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# ELUTION ORDER–ABSOLUTE CONFIGURATION RELATIONSHIP OF K-REGION DIHYDRODIOL ENANTIOMERS OF BENZ[*a*]ANTHRACENE DERIVATIVES IN CHIRAL STATIONARY PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

SHEN K. YANG* and MOHAMMAD MUSHTAQ
*Department of Pharmacology, F. Edward Hébert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814 (U.S.A.)*
and
PETER P. FU
*National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR 72079 (U.S.A.)*

## SUMMARY

The direct resolution of K-region *cis*- and *trans*-dihydrodiol enantiomers of 14 unsubstituted and methyl- and bromo-substituted benz[*a*]anthracene (BA) derivatives was investigated by high-performance liquid chromatography with commercially available columns, packed with $\gamma$-aminopropylsilanized silica to which either (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine (*R*-DNBPG) or (*S*)-N-(3,5-dinitrobenzoyl)leucine (*S*-DNBL) is either ionically or covalently bonded. BA derivatives used in this study include: BA, 1-methyl-BA, 4-methyl-BA, 7-methyl-BA, 8-methyl-BA, 10-methyl-BA, 11-methyl-BA, 12-methyl-BA, 7,12-dimethyl-BA, 7-bromo-BA, 7-bromo-1-methyl-BA, 7-bromo-11-methyl-BA, 7-bromo-12-methyl-BA, and 3-methylcholanthrene. The enantiomers of BA *trans*-5,6-dihydrodiol were the only compounds not resolved by any of the four chiral stationary phases (CSPs) tested. The results indicate that conformational preference of the hydroxyl group is one of the most important factor in determining the elution order of dihydrodiol enantiomers. The presence and the location of a substituent and the molecular size and shape of the dihydrodiols can significantly affect the efficiency of enantiomeric resolution. In general, the ionically bonded *R*-DNBPG provides the best resolution of enantiomeric quasidiequatorial *trans*-dihydrodiols and the *R,R* enantiomers are consistently more strongly retained. In contrast, the enantiomeric pairs of quasidiaxial *trans*-dihydrodiols are generally better resolved by the covalently bonded *R*-DNBPG, and the *S,S* enantiomers are more strongly retained. The enantiomers of *cis*-dihydrodiols having hydroxyl groups that adopt quasiequatorial-quasiaxial and/or quasiaxial-quasiequa-

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torial conformations are more consistently resolved by the ionically bonded *S*-DNBL and in all cases the *S,R* enantiomers are more strongly retained. Thus, it is possible to choose a CSP which resolves the K-region dihydrodiol enantiomers with a predictable elution order.

## INTRODUCTION

Pirkle and co-workers[1–3] have successfully resolved the enantiomers of a large number of compounds by high-performance liquid chromatography (HPLC) with the chiral stationary phases (CSPs) that they have developed. Columns packed with covalently and ionically bonded CSPs, (*R* or *S*)-N-(3,5-dinitrobenzoyl)phenylglycine (*R*- or *S*-DNBPG) and (*R* or *S*)-N-(3,5-dinitrobenzoyl)leucine (*R*- or *S*-DNBL), are available commercially. Using these CSP columns, a solvent system (ethanol–acetonitrile–hexane) was developed which allowed the separation of enantiomers of relatively more polar compounds, such as diol derivatives of polycyclic aromatic hydrocarbons (PAHs)[4]. This CSP-HPLC method has been applied successfully to the resolution of mono-ol, epoxide, and diol enantiomers of PAHs including phenanthrene, chrysene, benz[*a*]anthracene (BA), monomethylbenz[*a*]anthracene (*x*-MBA), 7,12-dimethylbenz[*a*]anthracene (7,12-DMBA), dibenz[*a,h*]anthracene, cholanthrene, 3-methylcholanthrene (3-MC), and benzo[*a*]pyrene (BaP)[4–14].

Pirkle *et al.*[2] proposed a chiral recognition mechanism to predict the elution order of enantiomers of cyclic alcohols (mono-ols) as well as other types of compounds on an ionically bonded CSP. This chiral recognition mechanism has been adopted to interpret the results of enantiomeric separation of chiral PAH derivatives[5,8,13,15]. Due to limited information on the absolute configurations of resolved enantiomers, it has not been possible to establish a rule that correctly predicts the elution order–absolute configuration relationship of resolved enantiomers[8,13,15].

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 on an ionically bonded *R*-DNBPG column has been reported[13]. It was found that structural factors, such as conformation, presence and location of a methyl substituent, molecular size and shape, and ring saturation all contributed to chiral interactions between the CSP and the solutes. Furthermore, the enantiomers of 7,12-DMBA *trans*-5,6-dihydrodiol were found to have different elution orders on covalently and ionically bonded *R*-DNBPG[8]. Recently, the enantiomeric separation of some PAH diols which, due to steric constraint, adopt only one of two possible conformations, provided additional insight into the relationships of conformational preference, absolute configuration, and elution order of diol enantiomers[15]. The separation of K-region *trans*- and *cis*-dihydrodiol enantiomers of nine additional BA derivatives has been studied using both covalently and ionically bonded *R*-DNBPG and *S*-DNBL, and is the subject of this report. The results of this study and those reported earlier[8,13,15] indicate that it is possible to choose a CSP which provides a predictable elution order of resolved K-region dihydrodiol enantiomers.

K- and bay-region designation and numbering system of BA and 3-MC are indicated in Fig. 1.

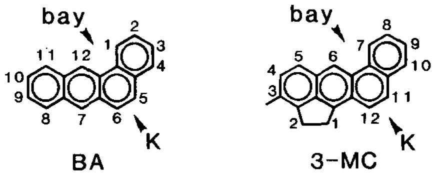

Fig. 1. K- and bay-region designation and numbering system of benz[*a*]anthracene (BA) and 3-methylcholanthrene (3-MC).

## EXPERIMENTAL

### *Materials and methods*

BA, 7,12-DMBA, 3-MC, and osmium tetroxide were purchased from Aldrich (Milwaukee, WI, U.S.A.). 1-MBA, 4-MBA, 7-MBA, 8-MBA, 10-MBA, 11-MBA, and 12-MBA were synthesized according to established procedures[14,16–18]. 7-Br-BA[7], 7-Br-1-MBA, 7-Br-11-MBA, 7-Br-12-MBA were synthesized by bromination of BA, 1-MBA, 11-MBA, and 12-MBA, respectively, with N-bromosuccinimide in dimethylformamide and their structures were determined by analysis of their UV–VIS absorption, mass, and proton nuclear magnetic resonance ($^1$H NMR) spectral data.

