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Evaluation of the Enantiomeric Resolution of 7,8-Dihydroxy-7,8-dihydrobenzo[*a*]-pyrene and Its 6-Fluoro and 6-Bromo Derivatives on Polysaccharide-Derived Stationary Phases

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Abstract: The enantiomeric resolution and the elution order of (±)-*trans*-7,8-dihydrodiols of benzo[*a*]pyrene and its 6-fluoro and 6-bromo derivatives were analyzed on three polysaccharide-based columns: Daicel Chiralcel CA-I (cellulose triacetate), OF, and OG [cellulose tris(4-chloro- and 4-methylphenylcarbamate)]. For comparison, the separation of (±)-1,1′-bi-2-naphthol was evaluated on the OG and OF columns. Possibly similar interactions of (*S*)-1,1′-bi-2-naphthol and (7*S*,8*S*)-isomers of 6-halo-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene with the chiral sorbent are suggested.

Benzo[*a*]pyrene (B*a*P) is a known environmental pollutant[1] that is metabolized to carcinogenic bay-region diol epoxides via dihydrodiols. Introduction of a halo substituent into position 6 of the B*a*P system dramatically affects its tumorigenic properties, resulting in diminished activity.[2] The peri C-6 substituent also influences the conformation of the dihydro and the tetrahydro ring in the dihydrodiol and diol epoxide metabolites. Thus, among the dihydrodiols, a preference for the quasi-diaxial orientation of vicinal hydroxyl groups is observed in the 6-bromo[3] and 6-fluoro[4] 7,8-dihydrodiols, whereas in the 6-H analogue, these are quasi-diequatorial. Also, among the diol epoxides, the (7*R*,8*S*)-diol (9*S*,10*R*)-epoxide of B*a*P is a potent tumorigen, whereas a lack of tumorigenic activity has been reported for the 6-fluoro analogue.[5] This has been attributed to the conformational differences.

Recently, the synthesis of the (±)-*trans*-7,8-dihydrodiol of 6-fluoro-benzo[*a*]pyrene was reported.[4] In light of the small quantities needed, direct resolution by chiral HPLC was preferred, rather than via diastereomer separation.[6] Separation of dihydro and tetrahydrodiol derivatives of several polycyclic aromatic hydrocarbons (PAHs) has been extensively studied on amino acid-based enantioselective columns.[7−13] Resolution of B*a*P dihydrodiol has been achieved on a column packed with (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine, ionically bonded to α-aminopropylsilanized silica (Regis Pirkle I-A).[7,8] On the basis of this result, the separation of B*a*P dihydrodiol, as well as the 6-fluoro analogue was attempted on the Regis Pirkle I-A column. Although the B*a*P dihydrodiol enantiomers separated as reported, the 6-fluoro B*a*P dihydrodiol enantiomers did not.

This led us to investigate other commercially available columns for the separation of the 6-fluoro B*a*P dihydrodiol. Although other amino acid-based columns are commercially available, we were surprised to find that no studies on the separation of dihydrodiol or other PAH metabolites have been reported using polysaccharide-based columns, despite their various successful applications.[14−20] Excellent separation of the (±)-*trans*-7,8-dihydrodiol of 6-fluoro-B*a*P on a Daicel Chiralcel OG column[4] initiated our present investigation.

Among the commercially available cellulose-based Daicel columns, three were chosen for the study: Chiralcel CA-I (microcrystalline cellulose triacetate), Chiralcel OG and OF (phenylcarbamate derivatives of cellulose coated on silica gel). As substrates, 6-H-B*a*P-DHD, 6-F-B*a*P-DHD and 6-Br-B*a*P-DHD (Figure 1) were chosen. These close structural analogues offer conformational diversity, which can be valuable in understanding chiral recognition. In the C-6 protio analogue, the hydroxyls are predominantly quasi-diequatorial, while in the C-6 halo analogues, these are predominantly quasi-diaxial. The syntheses of B*a*P dihydrodiol[21] and the C-6 fluoro analogue[4] have been reported, while the C-6 bromo analogue was synthesized from 6-bromo-9,10-dihydrobenzo[*a*]pyrene[4] (see Supporting Information). (±)-6-Br-B*a*P-DHD

