Investigation of the enantioselective separations of α-alkylarylcarboxylic acids on an amylose tris(3,5-dimethylphenylcarbamate) chiral stationary phase using quantitative structure–enantioselective retention relationships Identification of a conformationally driven chiral recognition mechanism

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Abstract

A series of 28 chiral α-alkyl arylcarboxylic acids were chromatographed on an amylose tris(3,5-dimethylphenyl-carbamate) chiral stationary phase (AD-CSP). The retention data were correlated to a series of molecular descriptors to produce quantitative structure–enantioselective retention relationships (QSERR) incorporating the hydrogen bonding ability and aromaticity of the solutes.

The QSERR equations were used to guide molecular modelling experiments designed to investigate the chiral recognition mechanism responsible for the observed enantioselective separations. The results of the study indicate that unlike the standard "three-point interaction" model of chiral recognition, enantioselectivity was due to a "conformationally driven" chiral recognition process.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Quantitative structure–retention relationships; Molecular modelling; Enantioselectivity; Benoxaprofen; Amylose tris(3,5-dimethylphenylcarbamate)

1. Introduction

A wide variety of chiral stationary phases (CSPs) have been developed for the enantioselective separation of chiral substances. The majority of the chiral separations achieved on CSPs are obtained based

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upon the accumulated trial and error knowledge of the analyst, and often simply by chance. In order to improve the utility of a number of the CSPs, some laboratories have compiled data banks containing reported enantioselective separations [1,2]. Others have developed predictive empirical rules based upon molecular structure [3–5]. A comprehensive quantitative approach to chiral separations has not been developed, although theoretical and chemometric attempts at understanding CSP structure and

recognition mechanisms have been receiving increased attention. This has been recently reviewed by Lipkowitz [6].

One strategy for the development of chemometrically driven predictions of retention and enantioselectivity is the construction of quantitative structure–enantioselective retention relationships (QSERR) [7]. Two types of data are needed for QSERR studies: (1) quantitatively comparable retention data for a set of solutes; (2) molecular descriptors reflecting the structural features of the solutes. Through the use of multiparameter regression analysis or factor analysis, the HPLC capacity factors and molecular descriptors are mutually related. The resulting relationships can then be used to predict retention and enantioselective separations based on the structures of the solute and HPLC-CSP.

In addition to predicting retention and enantioselectivity, QSERR correlations can also be used to describe the interactions which produce the observed chromatographic results [7,8]. Kaliszan and co-workers [9–11] have successfully used this approach to study the retention and chiral recognition mechanisms for a series of benzodiazepines chromatographed on a human serum albumin (HSA)-based CSP. The QSERR analysis also provided information on the structure of the HSA-benzodiazepine binding site [11], which was consistent with the structure derived from X-ray crystallographic studies. Analogous studies have been performed on a variety of other phases [12–15].

A key class of HPLC-CSPs are those based upon the derivatized polysaccharides, cellulose and amylose. While phases based upon both of these backbones are widely used in analytical and preparative enantioselective separations, little is known about the chiral recognition mechanisms operating on the amylose-based CSPs.

In contrast, a number of studies have investigated the foundations of the chiral recognition mechanisms on the cellulose-based CSPs. Francotte and co-workers [16–18] have utilised the cellulose triacetate (CTA I-CSP) and tribenzoate CSP to investigate the relationships between solute structure and enantioselective resolutions, as well as the effect of cellulose supramolecular structure on these separations [19]. Roussel et al. [20] and Isaksson et al.

[21] have also carried out computational studies of enantioselective separations on the CTA I-CSP.

Wainer and co-workers [22,23] have investigated the chiral recognition mechanisms responsible for enantioselective resolutions on a CSP composed of cellulose tribenzoate coated upon macroporous silica gel (OB-CSP). When a series of 29 related chiral amides were chromatographed on the OB-CSP, the data suggested the existence of an "attractive interaction-steric fit" chiral recognition mechanism involving the amide dipole on the solute and the ester-moiety on the CSP [22]. When the solutes were chiral aromatic alcohols, a similar mechanism was proposed where the first attractive interaction was a hydrogen bonding interaction between the alcohol moiety on the solute and the carbonyl oxygen on the ester moiety of the CSP [23].

Yashima et al. [24] recently calculated interaction energies between cellulose trisphenylcarbamate (CTPC) and (R,R)- and (S,S)-trans-stilbene oxide, as well as between CTPC and (R,R)- and (S,S)-1,2-diphenylcyclopropane. The results were more qualitative than quantitative but, when compared to HPLC retention data, demonstrated the potential of this approach. ^{1}H NMR spectroscopy has also been used to observe the chiral discrimination of trans-stilbene oxide and 2-butanol enantiomers by cellulose tris-4-methylsilylphenyl carbamate (CTSP) [25]. More information on the actual mechanism of recognition will possibly be obtained by the observation of nuclear Overhauser effect enhancements.

