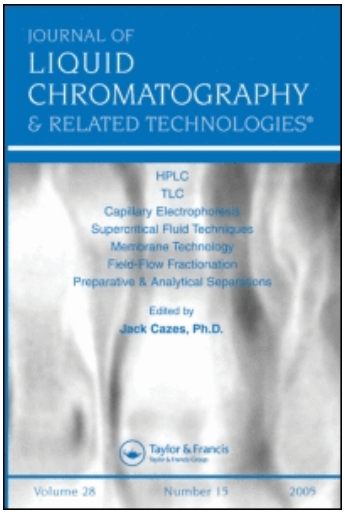

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Chiral Recognition Mechanisms in the Direct Resolution of Diol Enantiomers of Some Polycyclic Aromatic Hydrocarbons by High Performance Liquid Chromatography with Chiral Stationary Phases

Shen K. Yang[a]; Mohammad Mushtaq[a]; Henri B. Weems[a]; Peter P. Fu[b]

[a] Department of Pharmacology, F. Edward Hébert School of Medicine Uniformed Services University of Health Sciences, Bethesda, Maryland [b] National Center for Toxicological Research Food and Drug Administration, Jefferson, Arkansas

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CHIRAL RECOGNITION MECHANISMS IN THE DIRECT RESOLUTION OF DIOL ENANTIOMERS OF SOME POLYCYCLIC AROMATIC HYDROCARBONS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH CHIRAL STATIONARY PHASES

Shen K. Yang[1], Mohammad Mushtaq[1], Henri B. Weems[1], and Peter P. Fu[2]

[1]*Department of Pharmacology*
F. Edward Hébert School of Medicine
Uniformed Services University of Health Sciences
Bethesda, Maryland 20814-4799
[2]*National Center for Toxicological Research*
Food and Drug Administration
Jefferson, Arkansas 72079

ABSTRACT

The direct resolution of trans and cis bay region dihydrodiol and tetrahydrodiol enantiomers of benz[a]anthracene and benzo[a]pyrene, and trans and cis K-region dihydrodiol enantiomers of benz[a]anthracene, 4-methylbenz[a]anthracene, 7-methylbenz[a]anthracene, 7,12-dimethylbenz[a]anthracene, and 3-methylcholanthrene was evaluated by high-performance liquid chromatography using commercially available columns packed with (R)-N-(3,5-dinitrobenzoyl)phenylglycine and (S)-N-(3,5-dinitrobenzoyl)leucine either ionically or covalently bonded to γ-aminopropylsilanized silica. Except benz[a]anthracene trans-5,6-dihydrodiol, the diol enantiomers of other hydrocarbons were all resolved by at least one of the four chiral stationary phases tested. The absolute configurations of enantiomeric diols, whose hydroxyl groups' conforma-

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tions are restricted due to steric factors, have been established by the exciton chirality circular dichroism method. Based on the experimental results, the mechanisms of some chiral interactions between diol solutes and chiral stationary phases responsible for enantiomeric resolution can be rationalized.

INTRODUCTION

Pirkle et al. (1,2) have successfully resolved the enantiomers of a large number of compounds with the chiral stationary phases (CSPs) that they have developed. Columns packed with two different CSPs, (R)-N-(3,5-dinitrobenzoyl)phenylglycine ((R)-DNBPG) and (S)-N-(3,5-dinitrobenzoyl)leucine ((S)-DNBL), are available commercially. Using these CSP columns, a solvent system (ethanol/acetonitrile/hexane) was used to resolve enantiomers of relatively more polar compounds such as diol derivatives of polycyclic aromatic hydrocarbons (PAHs) (3). This CSP-HPLC method has been applied successfully in resolving some mono-ol, epoxide, and diol enantiomers of PAHs such as phenanthrene, chrysene, benz[a]anthracene (BA), monomethylbenz[a]anthracene (MBA), 7,12-dimethylbenz[a]anthracene (DMBA), dibenz[a,h]anthracene, cholanthrene, 3-methylcholanthrene (3-MC), and benzo[a]pyrene (BaP) (4-12).

The direct resolution of a large number of structurally related mono-ol, and trans and cis-diol enantiomers of unsubstituted and methyl-substituted BA and BaP by CSP-HPLC method using ionically bonded (R)-DNBPG column has been reported (12). It was found 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 could not be established due to complex structural factors that influence enantiomeric resolutions and the lack of data on the absolute configurations of the resolved enantiomers. In this report, we examined more closely the effect of conformation of the hydroxyl groups of some diols. Due to steric factors, these diols adopt only one of two possible conformations. The absolute configurations of these diols have been established. Thus it is possi-

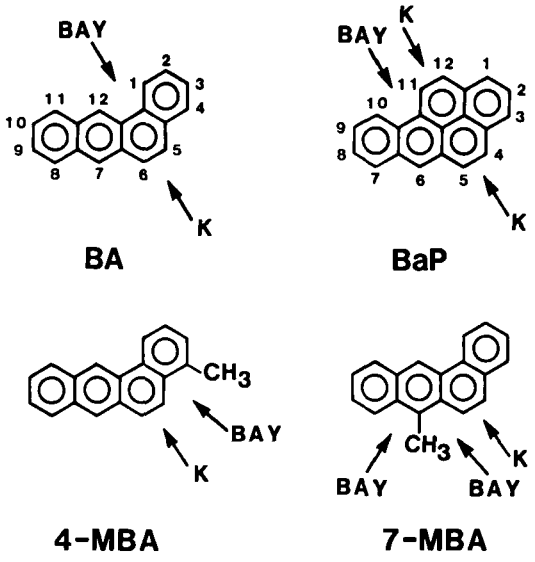

FIGURE 1. Numbering system and the bay- and K-region designations in BA, 4-MBA, 7-MBA, and BaP. K-region is the most electron-rich double bond of the molecule and bay region is the angular region such as the area between, and including, the C_1 and C_{12} positions of BA. For the purpose of designating the quasi-axial conformations of hydroxyl groups adjacent to a peri methyl group, the area adjacent to the peri methyl group are also designated as bay regions.

ble to assess the role of the hydroxyl groups in chiral interactions which contribute to enantiomeric resolutions. Various regions that affect the conformational preference of BA, 4-MBA, 7-MBA, and BaP are indicated in Figure 1.

