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DIRECT RESOLUTION OF MONO- AND DIOL ENANTIOMERS OF UNSUBSTITUTED AND METHYL-SUBSTITUTED BENZ[*a*]ANTHRACENE AND BENZO[*a*]PYRENE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH A CHIRAL STATIONARY PHASE*

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SUMMARY

The direct resolution of 86 structurally related monomethyl, mono-ol, and *trans*- and *cis*-diol enantiomers of unsubstituted and methyl-substituted benz[*a*]anthracene and benzo[*a*]pyrene was investigated by high-performance liquid chromatography with a commercially available column, packed with an (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine, ionically bonded to γ-aminopropylsilanized silica. The results indicate that structural factors, such as conformation, presence of a methyl substituent, molecular size and shape, and ring saturation all contribute to chiral interactions between the chiral stationary phase and the solutes. Detailed chiral recognition mechanisms can not yet be established, due to complex structural factors that influence enantiomeric resolutions and the lack of data on the absolute configurations of the resolved enantiomers. Nevertheless, the chromatographic method can be applied to the determination of enantiomeric purity of mono- and diol metabolites of polycyclic aromatic hydrocarbons. The absolute configurations of a limited number of resolved enantiomers have been established.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants and some are believed to contribute to the incidence of cancer in man. Carcinogenic PAHs require metabolic activation by the mammalian drug-metabolizing

* The opinions or assertions contained in this article are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences or the Food and Drug Administration. The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals", Institute of Animal Resources, National Research Council, DHEW Pub. No. (NIH) 78-23.

enzyme systems to exert their mutagenic and carcinogenic activities[1]. Most PAH metabolites, such as epoxides, dihydrodiols, phenol-dihydrodiols, dihydrodiol-epoxides, triols and tetrols are optically active[1,2]. The ultimate carcinogenic metabolite of benzo[*a*]pyrene (BaP), for instance, is a diastereomeric 7,8-dihydrodiol-9,10-epoxide of high optical purity[2]. We have been interested in the elucidation of the stereochemical pathways of metabolism in the detoxification and activation of PAHs. Research efforts include the resolution of optical isomers and the elucidation of their absolute configurations. The optical purity of BaP dihydrodiol metabolites has been previously determined by high-performance liquid chromatographic (HPLC) resolution of diastereomers, derivatized with (−)-menthoxyacetyl chloride[2–4] or (−)-α-methoxy-α-trifluoromethylphenylacetyl chloride[5,6]. Recently, we developed an HPLC method by using a commerically available column packed with a chiral stationary phase (CSP), (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine, ionically bonded to γ-aminopropylsilanized silica to resolve some dihydrodiol and tetrahydrodiol enantiomers of benz[*a*]anthracene (BA) and BaP directly[7]. The method has been applied to determine the optical purity of some dihydrodiols and mono-ols (cyclic alcohols) formed in the metabolism of several PAHs by hepatic microsomes[7–10]. In some cases, the absolute configurations of the enzymatically formed PAH dihydrodiols can be determined[9,10].

Pirkle *et al.*[11,12] have successfully resolved the enantiomers of a large number of compounds with the CSP that they have developed. A chiral recognition mechanism was proposed[12] to predict the enantiomer of cyclic alcohols (mono-ols) as well as other types of compounds more strongly retained by the ionically bonded CSP. However, the exact chiral recognitions that contribute to the enantiomeric resolutions[8,12–14] have not been established, because the absolute configurations of most of the mono-ol derivatives studied were not known. Furthermore, not all structurally similar mono-ols can be resolved[8,14].

It is necessary to know the absolute configurations of the resolved enantiomers before the exact chiral recognition mechanisms can be elucidated. It is also important to understand the structure–resolution relationships. For these purposes, we have studied the resolution of a series of structurally related mono-ol and diol derivatives of BA and BaP by an ionically bonded CSP. Various regions that affect the conformational preference of diol derivatives of BA and BaP are indicated in Fig. 1. For the purpose of indicating the conformation of the diols, the double bonds adjacent

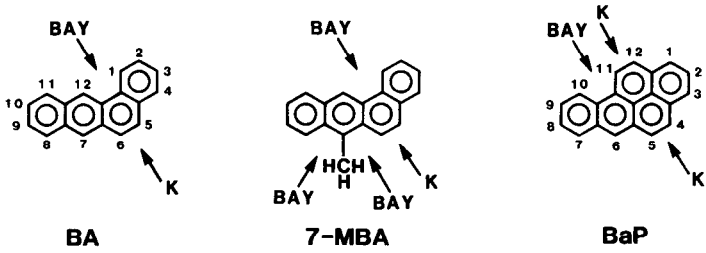

Fig. 1. Numbering system and the bay- and K-region designations in BA, 7-MBA, and BaP. K-region is the most electron-rich region of the molecule, and bay region is the angular region, such as the area between and including the C_1 and C_{12} positions of BA. For the purpose of designating the quasiaxial conformations of hydroxyl groups adjacent to a *peri* methyl group, the area adjacent to the *peri* methyl group are also designated as bay regions.

to a *peri* methyl group are also designated as bay regions. The results described in this report reveal many structural factors that influence the resolution of enantiomers.

MATERIALS AND METHODS

Materials

Mono- and diols of unsubstituted and methyl-substituted BA and BaP were obtained by (i) reduction of ketone precursors by $NaBH_4$, (ii) methylation of ketone precursors by CH_3Li, (iii) oxidation of appropriate precursors by OsO_4, (iv) catalytic hydrogenation of appropriate precursors in the presence of either PtO_2 or Pd powder, (v) acid-catalyzed dehydration of appropriate mono-ols followed by PtO_2-catalyzed hydrogenation, (vi) HPLC isolation of metabolites formed in the metabolism of unsubstituted and methyl-substituted BAs by rat liver microsomes[15–17]. Some dihydrodiols of BA and BaP were obtained from the Chemical Repository of the National Cancer Institute. Synthetic procedures of chemicals used in this study are either available in the literature[6,10,18–22] or to be published elsewhere.

