Direct Enantiomeric Resolution of Some 7,12-Dimethylbenz[a]anthracene Derivatives by High-Performance Liquid Chromatography with Ionically and Covalently Bonded Chiral Stationary Phases

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The direct resolution of the enantiomers of *trans*-3,4-dihydrodiol, *trans*-5,6-dihydrodiol, *cis*-5,6-dihydrodiol, *trans*-8,9-dihydrodiol, and 8,9,10,11-tetrahydro-*trans*-8,9-diol of 7,12-dimethylbenz[*a*]anthracene was evaluated by high-performance liquid chromatography using commercially available columns packed with (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine and (*S*)-*N*-(3,5-dinitrobenzoyl)leucine either ionically or covalently bonded to γ-aminopropylsilanized silica. The enantiomers of all diol derivatives were resolved by one or more, but not all, of the chiral stationary phases tested. Resolution of enantiomers was confirmed by ultraviolet–visible absorption, mass, and circular dichroism spectral analyses.

7,12-Dimethylbenz[a]anthracene (DMBA) is a potent carcinogen (*1*) and is stereoselectively metabolized by mammalian drug-metabolizing enzyme systems to optically active products (*2–4*). DMBA derivatives vary substantially in their mutagenic and carcinogenic activities (*5–8*). Enantiomeric epoxides and dihydrodiols as well as diastereomeric dihydrodiol-epoxides of some polycyclic aromatic hydrocarbons (PAHs) have been shown to possess different biological activities (*9*). The dihydrodiol enantiomers of some PAHs have been resolved by HPLC as diastereomers (*10, 11*). Kim et al. described the HPLC resolution of some dihydrodiol enantiomers of benzo[a]pyrene (BaP) and benz[a]anthracene (BA) by a covalently bonded CSP (*12*). Recently, a high-performance liquid chromatographic (HPLC) method using the chiral stationary phase (CSP) (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine ionically bonded to γ-aminopropylsilanized silica was developed to resolve some dihydrodiol and tetrahydrodiol enantiomers of BA and BaP (*13*) as well as certain cyclic alcohol derivatives of many different PAH structures (*14*). This method has been applied to determine the optical purity and absolute configuration of dihydrodiols and cyclic alcohols formed in the metabolism of several PAHs by hepatic microsomal enzymes (*4, 13–16*).

Leaching of ionically bonded CSP occurs when a polar solvent is used as the mobile phase. The resolving power of an HPLC column with ionically bonded CSP column rapidly deteriorates if solute elution requires relatively high solvent polarity. Further measurement of UV–vis absorption and circular dichroism spectra of the resolved enantiomers requires removal of the leached CSP by other chromatographic methods. The disadvantages of the ionically bonded CSP may be overcome by using covalently bonded CSP. However, covalently bonded CSP may not necessarily interact sufficiently with the enantiomers to effect resolution if the ionic bonding of the ionically bonded CSP plays a role in the chiral recognitions between the CSP and the enantiomers.

In this study, the resolution of some diol enantiomers of DMBA is compared by using ionically and covalently bonded CSP (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine orginally developed by Pirkle and associates (*17*). The resolution of diol enantiomers by ionically and covalently bonded CSP (*S*)-*N*-(3,5-dinitrobenzoyl)leucine (*18*) is also described.

EXPERIMENTAL SECTION

Materials. Racemic DMBA *trans*-3,4-dihydrodiol was prepared in low yield from DMBA 3,4-dione as described (*19*) and was purified by the combined uses of normal-phase and reversed-phase HPLC (*3*). DMBA 3,4-dione was generously provided by Melvin Newman of The Ohio State University, Columbus, OH. DMBA *trans*-5,6-dihydrodiol was prepared by incubation of DMBA 5,6-dihydroepoxide in 0.1 M Tris–HCl buffer (pH 8.9) with liver microsomes from phenobarbital-treated male Sprague-Dawley rats in the absence of NADPH. Racemic DMBA *trans*-8,9-dihydrodiol and DMBA 8,9,10,11-tetrahydro-*trans*-8,9-diol were obtained as described (*3*). DMBA 8,9,10,11-tetrahydro-(8*S*,9*S*)-diol was prepared by catalytic hydrogenation (tetrahydrofuran, PtO_2/H_2, 30 min) of DMBA (8*S*,9*S*)-dihydrodiol. DMBA *cis*-5,6-dihydrodiol and DMBA 5,6-dihydroepoxide were obtained from the Chemical Repository of the National Cancer Institute. DMBA *trans*-10,11-dihydrodiol was purified by normal-phase and reversed-phase HPLC from a metabolite mixture obtained by incubation of DMBA with liver microsomes from phenobarbital-treated rats (*3*).