MATERIALS

The following racemic compounds (abbreviations in parentheses) were obtained from the Chemical Repository of the National Cancer Institute: trans-1,2-dihydroxy-1,2-dihydrobenz[a]anthracene (BA t-1,2-H_2diol), trans-5,6-dihydroxy-5,6-dihydrobenz[a]anthracene (BA t-5,6-H_2diol), trans-9,10-dihydroxy-9,10-dihydrobenzo[a]pyrene (BaP t-9,10-H_2diol), cis-9,10-dihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BaP c-9,10-H_4diol), trans-9,10-dihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BaP t-9,10-H_4diol), 5,6-epoxy-

5,6-dihydro-7,12-dimethylbenz[a]anthracene (DMBA 5,6-epoxide), 11,12-epoxy-11,12-dihydro-3-methylcholanthrene (3-MC 11,12-epoxide), cis-5,6-dihydroxy-5,6-dihydrobenz[a]anthracene (BA c-5,6-H_2diol), cis-5,6-dihydroxy-5,6-dihydro-7,12-dimethylbenz[a]anthracene (DMBA c-5,6-H_2diol), cis-11,12-dihydroxy-11,12-dihydro-3-methylcholanthrene (3-MC c-11,12-H_2diol).

Trans-1,2-dihydroxy-1,2,3,4-tetrahydrobenz[a]anthracene (BA t-1,2-H_4diol) was prepared by catalytic hydrogenation (tetrahydrofuran, PtO_2/H_2, 1 atm, 30 min) of BA t-1,2-H_2diol. Trans-5,6-dihydroxy-5,6-dihydro-7,12-dimethylbenz[a]anthracene (DMBA t-5,6-H_2diol) and trans-11,12-dihydroxy-11,12-dihydro-3-methylcholanthrene (3-MC t-11,12-H_2diol) were each obtained by incubation of the corresponding racemic K-region epoxide in 0.1 M Tris-HCl (pH 8.9) with liver microsomes from phenobarbital-treated male Sprague Dawley rats in the absence of NADPH (11).

Cis-5,6-dihydroxy-5,6-dihydro-7-methylbenz[a]anthracene (7-MBA c-5,6-H_2diol) was synthesized as described (13). 4-Methylbenz-[a]anthracene (4-MBA) was prepared by reacting 1,2-dihydrobenz-[a]anthracene-4(3H)-one (provided by Dr. Robert Roth, Midwest Research Institute, Kansas City, MO) with methyllithium followed with acid-catalyzed dehydration and aromatization of the resulting 1,2-dihydro-4-methyl-BA with 2,3-dichloro-5,6-dicyano-1,6-benzoquinone. Cis-5,6-dihydroxy-5,6-dihydro-4-methylbenz[a]anthracene (4-MBA c-5,6-H_2diol) were synthesized from 4-MBA similarly as described for the synthesis of 7-MBA c-5,6-H_2diol (13). 500 MHz proton NMR spectrum of 4-MBA c-5,6-H_2diol (measured in acetone-d_6 with a trace of D_2O): 2.51 (s, 3, CH_3), 4.91 (d, 1, H_5), 5.08 (d, 1, H_6; $J_{5,6}$ = 3.5 Hz), 7.22 (d, 1, H_3; $J_{2,3}$ = 7.5 Hz), 7.37 (t, 1, H_2), 7.47 (m, 2, $H_{9,10}$), 7.94 (m, 3, $H_{1,8,11}$), 8.19 (s, 1, H_7), and 8.30 ppm (s, 1, H_{12}).

Cis-1,2-dihydroxy-1,2,3,4-tetrahydrobenz[a]anthracene (BA c-1,2-H_4diol) was synthesized by reduction of of 3,4-dihydrobenz-[a]anthracene-1(2H)-one (Aldrich Chemical Co., Milwaukee, WI) with sodium borohydride, followed with acid-catalyzed dehydration of the alcohol and reaction of the resulting 3,4-dihydro-BA with osmium tetroxide.

Trans-5,6-dihydroxy-5,6-dihydro-4-methylbenz[a]anthracene (4-MBA t-5,6-H_2diol) and trans-5,6-dihydroxy-5,6-dihydro-7-methylbenz[a]anthracene (7-MBA t-5,6-H_2diol) were each isolated, by reversed-phase and normal-phase HPLC, from a metabolite mixture obtained by incubation of the parent hydrocarbon with liver microsomes from phenobarbital-treated male Sprague-Dawley rats and a NADPH regenerating system (13).

METHODS

Chromatography: Chemicals were analyzed with HPLC columns (4.6 mm x 25 cm; Regis Chemical, Morton Grove, IL) packed with an (R)-N-

(3,5-dinitrobenzoyl)phenylglycine ionically bonded ((R)-DNBPG-I) or covalently bonded ((R)-DNBPG-C) and an (S)-N-(3,5-dinitrobenzoyl)leucine either ionically bonded ((S)-DNBL-I) or covalently bonded ((S)-DNBL-C) to spherical particles of 5 micrometer diameter of γ-aminopropylsilanized silica (1,14). HPLC was performed using a Waters Associates (Milford, MA) 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 (254 nm) detector. Samples were injected via a Valco model N60 loop injector (Valco, Houston, TX). Separation of enantiomeric diols was achieved isocratically with a flow rate of 2 ml/min using premixed solvents of up to 15% (v/v) of solvent A (ethanol/acetonitrile, 2:1, v/v) in hexane at ambient temperature. Optically pure enantiomers were obtained by repetitive chromatography. Solvent was removed from the resolved enantiomers by evaporation under nitrogen. CSP leached from the ionically bonded CSP column into the resolved enantiomers was removed prior to circular dichroism (CD) spectral measurement by reversed-phase HPLC with a DuPont Zorbax ODS column as described previously (3).

