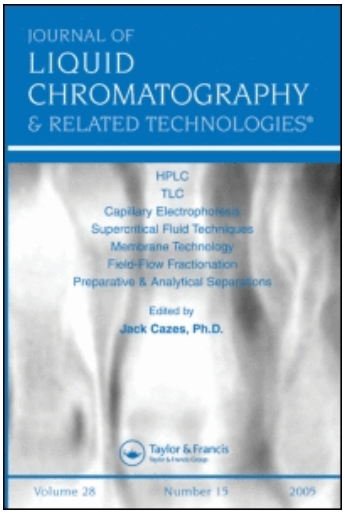

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Comparison of the Enantioselectivity of β-Cyclodextrin vs. Heptakis-2,3-O-dimethyl-β-cyclodextrin LC Stationary Phases

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COMPARISON OF THE ENANTIOSELECTIVITY OF β-CYCLODEXTRIN VS. HEPTAKIS-2,3-O-DIMETHYL-β-CYCLODEXTRIN LC STATIONARY PHASES

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ABSTRACT

The enantioselectivity of native β-cyclodextrin and its 2,3-methylated analogue were examined in both the "polar-organic" mode and the reversed phase mode. By comparing these two chiral selectors, the function of the secondary 2,3-hydroxyl groups at the mouth of the cyclodextrin can be examined (in regard to selectivity and retention). In the "polar-organic" mode, compounds that are easily resolved on the native β-cyclodextrin chiral stationary phase (CSP) cannot be resolved on the methylated CSP. These results, as well as the retention data, lend support to the previously proposed "noninclusion" mechanism. In the reversed phase mode, many more compounds could be resolved on the native β-cyclodextrin CSP than on the methylated analogue. However, a few compounds were resolved only on the 2,3-methylated cyclodextrin CSP. In these cases, steric interactions at the mouth of the cyclodextrin cavity may be more important to chiral recognition than hydrogen bonding.

INTRODUCTION

The β-cyclodextrin bonded stationary phase was the first broadly useful, reversed-phase chiral stationary phase (CSP).[1,2] When using aqueous or hydro-organic solvents the enantioselective retention mechanism was shown to involve inclusion complexation along with hydrogen bonding and/or steric interactions at the outer edge of the cyclodextrin cavity.[2-7] Conversely traditional normal phase enantioseparations with native β-cyclodextrin columns are quite rare.

Two very different approaches were utilized in order to extend the utility and selectivity of cyclodextrin-based CSPs. One approach was to use the "polar organic mode" in which the mobile phase consisted mainly of an aprotic polar organic solvent such as acetonitrile.[8-10] Smaller amounts of other nonaqueous additives (e.g., methanol, triethylamine, glacial acetic acid, etc.) were used to regulate retention and analyte charge. Inclusion complex formation was not thought to occur in this mode since the dominant acetonitrile component of the mobile phase occupied the cyclodextrin cavity.[8-10] Retention was thought to be due to hydrogen bonding and dipolar interactions between the analyte and the secondary hydroxyl groups at the mouth of the cyclodextrin cavity.[8-10] Steric interactions could also play a role in chiral recognition in this mode (see Figure 1). Since inclusion complexation does not occur, this mechanism is very different from that which predominates in the reversed-phase mode. This explains why:

1. The β-cyclodextrin enantioselectivity is different in the two modes,

2. non-hydrogen bonding solvents such as acetonitrile are necessary to accentuate retention and selectivity,

3. hydrogen bonding solvents such as methanol and water decrease retention and selectivity, and

4. analytes that are resolved in this mode usually have a minimum of two hydrogen bonding groups (one of them near to the stereogenic center) and an aromatic ring.[8-10]

A completely different way to alter the enantioselectivity of β-cyclodextrin involves derivatizing it with a variety of different groups.[11-14] Early on, both acetyl and hydroxypropyl derivatized cyclodextrin CSPs were shown to produce unique selectivities compared to native β-cyclodextrin.[11,12] These were followed by a variety of aromatic cyclodextrin derivatives and most recently with charged derivatives (e.g., sulfate, etc.).[13-15] The functionalized cyclodextrins greatly expanded the utility and expanded the enantioselectivity of this class of CSPs.

