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IMPROVED ENANTIOMERIC SEPARATION OF DIHYDRODIOLS OF POLYCYCLIC AROMATIC HYDROCARBONS ON CHIRAL STATIONARY PHASES BY DERIVATIZATION TO O-METHYL ETHERS

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SUMMARY

K-region *trans*-dihydrodiol derivatives of phenanthrene, 1-methylphenanthrene, 4,5-methylenephenanthrene, pyrene, 1-bromopyrene, chrysene, benzo[*c*]phenanthrene, benz[*a*]anthracene, 1-, 4-, 6-, 7-, 11- and 12-methylbenz[*a*]anthracenes, 7,12-dimethylbenz[*a*]anthracene, 3-methylcholanthrene, and benzo[*a*]pyrene, and non-*K*-region *trans*-3,4-dihydrodiols of benz[*a*]anthracene, chrysene, and 7,12-dimethylbenz[*a*]anthracene are converted to O-methyl ethers. Enantiomers of these O-methyl ethers are generally more efficiently separated on Pirkle's chiral stationary phases than the enantiomers of underivatized dihydrodiols. O-Methyl ethers are substantially less polar than dihydrodiols, and O-methyl ethers are eluted with shorter retention times. Eluents of lower polarity can hence be used. This enhances chiral interactions between chiral stationary phase and solutes, allowing improved separation of enantiomers.

INTRODUCTION

Separation of dihydrodiol enantiomers of many polycyclic aromatic hydrocarbons (PAHs) has recently been studied by using columns packed with Pirkle's chiral stationary phases (CSP)[1–9]. Although enantiomers of many dihydrodiols are efficiently resolved, enantiomers of some dihydrodiols are either poorly resolved or not resolved at all. In order to explore means to improve enantiomeric resolution, unresolvable or poorly resolved dihydrodiols are derivatized to O-methyl ethers. Conversion to O-methyl ethers substantially reduces the polarity of dihydrodiols, hence an eluent with lower polarity can be used. The use of eluents with lower polarity helps to extend the useful lifetime of ionically bonded chiral stationary phase columns. The compounds studied are *K*-region *trans*-dihydrodiols of phenanthrene, 1-methylphenanthrene, 4,5-methylenephenanthrene, pyrene, 1-bromopyrene, chrysene, benzo[*c*]phenanthrene, benz[*a*]anthracene, 1-, 4-, 6-, 7-, 11-, and 12-methylbenz[*a*]anthracenes, 7,12-dimethylbenz[*a*]anthracene, 3-methylcholanthrene, and benzo[*a*]pyrene, and non-*K*-region *trans*-3,4-dihydrodiols of benz[*a*]anthracene, chrysene, and 7,12-

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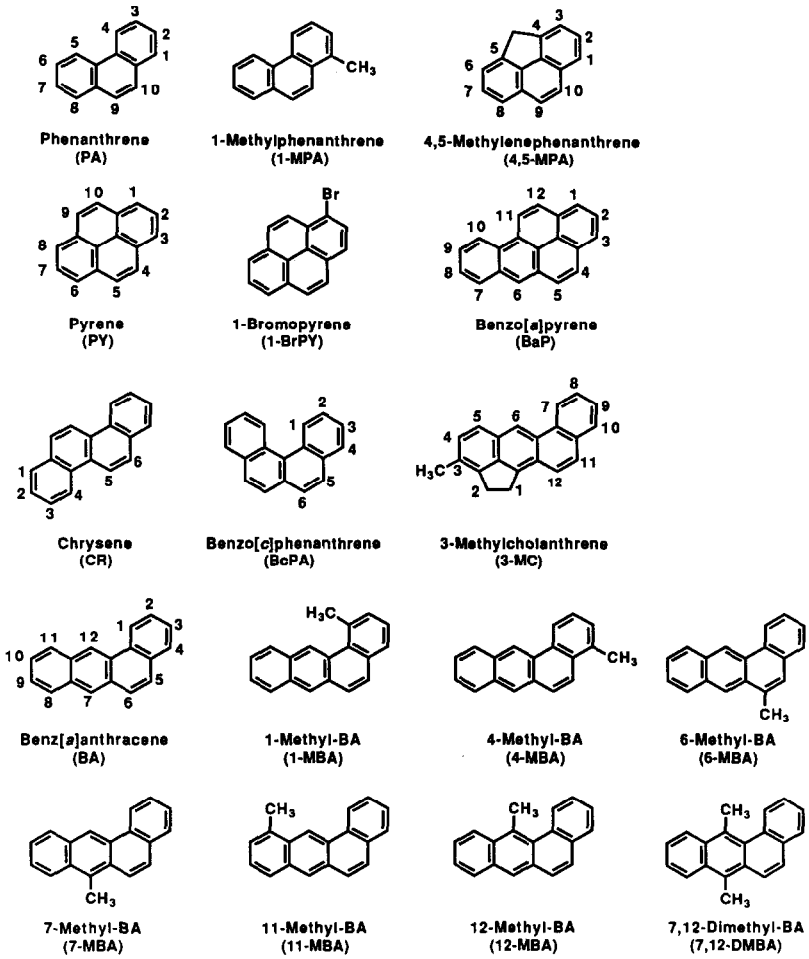


Fig. 1. Structures, numbering systems and abbreviations of PAHs described in this study.

dimethylbenz[*a*]anthracene. Structures, numbering systems, and abbreviations of parent PAHs included in this study are shown in Fig. 1. In the majority of cases, conversion of dihydrodiols to O-methyl ethers substantially improves enantiomeric separations.

EXPERIMENTAL

Chemicals

Epoxides and *trans*-dihydrodiols of PAHs were obtained either from the Chemical Repository of the National Cancer Institute or by incubation of the respective parent PAH with rat liver microsomes and an NADPH-regenerating system in the presence or in the absence of an epoxide hydrolase inhibitor[7,10,11].

High-performance liquid chromatography (HPLC)

HPLC was performed with a Waters Assoc. (Milford, MA, U.S.A.) liquid chromatograph, consisting of a Model 6000A solvent delivery system, a Model M45 solvent delivery system, a Model 660 solvent programmer, and a Model 440 absorbance detector (254 nm). Samples were injected via a Valco (Houston, TX, U.S.A.) Model N60 loop injector. Retention times and ratios of areas under the chromatographic peaks were recorded with a Hewlett-Packard (Palo Alto, CA, U.S.A.) Model 3390A integrator.

Normal-phase HPLC

O-Methyl ethers derived from *K*-region epoxides were separated on either a DuPont Golden Series SIL column (80 mm × 6.2 mm I.D.) or a DuPont Zorbax SIL column (250 mm × 6.2 mm I.D.). Eluents used for separation of isomeric O-methyl ethers are indicated in Table I.

TABLE I

NORMAL-PHASE HPLC SEPARATION OF ISOMERIC O-METHYL ETHERS OF DIHYDRODIOL DERIVATIVES OF SOME POLYCYCLIC AROMATIC HYDROCARBONS

*Dihydrodiol**	*Length of column (cm)***	*Eluent**** *(%)*	*Retention time (min) of O-methyl ether*§	
			Isomer 1	*Isomer 2*
PA 9,10-DHD§§	8	3.5% Eluent A	4.2 (9-O-methyl)	
1-MPA 9,10-DHD (AA)	8	1.8% Eluent A	7.2 (9-O-methyl)	9.4 (10-O-methyl)
4,5-MPA 9,10-DHD§§	25	5% Eluent A	14.1 (9-O-methyl)	
PY 4,5-DHD§§	8	3.5% Eluent A	5.2 (4-O-methyl)	
1-BrPY 4,5-DHD	25	2.5% Eluent A	14.8§§§	15.6§§§
1-BrPY 9,10-DHD (AA)	25	2.5% Eluent A	20.1§§§	22.6§§§
BaP 4,5-DHD	8	THF–MeOH–EtOAc–Hex (0.2:0.2:5:94.6)	17.1 (4-O-methyl)	18.0 (5-O-methyl)
CR 5,6-DHD (AA)	25†	EtOAc–Hex (7:13)	15.9 (6-O-methyl)	22.3 (5-O-methyl)
BcPA 5,6-DHD	8	0.5% Eluent A	9.8 (5-O-methyl)	10.8 (6-O-methyl)
3-MC 11,12-DHD (AA)	8	MeOH–THF–Hex (1.5:5:93.5)	6.5 (11-O-methyl)	7.3 (12-O-methyl)
BA 5,6-DHD	8	THF–MeOH–EtOAc–Hex (0.2:0.2:5:94.6)	13.3 (5-O-methyl)	14.0 (6-O-methyl)
1-MBA 5,6-DHD	25	EtOAc–EtOH–Hex (8:0.4:91.6)	20.4 (5-O-methyl)	21.5 (6-O-methyl)
4-MBA 5,6-DHD (AA)	8	2.5% Eluent A	7.1 (6-O-methyl)	8.6 (5-O-methyl)
6-MBA 5,6-DHD	8	0.5% Eluent A	7.0 (5-O-methyl)	9.4 (6-O-methyl)
7-MBA 5,6-DHD (AA)	8	2.5% Eluent A	7.2 (5-O-methyl)	8.9 (6-O-methyl)