Chromatography. Chemicals were analyzed with HPLC columns (25 cm × 4.6 mm i.d., Regis Chemical, Morton Grove, IL) packed with an (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine ionically bonded ((*R*)-DNBPG-I, serial no. 600158) or covalently bonded ((*R*)-DNBPG-C, serial no. 440407) and ionically bonded (*S*)-*N*-(3,5-dinitrobenzoyl)leucine ((*S*)-DNBLeu-I, serial no. 700225) to spherical particles of 5 μm diameter of γ-aminopropylsilanized silica (*17, 18*). A column (25 cm × 4.6 mm i.d.) packed with an (*S*)-*N*-(3,5-dinitrobenzoyl)leucine covalently bonded to γ-aminopropylsilanized silica ((*S*)-DNBLeu-C, serial no. 332133-1) was purchased from J. T. Baker (Phillipsburg, NJ). HPLC was performed with a Waters Associates (Milford, MA) liquid chromatograph consisting of a Model 6000A solvent delivery system, a Model M45 solvent delivery system, a Model 660 solvent programmer, and a Model 440 absorbance detector. Samples were injected via a Valco Model N60 loop injector (Valco, Houston, TX). Ratios of enantiomers were determined by areas under the peak with a Hewlett-Packard Model 3390A integrator. Separation of diols was achieved isocratically with a flow rate of 2 mL/min using premixed solvents of up to 15% (v/v) of solvent A (ethanol:acetonitrile, 2:1, v/v) in hexane at ambient temperature. Optically pure enantiomers were obtained by repetitive chromatography. Solvent was removed from the resolved enantiomers by evaporation under nitrogen. CSP leached from the ionically bonded CSP column into the resolved enantiomers was removed prior to circular dichroism (CD) spectral measurement by reversed-phase HPLC with a Du Pont Zorbax ODS column as described previously (*13*).

Spectral Analysis. Ultraviolet–visible absorption spectra of samples in methanol were determined by 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 by using a Jasco Model 500A spectropolarimeter equipped with a Model DP-500 data

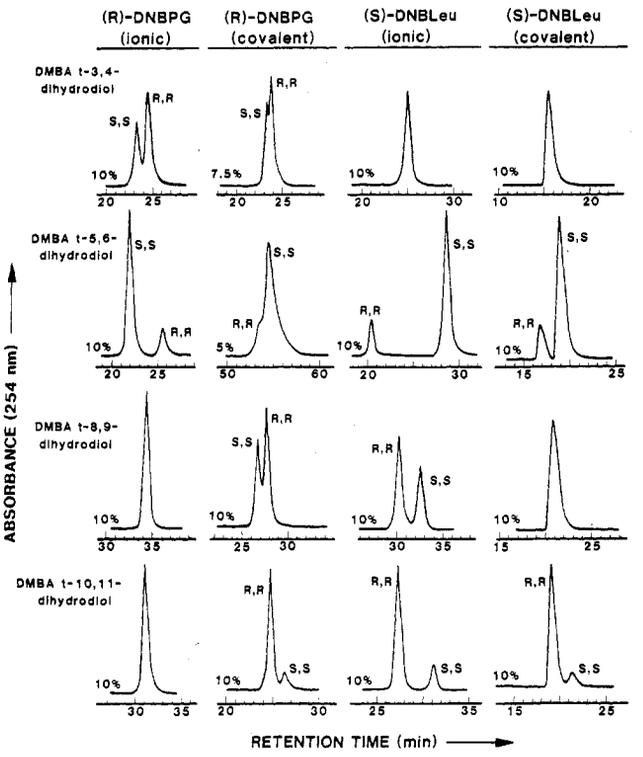

Figure 1. Resolutions of DMBA *trans*-dihydrodiol enantiomers by ionically and covalently bonded chiral stationary phases. Abbreviations of the CSPs are described in Materials and Methods. The percentages of solvent A (ethanol/acetonitrile, 2:1, v/v) in hexane used in chromatography are indicated in the chromatograms. The solvent flow rate was 2 mL/min. Different peak heights of resolved enantiomers are shown to indicate elution orders of the resolved enantiomers by different CSPs. See text for the assignments of the absolute configuration of the other resolved enantiomers.

processor. The concentration of the sample is indicated by A_λ/mL (number of absorbance units at λ_{max} per mL of methanol). CD spectra are expressed by ellipticity (in millidegrees) for methanol solutions that have an absorbance of 1.0 unit at the wavelength of maximal absorption (*15*).

RESULTS AND DISCUSSION

The resolutions of the enantiomers of DMBA *trans*-3,4-*trans*-5,6-, *trans*-8,9-, and *trans*-10,11-dihydrodiols by the ionically and covalently bonded (*R*)-DNBPG and (*S*)-DNBLeu are shown in Figure 1. The retention times and resolution values of these diols and those of DMBA 8,9,10,11-tetrahydro-*trans*-8,9-diol and DMBA *cis*-5,6-dihydrodiol are listed in Table I. The structures of the resolved enantiomers were confirmed by UV–vis absorption and mass spectral analyses.

