Assignment of Absolute Configuration to Metabolically Formed *trans*-Dihydrodiols of Dibenz[*a*,*h*]anthracene by Two Distinct Spectroscopic Methods

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Absolute configuration was assigned to the enantiomers of three metabolically formed *trans*-dihydrodiols of dibenz[*a*,*h*]anthracene by application of NMR and CD spectroscopic methods. Racemic *trans*-1,2-dihydrodiol (**1a**), *trans*-3,4-dihydrodiol (**2a**), and *trans*-5,6-dihydrodiol (**3a**) were transformed to their diastereomeric bis(menthoxyacetates) **1b**, **2b**, and **3b** followed by preparative separation of the diastereomers on silica gel. Correlation of the isolated diastereomers to the corresponding dihydrodiols was achieved by comparison of their chromatographic behavior with that of diastereomeric bis(menthoxyacetates) obtained from enantiomeric pure dihydrodiols on an analytical scale. Determination of absolute configuration of dihydrodiols **1a**, **2a**, and **3a** was achieved by analysis of coupling patterns of $OCOCH_2O$ methylene proton resonances in the NMR spectra of bis(menthoxyacetates) **1b**, **2b**, and **3b**. The empirical rule that the less polar diastereomer with larger negative optical rotation and minor magnetical nonequivalence of methylene protons has (*R*,*R*) absolute configuration was found to be valid for all three dihydrodiols. The configurational assignment by the empirical NMR method was fully confirmed by application of the exciton chirality method to esters of enantiomeric pure dihydrodiols with *p*-(dimethylamino)cinnamic acid as a new red-shifted chromophore. This cinnamic acid derivative seems to be a powerful tool for use in the exciton chirality method in all these cases of polycyclic aromatic hydrocarbons, where the commonly used chromophore *p*-(dimethylamino)benzoic acid leads to noninterpretable split CD Cotton effects and therefore requires troublesome and time-consuming partial hydrogenation of the polycyclic aromatic system. The present study revealed that (*R*,*R*) absolute configuration is associated with (−)-*trans*-1,2-dihydrodiol, (−)-*trans*-3,4-dihydrodiol, and (+)-*trans*-5,6-dihydrodiol of dibenz[*a*,*h*]anthracene. In addition, these enantiomers were found to be preferentially formed by liver microsomes from rats pretreated with 3-methylcholanthrene.

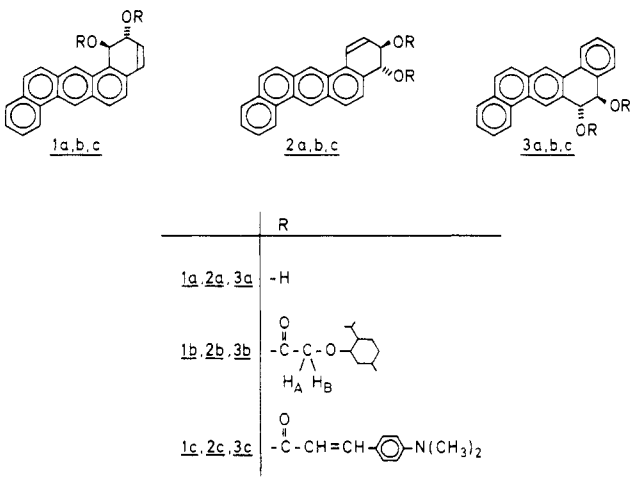
Polycyclic aromatic hydrocarbons (PAH) are widespread environmental pollutants[1] that exhibit mutagenic and carcinogenic properties upon metabolic conversion to reactive metabolites.[2-4]

Biologically significant metabolites are *trans*-dihydrodiols[5-9] since some of them can be enzymatically transformed to highly reactive dihydrodiol epoxides that are ultimate mutagens and carcinogens.[10-12]

The *trans*-dihydrodiols are metabolically obtained from PAH with high and remarkably similar stereoselectivity.[4]

In order to study the metabolism of PAH leading to enantiomeric enriched *trans*-dihydrodiols, their absolute configuration has to be determined. This has been achieved in the case of phenanthrene,[13,14] chrysene,[14-16] benz[*a*]anthracene,[14,16] benzo[*c*]phenanthrene,[14,17] and benzo[*a*]pyrene[21,23] by the application of two spectroscopic methods.

Chart I



The first, an empirical method, uses NMR spectral analysis of methylene protons in diastereomeric bis(menthoxyacetates) of *trans*-dihydrodiols for elucidation of absolute configuration; in the second, nonempirical exciton chirality method, the determination of Cotton effects in CD spectra of enantiomeric pure bis[*p*-(dimethylamino)benzoates] of *trans*-dihydrodiols or of their partially saturated derivatives, reveals their absolute configuration.

The carcinogenic PAH dibenz[*a*,*h*]anthracene (DBA) is metabolically transformed to three *trans*-dihydrodiols (cf. Chart I) whose enantiomeric composition and absolute configuration are yet unknown.