In this paper, we report the results of a series of studies into the chiral recognition mechanism responsible for the enantioselective resolutions of chiral α-alkyl aromatic carboxylic acids on a CSP based upon amylose tris(3,5-dimethylphenylcarbamate), the AD-CSP. The AD-CSP was chosen due to the numerous reported applications using this CSP, c.f. [26–37], and the lack of a clear description of the chromatographic chiral recognition processes operating on this phase.

In the reported studies, QSERR equations were developed by comparing the retention and enantioselectivity data from 28 solutes with a variety of molecular descriptors. Molecular modelling studies were then carried out using the QSERR results to guide the docking of the solutes with a representative

structure of the AD-CSP. The results of these studies indicate that the chiral recognition process involves initial hydrogen bonding interactions between the solute and CSP, insertion of the solute into a ravine on the surface of the CSP, and stabilisation of the solute–CSP complex by formation of an additional hydrogen bond within the ravine. Both enantiomers of the solute can create the same hydrogen bonding interactions with the CSP although one of the enantiomers must expend a greater amount of energy to produce the conformation required for this interaction. Thus, unlike the standard "three-point interaction" model of chiral recognition, enantioselectivity in the system investigated in this study is due to a "conformationally driven" chiral recognition process.

2. Experimental

2.1. Compounds

Compounds 1, 2, 3, 9, 10, 13, 17–21, 27 and 28 were gifts from Upjohn (Kalamazoo, MI, USA). Compounds 4 (Ketoprofen) and 12 (suprofen) were obtained from Sigma (St. Louis, MO, USA). 8 (Benoxaprofen) was obtained from Eli Lilly (Indianapolis, IN, USA). Compounds 5, 6, 11, 14, 16, 22–26, benzoic acid and phenylacetic acid were obtained from Aldrich (Milwaukee, WI, USA). Compounds 7 and 15 were kindly provided by Dr. W.J. Lough (University of Sunderland, Tyne and Wear, UK).

2.2. Preparation of esters

Esters were prepared by dissolving 100 mg of acid in 25 ml dry methanol. A few drops of concentrated hydrochloric acid were added and the reaction mixture left to reflux. All reactions were complete in under 3 h as determined by TLC. The methanol was then removed under vacuum and the ester reconstituted in 25 ml dichloromethane. The organic layer was then washed, 3×30 ml water, 3×30 ml saturated sodium bicarbonate solution and 3×30 ml saturated sodium chloride solution. The organic layer was collected, dried over magnesium sulphate and evapo-

rated to dryness. All compounds were identified by GC–MS.

2.3. Chemicals

HPLC-grade methanol, hexane and isopropanol were obtained from Anachemia Science (Montreal, Canada). Trifluoroacetic acid was obtained from Aldrich.

2.4. Chromatography

The chromatographic system was composed of a Spectra-Physics P1500 binary pump, a Spectra-Physics UV100 variable-wavelength detector and a Spectra-Physics SP8875 autosampler equipped with a 20-μl loop (Thermo Separation Products, Toronto, Canada). Separation was performed on a Chiralpak AD chiral column, 250×4.6 mm I.D. (Chiral Technologies, Exton, PA, USA). Elution order was determined on a Chiramonitor 2000 optical rotation detector (Interscience, Markham, Canada). A Spectra-Physics Datajet integrator acted as an interface for electronic data collection using Winner on Windows software run on a 386 personal computer.

Column temperature regulation was achieved by using a Haake D1-G refrigerated bath/circulator (Fischer Scientific, Montreal, Canada) and a column water jacket.

The mobile phase consisted of hexane–isopropanol (95:5, v/v) plus 1% trifluoroacetic acid, filtered and degassed. All samples were prepared in mobile phase.

Column performance was monitored daily by injecting compound 9 (flurbiprofen). Capacity factors and α values were compared to those obtained when the column was first used, to ensure no variation in column performance over the period of the study.

2.5. Computational chemistry

Molecular models were created using Insight II release 235 (Biosym, San Diego, CA, USA) run on an IBM RS6000 RISC workstation (IBM, Austin, TX, USA). Conformational searching was performed using Search-Compare ver. 2.3 (Biosym) and all energy minimisations were performed using Discov-

er ver. 2.9.5 (Biosym), both running within Insight II.

Statistical analysis was performed using CSS (Statsoft, Tulsa, OK, USA) run on a 486 personal computer.