The CD spectrum of the optically pure DMBA *trans*-3,4-dihydrodiol enantiomer, which was less strongly retained by the ionically bonded (*R*)-DNBPG, is shown in Figure 2. Due to the presence of the 7- and 12-methyl groups, the UV–vis absorption peaks of DMBA *trans*-3,4-dihydrodiol are shifted toward longer wavelengths relative to those of BA *trans*-3,4-dihydrodiol (*2*). Consequently the CD Cotton effects of DMBA *trans*-3,4-dihydrodiol enantiomer are also shifted toward longer wavelengths (Figure 2). The hydroxyl groups of both DMBA *trans*-3,4-dihydrodiol (NMR coupling constant $J_{3,4}$ = 11.9 Hz) (*2*) and BA *trans*-3,4-dihydrodiol (NMR coupling constant $J_{3,4}$ = 11.5 Hz) (*20*) are preferentially in quasi-diequatorial conformations. Because of their quasi-diequatorial conformations, the CD spectra of the (3*S*,4*S*)-dihydrodiols of both BA and DMBA are expected to have similar characteristics (*4*, *15*, *16*). As indicated in Figure 2, except the differences of CD Cotton effects between 280 nm and 310 nm, the characteristic CD Cotton effects of the two enantiomers are similar. On the basis of its CD Cotton effects, the

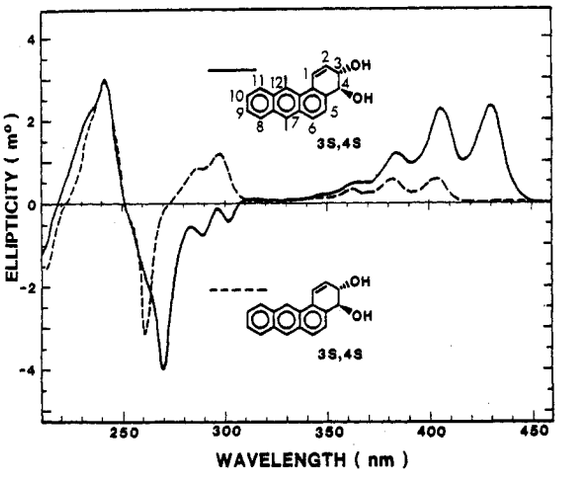

Figure 2. CD spectra of the optically pure DMBA *trans*-3,4-dihydrodiol enantiomer less strongly retained by the ionically bonded (*R*)-DNBPG (—, concentration 1.0 A_{271}/mL) and an optically pure BA (3*S*,4*S*)-dihydrodiol; (- - -, concentration 0.9 A_{261}/mL). BA (3*S*,4*S*)-dihydrodiol was obtained as described previously (*25*).

DMBA *trans*-3,4-dihydrodiol less strongly retained by the ionically bonded (*R*)-DNBPG is deduced to have 3*S*,4*S* absolute configuration.

The *R*,*R* enantiomer of DMBA *trans*-3,4-dihydrodiol is more strongly retained by both the ionically and covalently bonded (*R*)-DNBPG (Figure 1). However, the covalently bonded (*R*)-DNBPG is less efficient in resolving the enantiomers. Neither the ionically nor the covalently bonded (*S*)-DNBLeu resolved the enantiomers of DMBA *trans*-3,4-dihydrodiol.

The absolute configuration of DMBA *trans*-5,6-dihydrodiol enantiomers has been elucidated by the exciton chirality method (*4*). Both ionically and covalently bonded (*R*)-DNBPG and (*S*)-DNBLeu resolved the enantiomers of DMBA *trans*-5,6-dihydrodiol (Figure 1). Surprisingly, the elution order of the enantiomers on the covalently bonded (*R*)-DNBPG was reversed from that on the ionically bonded (*R*)-DNBPG. Similar to that found for DMBA *trans*-3,4-dihydrodiol, the covalently bonded (*R*)-DNBPG was less efficient than the ionically bonded (*R*)-DNBPG in resolving the enantiomers of DMBA *trans*-5,6-dihydrodiol. The 5*S*,6*S* enantiomer of DMBA *trans*-5,6-dihydrodiol was more strongly retained by both the ionically and covalently bonded (*S*)-DNBLeu but was less strongly retained on the (*R*)-DNBPG (Figure 1). The enantiomers of DMBA *trans*-5,6-dihydrodiol were more efficiently resolved by the ionically bonded (*S*)-DNBLeu (Figure 1 and Table I).