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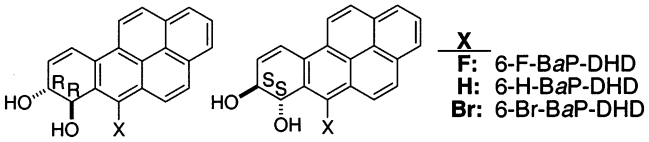

FIGURE 1. Structures of B*a*P dihydrodiol enantiomers as well as the 6-halo analogues.

TABLE 1. Enantiomeric Resolution of 6-Br, 6-F, and 6-H-B*a*P-DHD on Chiralcel CA-I, OF, and OG

	Chiralcel CA-I[a]			Chiralcel OF[f,g]			Chiralcel OG[f,j]		
X	α[b]	R[c]	k_1'[d]	α[b]	R[h]	k_1'[d]	α[b]	R[h]	k_1'[d]
Br	1.45	0.6[e]	1.62 (7*R*,8*R*)	u[i]	u[i]	4.12[i] (±)	1.36	1.4	3.97 (7*R*,8*R*)
F	1.47	0.7	2.20 (7*R*,8*R*)	1.23	0.9	3.21 (7*S*,8*S*)	1.41	1.8	2.56 (7*R*,8*R*)
H	1.74	1.5	3.70 (7*R*,8*R*)	1.27	0.8	1.31 (7*S*,8*S*)	1.33	1.3	1.51 (7*S*,8*S*)

[a] Analytical column, 0.46 × 25 cm; solvent system, 15% H_2O in MeOH; flow rate, 0.5 mL/min. [b] $\alpha = k_2'/k_1'$. [c] Resolution factor, as defined by Cai and Wu,[23] was used. [d] k_1': retention factor, *early*-eluted isomer. [e] Value = 0.8 when 20% H_2O in MeOH was used. [f] Analytical column, 0.46 cm × 25 cm with a 0.46 cm × 5 cm precolumn; column temperature, 30 °C; solvent, 1:1 *n*-hexane−*i*-PrOH; t_o was measured by the use of 1,3,5-tri-*tert*-butylbenzene.[26] [g] Flow rate: 0.8 mL/min. [h] Resolution factor: $R = 1.18\,(t_{R(2)} - t_{R(1)})/(w_{1/2(1)} + w_{1/2(2)})$, where $w_{1/2}$ is the peak width on half-height. [i] Unresolved. [j] Flow rate: 0.9 mL/min.

was resolved by chiral HPLC (Daicel Chiralcel OG) and chirality of the enantiomers was established by comparison of their CD spectra to that of *trans*-(7*R*,8*R*)-6-bromo-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene[3] (Figure A in Supporting Information).

Enantiomeric Separation by Chiralcel CA-I. Cellulose triacetate (CTA-I), was first reported by Hesse and Hagel[22] to possess chiral resolving properties, and its microcrystalline structure was postulated to be crucial for enantioselection. Table 1 shows the resolution of all three substrates on Chiralcel CA-I, a microcrystalline cellulose triacetate.

Comparison of k' values under identical chromatographic conditions (15% water in methanol) revealed that, in each set of *early*- and *late*-eluting dihydrodiols, the bromo is the fastest eluting, followed by the fluoro and then by the protio analogue. This order is in agreement with the presumption that the flat molecules fit better into chiral cavities of CTA-I.[24] In solution, 6-H-B*a*P-DHD is closer to planarity, possibly due to an intramolecular hydrogen bond between the two equatorial hydroxyls,[25] which contrasts to the 6-F- and 6-Br-B*a*P-DHD, where the hydroxyl groups are predominantly diaxial. Better separation of the 6-Br derivative was obtained when 20% water in methanol was used as the eluent. In each case the (7*R*,8*R*)-isomer eluted *early*.