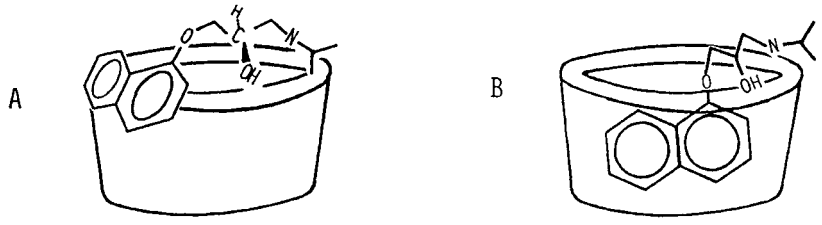


Figure 1. Simplified schematics illustrating two different enantioselective retention mechanisms for the native β-cyclodextrin/propanolol system. Case "A" is the polar-organic mode where acetonitrile occupies the hydrophobic cavity and the analyte is retained via a combination of hydrogen bonding and dipolar interactions at the mouth of the cyclodextrin. Steric interactions also can contribute to chiral recognition.[8,9] In case "B", (the reversed phase mode) retention is mainly due to hydrophobic inclusion complexation, while enantioselectivity also requires hydrogen bonding and steric interactions at the mouth of the cyclodextrin cavity.[1-4]

Subsequently, β-cyclodextrin and its derivatives were utilized as chiral run buffer additives in capillary electrophoresis.[16] One derivative that has been used in CE but not tested extensively in LC are simple methylated cyclodextrins. In this work we synthesized a heptakis-2,3-di-O-methyl-β-cyclodextrin bonded CSP and compared it to the native β-cyclodextrin CSP in both the reversed phase and "polar-organic" LC modes. This exercise was useful for at least three reasons: 1. It provided a stringent test for the proposed enantioselective retention mechanism in the "polar-organic mode", 2. it could help to elucidate the role of hydrogen bonding and steric interactions in the reversed phase mode, and 3. it indicated whether or not the selectivity of this particular derivatized cyclodextrin and its overall usefulness exceeds that of other cyclodextrins or cyclodextrin derivatives that are used as chiral selectors in LC.

EXPERIMENTAL

Materials

The analytes used in this study are given in the tables. Coumafuryl was obtained from Chem Service (West Chester, PA). Idazoxan and derivatives are drugs under investigation at Reckitt & Colman. They were supplied by N. A. Hyde (Danson Lane, Kingston-upon-Hull, UK). The crown ether analogues used were prepared for a previous study.[17] All other compounds were purchased from Sigma

Chemical Co. (St. Louis, MO) and Aldrich Chemical Co. (Milwaukee, WI). HPLC grade water acetonitrile, methanol, triethylamine, and glacial acetic acid were obtained from Fisher Scientific (St. Louis, MO).

The first step in the synthesis of heptakis-2,3-di-O-methyl-β-cyclodextrin was the protection of the primary hydroxy groups of β-CD with *t*-butyl dimethylsilylchloride in pyridine according to a procedure described by Fugedi.[18] The intermediate 6-O-*t*-butyldimethylsilyl β-cyclodextrin was made as described by Dietrich, et al.[19] The removal of the protecting group was achieved by refluxing 6-O-*t*-butyldimethylsilyl-2,3-di-O-methyl-β-cyclodextrin (25g) with ammonium fluoride (16 g) in methanol (250 mL) for 24 hours. The reaction was concentrated and ethyl acetate (150 mL) was added. The mixture was filtered through a pad of silica gel and the solvents were removed by distillation under vacuum. The resulting 2,3-di-O-methyl β-cyclodextrin was used without further purification. Binding of 2,3-di-O-methyled-β-cyclodextrin to epoxysilica was done as described previously.[1]

Methods

The Cyclobond I 2000 columns (25 cm x 4.6 mm i.d.) were obtained from Advanced Separation Technologies, Inc. (Whippany, NJ). All separations were done at room temperature using a Shimadzu model LC-6A solvent delivery module and SPD-6A UV detector.