The K-region *trans*-dihydrodiols were isolated, by a combination of reversed-phase and normal-phase HPLC, from a mixture of metabolites, obtained by incubation of each parent hydrocarbon with liver microsomes from male Sprague-Dawley rats which had been treated with either 3-methylcholanthrene or phenobarbital and a reduced nicotinamide-adenine dinucleotide phosphate (NADPH) regenerating system[12,14,19]. The (*R,R*)/(*S,S*) enantiomer ratios of K-region *trans*-dihydrodiols formed in the metabolisms of the parent hydrocarbons by liver microsomes from 3-MC-treated male Sprague-Dawley rats were: 1-MBA, 55:45; 4-MBA, 82:18; 7-MBA, 51:49; 8-MBA, 90:10; 10-MBA, 57:43; 11-MBA, 73:27; 12-MBA, 5:95; 7,12-DMBA, 6:94; 7-Br-BA, 99:1; and 7-Br-1-MBA, 94:6. The (*R,R*)/(*S,S*) enantiomer ratios of K-region *trans*-dihydrodiols formed in the metabolisms of 7-Br-11-MBA and 7-Br-12-MBA by liver microsomes from phenobarbital-treated male Sprague-Dawley rats were 99:1 and 87:13, respectively. Racemic K-region *cis*-dihydrodiols of methylated and brominated BA were synthesized by reaction of each parent hydrocarbon with osmium tetroxide as described[20].

### *Chromatography*

Dihydrodiols were analyzed with HPLC columns (25 cm × 4.6 mm I.D.; Regis Chemical Co., Morton Grove, IL, U.S.A.) packed with spherical particles of 5-$\mu$m diameter of $\gamma$-aminopropylsilanized silica to which either an (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine (*R*-DNBPG) or an (*S*)-N-(3,5-dinitrobenzoyl)-leucine (*S*-DNBL) was either ionically or covalently bonded[1,3]. HPLC was performed on 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 (254 nm or 280 nm) detector. Samples were injected via a Valco Model N60 loop injector (Valco, Houston, TX, U.S.A.). 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 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 by reversed-phase HPLC with a DuPont Zorbax ODS column, as described previously[4], prior to circular dichroism (CD) spectral measurement.

### *Absolute configuration of dihydrodiol enantiomers*

The absolute configurations of dihydrodiol enantiomers were determined by the exciton chirality CD method[21] similarly as described[15]. Each dihydrodiol (0.1–0.3 mg) in a test tube was dissolved in 1 ml of ethyl acetate that had been dried by sodium hydride treatment. Sodium hydride (*ca.* 1 mg) was added, followed by *p*-N,N-dimethylaminobenzoyl chloride (*ca.* 5 mg). After being cooled in ice water for about 5 min, two drops of *p*-N,N-dimethylaminopyridine (10 mg/ml of ethyl acetate) were added and the reaction mixture was stirred for 16 h. Solid material was removed by centrifugation at 4000 *g* and the supernatant was dried, redissolved in tetrahydrofuran–methanol (1:1) and injected onto a DuPont Zorbax ODS column (25 cm × 4.6 mm I.D.) and was eluted with a linear gradient of methanol–water (3:1, v/v) to methanol at 1.5 ml/min over a period of 15 min. The bis-*p*-N,N-dimethylaminobenzoate was eluted between 16–20 min.

### *Spectral analysis*

UV–VIS 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 $A_{\lambda}$/ml (number of absorbance units at wavelength $\lambda$ 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[6]. Unless stated otherwise, all CD spectral data were obtained with optically pure enantiomers.

## RESULTS AND DISCUSSION

Enantiomeric separations of K-region *trans*- and *cis*-dihydrodiols of BA, 4-MBA, 7-MBA, 7,12-DMBA, and 3-MC using both ionically and covalently bonded *R*-DNBPG and *S*-DNBL columns were reported recently[15]. These data are included in Tables I–IV for comparison. The more structurally related K-region dihydrodiols that are available for study, the greater the likelihood that the essential structural feature(s) responsible for enantiomeric separations can be revealed.

The conformation of PAH *trans*-dihydrodiols can be determined by NMR spectroscopy. The coupling constant between the carbinol protons is *ca.* 2–4 Hz for quasidiaxial *trans*-dihydrodiols and *ca.* 9–10 Hz for quasidiequatorial *trans*-dihydrodiols[22]. Due to steric and/or electronic repulsion, *trans*-dihydrodiols with a *peri*-

methyl or a *peri*-halogen substituent preferentially adopt quasidiaxial conformations. Those that do not have a *peri* substituent adopt preferentially quasidiequatorial conformations, due to intramolecular hydrogen bonding[23].

The coupling constants between the carbinol protons of K-region *cis*-dihydrodiols are *ca.* 3.5 Hz[14,15,23]. The two possible conformations of K-region *cis*-dihydrodiols are quasiequatorial-quasiaxial and quasiaxial-quasiequatorial. In the presence of a *peri*-methyl or a halogen substituent, its steric and/or electronic effect causes the *peri*-hydroxyl group to adopt preferentially a quasiaxial conformation. Thus 1-MBA *cis*-5,6-dihydrodiol adopts both 5-quasiequatorial-6-quasiaxial (5e, 6a) and 5-quasiaxial-6-quasiequatorial (5a, 6e) conformations, 4-MBA *cis*-5,6-dihydrodiol adopts preferentially a 5a, 6e conformation, and 7-Br-1-MBA *cis*-5,6-dihydrodiol adopts preferentially a 5e, 6a conformation, respectively.

### *Determination of absolute configuration of dihydrodiol enantiomers*

The absolute configuration of quasidiequatorial *trans*-5,6-dihydrodiol enantiomer of 1-MBA less strongly retained by the ionically bonded *R*-DNBPG (Table I) was established by the exciton chirality CD method[21]. The bis-*p*-N,N-dimethylaminobenzoate derivative exhibits a strong and positive CD band at 323 nm (Fig. 2) which indicates that the benzoate groups have a positive chirality, hence, the dihydrodiol from which the bis-ester was derived has a 5*S*,6*S* absolute stereochemistry[21].

The quasidiaxial 7-Br-11-MBA *trans*-5,6-dihydrodiol enantiomer less strongly retained by both ionically and covalently bonded *R*-DNBPG (Tables I and II) is established to have a 5*R*,6*R* absolute stereochemistry because its CD Cotton effects (Fig. 3) are similar to those of quasidiaxial 7-Br-BA (*trans*)-5*R*,6*R*-dihydrodiol[7] (Fig. 3), 7-MBA (*trans*)-5*R*,6*R*-dihydrodiol[14], 3-MC (*trans*)-11*R*,12*R*-dihydrodiol[15], and 4-MBA (*trans*)-5*R*,6*R*-dihydrodiol (Fig. 4). The quasidiaxial *trans*-5,6-dihydrodiol enantiomers of 7-Br-1-MBA and 7-Br-12-MBA less strongly retained by the covalently bonded *R*-DNBPG are established to have 5*R*,6*R* absolute stereochemistries because their CD Cotton effects (Fig. 3) are similar to those of quasidiaxial 7,12-DMBA (*trans*)-5*R*,6*R*-dihydrodiol[12] (Fig. 3). When the bromo substituents of 7-Br-BA 5*R*,6*R*-dihydrodiol[7], 7-Br-1-MBA 5*R*,6*R*-dihydrodiol, 7-Br-11-MBA 5*R*,6*R*-dihydrodiol, 7-Br-12-MBA 5*R*,6*R*-dihydrodiol are removed by hydrogenolysis (tetrahydrofuran, $PtO_2/H_2$, 1 atm, 30 min), the CD spectra of the resulting *trans*-5,6-dihydrodiols are identical to those of BA 5*R*,6*R*-dihydrodiol[7,12,24], 1-MBA 5*R*,6*R*-dihydrodiol (Fig. 2), 11-MBA 5*R*,6*R*-dihydrodiol[17], and 12-MBA 5*R*,6*R*-dihydrodiol[18], respectively.