(*Continued on p. 380*)

TABLE I (*continued*)

*Dihydrodiol**	*Length of column (cm)***	*Eluent**** *(%)*	*Retention time (min) of O-methyl ether*§*	
			Isomer 1	*Isomer 2*
11-MBA 5,6-DHD	25	1% Eluent A	22.0 (5-O-methyl)	22.6 (6-O-methyl)
12-MBA 5,6-DHD	25	EtOAc–MeOH–Hex (5:0.5:94.5)	19.8 (5-O-methyl)	20.3 (6-O-methyl)
7,12-DMBA 5,6-DHD (AA)	25	THF–Hex (1:4)	16.6 (5-O-methyl)	21.5 (6-O-methyl)
CR 3,4-DHD	8	3.6% Eluent A	9.0 (3-O-methyl)	10.7 (4-O-methyl)
BA 3,4-DHD	8	3.6% Eluent A	4.5 (3-O-methyl)	5.9 (4-O-methyl)
7,12-DMBA 3,4-DHD	8	3.6% Eluent A	4.4 (3-O-methyl)	5.8 (4-O-methyl)

* DHD = *trans*-dihydrodiol. Absolute configurations of resolved enantiomers are assigned as described in refs. 5, 7, 10 and 11. Dihydrodiols with hydroxyl groups preferentially in quasidiaxial conformation are indicated by AA in parenthesis.

** Unless otherwise stated, the column is indicated by its length and is either the DuPont Zorbax SIL column (250 × 6.2 mm I.D.) or DuPont Golden SIL column (80 × 6.2 mm I.D.).

*** The percentage of eluent A (ethanol–acetonitrile; 2:1, v/v) in hexane is indicated. The compositions of other elution solvents are as indicated. The flow-rate was 2 ml/min. Abbreviations for solvents are: THF = tetrahydrofuran; EtOAc = ethyl acetate; Hex = hexane, MeOH = methanol.

§ O-Methyl ethers are designed as isomers 1 and 2, according to their elution order on NP-HPLC. Normal-phase HPLC separations of O-methyl ethers, derived from *K*-region dihydrodiols of BaP[10], CR[5], BcPA[7], 3-MC[10], 12-MBA[11] and 7,12-DMBA[13], were partially described in earlier reports. The identity of each O-methyl ether is indicated in parenthesis. Bis-O-methyl ethers are eluted with much shorter retention times than O-methyl ethers.

§§ Due to symmetry, there is only one O-methyl ether.

§§§ The location of O-methyl group has not been established.

† A Resolvex SIL column (250 × 4.6 mm I.D.; Fisher Scientific) was used[5].

CSP HPLC

Enantiomeric separation of each dihydrodiol and O-methyl ether was tested by using a CSP column (250 mm × 4.6 mm I.D.; Regis, Morton Grove, IL, U.S.A.), packed with spherical particles of 5 μm diameter of γ-aminopropylsilanized silica to which either (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine (*R*-DNBPG-I or *R*-DNBPG-C) or (*S*)-N-(3,5-dinitrobenzoyl)leucine (*S*-DNBL-I or *S*-DNBL-C) was bonded, either ionically (I) or covalently (C). The eluent was 0.1–10% (v/v) of eluent A (ethanol–acetonitrile; 2:1, v/v) in hexane at 2 ml/min.

Methoxylation of K-region epoxides

Racemic or enantiomeric epoxide was dissolved in methanol alone or in methanol, saturated with sodium methoxide and heated at 50°C for 1 h and then stored overnight at room temperature. The resulting two isomeric O-methyl ethers were separated by normal-phase HPLC. Enantiomers of each O-methyl ether were resolved by CSP-HPLC.

Methylation of trans-dihydrodiol

trans-Dihydrodiols were each methylated to a pair of isomeric O-methyl ethers by dissolving in sodium hydride-treated tetrahydrofuran (THF; 1 ml), methyl iodide (methyl iodide/O-methyl ether $\simeq$ 500, molar ratio), and a catalytic amount of sodium hydride, added at 0, 15, 30 and 45 min at room temperature in the dark. Fifteen minutes after the last addition of methyl iodide, the reaction was stopped by dropwise additions of methanol. The resulting isomeric O-methyl ethers were separated by normal-phase HPLC, as described above. Due to the use of excess amount of methyl iodide, a bis-O-methyl ether was formed in addition to O-methyl ethers. Relative amounts of O-methyl ethers and bis-O-methyl ether derived from each dihydrodiol varied greatly in repeated experiments under essentially the same experimental conditions.

Location of O-methyl group in O-methyl ethers

The location of O-methyl group in each O-methyl ether was established either by 500 MHz proton NMR spectroscopy[5,11] or by chemical methods[5,7,10,11] similarly as described in earlier reports.

Spectral analysis

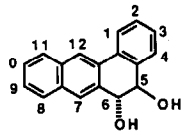
All PAH derivatives in this study were analyzed by mass, UV–VIS, and circular dichroism (CD) spectral analyses. Mass spectral analysis was performed on a Finnigan Model 4000 gas chromatograph–mass spectrometer–data system (Finnigan MAT, San Jose, CA, U.S.A.) by electron impact with a solid probe at 70 eV and 250°C ionizer temperature. UV–VIS absorption spectra of samples in methanol were determined using a 1-cm path length quartz cuvette with a Varian Model Cary 118C spectrophotometer. CD spectra of samples in methanol in a quartz cell of 1-cm path length at room temperature were measured with a Jasco (Japan Scientific, Tokyo, Japan) Model 500A spectropolarimeter, equipped with a Model DP500 data processor.

RESULTS AND DISCUSSION

Normal-phase HPLC separation of O-methyl ethers

Due to symmetry, methoxylation of PA 9,10-epoxide, 4,5-MPA 9,10-epoxide, and PY 4,5-epoxide (or monomethylation of the corresponding dihydrodiol) each results in only one dihydrodiol O-methyl ether. Methoxylation of an epoxide (or monomethylation of a *trans*-dihydrodiol) of other PAHs each results in a pair of isomeric O-methyl ethers which can be separated by normal-phase HPLC (Table I). Structures, numbering systems, and abbreviations of dihydrodiols, O-methyl ethers, and bis-O-methyl ethers are shown in Figs. 2 and 3. Relative amounts of O-methyl ethers obtained by methoxylation of an epoxide and by methylation of a dihydrodiol are shown in Table II. Distribution of isomeric O-methyl ethers derived by methoxylation of an epoxide is reproducible. However, product ratios resulting from the reaction of methyl iodide and dihydrodiols are highly variable among repeated experiments.