Enantiomeric Separation by Cellulose Phenylcarbamates. Among polysaccharide derivatives coated on silica gel, cellulose and amylose phenylbenzoates as well as phenylcarbamates have proven to possess very good resolving qualities.[16−20] A wide range of cellulose tris(phenylcarbamate) derivatives have thus been studied as HPLC chiral stationary phases (CSPs).[26] Introduction of a methyl or halo substituent at the meta and/or para position of the phenylcarbamate increases the enantioseparating power of the CSP. We therefore chose for our study a Daicel Chiralcel OF column (a 4-chloro substituted phenylcarbamate) and a Daicel Chiralcel OG column (a 4-methyl-substituted phenylcarbamate).

Chromatography on a Daicel Chiralcel OF Column. HPLC of the three racemates was performed at 30 °C and under identical conditions (1:1 2-propanol−*n*-hexane). Only 6-H and 6-F-B*a*P-DHD were resolved, while 6-Br was not (Table 1).

As evident from Table 1, the elution times of the enantiomers of 6-H-B*a*P-DHD were shorter than those of the 6-F, as well as that of the unresolved 6-Br-B*a*P-DHD. This could be explained on the basis of the conformation of the cyclohexadienyl moiety in the dihydrodiols. Such an effect on the HPLC elution orders of isomeric PAH dihydrodiols was first suggested by Grover et al.,[27] who studied their chromatographic behavior on an achiral silica column. Within a series of dihydrodiols derived from a hydrocarbon, trans isomers with quasi-diequatorial hydroxyl groups eluted much earlier, possibly due to an intramolecular hydrogen bond.[27] Such a phenomenon may be responsible for the observed retention times in the chiral resolution as well. As a consequence, there are possibly fewer hydrogen-bonding interactions between the solute and the CSP in the case of 6-H-B*a*P-DHD enantiomers, which is apparently not the case with the 6-F and 6-Br derivatives. For both resolved substrates the (7*S*,8*S*)-isomer eluted early.

Chromatography on a Daicel Chiralcel OG Column. The Daicel Chiralcel OG column was found to be the best for the resolution of the dihydrodiols (Table 1, 30°C, 1:1 2-propanol-*n*-hexane, also see Figure B in Supporting Information). Comparison of the retention times of the enantiomers of the three substrates shows that the 6-H-B*a*P-DHD enantiomers had the shortest elution times, followed by the 6-F derivative. The retention times of the 6-Br-B*a*P-DHD enantiomers were approximately twice as long as those of 6-H-B*a*P-DHD. This could again be explained by lesser solute−CSP hydrogen bond interactions in the case of 6-H-B*a*P-DHD, due to intramolecular hydrogen bonding.[27] The elution order of the 6-H-B*a*P-DHD enantiomers was the same as on the Chiralcel OF column, with the (7*S*,8*S*)-enantiomer eluting first. On the other hand, in the C-6 halo analogues, the (7*R*,8*R*)-isomer eluted early.

Evaluation of CSP−PAH Solute Interactions on OF and OG Columns and Comparison to CSP−Binol Interactions. Attractive interactions between the solute and the polar carbamate groups of the CSP have been suggested as the primary mechanism for chiral recognition by phenylcarbamate derivatives of cellu-

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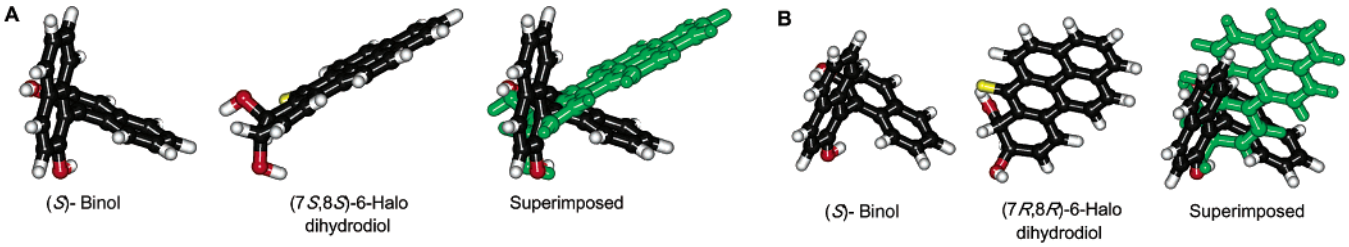

