90(−)	1.47	1.72	9.86
6 4-F	0.95(+)	~1		0.65(+)	1.21	0.80	2.73(−)	1.16	0.84	1.15(−)	1.09		1.48(−)	1.08		2.66(−)	~1		10.22
7 4-CF₃	0.29(+)	1.19		0.36(+)	1.53	1.33	1.45(−)	1.20	1.08	0.56(−)	~1		1.32(−)	1.15	0.77	2.36(−)	~1		11.50
8 3,5-Cl₂	0.60(+)	~1		0.47(+)	1.32	0.90	1.92(−)	1.08		0.58	1.00		3.03(−)	1.33		4.50(+)	1.11		12.20

* Eluent: hexane–2-propanol (98:2).

13   14   15   16   17

18   19   20   21
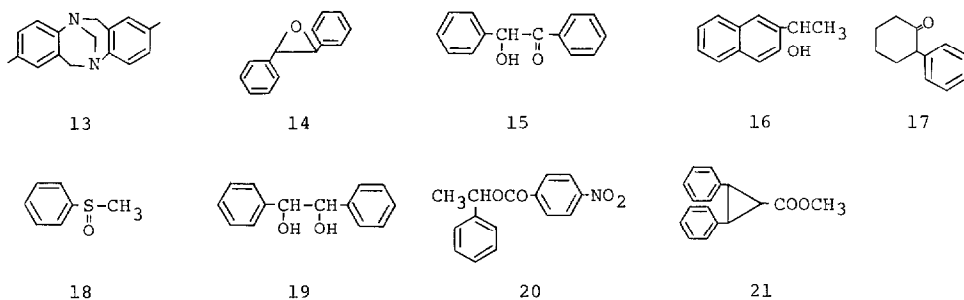
with electron-donating substituents, and the 4-methyl group appeared best. Unsubstituted benzoate (5) showed good separation for 14, 17, and particularly 18.

The retention times (t_a) of acetone on these columns roughly increased with an increase of the electron-withdrawing power of subsituents. Table III shows characteristics of IR and ^{13}C NMR spectra of CTB derivatives 4, 5, 6 and 8. In the IR spectra, the absorbance of carbonyl groups shifts to higher wave number as the electron-withdrawing power of the substituents increases. In the ^{13}C NMR spectra, three carbonyl resonances shift to higher magnetic field with an increase of electron-withdrawing power. These results suggest that the electron density and/or the dipole

TABLE III

IR AND ^{13}C NMR DATA OF CELLULOSE TRIBENZOATE DERIVATIVES 4, 5, 6 AND 8

	Derivative			
	4	5	6	8
IR (cm^{-1})*	1730	1732	1734	1740
^{13}C NMR (ppm)**	165.10	165.07	164.07	162.71
	164.87	164.87	163.81	162.66
	164.54	164.48	163.59	162.39

* Absorbance of carbonyl group.
** Chemical shift of carbonyl carbon.

TABLE IV

RETENTION TIME FOR BENZENE AND MONOSUBSTITUTED BENZENE ON A CELLULOSE TRIBENZOATE COLUMN (5)

Eluent, hexane–2-propanol (90:10).

Solute	Retention time (min)
C_6H_5–OCH_3	9.71
C_6H_5–$C(CH_3)_3$	7.37
C_6H_5–CH_3	7.94
C_6H_6	8.10
C_6H_5–CF_3	8.00
C_6H_5–Cl	8.08

TABLE V

RESOLUTION OF ENANTIOMERS (13–17, 19) ON METHYL-SUBSTITUTED CTB DERIVATIVES (4, 9–11)