Absolute Configuration of Diols: The absolute configurations of diol enantiomers were determined by the exciton chirality circular dichroism method (15) similarly as described (11,13) except ethyl acetate was used as the solvent instead of tetrahydrofuran. Each diol (0.1-0.3 mg) was dissolved in a test tube in 1 ml of ethyl acetate that has been dried by treating with NaH. A small amount (ca. 1 mg) of NaH was added, followed by the addition of p-N,N-dimethylaminobenzoyl chloride (ca. 5 mg). The test tube was kept in ice for about 5 min and 2 drops of N,N-dimethylaminopyridine (10 mg/ml of ethyl acetate) was added. The solution was stirred for 16 h. Solid material was removed by centrifugation and the supernatant was injected onto a DuPont Zorbax ODS column (4.6 mm x 25 cm). The ODS column was eluted with a linear gradient of methanol/water (3:1, v/v) to methanol over a period of 15 min at a flow rate of 1.5 ml/min. The bis-p-N,N-dimethylaminobenzoate was eluted between 16-20 min.

Spectral Analysis: Ultraviolet-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. 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. The concentration of the sample is indicated by A_λ/ml (number of absorbance unit at a specific wavelength per ml of methanol). CD spectra are expressed by ellipticity (in millidegrees) for methanol solutions that have an absorbance of 1.0 unit at a specified wavelength (15).

RESULTS

Due to steric hindrance, the non-K and bay region trans and cis diols of both BA and BaP can exist in only one of two possible conformations. Enantiomers of these diols can be resolved with varying degrees of efficiency by at least two of the four CSPs tested (Table 1). The enantiomers of BA t-1,2-H_2diol and BaP t-9,10-H_2diol are less efficiently resolved. Except the marginal enantiomeric resolution of BA t-1,2-H_4diol on (S)-DNBL-C column, the enantiomeric resolutions of other diols were all improved when a dihydrodiol is converted to a tetrahydrodiol. In general, higher degree of ring saturation favors the resolution of the enantiomeric mono-ols and diols (12). The enantiomers of non-K and bay region diols are more efficiently resolved by (R)-DNBPG than by (S)-DNBL. For some diols, the enantiomers are more efficiently resolved by covalently bonded CSP than by ionically bonded CSP (e.g., BA c-1,2-H_4diol and BaP c-9,10-H_4diol; Table 1).

With the exception that the elution order of DMBA t-5,6-H_2diol enantiomers on (R)-DNBPG-I is reversed from that on (R)-DNBPG-C (Table 2 and ref. 12), all other diols examined to date have the same elution order on a CSP either ionically or covalently bonded to the stationary phase. In the discussion that follows, the same chiral recognition mechanism is assumed for both ionically and covalently bonded CSP of either (R)-DNBPG or (S)-DNBL.

The R,R enantiomers of BA t-1,2-H_2diol, BA t-1,2-H_4diol, and BaP t-9,10-H_2diol are more strongly retained on both (R)-DNBPG and (S)-DNBL columns (Table 1). The elucidation of the absolute configurations of enantiomeric BA t-1,2-H_2diol (16), BA t-1,2-H_4diol (16), BaP t-9,10-H_2diol (17), and BaP t-9,10-H_4diol (18) was reported earlier. As indicated in Table 1, the elution orders of the enantiomers of BA c-1,2-H_2diol, BaP t-9,10-H_4diol, and BaP c-9,10-H_4diol on (R)-DNBPG columns are reversed from those on (S)-DNBL columns.

The absolute configuration of the BA c-1,2-H_4diol enantiomer less strongly retained by (R)-DNBPG columns was determined by the

TABLE 1.

CSP-HPLC Resolution of Bay Region Diol Enantiomers of Benz[a]-anthracene and Benzo[a]pyrene.

The conformation of hydroxyl groups are indicated by a (axial) and e (equatorial). t = trans, c = cis, H_2 = dihydro, H_4 = tetrahydro.

Chemical	CSP[a]	%A[b]	Retention Time[c] peak a	Retention Time[c] peak b	RV[d]
BA t-1,2-H_2diol (aa)	(R)-DNBPG-I	15	21.8 (S,S)	22.2 (R,R)	0.1
	(R)-DNBPG-C	15	17.2	17.2	0
	(S)-DNBL-I	15	17.8	17.8	0
		10	49.0 (S,S)	49.5 (R,R)	0.1
	(S)-DNBL-C	15	11.7	11.7	0
BA t-1,2-H_4diol (aa)	(R)-DNBPG-I	15	14.4 (S,S)	15.7 (R,R)	2.4
	(R)-DNBPG-C	15	11.8 (S,S)	12.5 (R,R)	1.4
	(S)-DNBL-I	15	12.3	12.3	0
		10	20.8	20.8	0
	(S)-DNBL-C	15	9.0	9.0	0
		10	14.2 (S,S)	14.4 (R,R)	0.1
BA c-1,2-H_4diol (1a,2e)	(R)-DNBPG-I	15	16.8 (S,R)	24.6 (R,S)	8.9
	(R)-DNBPG-C	15	10.5 (S,R)	13.7 (R,S)	5.5
	(S)-DNBL-I	10	19.2 (R,S)	19.7 (S,R)	0.6
	(S)-DNBL-C	10	10.7 (R,S)	11.2 (S,R)	0.8
BaP t-9,10-H_2diol (aa)	(R)-DNBPG-I	15	49.5 (S,S)	52.4 (R,R)	1.0
	(R)-DNBPG-C	15	33.1 (S,S)	35.0 (R,R)	0.8
	(S)-DNBL-I	15	32.5 (S,S)	34.1 (R,R)	1.0
	(S)-DNBL-C	15	20.1	20.1	0
BaP t-9,10-H_4diol (aa)	(R)-DNBPG-I	15	35.1 (S,S)	39.5 (R,R)	3.7
	(R)-DNBPG-C	15	26.6 (S,S)	28.5 (R,R)	2.1
	(S)-DNBL-I	15	24.2 (R,R)	26.7 (S,S)	2.7
	(S)-DNBL-C	15	17.2 (R,R)	18.0 (S,S)	1.0
BaP c-9,10-H_4diol (9e,10a)	(R)-DNBPG-I	15	43.0 (R,S)	53.5 (S,R)	4.5
	(R)-DNBPG-C	15	22.4 (R,S)	30.1 (S,R)	5.6
	(S)-DNBL-I	15	25.1 (S,R)	26.1 (R,S)	0.8
	(S)-DNBL-C	15	13.6 (S,R)	15.0 (R,S)	2.0

