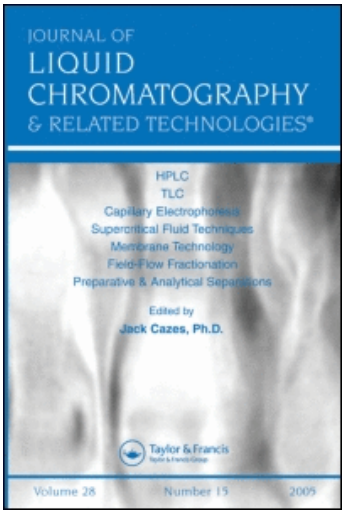

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Comparison of the Selectivity and Retention of β-Cyclodextrin vs. Heptakis-2,3-O-dimethyl-β-cyclodextrin LC Stationary Phases for Structural and Geometric Isomers

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COMPARISON OF THE SELECTIVITY AND RETENTION OF β-CYCLODEXTRIN VS. HEPTAKIS-2,3-O-DIMETHYL-β-CYCLO-DEXTRIN LC STATIONARY PHASES FOR STRUCTURAL AND GEOMETRIC ISOMERS

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ABSTRACT

Both β-cyclodextrin and 2,3-methylated-β-cyclodextrin bonded stationary phases effectively separate a variety of structural and geometrical isomeric compounds in the reversed phase mode. The retention of neutral structural isomeric compounds, as well as substituted phenols and anilines, was usually longer on the methylated cyclodextrin stationary phase. Conversely, the retention of all substituted carboxylic acids was greater on the native β-cyclodextrin stationary phase. The selectivity differences between the native β-cyclodextrin and its 2,3-methylated analogue were not as great as expected for the structural isomers. In general, the structural isomer that was retained longest on the native β-cyclodextrin stationary phase also was retained longest on the 2,3-methylated cyclodextrin stationary phase.

The *para* substituted isomer was often the longest retained, except for certain neutral compounds containing nitro substituents. In these cases, the *ortho* isomer was retained to the greatest extent. The greatest selectivity differences (i.e., retention reversals) were for geometrical isomers and the less retained structural isomers.

INTRODUCTION

β-Cyclodextrin bonded stationary phases are well known as one of the earlier and more successful chiral stationary phases for the resolution of enantiomers.[1-4] However, they also have been utilized as effective stationary phases for routine reversed phase separations.[5-10] The selectivity often is different from C_{18} and C_8 reversed phase columns. Also, it has been shown that β-cyclodextrin columns are usually more effective for the reversed phase separation of structural and geometrical isomers.[11-16] The formation of a host-guest inclusion complex is known to be the most important retention factor when using water-based mobile phase systems. However, the role (if any) of hydrogen bonding and/or steric interactions at the mouth of the cyclodextrin cavity is not well understood for achiral analytes and has only been considered in a few cases.[11,13]

In this work, a series of structural isomeric compounds and geometrical isomers are separated on both the native β-cyclodextrin bonded stationary phase and a 2,3-methylated-β-cyclodextrin bonded stationary phase. Differences in the reversed-phase selectivity and retention are examined and discussed.

EXPERIMENTAL

Methods

All separations were done at room temperature (21°C) either on a Shimadzu LC-6A or a Waters 590 liquid chromatograph. The compounds were detected with a Waters R401 Differential refractometer or, more frequently, with a variable-wavelength detector at 254 nm or 195 nm. All samples were dissolved in methanol prior to manual injection. The native β-cyclodextrin column (*i.e.*, Cyclobond I 2000) was obtained from Advanced Separation Technologies, Inc. (Whippany, NJ). The void volumes of the columns were determined by injecting neat methanol. The mobile phase consisted of mixtures (by volume) of methanol with water or 0.1 % buffer of pH 6.5 or 4.

The buffer solutions were made by dissolving pure triethylamine in water to form a 0.1% (by volume) solution. Then, glacial acetic acid was added drop-wise to achieve the desired pH. A silica-gel precolumn was used before the injector to saturate the mobile phase. The flow rate was 1 mL/min. for all separations.