The enantiomers of DMBA *cis*-5,6-dihydrodiol were resolved by three out of the four CSPs tested (Table I). The elution orders of the resolved enantiomers were the same on all three CSPs. The CD spectrum of the enantiomer less strongly retained by the CSP is similar to that of DMBA *trans*-5*S*,6*S*-dihydrodiol (Figure 3). Due to steric hindrance of the 7-methyl group, the hydroxyl groups at C_5 and C_6 of DMBA *cis*-5,6-dihydrodiol are in quasi-equatorial and quasi-axial positions, respectively (*21*). Both of the hydroxyl groups of DMBA *trans*-5,6-dihydrodiol are preferentially in quasi-axial positions (*4*). Thus a common structural feature exists between the *trans*- and *cis*-5,6-dihydrodiols of DMBA; the C_6-hydroxyl groups of both diols are quasi-axial. Since the less strongly retained enantiomer of DMBA *cis*-5,6-dihydrodiol (Figure 1) has similar CD Cotton effects to the DMBA *trans*-5*S*,6*S*-dihydrodiol (Figure 3), the C_6-hydroxyl group of the less strongly retained enantiomer of DMBA *cis*-5,6-dihydrodiol must also have *S* configuration. The less strongly retained enantiomer of DMBA *cis*-5,6-dihydrodiol

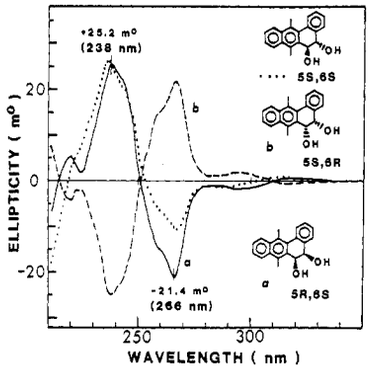

Figure 3. CD spectra of the resolved enantiomers of DMBA *cis*-5,6-dihydrodiol (--- and —, concentration 1.0 A_{268}/mL) and of the optically pure DMBA *trans*-(5*S*,6*S*)-dihydrodiol less strongly retained by the (*R*)-DNBPG-I (···, concentration 1.0 A_{269}/mL). The enantiomers were resolved as described in Figure 1. The enantiomers of DMBA *cis*-5,6-dihydrodiol are also designated as a and b as indicated in Table I.

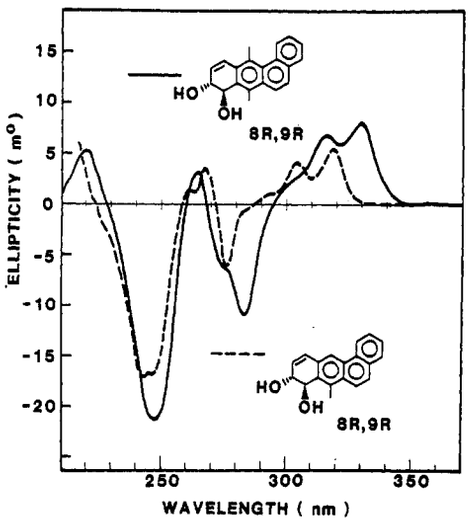

Figure 4. CD spectra of the optically pure DMBA *trans*-8,9-dihydrodiol enantiomer less strongly retained by the ionically bonded (*S*)-DNBLeu (—, concentration 1.0 A_{268}/mL) and 7-MBA (8*R*,9*R*)-dihydrodiol (---, concentration 1.0 A_{266}/mL) formed from 7-MBA metabolism by liver microsomes of 3-methylcholanthrene-treated rats (*24*).

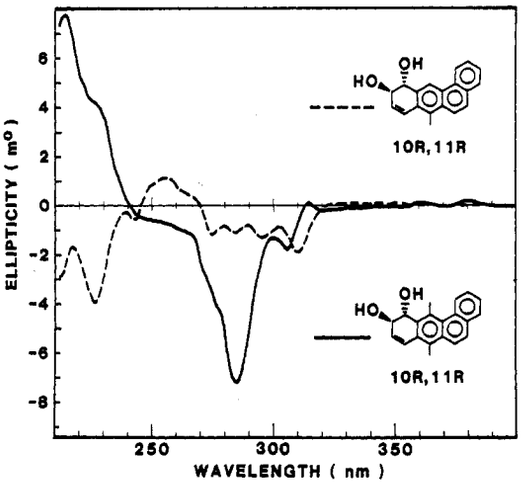

Figure 5. CD spectra of the optically pure DMBA *trans*-10,11-dihydrodiol enantiomer less strongly retained by the covalently bonded (*R*)-DNBPG (—, concentration 1.0 A_{278}/mL) and 7-MBA (10*R*,11*R*)-dihydrodiol (---, concentration 1.0 A_{276}/mL) formed from 7-MBA metabolism by liver microsomes of 3-methylcholanthrene-treated rats (*24*).

by both the (*R*)-CSP and the (*S*)-CSP is therefore deduced to have 5*R*,6*S* absolute configuration.