[a]CSPs are defined in MATERIALS.
[b]Percent of solvent A (ethanol/acetonitrile; 2:1,v/v) in hexane. The flow rate was 2 ml/min.
[c]See text for the absolute configurations of the resolved enantiomers. The enantiomers are also designated as a and b according to their elution order.
[d]RV = resolution value = $2(V_2-V_1)/(W_2+W_1)$, where V is retention volume and W is peak width at base. The void time was 1.2 min.

TABLE 2

CSP-HPLC Resolution of K-region Diol Enantiomers of Unsubstituted and Methyl-substituted Hydrocarbons with a Benz[a]anthracene Nucleus.

See TABLE 1 and MATERIALS for chromatographic conditions and abbreviations.

Chemical	CSP	%A	Retention Time peak a		peak b		RV
BA t-5,6-H_2diol (ee)	(R)-DNBPG-I	10	15.8		15.8		0
	(R)-DNBPG-C	10	8.6		8.6		0
	(S)-DNBL-I	10	13.6		13.6		0
	(S)-DNBL-C	10	8.2		8.2		0
BA c-5,6-H_2diol (5e,6a & 5a,6e)	(R)-DNBPG-I	10	25.1	(S,R)[a]	25.8	(R,S)[a]	0.6
	(R)-DNBPG-C	10	16.0		16.0		0
	(S)-DNBL-I	10	20.7	(R,S)	21.9	(S,R)	1.1
	(S)-DNBL-C	10	12.0	(R,S)	12.5	(S,R)	0.6
4-MBA t-5,6-H_2diol (aa)	(R)-DNBPG-I	10	23.4	(R,R)	24.1	(S,S)	0.6
	(R)-DNBPG-C	10	19.6	(R,R)	20.7	(S,S)	1.1
	(S)-DNBL-I	10	19.3	(R,R)	20.2	(S,S)	1.2
	(S)-DNBL-C	10	13.8	(R,R)	14.3	(S,S)	0.1
4-MBA c-5,6-H_2diol (5a,6e)	(R)-DNBPG-I	10	17.2	(S,R)	19.1	(R,S)	2.3
	(R)-DNBPG-C	10	12.6	(S,R)	13.6	(R,S)	1.5
	(S)-DNBL-I	10	14.7	(R,S)	15.3	(S,R)	0.8
	(S)-DNBL-C	10	9.4	(R,S)	10.0	(S,R)	1.1
7-MBA t-5,6-H_2diol (aa)	(R)-DNBPG-I	10	30.9	(R,R)	32.6	(S,S)	1.1
	(R)-DNBPG-C	10	24.1	(R,R)	30.0	(S,S)	5.4
	(S)-DNBL-I	10	25.6	(R,R)	27.2	(S,S)	0.9
	(S)-DNBL-C	10	17.1	(R,R)	17.5	(S,S)	0.4
7-MBA c-5,6-H_2diol (5e,6a)	(R)-DNBPG-I	10	22.3	(R,S)	23.5	(S,R)	1.0
	(R)-DNBPG-C	10	14.6	(R,S)	15.9	(S,R)	1.7
	(S)-DNBL-I	10	17.1	(R,S)	18.9	(S,R)	2.2
	(S)-DNBL-C	10	10.3	(R,S)	10.5	(S,R)	0.4
3-MC t-11,12-H_2diol (aa)	(R)-DNBPG-I	10	26.4	(R,R)	27.0	(S,S)	0.1
	(R)-DNBPG-C	10	16.9	(R,R)	19.2	(S,S)	2.8
	(S)-DNBL-I	10	23.2	(R,R)	24.8	(S,S)	1.3
	(S)-DNBL-C	10	13.2		13.2		0

Table 2 is continued on next page.

TABLE 2 - continued.

Chemical	CSP	%A	Retention Time peak a	Retention Time peak b	RV[d]
3-MC c-11,12-H_2diol (11e,12a)	(R)-DNBPG-I	10	37.0	37.0	0
	(R)-DNBPG-C	10	19.2 (R,S)	21.1 (S,R)	1.8
	(S)-DNBL-I	15	15.8 (R,S)	19.0 (S,R)	3.4
	(S)-DNBL-C	10	13.2 (R,S)	13.6 (S,R)	0.4
DMBA t-5,6-H_2diol[b] (aa)	(R)-DNBPG-I	10	21.8 (S,S)	25.4 (R,R)	2.6
	(R)-DNBPG-C	10	20.3 (R,R)	20.8 (S,S)	0.5
	(S)-DNBL-I	10	20.6 (R,R)	28.6 (S,S)	6.9
	(S)-DNBL-C[c]	10	14.8 (R,R)	18.1 (S,S)	4.1
DMBA c-5,6-H_2diol[b] (5e,6a)	(R)-DNBPG-I	10	15.2 (R,S)	16.0 (S,R)	1.0
	(R)-DNBPG-C	10	10.9 (R,S)	11.6 (S,R)	1.3
	(S)-DNBL-I	10	16.2 (R,S)	17.7 (S,R)	1.9
	(S)-DNBL-C[c]	10	8.8 (R,S)	9.1 (S,R)	0.5