Chromatography

Chemicals were analyzed on HPLC columns (25 cm × 4.6 mm I.D., Regis Chemical, Morton Grove, IL, U.S.A.), packed with an (*R*)-N-(3,5-dinitrobenzoyl) phenylglycine, ionically bonded to spherical particles of 5 μm diameter of γ-aminopropylsilanized silica[23]. HPLC was performed by using a Waters Assoc. (Milford, MA, U.S.A.) liquid chromatograph, consisting of a Model 6000A solvent delivery system, a Model M45 solvent delivery system, a Model 660 solvent programmer and a Model 440 absorbance detector. Samples were injected via a Valco model N60 loop injector (Valco, Houston, TX, U.S.A.). Samples were analyzed isocratically with a flow-rate of 2 ml/min using premixed solvents of up to 18% (v/v) of solvent A (ethanol–acetonitrile, 2:1, v/v) in hexane at ambient temperature. Resolved enantiomers were purified and their circular dichroism spectra were obtained as described[7–10]. The enantiomers of some diol derivatives previously found unstable or not resolved[7] are resolved by conditions described in this report. Better resolutions of enantiomers than previously obtained have been achieved with the new columns[7]. The prolonged usage of a column and the use of a less sensitive detector of a larger volume flow cell may have contributed to the undesirable results observed in our earlier study[7].

Spectral analysis

UV–visible absorption spectra of samples in methanol were determined using a 1-cm path length quartz cuvette with a Varian Model 118C spectrophotometer. Mass spectral analysis was performed on a Finnigan Model 4000 gas chromatograph–mass spectrometer–data system by electron impact with a solid probe at 70 eV and 250°C ionizer temperature. Circular dichroism (CD) spectra of samples in methanol were measured in a cell of 1-cm path length at room temperature using a Jasco Model 500A spectropolarimeter equipped with a Model DP-500 data processor. CD spectra are expressed by ellipticity, as described[8–10]. ^1H NMR spectra of samples in acetone-d_6 with a trace amount of ^2H_2O were obtained on a Bruker WM270 Fourier transform high-resolution spectrometer.

RESULTS AND DISCUSSION

The resolutions of mono- and diol enantiomers derived from the 1,2,3,4-ring of BA and methyl-BAs by CSP–HPLC are summarized in Fig. 2 and Table I. The chromatographic properties of the compounds derived from the 8,9,10,11-ring of BA and 7,8,9,10-ring of BaP are indicated in Tables I–III. The resolutions of mono-ol and diol enantiomers derived from the K-regions of BA, monomethyl-BA (MBA), and BaP as well as the mono-ol at C_3 position of BaP are shown in Tables IV and V. The results shown in Fig. 2 and Tables I–V indicate that many structural factors influence the resolutions of enantiomers. The discernible structural factors which influence the resolution of enantiomers are described below.

Benzylic chiral center bearing a methyl group

The resolution of the enantiomers of six methylated compounds, each containing a methyl group on a benzylic chiral center, were tested. The enantiomers of 10-methyl-7,8,9,10-H_4BaP were resolved (Table III) and were confirmed by CD (Fig. 3), UV absorption, and mass spectral analyses. The enantiomers of 1-methyl-1,2,3,4-H_4BA (1,2,3,4-H_4-1-MBA; other methyl derivatives are similarly abbreviated) were marginally resolved, with a resolution value of less than 0.1 (Table I). As far as we know, these are the only known examples indicating that enantiomers of compounds with a methyl group on a benzylic chiral center can be resolved by the CSP used. It is interesting to note that the methyl group in both compounds are in the bay region. Other non-bay region methylated H_4BA and H_4BaP antipodes were not resolved (Tables I–III). These results indicate that a bay region methyl group can interact with the CSP to effect enantiomeric resolution.

Presence of a methyl group at the benzylic carbinol position greatly improves the resolution of mono- and diol enantiomers. For example, 1-methyl-1-OH-1,2,3,4-H_4BA (1-OH-1,2,3,4-H_4-1-MBA; other mono-ol derivatives are similarly abbreviated) and 4-methyl-1,2,3,4-H_4BA *trans*-3,4-diol (1,2,3,4-H_4-4-MBA *trans*-3,4-

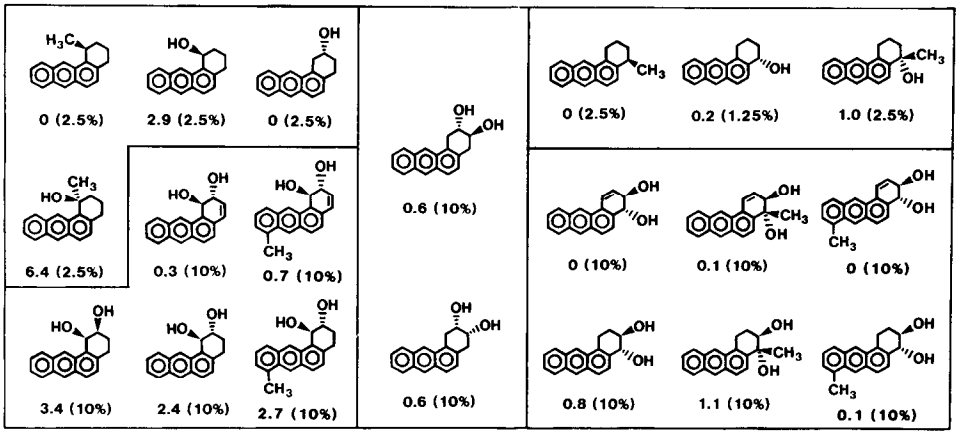

Fig. 2. Structure and CSP–HPLC resolution of monomethyl, mono-ol, and diol enantiomers derived from the 1,2,3,4-ring of unsubstituted and methyl-substituted BA. The numbers below each structure indicate the resolution value and the solvent (percentage of solvent A in hexane) used for chromatography.

TABLE I

CSP-HPLC RESOLUTION OF MONO- AND DIOL ENANTIOMERS DERIVED FROM THE 1,2,3,4-RING OF BENZ[*a*]ANTHRACENE AND ITS METHYLATED DERIVATIVES

Column packing, (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine, ionically bonded to γ-aminopropylsilanized silica; column size, (250 × 4.6 mm I.D.); solvent A, ethanol–acetonitrile (2:1, v/v); flow-rate, 2 ml/min; void time, 1.2 min.