The enantiomers of DMBA *trans*-8,9-dihydrodiol and 8,9,10,11-tetrahydro-*trans*-8,9-diol were not resolved by the ionically bonded (*R*)-DNBPG but were resolved by the covalently bonded (*R*)-DNBPG (Figure 1 and Table I). Conversely, the ionically bonded (*S*)-DNBLeu resolved the enantiomers, while the covalently bonded (*S*)-DNBLeu did not. The enantiomers were more efficiently resolved by the ionically bonded (*S*)-DNBLeu than by the covalently bonded (*R*)-DNBPG. The elution order of the resolved enantiomers on the covalently bonded (*R*)-DNBPG was reversed from that on the ionically bonded (*S*)-DNBLeu (Figure 1).). Due to steric hindrance of the 7-methyl group, the *trans*-8,9-dihydrodiols of both DMBA and 7-MBA preferentially adopt quasi-diaxial conformations (*22*–*24*). The CD spectrum of the less strongly retained enantiomer of DMBA *trans*-8,9-dihydrodiol by the ionically bonded (*S*)-DNBLeu is, except the bathochromic shifts of some CD Cotton effects due to the presence of the 12-methyl group, similar to that of 7-MBA 8*R*,9*R*-dihydrodiol (Figure 4). On the basis of its characteristic CD Cotton effects, the less strongly retained enantiomer of DMBA *trans*-8,9-dihydrodiol by the ionically bonded (*S*)-DNBLeu is deduced to have 8*R*,9*R* absolute configuration. The elution orders of DMBA 8,9,10,11-tetrahydro-*trans*-8,9-diol enantiomers were established by chromatography of DMBA 8,9,10,11-tetrahydro-(8*S*,9*S*)-diol derived from DMBA (8*S*,9*S*)-dihydrodiol by catalytic hydrogenation.

The enantiomers of DMBA *trans*-10,11-dihydrodiol were not resolved by the ionically bonded (*R*)-DNBPG but were resolved by the other three CSPs tested and the more strongly retained enantiomer is the same in each case (Figure 1). Like those of DMBA *trans*-8,9-dihydrodiol, the enantiomers of DMBA *trans*-10,11-dihydrodiol were also more efficiently resolved by the ionically bonded (*S*)-DNBLeu. Due to steric hindrance by the 12-methyl group, the hydroxyl groups of DMBA *trans*-10,11-dihydrodiol preferentially adopt quasi-diaxial conformations (*22*). The CD spectrum of the less retained enantiomer of DMBA *trans*-10,11-dihydrodiol is different from that of the quasi-diequatorial 7-MBA (10*R*,11*R*)-dihydrodiol (*24*) (Figure 5). The CD Cotton effects of the two *trans*-10,11-dihydrodiols between 220 nm and 240 nm are opposite in sign (Figure 5) which are characteristic of dihydrodiols that have the same absolute configurations but with different conformations (*4*, *15*, *16*, *24*). If the conformation of the DMBA *trans*-10,11-dihydrodiol were quasi-diequatorial, the CD spectrum is expected to be either similar to or mirror image of that of the quasi-diequatorial 7-MBA (10*R*,10*R*)-dihydrodiol (*15*, *16*). For example, the CD Cotton effects of the quasi-diequatorial BA (10*R*,11*R*)-dihydrodiol are closely similar to those of the quasi-diequatorial 7-MBA (10*R*,11*R*)-dihydrodiol (*24*, *25*). Based on these considerations, we concluded that the differences of the two CD spectra shown in Figure 5 were mainly due to differences in the conformations of their hydroxyl groups, not due to their absolute configurations. The major enantiomer of DMBA *trans*-10,11-dihydrodiol formed from the metabolism of DMBA by liver microsomes from phenobarbital-treated rats is therefore deduced to have *R*,*R* absolute configuration and is less strongly retained by three of the four CSPs tested (Figure 1).

The enantiomers of each of the six DMBA diol derivatives can be resolved by at least two of the four CSPs tested. The efficiency of resolution, however, varies greatly. Among the four kinds of CSPs tested, only covalently bonded (*R*)-DNBPG resolves all six DMBA diol derivatives. In general, when an enantiomeric pair is resolved, the resolution achieved with the covalently bonded (*R*)-DNBPG is poorer than that obtained with the other CSPs (Table I). A rather surprising finding in this study is that the DMBA *trans*-(5*S*,6*S*)-dihydrodiol is

Table I. CSP-HPLC Resolution of Some Diol Enantiomers of 7,12-Dimethylbenz[*a*]anthracene (DMBA)[a]