[a]The absolute configurations of these resolved enantiomers have not been established and are arbitrarily designated for the purpose of indicating their relative elution orders on different CSP columns.
[b]The resolution of these enantiomers were reported earlier (7) and are included for comparison.
[c]Data obtained using a column purchased from Regis Chemical Co.

exciton chirality CD method (15). A relatively large amount (ca. 0.3 mg) of enantiomerically pure BA c-1,2-H_4diol was prepared by repetitive chromatography using (R)-DNBPG-I column. The less strongly retained enantiomer was allowed to react with p-N,N-dimethylaminobenzoyl chloride in dried ethyl acetate in the presence of NaH (ca. 1 mg). The resulting bis-p-N,N-dimethylaminobenzoate, purified by reversed-phase HPLC, showed a pair of strong, symmetric Cotton effects; positive at 324 nm and negative at 299 nm which passed through zero at 315 nm (Figure 2). This exciton chirality CD spectrum indicates that the BA c-1,2-H_4diol enantiomer less strongly retained by (R)-DNBPG has a 1S,2R absolute stereochemistry (15). The BaP c-9,10-H_4diol enantiomer more strongly retained by (R)-DNBPG columns was similarly determined to

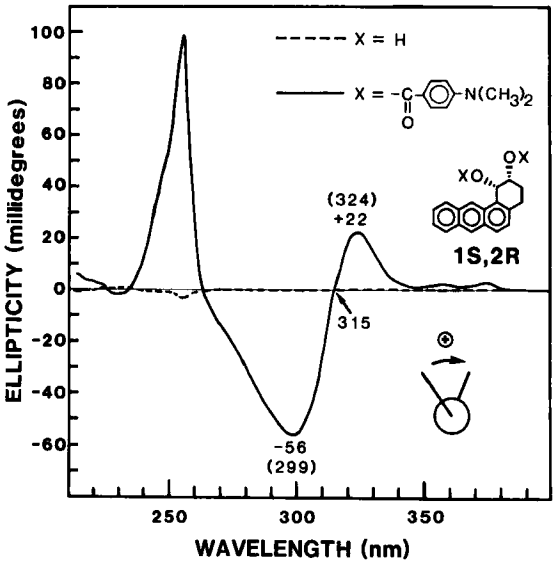

FIGURE 2. CD spectra of the optically pure BA c-1,2-H_4diol enantiomer less strongly retained by (R)-DNBPG-I (----, conc. 1.0 A_{256}/ml) and its bis-p-N,N-dimethylaminobenzoate derivative (——, conc. 1.0 A_{310}/ml).

have a 9S,10R absolute stereochemistry (P.L. Chiu and S.K. Yang, unpublished results).

The enantiomers of K- and non-bay region BA t-5,6-H_2diol, whose hydroxyl groups adopt preferentially quasiequatorial conformations, were not resolved by any CSPs tested (Table 2). However, the enantiomers of BA c-5,6-H_2diol were resolved by three of the four CSPs (Table 2). Because the hydroxyl groups can adopt either a 5-quasiequatorial-6-quasiaxial or a 5-quasiaxial-6-quasiequatorial conformation, we found that the absolute configuration of an enantiomeric BA c-5,6-H_2diol cannot be determined by the exciton chirality method described by Harada and Nakanishi (15). The absolute stereochemistries of the resolved enantiomers are arbitrarily designated in Table 2 in order to indicate the relative elution order of enantiomers on different CSPs.

One of the two hydroxyl groups in K-region cis-dihydrodiols of 4-MBA, 7-MBA, 3-MC, and DMBA is peri to either a methyl or a methylene group and consequently adopts a quasiaxial conformation due to steric hindrance imposed by the substituent (19). The other hydroxyl group of these cis-dihydrodiols adopts a quasiequatorial conformation (19). Both hydroxyl groups of K-region trans-dihydrodiols of 4-MBA, 7-MBA, 3-MC, and DMBA adopt quasiaxial conformations (11,13). Thus the K-region cis-dihydrodiols of 4-MBA, 7-MBA, 3-MC, and DMBA can exist in either a quasiequatorial-quasiaxial or a quasiaxial-quasiequatorial conformation depending on the location of the peri substituent.

With the exception that DMBA t-(5R,6R)-H_2diol is more strongly retained by (R)-DNBPG-I column (7), the (S,S)-dihydrodiol enantiomers of all K-region trans-dihydrodiols of 4-MBA, 7-MBA, and 3-MC are more strongly retained by either (R)-DNBPG-I or (R)-DNBPG-C (Table 2). For K-region cis-dihydrodiols of 7-MBA, 3-MC, and DMBA, the (S,R)-dihydrodiol enantiomers are more strongly retained by both (R)-DNBPG and (S)-DNBL (Table 2). In contrast, 4-MBA cis-(5S,6R)-dihydrodiol is less strongly retained by (R)-DNBPG and is more strongly retained by (S)-DNBL (Table 2).

