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Resolution of enantiomeric triols, triol-hydroxyethylthioethers, and methoxy-triols derived from three benzo[*a*]pyrene diol-epoxides by chiral stationary phase high-performance liquid chromatography

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ABSTRACT

Benzo[*a*]pyrene 7,8-diol-*anti*-9,10-epoxide, 7,8-diol-*syn*-9,10-epoxide, and 9,10-diol-*anti*-7,8-epoxide were converted to triol, triol-hydroxyethylthioether, and methoxy-triol derivatives. Enantiomeric pairs of these derivatives were resolved by high-performance liquid chromatography with Pirkle's π-electron acceptor chiral stationary phases. Resolution of enantiomers was confirmed by ultraviolet-visible absorption, circular dichroism, and mass spectral analyses. Relative to those of tetrols, these derivatives are less polar and have shorter retention times and improved enantiomeric resolution on chiral stationary phases. Absolute stereochemistries of most enantiomeric derivatives were deduced by comparing their circular dichroism spectra to those of similar compounds derived from enantiomeric diol-epoxides of known absolute stereochemistry.

INTRODUCTION

Benzo[*a*]pyrene (BP) 7,8-diol-*anti*-9,10-epoxide (see structures and abbreviations in Fig. 1) is the predominant ultimate carcinogenic metabolite of BP [1,2]. The metabolically formed BP 7,8-diol-*anti*-9,10-epoxide is enriched in the (7*R*,8*S*,9*S*,10*R*)-enantiomer and reacts covalently with cellular macromolecules [1,2]. BP 7,8-diol-*anti*-9,10-epoxide is hydrolyzed to form 7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydro derivatives (tetrols) and reduced by NADPH (or NADH) to 7,8,9-trihydroxy-7,8,9,10-tetrahydro derivatives (triols) [2–4].

Since the BP 7,8-diol-*anti*-9,10-epoxide is formed by three stereoselective enzymatic reactions in the metabolism of BP [1,2], the hydrolysis products (tetrols) and reduction products (triols) are also highly enriched in one enantiomer. We have recently reported the resolution of the enantiomeric pairs of BP 7,8-diol-9,10-epoxides (*anti* and *syn* isomers), BP 9,10-diol-*anti*-7,8-epoxide, and their hydrolysis products (tetrols) by chiral stationary phase (CSP) high-performance liquid chromatography (HPLC) and have established their absolute configurations [5]. In this paper, we report the resolution of enantiomeric pairs of triols, triol-hydroxyethylthioethers, and

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methoxy-triols derived from BP 7,8-diol-9,10-epoxides (*anti* and *syn* isomers) and BP 9,10-diol-*anti*-7,8-epoxide by HPLC using covalently bonded Pirkle's π-electron acceptor CSPs. Reduction of diol-epoxides with $NaBH_4$ yields triols. Reactions of diol-epoxides with 2-mercaptoethanol (β-ME) in an alkaline aqueous solution or methanolic sodium methylate produce triol-hydroxyethylthioethers or methoxy-triols, respectively. Relative to tetrols, conversion of diol-epoxides to the triolic derivatives reduces the polarity and improves the enantiomeric resolution of enantiomers.

EXPERIMENTAL

Materials

Racemic BP 7,8-diol-*anti*-9,10-epoxide, BP 7,8-diol-*syn*-9,10-epoxide, and BP 9,10-diol-*anti*-7,8-epoxide were obtained from the Chemical Repository of the National Cancer Institute. BP (7,10/8)-triol was a gift from Dr. Peter Fu of the National Center for Toxicological Research, Jefferson, AR, U.S.A. Sodium borohydride ($NaBH_4$) and sodium methylate ($NaOCH_3$) were obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Lithium aluminium hydride ($LiA1H_4$) was purchased from Aldrich (Milwaukee, WI, U.S.A.). 2-Mercaptoethanol (β-ME) was purchased from Sigma (St. Louis, MO, U.S.A.). Solvents were HPLC grade (methanol, Baker, Phillipsburg, NJ, U.S.A.); hexane, tetrahydrofuran (THF), diethyl ether, acetonitrile, Mallinkrodt, Paris, KY, U.S.A.; ethanol, Midwest Grain, Perkin, IL, U.S.A.; dioxane, Aldrich).

Preparation of tetrols

Approximately 0.1 mg of either a racemic or an enantiomeric diol-epoxide was dissolved in 0.4 ml of THF, diluted with 4 ml of 0.1 *M* HCl and stored at ambient temperature overnight (16 h). The hydrolysis products were extracted twice with an equal volume of ethyl acetate, transferred to a new tube, and evaporated to dryness under nitrogen at 50°C. Tetrols were dissolved in methanol and isolated by gradient reversed-phase HPLC.

Preparation of triols

Approximately 0.1 mg of an enantiomeric or racemic diol-epoxide was dissolved in 4 ml of ethanol. Since the presence of water will hydrolyze the diol-epoxide, the ethanol was dried with $NaBH_4$ prior to use. An excess amount of $NaBH_4$ ($\approx$ 5 mg) was added, mixed, and stored at 50°C overnight (16 h). Reduction products were partitioned and extracted twice with ethyl acetate and water. The organic phase was transferred to a new tube and evaporated to dryness under nitrogen at 50°C. Triols (yield $\approx$ 93%) were dissolved in methanol and isolated by gradient reversed-phase HPLC.

Preparation of triol-hydroxyethylthioethers

Approximately 0.1 mg of an enantiomeric or racemic diol-epoxide was dissolved in 0.5 ml of a 2 *M* aqueous β-ME solution (containing 0.4 *M* NaOH) and allowed to react for 15 min at ambient temperature. Reaction products were extracted twice with an equal volume of ethyl acetate, transferred to a new tube, and dried under nitrogen at 50°C. The resulting triol-hydroxyethylthioethers (yield $\approx$ 90%) were dissolved in methanol and isolated by gradient reversed-phase HPLC.

Preparation of methoxy-triols

Approximately 0.1 mg of an enantiomeric or racemic diol-epoxide was dissolved in 1 ml of methanol containing 2% (w/v) of $NaOCH_3$ and allowed to react overnight (16 h) at ambient temperature. Products were partitioned and extracted with equal volumes of ethyl acetate and water. The organic phase was transferred to a new tube and dried under nitrogen at 50°C. Methoxy-triols (yield $\approx$ 80%) were dissolved in methanol and isolated by gradient reversed-phase HPLC.

High-performance liquid chromatography

HPLC was performed using a Waters Assoc. (Milford, MA, U.S.A.) Model 510 solvent delivery system, an Autochrom (Milford, MA, U.S.A.) Model OPG/S gradient system, a Kratos (Ramsey, NJ, U.S.A.) Model 757 variable absorbance detector, a Valco (Houston, TX, U.S.A.) Model N60 loop injector, and a Hewlett-Packard (Palo Alto, CA, U.S.A.) Model 3390A integrator.