Rheodyne's model 7125 sample injection valve (Cotati, CA) with a 20-μL loop was used. The flow rate was 1 mL/min. Supporting evidence for chiral separation was supplied by repeating the separation with detection accomplished at different UV wavelengths (e.g., 254 nm, 275 nm, etc.).

The mobile phases of reversed phase mode were mixtures of buffer and methanol or acetonitrile by volume ratios. The aqueous portion of the mobile phase (i.e., the buffer) was made by dissolving the desired amount of pure triethylamine in water and then adding glacial acetic acid to achieve the desired pH. The high water content of the mobile phase required the use of a silica pre-saturator column to saturate the mobile phase with silica in order to extend the life of the silica-based columns. The presaturated column consisted of a 10 cm x 0.46 stainless steel tube packed with 30-40 μm silica and was placed in-line before the injector.

The polar organic mode was also studied. The mobile phase conditions are given as the desired volume ratios acetonitrile/methanol/glacial acetic acid/triethylamine.

RESULTS AND DISCUSSION

Polar-Organic Mode

Native cyclodextrin bonded phase LC columns have been shown to resolve a large number of compounds in the "polar organic mode" even though inclusion complex formation is not believed to occur.[8-10] Table 1 lists typical compounds that have been resolved on β-cyclodextrin CSPs in the "polar organic mode." These include β-blockers, dansyl amino acids and a variety of other biologically active compounds that contain at least two hydrogen bonding groups and an aromatic moiety. However, none of these compounds could be resolved in the "polar organic mode" when using the 2,3-methylated-β-cyclodextrin CSP. They eluted as single peaks and their retention times were generally less than those for the equivalent separation done on native β-cyclodextrin CSPs. Thus far, no racemic compounds have been found that resolve on this methylated-β-cyclodextrin CSP in the "polar-organic-mode." This indicates that methylating the secondary hydroxyl groups at the mouth of the cyclodextrin cavity effectively eliminates chiral recognition and reduces retention for these compounds in the "polar organic mode." Further, this lends support to the proposed enantioselective retention mechanism in which chiral recognition and resolution is due to a combination of hydrogen bonding, dipolar and steric interactions at the mouth of the cyclodextrin cavity (see Figure 1A).

Reversed Phase Mode

Out of hundreds of compounds tested in the reversed phase mode, only a few were found that resolved exclusively on the 2,3-methylated-β-cyclodextrin CSP. These are listed in Table 2. About twice as many compounds could be resolved on both columns (Table 3). However, the greatest number and variety of compounds were found to resolve only on the native β-cyclodextrin CSP (Table 4). A number of interesting facts can be gleaned from this data. However, it would be useful to first briefly review and compare the factors that affect the strength of a cyclodextrin inclusion complex and chiral recognition.[27] (a) First and foremost (when in aqueous solution), organic solutes tend to form a hydrophobic association with the less polar interior of the cyclodextrin cavity (i.e., van der Waals-London dispersion forces). This can occur with both the native and 2,3-methylated β-cyclodextrin. However, the methyl groups at the mouth of the derivatized cyclodextrin would tend to enlarge the cavity somewhat and make the entire cyclodextrin more hydrophobic.[28,29] (b) Hydrogen binding between the guest molecule and the cyclodextrin (particularly the secondary hydroxyls at the mouth of the cavity) tends to enhance the strength of an inclusion complex. Clearly, this important interaction