The absolute configurations of the less strongly retained enantiomer of 3-MC t-11,12-H_2diol and the more strongly retained enantiomer of DMBA c-5,6-H_2diol were determined by the exciton chirality CD method (15). The CD spectrum of the bis-p-N,N-dimethylaminobenzoate derived from the less strongly retained enantiomer of 3-MC t-11,12-H_2diol showed a negative CD band at 322 nm (Figure 3) which indicates that the dihydrodiol enantiomer has a 11R,12R absolute stereochemistry (15). The CD spectrum of the bis-p-N,N-dimethylaminobenzoate derived from the more strongly retained enantiomer of DMBA c-5,6-H_2diol showed a pair of symmetric Cotton effects; negative at 327 nm and positive at 303 nm which passed through zero at 319 nm (Figure 4). This exciton chirality CD spectrum requires that the dihydrodiol enantiomer has a 5S,6R absolute stereochemistry (15). This result confirms our earlier conclusion which was based on the conformation of the

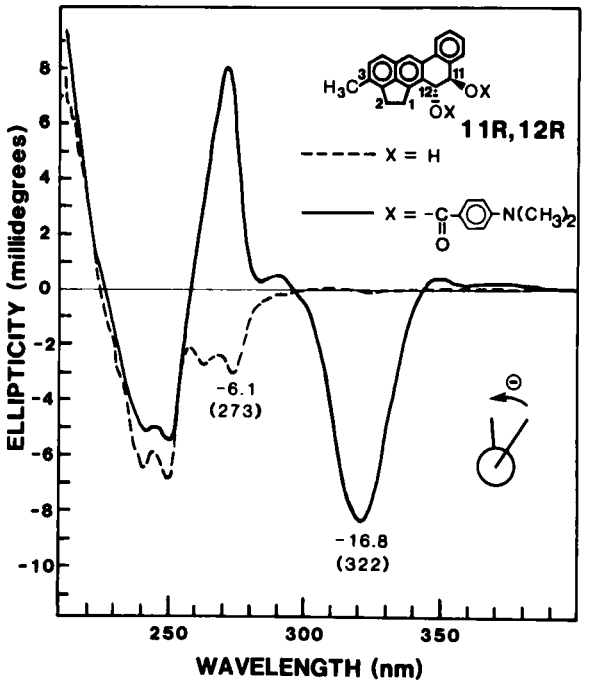

FIGURE 3 (LEFT). CD spectra of optically pure enantiomer of 3-MC t-11,12-H_2diol less strongly retained by (R)-DNBPG-I (----, conc. 1.0 A_{274}/ml) and its bis-p-N,N-dimethylaminobenzoate derivative (——, conc. 1.0 A_{310}/ml).

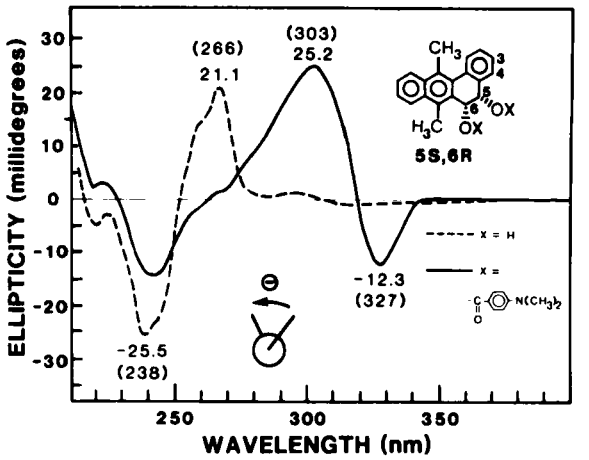

FIGURE 4 (RIGHT). CD spectra of optically pure enantiomer of DMBA c-5,6-H_2diol more strongly retained by (R)-DNBPG-I (----, conc. 1.0 A_{268}/ml) and its bis-p-N,N-dimethylaminobenzoate derivative (——, conc. 1.0 A_{314}/ml).

hydroxyl groups and the CD spectrum of a DMBA cis-5,6-dihydrodiol enantiomer (7).

The absolute configurations of enantiomeric trans-5,6-dihydrodiols of 7-MBA (13) and DMBA (11) have been reported. The absolute configurations of enantiomeric trans and cis 5,6-dihydrodiols of 4-MBA, 7-MBA cis-5,6-dihydrodiol, and 3-MC cis-11,12-dihydrodiol were also determined by the exciton chirality CD method and the experimental results will be described elsewhere.

DISCUSSION

Pirkle et al. (2) suggested that the chiral interactions between the CSP such as (R)-DNBPG and cyclic alcohols (mono-ols) such as 1-hydroxy-1,2,3,4-H_4BA are: (i) $\pi-\pi$ 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 are applicable to the separation of enantiomeric diols of polycyclic aromatic hydrocarbons. The results of this study are consistent with the chiral recognition mechanisms proposed by Pirkle et al. (2). Our results also indicate that the non-amide carbonyl oxygen of the CSPs may also be involved in hydrogen bonding with the allylic hydroxyl group of some diols.

Non-K & Bay Region Diols

The enantiomers of the non-K and bay region dihydrodiols and tetrahydrodiols of BA and BaP which are more strongly retained by (R)-DNBPG have one structural feature in common (Figure 5); all benzylic hydroxyl groups have the same absolute configuration (i.e., the benzylic hydroxyl groups point toward the viewer). In Figure 5, structures are drawn so that the aromatic nuclei of the diols responsible for $\pi-\pi$ interaction with the 3,5-dinitrobenzoyl moiety of the CSPs are all placed at the lower left hand corner.