It is interesting to note that the signs of Cotton effects at *ca.* 265 nm in the CD spectra of the quasidiaxial 5*R*,6*R* dihydrodiol enantiomers of 7-Br-1-MBA, 7-Br-12-MBA, and 7,12-DMBA are opposite to those of quasidiaxial K-region *R*,*R*-dihydrodiol enantiomers of 7-Br-BA (Fig. 3), 7-Br-11-MBA (Fig. 3), 4-MBA (Fig. 4), 7-MBA[14], and 3-MC[15]. Apparently, these differences are due to the presence (or the absence) of a methyl group in the bay region of the molecules. Substitution with a methyl group at C-1 or C-12 of BA is known to cause an out-of-plane distortion (an *ca.* 21° angle between the 1,2,3,4-ring and the 8,9,10,11-ring) of the otherwise planar BA molecule[25].

The absolute configuration of quasidiaxial 4-MBA *trans*-5,6-dihydrodiol enantiomer less strongly retained by both ionically and covalently bonded *R*-DNBPG

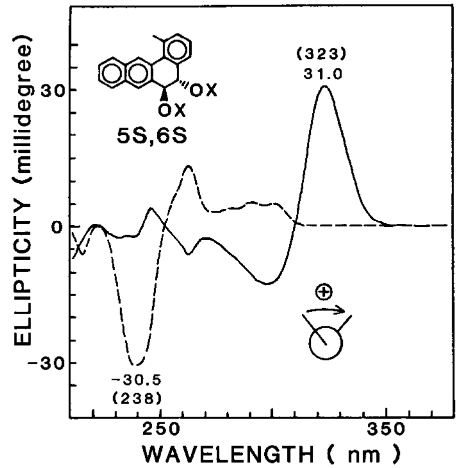

Fig. 2. CD spectra of 1-MBA *trans*-5,6-dihydrodiol enantiomer less strongly retained by the ionically bonded *R*-DNBPG (— — —, X = H; 1.0 $A_{264}$/ml) and its bis-*p*-N,N-dimethylaminobenzoate derivative (———, X = *p*-N,N-dimethylaminobenzoyl; 1.0 $A_{307}$/ml).

was elucidated by the exciton chirality CD method[19] (Fig. 4). Its bis-N,N-dimethylaminobenzoate derivative exhibits a negative CD band at 320 nm (Fig. 4, left panel), which indicates that the *trans*-dihydrodiol under consideration has a 5*R*,6*R* absolute stereochemistry[21]. The absolute configuration of the 5a,6e 4-MBA *cis*-5,6-dihydro-

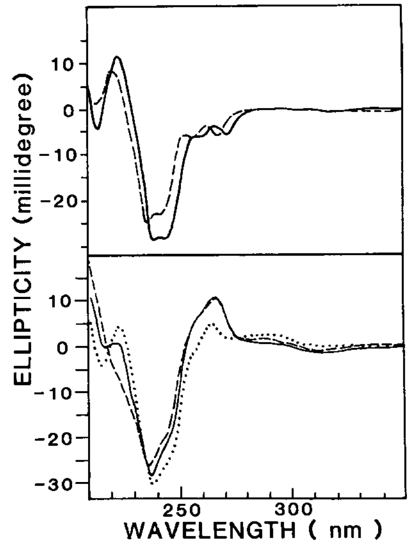

Fig. 3. CD spectra of 7-Br-BA (*trans*)-5*R*,6*R*-dihydrodiol[7] (— — —, 1.0 $A_{268}$/ml; upper panel), 7-Br-11-MBA *trans*-5,6-dihydrodiol enantiomer less strongly retained by both ionically and covalently bonded *R*-DNBPG (———, 1.0 $A_{271}$/ml; upper panel), 7,12-DMBA (*trans*)-5*R*,6*R*-dihydrodiol[12] (— — —, lower panel; 1.0 $A_{269}$/ml), and the *trans*-5,6-dihydrodiol enantiomers of 7-Br-1-MBA (......, lower panel; 1.0 $A_{267}$/ml) and 7-Br-11-MBA (———, lower panel; 1.0 $A_{268}$/ml) less strongly retained by the covalently bonded *R*-DNBPG, methanol.

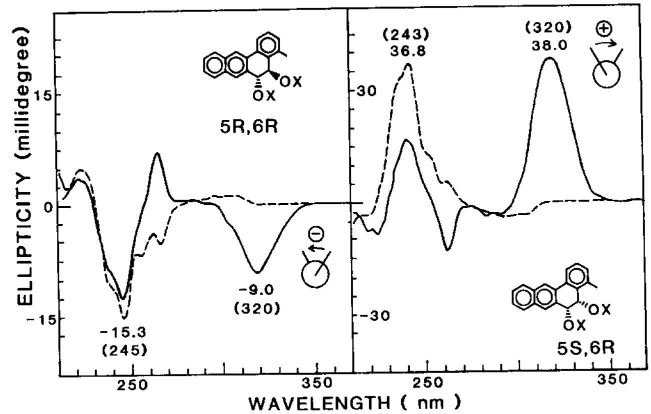

Fig. 4. CD spectra of 4-MBA *trans*-5,6-dihydrodiol enantiomer (enantiomeric excess 64%) less strongly retained by the covalently bonded *R*-DNBPG (— — —, X = H, left panel; 1.0 $A_{266}$/ml) and its bis-*p*-N,N-dimethylaminobenzoate derivative (———, X = *p*-N,N-dimethylaminobenzoyl, left panel; 1.0 $A_{313}$/ml), and the CD spectra of 4-MBA *cis*-5,6-dihydrodiol enantiomer less strongly retained by the ionically bonded *R*-DNBPG (— — —, X = H, right panel; 1.0 $A_{265}$/ml) and its bis-*p*-N,N-dimethylaminobenzoate derivative (———, X = *p*-N,N-dimethylaminobenzoyl, right panel; 1.0 $A_{307}$/ml).

diol enantiomer less strongly retained by the ionically bonded *R*-DNBPG was also elucidated by the exciton chirality CD method[19] (Fig. 4, right panel). Its bis-N,N-dimethylaminobenzoate derivative exhibits a strong and positive CD band at 320 nm (Fig. 4, left panel), which indicates that the *cis*-dihydrodiol enantiomer under consideration has a 5*S*,6*R* absolute stereochemistry[21].