FIGURE 2. Geometrically optimized (HyperChem) structures of (A) (*S*)-Binol, (7*S*,8*S*)-6-halo dihydrodiol, and the manually superimposed structures and (B) (*S*)-Binol, (7*R*,8*R*)-6-halo dihydrodiol, and the manually superimposed structures.

TABLE 2. Comparison of the *k*′ of (7*S*,8*S*)- and (7*R*,8*R*)-Isomers of the B*a*P Dihydrodiols on Chiralcel OG and OF[a]

	Chiralcel OG		Chiralcel OF	
substituent X[a]	*k*′: (7*S*,8*S*)	*k*′: (7*R*,8*R*)	*k*′: (7*S*,8*S*)	*k*′: (7*R*,8*R*)
H	1.51	2.01	1.31	1.66
	$\Delta k'$: $(k'_F - k'_H) = 2.1$	$\Delta k'$: $(k'_F - k'_H) = 0.5$	$\Delta k'$: $(k'_F - k'_H) = 1.9$	$\Delta k'$: $(k'_F - k'_H) = 2.3$
F	3.61	2.56	3.21	3.95
	$\Delta k'$: $(k'_{Br} - k'_H) = 3.9$	$\Delta k'$: $(k'_{Br} - k'_H) = 2.0$		
Br	5.40	3.97	4.12[b]	4.12[b]

[a] Substituent at position 6 of the 7,8-dihydrodiol of B*a*P. [b] Unresolved.

lose.[16−20] These include H-bonding with the NH and C=O groups, dipole−dipole stacking,[26,28] and $\pi-\pi$ interactions.[15,29] With solutes containing hydroxyl groups, hydrogen bonding between the −OH and the carbamate residues is mainly implicated for chiral recognition.[19,20,30,31]

For a better understanding of PAH dihydrodiol interactions with phenylcarbamate-derived cellulose, we evaluated the literature for reports on the resolution and chiral recognition studies of dihydroxylated substrates. Okamoto et al. have performed a thorough study of solute−CSP interactions on 1,1′-bi-2-naphthol (Binol) and the chloroform-soluble cellulose tris(5-fluoro-2-methylphenylcarbamate).[30] The cellulose polymer used in the study was suggested to have a left-handed 3/2 helical structure[30] such as the crystalline cellulose tris(phenylcarbamate)[32] and tris(4-chlorophenylcarbamate). The latter is the OF CSP. In these studies, a model showing a CSP:(*S*)-Binol complex with two simultaneous hydrogen bonds between the two hydroxyls of bi-2-naphthol and CO groups of the carbamate moieties of two neighboring glucose units was proposed. On the other hand, in the case of (*R*)-Binol, hydrogen bonding between only one hydroxyl group of the solute and CO of the CSP was suggested.

We became interested in assessing whether there were similarities in the CSP−solute interactions observed in Binol to those of dihydrodiols with diaxially oriented hydroxyls. We therefore evaluated the intramolecular distance of the OH groups in Binol as well as 6-Br- and 6-F-B*a*P-DHD through the use of the HyperChem suite of computational programs (see Supporting Information). The (7*S*,8*S*)-isomers of 6-Br- and 6-F-B*a*P-DHD were geometrically optimized and manually superimposed with that of (*S*)-Binol. Nearly equivalent oxygen−oxygen distances were found in both, 6-Br- and 6-F-B*a*P-DHD and (*S*)-Binol (Figure 2). In (7*S*,8*S*)-isomers of 6-Br- and 6-F-B*a*P-DHD, the rest of the polyaromatic moiety is extended in such a fashion that, according to the model reported for (*S*)-Binol,[30] it could fit into the CSP groove (which is not the case with the (7*R*,8*R*)-isomers, Figure 2).