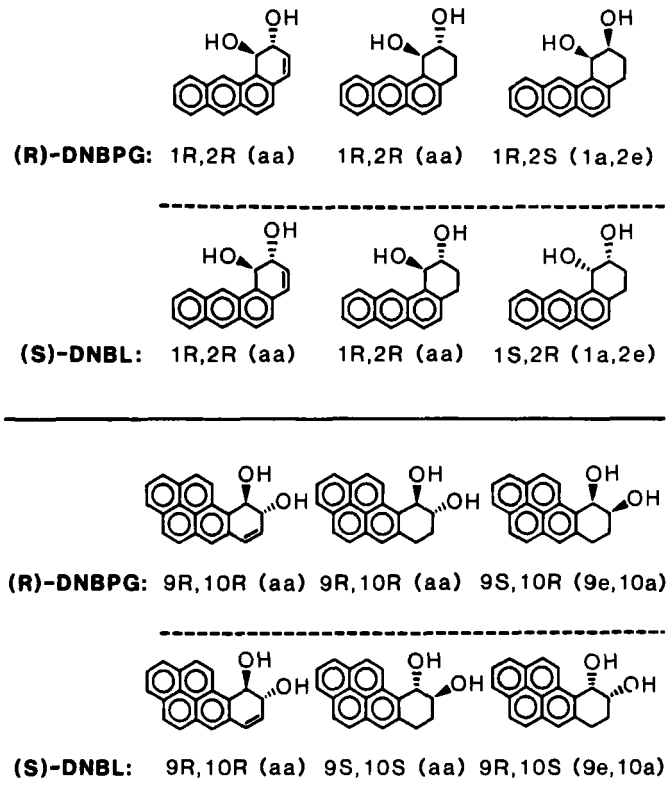

FIGURE 5. Structures of the non-K and bay region diol enantiomers of BA and BaP that are more strongly retained by CSPs (R)-DNBPG and (S)-DNBL. Elution orders of enantiomers on a CSP are the same regardless whether the CSP is ionically bonded or covalently bonded (see Table 1).

When we examine the chiral interactions between (R)-DNBPG and the bay region diols (Figure 6) in the same manner as described by Pirkle et al. (2), it is clear that, in addition to the hydrogen bonding between the benzylic hydroxyl group of the diol and the amide hydrogen of the CSP, the allylic hydroxyl group of the diols may also interact with the non-amide carbonyl oxygen of (R)-DNBPG (Figure 6). When the allylic hydroxyl group is in quasiequatorial conformation (e.g., BA cis-(1R,2S)-H_4diol and BaP cis-(9S,10R)-

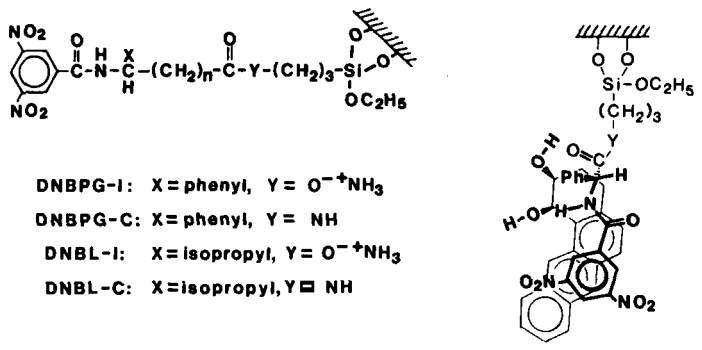

FIGURE 6. Structure of Pirkle's CSPs (n=0) used in this study. A chiral recognition model showing the relative arrangement between (R)-DNBPG and the more strongly retained enantiomer of BA c-1,2-H_4diol (modified from ref. 12). The potential interaction between allylic hydroxyl group and non-amide carbonyl oxygen may be eliminated if the CSP is modified by inserting a hydrocarbon chain ($n \geq 1$).

H_4diol), hydrogen bonding with non-amide carbonyl oxygen apparently contributes to the chiral interactions and significantly enhances the separation of enantiomers (Table 1). This is more apparent when we compare the efficiencies of enantiomeric resolution between BA t-1,2-H_4diol and BA c-1,2-H_4diol, and also between BaP t-9,10-H_4diol and BaP c-9,10-H_4diol (Table 1 and Figure 5).

The quasiaxial allylic hydroxyl group of BA t-1R,2R-H_2diol, BA t-1R,2R-H_4diol, BaP t-9R,10R-H_2diol and BaP t-9R,10R-H_4diol is away from, and does not interact with, the non-amide carbonyl oxygen of (R)-DNBPG. Enantiomers of these bay region trans dihydrodiols and tetrahydrodiols of BA and BaP are resolved in the absence of the hydrogen bonding between the allylic hydroxyl group of the diol and non-amide carbonyl oxygen of the CSP. These results indicate that the interaction between benzylic hydroxyl group and the amide hydrogen, along with the π-π and stereochemical interactions, are sufficient to effect enantiomeric resolutions. For example, enantiomers of 1-hydroxy-1,2,3,4-H_4BA, 4-

hydroxy-1,2,3,4-H_4BA, 7-hydroxy-7,8,9,10-H_4BaP, and 10-hydroxy-7,8,9,10-H_4BaP were all resolved by (R)-DNBPG-I (12).

The enantiomers of allylic cyclic alcohols such as 2-hydroxy-1,2,3,4-H_4BA, 8-hydroxy-7,8,9,10-H_4BaP, and 9-hydroxy-7,8,9,10-H_4BaP are not resolved by (R)-DNBPG-I (12). These results indicate that the interaction between the allylic hydroxyl group and either the amide hydrogen or the non-amide carbonyl oxygen is very weak and by itself is insufficient to effect enantiomeric resolutions. Partial resolutions of enantiomeric diols such as 1,2,3,4-H_4BA *trans* and *cis* 2,3-diols (12) indicate that interactions between (R)-DNBPG and two allylic hydroxyl groups of the enantiomeric diols are sufficiently different to effect enantiomeric resolutions.

The enantiomers of tetrahydrodiols such as BA t-1,2-H_4diol and BaP t-9,10-H_4diol are more efficiently resolved than the corresponding dihydrodiols such as BA t-1,2-H_2diol and BaP t-9,10-H_2diol. This is probably due to the simple reason that a more saturated ring provides a more flexible, hence stronger stereochemical repulsive interaction.

When (R)-DNBPG is replaced by (S)-DNBPG, the elution orders of resolved enantiomeric diols should be reversed. It is tempting to assume that resolved enantiomeric diols have different elution orders on (R)-DNBPG and (S)-DNBL. However, it is important to recognize that the steric crowding imposed by the isopropyl group in (S)-DNBL is different from that by the phenyl group in (R)-DNBPG. Thus the enantiomer more strongly retained by (R)-DNBPG may not necessarily be the less strongly retained enantiomer by (S)-DNBL.