K-region *trans*-dihydrodiols with hydroxyl groups preferentially in quasidiaxial conformation[12] are indicated by "AA" in Table I. The less strongly retained (hence, less polar) O-methyl ethers derived from quasidiaxial dihydrodiols (*e.g.*, 1-MPA



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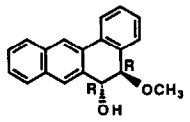

BA 5,6-DHD
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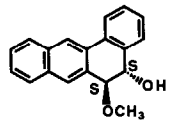

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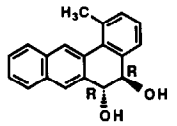

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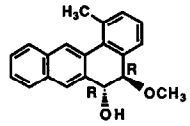

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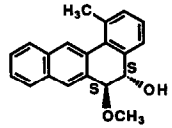

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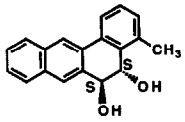

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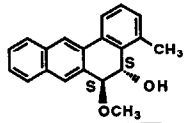

4-MBA 5,6-DHD
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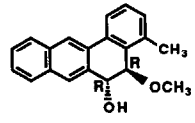

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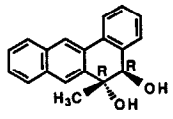

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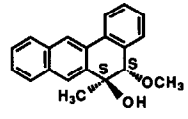

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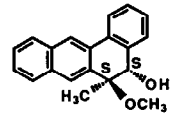

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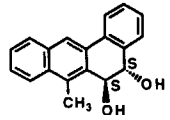

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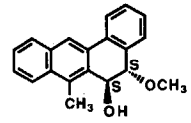

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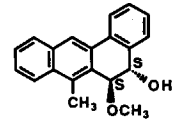

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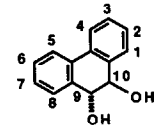

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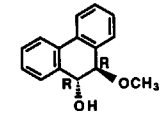
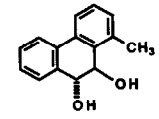


PA 9,10-DHD
9-O-methyl



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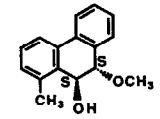

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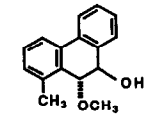
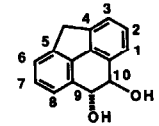
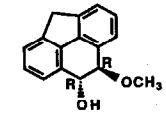
9-O-methyl (#1)



1-MPA 9,10-DHD
10-O-methyl (#2)



4,5-MPA 9,10-DHD



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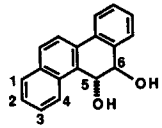
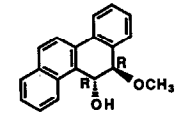
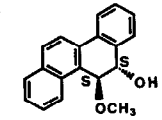
9-O-methyl



CR 5,6-DHD
(5S,6S on PG-C)



CR 5,6-DHD
6-O-methyl (#1)
(RV=0 on PG-C)



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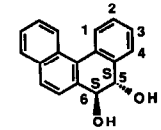
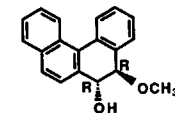
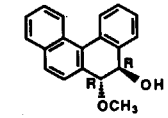


BcPA 5,6-DHD
(RV=0 on PG-C)



BcPA 5,6-DHD
5-O-methyl (#1)



BcPA 5,6-DHD
6-O-methyl (#2)

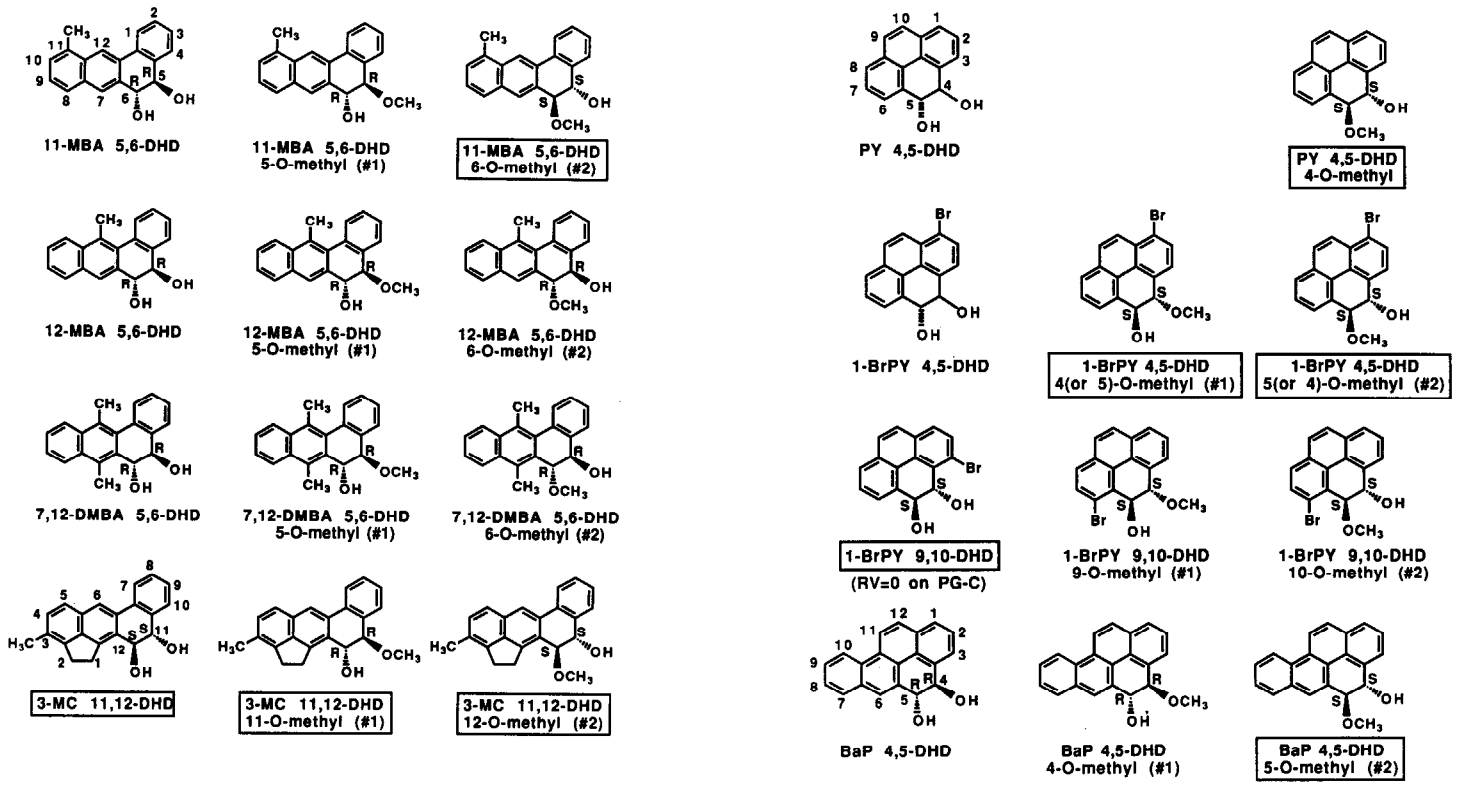


Fig. 2. Directions for reading this figure: (i) abbreviations of dihydrodiols (DHD) and O-methyl ethers are shown; (ii) two isomeric O-methyl ethers of each dihydrodiol are designated as 1 and 2 according to their elution order in normal-phase HPLC (Table I). Relative amounts of O-methyl ethers, obtained either by methoxylation of an epoxide or by methylation of a dihydrodiol, are indicated in Table II; (iii) absolute configurations of the more strongly retained enantiomers on the ionically bonded *R*-DNBPG column are shown; (iv) structures that do not have associated *R* and/or *S* designations indicate that enantiomers of dihydrodiol and/or O-methyl ether are not resolved by anyone of the four CSP columns used in this study; (v) enantiomeric separation on *R*-DNBPG-C, if different from that on *R*-DNBPG-I, is shown in parenthesis under the structure; (vi) a boxed-in name under the structure indicates that the same enantiomer is more strongly retained on *S*-DNBL-I and/or *S*-DNBL-C column; (vii) data on enantiomeric separations on four CSPs are shown in Table III.

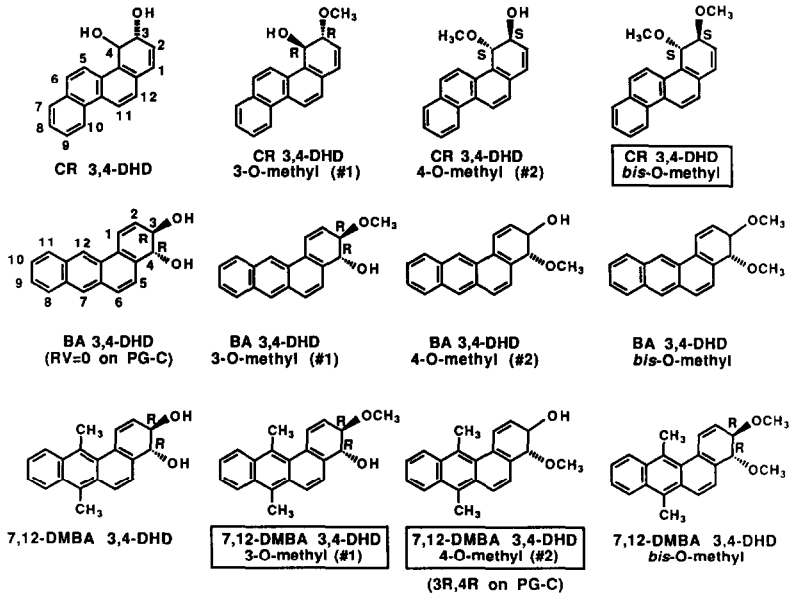


Fig. 3. Directions for reading this figure are similar to those described in the legend for Fig. 2.