The absolute configurations of 11e,12a 3-MC *cis*-11,12-dihydrodiol and 5e,6a 7-MBA *cis*-5,6-dihydrodiol enantiomers less strongly retained by the covalently bonded *R*-DNBPG were similarly established by the exciton chirality CD method[19] (Fig. 5). Both bis-N,N-dimethylaminobenzoate derivatives exhibit positive CD bands at 324 nm (Fig. 5), which indicate that both *cis*-dihydrodiol enantiomers under considerations have *R*,*S* absolute stereochemistries[21].

The absolute configurations of enantiomeric *cis*-5,6-dihydrodiols of 8-MBA and 10-MBA less strongly retained by the ionically bonded *S*-DNBL were established to be the 5*R*,6*S* enantiomers, because their CD Cotton effects are similar to those of the 12-MBA (*cis*)-5*R*,6*S*-dihydrodiol enantiomer[26] (Fig. 6). The shifts in the wavelengths of CD maxima are due to effects of the methyl substituent at various positions of BA. The absolute configurations of K-region *cis*-dihydrodiol enantiomers of BA, 1-MBA, 11-MBA, 7-Br-BA, 7-Br-1-MBA, and 7-Br-11-MBA have been determined similarly according to the methods reported for K-region *cis*-5,6-dihydrodiol enantiomers of 12-MBA and 7-Br-12-MBA[26]. The results in detail will be reported elsewhere[27].

It is interesting to note that CD bands of all quasidiaxial K-region *trans*-*R*,*R*-dihydrodiol enantiomers have negative signs between approximately 230–250 nm (Figs. 3 and 4 and refs. 7, 12, 14 and 15). In contrast, CD bands of all quasidiequatorial K-region *trans*-*R*,*R*-dihydrodiol enantiomers have positive signs between approximately 230–250 nm (Fig. 2 and refs. 7, 12, 14, 17, 18, and 28). The relationships between conformational preference of hydroxyl groups, CD Cotton effects, and

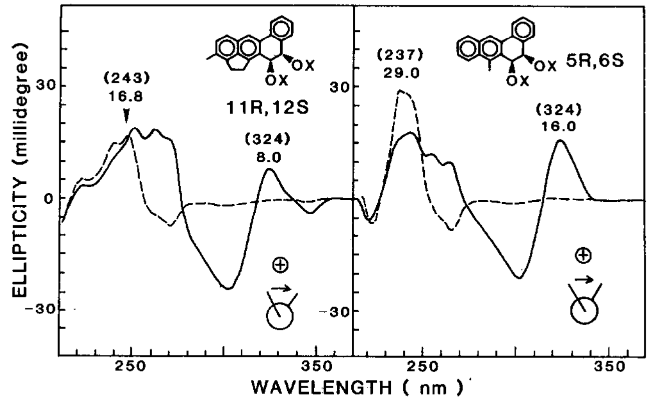

Fig. 5. CD spectra of 3-MC *cis*-11,12-dihydrodiol enantiomer less strongly retained by the covalently bonded *R*-DNBPG (— — —, X = H, left panel; 1.0 $A_{273}$/ml) and its bis-*p*-N,N-dimethylaminobenzoate derivative (———, X = *p*-N,N-dimethylaminobenzoyl, left panel; 1.0 $A_{312}$/ml), and the CD spectra of 7-MBA *cis*-5,6-dihydrodiol enantiomer less strongly retained by the covalently bonded *R*-DNBPG (— — —, X = H, right panel; 1.0 $A_{268}$/ml) and its bis-*p*-N,N-dimethylaminobenzoate derivative (———, X = *p*-N,N-dimethylaminobenzoyl, right panel; 1.0 $A_{314}$/ml).

absolute configurations of K-region and non-K-region dihydrodiol enantiomers will be described in detail in a separate report.

*Elution order of dihydrodiol enantiomers on ionically bonded R-DNBPG*

The results of enantiomeric separation of K-region dihydrodiols by the ionically bonded *R*-DNBPG are shown in Table I.

Except for the enantiomers of BA *trans*-5,6-dihydrodiol which are not resolved, the *R*,*R* enantiomers of all other quasidiequatorial *trans*-dihydrodiols are more strongly retained by the ionically bonded *R*-DNBPG. However, most enantiomeric pairs of quasidiequatorial *trans*-dihydrodiols are not efficiently resolved. A methyl substituent at either C-1 or C-12 of BA (*e.g.*, 1-MBA and 12-MBA) facilitates the

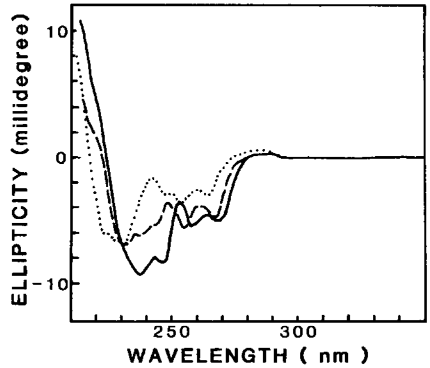

Fig. 6. CD spectra of 12-MBA (*cis*)-5*R*,6*S*-dihydrodiol[26] (....., 1.0 $A_{265}$/ml) and the *cis*-5,6-dihydrodiol enantiomers of 8-MBA (———, 1.0 $A_{269}$/ml) and 10-MBA (— — —, 1.0 $A_{264}$/ml) less strongly retained by the ionically bonded *S*-DNBL.

resolution of *trans*-5,6-dihydrodiol enantiomers (with resolution value > 1).

Six of eight quasidiaxial K-region *trans*-dihydrodiols are resolved with various efficiencies and the *S,S* enantiomers are more strongly retained. However, the *R,R* enantiomers of K-region *trans*-5,6-dihydrodiols of 7-Br-12-MBA and 7,12-DMBA are more strongly retained. Apparently the combined effects of substituents at both C-7 and C-12 positions of BA changed the chiral interactions between the solute molecules and the CSP.

Among the K-region *cis*-dihydrodiols that can adopt both 5a,6e and 5e,6a conformations, only the enantiomers of 8-MBA *cis*-5,6-dihydrodiol are not resolved. All other dihydrodiol enantiomers are resolved, the *R,S* enantiomers being more strongly retained.

Because of the steric hindrance due to a *peri* C-4 methyl substituent, 4-MBA *cis*-5,6-dihydrodiol adopts only the 5a,6e conformation. The 5*R*,6*S* enantiomer of this dihydrodiol is more strongly retained. Among all the dihydrodiols tested, 4-MBA *cis*-5,6-dihydrodiol is the only dihydrodiol with a 5a,6e conformation. Testing of additional dihydrodiols with the same (5a,6e) conformational preference should provide more insight into the elution order–absolute configuration relationship.