Chemicals

HPLC-grade methanol, triethylamine, glacial acetic acid were obtained from Fischer Scientific (St. Louis, MO). All the compounds tested were purchased from Aldrich Chemical Co. (Milwaukee, WI), Sigma Chemical Co. (St. Louis, MO) or Fluka Chemical Co. (Ronkonkoma, NY). The heptakis, 2,3-di-O-methyl-β-cyclodextrin was synthesized as follows. First, the primary hydroxy groups of β-CD were protected with *t*-butyldimethylsilychloride (in pyridine solution), and the methylated analogue was made as indicated in the previous paper of this series.[18] 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 (16g) in methanol (250 mL) for 25 hours. The reaction was concentrated and ethyl acetate (150 mL) was added. The mixture was filtered through a pad of silica gel and the solvent was 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-methylated-β-cyclodextrin to epoxy silica was done by the same method reported previously.[1]

RESULTS AND DISCUSSION

The strength of a cyclodextrin inclusion complex is determined by several factors including: the guest molecules's hydrophobic association with the less polar interior of the cyclodextrin cavity, hydrogen bonding between a guest and the cyclodextrin hydroxyl groups, the release of "high energy water molecules" from the cyclodextrin cavity during complex formation, and conformation changes in the cyclodextrin ring system upon complex formation.[17] Steric repulsion between the guest and the cyclodextrin can effect the strength of an inclusion complex as well (in a negative fashion). Even though steric repulsion can decrease the strength of an inclusion complex, it frequently increases the selectivity of a separation.[18]

When comparing the inclusion complex formed between a compound and native β-cyclodextrin or the 2,3-methylated cyclodextrin, major differences in hydrogen bonding effects at the mouth of the cyclodextrin cavity would be expected. Although inclusion complexation occurs with both β-cyclodextrin and its methylated analogue,[18] the 2,3-methylated cyclodextrin would be more hydrophobic.

Table 1

Comparison of Chromatographic Retention Data (k′s) for a Series of Structural Isomers on a Native β-Cyclodextrin Bonded Stationary Phase and a 2,3-Methylated-β-Cyclodextrin Bonded Stationary Phase[a]