9,10-DHD 9-O-methyl, CR 5,6-DHD 6-O-methyl, 4-MBA 5,6-DHD 6-O-methyl, 7-MBA 5,6-DHD 5-O-methyl, 7,12-DMBA 5,6-DHD 5-O-methyl, and 3-MC 11,12-DHD 11-O-methyl) all have their O-methyl groups located away from the sterically hindered region (Fig. 2a–d). Based on these observations, the less strongly retained O-methyl ether of 1-BrPY 9,10-DHD is tentatively assigned as the 9-O-methyl derivative (Fig. 2b). The less strongly retained O-methyl ethers derived from quasidiequatorial dihydrodiols with either a benz[*a*]anthracene or a benzo[*c*]phenanthrene nucleus all have their O-methyl groups at the C-5 position (Fig. 2a, c and d); the O-methyl group of BaP 4,5-DHD O-methyl No. 1 is similarly located at the C-4 position (Fig. 2b). However, structures of two isomeric O-methyl ethers derived from 1-BrPY 4,5-DHD cannot be established on the basis of the observation described above.

CSP HPLC resolution of enantiomers

Retention times, absolute configurations, and resolution values in the separation of enantiomeric dihydrodiols and O-methyl ethers, derived either by methoxylation of *K*-region epoxides or by methylation of *trans*-dihydrodiols by ionically and covalently bonded *R*-DNBPG and *S*-DNBL columns, are shown in Tables III and IV. Data on the enantiomeric resolutions of some *K*-region *trans*-dihydrodiols were partially reported earlier[1–10] and are included in Table III for comparison; new chromatographic data were obtained with different eluent compositions and/or CSP columns which were not previously reported. The major emphasis of the data in Tables III and IV is on the improvement of enantiomeric separation of O-methyl ethers as compared with that of dihydrodiols.

TABLE II

PERCENTAGES OF O-METHYL ETHERS DERIVED FROM METHOXYLATION OF EPOXIDES AND FROM METHYLATION OF DIHYDRODIOLS

*Epoxide or dihydrodiol**	*Methoxylation or methylation product***		
	Bis-O-methyl	*O-Methyl 1*	*O-Methyl 2*
1-MPA 9,10-epoxide	NF	36	64
1-MPA 9,10-DHD	ND	72	28
1-BrPY 4,5-epoxide	NF	51	49
1-BrPY 9,10-epoxide	NF	72	28
BaP 4,5-epoxide	NF	53	47
BaP 4,5-DHD	ND	52	48
	ND	66	34
CR 5,6-epoxide	NF	50	50
CR 5,6-DHD****	25	50	25
	50	44	6
BcPA 5,6-epoxide	NF	30	70
BcPA 5,6-DHD	ND	49	51
3MC 11,12-epoxide	NF	32	68
3MC 11,12-DHD	ND	87	13
	ND	83	17
BA 5,6-epoxide	NF	50	50
BA 5,6-DHD****	7	44	49
	18	40	42
1-MBA 5,6-epoxide	NF	58	42
1-MBA 5,6-DHD	ND	47	53
4-MBA 5,6-epoxide	NF	41	59
4-MBA 5,6-DHD	ND	82	18
6-MBA 5,6-epoxide	NF	85	15
7-MBA 5,6-epoxide	NF	42	58
11-MBA 5,6-epoxide	NF	48	52
11-MBA 5,6-DHD	ND	53	47
12-MBA 5,6-epoxide	NF	23	77
12-MBA 5,6-DHD****	ND	43	57
	29	55	16
7,12-DMBA 5,6-epoxide	NF	15	85
7,12-DMBA 5,6-DHD§	ND	80	20
CR 3,4-DHD	14	47	39
BA 3,4-DHD	10	26	64
7,12-DMBA 3,4-DHD	1	47	52

* Data on *K*-region derivatives of BaP[10], CR[5], BcPA[7], 3-MC[10], BA[11], 12-MBA[11] and 7,12-DMBA[13,17] are taken from earlier reports and are included for comparison.

** NF = not formed; ND = not detected in that particular experiment. Identities of O-methyl ethers are indicated in Table I.

*** Examples indicating that the relative amounts of O-methyl ethers by methylation of dihydrodiols varied substantially among repeated experiments.

§ Data from ref. 17.

TABLE III

CSP-HPLC RESOLUTION OF *K*-REGION *TRANS*-DIHYDRODIOLS AND THEIR MONOMETHYL ETHERS OF SOME POLYCYCLIC AROMATIC HYDROCARBONS

*Chemical**	*CSP***	*A (%)****	*Retention time (min)*§		*RV*§§
			Enantiomer 1	*Enantiomer 2*	
PA 9,10-DHD	*R*-DNBPG-I	2.5	37.5	37.5	0
	R-DNBPG-C	2.5	25.2	25.2	0
	S-DNBL-I	2.5	34.8	34.8	0
	S-DNBL-C	2.5	19.7	19.7	0
PA 9,10-DHD 9-O-methyl	*R*-DNBPG-I	0.5	25.6 (*S,S*)	26.1 (*R,R*)	0.3
	R-DNBPG-C	0.5	30.5 (*S,S*)	30.9 (*R,R*)	0.2
	S-DNBL-I	0.5	20.7	20.7	0
	S-DNBL-C	0.5	17.7 (*R,R*)	18.1 (*S,S*)	0.3
1-MPA 9,10-DHD	*R*-DNBPG-I	5.0	34.9	34.9	0
	R-DNBPG-C	5.0	30.5	30.5	0
	S-DNBL-I	5.0	31.3	31.3	0
	S-DNBL-C	5.0	22.9	22.9	0
1-MPA 9,10-DHD 9-O-methyl (1)	*R*-DNBPG-I	2.5	11.7 (*R,R*)	12.2 (*S,S*)	1.0
	R-DNBPG-C	2.5	12.0 (*R,R*)	12.7 (*S,S*)	1.2
	S-DNBL-I	2.5	9.9	9.9	0
	S-DNBL-C	2.5	8.6	8.6	0
1-MPA 9,10-DHD 10-O-methyl (2)	*R*-DNBPG-I	2.5	15.8	15.8	0
	R-DNBPG-C	2.5	16.6	16.6	0
	S-DNBL-I	2.5	13.6	13.6	0
	S-DNBL-C	2.5	11.3	11.3	0
4,5-MPA 9,10-DHD	*R*-DNBPG-I	5.0	31.4	31.4	0
	R-DNBPG-C	5.0	20.9	20.9	0
	S-DNBL-I	5.0	24.7	24.7	0
	S-DNBL-C	5.0	15.0	15.0	0
4,5-MPA 9,10-DHD 9-O-methyl	*R*-DNBPG-I	1.0	23.2	23.2	0
		0.5	66.6 (*S,S*)	69.7 (*R,R*)	1.0
	R-DNBPG-C	1.0	36.0 (*S,S*)	37.7 (*R,R*)	0.9
	S-DNBL-I	1.0	23.0	23.0	0
		0.5	37.2 (*R,R*)	38.0 (*S,S*)	0.4
	S-DNBL-C	1.0	32.7 (*R,R*)	35.3 (*S,S*)	1.5
CR 5,6-DHD	*R*-DNBPG-I	10	25.3	25.3	0
	R-DNBPG-C	10	20.2 (*R,R*)	20.8 (*S,S*)	0.7
	S-DNBL-I	10	24.1 (*R,R*)	25.1 (*S,S*)	1.1
	S-DNBL-C	10	14.2 (*R,R*)	14.6 (*S,S*)	0.5
CR 5,6-DHD 6-O-methyl (1)	*R*-DNBPG-I	5	16.6 (*S,S*)	17.4 (*R,R*)	1.1
	R-DNBPG-C	5	15.2	15.2	0
	S-DNBL-I	5	10.0 (*R,R*)	10.3 (*S,S*)	0.6
	S-DNBL-C	5	9.0	9.0	0
CR 5,6-DHD 5-O-methyl (2)	*R*-DNBPG-I	5	18.7 (*R,R*)	19.9 (*S,S*)	1.5
	R-DNBPG-C	5	17.1 (*R,R*)	18.6 (*S,S*)	1.9
	S-DNBL-I	5	11.7 (*R,R*)	12.2 (*S,S*)	0.9
	S-DNBL-C	5	10.8 (*R,R*)	10.9 (*S,S*)	0.1