Reversed-phase HPLC

Derivatives of diol-epoxides were separated on a Zorbax ODS column (80 × 6.2 mm I.D., 3-μm particle, MAC-MOD, Chadds Ford, PA, U.S.A.). Compounds were eluted with 30 min linear gradient from methanol–water (1:1, v/v) to methanol at a flow-rate of 1 ml/min and monitored at 247 nm. For the purpose of comparing retention times to those of tetrols, the column was eluted isocratically at ambient temperature with premixed methanol–water (1:1, v/v) at a flow-rate of 1 ml/min.

Chiral stationary phase HPLC

Resolution of enantiomeric diol-epoxides or triolic derivatives of diol-epoxides was carried out on CSP columns, 250 × 4.6 mm I.D., Regis Chemical (Morton Grove, IL, U.S.A.) packed with spherical particles of 5 μm diameter of γ-aminopropylsilanized silica to which either (*S*)-N-(3,5-dinitrobenzoyl)leucine [(*S*)-DNBL] or (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine [(*R*)-DNBPG] was bonded ionically (I) or covalently (C). The (*R*)-DNBPG-C column used in this study was labeled as "Hi-Chrom Pirkle covalent phenylglycine" by Regis Chemical. This is different from the more recent "Rexchrom Pirkle covalent D-phenylglycine" column marketed by Regis Chemical. BP diol-epoxides and derivatives were resolved on CSP columns using from 10 to 30% of solvent A (ethanol–acetonitrile, 2:1, v/v) in hexane at a flow-rate of 2 ml/min. Since leaching of stationary phases occurs with ionically bonded columns with mobile phases of high polarity, solvents containing greater than 15% of solvent A in hexane were avoided when using ionic bonded CSPs [6]. For this reason, separations which required 20–30% of solvent A were restricted to covalently bonded CSPs. Elution order of enantiomeric pairs on different CSPs was established by chromatographing a sample containing unequal amounts of two enantiomers.

Absolute configuration of enantiomeric triolic derivatives

Racemic and enantiomeric BP diol-epoxides were converted to triols, triol-hydroxyethylthioethers, or methoxy-triols as described above and resolution of each enantiomeric pair of the triolic derivatives was tested by CSP-HPLC. Triolic derivatives were purified by reversed-phase HPLC prior to CSP-HPLC separation. Circular dichroism (CD) spectra of triolic derivatives enriched in one enantiomer were

obtained. Since the absolute configuration of enantiomeric BP 7,8-diol-*anti*-9,10-epoxides has been established [5], the absolute stereochemistry of their enantiomeric triol derivatives can be readily deduced. CD spectra of some enantiomeric triolic derivatives resolved by CSP-HPLC were compared to those of similar derivatives from enantiomeric BP diol-epoxides. However, all reduction reactions of resolved BP 7,8-diol-*syn*-9,10-epoxide enantiomers did not give identifiable products. The CSP-HPLC and CD spectral analyses have enabled us to establish the elution order-absolute configuration-CD spectrum relationship of most of the enantiomeric triolic derivatives described in this report.

Spectral analysis

Mass spectral analysis was performed on a Finnigan 4000 gas chromatograph–mass spectrometer with a Technivent 1050 data system. Samples were introduced by a solid probe in the electron impact mode at 70 eV with a 250°C ionizer temperature or by a Vacumetrics DCI desorption probe with a 150°C ionizer temperature. Ultraviolet-visible absorption spectra of samples (in methanol) were determined using 1 cm path length quartz cuvette with either a Cary 118C (Varian, Palo Alto, CA, U.S.A.) spectrophotometer or a DW2000 UV/VIS scanning spectrophotometer (slit 2 nm and scan rate 2 nm/sec; SLM Instruments, Urbana, IL, U.S.A.). Circular dichroism (CD) spectra of samples (in methanol) in a quartz cell of 1 cm path length were measured at ambient temperature with a Jasco 500A spectropolarimeter equipped with a Model DP500 data processor. The concentration of the sample is indicated by $A_{\lambda 2}$/ml (absorbance units at wavelength $\lambda 2$ per ml of solvent). CD spectra are expressed by ellipticity ($\Phi_{\lambda 1}/A_{\lambda 2}$, in millidegrees) for solutions that have an absorbance of $A_{\lambda 2}$ unit per ml of solvent at wavelength $\lambda 2$ (usually the wavelength of maximal absorption). Under conditions of measurements indicated above, the molar ellipticity ($[\Theta]_{\lambda 1}$, in deg cm^2 dmole^{-1}) and ellipticity ($\Phi_{\lambda 1}/A_{\lambda 2}$, in millidegrees) are related to the extinction coefficient ($\varepsilon_{\lambda 2}$, in cm^{-1} M^{-1}) as follows:

$$[\theta]_{\lambda 1} = 0.1\ \varepsilon_{\lambda 2}\ (\Phi_{\lambda 1}/A_{\lambda 2})$$

RESULTS

Triols

The structures of triols derived by reduction of each of the three BP diol-epoxides with $NaBH_4$ are shown in Fig. 1. Reduction of BP 7,8-diol-*anti*-9,10-epoxide produced only one triol, whereas reduction of either BP 7,8-diol-*syn*-9,10-epoxide or BP 9,10-diol-*anti*-7,8-epoxide gave two stereoisomeric triols. The elution order and retention time (in min) of BP tetrols and triols on reversed-phase HPLC using a Zorbax ODS column (80 × 6.2 mm I.D.) and methanol–water (1:1, v/v) at a flow-rate of 1 ml/min are: (7,10/8,9)-tetrol, 10.9; (7,8,9/10)-tetrol, 12.7; (7,9/8,10)-tetrol, 13.6; (7/8,9,10)-tetrol, 15.1; (8,9/10)-triol, 18.9; (7,9/8)-triol, 20.2; (7,9,10/8)-tetrol, 22.6; (7/8,9)-triol, 22.8; (7,9/10)-triol, 35.3; (7,10/8)-triol, 45.4. All triols exhibited M^+ at m/z 304 with fragment ions at m/z 286 (loss of H_2O), and 268 (loss of two H_2O) by mass spectral analysis.

The retention times and resolution values of some enantiomeric triol derivatives by HPLC using (*R*)-DNBPG-C and (*S*)-DNBL-C columns are listed in Table I.