No.	Compound	Mobile Phase Composition[b] Methanol/H_2O		Methanol/pH6.5 Buffer		Methanol/pH4.0 Buffer	
		β-CD[c]	DM-β-CD[d]	β-CD[c]	DM-β-CD[d]	β-CD[c]	DM-β-CD[d]
1	Anthracene	1.59[e]	2.39[e]	1.47[f]	3.36[f]	0.90[f]	2.23[f]
	Phenanthrene	0.85[e]	1.62[e]	0.69[f]	1.94[f]	0.48[f]	1.50[f]
2	Benz[a]anthracene	2.88[g]	9.53[g]	2.88[g]	9.72[G]	3.41[G]	5.57[g]
	Benz[b]anthracene	2.15[g]	4.4[g]	1.91[g]	3.26[g]	1.14[g]	3.08[g]
	Chrysene	2.80[g]	9.53[g]	2.68[g]	8.57[g]	3.22[g]	4.81[g]
3	Benzo[a]pyrene	2.57[g]	14.58[g]	3.46[g]	13.28[g]	3.13[g]	13.46[g]
	Benzo[e]pyrene	2.36[g]	14.08[g]	3.01[g]	12.95[g]	2.65[g]	12.71[g]
4	Dibenz[a,c]-anthracene	1.62[f]	6.79[f]	4.14[g]	2.32[g]	4.40[g]	2.55[g]
	Dibenz[a,h]-anthracene	0.32[f]	1.08[f]	0.73[g]	0.79[g]	0.71[g]	0.68[g]
5	o-Nitrotoluene	1.27[h]	1.94[h]	0.88[h]	1.42[h]	0.79[h]	1.18[h]
	m-Nitrotoluene	0.99[h]	2.08[h]	0.66[h]	1.42[h]	0.59[h]	1.26[h]
	p-Nitrotoluene	2.10[h]	2.67[h]	1.43[h]	1.88[h]	1.22[h]	1.61[h]
6	o-Iodotoluene	0.36[e]	0.94[e]	1.19[g]	2.00[g]	4.70[h]	6.17[h]
	m-Iodotoluene	0.36[e]	1.09[e]	1.26[g]	2.00[g]	5.27[h]	7.34[h]
	p-Iotoluene	0.58[e]	1.50[e]	1.72[g]	2.26[g]	8.67[h]	9.63[h]
7	o-Xylene	1.97[h]	2.47[h]	1.23[h]	1.57[h]	1.16[h]	1.39[h]
	m-Xylene	1.97[h]	2.75[h]	0.96[h]	1.78[h]	1.10[h]	1.50[h]
	p-Xylene	3.09[h]	3.34[h]	2.01[h]	2.16[h]	1.81[h]	1.84[h]
8	o-Fluoronitrobenzene	1.10[h]	1.39[h]	0.77[h]	0.99[h]	0.69[h]	0.89[h]
	m-Fluoronitrobenzene	0.68[h]	1.21[h]	0.48[h]	0.85[h]	0.49[h]	0.78[h]
	p-Fluoronitrobenzene	0.54[h]	1.04[h]	0.43[h]	0.72[h]	0.40[h]	0.66[h]
9	o-Chloronitrobenzene	3.94[I]	4.34[I]	1.63[h]	1.93[h]	1.79[h]	1.91[h]
	m-Chloronitrobenzene	2.52[I]	3.08[I]	0.76[h]	1.22[h]	0.91[h]	1.28[h]
	p-Chloronitrobenzene	2.52[I]	2.83[I]	0.76[h]	1.22[h]	0.91[h]	1.28[h]
10	o-Bromonitrobenzene	2.70[h]	3.11[h]	1.67[h]	2.17[h]	1.60[h]	1.99[h]
	m-Bromonitrobenzene	1.30[h]	2.02[h]	0.81[h]	1.41[h]	0.81[h]	1.33[h]
	p-Bromonitrobenzene	1.30[h]	2.30[h]	0.81[h]	1.60[h]	0.87[h]	1.49[h]
11	o-Iodonitrobenzene	2.43[h]	3.24[h]	2.57[h]	3.17[h]	2.72[h]	3.12[h]
	m-Iodonitrobenzene	1.32[h]	2.31[h]	1.39[h]	2.23[h]	1.51[h]	2.27[h]
	p-Iodonitrobenzene	1.53[h]	2.91[h]	1.60[h]	2.23[h]	1.73[h]	2.87[h]
12	o-Dinitrobenzene	2.33[I]	2.67[I]	1.05[h]	1.24[h]	2.35[I]	2.67[I]
	m-Dinitrobenzene	0.79[I]	1.12[I]	0.27[h]	0.46[h]	0.79[I]	1.03[I]
	p-Dinitrobenzene	0.68[I]	1.01[I]	0.27[h]	0.46[h]	0.70[I]	0.94[I]
13	o-Nitroanisole	0.53[h]	0.89[h]	0.47[h]	0.83[h]	0.59[h]	0.88[h]
	m-Nitroanisole	1.29[h]	1.74[h]	1.08[h]	1.56[h]	1.35[h]	1.72[h]
	p-Nitroanisole	1.23[h]	1.88[h]	1.08[h]	1.69[h]	1.27[h]	1.86[h]
14	α-Naphthol	1.61[g]	1.64[g]	0.45[f]	1.01[f]	1.23[g]	2.19[g]
	β-Naphthol	2.38[g]	2.46[g]	0.32[f]	0.92[f]	0.93[g]	1.91[g]

Table 1 (continued)

Comparison of Chromatographic Retention Data (k′s) for a Series of Structural Isomers on a Native β-Cyclodextrin Bonded Stationary Phase and a 2,3-Methylated-β-Cyclodextrin Bonded Stationary Phase[a]

