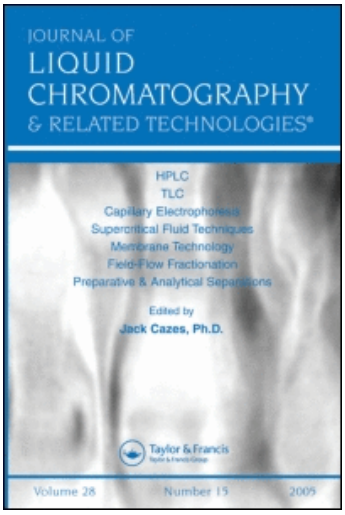

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ENANTIORECOGNITION MECHANISMS FOR DERIVATIZED CELLULOSE UNDER REVERSED PHASE CONDITIONS

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ABSTRACT

The chiral recognition mechanism for a derivatized polysaccharide chiral stationary phase (CSP) under reversed phase conditions was investigated (Chiralcel OJ-R). While enantiorecognition mechanisms have been proposed for these types of phases under normal phase conditions, it is unlikely that they would apply to reversed phase conditions due to the high polarity of the eluent. Indandiol was utilized as the main probe. The effect of organic modifier, eluent anion, and temperature on enantioselectivity for indandiol was determined. Additionally, a number of structurally related probes were also investigated to define the role of various functional groups on enantioselectivity.

The investigation revealed that hydrogen bonding did not have a gross direct effect on enantiorecognition but may contribute through it's effect on the tertiary structure of the CSP. It appears that inclusion is the major contributor to enantioselectivity with the size and shape of the probe being the determinant as to the extent of enantioselectivity rather than electrostatic interactions involving the functional groups of the solute.

INTRODUCTION

Chromatography has become a common analytical tool for enantiomeric quantitation as evidenced by the proliferation of commercially available chiral stationary phases (CSP). The more popular CSP can be classified under two groups. The first group consists of small chiral molecules and includes Pirkle phases, cyclodextrins, crown ethers, and ligand exchange selectors. The second group is polymeric in nature and includes proteins and polysaccharides. Macrocyclic antibiotics fall somewhere between these two groups.

Chiral recognition mechanisms for cyclodextrin based CSP and Pirkle type CSP have been extensively investigated using such tools as NMR, X-Ray analysis, and molecular modeling.[1-5] As a consequence, a number of rational models have been developed to describe the interaction between the chiral solute and the CSP. These models are very useful for the development of methods to resolve species on these CSPs.

Few attempts at the elucidation of the chiral recognition mechanisms for polymeric CSP have been undertaken.[6-14] Difficulties arise as a consequence of the multiplicity and diversity of binding sites. Additionally, in many cases, the supra-molecular structure contributes to enantioselectivity. The supra-molecular structures of these CSPs are difficult to determine whether in solid state or solution and, hence, the extent of its contribution to enantioselectivity is difficult to quantify.

For derivatized polysaccharide CSP under normal phase conditions, chiral recognition is proposed to occur through solute interactions at the derivatized ester or carbamate functionalities. These interactions are typically hydrogen bonding and dipole interactions. There is also the possibility of π-π interactions between the aromatic moieties of the solute and the CSP. The secondary structure of the CSP is characterized by the presence of chiral ravines that can also lead to discrimination through inclusion.[10-14]

While derivatized polysaccharide CSPs have demonstrated enantioselectivity for a broad range of solutes under normal phase conditions, there has been an increasing trend for their utilization under reversed phase conditions.[15-19] It would be anticipated that, as in the case for achiral separations, the retention mechanisms will differ in going from normal to reversed phase conditions. In a reversed phase medium the more polar solvent molecules should compete strongly with the solute for the derivatized carbamate or ester moieties. The enantioselective mechanisms for a commercially available derivatized cellulose CSP (Chiralcel OJ-R) were investigated. This CSP is an ester derivatized phase with a methyl group attached in the para position of the benzene ring.