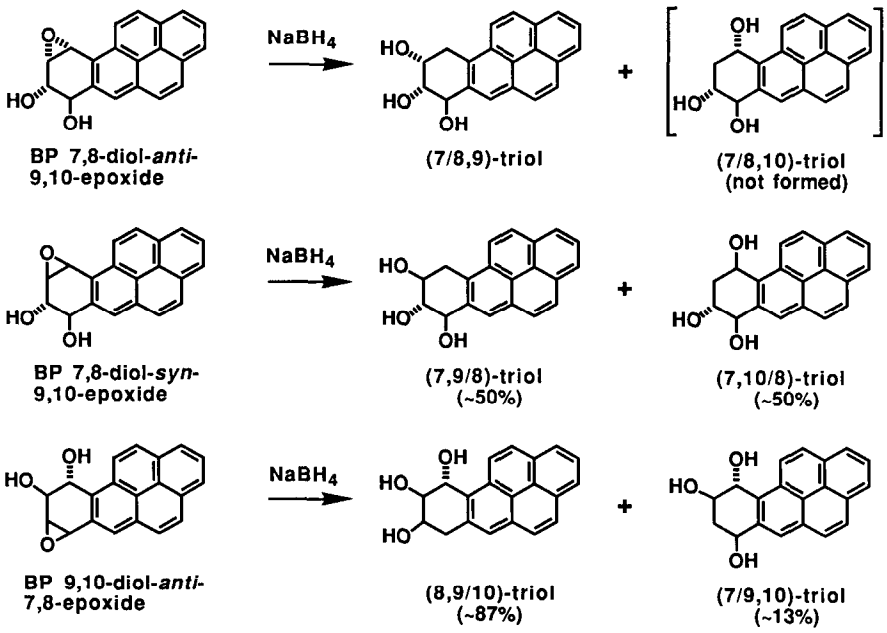

Fig. 1. Structures, abbreviations, and relative configurations of stereoisomeric BP diol-epoxides and their reduction triol products.

Absolute configurations of resolved enantiomers, whenever established, are also indicated in Table I. Each of the resolved enantiomeric triols was confirmed by UV-VIS absorption, CD, and mass spectral analyses.

BP 7,8-diol-*anti*-9,10-epoxide was reduced with $NABH_4$ to produce a (7/8,9)-triol. The second possible reduction product [(7/8,10)-triol, Fig. 1], which may be formed by reduction at the non-benzylic C9 position, was not detected. The (7/8,9)-triol was identical to the (7/8,9)-triol formed by reduction of BP 7,8-diol-*anti*-9,10-epoxide with either NADPH or NADH reported earlier [3,4]. Elution order of (7/8,9)-triol enantiomers is the same on both (*R*)-DNBPG-C and (*S*)-DNBL-C columns (Table I). A BP 7,8-diol-*anti*-9,10-epoxide enriched in the (7*R*,8*S*,9*S*,10*R*)-enantiomer was obtained by CSP-HPLC on a (*R*)-DNBPG-C column [5] and was reduced to a (7/8,9)-triol enriched in the (7*R*,8*R*,9*R*)-enantiomer. The UV absorption and CD spectra of this (7*R*,8*R*,9*R*)-triol is shown in Fig. 2A. This (7*R*,8*R*,9*R*)-triol enantiomer is more strongly retained on both (*R*)-DNBPG-C and (*S*)-DNBL-C columns (Table I). The CD spectrum of the (7/8,9)-triol enantiomer more strongly retained on the (*S*)-DNBL-C column was identical to that of (7*R*,8*R*,9*R*)-triol shown in Fig. 2A. The CD spectrum of (7*R*,8*R*,9*R*)-triol is similar to that of BP 7,8,9,10-tetrahydro-(7*R*,8*R*)-diol [6]. Hence the elution order of (7/8,9)-triol enantiomers on the CSP columns was established (Table I).

BP 7,8-diol-*syn*-9,10-epoxide was reduced with $NaBH_4$ in ethanol at ambient temperature to form two triols. Either dioxane or diethyl ether may be used to replace ethanol in the reduction reaction. Reduction with $LiA1H_4$ in fresh THF at ambient

TABLE I

CSP-HPLC RESOLUTION OF BP TRIOLS

Chemical[a]	CSP[b]	%A[c]	Retention time (min)		*RV*[e]
			Enantiomer 1[d]	Enantiomer 2[d]	
From BP 7,8-diol-anti-9,10-epoxide:					
BP (7/8,9)-triol	(*R*)-DNBPG-C	30	16.5 (7*S*,8*S*,9*S*)	16.9 (7*R*,8*R*,9*R*)	0.2
		20	28.7 (7*S*,8*S*,9*S*)	29.4 (7*R*,8*R*,9*R*)	0.3
	(*S*)-DNBL-C	30	12.5 (7*S*,8*S*,9*S*)	13.5 (7*R*,8*R*,9*R*)	0.6
		20	22.6 (7*S*,8*S*,9*S*)	24.5 (7*R*,8*R*,9*R*)	0.7
From BP 7,8-diol-syn-9,10-epoxide:					
BP (7,9/8)-triol	(*R*)-DNBPG-C	30	7.9 (7*S*,8*S*,9*R*)	8.4 (7*R*,8*R*,9*S*)	0.8
		20	18.3 (7*S*,8*S*,9*R*)	19.5 (7*R*,8*R*,9*S*)	1.2
	(*S*)-DNBL-C	30	5.3 (7*S*,8*S*,9*R*)	5.6 (7*R*,8*R*,9*S*)	0.8
		20	11.2 (7*S*,8*S*,9*R*)	12.0 (7*R*,8*R*,9*S*)	1.6
BP (7,10/8)-triol	(*R*)-DNBPG-C	30	13.3	13.3	0
		20	26.2	26.2	0
		15	43.1	43.1	0
	(*S*)-DNBL-C	30	7.2	7.2	0
		20	13.5	13.7	~0.1
		15	23.0	23.0	0
From BP 9,10-diol-anti-7,8-epoxide:					
BP (8,9/10)-triol	(*R*)-DNBPG-C	30	16.5	16.5	0
		20	41.4	41.4	0
	(*S*)-DNBL-C	30	7.3 (8*S*,9*S*,10*S*)	7.7 (8*R*,9*R*,10*R*)	0.9
		20	16.8 (8*S*,9*S*,10*S*)	17.8 (8*R*,9*R*,10*R*)	1.2
BP (7,9/10)-triol	(*R*)-DNBPG-C	30	7.2 (A)[f]	7.4 (B)[f]	0.4
		20	14.1 (A)	14.7 (B)	0.6
	(*S*)-DNBL-C	30	5.1 (B)	5.3 (A)	0.7
		20	9.4 (B)	9.8 (A)	1.0

[a] Relative configuration for stereoisomers is designated as described in Fig. 1.

[b] CSPs are described in Experimental section.

[c] Percent of solvent A [ethanol–acetonitrile, 2:1 (v/v)] in hexane at a flow-rate of 2 ml/min and a void volume of 2.4 ml.

[d] Enantiomers are designated 1 and 2 according to elution order.

[e] RV = resolution value = $2(V_2 - V_1)/(W_2 + W_1)$, where V is retention volume and W is peak width at base.