Except for the *cis*-5,6-dihydrodiol enantiomers of 7-MBA and 7,12-DMBA, the enantiomeric pairs of all other *cis*-dihydrodiols with 5e,6a (or 11e,12a) conformations are either not resolved at all (*e.g.*, 7-Br-12-MBA *cis*-5,6-dihydrodiol) or poorly resolved. The *S,R* enantiomers of five out of seven *cis*-dihydrodiols with 5e,6a (or 11e,12a) conformations are more strongly retained by the CSP. However, the *R,S* enantiomer of 7-Br-11-MBA *cis*-5,6-dihydrodiol is more strongly retained.

### *Elution order of dihydrodiol enantiomers on covalently bonded R-DNBPG*

The results of enantiomeric separation of K-region dihydrodiols by covalently bonded *R*-DNBPG are shown in Table II. In contrast to the results obtained by using the ionically bonded *R*-DNBPG, none of the enantiomeric pairs of six quasidiequatorial *trans*-dihydrodiols are resolved by the covalently bonded *R*-DNBPG. However, enantiomeric pairs of eight quasidiaxial *trans*-dihydrodiols are all resolved (resolution value 0.5–5.4) and the *S,S* enantiomers are more strongly retained by the CSP.

Among the K-region *cis*-dihydrodiols that can adopt both 5a,6e and 5e,6a conformations, only the enantiomers of 12-MBA *cis*-5,6-dihydrodiol are resolved with resolution value greater than 1. All other *cis*-dihydrodiol enantiomers are either poorly resolved or not resolved at all. Among those resolved, the *R,S* enantiomers are more strongly retained. The *R,S* enantiomer of 4-MBA *cis*-5,6-dihydrodiol (5a,6e conformation) is more strongly retained by the CSP.

The *S,R* enantiomers of all seven *cis*-dihydrodiols with 5e,6a (or 11e,12a) conformations are more strongly retained by the CSP. In comparison with the results obtained by using the ionically bonded *R*-DNBPG, the elution orders of enantiomeric *cis*-dihydrodiols with 5e,6a (or 11e,12a) conformations are more consistent when covalently bonded *R*-DNBPG is used (Table I *vs.* Table II).

### *Elution order of dihydrodiol enantiomers on ionically bonded S-DNBL*

The results of enantiomeric separation of K-region dihydrodiols obtained by using the ionically bonded *S*-DNBPG are shown in Table III.

TABLE I

CSP-HPLC RESOLUTION OF K-REGION *trans*- AND *cis*-DIHYDRODIOL ENANTIOMERS OF UNSUBSTITUTED AND METHYL- AND BROMO-SUBSTITUTED BENZ[*a*]ANTHRACENE DERIVATIVES WITH AN IONICALLY BONDED *R*-DNBPG COLUMN

The preferred conformation of the hydroxyl group is indicated by either "a" (quasiaxial) or "e" (quasiequatorial). DHD = dihydrodiol.

| *Dihydrodiol* | *Preferred conformation* | *A (%)** | *Retention time* | | *RV*** | Ref.*** |
|---|---|---|---|---|---|---|
| | | | *Peak 1* | *Peak 2* | | |
| BA *trans*-5,6-DHD | 5e,6e | 10 | 15.8 | 15.8 | 0 | 12, 24 |
| 1-MBA *trans*-5,6-DHD | 5e,6e | 10 | 10.8 (*S,S*) | 11.2 (*R,R*) | 0.7 | This paper |
| | | 5 | 23.0 (*S,S*) | 24.1 (*R,R*) | 1.1 | |
| 8-MBA *trans*-5,6-DHD | 5e,6e | 10 | 14.3 (*S,S*) | 14.6 (*R,R*) | 0.3 | 28 |
| 10-MBA *trans*-5,6-DHD | 5e,6e | 10 | 15.8 (*S,S*) | 16.1 (*R,R*) | 0.1 | This paper |
| | | 5 | 39.5 (*S,S*) | 40.0 (*R,R*) | 0.2 | |
| 11-MBA *trans*-5,6-DHD | 5e,6e | 10 | 16.4 (*S,S*) | 17.0 (*R,R*) | 0.5 | 17 |
| 12-MBA *trans*-5,6-DHD | 5e,6e | 10 | 13.4 (*S,S*) | 14.5 (*R,R*) | 1.8 | 18 |
| 4-MBA *trans*-5,6-DHD | 5a,6a | 10 | 23.4 (*R,R*) | 24.1 (*S,S*) | 0.6 | This paper |
| 7-MBA *trans*-5,6-DHD | 5a,6a | 10 | 30.9 (*R,R*) | 32.6 (*S,S*) | 1.1 | 14 |
| 7-Br-BA *trans*-5,6-DHD | 5a,6a | 15 | 16.4 (*R,R*) | 17.9 (*S,S*) | 1.8 | 7 |
| 7-Br-11-MBA *trans*-5,6-DHD | 5a,6a | 15 | 17.3 (*R,R*) | 20.6 (*S,S*) | 3.5 | This paper |
| 7-Br-1-MBA *trans*-5,6-DHD | 5a,6a | 10 | 20.6 (*R,R*) | 21.3 (*S,S*) | 0.1 | This paper |
| 3-MC *trans*-11,12-DHD | 11a,12a | 10 | 26.4 (*R,R*) | 27.0 (*S,S*) | 0.1 | 15 |
| 7-Br-12-MBA *trans*-5,6-DHD | 5a,6a | 10 | 28.0 (*S,S*) | 28.3 (*R,R*) | 0.2 | This paper |
| 7,12-DMBA *trans*-5,6-DHD | 5a,6a | 10 | 21.8 (*S,S*) | 25.4 (*R,R*) | 2.6 | 12 |
| BA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 25.1 (*S,R*) | 25.8 (*R,S*) | 0.6 | 27 |
| 1-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 16.3 (*S,R*) | 17.0 (*R,S*) | 0.8 | 27 |
| 8-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 23.8 | 23.8 | 0 | This paper |
| 10-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 26.0 (*S,R*) | 26.4 (*R,S*) | 0.3 | This paper |
| 11-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 27.0 (*S,R*) | 27.8 (*R,S*) | 0.5 | 27 |
| 12-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 21.9 (*S,R*) | 23.5 (*R,S*) | 1.6 | 26 |
| 4-MBA *cis*-5,6-DHD | 5a,6e | 10 | 17.2 (*S,R*) | 19.1 (*R,S*) | 2.3 | This paper |
| 7-Br-BA *cis*-5,6-DHD | 5e,6a | 10 | 23.9 (*R,S*) | 24.6 (*S,R*) | 0.7 | 27 |
| 7-MBA *cis*-5,6-DHD | 5e,6a | 10 | 22.3 (*R,S*) | 23.5 (*S,R*) | 1.0 | This paper |
| 7-Br-1-MBA *cis*-5,6-DHD | 5e,6a | 10 | 14.9 (*R,S*) | 15.1 (*S,R*) | 0.1 | 27 |
| 7-Br-11-MBA *cis*-5,6-DHD | 5e,6a | 10 | 24.5 (*S,R*) | 24.8 (*R,S*) | 0.1 | 27 |
| 7-Br-12-MBA *cis*-5,6-DHD | 5e,6a | 10 | 20.9 | 20.9 | 0 | 26 |
| | | 7.5 | 28.4 | 28.4 | 0 | |
| 7,12-DMBA *cis*-5,6-DHD | 5e,6a | 10 | 15.2 (*R,S*) | 16.0 (*S,R*) | 1.0 | 8, 15 |
| 3-MC *cis*-11,12-DHD | 11e,12a | 10 | 44.4 (*R,S*) | 45.2 (*S,R*) | 0.3 | This paper |

* Percent of solvent A (ethanol–acetonitrile; 2:1, v/v) in hexane. The flow-rate was 2 ml/min.