chemical	CSP	% solv A[b]	retention time[c] peak a	retention time[c] peak b	*R* value[d]
DMBA *trans*-3,4-dihydrodiol	(*R*)-DNBPG-I	15	14.1 (3*S*,4*S*)	14.8 (3*R*,4*R*)	0.9
		10	23.2 (3*S*,4*S*)	24.5 (3*R*,4*R*)	1.1
	(*R*)-DNBPG-C	10	16.5 (3*S*,4*S*)	16.8 (3*R*,4*R*)	0.1
		7.5	23.1 (3*S*,4*S*)	23.6 (3*R*,4*R*)	0.4
	(*S*)-DNBLeu-I	10	24.9	24.9	0
	(*S*)-DNBLeu-C	15	10.5	10.5	0
		10	15.6	15.6	0
DMBA *trans*-5,6-dihydrodiol	(*R*)-DNBPG-I	15	12.2 (5*S*,6*S*)	13.8 (5*R*,6*R*)	2.4
		10	21.8 (5*S*,6*S*)	25.4 (5*R*,6*R*)	2.6
	(*R*)-DNBPG-C	15	11.4 (5*R*,6*R*)	11.7 (5*S*,6*S*)	0.2
		10	20.3 (5*R*,6*R*)	20.8 (5*S*,6*S*)	0.5
		5	53.3 (5*R*,6*R*)	54.8 (5*S*,6*S*)	0.7
	(*S*)-DNBLeu-I	15	12.2 (5*R*,6*R*)	16.3 (5*S*,6*S*)	5.8
		10	20.6 (5*R*,6*R*)	28.6 (5*S*,6*S*)	6.9
	(*S*)-DNBLeu-C	15	9.7 (5*R*,6*R*)	10.8 (5*S*,6*S*)	1.2
		10	16.8 (5*R*,6*R*)	18.9 (5*S*,6*S*)	1.3
DMBA *cis*-5,6-dihydrodiol	(*R*)-DNBPG-I	15	9.4 (5*R*,6*S*)	9.8 (5*S*,6*R*)	0.7
		10	15.2 (5*R*,6*S*)	16.0 (5*S*,6*R*)	1.0
	(*R*)-DNBPG-C	15	7.4 (5*R*,6*S*)	7.7 (5*S*,6*R*)	0.8
		10	10.9 (5*R*,6*S*)	11.6 (5*S*,6*R*)	1.3
		5	14.9 (5*R*,6*S*)	15.9 (5*S*,6*R*)	1.4
	(*S*)-DNBLeu-I	15	10.6 (5*R*,6*S*)	11.4 (5*S*,6*R*)	1.4
		10	16.2 (5*R*,6*S*)	17.7 (5*S*,6*R*)	1.9
	(*S*)-DNBLeu-C	15	6.6	6.6	0
		10	9.8	9.8	0
DMBA *trans*-8,9-dihydrodiol	(*R*)-DNBPG-I	10	34.4	34.4	0
	(*R*)-DNBPG-C	10	26.7 (8*S*,9*S*)	27.7 (8*R*,9*R*)	0.8
		5	43.6 (8*S*,9*S*)	45.4 (8*R*,9*R*)	0.9
	(*S*)-DNBLeu-I	15	16.3 (8*R*,9*R*)	17.6 (8*S*,9*S*)	1.5
		10	30.1 (8*R*,9*R*)	32.3 (8*S*,9*S*)	1.6
	(*S*)-DNBLeu-C	15	12.3	12.3	0
		10	20.9	20.9	0
DMBA 8,9,10,11-tetrahydro-*trans*-8,9-diol	(*R*)-DNBPG-I	10	23.7	23.7	0
	(*R*)-DNBPG-C	10	19.4 (8*S*,9*S*)	19.8 (8*R*,9*R*)	0.3
		7.5	30.0 (8*S*,9*S*)	30.9 (8*R*,9*R*)	0.6
	(*S*)-DNBLeu-I	10	22.1 (8*R*,9*R*)	22.5 (8*S*,9*S*)	0.4
	(*S*)-DNBLeu-C	15	9.9	9.9	0
		10	15.7	15.7	0
DMBA *trans*-10,11-dihydrodiol	(*R*)-DNBPG-I	10	31.0	31.0	0
	(*R*)-DNBPG-C	10	24.8 (10*R*,11*R*)	26.3 (10*S*,11*S*)	1.3
	(*S*)-DNBLeu-I	15	14.9 (10*R*,11*R*)	16.9 (10*S*,11*S*)	2.7
		10	27.2 (10*R*,11*R*)	31.2 (10*S*,11*S*)	2.9
	(*S*)-DNBLeu-C	15	11.5 (10*R*,11*R*)	11.9 (10*S*,11*S*)	0.3
		10	19.0 (10*R*,11*R*)	21.3 (10*S*,11*S*)	1.1

[a] Columns (25 cm × 4.6 mm i.d.) were packed with either (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine ((*R*)-DNBPG) or (*S*)-*N*-(3,5-dinitrobenzoyl)leucine ((*S*)-DNBLeu) which were either ionically (I) or covalently (C) bonded to γ-aminopropylsilanized silica. [b] Percentage of solvent A (ethanol/acetonitrile, 2:1, v/v) in hexane. The flow rate was 2 mL/min. [c] See text for the assignments of the absolute configurations of the resolved enantiomers. The enantiomers are also designated as a and b according to the orders of elution. [d] *R* (resolution) = $(V_2 - V_1)/[1/2(W_2 + W_1)]$, where *V* is retention volume and *W* is peak width at base. The void time was 1.2 min.

more strongly retained by the covalently bonded (*R*)-DNBPG whereas it is less strongly retained by the ionically bonded (*R*)-DNBPG.