*Chemical**	*Solvent A in hexane (%)*	*Retention time*		*Resolution value***
		Peak a	*Peak b*	
1,2,3,4-H_4-1-MBA	2.5	2.4	2.4	0
	0	6.7	6.8	0.1 > RV > 0
1-OH-1,2,3,4-H_4BA	5	8.9	9.6	1.4
	2.5	19.5	22.9	2.9
1-OH-1,2,3,4-H_4-1-MBA	10	3.8	4.2	1.7
	5	7.2	9.3	3.5
	2.5	10.5	15.0	6.4
2-OH-1,2,3,4-H_4BA	5	15.1	15.1	0
	2.5	31.7	31.7	0
BA *trans*-1,2-H_2diol	10	39.8(*S,S*)	40.6(*R,R*)	0.3
BA 1,2,3,4-H_4-*trans*-1,2-diol	10	24.8(*S,S*)	27.6(*R,R*)	2.4
8-MBA *trans*-1,2-H_2diol	18	17.5(*S,S*)	18.0(*R,R*)	0.4
	15	24.0(*S,S*)	24.7(*R,R*)	0.5
	10	50.8(*S,S*)	52.5(*R,R*)	0.7
8-MBA 1,2,3,4-H_4-*trans*-1,2-diol	18	12.2(*S,S*)	13.6(*R,R*)	1.7
	15	16.3(*S,S*)	18.3(*R,R*)	2.0
	10	31.8(*S,S*)	36.4(*R,R*)	2.7
BA 1,2,3,4-H_4-*cis*-1,2-diol	18	14.0	18.6	2.7
	15	15.8	21.3	3.0
	10	28.1	38.8	3.4
BA 1,2,3,4-H_4-*trans*-2,3-diol	10	38.9	40.2	0.6
BA 1,2,3,4-H_4-*cis*-2,3-diol	10	39.8	41.1	0.6
1,2,3,4-H_4-4-MBA	2.5	2.7	2.7	0
	0	12.6	12.6	0
4-OH-1,2,3,4-H_4BA	1.25	54.5	55.0	0.2
4-OH-1,2,3,4-H_4-4-MBA	5	12.4	12.7	0.2
	2.5	21.8	22.9	1.0
BA *trans*-3,4-H_2diol	10	28.0	28.0	0
	5	71.5	71.5	0
BA 1,2,3,4-H_4-*trans*-3,4-diol	10	30.4(*S,S*)	31.7(*R,R*)	0.8
	5	78.9(*S,S*)	82.7(*R,R*)	0.9
4-MBA *trans*-3,4-H_2diol	10	16.0	16.2	0.1
	7.5	20.0	20.4	0.3
4-MBA 1,2,3,4-H_4-*trans*-3,4-diol	10	18.6	19.7	1.1
8-MBA *trans*-3,4-H_2diol	15	20.4	20.4	0
	10	33.9	33.9	0
8-MBA 1,2,3,4-H_4-*trans*-3,4-diol	18	16.4	16.4	0
	15	21.0	21.0	0
	10	37.8(*S,S*)	39.0(*R,R*)	0.1

* Chemicals are abbreviated as indicated in the text.

** Resolution value = $(V_2 - V_1)/[(W_1 + W_2)/2]$ where V is the retention volume and W is the peak width at base.

TABLE II

CSP-HPLC RESOLUTION OF MONO- AND DIOL ENANTIOMERS DERIVED FROM THE 8,9,10,11-RING OF BENZ[*a*]ANTHRACENE AND ITS METHYLATED DERIVATIVES

Chromatographic conditions and abbreviations are the same as indicated in Table I.

Chemical	*Solvent A in hexane (%)*	*Retention time*		*Resolution value*
		Peak a	*Peak b*	
8,9,10,11-H_4-8-MBA	1	3.4	3.4	0
	0	10.0	10.0	0
8-OH-8,9,10,11-H_4BA	3	16.5	17.6	1.3
8-OH-8,9,10,11-H_4-8-MBA	5	10.5	10.6	0.1
	2.5	19.0	19.6	0.5
BA *trans*-8,9-H_2diol	15	19.2(*S,S*)	20.6(*R,R*)	1.3
	10	35.3(*S,S*)	38.1(*R,R*)	1.6
BA 8,9,10,11-H_4-*trans*-8,9-diol	10	39.4(*S,S*)	46.5(*R,R*)	3.6
	15	22.1(*S,S*)	25.7(*R,R*)	3.4
	18	14.4(*S,S*)	17.5(*R,R*)	3.4
8-MBA *trans*-8,9-H_2diol	15	10.6(*S,S*)	12.1(*R,R*)	2.3
	10	16.6(*S,S*)	19.6(*R,R*)	3.4
8-MBA 8,9,10,11-H_4-*trans*-8,9-diol	18	10.0(*S,S*)	12.3(*R,R*)	3.3
	15	12.3(*S,S*)	15.1(*R,R*)	3.5
	10	21.1(*S,S*)	26.9(*R,R*)	4.7
11-MBA *trans*-8,9-H_2diol	10	27.8(*S,S*)	29.9(*R,R*)	1.0
8,9,10,11-H_4-11-MBA	2.5	3.1	3.1	0
	0	9.7	9.7	0
11-OH-8,9,10,11-H_4BA	5	13.3	14.0	1.3
11-OH-5,6,8,9,10,11-H_6BA	1	26.4	28.9	2.0
11-OH-5,6,8,9,10,11-H_6-10-MBA (*trans*)*	1	13.3	14.1	1.2
11-OH-8,9,10,11-H_4-11-MBA	5	11.0	11.9	1.6
11-OH-5,6,8,9,10,11-H_6-11-MBA	5	4.0	4.0	0
	1	8.7	9.8	2.1
BA *trans*-10,11-H_2diol	10	28.1(*S,S*)	30.4(*R,R*)	1.5
BA 8,9,10,11-H_4-*trans*-10,11-diol	18	16.2(*S,S*)	18.9(*R,R*)	3.7
	10	34.4(*S,S*)	42.4(*R,R*)	4.8
8-MBA *trans*-10,11-H_2diol	15	16.0	16.0	0
	10	26.4	26.4	0
8-MBA 8,9,10,11-H_4-*trans*-10,11-diol	18	13.9	13.9	0
	15	17.6	17.6	0
	10	31.4	31.4	0
11-MBA *trans*-10,11-H_2diol	18	8.1(*S,S*)	9.2(*R,R*)	2.5
	15	9.6(*S,S*)	11.1(*R,R*)	2.7
	10	13.4(*S,S*)	15.8(*R,R*)	3.1