[f] A and B indicate two enantiomers whose absolute configurations have not been established.

temperature gave identical results. Two triols ($\approx$ 1:1) were separated by reversed-phase HPLC. The late eluting triol had the same retention time as that of an authentic standard (7,10/8)-triol on both reversed-phase HPLC and CSP-HPLC. The enantiomers of (7,10/8)-triol were not resolved by CSP-HPLC (Table I). The earlier eluting triol had the 7,9/8 relative stereochemistry (Fig. 1) and its enantiomers were resolved by CSP-HPLC (Table I). Elution order of (7,9/8)-triol enantiomers is the same on both (*R*)-DNBPG-C and (*S*)-DNBL-C columns (Table I). The CD spectrum of the less strongly retained (7,9/8)-triol enantiomer (Fig. 2B) is similar to the major (7*S*,8*R*,9*R*,10*R*)-tetrol derived from BP 7*S*,8*R*-diol-*syn*-9*S*,10*S*-epoxide [5]. Because the benzylic hydroxyl group is responsible for CSP recognition [7] and the BP 7,8,9,10-tetrahydro-(7*R*,8*R*)-diol is more strongly retained on the (*R*)-DNBPG-C [8],

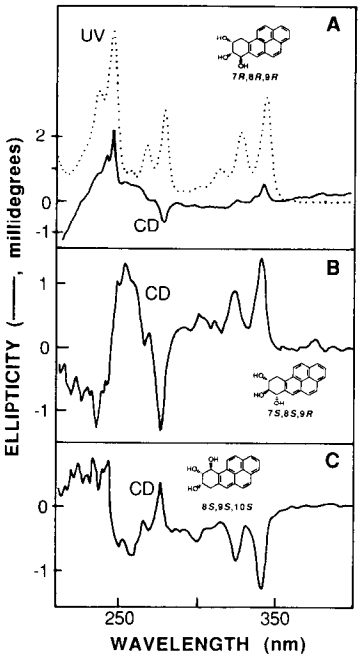

Fig. 2. CD spectra of (A) (7*R*,8*R*,9*R*)-triol, (B) (7*S*,8*S*,9*R*)-triol, and (C) (8*S*,9*S*,10*S*)-triol. The (7*R*,8*R*,9*R*)-triol [concn. 1.0 A_{246}/ml methanol; Φ_{342} = + 0.47 mdeg, ≈90% enantiomer excess (ee)] in A was derived from BP 7*R*,8*S*-diol-*anti*-9*S*,10*R*-epoxide. The (7*S*,8*S*,9*R*)-triol (concn. 1.0 A_{246}/ml methanol; Φ_{340} = + 1.4 mdeg, ≈99% ee) in B was the (7,9/8)-triol enantiomer less strongly retained on the (*S*)-DNBL-C. The (8*S*,9*S*,10*S*)-triol (concn. 1.0 A_{245}/ml methanol; Φ_{341} = − 1.30 mdeg, ≈91% ee) in C was derived from BP 9*R*,10*S*-diol-*anti*-7*S*,8*R*-epoxide.

the (7,9/8)-triol enantiomer more strongly retained on the CSP is therefore assigned to have the 7*R*,8*R*,9*S* absolute stereochemistry.

BP 9,10-diol-*anti*-7,8-epoxide was reduced with $NaBH_4$ in ethanol at ambient temperature to form two triols (Fig. 1). Two triols were separated by reversed-phase HPLC and the areas under chromatographic peaks indicated that the two triols were formed in a ratio of ≈7:1, with the major triol eluting earlier. The result on the reduction of 7,8-diol-*anti*-9,10-epoxide by $NaBH_4$ indicated that the benzylic C10 position is the predominant site of reduction (see Fig. 1 and description above). Hence the major site of reduction of 9,10-diol-*anti*-7,8-epoxide is also expected to be at the benzylic C7 position, resulting in the formation of (8,9/10)-triol as the major product. The minor reduction product was therefore assigned to be the (7,9/10)-triol. The enantiomers of the major (8,9/10)-triol were resolved on (*S*)-DNBL-C, but not on (*R*)-DNBPG-C (Table I). A BP 9,10-diol-*anti*-7,8-epoxide enriched in the (7*R*,8*S*,9*S*,10*R*)-enantiomer was obtained by CSP-HPLC on a (*R*)-DNBPG-C column [5] and was reduced with $NaBH_4$ to form two triols. The CD spectrum of the major (8,9/10)-triol, enriched in the (8*S*,9*S*,10*S*)-enantiomer, is shown in Fig. 2C. The CD spectrum of the less strongly retained enantiomer of the (8,9/10)-triol on the (*S*)-DNBL-C was identical to that shown in Fig. 2C. Hence the (8,9/10)-triol enantiomer with a CD spectrum shown in Fig. 2C is deduced to have the 8*S*,9*S*,10*S*

absolute stereochemistry. The CD spectrum of (8*S*,9*S*,10*S*)-triol is also similar to that of BP 7,8,9,10-tetrahydro-(9*S*,10*S*)-diol [6,9]. The enantiomers of the minor (7,9/10)-triol were resolved on both the (*R*)-DNBPG-C and (*S*)-DNBL-C columns (Table I). However, the absolute configurations of the (7,9/10)-triol enantiomers have not been established.

Triol-hydroxyethylthioethers

The structures of triol-hydroxyethylthioethers derived by reaction of each of the three BP diol-epoxides with β-ME in an alkaline aqueous solution are shown in Fig. 3. Only one triol-hydroxyethylthioether was formed by *trans*-addition of the thiol group at the benzylic position of each BP diol-epoxide. It is known that compounds such as *tert.*-butyl mercaptan [10] and β-ME [11] react with BP 7,8-diol-*anti*-9,10-epoxide by *trans*-addition at the benzylic C10 position. Since hydrolysis of BP 9,10-diol-*anti*-7,8-epoxide occurs predominantly by *trans*-addition at the benzylic C7 position [5], it is reasonable to assume that reaction of BP 9,10-diol-*anti*-7,8-epoxide with β-ME in an alkaline aqueous solution also occurs by *trans*-addition at the benzylic C7 position, resulting in the formation of 8,9,10-triol-7-hydroxyethylthioether with a 7,10/8,9 relative stereochemistry (Fig. 3). In comparison, hydrolysis of BP 7,8-diol-*syn*-9,10-epoxide occurs predominantly by *cis*-addition at C10 position [4]. Hence we assume that the reaction of BP 7,8-diol-*syn*-9,10-epoxide with β-ME in an alkaline aqueous solution also occurs by *cis*-addition at the benzylic C10 position, resulting in the formation of 7,8,9-triol-10-hydroxyethylthioether with a 7,9,10/8 relative stereochemistry (Fig. 3).

Each triol-hydroxyethylthioether was purified by reversed-phase HPLC prior to CSP-HPLC separation of enantiomers. The retention times and resolution values of enantiomeric triol-hydroxyethylthioethers by HPLC on either (*R*)-DNBPG-C or (*S*)-DNBL-C are listed in Table II. Absolute configurations of resolved enantiomers, whenever established, are also indicated in Table II. Each triol-hydroxyethylthioether was confirmed by UV-VIS absorption, CD, and mass spectral analyses. All triol-hydroxyethylthioethers exhibited M^+ at *m/z* 380 by mass spectral analysis.