2.6. Construction of the AD-CSP

A model of the amylose tris(3,5-dimethylphenyl-carbamate) stationary phase was constructed following an approach described by Francotte and Wolf [18]. A molecule of α-D-glucose was retrieved from the molecular fragment library within Insight II. This was appropriately derivatised with pre-geometry optimised 3,5-dimethylphenyl groups. Geometry optimisation was performed in three steps: steepest descents followed by conjugate gradients and then the Broyden-Fletcher-Goldfarb-Shanno (BFGS) algorithm. All steps used the constant valence force field (cvff). A convergence criterion of 0.001 kcal mol^{-1} RMS deriv. was used for the final step. All three derivatives were subjected to a systematic conformational search (e.g. for two rotatable bonds using a 1° interval, 129 600 conformations would be sampled), followed by geometry optimisation to give the lowest energy conformation. This monomer was duplicated and the two glucose units joined via the oxygen at C_1 and C_4. The dimer was relaxed and a second conformational search performed to find the values of ϕ and ψ (torsion angles in the linkage between two glucose units) corresponding to the lowest energy structure. These were found to be $\phi = -28.0°$ and $\psi = -13.8°$. Using these values and the previous convergence criterion, a highly relaxed polymer consisting of 20 glucose units was created.

The low energy conformations of (R)- and (S)-benoxaprofen were also created by systematic searches and geometry optimisation. Both enantiomers obtained exactly the same total energy (E_T) value of 96.078 kcal mol^{-1}.

An 8 Å radius subset of the stationary phase was created. (R)- and (S)-benoxaprofen enantiomers were docked into the subset in real time whilst monitoring the interaction energies and hydrogen bonds. A molecule was assumed to be optimally docked when the maximum number of possible hydrogen bonds had formed, combined with the lowest achievable interaction energy.
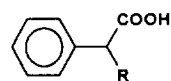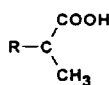
3. Results

A series of 28 α-alkyl arylcarboxylic acids and related compounds were chromatographed on an amylose tris(3,5-dimethylphenylcarbamate) chiral stationary phase; the structures of these compounds are presented in Fig. 1. The contributions of the substituents at the stereogenic centre i.e. the aromatic, alkyl, and acidic moieties, to relative retention (k') and enantioselectivity (α) were investigated by systematically varying these groups. The results are presented below.

3.1. Carboxylic acid moiety

In order to study the contribution of the carboxylic acid moiety, selected compounds were converted into their methyl ester derivatives. Table 1 lists the retention data for the acids and their corresponding methyl esters. In all cases a substantial reduction in retention was observed for the ester as compared to the acid. Separation was only achieved for two of the esters (compound 8, benoxaprofen methyl ester, and compound 25, methyl mandelate) as compared to separation for all of the acids.

A decrease in retention is to be expected in normal-phase chromatography when the hydrophobicity of a solute is increased, thus increasing solute solubility in the mobile phase. In addition, reduction of non-specific interactions between the solute and the more polar stationary phase will also decrease retention. In this case, replacement of the acidic proton with a methyl group reduced the ability of the solute to act as a hydrogen bond donor. The formation of hydrogen bonds between the solute and stationary phase appears to be a key factor in the general retention, as is demonstrated by the drastic reductions in k' following conversion from acid to ester.

Although separation was not obtained for most of the esters, this does not necessarily indicate that the structural requirements for enantiorecognition are no longer present. Rather, solute stationary phase interactions governing general retention, were insufficient to permit noticeable discrimination between enantiomers. Benoxaprofen methyl ester and methyl mandelate both retain a higher degree of retention ($k'_2 = 2.09$ and 3.18, respectively) thus allowing observable

Fig. 1. Structures of the compounds used in this study.

separation. This indicates that other factors can play a significant role in the determination of k' and α.

3.2. α-Alkyl moiety

The effects of variations in the alkyl substituent on k' and α are presented in Table 2. The observed retention values can be grouped into three distinct categories, $k'<4$, $4<k'<8$ and $k'>8$. The compounds whose k' values were less than 4 contained only a straight chained or branched alkyl moiety. The compounds which lay within the $4< k'<8$ group contained meta methoxy phenyl moieties and the compounds which comprised the $k'>8$ group

Table 1
Effect on k' and α following conversion from acid to methyl ester

Compound methyl ester	k'_2	Decrease in k'_2 (%) on conversion from acid to ester	α Ester	α Acid
5	0.41	78.9	1.00	1.23
6	0.38	83.2	1.00	1.33
8	2.09	91.6	1.17	1.82
9	0.28	92.4	1.00	1.48
11	0.27	86.9	1.00	1.10
14	0.48	83.5	1.00	1.27
25	3.18	68.7	1.09	1.17

See Experimental for chromatographic conditions.