* 10-Methyl group is *trans* to the 11-hydroxyl group (^{1}H NMR $J_{10,11}$ = 8.6 Hz).

diol; other diol derivatives are similarly abbreviated) are both more efficiently resolved than 1-OH-1,2,3,4-H_4BA and 1,2,3,4-H_4BA *trans*-3,4-diol, respectively (Table I). Many other examples of improved enantiomeric resolution due to the presence of a methyl group on a benzylic chiral center are listed in Tables I–IV. These results indicate that a methyl group, either in the bay region or in the non-bay region, contributes to the chiral interactions of mono- and diols that effect enantiomer separation.

TABLE III

CSP-HPLC RESOLUTION OF MONO- AND DIOL ENANTIOMERS DERIVED FROM THE 7,8,9,10-RING OF UNSUBSTITUTED AND METHYL-SUBSTITUTED BENZO[*a*]PYRENE

Chromatographic conditions and abbreviations are the same as indicated in Table I.

Chemical	*Solvent A in hexane (%)*	*Retention time*		*Resolution value*
		Peak a	*Peak b*	
7,8,9,10-H_4-7-MBaP	1	6.6	6.6	0
	0	24.9	24.9	0
7-OH-7,8,9,10-H_4-BaP	10	17.1	17.7	0.6
	5	28.0	29.1	0.8
7-OH-7,8,9,10-H_4-7-MBaP	5	21.8	22.5	0.5
	2.5	44.0	46.0	0.9
7-OH-7,8,9,10-H_4-10-MBaP	2.5	35.4	36.0	0.2
8-OH-7,8,9,10-H_4BaP	5	47.5	47.5	0
BaP *trans*-7,8-H_2diol	18	28.5(*S,S*)	32.0(*R,R*)	2.0
	15	37.3(*S,S*)	41.6(*R,R*)	2.1
BaP 7,8,9,10-H_4 *trans*-7,8-diol	18	33.4(*S,S*)	41.3(*R,R*)	3.2
BaP *cis*-7,8-H_2diol	18	40.7	41.9	0.5
BaP 7,8,9,10-H_4-*cis*-7,8-diol	18	32.8	34.2	0.8
7-MBaP *trans*-7,8-H_2diol	15	17.5(*S,S*)	22.5(*R,R*)	4.5
7-MBaP 7,8,9,10-H_4-*trans*-7,8-diol	15	24.7(*S,S*)	33.2(*R,R*)	5.3
7-MBaP *cis*-7,8-H_2diol	15	18.1	18.6	0.4
	10	29.7	30.6	0.5
7-MBaP 7,8,9,10-H_4-*cis*-7,8-diol	18	14.3	15.7	1.6
	15	17.3	18.9	1.7
9-OH-7,8,9,10-H_4BaP	5	35.0	35.0	0
10-OH-7,8,9,10-H_4BaP	10	13.3	13.3	0
	5	29.6	30.4	0.4
	2	77.3	80.4	1.0
7,8,9,10-H_4-10-MBaP	1	5.8	6.1	0.4
	0	19.7	20.6	0.6
10-OH-7,8,9,10-H_4-10-MBaP	10	7.5	8.5	1.7
	5	17.8	22.0	4.2
7,8,9,10-H_4-10-MBaP *trans*-9,10-diol	18	15.0	21.5	6.5
	15	18.9	26.2	7.3
BaP *trans*-9,10-H_2diol	18	34.0(*S,S*)	35.7(*R,R*)	1.0
BaP 7,8,9,10-H_4-*trans*-9,10-diol	18	24.4(*S,S*)	27.1(*R,R*)	2.2
BaP 7,8,9,10-H_4-*cis*-9,10-diol	18	32.6	39.4	4.0

Effect of ring saturation

Enantiomeric resolution is greatly improved when the vicinal olefinic double bond of either a *cis* or a *trans* non-K-region dihydrodiol is saturated. For example, when the vicinal double bonds of BA *trans*-1,2-H_2 diol and BA *trans*-3,4-H_2 diol are saturated (Table I), the enantiomers of the resulting tetrahydrodiols are much more efficiently resolved. Many other examples are shown in Tables I–III.

Saturation of the 8,9,10,11-ring of BA *trans*-5,6-H_2 diol does not improve the resolution of enantiomers (Table IV). In contrast, saturation of the 8,9,10,11-ring of BA *cis*-5,6-dihydrodiol (BA *cis*-5,6-H_2 diol) greatly improves the enantiomeric resolution (Table IV). When the 7,8,9,10-ring of BaP *cis*-4,5-H_2 diol is saturated, the enantiomers of the resulting hexahydrodiol are also more efficiently resolved (Table

TABLE IV

CSP-HPLC RESOLUTION OF K-REGION DIHYDRODIOL ENANTIOMERS OF UNSUBSTITUTED AND METHYL-SUBSTITUTED BENZ[*a*]ANTHRACENE

Chromatographic conditions and abbreviations are the same as indicated in Table I.