Since we have been studying the regio- and stereoselective metabolism of DBA, we were interested in the absolute configuration of these *trans*-dihydrodiols. We therefore applied the two spectroscopic methods men-

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Table I. Specific Rotations and Chromatographic Behavior of Enantiomerically Pure *trans*-Dihydrodiols 1a, 2a, and 3a of Dibenz[*a*,*h*]anthracene and of Their Diastereomeric Bis(menthoxyacetates) 1b, 2b, and 3b; Enantiomeric *trans*-Dihydrodiols and Their Corresponding Bis(menthoxyacetates) Arranged in Same Columns

	trans-1,2-dihydrodiol		*trans*-3,4-dihydrodiol		*trans*-5,6-dihydrodiol	
	(−)-**1a**	(+)-**1a**	(−)-**2a**	(+)-**2a**	(+)-**3a**	(−)-**3a**
$[\alpha]^{23}_D$,[a] deg	−468	+406	−193	+202	+183	−185
concn, g/100 mL	0.22	0.23	0.06	0.06	0.20	0.20
	(−)-**1b**	(+)-**1b**	(−)-**2b**	(+)-**2b**	(−)-**3b**	(+)-**3b**
$[\alpha]^{23}_D$,[b] deg	−360	+273	−333	+174	−246	+73
concn, g/100 mL	0.25	0.10	0.39	0.36	0.17	0.14
t_R,[c] min	10.8	11.8	10.0	11.6	7.8	8.8

	(±)-**1b**		(±)-**2b**		(±)-**3b**	
	E_1[d]	E_2[e]	E_1	E_2	E_1	E_2
t_R,[c] min	10.8	11.9	10.0	11.7	7.8	8.9
R_s[f]	1.1		1.0		1.1	

[a] Rotations determined in tetrahydrofuran. [b] Rotations determined in chloroform. [c] Retention times on silica gel (Polygosil 60, 5 μm, 4 × 250 mm) eluted with *n*-hexane/Et_2O (9:1, v/v) at 1 mL/min. [d] Early-eluting diastereomer. [e] Late-eluting diastereomer. [f] Chromatographic resolution.

tioned above to synthetic *trans*-1,2-, -3,4-, and -5,6-dihydrodiols of DBA.

The use of the *p*-(dimethylamino)benzoic chromophore in the exciton chirality method is often hampered by undesired dipole–dipole interactions of the aromatic ring system of the PAH with the benzoic chromophore, thus leading to noninterpretable CD spectra. This problem could only be solved so far by partial hydrogenation of the PAH, thus adding a cumbersome synthetic step. The recently introduced *p*-(dimethylamino)cinnamic chromophore,[18] in contrast to *p*-(dimethylamino)benzoic acid, has an extended π-electron system that causes a bathochromic shift in UV absorption into a region where the aromatic system of the PAH no longer exhibits optical transitions.

We can now report successful application of the *p*-(dimethylamino)cinnamic chromophore in determining absolute configuration of *trans*-dihydrodiols with the exciton chirality method.

Results

In order to determine the absolute configuration empirically by NMR spectroscopy, the three *trans*-dihydrodiols of DBA had to be transformed to bis(menthoxyacetates). Esterification of racemic **1a**, **2a**, and **3a** (cf. Chart I) with (−)-menthoxyacetic acid chloride proceeded smoothly yielding diastereomeric mixtures of bis(menthoxyacetates) **1b**, **2b**, and **3b** (cf. Chart I), which were separated satisfactorily into the diastereomers (i.e. E_1 and E_2 in Figure 1 and Table I) by preparative HPLC on silica gel. For the determination of the enantiomeric *trans*-dihydrodiol from which a given diastereomeric bis(menthoxyacetate) originated, the racemic *trans*-dihydrodiols were separated into their enantiomers (+)-**1a**, (−)-**1a**, (+)-**2a**, (−)-**2a**, and (+)-**3a**, (−)-**3a** by HPLC on chiral stationary phases.[19] Comparative chromatography of bis(menthoxyacetates) from racemic and enantiomeric *trans*-dihydrodiols on silica gel under identical running conditions led to the results in Table I, which show that in all three cases the bis(menthoxyacetate) with negative optical rotation elutes first from the silica gel column. Furthermore, the sign of optical rotation of the bay-region (1,2-) dihydrodiol, **1a**, and the M-region (3,4-) dihydrodiol, **2a**, is maintained in the corresponding bis(menthoxyacetates) **1b** and **2b**, respectively, while in the case of the K-region (5,6-) dihydrodiol, **3a**, the sign of rotation changes upon esterification with (−)-menthoxyacetic acid chloride; i.e. (+)-**3a** is transformed to (−)-**3b** and (−)-**3a** to (+)-**3b**.