Table 2
Effect of variations in the α-alkyl moiety on k' and α

Compound	k'_1	k'_2	α	Elution
11	1.88	2.06	1.10	R(−):S(+)
16	1.80	2.04	1.14	R(−):S(+)
17	1.55	1.75	1.13	(−):(+)
18	1.36	1.52	1.12	(−):(+)
19	1.66	1.82	1.10	(−):(+)
20	1.56	1.78	1.14	(−):(+)
21	1.42	1.71	1.21	(−):(+)
22	2.31	2.51	1.09	S(−):R(+)
23	2.84	3.20	1.13	(−):(+)
24	2.74	3.01	1.10	R(−):S(+)
25	8.68	10.17	1.17	(+):(−)
26	10.06	12.25	1.22	(+):(−)
27	6.11	7.28	1.19	(−):(+)
28	5.44	5.53	1.02	(−):(+)

See Experimental for chromatographic conditions.

contained hydroxyl moieties. This suggests that additional hydrogen bonding interactions are taking place between the oxygen and hydroxyl moieties in the solute and CSP.

When the correlation between hydrophobicity (log P) of these substituents and their associated k' values was tested, a correlation factor of $r = -0.5941$ ($p = 0.03$) was determined. This indicates that a correlation exists between the structure of the side chain and retention; an increase in hydrophobicity causing a decrease in retention. Similar effects have been identified on Pirkle-type [38] and cellulosic [22] CSPs.

Although enantioselective separations were achieved for all compounds, α values were low and fell within a fairly narrow range (1.02–1.22). Previous studies [22,38] have indicated that the size of an alkyl substituent at the stereogenic centre affects α, the α value increasing with increasing substituent size. In this study, when the correlation between alkyl substituent molecular volumes and their associated α values was tested, a correlation factor of $r = -0.1443$ ($p =$ not significant) was determined. This indicates that in the whole series, molecular volume does not appear to be correlated with enantioselectivity.

However, when only the unsubstituted alkyl side chains (compounds 11, 16–21) were considered, a statistically significant correlation, $r = 0.7842$ ($p =$

0.04), was observed. Further analysis of this subset, indicated that removal of compound 21 from the analysis reduced the correlation factor to $r = 0.3938$ ($p =$ not significant) and the comparison lost significance. These results, coupled with observation that compound 28 has the α-substituent with the largest molecular volume but the lowest enantioselectivity ($\alpha = 1.02$), suggests that steric limitations exist within the interaction site on the AD-CSP.

3.3. Aromatic moiety

The effect of the structure of the aromatic substituent on k' and α is presented in Table 3. From this data, it is evident that this moiety plays a key factor in the determination of k' and α. The magnitude of this effect is dependent upon the position, nature and number of hydrogen bonding groups within the substituent. Compounds 2, 8 and 9 which contain *meta* acceptors, exhibit the highest enantioselectivities (1.48–1.82), and compounds with acceptors α to the stereogenic centre have enantioselectivities in the 1.23–1.33 range.

For those compounds where absolute configuration is known, the elution order $R:S$ is prevalent. Compounds 5, 6 and 14 elute $S:R$ only as a result of a change in priority of the Cahn, Ingold, Prelog

Table 3
Effect of variations in the aromatic moiety on k' and α

Compound	k'_1	k'_2	α	Elution
1	1.48	1.48	1.00	R(−):S(+)
2	10.48	16.99	1.62	(+):(−)
3	1.26	1.65	1.31	(−):(+)
4	8.51	9.52	1.12	R(−):S(+)
5	1.58	1.94	1.23	S(−):R(+)
6	1.70	2.26	1.33	S(−):R(+)
7	2.22	2.46	1.11	(−):(+)
8	13.71	24.89	1.82	R(−):S(+)
9	2.48	3.67	1.48	R(−):S(+)
10	3.06	3.37	1.10	(−):(+)
11	1.88	2.06	1.10	R(−):S(+)
12	12.11	14.01	1.16	S(+):R(−)
13	3.42	4.30	1.26	(+):(−)
14	2.29	2.91	1.27	S(−):R(+)
15	3.42	4.17	1.22	(+):(−)

See Experimental for chromatographic conditions.

naming system for chiral molecules. Column efficiency for compound 1, was too low to directly observe the enantioselective separation by UV however, determination of the enantiomeric elution order was possible using optical rotatory dispersion (ORD).