Chemical	*Solvent A in hexane (%)*	*Retention time*		*Resolution value*
		Peak a	*Peak b*	
BA *trans*-5,6-H_2diol	10	16.8	16.8	0
	5	36.8	36.8	0
BA *cis*-5,6-H_2diol	10	26.4	27.1	0.5
5,6,8,9,10,11-H_6BA *trans*-5,6-diol	10	8.1	8.1	0
	5	26.5	26.5	0
5,6,8,9,10,11-H_6BA *cis*-5,6-diol	10	11.4	12.0	1.2
	5	22.5	23.8	1.4
1-MBA *trans*-5,6-H_2diol	10	10.8(*S,S*)	11.1(*R,R*)	0.6
	5	20.3(*S,S*)	21.1(*R,R*)	0.9
1-MBA *cis*-5,6-H_2diol	10	16.9	17.4	0.7
	5	38.8	40.5	1.0
5-MBA *cis*-5,6-H_2diol	10	8.8	9.3	1.3
	5	18.4	19.9	1.7
7-MBA *trans*-5,6-H_2diol	15	15.6(*R,R*)	16.4(*S,S*)	0.8
	10	31.0(*R,R*)	32.4(*S,S*)	0.9
7-MBA *cis*-5,6-H_2diol	15	13.6(*R,S*)*	14.2(*S,R*)*	0.9
	10	23.1(*R,S*)	24.3(*S,R*)	1.0
8-MBA *trans*-5,6-H_2diol	10	14.1	14.1	0
	5	31.4(*S,S*)	32.0(*R,R*)	0.2
	2.5	81.7(*S,S*)	82.7(*R,R*)	0.3
8-MBA *cis*-5,6-H_2diol	15	14.2	14.2	0
	10	22.7	22.7	0
11-MBA *trans*-5,6-H_2diol	10	15.7(*S,S*)	16.0(*R,R*)	0.2
	6	25.0(*S,S*)	25.7(*R,R*)	0.6
11-MBA *cis*-5,6-H_2diol	10	24.9	25.4	0.5
	5	60.7	62.5	0.6
7,12-DMBA *trans*-5,6-H_2diol	15	12.2(*S,S*)	13.8(*R,R*)	2.4
	10	21.8(*S,S*)	24.5(*R,R*)	2.6
7,12-DMBA *cis*-5,6-H_2diol	15	9.4(*R,S*)*	9.8(*S,R*)*	0.7
	10	15.2(*R,S*)	16.0(*S,R*)	1.0

* The absolute configurations of the resolved enantiomers are tentatively assigned on the basis of their CD spectral data (not shown).

V). The mono- and diol enantiomers of several hexahydro and decahydro derivatives of BaP were also more efficiently resolved than the corresponding compounds with lower degree of ring saturation (Table V).

Molecular size and shape

The results of this study suggest that molecular size and shape also play important roles in the separation of some enantiomers. However, no general rule can be found to predict the enantiomeric resolutions. The indications that molecular size and shape play some roles in chiral recognitions came from the enantiomeric resolutions of some structurally more closely related compounds. Among the antipodes derived from the 1,2,3,4-ring of BA, the enantiomers of elongated molecules are

TABLE V

CSP-HPLC RESOLUTION OF MONO- AND DIOL ENANTIOMERS DERIVED FROM POSITIONS OTHER THAN THE 7,8,9,10-RING OF BENZO[*a*]PYRENE

Chromatographic conditions and abbreviations are the same as indicated in Table I.

Chemical	*Solvent A in hexane (%)*	*Retention time*		*Resolution value*
		Peak a	*Peak b*	
3-OH-1,2,3,6,10b,11,12,12a-H_8BaP	5	17.2	18.3	1.0
BaP *trans*-4,5-H_2diol	10	28.8(*S,S*)	29.7(*R,R*)	0.5
	7.5	40.3(*S,S*)	41.5(*R,R*)	0.5
BaP *cis*-4,5-H_2diol	18	15.5	15.5	0
	15	20.1	20.1	0
	10	35.6	35.6	0
BaP 4,5,7,8,9,10-H_6-*cis*-4,5-diol	10	21.8	24.5	2.2
7-MBP *trans*-4,5-H_2diol	10	24.6(*S,S*)	25.3(*R,R*)	0.5
7-MBaP *cis*-4,5-H_2diol	10	38.6	38.6	0
11-OH-1,2,3,7,8,9,10,11,12,12a-H_{10}BaP	5	10.4	10.9	0.8
	2.5	19.3	20.6	1.6
	1	46.9	53.1	2.8
11-OH-1,2,3,11,12,12a-H_6BaP	5	10.2	10.2	0
	2.5	19.0	19.5	0.3
BaP *trans*-11,12-H_2diol	7.5	54.4	56.3	0.6
BaP 4,5,11,12-H_4-*cis*-11,12-diol	18	11.1	11.5	0.5
	15	12.5	13.0	0.7
	10	22.8	23.8	0.8
BaP 7,8,9,10,11,12-H_6-*cis*-11,12-diol	10	15.5	20.7	5.2

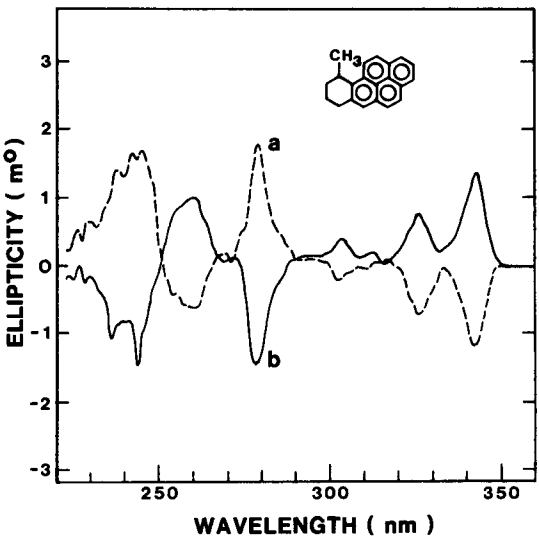

Fig. 3. CD spectra of the enantiomers of 10-methyl-7,8,9,10-H_4BaP (concentration 1.0 A_{247}/ml). The chromatographic peaks of the partially resolved enantiomers were used for CD spectral measurements and were not optically pure. Enantiomers a and b are designated according to their elution order (Table III).

either less efficiently resolved or not resolved at all when compared to the more "compact" molecules. For example, the enantiomers of 4-OH-1,2,3,4-H_4BA are less efficiently resolved than those of 1-OH-1,2,3,4-H_4BA (Fig. 2 and Table I). The enantiomers of BA *trans*-3,4-H_2diol are less efficiently resolved than those of BA *trans*-1,2-H_2diol. The enantiomers of 1,2,3,4-H_4BA *trans*-3,4-diol are also less efficiently resolved than those of 1,2,3,4-H_4BA *trans*-1,2-diol (Fig. 2). The fact that the enantiomers of 8-methyl-1,2,3,4-H_4BA *trans*-3,4-diol are less efficiently resolved than those of 1,2,3,4-H_4BA *trans*-3,4-diol (Fig. 2) further indicates that chiral interactions are less efficient between the CSP and the enantiomers of elongated molecules, especially at the 3 and 4 positions of BA.