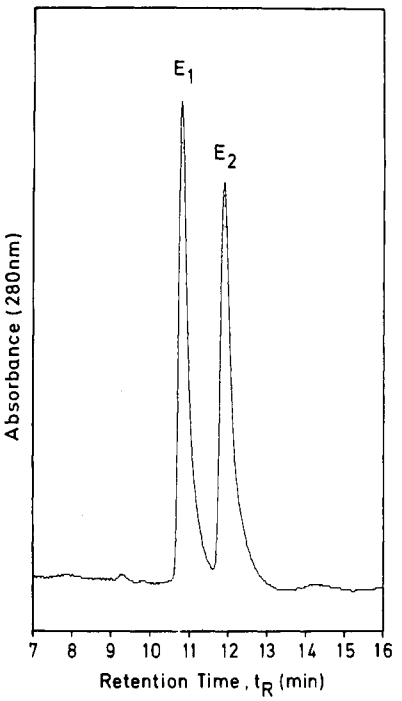

Figure 1. Analytical separation of the mixture of diastereomeric bis(menthoxyacetates) (±)-**1b** of (±)-*trans*-1,2-dihydroxy-1,2-dihydrodibenz[*a*,*h*]anthracene [(±)-**1a**] by HPLC. Stationary phase: Polygosil Si 60, 5 μm, 4 × 250 mm. Mobile phase: *n*-hexane/Et_2O (9:1, v/v). Flow: 1 mL/min (E_1, early-eluting diastereomer; E_2, late-eluting diastereomer).

Table II. Chemical Shifts δ and Coupling Constants J_{gem} for Methylene Protons H_A and H_B in NMR Spectra of Diastereochemically Pure Bis(menthoxyacetates) 1b, 2b, and 3b

	(−)-**1b** E_1[a]	(+)-**1b** E_2[b]	(−)-**2b** E_1	(+)-**2b** E_2	(−)-**3b** E_1	(+)-**3b** E_2
δ(AB)	4.05	4.06	4.15[c]	4.16[c]	4.00[c]	4.01[c]
δ(A′B′)	4.01	4.03	4.08[c]	4.09[c]	3.94[c]	3.96[c]
J_{gem}(AB)[d]	0	39.88	16.24	23.97	13.25	34.86
J_{gem}(A′B′)[d]	0	48.40	17.37	33.28	16.42	37.10

[a] Early-eluting diastereomer on silica gel. [b] Late-eluting diastereomer on silica gel. [c] Center of quartet (ppm). [d] Geminal coupling constants, J_{gem}, in hertz (Hz).

Since NMR coupling patterns of the $OCOCH_2O$ hydrogens in the bis(menthoxyacetates) are diagnostic of their absolute configuration,[14,16] highly resolved proton NMR spectra of **1b**, **2b**, and **3b** were obtained. The relevant part of these spectra is shown in Figure 2, while chemical shifts and coupling constants of geminal methylene protons H_A and H_B (cf. Chart I), treated as an AB system, are summarized in Table II.

There are striking differences between the NMR coupling patterns of both diastereomeric bis(menthoxyacetates) in all *trans*-dihydrodiols under investigation. In the diastereomers with negative optical rotation, i.e. (−)-**1b**, (−)-**2b**, and (−)-**3b**, the methylene protons H_A, H_B and $H_{A'}$, $H_{B'}$ in each ester moiety exhibit less magnetic nonequivalence (cf. Table II) than in the case of the diastereomers

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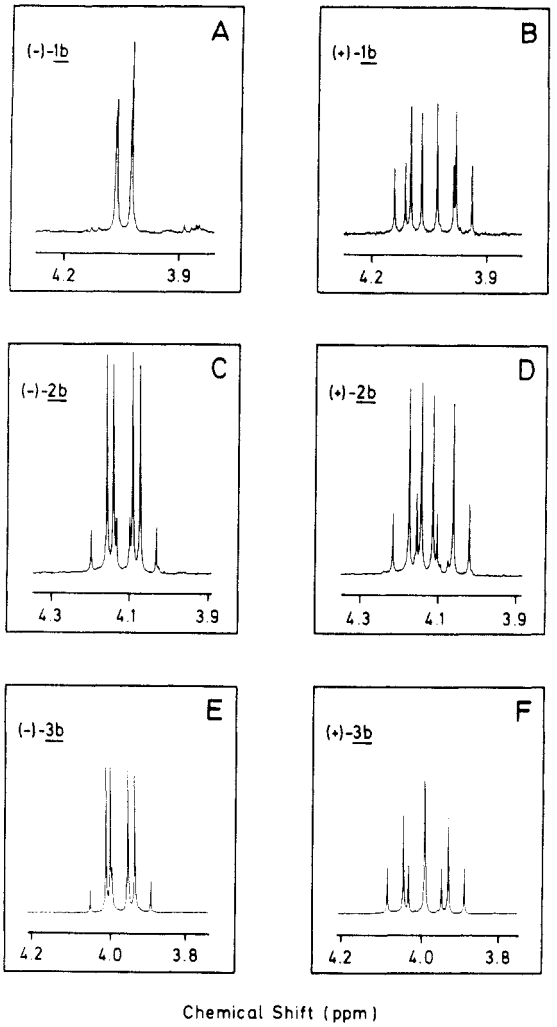