3.4. QSERR analysis

The retention data were subjected to multiparameter regression analysis against various non-empirical molecular descriptors. The simplest possible relationships between these descriptors were established and are presented in Eq. 1 and Eq. 2.

$$\ln k'_1 = -2.499 + 1.369X(\pm 0.177)$$
$$p = 0.00000$$

$$+ 0.791Y(\pm 0.121) + 0.415Z(\pm 0.094) \quad (1)$$
$$p = 0.00000 \qquad p = 0.00022$$

$n = 26$, Multiple $R = 0.9469$, $p < 0.0000$,

$F_{(3,22)} = 63.63$, $F_{(3,22), \ \alpha=0.01} = 4.82$

$$\ln k'_2 = -2.659 + 1.498X(\pm 0.151)$$
$$p = 0.00000$$

$$+ 0.896Y(\pm 0.103) + 0.439Z(\pm 0.080) \quad (2)$$
$$p = 0.00000 \qquad p = 0.00002$$

$n = 26$, Multiple $R = 0.9670$, $p < 0.0000$,

$F_{(3,22)} = 105.58$, $F_{(3,22), \ \alpha=0.01} = 4.82$

X and Y are the numbers of hydrogen bond donors and acceptors, respectively. Z is the degree of aromaticity in the molecule.

3.5. Molecular modelling studies

Directed by the initial QSERR equations, molecular modelling was used to either expand or reject the hypotheses proposed via the equations. Benoxaprofen (BP, compound 8), was chosen as the test molecule as it demonstrated the highest retention and enantioselectivity of the series. A conformation for the amylose tris(3,5-dimethylphenylcarbamate) chiral stationary phase (AD-CSP) has been proposed (Fig. 2). From this model it would appear that while the helix is much larger than those proposed for related cellulosic phases [39], it is consistent with other reports [40]. It is recognised that this may not be a true representation of the actual stationary phase, however, in the absence of a crystal structure, this simulation was accepted as the working model.

The model AD-CSP also emphasises the number of possible hydrogen bonding sites covering the surface of the helical cavity. The modelling study was not aimed at trying to reproduce the energies of interaction between the two enantiomers of BP and the stationary phase, but rather probe the possible modes of binding. It is clear that there are many sites for acid interactions of a non-enantiospecific nature thus contributing to general retention.

3.6. Chiral recognition process

A site was located within the helical ravine (Fig. 3) at which it is believed that enantioselective discrimination occurs. When BP is docked in this ravine, there is a potential for three simultaneous hydrogen bonding interactions (Fig. 4). Two of these interactions can occur between the carbonyl oxygen and acid hydroxyl proton on the acid moiety of BP and the amide proton and ether oxygen on the CSP. A third hydrogen bond can be formed between an amide proton on the CSP and the oxygen and/or nitrogen atom in the five-membered oxazole ring.

The first two interactions are presumably the initial solute–stationary phase interactions and serve to tether BP to the CSP. These interactions constitute a major portion of the chromatographic retention (see Table 1), but are not the primary driving force behind the chiral recognition process. The third hydrogen bonding interaction also contributes to the chromatographic retention. In the case of BP, complementary distances between interacting groups in BP and CSP and the ability of the solute to fit the contour of the stationary phase contribute to the high retention of this solute. This is suggested by the relatively high retention of BP after the primary hydrogen bonding interactions are reduced by conversion of the acid to the methyl ester (Table 1).
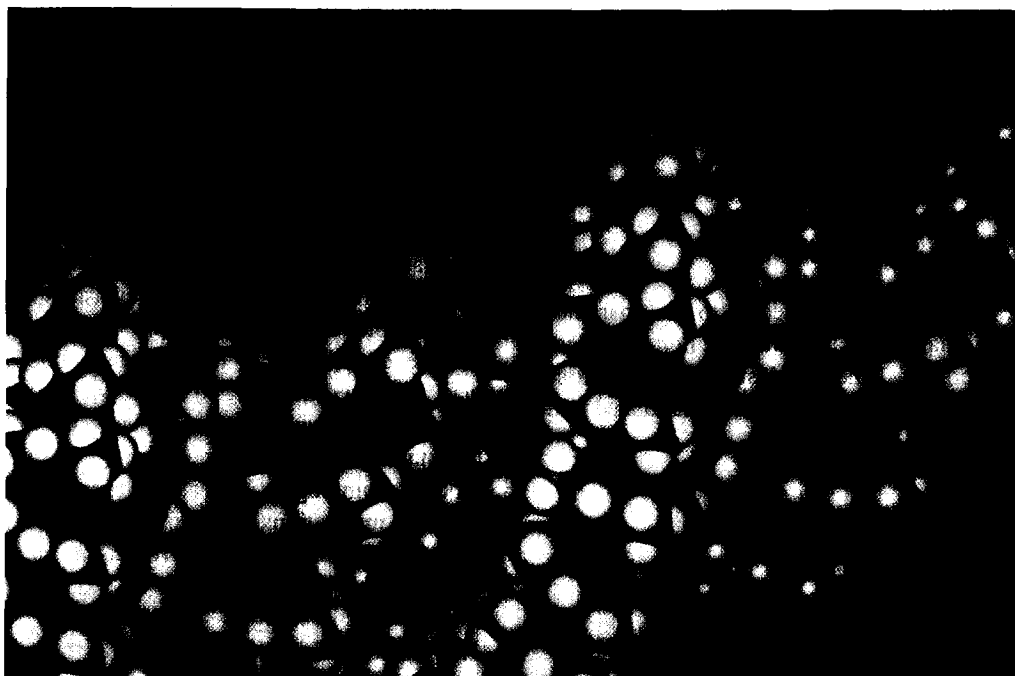
Fig. 2. Model of the proposed amylose tris(3,5-dimethylphenylcarbamate) chiral selector.