Among the enantiomers of the PAH *trans*-dihydrodiols and tetrahydrodiols of known absolute configurations that are resolved by the ionically bonded (*R*)-DNBPG, the *R,R* enantiomers are all more strongly retained (Table I and ref 12). However, no rule is apparent regarding the chiral recognition mechanisms between the DMBA diols and the covalently bonded (*R*)-DNBPG. In contrast, the *S,S* enantiomers of PAH *trans*-diols appear to be the more strongly retained by both the ionically and the covalently bonded (*S*)-DNBLeu (Figure 1). It remains to be established if a general rule can emerge by investigating the enantiomeric resolution of a large number of PAH diols. Only when the exact chiral recognition mechanisms between the CSP and the solute are understood, will it then be possible to correctly predict the enantiomer that interacts more strongly with a particular CSP. It is also important to understand the structural factors which render some PAH diols unable to be resolved by the CSPs employed.

It should be noted that the actual structure of the enantiomeric PAH dihydrodiols and tetrahydrodiols, rather than the *R* and *S* designations, is important in considering the chiral recognitions between the CSP and the solute. For example, if the 10-hydroxyl group of DMBA (10*R*,11*R*)-dihydrodiol were replaced by a hydrogen, the 11-hydroxyl group would be designated by *S* rather than by *R*.

Registry No. (*R*)-DNBPG, 74927-72-3; (*S*)-DNBLeu, 7495-01-4; DMBA *trans*-3,4-dihydrodiol racemate, 74938-86-6; DMBA *trans*-3,4-dihydro-(3*S*,4*S*)-diol, 92693-62-4; DMBA *trans*-3,4-dihydro-(3*R*,4*R*)-diol, 92693-63-5; DMBA *trans*-5,6-dihydrodiol racemate, 75262-88-3; DMBA *trans*-5,6-dihydro-(5*S*,6*S*)-diol, 92693-64-6; DMBA *trans*-5,6-dihydro-(5*R*,6*R*)-diol, 92693-65-7; DMBA *cis*-5,6-dihydrodiol racemate, 64265-59-4; DMBA *cis*-5,6-dihydro-(5*R*,6*R*)-diol, 92693-66-8; DMBA *cis*-5,6-dihydro-(5*S*,6*S*)-diol, 92693-67-9; DMBA *trans*-8,9-dihydrodiol racemate, 74938-87-7; DMBA *trans*-8,9-dihydro-(8*S*,9*S*)-diol, 92693-68-0; DMBA *trans*-8,9-dihydro-(8*R*,9*R*)-diol, 92693-69-1; DMBA 8,9,10,11-tetrahydro-*trans*-8,9-diol racemate, 92622-78-1; DMBA 8,9,10,11-tetrahydro-*trans*-(8*S*,9*S*)-diol, 92693-70-4; DMBA 8,9,10,11-tetrahydro-*trans*-(8*R*,9*R*)-diol, 92693-71-5; DMBA

trans-10,11-dihydrodiol racemate, 92622-79-2; DMBA *trans*-10,11-dihydro-(10*R*,11*R*)-diol, 92693-72-6; DMBA *trans*-10,11-dihydro-(10*S*,11*S*)-diol, 92693-73-7; *trans*-benz[*a*]anthracene 3,4-dihydro-(3*S*,4*S*)-diol, 67335-43-7; *trans*-7-methylbenz[*a*]-anthracene 8,9-dihydro-(8*R*,9*R*)-diol, 88244-40-0.