The mechanisms were investigated using indandiol (Figure 1) as a probe. Indandiol has two chiral centers contained within a five-member ring that contributes rigidity around the chiral centers. There are four optical isomers, trans and cis enantiomeric pairs. The molecule is neutral, thereby eliminating the possibility of non-specific electrostatic silanophilic interactions. Enantioselectivity was monitored as a function of organic modifier, eluent anion, and temperature. A comparison was then made between indandiol and a number of structurally related compounds (Figure 1) in order to determine what structural parameters of the solute affect enantioselectivity.

EXPERIMENTAL

Chemicals

The HPLC grade solvents (Optima) were purchased from Fisher Scientific, Fairlawn NJ. HPLC water was obtained from a Picosystem Pump HIDRO (Garfield, NJ) water purification system. Indandiol was provided by Bioprocess Research of Merck & Co. (Rahway, NJ). Other probe molecules were purchased from Aldrich (Milwaukee, WI) and Acros Organics (Geel, Belgium). Sodium perchlorate and ammonium acetate was purchased from Fisher Scientific (Fairlawn, NJ). Potassium phosphate, mono and dibasic, was purchased from J. T. Baker (Phillipsburg, NJ).

Chromatographic Equipment

The HPLC equipment consisted of a Spectra-Physics SP8800 ternary pump, a Spectra-Physics SP8775 autosampler (Thermoseparations Products, Piscataway, NJ), and a Kratos Spectroflow 757 absorbance detector (ABI Analytical, Foster City, CA). The column cooler was a Column Chiller Model 7955 (Jones Chromatography, Lakewood, CA). Chromatograms were processed using a Perkin Elmer Nelson Analytical 900 Series Interface. The Diacel Chiralcel OJ-R column (150 x 4.6 mm, 5 μ particle size) was purchased from Chiral Technologies.

Chromatographic Conditions

The probe molecules were all dissolved in 50/50 acetonitrile/water and injected onto the column through a 10 μL loop. A flow rate of 1.0 mL/min was employed. All runs were temperature controlled at 25°C, except for the thermodynamic studies in which the temperature was varied. Detection was by UV-Vis at 220 nm for all of the studies. For the thermodynamic studies, due to column limitations, the range studied was from 0 to 40°C at 5° intervals.