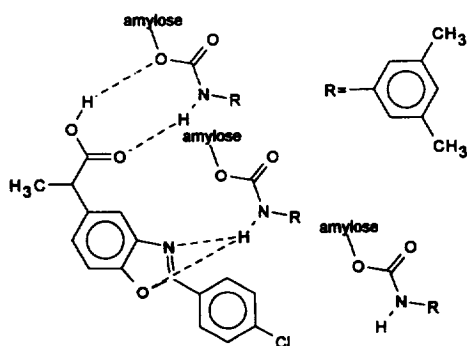Fig. 3. Model showing $R(-)$-benoxaprofen docked into the helical cavity within the chiral selector.

Fig. 4. Proposed mechanism of binding showing hydrogen bonding sites for benoxaprofen.

When (S)-BP was docked in the ravine in its lowest energy conformation, all three simultaneous hydrogen bonding interactions were formed without significant molecular deformations. (R)-BP has a mirror image lowest energy conformation. The docking of this conformation in the ravine produced significant steric interactions between the solute and stationary phase and the same hydrogen bonding interactions could not be obtained. However, a slight rotational change in the conformation of (R)-BP produced a structure which was capable of forming the identical solute–CSP interactions obtained by (S)-BP.

Even though the two enantiomers form identical hydrogen bonding interactions, and presumably the same hydrophobic interactions as well, the diastereomeric BP–CSP complexes differ in their stabilities leading to chiral discrimination. The difference arises from the internal energies of the two enantiomer conformations. The bonding conformation of (R)-BP is approximately 250 cal mol^{-1} higher in energy than that of (S)-BP. The theoretical enantioselectivity arising from this energy difference can be estimated using the equation $\Delta\Delta G = -RT\ln\alpha$ [41], and the calculated α (1.52) is consistent with the observed α (1.82).

The proposed conformationally driven chiral recognition mechanism is also consistent with the observed minimal effect of the steric bulk of the α-substituent (Table 2). When the solutes are docked in the ravine, the α-alkyl substituents are directed

away from the stationary phase, out of the ravine and into the mobile phase (Fig. 4). Since differences in molecular conformational energy and not steric interactions appear to be the key driving forces behind chiral discrimination of the majority of the solutes, it would be assumed that in a series of 14 α-alkyl phenylacetic acids, there would be relatively little variation in the magnitude of the observed enantioselectivities. This is indeed the case.

In the proposed chiral recognition mechanism, the stronger the bonding interactions between the solute and CSP, the larger are the associated decreases in the entropic freedoms of the two entities, together with a greater increase in the conformational (and therefore, energetic) demands. In the system studied, the key determinant of the strength of the solute–CSP complex is the third hydrogen bonding interaction between an amide hydrogen on the CSP and a hydrogen bond acceptor in or on the aromatic moieties. The importance of this interaction is illustrated by the variation in the observed enantioselectivities for the series of 15 α-methyl arylcarboxylic acids (Table 3).

It should be noted that the chromatographic process utilised in this study involves distribution from a non-polar mobile phase to a polar stationary phase. Thus, general molecular hydrophobicity should reduce chromatographic retention and not play a major role in the chiral recognition process. However, once the solute has been tethered to the CSP via the hydrogen bonding interactions between the carbonyl oxygen and acid hydroxyl proton on the acid moiety of BP and the amide proton and ether oxygen on the CSP, the hydrophobicity of the aromatic portion of the solute may play a role in the insertion of the solute into the ravine in the CSP. Thus the enantioselective separations of the α-alkylphenyl acetic acids are a result of either the hydrophobicity of the phenyl ring, or π-facial hydrogen bonding interactions [42] between the aromatic ring on the solute and an amide hydrogen on the CSP. In either case, the interactions are weak and the resultant enantioselectivity relatively low (Table 2 and Table 3).