No.	Compound	Mobile Phase Composition[b]					
		Methanol/H_2O		Methanol/pH6.5 Buffer		Methanol/pH4.0 Buffer	
		β-CD[c]	DM-β-CD[d]	β-CD[c]	DM-β-CD[d]	β-CD[c]	DM-β-CD[d]
15	o,o'-Biphenol	0.51[e]	0.69[e]	0.23[f]	0.49[f]	0.40[g]	1.25[g]
	p,p'-Biphenol	0.78[e]	3.73[e]	0.85[f]	5.11[f]	2.47[g]	10.90[G]
16	o-Cresol	0.53[h]	1.16[h]	0.43[h]	1.00[h]	0.39[h]	0.86[h]
	m-Cresol	0.63[h]	1.25[h]	0.54[h]	1.10[h]	0.47[h]	0.95[h]
	p-Cresol	1.01[h]	1.55[h]	0.83[h]	1.36[h]	0.72[h]	1.16[h]
17	o-Nitrophenol	1.01[g]	0.86[g]	0.74[h]	0.50[h]	0.66[h]	0.88[h]
	m-Nitrophenol	0.61[g]	1.49[g]	0.74[g]	1.03[g]	0.59[h]	1.56[h]
	p-Nitrophenol	2.35[g]	1.82[g]	1,84[h]	1.03[h]	0.88[h]	1.95[h]
18	o-Bromophenol	0.72[h]	1.74[h]	1.00[h]	2.35[h]	1.20[h]	1.36[h]
	m-Bromophenol	1.32[h]	3.15[h]	1.62[h]	4.02[h]	2.06[h]	2.34[h]
	p-Bromophenol	1.74[h]	3.73[h]	1.99[h]	4.41[h]	2.52[h]	2.44[h]
19	o-Ethylphenol	0.69[h]	1.35[h]	0.72[h]	1.45[h]	0.82[h]	1.70[h]
	m-Ethylphenol	1.10[h]	1.99[h]	1.14[h]	2.12[h]	1.29[h]	2.42[h]
	p-Ethylphenol	1.80[h]	2.55[h]	1.93[h]	2.92[h]	2.21[h]	3.15[h]
20	o-Chlorophenol	0.60[h]	1.14[h]	0.89[h]	1.84[h]	1.08[h]	2.29[h]
	m-Chlorophenol	1.03[h]	2.32[h]	1.20[h]	2.89[h]	1.53[h]	3.51[h]
	p-Chlorophenol	1.22[h]	2.56[h]	1.37[h]	2.89[h]	1.77[h]	3.69[h]
21	o-Nitroaniline	0.85[h]	1.10[h]	0.59[h]	1.27[h]	0.55[h]	1.14[h]
	m-Nitroaniline	0.54[h]	1.59[h]	0.47[h]	1.67[h]	0.43[h]	1.49[h]
	p-Nitroaniline	1.17[h]	2.19[h]	1.48[h]	2.53[h]	1.34[h]	2.35[h]
22	o-Chloroaniline	0.74[h]	0.99[h]	0.67[h]	1.10[h]	1.23[j]	1.84[j]
	m-Chloroaniline	0.83[h]	1.48[h]	0.77[h]	1.68[h]	1.31[j]	2.62[j]
	p-Chloroaniline	1.22[h]	1.48[h]	1.12[h]	1.61[h]	1.70[j]	2.37[j]
23	o-Bromoaniline	1.06[h]	0.96[h]	0.79[h]	0.91[h]	1.00[h]	1.59[h]
	m-Bromoaniline	1.41[h]	1.46[h]	1.00[h]	1.51[h]	1.15[h]	2.92[h]
	p-Bromoaniline	2.41[h]	1.46[h]	2.41[h]	1.51[h]	1.71[h]	2.53[h]
24	o-Iodoaniline	0.46[h]	0.97[h]	0.15[f]	0.60[f]	0.36[f]	0.78[f]
	m-Iodoaniline	0.81[h]	2.99[h]	0.27[f]	1.37[f]	0.54[f]	2.09[f]
	p-Iodoaniline	1.65[h]	3.16[h]	0.58[f]	1.24[f]	1.04[f]	1.81[f]
25	o-Anisidine	1.05[g]	2.57[g]	0.94[h]	0.94[h]	0.24[j]	0.68[j]
	m-Anisidine	1.22[g]	1.51[g]	1.21[h]	1.11[h]	0.52[j]	0.85[j]
	p-Anisidine	1.56[g]	2.84[g]	1.48[h]	1.21[h]	0.00[j]	0.50[j]
26	o-Toluidine	0.51[h]	1.26[h]	0.87[I]	0.94[I]	0.49[I]	0.80[I]
	m-Toluidine	0.61[h]	1.97[h]	1.04[I]	1.14[I]	0.27[I]	0.80[I]
	p-Toluidine	1.02[h]	2.18[h]	1.92[I]	1.43[I]	0.27[I]	0.80[I]
27	o-Anisic Acid	0.67[I]	0.00[I]	0.29[h]	0.00[h]	3.97[h]	0.13[h]
	m-Anisic Acid	1.26[I]	0.10[I]	0.51[h]	0.04[h]	6.10[h]	0.79[h]
	p-Anisic Acid	1.85[I]	0.10[I]	0.68[h]	0.07[h]	5.29[h]	1.46[h]

(continued)

Table 1 (continued)

Comparison of Chromatographic Retention Data (k´s) for a Series of Structural Isomers on a Native β-Cyclodextrin Bonded Stationary Phase and a 2,3-Methylated-β-Cyclodextrin Bonded Stationary Phase[a]