Figure 2. Partial proton NMR spectra (400 MHz, CD_2Cl_2) of diastereomeric bis(menthoxyacetates) (−)-**1b** (A), (+)-**1b** (B), (−)-**2b** (C), (+)-**2b** (D), (−)-**3b** (E), and (+)-**3b** (F) of enantiomeric *trans*-1,2-dihydrodiol (**1a**), *trans*-3,4-dihydrodiol (**2a**), and *trans*-5,6-dihydrodiol (**3a**) of dibenz[*a*,*h*]anthracene.

with positive optical rotation, i.e. (+)-**1b**, (+)-**2b**, and (+)-**3b**. This is expressed in a pair of singlets for the methylene protons in (−)-**1b** or a pair of quartets in the cases of (−)-**2b** and (−)-**3b** with J_{gem} = 13–17 Hz, whereas (+)-**1b**, (+)-**2b**, and (+)-**3b** yield pairs of quartets in all three cases in which J_{gem} is increased almost twofold to J_{gem} = 24–48 Hz (Figure 2; Table II).

In order to assign the absolute configuration to *trans*-dihydrodiols of DBA by the nonempirical exciton chirality method of Nakanishi and Harada,[20] enantiomerically pure **1a**, **2a**, and **3a** had to be esterified with suitable chromophores to bis(esters) exhibiting strong absorption at longer wavelengths. Chiral interaction of the two chromophores should then result in split CD curves with extrema of opposite signs, required for deduction of the absolute configuration. Since the generally applied *p*-(dimethylamino)benzoic chromophore,[21] which absorbs at 311 nm, would interact with the aromatic chromophore of the *trans*-dihydrodiols of DBA, a recently described new chromophore, *p*-(dimethylamino)cinnamic acid,[18] that absorbs at 375 nm was used.

Enantiomeric pure *trans*-dihydrodiols (−)-**1a**, (−)-**2a**, and (−)-**3a**[19] were transformed to bis[*p*-(dimethylamino)-cinnamates] by treatment with *p*-(dimethylamino)-cinnamic acid chloride, which was best obtained from the corresponding cinnamic acid and cyanuric chloride,[22] while the published method for preparation of the acid chloride[18] proved less successful. Chromatographic purification by HPLC on silica gel yielded bis[*p*-(dimethylamino)-cinnamates] **1c**, **2c**, and **3c** (cf. Chart I) in fair amounts yet in high purity.

The CD spectra of bis[*p*-(dimethylamino)cinnamates] of (−)-**1a**, (−)-**2a**, and (−)-**3a** exhibited split Cotton effects as expected (cf. Figure 3). The most striking circular dichroic behavior was observed in the case of (−)-**1c**, i.e. very low symmetry of the split Cotton effect and one of the largest molar circular dichroisms ever before reported ($\Delta\epsilon$ = −108.9 L mol^{-1} cm^{-1} for the first, negative Cotton effect).

The CD spectrum of (−)-**2c** was characterized by a pair of strong and almost symmetric Cotton effects where the longest wavelength band was negative (cf. Figure 3). In the CD spectrum of (+)-**3c**, the first Cotton effect was remarkably positive (cf. Figure 3). Thus, the Cotton effects at longer wavelengths in the case of the bay-region (1,2-) dihydrodiol and the M-region (3,4-) dihydrodiol of negative optical rotation, i.e. (−)-**1a** and (−)-**2a**, were negative, whereas in the case of the K-region (5,6-) dihydrodiol with negative optical rotation, i.e. (−)-**3a**, it was positive. In all three cases, the two Cotton effects were centered around the UV absorption maximum of the *p*-(dimethylamino)-cinnamic moiety as predicted by theory.[20]

Discussion

Chemical shifts and signal patterns of methylene protons H_A and H_B (cf. Chart I) in the NMR spectra of each pair of diastereomeric bis(menthoxyacetates) (+)-/(−)-**1b**, (+)-/(−)-**2b**, and (+)-/(−)-**3b**, differ considerably due to distinct stereochemical alignments in underlying *trans*-dihydrodiols **1a**, **2a**, and **3a**. Thus, analysis of methylene proton resonances (Figure 2; Table II) allows a tentative assignment of absolute configuration to enantiomeric **1a**, **2a**, and **3a**, as it has been reported on *trans*-dihydrodiols of other PAH.[14,16,17,23,24]

In (−)-**1b**, methylene protons H_A and H_B of each ester group appear as a singlet (A, Figure 2). Magnetic equivalence between H_A and H_B is suggestive of (*R*,*R*) absolute configuration in (−)-*trans*-1,2-dihydrodiol [(−)-**1a**] whereas magnetic nonequivalence between H_A and H_B in (+)-**1b** leading to a pair of quartets (B, Figure 2) indicates (*S*,*S*) absolute configuration for (+)-*trans*-1,2-dihydrodiol [(+)-**1a**].