The computer-simulated docking of (S)-BP and (R)-BP suggested that the interaction of (S)-BP with the CSP takes place via a concerted mechanism while the (R)-BP–CSP interaction involves a two-step process. In order to investigate this possibility, a

study was conducted to determine the effect of temperature on the chromatographic retentions of the enantiomers of BP, BP methyl ester and for the achiral phenylacetic and benzoic acids. Retention data were obtained at 10, 15, 20, 25, 30, 35 and 40°C, and Van 't Hoff plots created for the 6 compounds, Fig. 5.

From the Van 't Hoff plots it can be seen that the two enantiomers of BP exhibit different behaviour. (S)-BP displayed linear behaviour while distinct curvature was observed for (R)-BP. Linearity within these graphs is attributed to enthalpy–entropy compensation with an invariant retention mechanism over the temperature range studied [43]. Non linearity is attributed to multiple retention mechanisms. Curvatures in Van 't Hoff plots have been observed in achiral RPLC systems [44], and attributed to transition within the stationary phase, from a less ordered to a more ordered state at lower temperatures. In this system, since a curvature is only noted for one enantiomer, then it is the solute not the CSP

which is undergoing a temperature-dependent transition. This is consistent with the results of the docking interactions and is supported by the Van 't Hoff plots for the achiral phenylacetic and benzoic acids which are both linear over the temperature range studied.

It is interesting to note that both enantiomers of BP methyl ester exhibit the same characteristics as (R)-BP, but with a substantial reduction in retention. The absence of two simultaneous hydrogen bonding interactions between the acid moiety of the solute and the CSP obviously changes the relative initial positions of the two entities and reduces the stability of the initial complex. Both the (S)-BP methyl ester and (R)-BP methyl ester must undergo conformational changes to produce the additional hydrogen bond involving the aromatic moiety. Thus, the retention mechanisms of both enantiomers should show temperature-dependent transitions and lower relative energy differences for the resultant diastereomeric complexes, i.e. lower enantioselectivities.
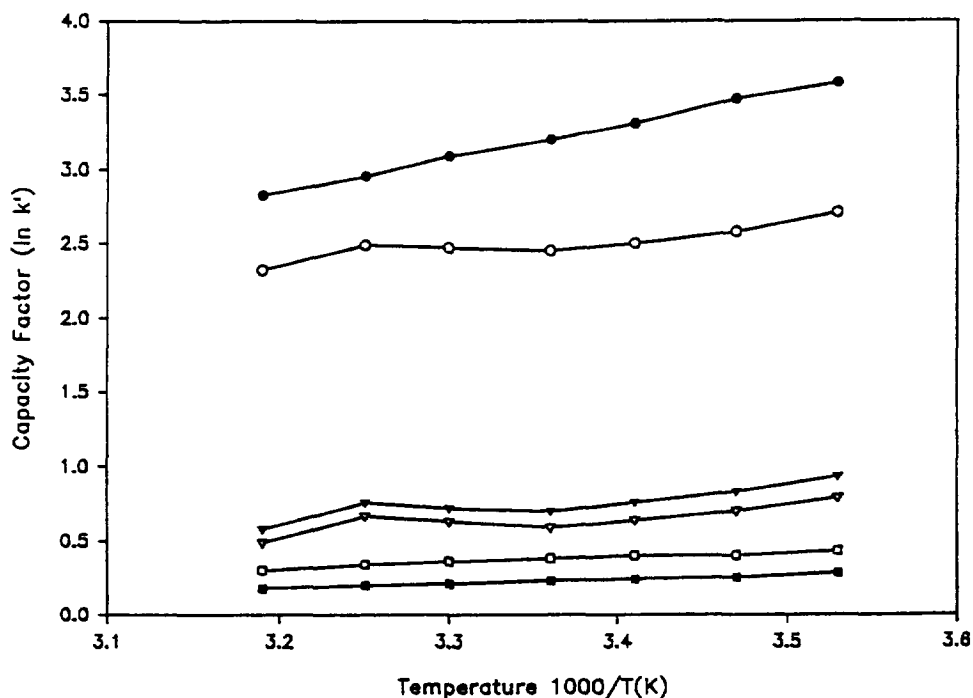

Fig. 5. Van 't Hoff plots for benoxaprofen, benoxaprofen methyl ester, benzoic acid and phenylacetic acid. (●) S(+)-benoxaprofen, (○) R(−)benoxaprofen, (■) benzoic acid, (□) phenylacetic acid, (▼) benoxaprofen methylester k'_1, (▽) benoxaprofen methylester k'_2.