TABLE III (*continued*)

*Chemical**	*CSP***	*A (%)****	*Retention time (min)*§		*RI*§§
			Enantiomer 1	*Enantiomer 2*	
BcPA 5,6-DHD	*R*-DNBPG-I	2.5	49.5 (*S,S*)	51.8 (*R,R*)	1.1
	R-DNBPG-C	2.5	30.3	30.3	0
	S-DNBL-I	2.5	38.8	38.8	0
	S-DNBL-C	2.5	22.6	22.6	0
BcPA 5,6-DHD 5-O-methyl (1)	*R*-DNBPG-I	0.5	27.8 (*S,S*)	31.3 (*R,R*)	2.5
	R-DNBPG-C	0.5	29.1 (*S,S*)	30.4 (*R,R*)	0.8
	S-DNBL-I	0.5	19.4 (*R,R*)	20.0 (*S,S*)	0.7
	S-DNBL-C	0.5	18.1 (*R,R*)	19.1 (*S,S*)	0.9
BcPA 5,6-DHD 6-O-methyl (2)	*R*-DNBPG-I	0.5	28.5 (*S,S*)	29.7 (*R,R*)	0.9
	R-DNBPG-C	0.5	28.8 (*S,S*)	29.0 (*R,R*)	0.2
	S-DNBL-I	0.5	21.7 (*R,R*)	22.7 (*S,S*)	0.8
	S-DNBL-C	0.5	21.1 (*R,R*)	21.8 (*S,S*)	0.5
PY 4,5-DHD	*R*-DNBPG-I	5.0	32.3	32.3	0
	R-DNBPG-C	5.0	20.3	20.3	0
	S-DNBL-I	5.0	22.3	22.3	0
	S-DNBL-C	5.0	17.2 (*R,R*)	18.0 (*S,S*)	0.6
		2.5	48.0 (*R,R*)	51.3 (*S,S*)	1.1
PY 4,5-DHD 4-O-methyl	*R*-DNBPG-I	2.0	21.7 (*R,R*)	22.4 (*S,S*)	0.7
	R-DNBPG-C	2.0	21.5 (*R,R*)	22.0 (*S,S*)	0.5
	S-DNBL-I	2.0	13.5	13.5	0
		1.0	25.7 (*R,R*)	26.3 (*S,S*)	0.4
	S-DNBL-C	2.0	12.7 (*R,R*)	13.2 (*S,S*)	0.6
		1.0	21.5 (*R,R*)	22.7 (*S,S*)	1.0
1-BrPY 4,5-DHD	*R*-DNBPG-I	5.0	16.0	16.0	0
		2.5	41.0	41.0	0
	R-DNBPG-C	5.0	15.7	15.7	0
	S-DNBL-I	5.0	23.0	23.0	0
	S-DNBL-C	2.5	34.0 (*R,R*)	35.2 (*S,S*)	0.6
1-BrPY 4,5-DHD 4 (or 5)-O-methyl (1)	*R*-DNBPG-I	2.5	14.1 (*R,R*)	14.6 (*S,S*)	0.6
	R-DNBPG-C	2.5	12.6 (*R,R*)	13.0 (*S,S*)	0.5
	S-DNBL-I	1.0	22.4	22.4	0
	S-DNBL-C	1.0	17.5 (*R,R*)	18.5 (*S,S*)	1.0
1-BrPY 4,5-DHD 5 (or 4)-O-methyl (2)	*R*-DNBPG-I	2.5	14.1 (*R,R*)	14.7 (*S,S*)	0.9
	R-DNBPG-C	2.5	12.9 (*R,R*)	13.4 (*S,S*)	0.6
	S-DNBL-I	1.0	23.2	23.2	0
	S-DNBL-C	1.0	17.3 (*R,R*)	18.0 (*S,S*)	0.7
1-BrPy 9,10-DHD	*R*-DNBPG-I	5.0	78.9(*R,R*)	80.1(*S,S*)	0.3
	R-DNBPG-C	2.5	31.9	31.9	0
	S-DNBL-I	5.0	54.2	54.2	0
	S-DNBL-C	5.0	40.9	40.9	0
		2.5	118.0(*R,R*)	124.0(*S,S*)	0.8
1-BrPY 9,10-DHD 9-O-methyl (1)	*R*-DNBPG-I	2.5	23.2(*R,R*)	26.0(*S,S*)	2.4
	R-DNBPG-C	2.5	18.6(*R,R*)	22.0(*S,S*)	3.0
	S-DNBL-I	1.0	35.5	35.5	0
	S-DNBL-C	1.0	28.3	28.3	0

(*continued on p. 388*)

TABLE III (*continued*)

*Chemical**	*CSP***	*A (%)****	*Retention time (min)*§		*RV*§§
			Enantiomer 1	*Enantiomer 2*	
1-BrPY 9,10-DHD	*R*-DNBPG-I	2.5	23.6(*R,R*)	24.3(*S,S*)	0.8
10-O-methyl (2)	*R*-DNBPG-C	2.5	22.8(*R,R*)	23.5(*S,S*)	0.6
	S-DNBL-I	1.0	46.1	46.1	0
	S-DNBL-C	1.0	36.3	36.3	0
BaP 4,5-DHD	*R*-DNBPG-I	10.0	24.4(*S,S*)	25.2(*R,R*)	0.8
	R-DNBPG-C	5.0	34.9(*S,S*)	35.3(*R,R*)	0.2
	S-DNBL-I	10.0	17.3(*R,R*)	17.6(*S,S*)	0.3
	S-DNBL-C	10.0	10.9(*R,R*)	11.1(*S,S*)	0.3
BaP 4,5-DHD	*R*-DNBPG-I	2.5	36.2(*S,S*)	38.6(*R,R*)	1.4
4-O-methyl (1)	*R*-DNBPG-C	2.5	36.0(*S,S*)	36.7(*R,R*)	0.3
	S-DNBL-I	5.0	10.5(*R,R*)	11.2(*S,S*)	1.1
	S-DNBL-C	5.0	9.6(*R,R*)	9.9(*S,S*)	0.4
BaP 4,5-DHD	*R*-DNBPG-I	5.0	19.0(*R,R*)	20.6(*S,S*)	2.0
5-O-methyl (2)	*R*-DNBPG-C	5.0	15.4(*R,R*)	17.4(*S,S*)	2.4
	S-DNBL-I	5.0	10.3(*R,R*)	10.5(*S,S*)	0.2
	S-DNBL-C	5.0	9.4(*R,R*)	9.5(*S,S*)	~0.1
BA 5,6-DHD	*R*-DNBPG-I	5.0	33.7	33.7	0.0
	R-DNBPG-C	5.0	18.8	18.8	0.0
	S-DNBL-I	5.0	24.0	24.0	0.0
	S-DNBL-C	5.0	14.2	14.2	0.0
BA 5,6-DHD	*R*-DNBPG-I	2.5	20.5(*S,S*)	22.6(*R,R*)	2.5
5-O-methyl (1)	*R*-DNBPG-C	2.5	17.6(*S,S*)	18.5(*R,R*)	1.2
	S-DNBL-I	2.5	12.4(*R,R*)	13.4(*S,S*)	1.8
	S-DNBL-C	2.5	10.7	10.7	0.0
		1.0	20.2(*R,R*)	21.5(*S,S*)	0.8
BA 5,6-DHD	*R*-DNBPG-I	2.5	18.4(*R,R*)	19.8(*S,S*)	1.7
6-O-methyl (2)	*R*-DNBPG-C	2.5	16.0(*R,R*)	17.8(*S,S*)	2.3
	S-DNBL-I	2.5	11.7(*R,R*)	12.2(*S,S*)	1.2
	S-DNBL-C	2.5	10.5	10.5	0.0
		1.0	19.4	19.4	0.0
1-MBA 5,6-DHD	*R*-DNBPG-I	5.0	23.0(*S,S*)	24.1(*R,R*)	1.1
	R-DNBPG-C	5.0	14.5	14.5	0
	S-DNBL-I	5.0	19.2(*R,R*)	19.6(*S,S*)	0.4
	S-DNBL-C	5.0	11.5	11.5	0
1-MBA 5,6-DHD	*R*-DNBPG-I	1.0	20.6(*S,S*)	24.3(*R,R*)	5.1
5-O-methyl (1)	*R*-DNBPG-C	1.0	17.6(*S,S*)	19.9(*R,R*)	3.1
	S-DNBL-I	1.0	13.1(*R,R*)	14.8(*S,S*)	3.2
	S-DNBL-C	1.0	9.4(*R,R*)	10.3(*S,S*)	1.8
1-MBA 5,6-DHD	*R*-DNBPG-I	2.5	10.6(*R,R*)	11.1(*S,S*)	0.9
6-O-methyl (2)	*R*-DNBPG-C	2.5	9.8(*R,R*)	10.5(*S,S*)	1.7
	S-DNBL-I	2.5	8.2(*R,R*)	8.6(*S,S*)	1.1
	S-DNBL-C	1.0	10.5(*R,R*)	11.0(*S,S*)	1.1
4-MBA 5,6-DHD	*R*-DNBPG-I	10.0	22.7(*R,R*)	23.4(*S,S*)	0.8
	R-DNBPG-C	10.0	20.8(*R,R*)	22.0(*S,S*)	1.2
	S-DNBL-I	10.0	18.4(*R,R*)	18.8(*S,S*)	~0.1
	S-DNBL-C	10.0	15.7(*R,R*)	16.2(*S,S*)	~0.1