To evaluate whether any qualitative indication of the similarity of the CSP−PAH complex to the model proposed for Binol could be derived from the data obtained with the dihydrodiols, we decided to analyze the data based on the conformational differences. Since separations of these closely related substrates were performed under identical conditions, a comparison of the *k*′ values within the series of (7*S*,8*S*)- and (7*R*,8*R*)-isomers was possible with each column. The *k*′ values are presented in Table 2.

Comparison of *k*′ Values for (7*S*,8*S*)-Isomers: On the OG CSP. Inspection of *k*′ of 6-H and 6-F derivatives for the (7*S*,8*S*)-isomers on the OG column reveals a $\Delta k'$ of 2. This difference in *k*′ may be a consequence of the differences in hydrogen bonding interactions of the substrates, allowing for an intramolecular hydrogen bond in the protio analogue.[27] This is not possible in 6-F-B*a*P-DHD, leaving both hydroxyl groups available for interactions with the CSP. Comparison of the protio analogue to the 6-Br revealed a $\Delta k'$ of ∼4. This could indicate that the (7*S*,8*S*)-isomer of the 6-bromo derivative forms a CSP−solute complex with more concomitant interactions than the 6-fluoro derivative, possibly including a bromine atom.

On the OF CSP. The same trend is observed in the series of (7*S*,8*S*)-isomers on the OF CSP as well, though the values of *k*′ are lower as compared to those on the OG CSP, indicating weaker CSP−solute complexes. An approximately equal difference of 2 is observed between the *k*′ of the protio and the fluoro analogue, with the latter being more retained. This again points to the possible solute−CSP complex with two simultaneous interactions in the case of 6-F-B*a*P-DHD as described above. (±)-6-Br-B*a*P-DHD, however, was not resolved on this column.

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Comparison of *k′* Values for (7*R*,8*R*)-Isomers: On the OG CSP. Interestingly, within the series of (7*R*,8*R*)-isomers, there is a small difference between the *k′* of the 6-H and 6-F analogues on the OG CSP, indicating a similar number of attractive interactions in the CSP−solute complex, despite different conformational preferences, while a difference of ∼2 between the *k′* of the 6-H and 6-Br derivatives is observed. Although there is an increase in Δ*k′* (∼2) between the protio and the bromo derivative, it is only half of what was observed for the corresponding (7*S*,8*S*)-isomer on the OG CSP. Thus, the (7*R*,8*R*)-isomers of both 6-halo derivatives show fewer simultaneous attractive interactions to the OG CSP than the corresponding (7*S*,8*S*)-isomers.

On the OF CSP. On the OF CSP, 6-Br-B*a*P-DHD was not resolved. There is a difference of ∼2 between the *k′* of the 6-H and 6-F derivatives, with the latter more retained. Here again, the Δ*k′* could suggest two simultaneous interactions between the solute and the CSP in the case of 6-F and not in the protio analogue, as described earlier. This is in contrast to the observation obtained on the OG CSP for (7*R*,8*R*)-isomers of the 6-H and 6-F derivatives, mentioned above, where a similar number of simultaneous solute−CSP interactions was suggested for both.

The polarity of the carbamate moiety is modulated by the substituents on the phenyl ring, which consequently influences the separating properties of the CSP.[26] The effect of the substituents on the adsorption properties of 4-substituted phenylcarbamate CSPs has been quantitatively evaluated by plotting the retention times of the first-eluted isomer of substrates containing a carbonyl group or a hydroxy group, against the Hammett σ_p substituent values.[17a,b] With increasing electron-withdrawing power of substituents on the phenyl ring, the substrate containing the carbonyl group was retained more. This was explained by increased acidity of the carbamate NH moiety leading to stronger hydrogen bonds to the basic site of the substrate. On the other hand, increasing electron-withdrawing power of the substituents diminished the basicity of the carbamate carbonyl, resulting in lower retention of the hydroxyl group containing substrate. This was explained by weaker hydrogen bonds between the carbonyl of the CSP and the hydroxyl of the substrate.[17a,b]