The enantiomers of *trans*-10,11-H_2diol and 8,9,10,11-H_4-*trans*-10,11-diol of BA and 11-MBA were all efficiently resolved (Table II). However, the enantiomers of *trans*-10,11-H_2diol and 8,9,10,11-H_4-*trans*-10,11-diol of 8-MBA were not resolved. The 8-methyl group apparently abolishes the chiral interactions between the hydroxyl groups of the 10,11-diols and the CSP. However, the 11-methyl group of 11-MBA *trans*-8,9-H_2diol, whose enantiomers are resolved (Table III), does not interfere with the chiral interactions between the hydroxyl groups and the CSP.

The effects of molecular size and shape on the resolution of enantiomers are not as pronounced among the diol derivatives of unsubstituted and methylsubstituted BaP (Table V). For example, the 7,8-diols of BaP are both longer molecules than the 9,10-diols of BaP. The *trans*-7,8-diols and 9,10-diols are all efficiently resolved. Only the enantiomers of the *cis*-7,8-diols were found less efficiently resolved than those of the *cis*-9,10-diol (Table III).

Conformation and polarity of diols

The polarity of mono- and diol derivatives of any given PAH is dependent on the conformational preference of the hydroxyl groups as well as the molecular size and shape. Mono-ols and diols may be retained by the CSP–HPLC column due to adsorption by the γ-aminopropylsililanized silica as well as interactions with the CSP. Other than the possible chiral interactions between the CSP and the solute, the elution order of the enantiomers of mono- and diols on the CSP column is similar to that on a silica gel column. The relative polarity of compounds used in this study can be ranked by the retention times of the less retained enantiomers when a defined composition of eluent is used; the longer the retention time, the more polar is the compound. The effects of conformation and polarity on the resolution of enantiomers have been evaluated by comparing the results of *cis*- and *trans*-diols with varying degree of ring saturation. Ring saturation can alter the retention time of diols; even the conformational preference of the hydroxyl groups is not significantly changed. Depending on the location of the hydroxyl groups, diols of BA and BaP can be grouped into four categories. The effects of conformation on enantiomeric resolutions are illustrated in the examples described below.

Non-K and non-bay region diols

Trans-H_2diols of this category preferentially adopt quasidiequatorial conformations[24]. The hydroxyl groups of *trans*-H_4diols are more flexible, resulting in a more polar compound than the *trans*-H_2diol and its enantiomers are more efficiently resolved (Fig. 4). Each of the two hydroxyl groups of *cis*-H_2diols adopt either a

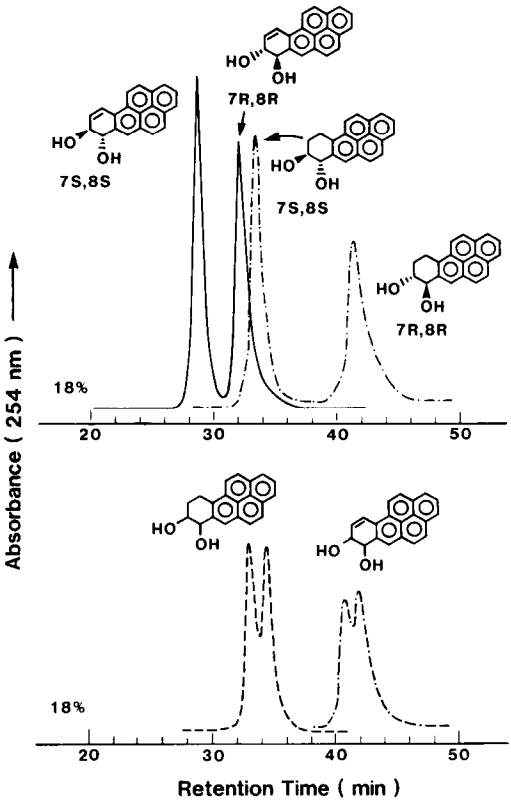

Fig. 4. Resolution of the enantiomers of non-K and non-bay region diols. Examples are shown for BaP dihydro and tetrahydro 7,8-diols. Other examples are listed in Tables I–III. Absolute configurations of the resolved enantiomers, if known, are indicated. The percentage of solvent A (ethanol–acetonitrile, 2:1, v/v) in hexane used in chromatography is indicated at the lower left of each chromatogram.

quasiequatorial or a quasiaxial conformation[25]. The *cis*-H_4diols are less polar, due to the more flexible tetrahydro ring structure. Resolution of enantiomers of the *cis*-dihydrodiol and *cis*-tetrahydrodiol is similar to, although less efficient than, the corresponding *trans* derivatives (Fig. 4). It is interesting to note that, due to flexible ring structures, the polarities of *cis*- and *trans*-tetrahydro-7,8-diols are similar (Fig. 4).

Non-K and bay region diols

The hydroxyl groups of all bay region (including both K- and non-K-regions) *trans*-H_2diols adopt quasidiaxial conformations[20,24]. The enantiomers of non-K and bay region *trans*-H_2diols such as BA *trans*-1,2-H_2diol and BaP *trans*-9,10-H_2diol were resolved (Figs. 2 and 5). Saturation of the vicinal double bonds of these bay region *trans*-diols substantially improved the enantiomeric resolution (Fig. 5). It is interesting to note that the hydroxyl groups of both BaP *trans*-9,10-H_2diol[25] and 7,8,9,10-H_4BaP *trans*-9,10-diol ($J_{9,10}$ = 3.0 Hz) are in quasidiaxial conformations. However, the retention time of 7,8,9,10-H_4BP *trans*-9,10-diol is shorter than those of both BaP *trans*-9,10-H_2diol and 7,8,9,10-H_4BP *cis*-9,10-diol (Fig. 5). The C_9 and