Table 1

List of Racemic Compounds That Can Easily Be Resolved in the "Polar Organic" Mode on a Native β-Cyclodextrin CSP But Cannot Be Resolved on Its 2,3-O-Methylated Analogue

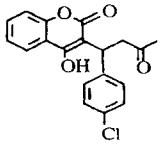
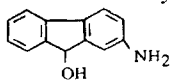
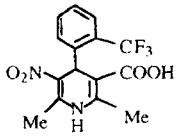
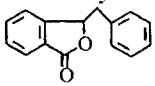
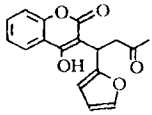
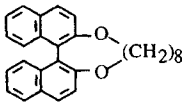
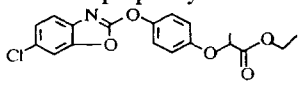
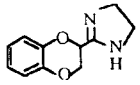
Compound	Reference
1. Alprenolol	8,9
2. Bendroflumethiazide	9
3. Homatropine	9
4. Metoprolol	8,9
5. Nadolol	8,9
6. Pindolol	8,9
7. Proglumide	9
8. Propranolol	8,9
9. Reuelene	9
10. Timolol	8,9
11. Trihexylphenidyl	9
12. Dansyl-D,L-phenylalanine	This work[a], 20
13. Dansyl-D,L-tryptophan	This work[a], 20
14. Dansyl-D,L-norleucine	20
15. Dansyl-D,L-norvaline	20

[a] The mobile phase condition for β-cyclodextrin was acetonitrile:methanol: acetic acid:triethylamine = 98:2:0.8:0.2 (v/v/v/v).

has been eliminated (in the case of the secondary hydroxyl groups) for the 2,3-methylated cyclodextrins. However, there is some possibility of hydrogen bonding with the remaining, less accessible primary hydroxyl groups at the bottom of the cyclodextrin cavity. (c) The release of "high energy water molecules" from the cyclodextrin cavity during complex formation also is beneficial.[27] This can occur for both the native and methylated cyclodextrins, however the number and energy of the water molecules may be different in the two cases. (d) Decreasing the strain energy of the cyclodextrin macrocyclic ring system upon complex formation can be a factor. However, it has been noted that these effects seem to be more important for the smaller α-cyclodextrin. (e) Steric effects (repulsion) tends to decrease the strength of an inclusion complex. These effects can be quite varied. One is an extreme case where the guest molecule is to large to form an inclusion complex. A more common scenario involves inclusion of part of a guest while other parts project out of the cyclodextrin cavity.

Table 2

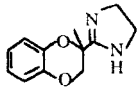
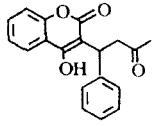
Compounds Resolved Only by 2,3-O-Methylated-β-Cyclodextrin CSP in the Reversed Phase Mode

Compound & Structure	k'[a]	α[b]	Rs[c]	Mobile Phase
1. Coumachlor 	3.06	1.37	3.38	A
2. 2-Amino-9-hydroxyfluorene 	2.40	1.29	2.36	B
3. BAY COOH 	1.06	1.18	1.0	C
4. 3-Benzyl Phthalide 	6.48	1.09	0.9	D
5. Coumafuryl 	3.08	1.20	1.25	E
6. Crown Ether Analogue #19 	1.25	1.30	2.06	F
7. Fenoxaprop-ethyl 	13.1	1.13	1.00	G
8. Idazoxan 	1.11	1.42	1.93	H

(continued)

Table 2 (continued)

Compounds Resolved Only by 2,3-O-Methylated-β-Cyclodextrin CSP in the Reversed Phase Mode