Of the six enantiomeric diols resolved by (S)-DNBL (Table 1 and Figure 5), the allylic hydroxyl groups of five diols more strongly retained have the same absolute stereochemistry (the allylic hydroxyl group points away from the viewer, Figure 5). Only BaP 9S,10S-H_4diol is more strongly retained by (S)-DNBL and its allylic hydroxyl group is pointing toward the viewer (Figure 5). Three of the diols had the same enantiomer more strongly

retained by both (R)-DNBPG and (S)-DNBL. It is apparent that an elution order-absolute configuration relationship does not exist for (S)-DNBL in the resolution of non-K and bay region diols.

K- & Bay Region Diols

Among the K- and bay region trans and cis dihydrodiols of 4-MBA, 7-MBA, 3-MC, and DMBA the enantiomers more strongly retained by (S)-DNBL have one structural feature in common; the enantiomers whose hydroxyl groups that are allylic to the naphthyl ring (e.g., the C_5 hydroxyl group in K-region dihydrodiols of 4-MBA and 7-MBA) and benzylic to the angular benzo ring (e.g., the C_5 hydroxyl group in K-region dihydrodiols of 4-MBA and 7-MBA) have the same S absolute configuration (the hydroxyl group points away from the viewer; Figures 7 and 8). The allylic (to the naphthyl ring) hydroxyl group of a dihydrodiol may exist in either quasiaxial or quasiequatorial conformation and may interact with the amide hydrogen of the CSP in effecting the separation of enantiomers.

Six of eight K- and bay region diol enantiomers more strongly retained by (S)-DNBL are also more strongly retained by (R)-DNBPG (Table 2, Figures 7 and 8). The allylic (to the naphthyl ring) hydroxyl group may interact with either the amide hydrogen or the non-amide carbonyl oxygen to effect enantiomeric resolution. The trans-(5S,6S)-dihydrodiol enantiomers of 7-fluoro-BA (data not shown), 7-chloro-BA (20), and 7-bromo-BA (20) are also more strongly retained by (R)-DNBPG-I. The (R)-DNBPG does not always retain enantiomers with the allylic (to the naphthyl ring) hydroxyl groups in S absolute configuration. For examples: (i) the 5R,6S enantiomer of 4-MBA c-5,6-H_2diol is retained more strongly by both the ionically and covalently bonded (R)-DNBPG and (ii) the elution order of DMBA t-5,6-H_2diol enantiomers on (R)-DNBPG-I is reversed from that on (R)-DNBPG-C (12 and Table 2).

In summary, by studying the enantiomeric resolutions of some trans and cis diols derived from benz[a]anthracene and benzo[a]-pyrene by CSPs (R)-DNBPG and (S)-DNBL, a clear insight is gained into the chiral recognition mechanisms responsible for the direct separation of enantiomers. The unique aspects of this study are

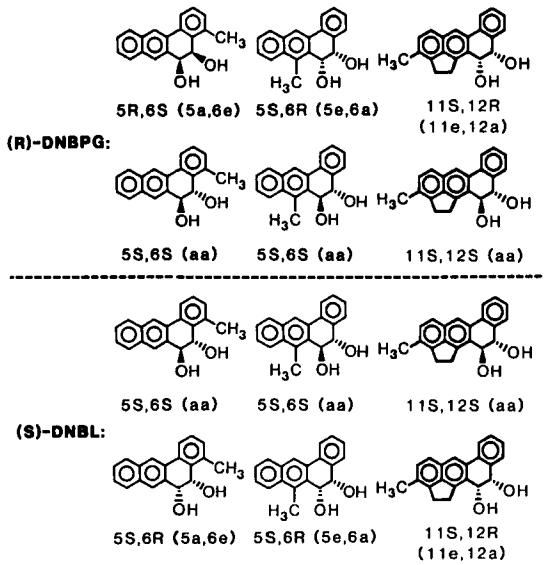

FIGURE 7. Structures of the K and bay region diol enantiomers of 4-MBA, 7-MBA, and 3-MC that are more strongly retained by CSPs (R)-DNBPG and (S)-DNBL. Elution orders of enantiomers, if resolved on a particular CSP, are the same regardless whether the CSP is ionically bonded or covalently bonded (see Table 2).

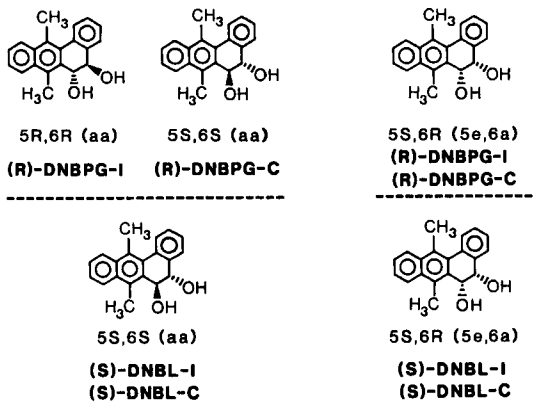

FIGURE 8. Structures of the K and bay region diol enantiomers of DMBA that are more strongly retained by the CSPs indicated.

that the conformational preferences and the absolute configurations of the enantiomeric diols have all been established. Thus the relationship regarding conformational preference, absolute configuration, as well as elution order of diol enantiomers can be clearly defined. The followings have been definitively established:

(i) The absolute configuration of benzylic hydroxyl group determines the elution orders of non-K and bay region diols on (R)-DNBPG.

(ii) The allylic hydroxy group of some non-K and bay region diols are involved in chiral interaction with the non-amide carbonyl oxygen of (R)-DNBPG.

(iii) The absolute configuration of the hydroxyl group allylic to the naphthyl ring determines the elution order of K-region trans and cis dihydrodiols derived from 4-MBA, 7-MBA, 3-MC, and DMBA on (S)-DNBL.

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