To gain an idea about the nature of the CSP−solute attractive forces operative in the resolution, the *k′* values of (7*S*,8*S*)- and (7*R*,8*R*)-isomers were compared on the two columns, in the light of the relative basicity/acidity of the sorbents resulting from the electronic effects of the 4-substituent on the phenylcarbamate.[17]

The OG CSP with the 4-methyl subsitutent (Hammett σ_p substituent value of −0.14) is a more basic sorbent and therefore a better hydrogen bond acceptor via the basic carbonyl moiety of the carbamate compared to the OF CSP. On the other hand, the OF CSP with the 4-chloro substituent (Hammett σ_p substituent value +0.24) contains more acidic NH groups and is likely a better proton donor than the OG CSP in hydrogen bonding interactions. Therefore, we reasoned that a comparison of the *k′* values (Table 2) of any given enantiomer on these two CSP systems could provide insight into the types of hydrogen bond donor−acceptor interactions. Since the (7*S*,8*S*)-enantiomers of the 6-H, 6-F, and 6-Br dihydrodiols as well as the (7*R*,8*R*)-isomer of the 6-H dihydrodiol show a greater retention on the OG CSP compared to the OF, it is possible that the solute−CSP interactions include those where the solute is a hydrogen bond donor via the hydroxyl protons. On the other hand, the 6-F (7*R*,8*R*)-dihydrodiol enantiomer is more retained on the OF CSP, and in this case, the solute−CSP interactions could involve the CSP as the hydrogen bond donor (through the carbamate NH) to the solute as well. It is difficult to make any predictions about the 6-Br dihydrodiols because these were not resolved on the OF CSP; however, the (7*S*,8*S*)-enantiomer is more retained on the more basic OG column compared to the racemate on the OF CSP.

Since the basis of our model for the enantiomeric separation of the 6-halo dihydrodiols was derived by analogy to (*S*)-Binol, we reasoned that if effects observed in the dihydrodiol separation translated to the separation of Binol, further support for the proposed model could be obtained. For this, we compared the separation of (±)-6-F-B*a*P-DHD on the OG and OF CSPs to that of (±)-Binol. As indicated earlier, (7*R*,8*R*)-6-F-B*a*P-DHD eluted early on the OG column and, on the OF CSP, this enantiomer eluted late. With (±)-Binol, (*R*)-(+)-Binol was early eluting on the OG CSP, whereas this elution order was reversed on the OF CSP, as was observed with the enantiomers of 6-F-B*a*P-DHD. Whereas only a small peak separation between Binol enantiomers was obtained under the HPLC conditions used to resolve the dihydrodiol enantiomers, co-injection of the racemic Binol with an enantiopure isomer enabled a clear distinction between the isomers. These results provide additional support to our proposed solute−CSP interactions in the enantioresolution of axially constrained non-bay-region dihydrodiols of PAHs.

In conclusion, various polysaccharide-based enantioselective columns can be successfully used for the resolution of chiral metabolites of PAHs. Although separation of all three substrates was attained on both the CA-I and the Chiralcel OG CSPs, significantly better resolution was observed on the latter (also partly due to peak broadening on the CA-I). A large set of close structural analogues such as a set of PAH dihydrodiols offers a good pool of substrates for further studies of solute−CSP interactions on chiral sorbents.

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Supporting Information Available: Scheme and description of synthesis of 6-Br-B*a*P-DHD, enantiomeric resolution of 6-Br-B*a*P-DHD, computational details, CD spectra of (7*R*,8*R*)- and (7*S*,8*S*)-6-Br-B*a*P-DHD, and HPLC traces for resolution of (±)-6-Br-B*a*P-DHD, (±)-6-F-B*a*P-DHD, and (±)-6-H-B*a*P-DHD on a Chiralcel OG column. This material is available free of charge via the Internet at http://pubs.acs.org.

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