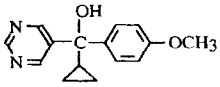
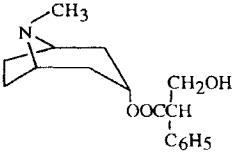
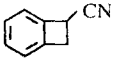
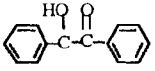
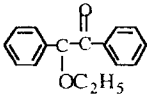
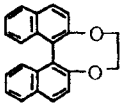
Compound & Structure	k′[a]	α[b]	Rs[c]	Mobile Phase
9. 1-Indanol 	1.34	1.18	1.25	I
10. Methylidazoxan 	1.31	1.26	1.03	D
11. 1,2,3,4-Tetrahydro-1-naphthol 	1.59	1.13	1.00	J
12. Warfarin 	2.72	1.58	2.11	A

[a] k′ = capacity factor of the first eluted enantiomer. [b] The selectivity factor, α, = k_1'/k_2'. [c] The resolution = $2(tr_2 - tr_1)/(w_1 + w_2)$. [d] Mobile phase compositions:
A=MeOH:1% triethyammonium acetate in water, pH 4.1=30:70 (v/v).
B=MeOH: 1% triethyammonium acetate in water, pH 7.1=15:85 (v/v).
C=acetonitrile: 1% triethyammonium acetate in water, pH 4.1=15:85 (v/v).
D=MeOH: 1% triethyammonium acetate in water, pH 7.1=10:90 (v/v).
E=MeOH: 1% triethyammonium acetate in water, pH 4.1=20:80 (v/v).
F=acetonitrile: 1% triethyammonium acetate in water, pH 4.1=40:60 (v/v).
G=MeOH: 1% triethyammonium acetate in water, pH 7.1=20:80 (v/v).
H=1% triethyammonium acetate in water, pH 4.1=100%.
I=MeOH: 1% triethyammonium acetate in water, pH 7.1=3:97 (v/v).
J=MeOH: 1% triethyammonium acetate in water, pH 4.1=10:90 (v/v).

Depending on the size, nature, and location of the projecting group, it can associate with the mouth of the cyclodextrin (hydrogen bonding for example) be repelled from the cyclodextrin (steric interaction) or have no effect. The large number and greater size of the methyl groups on the derivatized cyclodextrin would

Table 3

Compounds Resolved on Both the Native and the 2,3-O-Methylated β-Cyclodextrin Columns in the Reversed Phase Mode

Compound & Structure	Column	k'[a]	α[b]	Rs[c]	Mobile Phase
1. Ancymidol 	Methylated β-CD	7.92	1.08	0.90	A
	β-CD	3.21	1.09	0.80	B
2. Atropine 	Methylated β-CD	1.16	1.12	0.40	A
	β-CD	6.83	1.04	0.6	C
3. 1-Benzocyclobutene Carbonitrile 	Methylated β-CD	2.03	1.25	1.67	D
	β-CD	1.71	1.01	0.55	D
4. Benzoin 	Methylated β-CD	1.71	1.08	0.9	B
	β-CD	3.17	1.08	1.0	E
5. Benzoin Ethyl Ether 	Methylated β-CD	3.65	1.04	0.6	D
	β-CD	6.63	1.30	2.60	B
6. 2,2'-Binaphthyldiyl-8-crown-2 	Methylated β-CD	5.16	1.16	1.60	F
	β-CD	4.18	1.24	1.60	F

(continued)

Table 3 (continued)

Compounds Resolved on Both the Native and the 2,3-O-Methylated β-Cyclodextrin Columns in the Reversed Phase Mode