No.	Compound	Mobile Phase Composition[b] Methanol/H_2O β-CD[c]	Methanol/H_2O DM-β-CD[d]	Methanol/pH6.5 Buffer β-CD[c]	Methanol/pH6.5 Buffer DM-β-CD[d]	Methanol/pH4.0 Buffer β-CD[c]	Methanol/pH4.0 Buffer DM-β-CD[d]
28	o-Toluic Acid	0.48[h]	0.00[h]	0.88[I]	0.00[I]	2.25[f]	0.38[f]
	m-Toluic Acid	0.67[h]	0.07[h]	0.44[I]	0.14[I]	2.09[f]	0.62[f]
	p-Toluic Acid	0.81[h]	0.18[h]	2.57[I]	0.23[I]	2.39[f]	0.96[f]
29	o-Bromobenzoic Acid	0.84[h]	0.00[h]	1.42[I]	0.00[I]	6.84[f]	0.15[f]
	m-Bromobenzoic Acid	1.15[h]	0.11[h]	4.50[I]	0.30[I]	5.36[f]	0.73[f]
	p-Bromobenzoic Acid	1.15[h]	0.11[h]	6.06[I]	0.55[I]	5.63[f]	1.34[f]
30	o-Chlorobenzioc Acid	0.60[h]	0.00[h]	1.95[I]	0.00[I]	6.49[f]	0.13[f]
	m-Chlorobenzoic Acid	0.95[h]	0.00[h]	4.25[I]	0.24[I]	4.91[f]	0.63[f]
	p-Chlorobenzoic Acid	0.95[h]	0.13[h]	4.75[I]	0.35[I]	4.59[f]	0.91[f]
31	o-Nitrobenzoic Acid	1.23[h]	0.00[h]	2.13[I]	0.00[I]	7.61[f]	0.05[f]
	m-Nitrobenzoic Acid	1.37[h]	0.12[h]	3.08[I]	0.13[I]	5.84[f]	0.22[f]
	p-Nitrobenzoi c Acid	1.84[h]	0.15[h]	3.88[I]	0.21[I]	6.74[f]	0.40[f]

[a] Both columns were25x0.44 [i.d.] cm. The silica gel support consisted of identical 5μ spherical particles. The linkage chain and bonding chemistry were identical (see Experimental section).

[b] Three compositions of mobile phase were used: methanol with water; methanol with 0.1% triethylammonium acetate buffer, pH 6.5 and methanol with 0.1% triethylammonium acetate buffer, pH4.0.

[c] Native β-Cyclodextrin Bonded Stationary Phase.

[d] 2, 3-methylated-β-Cyclodextrin Bonded Stationary Phase.

[e-j] Mobile phase ratios (methanol to water or 0.1% triethylammonium acetate buffer, pH6.5 and 4.0, v/v): [e]55/45; [f]50/50; [g]40/60; [h]30/70; [I]10/90; [j]20/80.

The methyl groups at the mouth of the cyclodextrin cavity can be thought of as extending the size of the cavity and/or providing sites for steric repulsion at the mouth of the cyclodextrin cavity. These differences have been shown to significantly alter the enantioselectivity of β-cyclodextrin.[18] However, the effect on the retention and selectivity of other achiral isomeric compounds has not been considered.

Table 1 gives the separation data for a series 31 different structural isomeric compounds separated on both the native β-cyclodextrin bonded stationary phase and its 2,3-methylated analogue. The compounds are listed in groups according to their class (*i.e.*, from top to bottom: polycyclic aromatic hydrocarbons, neutral disubstituted benzenoid compounds, substituted phenolic compounds, substituted

Table 2

Comparison of Chromatographic Retention Data (k's) for a Series of Geometric Isomers on a Native β-Cyclodextrin Bonded Stationary Phase and a 2,3-Dimethylated β-Cyclodextrin Bonded Stationary Phase[a]