A racemic BP 7,8-diol-*anti*-9,10-epoxide was reacted with β-ME in an alkaline aqueous solution to form a 7,8,9-triol-10-hydroxyethylthioether with a 7,10/8,9 relative stereochemistry (Fig. 3). Consistent with an earlier report [12], this 7,8,9-triol-10-hydroxyethylthioether has a characteristic absorption band with a maximum at 348 nm (Fig. 4). In comparison, BP triols and tetrols have absorption maxima at ≈344 nm in the same wavelength region [3,5]. The enantiomers of this triol-hydroxyethylthioether were resolved on both (*R*)-DNBPG-C and (*S*)-DNBL-C columns (Fig. 5 and Table II). The less strongly retained enantiomer on the (*S*)-DNBL-C column had a CD spectrum as shown in Fig. 4A. A 7,8-diol-*anti*-9,10-epoxide enantiomer enriched in the (7*S*,8*R*,9*R*,10*S*)-enantiomer was obtained by CSP-HPLC on a (*R*)-DNBPG-C column [5] and was reacted with β-ME in an alkaline aqueous solution to form a 7,8,9-triol-10-hydroxyethylthioether enriched in the (7*S*,8*R*,9*R*,10*R*)-enantiomer. This enantiomeric 7,8,9-triol-10-hydroxyethylthioether had a CD spectrum identical to that shown in Fig. 4A. Hence the elution order-CD spectrum-absolute configuration relationship of the enantiomeric 7,8,9-triol-10-hydroxyethylthioethers derived from BP 7,8-diol-*anti*-9,10-epoxide was established (Fig. 4A and Table II).

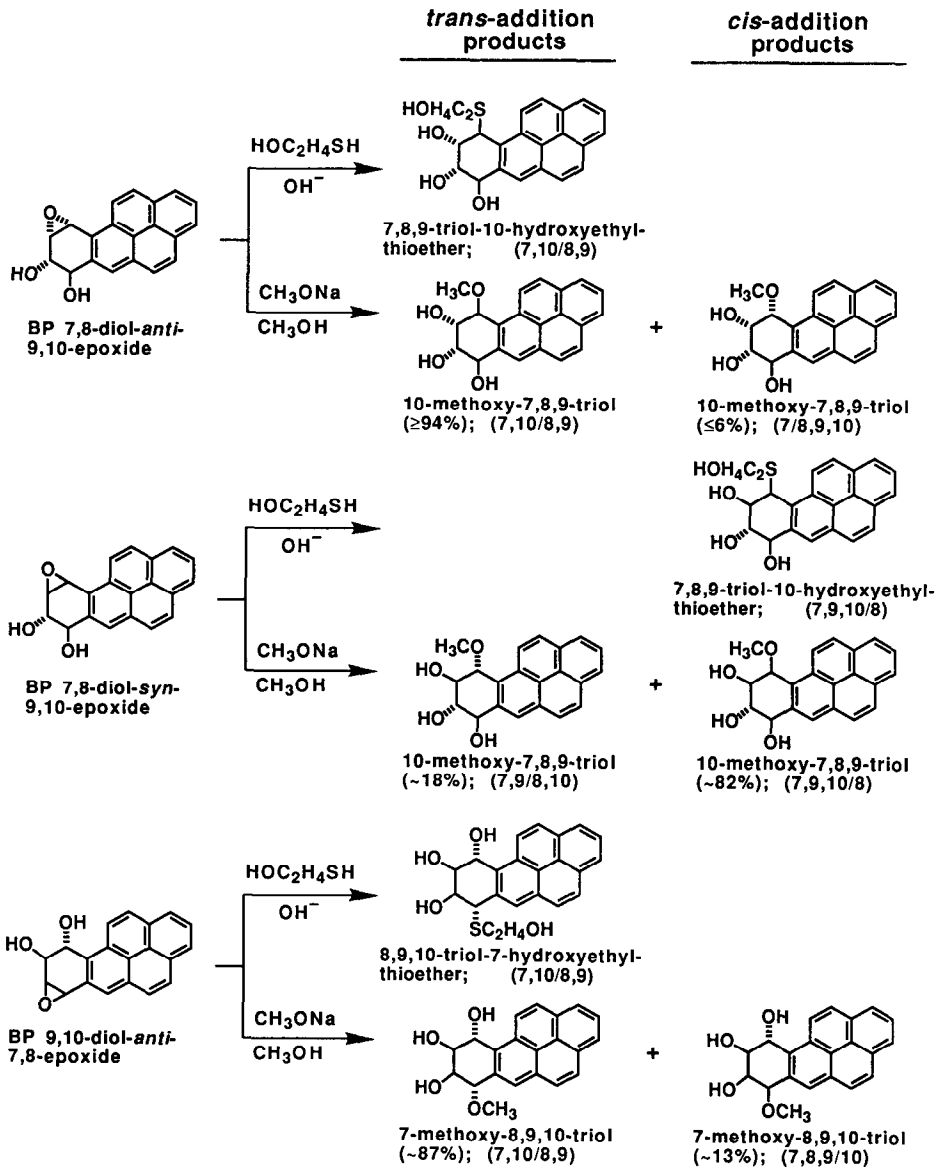

Fig. 3. Structures, abbreviations, and relative configurations of stereoisomeric BP diol-epoxides and their methoxylation and hydroxyethylthiolation products.

A racemic BP 7,8-diol-*syn*-9,10-epoxide was reacted with β-ME in an alkaline aqueous solution to produce a 7,8,9-triol-10-hydroxyethylthioether with a 7,9,10/8 relative stereochemistry (Fig. 3). This triol-hydroxyethylthioether has an absorption maximum identical to the 7,8,9-triol-10-hydroxyethylthioether derived from BP 7,8-diol-*anti*-9,10-epoxide (Fig. 4A). The enantiomers of this 7,8,9-triol-10-hydroxyethylthioether were resolved on both the (*R*)-DNBPG-C and (*S*)-DNBL-C columns