** 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.

*** Reference in which CD spectra and/or absolute configuration of enantiomers were reported.

Except for the enantiomers of BA *trans*-5,6-dihydrodiol which are not resolved, the *S,S* enantiomers of all other quasidiequatorial *trans*-dihydrodiols are more strongly retained by the ionically bonded *S*-DNBL. However, none of the quasidiequatorial *trans*-dihydrodiol enantiomers is efficiently resolved (resolution value < 1.0).

TABLE II

CSP-HPLC RESOLUTION OF K-REGION *trans*- AND *cis*-DIHYDRODIOL ENANTIOMERS OF UNSUBSTITUTED AND METHYL- AND BROMO-SUBSTITUTED BENZ[*a*]ANTHRACENE DERIVATIVES WITH A COVALENTLY BONDED *R*-DNBPG COLUMN

Conditions and abbreviations are the same as those indicated in Table I. References in which CD spectral data and/or absolute configurations of enantiomers are indicated in Table I.

| *Dihydrodiol* | *Preferred conformation* | *A (%)* | *Retention time* | | *RV* |
|---|---|---|---|---|---|
| | | | *Peak 1* | *Peak 2* | |
| BA *trans*-5,6-DHD | 5e,6e | 10 | 8.6 | 8.6 | 0 |
| 1-MBA *trans*-5,6-DHD | 5e,6e | 5 | 14.5 | 14.5 | 0 |
| 8-MBA *trans*-5,6-DHD | 5e,6e | 10 | 9.9 | 9.9 | 0 |
| 10-MBA *trans*-5,6-DHD | 5e,6e | 5 | 21.8 | 21.8 | 0 |
| 11-MBA *trans*-5,6-DHD | 5e,6e | 10 | 10.6 | 10.6 | 0 |
| 12-MBA *trans*-5,6-DHD | 5e,6e | 10 | 9.2 | 9.2 | 0 |
| 4-MBA *trans*-5,6-DHD | 5a,6a | 10 | 19.6 (*R,R*) | 20.7 (*S,S*) | 1.1 |
| 7-MBA *trans*-5,6-DHD | 5a,6a | 10 | 24.1 (*R,R*) | 30.0 (*S,S*) | 5.4 |
| 7-Br-BA *trans*-5,6-DHD | 5a,6a | 15 | 14.1 (*R,R*) | 15.9 (*S,S*) | 2.9 |
| 7-Br-11-MBA *trans*-5,6-DHD | 5a,6a | 15 | 14.1 (*R,R*) | 17.7 (*S,S*) | 4.7 |
| 7-Br-1-MBA *trans*-5,6-DHD | 5a,6a | 10 | 17.4 (*R,R*) | 19.8 (*S,S*) | 3.1 |
| 3-MC *trans*-11,12-DHD | 11a,12a | 10 | 16.9 (*R,R*) | 19.2 (*S,S*) | 2.8 |
| 7-Br-12-MBA *trans*-5,6-DHD | 5a,6a | 15 | 12.2 (*R,R*) | 13.9 (*S,S*) | 2.5 |
| | | 10 | 22.3 (*R,R*) | 26.1 (*S,S*) | 3.5 |
| 7,12-DMBA *trans*-5,6-DHD | 5a,6a | 10 | 20.3 (*R,R*) | 20.8 (*S,S*) | 0.5 |
| BA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 16.0 | 16.0 | 0 |
| 1-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 12.0 (*S,R*) | 12.2 (*R,S*) | 0.1 |
| | | 5 | 30.4 (*S,R*) | 31.3 (*R,S*) | 0.7 |
| 8-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 16.3 | 16.3 | 0 |
| 10-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 15.7 (*S,R*) | 16.0 (*R,S*) | 0.1 |
| 11-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 17.3 | 17.3 | 0 |
| 12-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 15.1 (*S,R*) | 15.6 (*R,S*) | 1.2 |
| 4-MBA *cis*-5,6-DHD | 5a,6e | 10 | 12.6 (*S,R*) | 13.6 (*R,S*) | 1.5 |
| 7-Br-BA *cis*-5,6-DHD | 5e,6a | 10 | 15.3 (*R,S*) | 16.2 (*S,R*) | 1.2 |
| 7-MBA *cis*-5,6-DHD | 5e,6a | 10 | 14.6 (*R,S*) | 15.9 (*S,R*) | 1.7 |
| 7-Br-1-MBA *cis*-5,6-DHD | 5e,6a | 10 | 10.5 (*R,S*) | 10.6 (*S,R*) | 0.1 |
| 7-Br-11-MBA *cis*-5,6-DHD | 5e,6a | 10 | 14.5 (*R,S*) | 15.4 (*S,R*) | 1.3 |
| 7-Br-12-MBA *cis*-5,6-DHD | 5e,6a | 10 | 13.2 (*R,S*) | 13.8 (*S,R*) | 1.0 |
| 7,12-DMBA *cis*-5,6-DHD | 5e,6a | 10 | 10.9 (*R,S*) | 11.6 (*S,R*) | 1.3 |
| 3-MC *cis*-11,12-DHD | 11e,12a | 10 | 19.2 (*R,S*) | 21.1 (*S,R*) | 1.8 |

Six out of eight quasidiaxial K-region *trans*-dihydrodiols are resolved, the *S,S* enantiomers being more strongly retained. However, the *R,R* enantiomers of *trans*-5,6-dihydrodiols of 7-Br-BA and 7-Br-11-MBA are more strongly retained with resolution values $\leqslant 0.2$ for the separation of enantiomers.

Regardless of the conformational preferences, the *S,R* enantiomers of all K-region *cis*-dihydrodiols are more strongly retained by the ionically bonded *S*-DNBL. Under the chromatographic conditions used, the resolution values for the enantiomeric separations range from 0.3 to 3.5 (Table III).