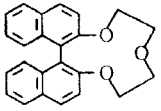
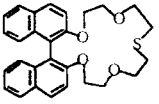
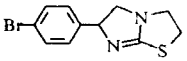
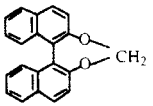
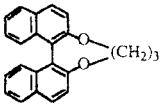
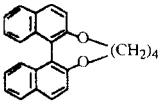
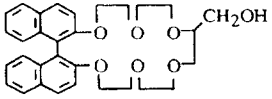
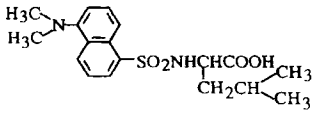
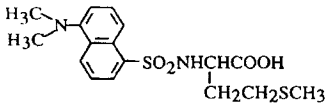
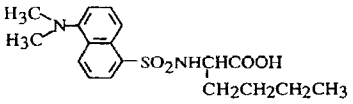
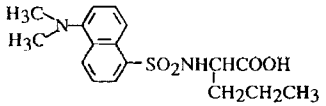
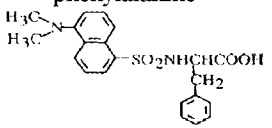
Compound & Structure	Column	k'[a]	α[b]	Rs[c]	Mobile Phase
7. 2,2'-Binaphthyldiyl-11-crown-3	Methylated β-CD	3.64	1.14	1.39	F
	Methylated β-CD	3.05	1.07	1.25	G
	β-CD	3.34-1.29	1.90		F
					
8. 2,2'-Binaphthyldiyl-17-thiacrown-5	Methylated β-CD	3.99	1.14	1.30	F
	β-CD	3.00	1.09	0.80	F
					
9. p-Bromotetramisole Oxalate	Methylated β-CD	5.44	1.07	0.75	H
	β-CD	5.22	1.17	1.25	B
					
10. Crown Ether Analogue #15	Methylated β-CD	7.01	1.14	1.27	F
	Methylated β-CD	5.24	1.12	1.22	G
	β-CD	4.56	1.26	1.61	F
					
11. Crown Ether Analogue #17	Methylated β-CD	5.17	1.22	2.47	F
	Methylated β-CD	3.96	1.18	2.24	G
	β-CD	4.42	1.21	1.41	F
					
12. Crown Ether Analogue #18	Methylated β-CD	5.32	1.22	1.86	F
	Methylated β-CD	5.03	1.20	1.73	G
	β-CD	5.85	1.10	0.85	F
					

Table 3 (continued)

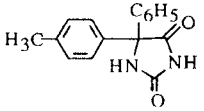
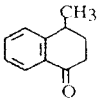
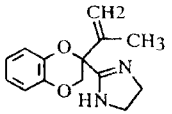
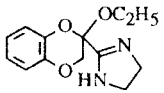
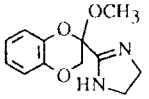
Compounds Resolved on Both the Native and the 2,3-O-Methylated β-Cyclodextrin Columns in the Reversed Phase Mode

Compound & Structure	Column	k'[a]	α[b]	Rs[c]	Mobile Phase
13. Crown Ether	Methylated β-CD	0.87	1.26	1.22	F
Analogue #24		1.93	1.22	1.36	
(2 enantiomers)	β-CD	1.81	1.10	0.80	F
					
14. Dansyl-D,L-Leucine	Methylated β-CD	2.52[D]	1.19	1.44	B
	β-CD	3.0[L]	1.4	2.4	I
					
15. Dansyl-D,L-Methionine	Methylated β-CD	2.29	1.06	0.6	B
	β-CD	3.18[L]	1.15	0.7	I
					
16. Dansyl-D,L-norleucine	Methylated β-CD	2.58	1.19	1.66	B
	β-CD	1.90[L]	1.26	2.30	I
					
17. Dansyl-D,L-norvaline	Methylated β-CD	2.08	1.06	0.6	B
	β-CD	2.80	1.13	0.83	I
					
18. Dansyl-D,L-	Methylated β-CD	1.61[D]	1.69	4.08	J
phenylalanine	β-CD	3.10[L]	1.23	1.10	K
					

(continued)

Table 3 (continued)

Compounds Resolved on Both the Native and the 2,3-O-Methylated β-Cyclodextrin Columns in the Reversed Phase Mode