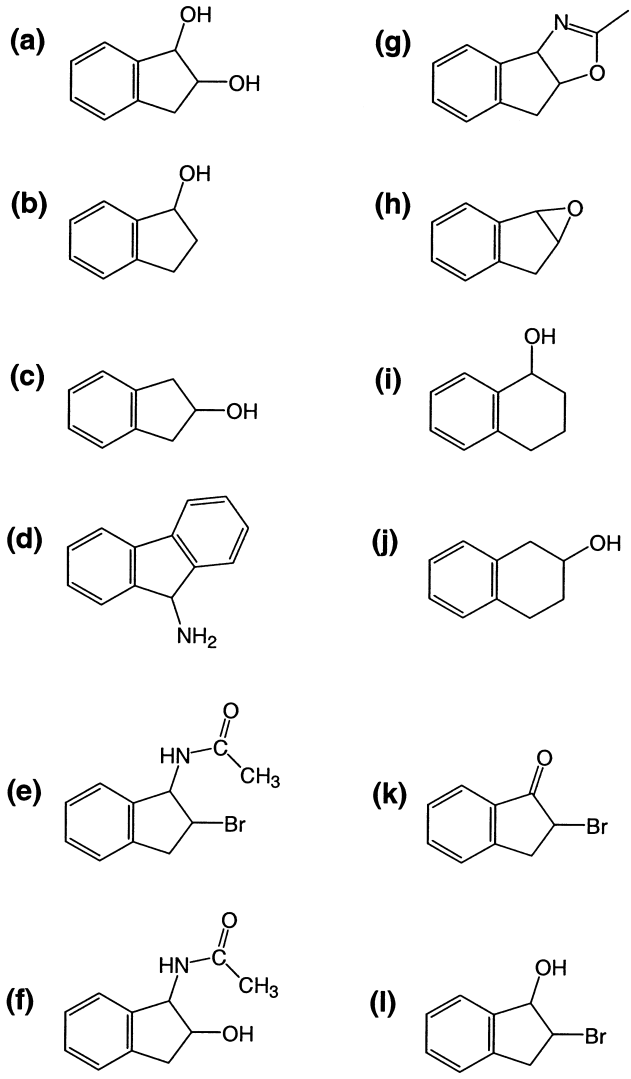

Figure 1. Structures of probes used in this study. (a) 1,2-Indandiol, (b) 1-Indanol, (c) 2-Indanol, (d) 9-Aminofluorene, (e) 1-Acetamido-2-bromoindene, (f) 1-Acetamido-2-indanol, (g) Oxazoline, (h) Indene Oxide, (i) 1,2,3,4-Tetrahydro-1-naphthol, (j) 1,2,3,4-Tetrahydro-2-naphthol, (k) 2-Bromoindanone, (l) 2-Bromoindanol.

RESULTS AND DISCUSSION

Influence of Organic Modifier

An investigation into the effect of organic modifiers on the interaction of the four optical isomers of indandiol with the chiral stationary phase was undertaken. Because of limitations due to solvent compatibility with the CSP, only methanol, ethanol, n-propanol, isopropanol, and acetonitrile were used to assess these effects.

Chiral separation was obtained with the alcohols (Figure 2), but acetonitrile did not resolve the enantiomers (Table 1). The alcohols are both hydrogen bond donors and acceptors, while acetonitrile is only a weak hydrogen bond acceptor. These differences in hydrogen bonding capability may have an effect on the tertiary structure of the CSP. The shapes of the chiral cavities may change as a consequence of these differences and account for the loss of enantioselectivity observed for acetonitrile.

A comparison of the capacity and selectivity factors as a function of the alcohols, indicates similar selectivity and a decrease in capacity factor as a function of the strength of the organic modifier. It should be noted, that the ratio of water to organic modifier was 90/10 in all cases, except for methanol. At a 90/10 ratio for methanol, there was substantial band broadening and concomitant loss of resolution, so a ratio of 85/15 was used for the study. The decrease in capacity factor indicates that the main contribution of the organic

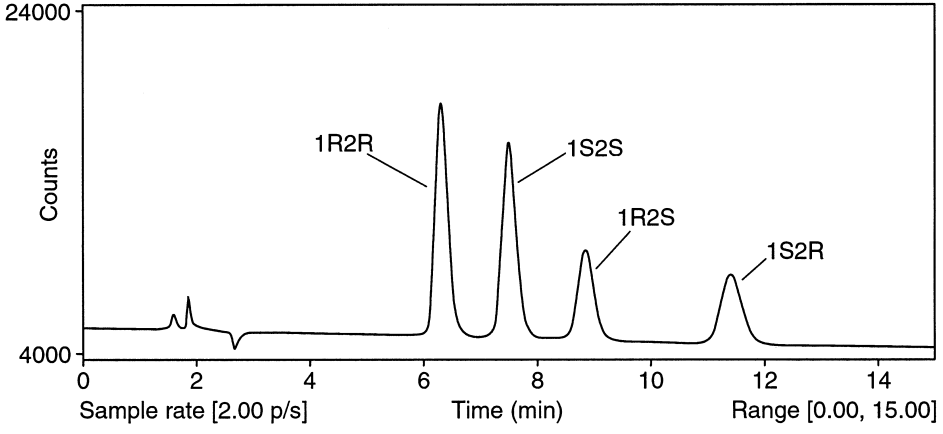

Figure 2. Chromatogram of the enantiomeric resolution of the four optical isomers of indandiol with 70% water and 30% methanol as eluent. Flow rate of 1.0 mL/min and UV detection at 220 nm. Elution order is as listed in Table 1.

Table 1

Retention Characteristics of Indandiol as a Function of Organic Modifier

Organic Modifier	k'_1	k'_2	α	k'_3	k'_4	α
Methanol (15%)	5.24	6.10	1.16	7.74	9.90	1.28
Ethanol (10%)	5.34	6.19	1.16	7.97	9.81	1.28
n-Propanol (10%)	3.05	3.63	1.19	4.28	5.44	1.27
i-Propanol (10%)	2.09	2.39	1.15	2.84	3.65	1.28
Acetonitrile (30%)	1.24	1.24	1.00	1.96	1.96	1.00

Water was used as co-eluent. Capacity factors corresponds to 1R2R (k'_1), 1S2S (k'_2), 1R2S (k'_3), and 1S2R (k'_4) isomers.

modifier is to affect the overall retention of the solute. This effect is opposite to that observed in normal phase chromatography, where the decrease in hydrogen bond capability of the organic modifier from methanol to isopropanol would result in increased retention.