TABLE II

CSP-HPLC RESOLUTION OF BP TRIOL-HYDROXYETHYLTHIOETHERS

Chemical[a]	CSP[b]	%A[c]	Retention time (min)		*RV*[e]
			Enantiomer 1[d]	Enantiomer 2[d]	
From BP 7,8-diol-anti-9,10-epoxide:					
BP 10-SE-7,8,9-triol	(*R*)-DNBPG-C	30	12.1 (7*R*,8*S*,9*S*,10*S*)	13.3 (7*S*,8*R*,9*R*,10*R*)	0.8
(7,10/8,9)		20	32.2 (7*R*,8*S*,9*S*,10*S*)	35.5 (7*S*,8*R*,9*R*,10*R*)	1.2
	(*S*)-DNBL-C	30	6.2 (7*S*,8*R*,9*R*,10*R*)	7.2 (7*R*,8*S*,9*S*,10*S*)	2.1
		25	8.2 (7*S*,8*R*,9*R*,10*R*)	9.7 (7*R*,8*S*,9*S*,10*S*)	2.5
		20	14.5 (7*S*,8*R*,9*R*,10*R*)	18.0 (7*R*,8*S*,9*S*,10*S*)	2.7
From BP 7,8-diol-syn-9,10-epoxide:					
BP 10-SE-7,8,9-triol	(*R*)-DNBPG-C	30	9.3 (7*R*,8*S*,9*R*,10*S*)	9.6 (7*S*,8*R*,9*S*,10*R*)	0.4
(7,9,10/8)		20	25.5 (7*R*,8*S*,9*R*,10*S*)	26.6 (7*S*,8*R*,9*S*,10*R*)	0.5
	(*S*)-DNBL-C	30	5.2 (7*S*,8*R*,9*S*,10*R*)	5.5 (7*R*,8*S*,9*R*,10*S*)	0.8
		20	12.8 (7*S*,8*R*,9*S*,10*R*)	13.7 (7*R*,8*S*,9*R*,10*S*)	1.3
		15	22.5 (7*S*,8*R*,9*S*,10*R*)	24.3 (7*R*,8*S*,9*R*,10*S*)	1.5
From BP 9,10-diol-anti-7,8-epoxide:					
BP 7-SE-8,9,10-triol	(*R*)-DNBPG-C	30	12.1 (7*S*,8*S*,9*S*,10*R*)	12.9 (7*R*,8*R*,9*R*,10*S*)	0.7
(7,10/8,9)		20	27.1 (7*S*,8*S*,9*S*,10*R*)	28.9 (7*R*,8*R*,9*R*,10*S*)	0.8
	(*S*)-DNBL-C	30	7.5	7.5	0
		20	15.7	15.7	0

[a] Relative configuration for stereoisomers is designated as described in Fig. 3. SE abbreviates for the $HOCH_2CH_2S$ group.

[b] CSPs are described in Experimental section.

[c] Percent of solvent A [ethanol–acetonitrile, 2:1 (v/v)] in hexane at a flow-rate of 2 ml/min and a void volume of 2.4 ml.

[d] Enantiomers are designated 1 and 2 according to elution order.

[e] RV = resolution value = $2\,(V_2 - V_1)/(W_2 + W_1)$, where V is retention volume and W is peak width at base.

(Table II). The CD spectrum of the less strongly retained enantiomer on the (*S*)-DNBL-C column is identical to that of the major (7*S*,8*R*,9*R*,10*R*)-tetrol derived by hydrolysis of BP 7*S*,8*R*-*syn*-9*S*,10*R*-epoxide [5]. Hence the elution order-CD spectrum-absolute configuration relationship of the enantiomeric 7,8,9-triol-10-hydroxyethylthioethers derived from BP 7,8-diol-*syn*-9,10-epoxide was established (Fig. 4B and Table II).

A racemic BP 9,10-diol-*anti*-7,8-epoxide was reacted with β-ME in an alkaline aqueous solution to produce a 8,9,10-triol-7-hydroxyethylthioether with a 7,10/8,9 relative stereochemistry (Fig. 3). This triol-hydroxyethylthioether has an absorption maximum at ≈344 nm (Fig. 4C). In contrast, the 10-hydroxyethylthioethers derived from BP 7,8-diol-*anti*-9,10-epoxide and 7,8-diol-*syn*-9,10-epoxide have an absorption maximum at 348 nm in the same wavelength region (Fig. 4A). Thus the absorption maximum at 348 nm is a unique property of 7,8,9-triol-10-hydroxyethylthioethers in which the 10-hydroxyethylthiol group is in the sterically crowded bay region. The CD spectrum of the more strongly retained enantiomer on (*R*)-DNBPG-C (Fig. 4C) is identical to that of the 8,9,10-triol-7-hydroxyethylthioether derived by reaction of β-ME with BP 9*R*,10*S*-diol-*anti*-7*S*,8*R*-epoxide. The latter was obtained from the

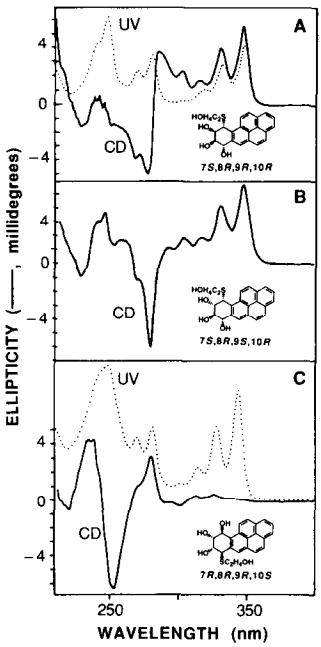

Fig. 4. CD spectra of (A) 7*S*,8*R*,9*R*-triol-10*R*-hydroxyethylthioether, (B) 7*S*,8*R*,9*S*-triol-10*R*-hydroxyethylthioether, and (C) 7*R*-hydroxyethylthioether-8*R*,9*R*,10*S*-triol. The 7*S*,8*R*,9*R*-triol-10*R*-hydroxyethylthioether (concn. 1.0 A_{248}/ml methanol; Φ_{347} = + 5.6 mdeg, ≈86% ee) in A was derived from BP 7*S*,8*R*-diol-*anti*-9*R*,10*S*-epoxide. The (7*S*,8*R*,9*S*)-triol-10*R*-hydroxyethylthioether (concn. 1.0 A_{247}/ml methanol; Φ_{346} = + 5.6 mdeg, ≈84% ee) in B was the less strongly retained enantiomer on the (*S*)-DNBL-C (Table II). The 7*R*-hydroxyethylthioether-8*R*,9*R*,10*S*-triol (concn. 1.0 A_{248}/ml methanol Φ_{254} = − 6.4 mdeg, ≈58% ee) in C was derived from BP 9*R*,10*S*-diol-*anti*-7*S*,8*R*-epoxide.

racemic compound by CSP-HPLC resolution on a (*R*)-DNBPG-C column [5]. The elution order-CD spectrum-absolute configuration relationship of the enantiomeric 8,9,10-triol-7-hydroxyethylthioethers derived from BP 9,10-diol-*anti*-7,8-epoxide was therefore established (Fig. 4C and Table II).