Compound & Structure	Column	k′[a]	α[b]	Rs[c]	Mobile Phase
19. 5-(4-Methylphenyl)-5-phenylhydantoin 	Methylated β-CD	5.22	1.09	1.25	M
	β-CD	10.17	1.12	2.0	E
20. 1-Methyl-4-tetralone 	Methylated β-CD	4.61	1.04	0.70	D
	β-CD	10.34	1.02	0.60	D
21. RX 811005A 	Methylated β-CD	2.89	1.31	1.75	D
	β-CD	3.20	1.30	2.30	N
22. RX 811059A 	Methylated β-CD	1.49	1.54	2.83	D
	β-CD	1.62	1.40	2.50	O
23. RX 821002A 	Methylated β-CD	1.50	1.45	2.33	D
	β-CD	1.21	1.40	2.5	N

[a] k′ = capacity factor of the firsat eluted enantiomer; configuration indicated as a superscript when known. [b] The selectivity factor, α, is equal to k_1'/k_2'. [c] The resolution is equal to $2(tr_2\text{-}tr_1)/(w_1+w_2)$. [d] Mobile phase compositions:

A=1% triethylammonium acetate in water, pH 4.1 = 100% (v/v).
B=MeOH: 1% triethylammonium acetate in water, pH 7.1=20:80 (v/v).
C=acetonitrile: 1% triethylammonium acetate in water, pH 4.1=2:98 (v/v).
D=MeOH: 1% triethylammonium acetate in water, pH 7.1=10:90 (v/v).

Table 3 (continued)

Compounds Resolved on Both the Native and the 2,3-O-Methylated β-Cyclodextrin Columns in the Reversed Phase Mode

E=MeOH: 1% triethylammonium acetate in water, pH 4.1=30:70 (v/v).
F=MeOH: 1% triethylammonium acetate in water, pH 7.1=40:60 (v/v).
G=acetonitrile: 1% triethylammonium acetate in water, pH 4.1=25:75 (v/v).
H=MeOH: 1% triethylammonium acetate in water, pH 7.1=15:85 (v/v).
I=MeOH:water =50:50 (v/v).
J=MeOH: 1% triethylammonium acetate in water, pH 7.1=30:70 (v/v).
K=MeOH:water=55:45 (v/v).
L=MeOH: 1% triethylammonium acetate in water, pH 4.1=20:80 (v/v).
M=MeOH: 1% triethylammonium acetate in water, pH 4.1=15:85 (v/v).
N=MeOH: 1% triethylammonium acetate in water, pH 4.2=10:90 (v/v).
O=MeOH: 1% triethylammonium acetate in water, pH 4.1=10:90 (v/v).

tend to increase the steric repulsion at the mouth cavity for most included guest molecules. Steric repulsion can contribute to chiral recognition and enantioseparation, even though they may decrease the overall strength of the inclusion complex.

A minimum of three simultaneous interactions must occur for enantioselective recognition between the cyclodextrin host and at least one of the two enantiomeric guest molecules. The most likely interactions or combination of interactions are believed to be: hydrophobic inclusion, hydrogen bonding, and steric types. When comparing the association of a chiral compound with native β-cyclodextrin versus the 2,3-methylated cyclodextrin, the hydrophobic inclusion interaction may be somewhat analogous for the two. However, the hydrogen bonding and steric interactions at the mouth of the cavity must be very different. To a first approximation, we assume that many of the differences in enantioselectivity (in the reversed phase mode) indicated in Tables 2, 3, and 4 are the result of the nonequivalent hydrogen bonding and steric interaction between the native and derivatized cyclodextrin. Clearly, native β-cyclodextrin resolves a much greater number and variety of compounds (Table 4 and Table 1) than does its 2,3-methylated analogue (Table 2). Blocking the hydrogen bonding groups (via methylation) at the mouth of the cyclodextrin cavity interfers with chiral recognition for a large number of compounds (Table 4). However, there are a small number of compounds (Table 2) in which methylation enhances chiral recognition. Those particular compounds appear to be either neutral, nonionizible molecules or larger, bulky compounds, often containing three rings. In these cases, hydrogen bonding interactions are not as important as a tight fit in the cyclodextrin cavity and steric