The interpretation of the NMR spectral data of bis-(menthoxyacetates) **2b** and **3b** is less unambiguous, since in all four diastereomers methylene protons H_A and H_B of each ester group yield pairs of quartets (C–F, Figure 2). However, tentative assignment of absolute configuration is still possible due to the lower magnetic nonequivalence observed in the case of (−)-**2b** as compared to (+)-**2b** and of (−)-**3b** as compared to (+)-**3b** (cf. Table II). Therefore, (*R*,*R*) absolute configuration was assigned to (−)-**2b** and (−)-**3b**, and consequently to (−)-*trans*-3,4-dihydrodiol [(−)-**2a**] and (+)-*trans*-5,6-dihydrodiol [(+)-**3a**], whereas

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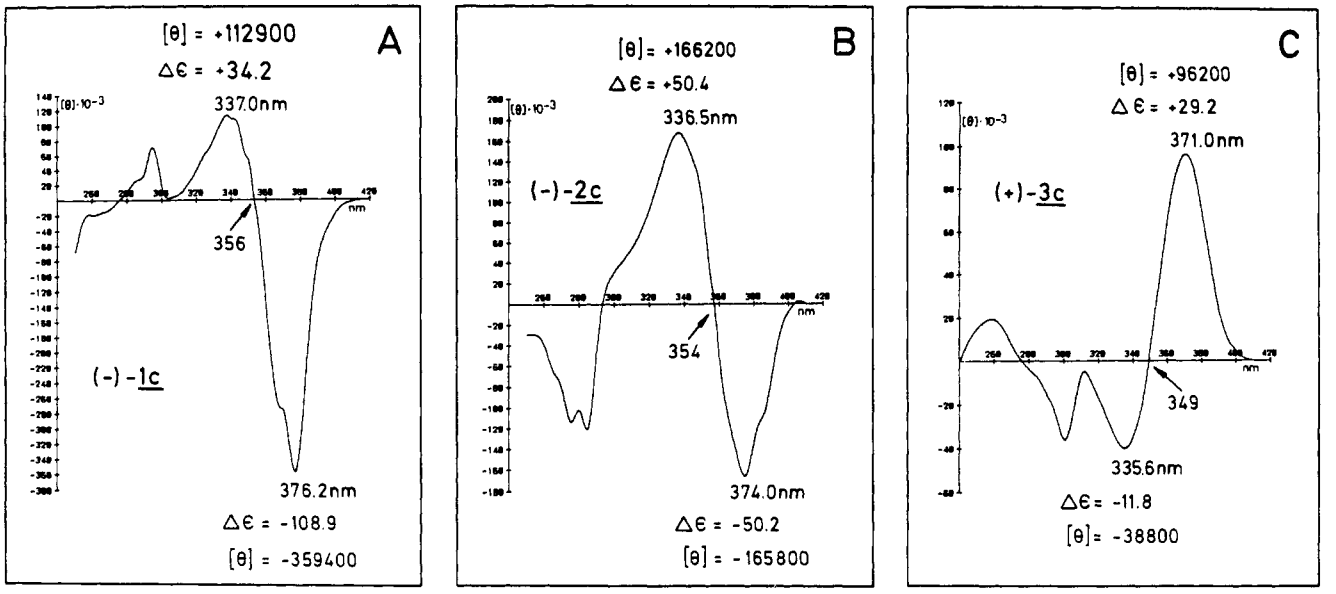

Figure 3. CD spectra in $CHCl_3$ of bis[*p*-(dimethylamino)cinnamates] (−)-**1c** (A), (−)-**2c** (B), and (+)-**3c** (C) of (−)-*trans*-1,2-dihydrodiol [(−)-**1a**], (−)-*trans*-3,4-dihydrodiol [(−)-**2a**], and (−)-*trans*-5,6-dihydrodiol [(−)-**3a**] of dibenz[*a*,*h*]anthracene. $\Delta\epsilon$ was calculated from the specific ellipticity $[\theta]$ with $\Delta\epsilon = [\theta]/3300$, where $\Delta\epsilon$ = molar circular dichroism in L mol^{-1} cm^{-1} and $[\theta]$ = molar ellipticity in L mol^{-1} cm^{-1}. UV absorption maxima for the cinnamic chromophore were 350 nm for (−)-**1c**, 367 nm for (−)-**2c**, and 370 nm for (+)-**3c**.

(*S*,*S*) absolute configuration was assigned to (+)-**2b** and (+)-**3b**, and consequently to (+)-*trans*-3,4-dihydrodiol [(+)-**2a**] and (−)-*trans*-5,6-dihydrodiol [(−)-**3a**].