4. Conclusions

4.1. Conformationally driven chiral recognition process

The chiral recognition process suggested by the results from this study is diagrammed in Fig. 6. In this scheme, step 1 is the distribution of the solute from the mobile phase to the CSP. This distribution is driven by hydrogen bonding interactions between the carbonyl oxygen and acid hydroxyl proton at the acid moiety of the solute and the amide proton and ether oxygen on the CSP. The tethering of the solute to the CSP results in the formation of two diastereomeric complexes [(R)-BP–CSP and (S)-BP–CSP] of equivalent stabilities. This is a retention step, not a chiral discrimination step. However, it is evident that without formation of the solute–CSP complexes, chiral recognition could not occur, and thus, these interactions are a key first step towards enantioselective separation.

Once the complex has been formed, the solutes and CSP form a third hydrogen bonding interaction produced after insertion of the solute into a ravine on the CSP. In this step the solutes and the CSP conformationally adjust to each other to maximise the interactions and, thereby, the stabilities of the diastereomeric complexes. It is this step that produces the different stabilities of the (R)-BP–CSP

Fig. 6. Proposed mechanism of chiral discrimination.

and (S)-BP–CSP complexes and ultimately the observed enantioselective separation.

Since three hydrogen bonding interactions are apparently involved in chiral discrimination, it is tempting to describe this process in terms of the "three-point" interaction model delineated by Pirkle et al. [45], for chiral chromatographic recognition. In this model, there must be at least three simultaneous interactions between the CSP and solute enantiomers, where at least one of the interactions is stereochemically dependent. Is this the case for the enantioselective resolutions achieved on the AD-CSP with this series of compounds?

(a) While three simultaneous hydrogen bonding interactions produced the largest enantioselectivities, the observed chiral resolution of (R,S)-BP methyl ester indicates that in this series, two simultaneous hydrogen bonding interactions were sufficient.

(b) One could also view the "three interactions" as involving three separate bonds to the stereogenic centre. In this case, the hydrogen bonding interactions involving the carboxylic and aromatic moieties of the solute would position the solutes relative to the CSP and the substituents on one or both of the remaining bonds to the stereogenic centre would provide the stereochemically dependent interaction(s). In the standard "three-point" interaction model, the third and defining factor is often due to steric interactions; this is illustrated by studies demonstrating a correlation between α and the size of alkyl side chains at the stereogenic centre [22,38,46]. This was not the case with this series of solutes on the AD-CSP, although steric structure of the interaction site on the CSP does play a role.

The results of this study suggest that another chiral recognition mechanism is responsible for the enantioselective separation of this series of solutes on the AD-CSP. It appears that the key factor in the chiral recognition mechanism is that both enantiomers can and do make the same hydrogen bonding interactions with the CSP. To make these interactions, the solute enantiomers must undergo conformational changes; the extent and energetic consequences of these changes are dictated by the chirality of the CSP. Thus, in this case, chiral recognition is not due to "point" interactions but "molecular" interactions and the process is conformationally driven.

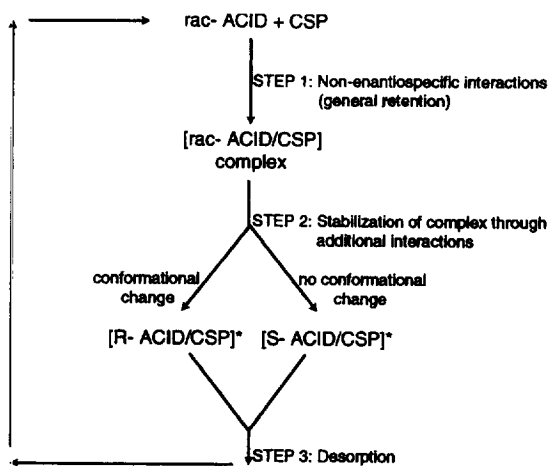This is an interesting direction for the study of

chiral recognition mechanisms, which will necessitate further investigations. A key aspect which is not yet available, is the elucidation of the CSP molecular conformation via such methods as NMR. Yashima et al. [47] has reported some initial NMR studies using a cellulose tris(4-trimethylsilylphenylcarbamate) CSP, and a few studies have been attempted on various amylose derivatives [48]. However, direct studies with the AD-CSP are difficult due to the insolubility of the phase [48].

Further studies with the AD-CSP and related phases are underway and will be reported elsewhere. A combination of the QSERR and molecular modelling results from this and the following studies, should help clarify the chiral recognition mechanism.

Acknowledgments

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