Hydrophobic interactions are not sterically driven and the similar selectivity observed with the alcohols indicates that hydrogen bonding does not appear to be a gross contributor to the enantiorecognition within the alcohols. However, the difference observed between the alcohols and acetonitrile does indicate that hydrogen bonding, through its effect on the tertiary structure of the CSP, does indirectly contribute to the enantiorecognition.

Influence of Counter Anion

Three anions were evaluated as the counter anion in the aqueous mobile phase in order to observe the effect of ions in the separation of indandiol on the CSP. Phosphate, perchlorate, and acetate solutions were all prepared at 100mM and pH 7. Methanol at 30% was used with these aqueous solutions for the mobile phase. The resulting capacity and selectivity factors (shown in Table 2) are similar for the three anions, which is in accordance with the observations of Ishikawa and Shibata for neutral compounds.[15]

Influence of Temperature

Temperature studies were performed with the CSP and indandiol between 0 and 40°C at 5° intervals. The van't Hoff equation expresses the relationship between the capacity factor ot the selectivity factor and temperature:

Table 2

Retention Characteristics of Indandiol as a Function of Anion Type

Anion	k'_1	k'_2	a	k'_3	k'_4	a
Phosphate	1.65	1.83	1.11	2.62	3.20	1.22
Perchlorate	1.61	1.80	1.12	2.55	3.13	1.23
Acetate	1.67	1.85	1.11	2.65	3.24	1.22

Eluent used was 100 mM of anion in water with 70% methanol. Capacity factors are as in Table 1.

$$\ln k' = -(\Delta H°/RT) + \Delta S°/R + \ln \Phi$$

$$\ln \alpha = -\Delta\Delta H°/RT + \Delta\Delta S°/R$$

Van't Hoff plots are used as a tool in determining the nature of the interactions occurring between the solute and CSP. Non-linearity of these plots is a good indication that multiple types of interactions are making significant contributions to either the overall interaction or the chiral recognition. Plots were generated for both cis and tran indandiol with an eluent of 70:30 water/methanol. All of the plots obtained were linear with correlation coefficients between 0.992 and 0.998.

The observed linearity is an indication that both the overall interaction and the chiral recognition are each dominated by one mechanism. The dominating mechanisms are not necessarily the same. The energy associated with the chiral recognition is usually much smaller than the energy associated with the overall interaction. Thus, the contribution of the chiral recognition to the overall retention may be small, while the mechanism which dominates the overall retention may have little or no contribution to the chiral recognition.

Influence of Solute Structure

The overall retention of an enantiomeric pair on a CSP in its simplest form can be characterized by two types of interactions. There is a primary, strong, non-specific interaction, which dominates the overall retention and a secondary, weaker interaction that induces chiral selectivity. Under reversed phase conditions, it is expected that the primary interaction is a hydrophobic interaction. The non-specificity of this type of interaction precludes it, however, from contributing to the chiral recognition. Candidates for the secondary interaction include hydrogen bonding, dipole interactions, and steric hindrance.

Table 3

Retention Characteristics of Probes With 70% Water and 30% Methanol as Eluent

Compound	k_1'	k_2'	α
Cis Indandiol	4.90	6.60	1.35
Tran Indandiol	3.21	4.00	1.25
1-Indanol	23.6	26.6	1.13
2-Indanol	31.4	31.4	1.00
Cis 1-Acetamido-2-Indanol	5.01	5.01	1.00
Trans 1-Acetamido-2-Indanol	3.13	3.72	1.19
Indene Oxide	22.9	35.8	1.56
Oxazoline	8.57	8.57	1.00

Observations on the effect of the organic modifier give a preliminary indication that hydrogen bonding is not a major contributor to chiral recognition.