These assignments were all confirmed by the results of the nonempirical exciton chirality method applied to CD spectra of bis[*p*-(dimethylamino)cinnamates] of (−)-**1a**, (−)-**2a**, and (−)-**3a**. All three enantiomeric esters (−)-**1c**, (−)-**2c**, and (+)-**3c** exhibit two separated Cotton effects of opposite signs at the absorption maximum of the cinnamic chromophore (A–C, Figure 3). In the case of (−)-**1c** and (−)-**2c**, the first Cotton effects at longer wavelengths are negative, thus requiring (*R*,*R*) absolute configuration; in the case of (+)-**3c**, the first Cotton effect is positive and hence indicates (*S*,*S*) absolute configuration.

The results presented above demonstrate the ease of using the *p*-(dimethylamino)cinnamic moiety as a chromophore with an extended π-electron system in the exciton chirality method. The great progress in determining the absolute configuration of *trans*-dihydrodiols of PAH is based on the fact that besides esterification no additional synthetic step is necessary. The use of the *p*-(dimethylamino)benzoic moiety, on the other hand, leads almost inevitably to undesired dipole–dipole interactions with the aromatic chromophore of the PAH, thus requiring a cumbersome hydrogenation step.[16]

The results of this study are another example of the observation that negative optical rotation of *trans*-dihydrodiols is associated with (*R*,*R*) absolute configuration only in the case of bay[24] and M-region dihydrodiols[15-17] [i.e., (−)-**1a** and (−)-**2a**], whereas the K-region dihydrodiols with negative optical rotation [i.e., (−)-**3a**] possess (*S*,*S*) absolute configuration as described for the corresponding dihydrodiols of phenanthrene,[14] benz[*a*]anthracene,[14,25] benzo[*c*]phenanthrene,[14] benzo[*a*]pyrene,[23] and dibenz[*c*,*h*]acridine.[14]

A striking correlation between optical rotation, absolute configuration, and chromatographic behavior, however, is obvious in the case of bis(menthoxyacetates) of enantiomeric dihydrodiols: early elution from a silica gel column, negative optical rotation, and (*R*,*R*) absolute configuration of the underlying *trans*-dihydrodiols are common for diastereomeric **1b**, **2b**, and **3b**, as has been described for benz[*a*]anthracene,[16,24] benzo[*c*]phenanthrene,[14,17] benzo[*a*]pyrene,[23] and dibenz[*c*,*h*]acridine.[14]

trans-Dihydrodiols constitute major microsomal metabolites of DBA[26,27] that are obtained as enantiomerically enriched mixtures due to the stereoselective action of the enzymes involved in their generation. The *trans*-3,4-dihydrodiol (**2a**) formed from DBA by liver microsomes from 3-methylcholanthrene-treated rats was determined to be 60% optically pure.[26] We could confirm this finding and furthermore observed that the enantiomeric excess of the metabolically formed *trans*-1,2-dihydrodiol (**1a**), and *trans*-5,6-dihydrodiol (**3a**), amounts to 66% and 91%, respectively.[27] On the basis of the present communication, we were able to determine that the preferentially formed *trans*-dihydrodiols of DBA possess (*R*,*R*) absolute configuration in all three cases. Thus, DBA is another example for the observation that metabolism of PAH to *trans*-dihydrodiols predominantly leads to (*R*,*R*)-configurated enantiomers.[27]

Experimental Section

General Procedures. Proton NMR spectra were obtained on a Bruker WM 400 connected with a Bruker Aspect 3000 computing unit using CD_2Cl_2 as solvent. UV spectra were recorded on a Shimadzu spectrophotometer MPS 2000 fitted to a Shimadzu graphic printer PR-7; CH_3CN served as a solvent in all cases. Specific rotations, as well as CD spectra, were measured in $CHCl_3$ at 23 °C, on a Perkin-Elmer 241 LC polarimeter and a Cary 61 spectrophotometer, respectively. Mass spectroscopic analysis was fully in accordance to the assumed structures of **1b**, **2b**, **3b**, **1c**, **2c**, and **3c**.

Preparative separation of (±)-**1b**, (±)-**2b**, and (±)-**3b** into pure diastereomers and purification of (−)-**1c**, (−)-**2c**, and (+)-**3c** were achieved by HPLC using a Constametric III pump (LDC/Milton Roy), a UV spectrophotometer (DuPont Instruments), and a silica gel column (Merck, LiChrosorb 60, 5 μm, 16 × 250 mm), whereas analytical HPLC was carried out on a Spectra Physics SP

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8700/8750 LC system, an LKB 2140 diode array detection unit, and a silica gel column (Macherey & Nagel, Polygosil 60, 5 μm, 4 × 250 mm). Bis(menthoxyacetates) **1b**, **2b**, and **3b** require 10 vol % Et_2O in *n*-hexane as eluent at flow rates of 9 and 1 mL/min for preparative and analytical separations, respectively. Bis[*p*-(dimethylamino)cinnamates] **1c**, **2c**, and **3c** were purified by HPLC with 10 vol % CH_3CN in CH_2Cl_2 as eluent, at a flow rate of 4 mL/min. The detection wavelength was 280 nm in all cases. Solvents for synthesis and HPLC were of analytical and HPLC grade, respectively. (−)-Menthoxyacetic acid (Aldrich; $[\alpha]_D$ −90°) was converted to its (−) acid chloride as described.[28] *p*-(Dimethylamino)cinnamic acid (Janssen) was transformed to its acid chloride as described by Venkataraman.[22] 4-Pyrrolidinopyridine was purchased from Janssen.