TABLE III

CSP-HPLC RESOLUTION OF K-REGION *trans*- AND *cis*-DIHYDRODIOL ENANTIOMERS OF UNSUBSTITUTED AND METHYL- AND BROMO-SUBSTITUTED BENZ[*a*]ANTHRACENE DERIVATIVES WITH AN IONICALLY *S*-DNBL COLUMN

Conditions and abbreviations are the same as those indicated in Table I. References in which CD spectral data and/or absolute configurations of enantiomers are indicated in Table I.

| *Dihydrodiol* | *Preferred conformation* | *A (%)* | *Retention time* | | *RV* |
|---|---|---|---|---|---|
| | | | *Peak 1* | *Peak 2* | |
| BA *trans*-5,6-DHD | 5e,6e | 10 | 13.6 | 13.6 | 0 |
| 1-MBA *trans*-5,6-DHD | 5e,6e | 10 | 9.8 (*R*,*R*) | 9.9 (*S*,*S*) | 0.1 |
| | | 5 | 19.2 (*R*,*R*) | 19.6 (*S*,*S*) | 0.4 |
| 8-MBA *trans*-5,6-DHD | 5e,6e | 10 | 11.9 (*R*,*R*) | 12.1 (*S*,*S*) | 0.1 |
| 10-MBA *trans*-5,6-DHD | 5e,6e | 5 | 28.9 (*R*,*R*) | 29.5 (*S*,*S*) | 0.2 |
| 11-MBA *trans*-5,6-DHD | 5e,6e | 10 | 14.0 (*R*,*R*) | 14.1 (*S*,*S*) | 0.1 |
| | | 5 | 38.6 (*R*,*R*) | 39.7 (*S*,*S*) | 0.5 |
| 12-MBA *trans*-5,6-DHD | 5e,6e | 10 | 11.1 (*R*,*R*) | 11.5 (*S*,*S*) | 0.1 |
| 4-MBA *trans*-5,6-DHD | 5a,6a | 10 | 19.3 (*R*,*R*) | 20.2 (*S*,*S*) | 1.2 |
| 7-MBA *trans*-5,6-DHD | 5a,6a | 10 | 25.6 (*R*,*R*) | 27.2 (*S*,*S*) | 0.9 |
| 7-Br-BA *trans*-5,6-DHD | 5a,6a | 10 | 25.8 (*S*,*S*) | 26.3 (*R*,*R*) | 0.2 |
| 7-Br-11-MBA *trans*-5,6-DHD | 5a,6a | 10 | 25.1 (*S*,*S*) | 25.6 (*R*,*R*) | 0.1 |
| 7-Br-1-MBA *trans*-5,6-DHD | 5a,6a | 10 | 16.8 (*R*,*R*) | 17.9 (*S*,*S*) | 2.0 |
| 3-MC *trans*-11,12-DHD | 11a,12a | 10 | 23.2 (*R*,*R*) | 24.8 (*S*,*S*) | 1.3 |
| 7-Br-12-MBA *trans*-5,6-DHD | 5a,6a | 15 | 11.1 (*R*,*R*) | 12.6 (*S*,*S*) | 3.0 |
| | | 10 | 20.1 (*R*,*R*) | 23.2 (*S*,*S*) | 2.8 |
| 7,12-DMBA *trans*-5,6-DHD | 5a,6a | 10 | 20.6 (*R*,*R*) | 28.6 (*S*,*S*) | 6.9 |
| BA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 20.7 (*R*,*S*) | 21.9 (*S*,*R*) | 1.1 |
| 1-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 15 | 9.1 (*R*,*S*) | 9.3 (*S*,*R*) | 0.5 |
| | | 10 | 15.4 (*R*,*S*) | 16.0 (*S*,*R*) | 0.7 |
| 8-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 17.4 (*R*,*S*) | 18.6 (*S*,*R*) | 1.4 |
| 10-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 11.7 (*R*,*S*) | 12.5 (*S*,*R*) | 1.3 |
| 11-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 20.2 (*R*,*S*) | 21.8 (*S*,*R*) | 1.7 |
| 12-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 15 | 11.1 (*R*,*S*) | 12.5 (*S*,*R*) | 2.3 |
| | | 10 | 17.7 (*R*,*S*) | 20.4 (*S*,*R*) | 3.3 |
| 4-MBA *cis*-5,6-DHD | 5a,6e | 10 | 14.7 (*R*,*S*) | 15.3 (*S*,*R*) | 0.8 |
| 7-Br-BA *cis*-5,6-DHD | 5e,6a | 10 | 17.4 (*R*,*S*) | 18.5 (*S*,*R*) | 1.5 |
| 7-MBA *cis*-5,6-DHD | 5e,6a | 10 | 17.1 (*R*,*S*) | 18.9 (*S*,*R*) | 2.2 |
| 7-Br-1-MBA *cis*-5,6-DHD | 5e,6a | 10 | 11.8 (*R*,*S*) | 12.1 (*S*,*R*) | 0.3 |
| | | 5 | 26.4 (*R*,*S*) | 27.4 (*S*,*R*) | 0.6 |
| 7-Br-11-MBA *cis*-5,6-DHD | 5e,6a | 10 | 16.5 (*R*,*S*) | 18.5 (*S*,*R*) | 2.2 |
| 7-Br-12-MBA *cis*-5,6-DHD | 5e,6a | 10 | 15.6 (*R*,*S*) | 17.2 (*S*,*R*) | 2.1 |
| 7,12-DMBA *cis*-5,6-DHD | 5e,6a | 10 | 16.2 (*R*,*S*) | 17.7 (*S*,*R*) | 1.9 |
| 3-MC *cis*-11,12-DHD | 11e,12a | 10 | 26.9 (*R*,*S*) | 32.7 (*S*,*R*) | 3.5 |

### *Elution order of dihydrodiol enantiomers on covalently bonded S-DNBL*

The results of enantiomeric separation of K-region dihydrodiols obtained by using the covalently bonded *S*-DNBL are shown in Table IV.

The elution orders of enantiomers, when resolved, are the same as those observed by using the ionically bonded *S*-DNBL. However, except for a few dihydrodiols, the enantiomeric separations of the majority of the dihydrodiols listed in Table IV are considerably less efficient than those in which the ionically bonded *S*-DNBL was used (Table III *vs*. Table IV).

Regardless of the conformational preferences, the *S,R*-enantiomers of all K-region *cis*-dihydrodiols are more strongly retained by the covalently bonded *S*-DNBL. Except for the *cis*-5,6-dihydrodiol enantiomers of 7-Br-BA and 7-Br-1-MBA, which are not resolved, the resolution values for the enantiomeric separations of all other *cis*-dihydrodiols range from 0.4 to 2.2 (Table IV).