Probe molecules with varying substituents on the indan ring (Figure 1), were utilized in order to obtain a firmer grasp on the secondary chiral interaction. Studies were performed with water/methanol eluent with either 70 (Table 3) or 50% water (Table 4).

Table 4

Retention Characteristics of Probes With 50% Water and 50% Methanol as Eluent

Compound	k_1'	k_2'	α
Cis Indandiol	1.80	2.04	1.14
Trans Indandiol	1.01	1.12	1.12
Cis 1-Acetamide Bromoindene	9.23	12.87	1.39
Trans 2-Bromoindanol	24.68	34.28	1.39
2-Bromoindanone	26.17	32.01	1.22
9-Aminofluorene	22.15	30.75	1.39
1,2,3,4-Tetrahydro-2-Naphthol	11.35	13.59	1.20
1,2,3,4-Tetrahydro-1-Naphthol	12.80	12.80	1.00

Studies performed with 70% water showed chiral recognition for both cis and trans indandiol. The cis indandiol had longer retention and higher enantioselectivity than the trans indandiol. The stronger interaction of the cis enantiomers is expected given that the two polar hydroxy groups are on the same side, allowing for hydrophobic interaction with an approach from the side away from the hydroxy groups. A comparison was made with one of the hydroxyl groups removed. Both 1- and 2- indanol exhibited stronger retention than the indandiols reflecting the decreased polarity of the probes. Enantioselectivity was observed for 1-indanol with a lower separation factor than the indandiols. Enantioselectivity was lost for 2-indanol, giving a preliminary indication that the presence of functionality at the 1 position of indane may enhance enantioselectivity.

Substitution of an acetamide group at the 1 position of indandiol led to chiral recognition for the trans enantiomers, but the more retained cis enantiomers exhibited no enantioselectivity. The indication is that the presence of functionality at the 1 position of indane is not the sole criteria for chiral selectivity, but that the molecular geometry rendered by the presence of that functionality may also be important.

The CSP, through its ester functionality, has the potential to undergo hydrogen bonding with a probe which possesses hydrogen bond donors. The necessity for hydrogen bonding for chiral recognition was investigated through the use of oxazoline and indene oxide. Chiral recognition was achieved with indene oxide with a separation factor much greater than that of the indandiols. Oxazoline did not show chiral separation. Since indene oxide does not possess a hydrogen donor, hydrogen bonding is obviously not a required interaction for chiral selectivity. The fact that chiral recognition was obtained for indene oxide and not oxazoline, further supports the theory that a shape related interaction is the primary contributor to chiral recognition.

Fifty percent water as the eluent was used for more strongly retained probes. Bromine substituted probes were used to further illustrate that hydrogen bonding was not a major contributor to the chiral interaction. The enantiomers of *cis* 1-acetamido-2-bromoindene, *trans* 2-bromoindanol, and 2-bromoindanone all were resolved with selectivity factors greater than those of the indandiols. Interestingly, 2-bromoindanone has a high selectivity factor, despite the lack of a chiral center at the 1-position. The inference is that the chiral interaction does not have to take place at the 1- position as long as there is some bulky group present.

The effect of changing the base ring structure was also investigated. 1,2,3,4, tetrahydro-1-naphthol showed chiral recognition, while 1,2,3,4, tetrahydro-2-naphthol did not, further establishing the importance of having a group present at the one position. Selectivity was somewhat larger than for 1-indanol,

which may be a reflection of the increased ring size. 9-Aminofluorene, with an even larger ring base, gave an even larger selectivity factor.

CONCLUSION

While not a comprehensive investigation into the mechanisms which determine enantioselectivity, this study provides some insight into the interactions occurring with cellulose derivatives under reversed phase conditions. For this group of structurally related compounds, the primary interaction which determines overall retention appears to be hydrophobic. The chiral recognition mechanism does not appear to involve hydrogen bonding, since no hydrogen bond donor was required for chiral recognition (as illustrated by the case of indene oxide).

Steric fit is proposed to be the major chiral recognition mechanism, with selectivity increasing with the bulk of the functional groups present. This is an effect which has been observed for the separation of diastereomers on achiral phases. For the probe molecules studied, the presence of a functional group at the one position also appeared to enhance chiral recognition.

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