TABLE III (*continued*)

*Chemical**	*CSP***	*A (%)****	*Retention time (min)*§		*RV*§§
			Enantiomer 1	*Enantiomer 2*	
4-MBA 5,6-DHD	*R*-DNBPG-I	2.5	20.7(*R*,*R*)	23.3(*S*,*S*)	2.7
6-O-methyl (1)	*R*-DNBPG-C	2.5	21.1(*R*,*R*)	25.1(*S*,*S*)	3.8
	S-DNBL-I	2.5	15.1(*R*,*R*)	15.4(*S*,*S*)	0.2
	S-DNBL-C	2.5	13.2(*R*,*R*)	14.2(*S*,*S*)	1.5
4-MBA 5,6-DHD	*R*-DNBPG-I	2.5	35.5(*S*,*S*)	36.3(*R*,*R*)	0.5
5-O-methyl (2)	*R*-DNBPG-C	2.5	35.6(*S*,*S*)	36.4(*R*,*R*)	0.5
	S-DNBL-I	2.5	21.2	21.2	0
	S-DNBL-C	2.5	20.4	20.4	0
6-MBA 5,6-DHD	*R*-DNBPG-I	5.0	16.8(*S*,*S*)	17.8(*R*,*R*)	1.3
	R-DNBPG-C	2.5	24.2(*S*,*S*)	25.4(*R*,*R*)	1.0
	S-DNBL-I	2.5	29.7	29.7	0
	S-DNBL-C	2.5	18.4(*S*,*S*)	19.0(*R*,*R*)	0.1
6-MBA 5,6-DHD	*R*-DNBPG-I	0.5	16.1(*R*,*R*)	16.4(*S*,*S*)	0.1
5-O-methyl (1)	*R*-DNBPG-C	0.5	17.7	17.7	0
	S-DNBL-I	0.5	11.9	11.9	0
	S-DNBL-C	0.5	10.6(*S*,*S*)	10.9(*R*,*R*)	0.1
		0.25	17.3(*S*,*S*)	18.0(*R*,*R*)	0.7
6-MBA 5,6-DHD	*R*-DNBPG-I	0.5	27.8(*R*,*R*)	29.6(*S*,*S*)	1.8
6-O-methyl (2)	*R*-DNBPG-C	0.5	23.6(*R*,*R*)	24.7(*S*,*S*)	1.0
	S-DNBL-I	0.5	20.4	20.4	0
	S-DNBL-C	0.5	15.4	15.4	0
7-MBA 5,6-DHD	*R*-DNBPG-I	10.0	30.9(*R*,*R*)	32.6(*S*,*S*)	1.1
	R-DNBPG-C	10.0	24.1(*R*,*R*)	30.0(*S*,*S*)	5.4
	S-DNBL-I	10.0	25.6(*R*,*R*)	27.2(*S*,*S*)	0.9
	S-DNBL-C	10.0	17.1(*R*,*R*)	17.5(*S*,*S*)	0.4
7-MBA 5,6-DHD	*R*-DNBPG-I	10.0	10.3(*R*,*R*)	11.3(*S*,*S*)	2.0
5-O-methyl (1)		5.0	20.5(*R*,*R*)	22.9(*S*,*S*)	3.2
	R-DNBPG-C	10.0	10.2(*R*,*R*)	11.8(*S*,*S*)	4.0
	S-DNBL-I	2.5	20.1	20.1	0
	S-DNBL-C	2.5	17.9(*R*,*R*)	18.2(*S*,*S*)	0.1
7-MBA 5,6-DHD	*R*-DNBPG-I	5.0	20.2(*R*,*R*)	23.6(*S*,*S*)	5.0
6-O-methyl (2)	*R*-DNBPG-C	5.0	20.0(*R*,*R*)	24.0(*S*,*S*)	5.7
	S-DNBL-I	5.0	12.4(*R*,*R*)	13.1(*S*,*S*)	1.1
		2.5	23.8(*R*,*R*)	25.3(*S*,*S*)	1.7
	S-DNBL-C	5.0	11.0(*R*,*R*)	11.7(*S*,*S*)	1.5
		2.5	18.2(*R*,*R*)	19.9(*S*,*S*)	2.1
11-MBA 5,6-DHD	*R*-DNBPG-I	6.0	25.0(*S*,*S*)	25.7(*R*,*R*)	0.6
	R-DNBPG-C	5.0	19.5	19.5	0
	S-DNBL-I	5.0	24.8(*R*,*R*)	25.5(*S*,*S*)	0.3
	S-DNBL-C	5.0	15.0(*R*,*R*)	15.0(*S*,*S*)	0.3
11-MBA 5,6-DHD	*R*-DNBPG-I	2.5	21.5(*S*,*S*)	24.2(*R*,*R*)	3.0
5-O-methyl (1)	*R*-DNBPG-C	2.5	19.4(*S*,*S*)	20.7(*R*,*R*)	1.7
	S-DNBL-I	2.5	12.2(*R*,*R*)	13.4(*S*,*S*)	1.7
	S-DNBL-C	2.5	12.1(*R*,*R*)	12.9(*S*,*S*)	1.5

(*continued on p. 390*)

TABLE III (*continued*)