(+)- and (−)-*trans*-1,2-Bis[(α-menthoxyacetyl)oxy]-1,2-dihydrodibenz[*a*,*h*]anthracene [(+)-1b, (−)-1b]. To a solution of (±)-**1a**[29] (20 mg, 64 μmol) in dry pyridine (5 mL) was added dropwise (−)-menthoxyacetic acid chloride[28] (75 mg, 322 μmol). The mixture was stirred under argon for 24 h at 4 °C and poured into an ice-cold saturated aqueous solution of $NaHCO_3$. After extraction with CH_2Cl_2 (4 × 15 mL), the organic phase was washed (H_2O, 10 mL), dried (Na_2SO_4), and evaporated under reduced pressure to leave a yellowish oil, which was subjected to silica gel chromatography (10 × 150 mm, 10 vol % MeOH in $CHCl_3$), yielding (±)-**1b** [39 mg (87%)] as a white semisolid. Preparative separation of (±)-**1b** into the diastereomers was achieved by HPLC (see General Procedures); chemical purity of (+)- and (−)-**1b** was ≥98% as judged by analytical HPLC. Preparation of diastereomeric **1b** from enantiomerically pure **1a**[19] (0.5 mg, 1.6 μmol) proceeded as described above. The early-eluting fraction (E_1 in Figure 1) afforded (−)-**1b**: 12 mg (26%); $[\alpha]^{23}_D$ −360° (*c* 0.25, $CHCl_3$); NMR (CD_2Cl_2) δ 4.01 (s, 2 H, $OCOCH_2O$, J_{gem} = 0 Hz), 4.05 (s, 2 H, $OCOCH_2O$, J_{gem} = 0 Hz), 5.55–5.57 (dd, 1 H, H_2, $J_{2,3}$ = 5.43 Hz), 6.29–6.33 (q, 1 H, H_3, $J_{3,2}$ = 5.43 Hz, $J_{3,4}$ = 9.53 Hz), 6.94–6.97 (d, 1 H, H_4, $J_{4,3}$ = 9.53 Hz). The late-eluting fraction (E_2 in Figure 2) afforded (+)-**1b**: 10 mg (22%; $[\alpha]^{23}_D$ +273° (*c* 0.10, $CHCl_3$); NMR (CD_2Cl_2) δ 4.03 (q, 2 H, $OCOCH_2O$, J_{gem} = 48.40 Hz), 4.06 (q, 2 H, $OCOCH_2O$, J_{gem} = 39.88 Hz), 5.56–5.58 (dd, 1 H, H_2, $J_{2,3}$ = 5.50 Hz), 6.30–6.34 (q, 1 H, H_3, $J_{3,2}$ = 5.50 Hz, $J_{3,4}$ = 9.39 Hz), 6.96–6.99 (d, 1 H, H_4, $J_{4,3}$ = 9.39 Hz).

(+)- and (−)-*trans*-3,4-Bis[(α-menthoxyacetyl)oxy]-3,4-dihydrodibenz[*a*,*h*]anthracene [(+)-2b, (−)-2b]. (±)-**2a**[30] (20 mg, 64 μmol) was transformed to (±)-**2b** [41 mg (91%)] as described for (±)-**1b**. Chemical purity of HPLC-separated (+)- and (−)-**2b** was ≥98% as judged by analytical HPLC. Preparation of diastereomeric **2b** from enantiomerically pure **2a**[19] (0.5 mg, 1.6 μmol) proceeded as described for **1b**. The early-eluting fraction (E_1) afforded (−)-**2b**: 9.6 mg (21%); $[\alpha]^{23}_D$ −333° (*c* 0.39, $CHCl_3$); NMR (CD_2Cl_2) δ 4.08 (q, 2 H, $OCOCH_2O$, J_{gem} = 17.37 Hz), 4.15 (q, 2 H, $OCOCH_2O$, J_{gem} = 16.24 Hz), 5.70–5.73 (t, 1 H, H_3, $J_{3,4}$ = 5.22 Hz, $J_{3,2}$ = 9.83 Hz), 6.34–6.37 (dd, 1 H, H_2, $J_{2,3}$ = 9.83 Hz, $J_{2,1}$ = 9.98 Hz), 6.39–6.41 (d, 1 H, H_4, $J_{4,3}$ = 5.22 Hz), 7.55–7.57 (d, 1 H, H_1, $J_{1,2}$ = 9.98 Hz). The late-eluting diastereomer (E_2) afforded (+)-**2b**: 8.8 mg (19%); $[\alpha]^{23}_D$ +174° (*c* 0.36, $CHCl_3$); NMR (CD_2Cl_2) δ 4.09 (q, 2 H, $OCOCH_2O$, J_{gem} = 33.28 Hz), 4.16 (q, 2 H, $OCOCH_2O$, J_{gem} = 23.97 Hz), 5.72–5.74 (t, 1 H, H_3, $J_{3,2}$ = 9.11 Hz, $J_{3,4}$ = 4.40 Hz), 6.34–6.37 (dd, 1 H, H_2, $J_{2,1}$ = 9.99 Hz, $J_{2,3}$ = 9.11 Hz), 6.39–6.41 (d, 1 H, H_4, $J_{4,3}$ = 4.40 Hz), 7.55–7.57 (d, 1 H, H_1, $J_{1,2}$ = 9.99 Hz).