Methoxy-triols

The structures of methoxy-triols derived by reaction of each of the three BP diol-epoxides with CH_3ONa in methanol are shown in Fig. 3. Similar to the formation of tetrols [4], methoxylation of each of the three BP diol-epoxides occurs by *trans*- and *cis*-addition at the benzylic position, forming two methoxy-triols (Fig. 3). The relative amount of the major and the minor methoxy-triols formed (Fig. 3) were similar to that of the tetrols formed [4]. The methoxy-triols were purified by reversed-phase HPLC prior to CSP-HPLC separation of enantiomers. The retention times and resolution values of enantiomeric methoxy-triols by HPLC using (*R*)-DNBPG-C and (*S*)-DNBL-C columns are listed in Table III. Absolute configurations of resolved enantiomers were established by comparing their CD spectra with those of tetrols of similar structure and known absolute stereochemistry [5]. Each of the resolved enantiomeric methoxy-triols was confirmed by UV-VIS absorption, CD, and mass spectral analyses. Unlike the enantiomers of triol-hydroxyethylthioethers, the CD

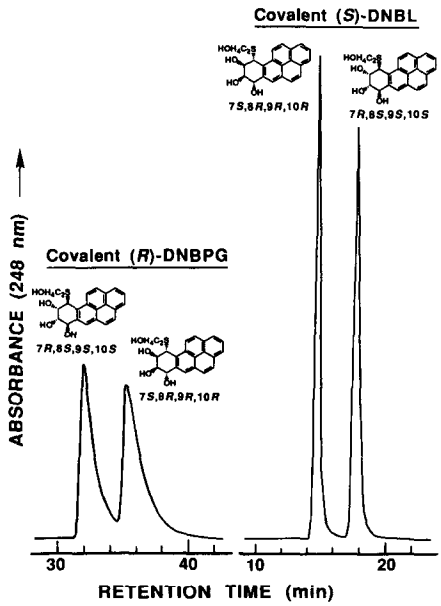

Fig. 5. CSP-HPLC resolution of enantiomeric BP 7,8,9-triol-10-hydroxyethylthioether (7,10/8,9 relative stereochemistry) on DNBPG-C (left chromatogram) and DNBL-C (right chromatogram). Both chromatograms were obtained using 20% of ethanol–acetonitrile (2:1, v/v) in hexane at a flow-rate of 2 ml/min. Column: 250 × 4.6 mm I.D.

spectra of enantiomeric methoxy-triols are closely similar to the corresponding tetrol enantiomers. All methoxy-triols exhibited M^+ at *m/z* 334 with fragment ions at *m/z* 316 (loss of H_2O), and 284 (loss of H_2O and CH_3OH) by mass spectral analysis. The retention times of each pair of methoxy-triols on the reversed-phase HPLC are: 12.8 min (major, $\geq$94%) and 17.3 min (minor, $\leq$6%) derived from BP 7,8-diol-*anti*-9,10-epoxide; 14,3 min (minor, $\approx$18%) and 16.0 min (major, $\approx$82%) derived from BP 7,8-diol-*syn*-9,10-epoxide; 13.3 min (major, $\approx$87%) and 15.3 min (minor, $\approx$13%) derived from BP 9,10-diol-*anti*-7,8-epoxide.

DISCUSSION

We have previously studied the enantiomeric separation of oxygenated polycyclic aromatic hydrocarbon (PAH) derivatives ranging from relatively less polar compounds containing one oxygen atom (epoxides and cyclic alcohols) to very polar compounds containing four oxygen atoms (tetrols) [9]. The recommended mobile phases for CSP-HPLC are a mixture of 2-propanol and hexane, which cannot be used to separate enantiomeric pairs of compounds with high polarity. For the purpose of resolving enantiomers of diol derivatives of PAH's, we developed a mobile phase {various percentages of solvent A [ethanol–acetonitrile (2:1, v/v) in hexane]} with relatively high polarity [6] and this solvent mixture has been used succesfully in resolving various kinds of PAH derivatives [9]. However, due to possible leaching of CSPs, mixtures containing greater than 15% of solvent A in hexane cannot be used

TABLE III

CSP-HPLC RESOLUTION OF BP METHOXY-TRIOLS AND TETROLS

Chemical[a]	CSP[b]	%A[c]	Retention time (min)		*RV*[e]
			Enantiomer 1[d]	Enantiomer 2[d]	
From BP 7,8-diol-anti-9,10-epoxide:					
BP 10-MeO-7,8,9-triol	(*R*)-DNBPG-C	30	12.6	12.6	0
(7,10/8,9), major		20	24.8	24.8	0
	(*S*)-DNBL-C	30	6.8 (7*S*,8*R*,9*R*,10*R*)	7.3 (7*R*,8*S*,9*S*,10*S*)	1.5
		20	12.7 (7*S*,8*R*,9*R*,10*R*)	13.7 (7*R*,8*S*,9*S*,10*S*)	1.7
		15	21.6 (7*S*,8*R*,9*R*,10*R*)	23.5 (7*R*,8*S*,9*S*,10*S*)	1.9
BP (7,10/8,9)-tetrol[f]	(*R*)-DNBPG-C	30	20.9 (7*S*,8*R*,9*S*,10*R*)	22.1 (7*R*,8*S*,9*R*,10*S*)	0.4
	(*S*)-DNBL-C	30	20.7 (7*S*,8*R*,9*S*,10*R*)	23.2 (7*R*,8*S*,9*R*,10*S*)	0.7
BP 10-MeO,7,8,9-triol	(*R*)-DNBPG-C	30	10.8 (7*S*,8*R*,9*R*,10*S*)	11.1 (7*R*,8*S*,9*S*,10*R*)	0.3
(7/8,9,10), minor		20	20.4 (7*S*,8*R*,9*R*,10*S*)	21.1 (7*R*,8*S*,9*S*,10*R*)	0.5
	(*S*)-DNBL-C	30	9.7 (7*S*,8*R*,9*R*,10*S*)	9.9 (7*R*,8*S*,9*S*,10*R*)	0.2
		20	17.7 (7*S*,8*R*,9*R*,10*S*)	18.2 (7*R*,8*S*,9*S*,10*R*)	0.3
BP (7/8,9,10)-tetrol[f]	(*R*)-DNBPG-C	30	17.4 (7*S*,8*R*,9*S*,10*S*)	17.9 (7*R*,8*S*,9*R*,10*R*)	0.3
	(*S*)-DNBL-C	30	17.5	17.5	0
From BP 7,8-diol-syn-9,10-epoxide:					
BP 10-MeO-7,8,9-triol	(*R*)-DNBPG-C	30	9.8	9.8	0
(7,9,10/8), major		20	17.9	17.9	0
	(*S*)-DNBL-C	30	5.7	5.7	0
		20	9.8 (7*S*,8*R*,9*S*,10*R*)	10.0 (7*R*,8*S*,9*R*,10*S*)	0.2
		15	14.9 (7*S*,8*R*,9*S*,10*R*)	15.2 (7*R*,8*S*,9*R*,10*S*)	0.4
		10	27.5 (7*S*,8*R*,9*S*,10*R*)	28.3 (7*R*,8*S*,9*R*,10*S*)	0.7
BP (7,9,10/8)-tetrol[f]	(*R*)-DNBPG-C	30	29.4 (7*R*,8*S*,9*S*,10*S*)	34.2 (7*S*,8*R*,9*R*,10*R*)	1.0
	(*S*)-DNBL-C	30	32.9 (7*R*,8*S*,9*S*,10*S*)	35.4 (7*S*,8*R*,9*R*,10*R*)	0.3
BP 10-MeO-7,8,9,-triol	(*R*)-DNBPG-C	30	27.3 (7*S*,8*R*,9*S*,10*S*)	29.7 (7*R*,8*S*,9*R*,10*R*)	0.6
(7,9/8,10), minor	(*S*)-DNBL-C	30	9.6 (7*R*,8*S*,9*R*,10*R*)	10.0 (7*S*,8*R*,9*S*,10*S*)	0.6
		20	17.7 (7*R*,8*S*,9*R*,10*R*)	18.5 (7*S*,8*R*,9*S*,10*S*)	0.7
BP (7,9/8,10)-tetrol[f]	(*R*)-DNBPG-C	30	20.6 (7*S*,8*R*,9*R*,10*S*)	22.4 (7*R*,8*S*,9*S*,10*R*)	0.7
	(*S*)-DNBL-C	30	28.6	28.6	0
From BP 9,10-diol-anti-7,8-epoxide:					
BP 7-MeO-8,9,10-triol	(*R*)-DNBPG-C	30	10.8	10.8	0
(7,10/8,9), major		20	21.0	21.0	0
	(*S*)-DNBL-C	30	6.2 (7*S*,8*S*,9*S*,10*R*)	6.5 (7*R*,8*R*,9*R*,10*S*)	0.6
		20	11.7 (7*S*,8*S*,9*S*,10*R*)	12.2 (7*R*,8*R*,9*R*,10*S*)	0.9
		15	20.8 (7*S*,8*S*,9*S*,10*R*)	21.9 (7*R*,8*R*,9*R*,10*S*)	1.3
BP 7-MeO-8,9,10-triol	(*R*)-DNBPG-C	30	10.2 (7*S*,8*R*,9*R*,10*S*)	10.7 (7*R*,8*S*,9*S*,10*R*)	0.5
(7,8,9/10), minor		20	19.9 (7*S*,8*R*,9*R*,10*S*)	21.0 (7*R*,8*S*,9*S*,10*R*)	0.8
	(*S*)-DNBL-C	30	6.0	6.0	0
		20	11.4	11.4	0
		15	18.5	18.5	0