No.	Compound	Mobile Phase Composition[b] Methanol/H_2O		Methanol/pH6.5 Buffer		Methanol/pH4.0 Buffer	
		β-CD[c]	DM-β-CD[d]	β-CD[c]	DM-β-CD[d]	β-CD[c]	DM-β-CD[d]
1	Cis-stilbene	1.63[e]	1.63[e]	0.90[e]	1.07[e]	0.26[f]	0.44[f]
	Trans-stilbene	0.84[e]	3.93[e]	0.58[e]	2.59[e]	0.15[f]	1.02[f]
2	Cis-3-hexene-1-ol	0.95[g]	0.65[g]	0.85[h]	0.69[h]	1.03[h]	0.72[h]
	Trans-3-hexene-1-ol	0.65[g]	0.57[g]	0.56[h]	0.46[h]	0.65[h]	0.63[h]
3	Cis-1,2-bis(phenyl sulfonyl) ethylene	2.47[I]	1.62[I]	2.15[I]	1.57[I]	1.39[I]	1.18[I]
4	trans-cis-decahydro-naphthalene	1.82[f]	1.16[f]				
5	trans-cis-1,4-dimethyl-cyclohexane	0.80[f]	0.52[f]				
	trans-	0.51[f]	0.36[f]				

[a] Both columns were25x0.44 [i.d.] cm. The silica gel support consisted of identical 5μ spherical particles. The linkage chain and bonding chemistry were identical (see Experimental section).

[b] Three compositions of mobile phase were used: methanol with water; methanol with 0.1% triethylammonium acetate buffer, pH 6.5 and methanol with 0.1% triethylammonium acetate buffer, pH4.0.

[c] Native β-Cyclodextrin Bonded Stationary Phase.

[d] 2, 3-methylated-β-Cyclodextrin Bonded Stationary Phase.

[e-i] Mobile phase ratios (methanol to water or 0.1% triethylammonium acetate buffer, pH6.5 and 4.0, v/v): [e]55/45; [f]70/30; [g]15/85; [h]10/90; [I]30/70.

anilines and substituted benzoic acids). Table 2 gives comparable separation data for several geometric isomers. Since all separations were done in the reversed phase mode (using hydro-organic solvents) it is assumed that inclusion complexation takes place.[1-4,18]

Retention

A number of interesting trends are evident from the data in Table 1. First, the retention of all neutral, nonionizable compounds (No.'s 1-13 in Table 1) is greater on the 2,3-methylated cyclodextrin stationary phase than on the native β-cyclodextrin stationary phase (when using comparable mobile phases and experimental conditions). Clearly, the methylated cyclodextrin stationary phase is more hydrophobic and nonpolar. Conversely, the retention of all carboxylic acid compounds (No.'s 27-31, Table 1) was greater on the native β-cyclodextrin

stationary phase than on its 2,3-methylated analogue. It is apparent that the interactions between the carboxylic acid moiety of the guest and the 2- or 3-hydroxyl groups at the mouth of the cyclodextrin cavity contributes significantly to the stability of these inclusion complexs.

The retention behavior of the aromatic amines (No.'s 21-26 Table 1) and the phenolic compounds, (No.'s 14-20, Table 1) is not as consistent as that of the aforementioned neutral compounds or the substituted carboxylic acids. Since the substituted phenols and anilines have ionizable functional groups that are able to act as hydrogen bond acceptors or donors, one might assume that their retention behavior would more closely resemble that of the substituted benzoic acids. However, this does not seem to be the case. Most of the time, the relative retentions of the substituted phenols and anilines resemble the neutral analytes in that they are retained to a greater extent on the 2,3-methylated-β-cyclodextrin stationary phase than on the native β-cyclodextrin stationary phase (Table 1). The main exceptions to this are a few *para* substituted compounds at specific pHs (see compounds 18, 25, and 26). It is not surprising that pH can have an effect, since it controls whether the compound is ionized or neutral. The pH effects on binding constants of substituted phenols and anilines has been studied previously.[19]

Adding buffer to the mobile phase can produce at least three different effects. First, it controls the ionization of analytes that have weak acidic or basic functional groups. Both the retention and selectivity of a compound depend on whether it is neutral or in an ionized state. For example, the retention of the substituted benzoic acids increases at pH 4.0 (when using comparable mobile phases). The opposite trend or variable retentions are observed for the substituted anilines (Table 1). If the ionization of a solute is not a factor, added buffer still sometimes reduces retention, in effect acting like additional organic modifier (see compounds 5-13, Table 1, where identical mobile phases are used). Finally, the buffer can sometimes enhance efficiency by interacting with and masking strong adsorption sites on the stationary phase. This trend was particularly evident for the separation of enantiomers[4,18] but did not seem to be as significant in this work for structural and geometrical isomers.