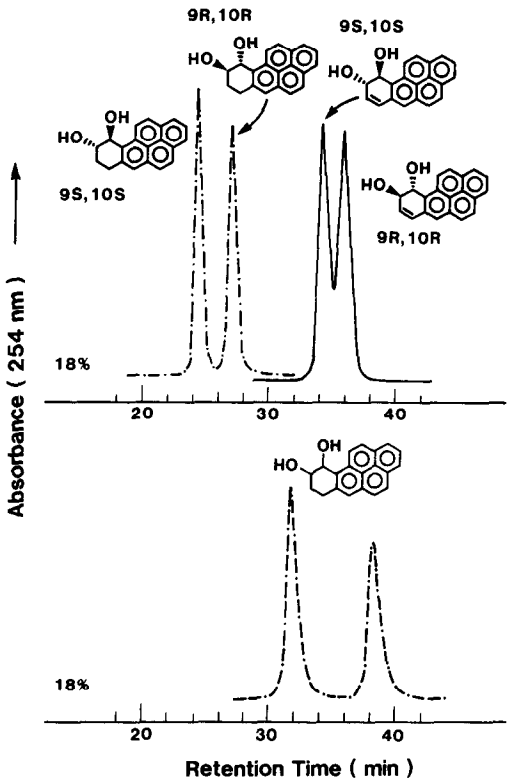

Fig. 5. Resolution of the enantiomers of non-K and bay region diols. Examples are shown for BaP dihydro and tetrahydro 9,10-diols. Other examples are listed in Tables I–III. BaP *cis*-9,10-H_2diol was not available for this study. The percentage of solvent A (ethanol–acetonitrile, 2:1, v/v) in hexane used in chromatography is indicated at the lower left of each chromatogram.

C_{10} hydroxyl groups of 7,8,9,10-H_4BaP *cis*-9,10-diol adopt quasiequatorial and quasiaxial conformation, respectively. The results shown in Figs. 4 and 5 indicate that the conformations of tetrahydro bay region *trans*-diols have unusual short retention time relative to the other non-bay region tetrahydrodiols. 7,8,9,10-H_4BaP *trans*-9,10-diol also had a shorter retention time than 7,8,9,10-H_4BaP *cis*-9,10-diol when the diols were analyzed on a silica gel column with a mixture of tetrahydrofuran–hexane as the eluent[17]. Similarly, the retention time of the quasidiaxial 1,2,3,4-H_4BA *trans*-1,2-diol ($J_{1,2}$ = 2.6 Hz) was also found to be shorter than that of quasiaxial–quasiequatorial 1,2,3,4-H_4BA *cis*-1,2-diol on both the CSP column (Table I) and the silica gel column.

The results in Figs. 4 and 5 and those shown in Tables I–III indicate that saturation of the vicinal double bond of bay region *trans*-dihydrodiols reduced the retention times. In contrast, saturation of the vicinal double bond of non-bay region *trans*-dihydrodiols increased the retention times.

K and non-bay region diols

The hydroxyl groups of all K-region *trans*-diols that are not in a bay region

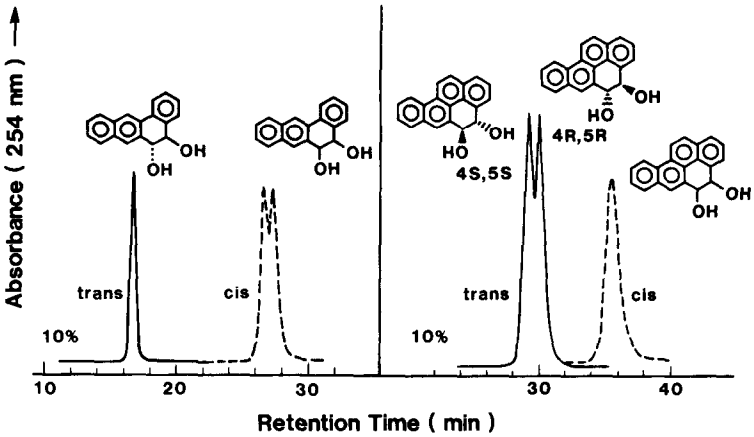

Fig. 6. Resolution of the enantiomers of K and non-bay region diols. Examples are shown for BA 5,6-H_2diols and BaP 4,5-H_2diols. Other examples are listed in Tables IV and V. The percentage of solvent A (ethanol–acetonitrile, 2:1, v/v) in hexane used in chromatography is indicated at the lower left of each chromatogram.

adopt quasidiequatorial conformations. Each of the two hydroxyl groups of *cis*-diols adopt either a quasiequatorial or a quasiaxial conformation. The quasidiequatorial K-region *trans*-H_2diols are all less polar and have shorter retention times than the corresponding *cis*-H_2diols (Fig. 6). Although many K and non-bay region diols were tested (Tables IV and V), no rule has emerged which can be used to predict the diols whose enantiomers can be resolved. For example, the enantiomers of BA *trans*-5,6-H_2diol were not resolved, whereas the enantiomers of BaP *trans*-4,5-H_2diol were partially resolved (Fig. 6). The enantiomeric resolution of other K- and non-bay region diols vary substantially (Tables IV and V). Saturation of the 8,9,10,11-ring of BA *cis*-5,6-H_2diol substantially improved the resolution of enantiomers (Table IV). In contrast, the enantiomers of 5,6,8,9,10,11-H_6BA *trans*-5,6-diol were not resolved (Table IV).

Among the *trans*-diols whose enantiomers were resolved, the *R,R* enantiomers were all more strongly retained by the CSP (Tables IV and V). The absolute configurations of resolved enantiomers were established by comparison of their CD spectra with those of known absolute configurations[5,6,15,16]. The absolute configurations of the enantiomers of the *cis*-diols in Tables IV and V are not known.