*Chemical**	*CSP***	*A (%)****	*Retention time (min)*§		*RI*§§
			Enantiomer 1	*Enantiomer 2*	
11-MBA 5,6-DHD	*R*-DNBPG-I	2.5	17.5(*R,R*)	19.4(*S,S*)	2.6
6-O-methyl (2)	*R*-DNBPG-C	2.5	15.3(*R,R*)	17.5(*S,S*)	3.3
	S-DNBL-I	2.5	11.2(*R,R*)	11.5(*S,S*)	0.7
	S-DNBL-C	2.5	10.5(*R,R*)	11.1(*S,S*)	1.3
12-MBA 5,6-DHD	*R*-DNBPG-I	10.0	13.4(*S,S*)	14.5(*R,R*)	1.8
	R-DNBPG-C	5.0	18.3	18.3	0
	S-DNBL-I	10.0	11.4(*R,R*)	11.5(*S,S*)	<0.1
	S-DNBL-C	5.0	13.8	13.8	0
12-MBA 5,6-DHD	*R*-DNBPG-I	1.0	28.3(*S,S*)	32.5(*R,R*)	3.6
5-O-methyl (1)	*R*-DNBPG-C	1.0	23.7(*S,S*)	25.2(*R,R*)	1.1
	S-DNBL-I	1.0	16.2	16.2	0
		0.5	25.5(*R,R*)	27.4(*S,S*)	1.4
	S-DNBL-C	1.0	13.8	13.8	0
		0.5	31.8(*R,R*)	34.3(*S,S*)	1.1
12-MBA 5,6-DHD	*R*-DNBPG-I	1.0	23.0(*S,S*)	24.7(*R,R*)	1.5
6-O-methyl (2)	*R*-DNBPG-C	1.0	21.6	21.6	0
	S-DNBL-I	1.0	13.7(*R,R*)	15.3(*S,S*)	2.0
	S-DNBL-C	1.0	11.9(*R,R*)	12.9(*S,S*)	1.4
7,12-DMBA 5,6-DHD	*R*-DNBPG-I	10.0	21.8(*S,S*)	25.4(*R,R*)	2.6
	R-DNBPG-C	10.0	20.3(*R,R*)	20.8(*S,S*)	0.5
	S-DNBL-I	10.0	20.6(*R,R*)	28.6(*S,S*)	6.9
	S-DNBL-C	10.0	16.8(*R,R*)	18.9(*S,S*)	1.3
7,12-DMBA 5,6-DHD	*R*-DNBPG-I	2.5	35.5(*S,S*)	39.4(*R,R*)	2.8
5-O-methyl (1)	*R*-DNBPG-C	2.5	33.0(*R,R*)	37.7(*S,S*)	3.0
	S-DNBL-I	5.0	10.5(*R,R*)	11.5(*S,S*)	1.9
	S-DNBL-C	2.5	17.9(*S,S*)	18.4(*R,R*)	0.3
7,12-DMBA 5,6-DHD	*R*-DNBPG-I	5.0	16.7(*S,S*)	18.1(*R,R*)	2.3
6-O-methyl (2)	*R*-DNBPG-C	5.0	17.1(*R,R*)	18.5(*S,S*)	1.8
	S-DNBL-I	5.0	10.0(*R,R*)	14.9(*S,S*)	8.1
	S-DNBL-C	5.0	10.6(*R,R*)	13.0(*S,S*)	2.8
3-MC 11,12-DHD	*R*-DNBPG-I	10.0	20.4(*R,R*)	21.2(*S,S*)	0.8
	R-DNBPG-C	10.0	16.1(*R,R*)	17.8(*S,S*)	2.1
	S-DNBL-I	10.0	23.2(*R,R*)	24.5(*S,S*)	1.3
	S-DNBL-C	5.0	26.9	26.9	0.0
3-MC 11,12-DHD	*R*-DNBPG-I	5.0	32.8(*S,S*)	33.7(*R,R*)	0.6
11-O-methyl (1)		2.5	58.2(*S,S*)	63.6(*R,R*)	1.8
	R-DNBPG-C	5.0	27.6(*R,R*)	32.7(*S,S*)	3.4
	S-DNBL-I	5.0	15.2(*S,S*)	15.7(*R,R*)	0.5
	S-DNBL-C	5.0	11.1(*S,S*)	11.7(*R,R*)	1.0

TABLE III (*continued*)

*Chemical**	*CSP***	*A (%)****	*Retention time (min)*§		*RV*§§
			Enantiomer 1	*Enantiomer 2*	
3-MC 11,12-DHD	*R*-DNBPG-I	5.0	18.5(*R,R*)	22.4(*S,S*)	4.2
12-O-methyl (2)	*R*-DNBPG-C	5.0	17.7(*R,R*)	23.8(*S,S*)	6.1
	S-DNBL-I	5.0	9.9(*R,R*)	10.3(*S,S*)	1.0
	S-DNBL-C	5.0	11.5	11.5	0.0
		2.5	21.1	21.1	0.0

* O-Methyl ethers are designated as 1 and 2 according to their elution order on NP-HPLC. DHD = *trans*-dihydrodiol. Part of the data on CSP-HPLC resolutions of DHD enantiomers were reported earlier[1-10] and data shown in this table were updated from reanalysis.

** CSPs are defined in Materials and Methods.

*** Percentage of eluent A (ethanol–acetonitrile, 2:1, v/v) in hexane. The flow-rate was 2 ml/min, with a void volume of 2.4 ml.

§ See text for assignments of absolute configurations of resolved enantiomers. Enantiomers are designated as 1 and 2 according to their elution order on CSP-HPLC and have CD spectra that are mirror images of each other.

§§ 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 IV

CSP-HPLC RESOLUTION OF SOME NON-*K*-REGION *TRANS*-DIHYDRODIOLS AND THEIR METHYL ETHERS OF CHRYSENE, BENZ[*a*]ANTHRACENE, AND 7,12-DIMETHYLBENZ[*a*]-ANTHRACENE

*Chemical**	*CSP***	*A (%)****	*Retention time* (min)§		*RV*§§
			Enantiomer 1	*Enantiomer 2*	
CR 3,4-DHD	*R*-DNBPG-I	10.0	38.1	38.1	0
	R-DNBPG-C	10.0	35.2	35.2	0
	S-DNBL-I	10.0	40.6(*R,R*)	42.0(*S,S*)	0.7
	S-DNBL-C	10.0	21.8	21.8	0
CR 3,4-DHD	*R*-DNBPG-I	5.0	26.4(*S,S*)	28.9(*R,R*)	1.9
3-O-methyl (1)	*R*-DNBPG-C	5.0	25.1(*S,S*)	26.1(*R,R*)	1.0
	S-DNBL-I	5.0	15.1(*R,R*)	15.9(*S,S*)	1.2
	S-DNBL-C	5.0	14.3	14.4	<0.1
		2.5	28.7(*R,R*)	30.1(*S,S*)	0.9
CR 3,4-DHD	*R*-DNBPG-I	5.0	29.7(*R,R*)	31.8(*S,S*)	2.2
4-O-methyl (2)	*R*-DNBPG-C	5.0	27.3(*R,R*)	29.6(*S,S*)	2.2
	S-DNBL-I	5.0	20.5	20.5	0
	S-DNBL-C	5.0	20.2	20.2	0
CR 3,4-DHD	*R*-DNBPG-I	1.25	11.0(*R,R*)	11.6(*S,S*)	1.1
bis-O-methyl	*R*-DNBPG-C	1.25	12.7(*R,R*)	13.3(*S,S*)	1.0
	S-DNBL-I	1.25	4.9	4.9	0
		0.5	10.0(*R,R*)	11.2(*S,S*)	1.6
	S-DNBL-C	1.25	6.0	6.0	0
		0.5	8.8(*R,R*)	9.1(*S,S*)	0.6

(*continued on p. 392*)

TABLE IV (*continued*)

*Chemical**	*CSP***	*A (%)****	*Retention time* (min)§		*RV*§§
			Enantiomer 1	*Enantiomer 2*	
BA 3,4-DHD	*R*-DNBPG-I	10.0	31.6(*S,S*)	32.1(*R,R*)	0.2
	R-DNBPG-C	10.0	19.6	19.6	0
	S-DNBL-I	10.0	20.6(*R,R*)	21.0(*S,S*)	0.2
	S-DNBL-C	10.0	14.0	14.0	0
BA 3,4-DHD	*R*-DNBPG-I	2.5	28.1(*S,S*)	28.9(*R,R*)	1.0
3-O-methyl (1)	*R*-DNBPG-C	2.5	33.7(*S,S*)	34.6(*R,R*)	0.8
	S-DNBL-I	2.5	15.5	15.6	<0.1
	S-DNBL-C	1.0	28.5	28.5	0
BA 3,4-DHD	*R*-DNBPG-I	2.5	35.7	35.7	0
4-O-methyl (2)	*R*-DNBPG-C	2.5	33.6	33.6	0
	S-DNBL-I	2.5	20.2	20.2	0
	S-DNBL-C	2.5	17.7	17.7	0
BA 3,4-DHD	*R*-DNBPG-I	0.25	30.6	30.6	0
bis-O-methyl§§§	*R*-DNBPG-C	0.25	36.1	36.1	0
	S-DNBL-I	0.25	13.9	13.9	0
		0.1	20.4	20.6	0.2
	S-DNBL-C	0.25	15.7	16.3	0.3
		0.1	21.8	22.8	0.8
7,12-DMBA 3,4-DHD	*R*-DNBPG-I	10.0	23.2(*S,S*)	24.5(*R,R*)	1.1
	R-DNBPG-C	10.0	16.5(*S,S*)	16.8(*R,R*)	0.1
	S-DNBL-I	10.0	24.9	24.9	0
	S-DNBL-C	10.0	15.6	15.6	0
7,12-DMBA 3,4-DHD	*R*-DNBPG-I	2.5	25.9(*S,S*)	27.2(*R,R*)	1.4
3-O-methyl (1)	*R*-DNBPG-C	2.5	24.2(*S,S*)	25.5(*R,R*)	1.4
	S-DNBL-I	1.0	27.7(*S,S*)	28.8(*R,R*)	0.9
	S-DNBL-C	1.0	26.7(*S,S*)	27.4(*R,R*)	0.6
7,12-DMBA 3,4-DHD	*R*-DNBPG-I	2.5	34.5	34.5	0
4-O-methyl (2)	*R*-DNBPG-C	2.5	33.8(*S,S*)	34.6(*R,R*)	0.5
	S-DNBL-I	2.5	18.8(*S,S*)	19.0(*R,R*)	~0.1
		1.0	46.2	46.2	0
	S-DNBL-C	2.5	17.7	17.7	0
7,12-DMBA 3,4-DHD	*R*-DNBPG-I	1.0	10.2(*S,S*)	10.6(*R,R*)	0.7
bis-O-methyl	*R*-DNBPG-C	1.0	9.8(*S,S*)	10.2(*R,R*)	0.9
	S-DNBL-I	0.1	14.6(*R,R*)	14.9(*S,S*)	<0.1
	S-DNBL-C	0.1	19.0(*R,R*)	19.8(*S,S*)	0.6