Selectivity

The *para*-isomer was generally the most retained isomer on both columns for the substituted phenols, anilines and carboxylic acids (Table 1). The one exception to this was for a few of the substituted anilines mainly at pH 4.0. Presumably, the shorter relative retention of the *para*-substituted anilines at this pH was because they are protonated, cationic species which do not form as strong inclusion complexes.

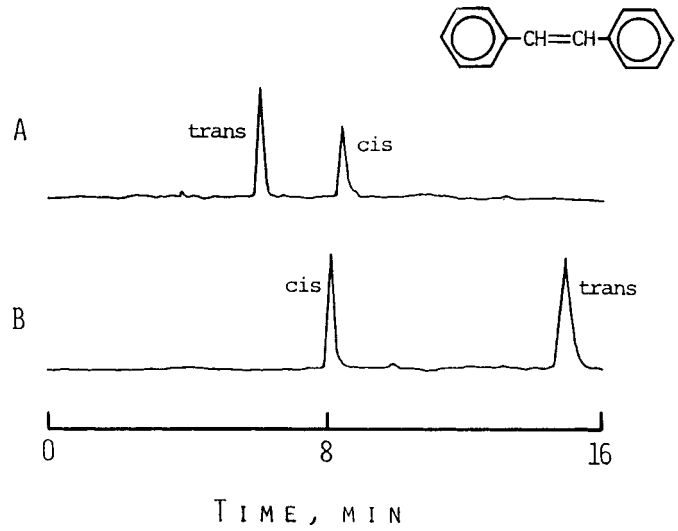

Figure 1. Chromatograms showing the difference in reversed phase selectivity for *cis* and *trans* stilbene of the A) native β-cyclodextrin bonded stationary phase, and the (B) 2,3-methylated-β-cyclodextrin bonded stationary phase. The mobile phase consisted of 45:55 (v:v) methanol:water in both cases. The flow rate was 1.0 mL/min. and UV detection (254 nm) was used.

Halogenated nitrobenzene and the dinitrobenzene (compounds 8-12, Table 1) were unique in that the *ortho* isomer was always the most strongly retained on both columns. In fact, the differences between the 2,3-methylated β-cyclodextrin and the native β-cyclodextrin column (in retention and selectivity) were not substantial for this particular group of neutral molecules. Apparently, shape discrimination during the hydrophopic inclusion complex process is the dominant factor determining selectivities for these compounds. For most of the other structural isomers, the ortho isomer was the least retained.

The *para*-isomers of most ionizable compounds (No.'s 15-31, Table 1) were retained to a greater extent than the ortho and meta isomers. The main exceptions to this were for some of the substituted anilines which were protonated at pH 4.0 (as discussed previously). It is interesting that the most retained structural isomer on the native β-cyclodextrin column also was most retained on the 2,3-methylated-β-cyclodextrin stationary phase. Selectivity differences between the two columns were more frequently observed for the less retained structural isomers (Table 1).

Geometrical isomers (Table 2) often gave the opposite retention order when separated on the native β-cyclodextrin column versus it 2,3-methylated analogue (see Figure 1). The retention trends for geometrical isomeric compounds were somewhat similar to that seen for the structural isomers. Neutral, hydrophobic compounds (stilbene, Table 2) tended to be retained more on the 2,3-methylated β-cyclodextrin stationary phase, whereas more polar or ionizable compounds were retained relatively longer on the native β-cyclodextrin stationary phase.

CONCLUSIONS

Methylation of the 2- and 3- hydroxyl groups on β-cyclodextrin produces a more nonpolar, hydrophobic stationary phase. Most of the isomeric compounds in this study were retained to a greater extent (in the reversed phase mode) on the methylated cyclodextrin stationary phase. The main exception to this was the substituted benzoic acids which were more strongly retained on the native β-cyclodextrin columns regardless of mobile phase conditions. The selectivity differences between the methylated and native β-cyclodextrin were not as significant for structural isomers as they were for the previously observed optical isomers (enantiomers).[18]

In general, the isomer that was retained longest on the native β-cyclodextrin stationary phase was also retained longest on its 2,3-methylated analogue. Retention reversals were most frequently seen for the less retained isomers. Retention reversals were also observed for pairs of geometrical isomers.

It appears that hydrogen bonding at the mouth of the cyclodextrin cavity is not as crucial to the selectivity of many structural isomers as it is for enantiomers. However, in some cases (*i.e.,* compounds with carboxy-functional groups) this hydrogen bonding, rim interaction remains very important.

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