Table 4

Racemic Compounds That Are Easily Resolved on the Native β-Cyclodextrin Column in the Reversed Phase Mode, But Which Could Not Be Resolved on the 2,3-O-Methylated β-Cyclodextrin CSP

Compound	Reference
1. Aminoglutethimide	4
2. 2,2′-Binaphthyldiyl-17crown-5	17,21
3. 2,2′-Binaphthyldiyl-20-crown-6	17,21
4. 2,2′-Binaphthyldiyl-23-crown-7	17,21
5. 2,2′-Binaphthyldiyl-17-thiacrown-5-sulphoxide	17,21
6. Crown Ether Analogue #9	17,21
7. Crown Ether Analogue #10	17,21
8. Crown Ether Analogue #11	17,21
9. Crown Ether Analogue #13	17,21
10. Crown Ether Analogue #14	17,21
11. Crown Ether Analogue #22	17,21
12. 1-[5-Chloro-2-(methylamino)-phenyl-1,2,3,4-tetrahydroisiquinoline	6
13. Chlorpheniramine	4
14. Chlorthalidone	4
15. Dansyl-D,L-α-amino-n-butyric Acid	22
16. Dansyl-D,L-serine	22
17. Dansyl-D,L-Threonine	22
18. Dansyl-D,L-valine	22
19. N-(3,5-Dinitrobenzoyl)-D,L-leucine	23
20. Ethylidazoxan	24
21. 5-Ethyl-5-(p-tolyl)-2-thiobarbituric Acid	6
22. Homatropine	25
23. 5-(4-Hydroxyphenyl)-5-phenylhydantoin	6
24. Ibuprofen	24
25. Ketoprofen	4
26. Methadone	4
27. 5-Methyl-phenylhydantoin	6
28. Metoprolol	4
29. Nicotine	26
30. Nisolidipene	4
31. Propranolol	4
32. Scopolamine	25
33. Tyrosine Methyl Ester	23
34. Verapramil	4

interactions at the mouth. It also is important to note that methylation of the 2- and 3-hydroxyl groups on β-cyclodextrin may not totally negate hydrogen bonding effects. It is still possible for an included compound to hydrogen bond with the primary hydroxyl groups at the narrow end of the cyclodextrin torus. This may be possible for smaller compounds such as 1-indanol (Table 2).

In some cases, chiral recognition was observed on both columns (Table 3). Compounds that contained hydrogen bonding or ionizible functional groups were usually retained longer on the native β-cyclodextrin CSP than on the 2,3-methylated analogue (when comparable mobile phases were used). However, neutral compounds containing no ionizible groups were usually retained longer on the 2,3-methylated-β-cyclodextrin column (see compounds 3, 6-8, and 10-11 in Table 3). Another interesting feature of the data in Table 3 is that the enantioselectivity of several compounds for which standards are available is reversed for the two columns. For example, the D-enantiomer of dansyl amino acids elute first on the methylated cyclodextrin column, but elute second on the native β-cyclodextrin column.

CONCLUSIONS

The 2 and 3-hydroxyl groups of β-cyclodextrin are essential for chiral recognition in the "polar-organic" LC mode. They are also very important for the enantioresolution of most compounds in the reversed phase mode. However, there are a few cases in the reversed phase mode where these hydroxyl groups are not essential. In fact, blocking them with methyl groups enhances the enantioseparation in these cases. The most likely reasons for the enhanced chiral recognition with 2,3-methylated-β-cyclodextrin are the increased steric interactions at the mouth of the cyclodextrin cavity and/or accentuated interaction with the primary 6-hydroxyl groups at the bottom of the torus (for those molecules with requisite geometry to reach them). Those compounds that can be resolved on both columns frequently show the opposite enantiomeric elution order. This indicates that there is a difference in some of the basic interactions that give rise to enantioselective retention.

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