Column	13			14			15			16			17			19		
	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
4 4-CH$_3$	0.98(+)	3.80	4.46	1.07(+)	1.17	0.81	3.30(−)	1.23	1.67	2.07(−)	1.23	1.45	1.22(−)	~1		2.65(+)	1.06	
9 3-CH$_3$	1.12(+)	2.06	3.21	0.98(+)	1.80	3.81	3.72(−)	1.09	0.70	2.17(−)	1.25	2.00	2.76(−)	1.28	1.88	2.63(+)	1.26	1.69
10 2-CH$_3$	0.85(+)	~1		0.70(+)	1.12		2.58(−)	1.30	1.63	1.01(−)	1.07		1.81(−)	~1		1.44(+)	~1	
11 3,4-(CH$_3$)$_2$	1.04(+)	2.14	2.89	0.97(+)	1.23	1.45	3.46(−)	1.08	0.72	1.85(−)	1.18	1.22	1.81(−)	1.19	0.67	2.30(+)	~1	

moment of carbonyl groups are influenced by the substituents. However, the reason why acetone was adsorbed more strongly on the benzoates with increasing electron-withdrawing power of the substituents is not clear at present.

The retention times of benzene and substituted benzenes were measured on 5 (Table IV). Almost the same retention times were observed for benzene, toluene, trifluoromethylbenzene, and chlorobenzene. This indicates that methyl, trifluoro-methyl, and chloro groups have no particular interaction with solutes when they are on the CTB derivatives. However, anisole showed a larger retention time than the others, suggesting that the methoxy group interacts with the benzoate 5. Therefore, if a stationary phase has methoxy groups like benzoates 1 and 2, the adsorption of solutes on these groups may occur. Because the substituents on benzoates 1 and 2 exist far from a chiral glucose unit, the interaction on these groups should lower the chiral recognition ability of the stationary phases. A more remarkable effect of a methoxy group has been observed on the chiral stationary phases prepared with cellulose triphenylcarbamate derivatives[1] probably because the urethane NHCOO bond is longer than the ester bond. On the other hand, tert.-butylbenzene eluted a little earlier than the other substituted-benzene. The bulky tert.-butyl group seems to prevent a solute from being adsorbed on stationary phases[7]. From these results, the main chiral adsorbing site is considered to be the ester group. The group can interact with a solute through the dipole–dipole interaction and/or hydrogen bonding. Its adsorbing power may be strongly influenced by the nature of substituents on the phenyl group.

Since the methyl-substituted CTB derivative (4) showed the highest chiral recognition ability in the resolution of 13, 15, and 16, the effect of the position and number of methyl substituent on the phenyl group were investigated. Table V summarizes the resolution of racemates 13–17 and 19 on four methyl-substituted CTB's. The chiral recognition of the stationary phases depended on the postion and the number of methyl groups. The 2-substituted derivative (10) showed the lowest chiral recognition for all the enantiomers except for compound 15. Analogous results have also been obtained on the cellulose triphenylcarbamate derivatives[1]. Other methyl substituted derivatives, particularly the 3-substituted derivative, seemed to show an high ability of chiral recognition. Unfortunately, the 3,5-dimethyl derivative (12) could not be used as a stationary phase because it was insoluble in common organic solvents, and could not be adsorbed on silica gel.

Most racemic compounds examined here and in the previous work[1] were better resolved on cellulose triphenylcarbamate derivatives. However, some compounds like 16, 19, 20 (column 5, $\alpha = 1.37$), and 21 (column 5, $\alpha = 1.33$) were better resolved on the CTB derivatives. The influence of the substituents of the CTB derivatives on their chiral recognition did not appear as clearly as in the case of cellulose triphen-ylcarbamate derivatives[1]. This may be due to the fact that the chiral recognition ability of CTB is affected greatly by other factors than chemical structure[*,6] such as morphology of CTB on silica. The influence of the substituents of the CTB derivatives

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* When we prepared the CTB stationary phase by evaporating the solvent (see Experimental section) instead of adding in hexane, the stationary phases exhibited chiral recognition different from that reported here. Rimböck et al.[8] also reported that microcrystalline CTB showed chiral recognition different from the CTB coated on silica gel.

on such factors may also depende greatly on the substituents. This is currently under investigation.

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