[a] Relative configuration for stereoisomers is designated as described in Fig. 3. MeO abbreviates for the methoxy group.

[b] CSPs are described in Experimental section.

[c] Percent of solvent A [ethanol–acetonitrile, 2:1 (v/v)] in hexane at a flow-rate of 2 ml/min and a void volume of 2.4 ml.

[d] Enantiomers are designated 1 and 2 according to elution order.

[e] RV = resolution value = $2\,(V_2 - V_1)/(W_2 + W_1)$, where V is retention volume and W is peak width at base.

[f] Data taken from ref. 5 for ready comparison.

when ionically bonded CSPs are used. Mobile phase of high polarity can be used with covalently bonded CSPs.

An approach to improve enantiomeric separation is to reduce the polarity of analytes by blocking polar groups via derivatization, thereby reducing the polarity of mobile phase and possibly increasing the efficiency of resolution. Derivatization of dihydrodiol derivatives of PAH's to O-methyl ethers, for example, has been shown to improve enantiomeric separation [13,14]. In this report, we compared the enantiomeric separation of highly polar derivatives of PAHs including tetrols, triols, triol-hydroxyethylthioethers, and methoxy-triols. All of these compounds were derived from BP diol-epoxides either by reduction with $NaBH_4$ or by reaction with nucleophiles such as water, CH_3O^-, and $HOC_2H_4S^-$.

Enantiomeric pairs of all triolic derivatives were separated with varying efficiency (resolution values 0–2.7) by one or both of the two covalent CSPs utilized (Tables I–III). Elution order of an enantiomeric pair, when resolved, may either be the same or different, depending on the compound. There is no apparent rule for predicting the elution order of enantiomers on any particular CSP column. Overall, the (*S*)-DNBL-C provided better resolution than the (*R*)-DNBPG-C. For example (Fig. 5), with identical mobile phase, the enantiomers of 7,8,9-triol-10-hydroxyethylthioether with a 7,10/8,9 relative stereochemistry are more efficiently separated on the (*S*)-DNBL-C than on the (*R*)-DNBPG-C.

The effects of substituents at C10 position of the triolic derivatives under study on the retention and enantiomeric resolution can be seen by the results obtained with derivatives (with a 7,10/8,9 relative stereochemistry) obtained from BP 7,8-diol-*anti*-9,10-epoxide (Fig. 6). The results were obtained by using 30% solvent A in hexane at a flow-rate of 2 ml/min on a (*S*)-DNBL-C column. The relative retention time of the more strongly retained enantiomer decreases depending on the structure of the C10 substituent: OH (23.2 min) >> H (13.5 min) >> OCH_3 (7.3 min) > SC_2H_4OH (7.2 min) (Fig. 6). This decrease in retention time (hence a decrease in polarity) is accompanied with an increased efficiency in the resolution of enantiomers

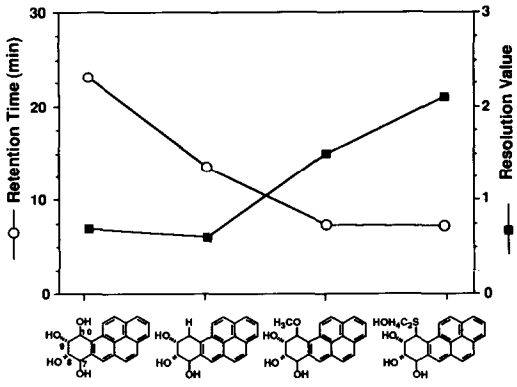

Fig. 6. Effects of substituent at C10 position of BP triolic derivatives on the retention and enantiomeric resolution on (*S*)-DNBL-C column. Samples were obtained as derivatives of BP 7,8-diol-*anti*-9,10-epoxide, isolated by reversed-phase HPLC, and resolved on (*S*)-DNBL-C using 30% of ethanol–acetonitrile (2:1, v/v) in hexane at a flow-rate of 2 ml/min. Data are taken from Tables I–III.

(Fig. 6). The C10 position is in the sterically crowded bay region. Any substituent at C10 adopts a quasiaxial conformation. It appears that the larger the quasiaxial substituent at C10 the better is the resolution of enantiomers on the (*S*)-DNBL-C column. Compared to tetrols, methoxy-triols are lower in polarity and the enantiomeric pairs are more efficiently resolved (Table III).

In conclusion, blocking one of the hydroxyl groups in BP 7,8,9,10-tetrols decreases retention time and increases enantiomeric resolution when compared to tetrol derivatives on Pirkle's covalently bonded CSPs. Changes in the retention and resolution are related to size and polarity of the substituent with the greatest changes noted in the bay region. The use of enantiomeric diol-epoxide enantiomers prior to derivatization has allowed elucidation of the absolute configurations of most of the triolic enantiomers.

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