TABLE IV

CSP-HPLC RESOLUTION OF K-REGION *trans*- AND *cis*-DIHYDRODIOL ENANTIOMERS OF UNSUBSTITUTED AND METHYL- AND HALOGEN-SUBSTITUTED BENZ[*a*]ANTHRACENE DERIVATIVES WITH A COVALENTLY BONDED *S*-DNBL COLUMN

Conditions and abbreviations are the same as those indicated in Table I. References in which CD spectral data and/or absolute configurations of enantiomers are indicated in Table I.

| *Dihydrodiol* | *Preferred Conformation* | *A (%)* | *Retention time* | | *RV* |
|---|---|---|---|---|---|
| | | | *Peak 1* | *Peak 2* | |
| BA *trans*-5,6-DHD | 5e,6e | 10 | 8.2 | 8.2 | 0 |
| 1-MBA *trans*-5,6-DHD | 5e,6e | 10 | 6.6 | 6.6 | 0 |
| | | 5 | 11.5 | 11.5 | 0 |
| 8-MBA *trans*-5,6-DHD | 5e,6e | 10 | 8.0 | 8.0 | 0 |
| 10-MBA *trans*-5,6-DHD | 5e,6e | 5 | 16.3 (*R,R*) | 16.7 (*S,S*) | 0.5 |
| 11-MBA *trans*-5,6-DHD | 5e,6e | 10 | 8.4 | 8.4 | 0 |
| | | 5 | 16.1 (*R,R*) | 16.2 (*S,S*) | 0.1 |
| 12-MBA *trans*-5,6-DHD | 5e,6e | 10 | 7.5 | 7.5 | 0 |
| 4-MBA *trans*-5,6-DHD | 5a,6a | 10 | 13.8 (*R,R*) | 14.3 (*S,S*) | 0.1 |
| 7-MBA *trans*-5,6-DHD | 5a,6a | 10 | 17.1 (*R,R*) | 17.5 (*S,S*) | 0.4 |
| 7-Br-BA *trans*-5,6-DHD | 5a,6a | 15 | 11.3 (*S,S*) | 11.6 (*R,R*) | 0.4 |
| 7-Br-11-MBA *trans*-5,6-DHD | 5a,6a | 10 | 19.3 (*S,S*) | 20.3 (*R,R*) | 0.9 |
| 7-Br-1-MBA *trans*-5,6-DHD | 5a,6a | 10 | 14.6 | 14.6 | 0 |
| 3-MC *trans*-11,12-DHD | 11a,12a | 10 | 13.2 | 13.2 | 0 |
| 7-Br-12-MBA *trans*-5,6-DHD | 5a,6a | 10 | 17.4 (*R,R*) | 18.5 (*S,S*) | 1.4 |
| 7,12-DMBA *trans*-5,6-DHD | 5a,6a | 10 | 14.8 (*R,R*) | 18.1 (*S,S*) | 4.1 |
| BA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 12.0 (*R,S*) | 12.5 (*S,R*) | 0.6 |
| 1-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 9.6 (*R,S*) | 9.9 (*S,R*) | 0.6 |
| | | 5 | 23.3 (*R,S*) | 24.4 (*S,R*) | 0.9 |
| 8-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 12.1 (*R,S*) | 12.8 (*S,R*) | 1.0 |
| 10-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 19.0 (*R,S*) | 20.5 (*S,R*) | 1.5 |
| 11-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 10 | 12.9 (*R,S*) | 13.8 (*S,R*) | 1.1 |
| 12-MBA *cis*-5,6-DHD | 5e,6a and 5a,6e | 15 | 7.1 (*R,S*) | 7.7 (*S,R*) | 1.4 |
| | | 10 | 11.2 (*R,S*) | 12.5 (*S,R*) | 2.2 |
| 4-MBA *cis*-5,6-DHD | 5a,6e | 10 | 9.4 (*R,S*) | 10.0 (*S,R*) | 1.1 |
| 7-Br-BA *cis*-5,6-DHD | 5e,6a | 10 | 12.1 | 12.1 | 0 |
| 7-MBA *cis*-5,6-DHD | 5e,6a | 10 | 10.3 (*R,S*) | 10.5 (*S,R*) | 0.4 |
| 7-Br-1-MBA *cis*-5,6-DHD | 5e,6a | 5 | 17.3 | 17.3 | 0 |
| 7-Br-11-MBA *cis*-5,6-DHD | 5e,6a | 5 | 25.0 (*R,S*) | 26.5 (*S,R*) | 1.1 |
| 7-Br-12-MBA *cis*-5,6-DHD | 5e,6a | 10 | 10.3 (*R,S*) | 10.6 (*S,R*) | 0.5 |
| 7,12-DMBA *cis*-5,6-DHD | 5e,6a | 10 | 8.8 (*R,S*) | 9.1 (*S,R*) | 0.5 |
| 3-MC *cis*-11,12-DHD | 11e,12a | 10 | 13.2 (*R,S*) | 13.6 (*S,R*) | 0.4 |

## CONCLUSIONS

When the K-region dihydrodiols are grouped together according to their conformational preferences, general trends emerged with respect to the elution orders of enantiomers separated by the CSPs. While exceptions may be found when more compounds are studied, the following conclusions can be made on the basis of results obtained in this study.

(1) The enantiomers of quasidiequatorial K-region *trans*-dihydrodiols are either not resolved at all or are poorly resolved [as indicated by the low ($\leqslant 1.0$) resolution values] on covalently bonded *R*-DNBPG and *S*-DNBL. When enantiomers are resolved, the *R,R* enantiomers are consistently more strongly retained by the ionically bonded *R*-DNBPG, and the *S,S* enantiomers are more strongly retained by the ionically bonded *S*-DNBL. Thus, when enantiomers are resolved, the *R,R* enantiomers of all quasidiequatorial K-region *trans*-dihydrodiols are more strongly retained by ionically bonded *R*-DNBPG and *R*-DNBL.

(2) Among four CSPs examined, the covalently bonded *R*-DNBPG is the only CSP which consistently retains more strongly the *S,S* enantiomers of quasidiaxial K-region *trans*-dihydrodiols of eight BA derivatives. Exceptions in elution orders of enantiomers exist when the other three CSPs are used.

(3) When enantiomeric pairs are resolved, the *R,S* enantiomers of K-region *cis*-dihydrodiols which adopt both 5e,6a and 5a,6e conformations are more strongly retained by *R*-DNBPG and less strongly retained by *S*-DNBL, regardless of whether the CSP is ionically or covalently bonded. The same elution order prevails for the enantiomers of 4-MBA *cis*-5,6-dihydrodiol which adopts only 5a,6e conformation. Thus, the *R,S* enantiomers of these K-region *cis*-dihydrodiols are more strongly retained by both *R*-DNBPG and *R*-DNBL, regardless of whether the CSPs are ionically or covalently bonded.

(4) When the enantiomers are resolved, the *S,R* enantiomers of K-region *cis*-dihydrodiols which adopt 5e,6a (or 11e,12a) conformations are consistently more strongly retained regardless of whether the CSPs have an *R* or an *S* configuration. The elution orders of enantiomers are the same regardless of whether the CSP is ionically or covalently bonded.

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