* Monomethyl ethers are designated as 1 and 2 according to their elution order on normal-phase HPLC.

** CSPs are defined in Materials and methods.

*** Percentage of eluent A (ethanol–acetonitrile, 2:1, v/v) in hexane. The flow-rate was 2 ml/min, with a void volume of 2.4 ml.

§ See text for the assignments of absolute configurations of resolved enantiomers. Enantiomers are designated as 1 and 2 according to their elution order on CSP-HPLC and have CD spectra that are mirror images of each other.

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

§§§ Absolute configurations of resolved enantiomers were not established due to limited amounts of samples obtainable.

Enantiomers can be considered to have a baseline separation if the chromatographic peaks of separated enantiomers are both perfectly symmetrical and have a resolution value $\geqslant 1.0$. In practice, however, two compounds are said to show baseline separation when the resolution value is 1.5 or greater. Identities of resolved enantiomers were confirmed by UV–VIS absorption, CD, and mass spectral analyses[9–11,13–15].

Elution order of enantiomers

Enantiomers of 3-MC 11-O-methyl ether[10], 7,12-DMBA 5,6-DHD[2], 7,12-DMBA 5- and 6-O-methyl ethers have different elution orders on *R*-DNBPG-I and *R*-DNBPG-C. These observations are similar to the one reported earlier indicating that lacinilene C and lacinilene C methyl ether have reversed elution order of enantiomers on *R*-DNBPG-I[16]. Furthermore, enantiomers of 7,12-DMBA 5,6-DHD 5-O-methyl ether have different elution orders on ionically and covalently bonded *S*-DNBL columns; this is the only example so far indicating that elution orders of enantiomers are different on ionically and covalently bonded *S*-DNBL columns. The enantiomers of other compounds in Tables III and IV, if resolved on a particular CSP, were found to have the same elution order regardless whether the CSP was ionically or covalently bonded. The following compounds (their names are boxed-in in Figs. 2 and 3) have the same elution order of enantiomers on *R*-DNBPG and *S*-DNBL columns: PY 4,5-DHD 4-O-methyl, two isomeric 1-BrPY 4,5-DHD O-methyl ethers, 1-BrPY 9,10-DHD, CR 5,6-DHD, CR 5,6-DHD 5-O-methyl, 3-MC 11,12-DHD and both of its isomeric O-methyl ethers, BA 5,6-DHD 6-O-methyl, 1-MBA 5,6-DHD 6-O-methyl, 4-MBA 5,6-DHD and its 6-O-methyl ether, 6-MBA 5,6-DHD, 7-MBA 5,6-DHD and both of its isomeric O-methyl ethers, 3-MC 11,12-DHD and both of its isomeric O-methyl ethers, 7,12-DMBA 5,6-DHD and both of its isomeric O-methyl ethers, BaP 4,5-DHD 5-O-methyl, CR 3,4-DHD bis-O-methyl, and 7,12-DMBA 3- and 4-O-methyl ethers.

Resolution of enantiomers

Enantiomeric pairs of 4 dihydrodiols (PA 9,10-DHD, 1-MPA 9,10-DHD, 4,5-MPA 9,10-DHD, and BA 5,6-DHD) are not resolved on any of the four CSP columns (Table III). Except for the unresolvable enantiomeric pairs of 1-MPA 9,10-DHD 10-O-methyl (Table III) and BA 3,4-DHD 4-O-methyl (Table IV, the enantiomer pairs of other O-methyl derivatives are resolved on two or all four CSP columns with resolution values as high as 2.5 (Tables III and IV). The enantiomers of other dihydrodiol O-methyl ethers, are all more efficiently resolved on one or more CSP columns than those of underivatized dihydrodiols (Tables III and IV). By successively decreasing the percentage of eluent A in hexane, separations of enantiomeric pairs of some O-methyl ethers which are not resolved at higher eluent polarity become apparent. Examples of these are: 4,5-MPA 9,10-DHD 9-O-methyl, PY 4,5-DHD 4-O-methyl, 1-BrPY 9,10-DHD, 12-MBA 5,6-DHD 5-O-methyl, CR 3,4-DHD 3-O-methyl, and CR 3,4-DHD bis-O-methyl (Tables III and IV). Decrease in the polarity of the eluent apparently enhanced the chiral interactions between the CSP and solutes, permitting separation of enantiomers.

Elution order/absolute configuration relationship

There are no definitive rules that govern the elution order/absolute configuration of enantiomers. The following observations are summarized for those dihydrodiols and O-methyl ethers the enantiomers of which can be resolved:

(a) The *S,S*-enantiomers of dihydrodiols and O-methyl ethers of PY and 1-BrPY are more strongly retained by both ionically and covalently bonded *R*-DNBPG.

(b) Except that the *S,S*-enantiomers of quasidiaxial 1-BrPY 9,10-DHD, 4-MBA 5,6-DHD, 7-MBA 5,6-DHD, and 3-MC 11,12-DHD are more strongly retained by the *R*-DNBPG columns, the *R,R*-enantiomers of other dihydrodiols in Tables III and IV are more strongly retained. In contrast, the *R,R*-enantiomer of the quasidiaxial 7,12-DMBA 5,6-DHD is more strongly retained on *R*-DNBPG[2].

(c) There are no definitive rules on the elution order/absolute configuration relationship among the O-methyl ethers (isomer 1), which are less strongly retained in normal-phase HPLC on a silica (SIL) column.

(d) The *S,S*-enantiomers of most O-methyl ethers (isomers 2 in normal-phase HPLC) derived from *K*-region dihydrodiols are more strongly retained on *R*-DNBPG (Figs. 2 and 3, Tables III and IV). However, the *R,R*-enantiomers of O-methyl ethers 2, derived from BcPA 5,6-DHD, 4-MBA 5,6-DHD, 12-MBA 5,6-DHD, and 7,12-DMBA 5,6-DHD, are more strongly retained on *R*-DNBPG.

(e) Elution orders of enantiomers on *R*-DNBPG are not always reversed on *S*-DNBL. Those enantiomeric pairs that have the same elution order on *R*-DNBPG and *S*-DNBL are indicated with their names boxed-in in Figs. 2 and 3.

CONCLUSION

Derivatization of dihydrodiols of PAHs to O-methyl ethers generally improves enantiomeric separation on one or more kinds of Pirkle's CSPs. However, general rules are not apparent that can be used to predict the relationship between elution order and absolute configuration of resolved enantiomers. Nonetheless, the findings that dihydrodiol enantiomeric pairs can be separated either directly or following conversion to O-methyl ethers are very useful and have been applied with considerable success in the understanding of the detailed stereoselective pathways of metabolism of PAHs catalyzed by drug-metabolizing enzyme systems[1,5,7,8,11,12,18].

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