LITERATURE CITED

(1) Dipple, D. In "Chemical Carcinogens"; Searle, C. E., Ed.; American Chemical Society: Washington, DC, 1982; ACS Monograph No. 173 pp 245-314.
(2) Yang, S. K.; Chou, M. W.; Roller, P. P. *J. Am. Chem. Soc.* **1979**, *101*, 237-239.
(3) Chou, M. W.; Yang, S. K. *J. Chromatogr.* **1979**, *185*, 635-654.
(4) Yang, S. K.; Fu, P. P. *Biochem J.* **1984**, *233*, 775-782.
(5) Malaveille, C.; Bartsch, H.; Tierney, B.; Grover, P. L.; Sims, P. *Biochem. Biophys. Res. Commun.* **1977**, *83*, 1468-1473.
(6) Slaga, T. J.; Gleason, G. C.; DiGiovanni, J. D.; Sukumaran, K. B; Harvey, R. G. *Cancer Res.* **1979**, *39*, 1934-1936.
(7) Huberman, E.; Chou, M. W.; Yang, S. K. *Proc. Natl. Acad. Sci. U. S.A.* **1979**, *76*, 862-866.
(8) Wislocki, P. G.; Juliana, M. M.; MacDonald, J. S; Chou, M. W.; Yang, S. K.; Lu, A. Y. H. *Carcinogenesis* **1981**, *2*, 511-514.
(9) Conney, A. H. *Cancer Res.* **1982**, *42*, 4875-4917.
(10) Yang, S. K.; Gelboin, H. V.; Weber, J. D.; Sankaran, V.; Fisher, D. L.; Engel, J. F. *Anal. Biochem.* **1977**, *78*, 520-526.
(11) Thakker, D. R.; Levin, W.; Yagi, H.; Turujman, S.; Kapadia, D.; Conney, A. H.; Jerina, D. M. *Chem.-Biol. Interact.* **1979**, *278* 145-161.
(12) Kim Y. H.; Tishbee, A.; Gil-Av, E. *J. Chem. Soc. Chem. Commun.* **1981**, 75-76.
(13) Weems, H. B.; Yang, S. K. *Anal. Biochem.* **1982**, *125*, 156-161.
(14) Yang, S. K.; Li, X. C. *J. Chromatogr.* **1984**, *291*, 265-273.
(15) Fu, P. P.; Yang, S. K. *Biochem. Biophys. Res. Commun.* **1982**, *109*, 927-934.
(16) Fu, P. P.; Yang, S. K. *Carcinogensis* **1983**, *4*, 979-984.
(17) Pirkle, W. H.; House, D. W.; Finn, J. M. *J. Chromatogr.* **1980**, *192*, 143-158.
(18) Pirkle, W. H.; Finn, J. M. *J. Org. Chem.* **1981**, *46*, 2935-2938.
(19) Sukumaran, K. B.; Harvey, R. G. *J. Am. Chem. Soc.* **1979**, *101*, 1353-1354.
(20) Lehr, R. E.; Schaefer-Ridder, M.; Jerina, D. M. *J. Org. Chem.* **1977**, *42*, 736-744.
(21) Zacharias, D. E.; Glusker, J. P.; Fu, P. P.; Harvey, R. G. *Cancer Res.* **1975**, *37*, 775-782.
(22) Tierney, B.; Hewer, A.; MacNicoll, A. D.; Gervasi, P. G; Rattle, H.; Walsh, C.; Grover, P. L.; Sims, P. *Chem.-Biol. Interact.* **1978**, *23*, 243-257.
(23) Tierney, B.; Abercrombie, B.; Walsh, C.; Hewer, A.; Grover, P. L.; Sims, P. *Chem.-Biol. Interact.* **1978**, *21* 289-298.
(24) Yang, S. K.; Fu, P. P. *Chem.-Biol. Interact.* **1984**, *49*, 71-88.
(25) Yang, S. K. *Drug Metab. Dispos.* **1982**, *10*, 205-211.

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Separation Strategy of Multicomponent Mixtures by Liquid Chromatography with a Single Stationary Phase and a Limited Number of Mobile Phase Solvents

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A methodology for the development of separation procedures for different groups of substances (mostly drugs) is described. Chromatography is performed on a cyanopropyl column with a limited number of mobile phase solvents to carry out normal-phase as well as reversed-phase HPLC. Initially, a gradient elution with two solvents is performed from which a starting isocratic mobile phase composition is selected to elute the substances in a suitable capacity factor range. Other binary and ternary solvent compositions, determined with an experimental design and having the same solvent strength but different selectivity, are tested to adjust the resolution of peak pairs and to improve the separation between the solutes. Benzamides, local anesthetics, phenothiazines, tricyclic antidepressants, benzodiazepines, corticosteroids, sulfonamides, barbiturates, and food preservatives are separated either in normal phase or in reversed phase or in both.

In the last few years, pioneering work on the optimization of HPLC separations has been carried out by Glajch, Kirkland, Snyder, and co-workers (*1–6*). We will call in this paper their collection of works the GKS strategy. An overview of the resulting methodology can be found in an article by Lehrer (*7*). In short, the philosophy of the GKS strategy is the following:

A limited number of solvents (a total of eight) and stationary phases (two) are sufficient to achieve the great majority of all HPLC separations.

The optimal constitution of the mobile phase can be found by using a formal optimization algorithm, which consists in determining first a suitable solvent strength and then in optimizing the solvent selectivity.

In our laboratory we have worked along the same lines on more limited sets of substances. Using information theory, we found that nearly every separation of pharmaceutically important bases can be achieved with a single stationary phase (i.e., a cyanopropyl bonded phase) and one of two preferred mobile phases, namely, *n*-hexane–dichloromethane–acetonitrile–propylamine (50/50/25/0.1) and acetonitrile–water–propylamine (90/10/0.01) (*8*). Optimization is then carried out with one of the two systems as a starting point and consists in fine tuning the volume ratio of the eluent components (*9*).

Because of the success of our strategy for basic drugs with a single column, we thought that it could be possible to develop a general strategy along the same lines as the GKS strategy, but with the cyanopropyl bonded phase as single phase instead of the two different columns. The CN bonded column is of intermediate polarity and can be used in the reversed-phase mode as well as in the normal mode. The GKS methodology

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