K and bay region diols

The hydroxyl groups of all *trans*-diols in this category adopt quasidiaxial conformations. For *cis*-diols, such as the *cis*-5,6-H_2diols of 7-MBA and 7,12-dimethyl-BA (7,12-DMBA), the bay region C_6-hydroxyl group adopts a quasiaxial conformation and the non-bay region C_5-hydroxyl group adopts a quasiequatorial conformation[26]. In contrast to the K and non-bay diols (Fig. 6), the K and bay region *trans*-diols are more polar and have longer retention times than the *cis*-diols (Fig. 7). The enantiomers of all K and bay region diols were resolved to varying extents (Fig. 7, Tables IV and V). Although both of the hydroxyl groups in the *trans*-5,6-H_2diols of 7-MBA and 7,12-DMBA adopt quasiaxial conformations, the *S,S* enantiomer of

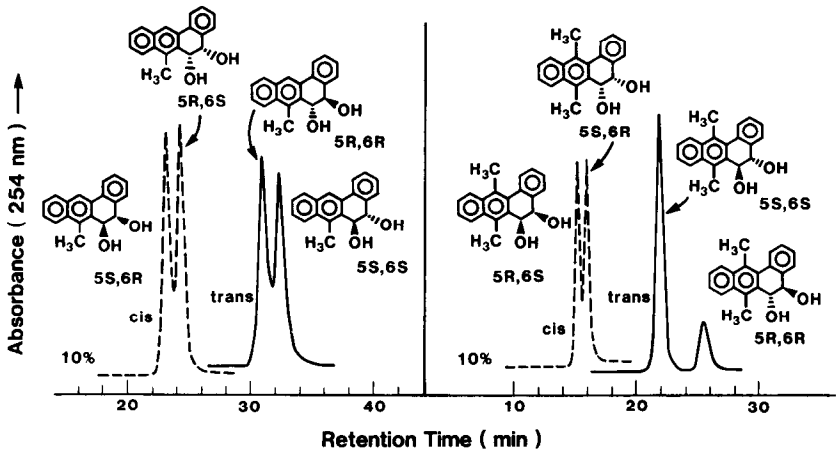

Fig. 7. Resolution of the enantiomers of K and bay region diols. Examples are shown for 7-MBA 5,6-H_2diols and 7,12-DMBA 5,6-H_2diols. The percentage of solvent A (ethanol–acetonitrile, 2:1, v/v) in hexane used in chromatography is indicated at the lower left of each chromatogram.

7-MBA *trans*-5,6-H_2diol[27] is more strongly retained, whereas the *R,R* enantiomer of 7,12-DMBA *trans*-5,6-H_2diol[28] is more strongly retained by the CSP. The only difference between the two *trans*-5,6-H_2diols is the presence of a bay region 12-methyl group in 7,12-DMBA *trans*-5,6-H_2diol. Due to steric crowding, the 1,2,3,4-ring of 7,12-DMBA *trans*-5,6-H_2diol is puckered. This ring puckering is absent in 7-MBA *trans*-5,6-H_2diol. The puckered ring structure in 7,12-DMBA *trans*-5,6-H_2diol apparently altered the chiral recognitions between the quasiaxial hydroxyl groups and the CSP, therefore reversing the elution order of the enantiomers from that of 7-MBA *trans*-5,6-H_2diol.

Chiral recognition mechanism

Pirkle *et al.*[12] suggested that the chiral interactions between the CSP and cyclic alcohols (mono-ols) such as 1-OH-1,2,3,4-H_4BA are: (i) π–π interaction between the π-basic aryl substituent of the cyclic alcohol and the π-acidic 3,5-dinitrobenzoyl ring, (ii) hydrogen bonding between the hydroxyl group of the cyclic alcohol and the amide hydrogen of the CSP, and (iii) a stereochemically dependent interaction probably due to repulsion between the steric barrier of the alicyclic ring and either the carboxylate or phenyl group of the CSP. These chiral interactions may be applicable to the separation of enantiomers of the mono-ols used in this study. It is difficult, however, to rationalize the chiral interactions that contribute to the enantiomer separations of PAH diols. In applying the chiral interaction mechanisms suggested by Pirkle and co-workers[11–13], it is not possible to account for the results obtained with the structurally related mono- and diol derivatives derived from the 1,2,3,4-ring of BA (Fig. 2 and Table I). The results on the enantiomeric resolution of mono- and diols derived from the 7,8,9,10-ring of BaP (Table III) further complicate the possible chiral recognition mechanisms. The results of a recent report[14] also cloud the exact chiral interactions responsible for enantiomeric separations of cyclic alcohols. Some interesting, albeit perplexing, results worthy of special mentions are: (a) the enan-

tiomers of 2-OH-1,2,3,4-H_4BA were not resolved, whereas those of 1-OH-1,2,3,4-H_4BA, 1,2,3,4-H_4BA *trans*- and *cis*-1,2-diols, 1,2,3,4-H_4BA *trans*- and *cis*-2,3-diols, and 1,2,3,4-H_4BA *trans*-3,4-diol were resolved, and (b) the enantiomers of 8-OH- and 9-OH-7,8,9,10-H_4BaP were not resolved, although the enantiomers of 7-OH- and 10-OH-7,8,9,10-H_4BaP as well as the *trans*- and *cis*-7,8- and 9,10-diols of 7,8,9,10-H_4BaP were all resolved. It is not clear why the non-benzylic hydroxyl groups in 2-OH-1,2,3,4-H_4BA, 8-OH- and 9-OH-7,8,9,10-H_4BaP do not have the required chiral interactions to effect enantiomer separations.

Elution order and absolute configurations

Among 27 *trans*-diols whose enantiomers are resolved and have known absolute configurations, the *R,R* enantiomers of 26 *trans*-diols are each more strongly retained by the CSP (Tables I–V). The only exception is 7-MBA *trans*-5,6-dihydrodiol; its *S,S* enantiomer is more strongly retained. The possible reason may be its quasidiaxial conformation and the absence of a bay region substituent. As shown in Fig. 7, the *R,R* enantiomer of the quasidiaxial 7,12-DMBA *trans*-5,6-dihydrodiol is more strongly retained.

Although the enantiomer separations of a large number of mono- and diol derivatives have been studied, there are two closely related areas that need further investigations. First, it is necessary to establish the absolute configurations of the resolved enantiomers, and secondly, it is necessary to establish the exact chiral recognition mechanisms required for enantiomer separations. In view of the results in this report and in an earlier report[14], it is obvious that many factors contribute to the chiral recognitions between the CSP and the solutes. Additional systematic study of structure-resolution relationships should provide more insight into the exact chiral recognition mechanisms and only then the elution order-absolute configuration relationships can be fully understood.

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