(+)- and (−)-*trans*-5,6-Bis[(α-menthoxyacetyl)oxy]-5,6-dihydrodibenz[*a*,*h*]anthracene [(+)-3b, (−)-3b]. (±)-**3a**[31] (20 mg, 64 μmol) was transformed to (±)-**3b** [40 mg (89%)] as described for (±)-**1b**. Chemical purity of HPLC-separated (+)- and (−)-**3b** was ≥98% as judged by analytical HPLC. Preparation of diastereomeric **3b** from enantiomerically pure **3a**[19] (0.5 mg, 1.6 μmol) proceeded as described for **1b**. The early-eluting fraction afforded (−)-**3b**: 14 mg (30%); $[\alpha]^{23}_D$ −246° (*c* 0.17, $CHCl_3$); NMR (CD_2Cl_2) δ 3.94 (q, 2 H, $OCOCH_2O$, J_{gem} = 13.25 Hz), 4.00 (q, 2 H, $OCOCH_2O$, J_{gem} = 16.42 Hz), 6.21–6.22 (d, 1 H, H_6, $J_{6,5}$ = 4.09 Hz), 6.42–6.43 (d, 1 H, H_5, $J_{5,6}$ = 4.09 Hz). The late-eluting fraction yielded (+)-**3b**: 13 mg (28%); $[\alpha]^{23}_D$ +110° (*c* 0.14, $CHCl_3$); NMR (CD_2Cl_2) δ 3.96 (q, 2 H, $OCOCH_2O$, J_{gem} = 37.10 Hz), 4.01 (q, 2 H, $OCOCH_2O$, J_{gem} = 34.86 Hz), 6.20–6.21 (d, 1 H, H_6, $J_{6,5}$ = 4.16 Hz), 6.40–6.41 (d, 1 H, H_5, $J_{5,6}$ = 4.17 Hz).

(−)-*trans*-1,2-Bis[[*p*-(dimethylamino)cinnamoyl]oxy]-1,2-dihydrodibenz[*a*,*h*]anthracene [(−)-1c]. (−)-**1a**[19] (11 mg, 35 μmol) and 4-pyrrolidinopyridine (2 mg, 13 μmol) were added to a solution of *p*-(dimethylamino)cinnamoyl chloride[22] (28 mg, 133 μmol). The mixture was stirred under argon for 72 h at 75 °C, diluted with water (10 mL), and stirred for another 1 h at room temperature. The precipitate was isolated by filtration, washed with water, a saturated aqueous solution of $NaHCO_3$, and again water, and dried under vacuum. Column chromatography (silica gel, 10 × 150 mm, 10 vol % CH_3CN in CH_2Cl_2) afforded a deep yellow solid, which was purified by HPLC (see General Procedures). Rechromatography of the first eluting fraction yielded (−)-**1c**: 4.6 mg (23%). Chemical purity was ≥97% as judged by analytical HPLC: $[\alpha]^{23}_D$ −1065° (*c* 0.25, $CHCl_3$); NMR (acetone-d_6) δ 2.97 [s, 6 H, $N(CH_3)_2$], 2.98 [s, 6 H, $N(CH_3)_2$], 5.64–5.66 (dd, 1 H, H_2, $J_{2,3}$ = 5.39 Hz, $J_{2,4}$ = 1.58 Hz), 6.18 (d, 2 H, ArCH=C*H*, *J* = 15.82 Hz), 6.21 (d, 2 H, ArC*H*=CH, *J* = 15.82 Hz), 6.40–6.43 (m, 1 H, H_3), 6.64–6.68 [m, 4 H, $(CH_3)_2NArH$], 7.10 (d, 1 H, H_4, $J_{3,4}$ = 9.61 Hz); UV (EtOH λ_{max} 350 nm, 307, 297, 260, 222.

(−)-*trans*-3,4-Bis[[*p*-(dimethylamino)cinnamoyl]oxy]-3,4-dihydrodibenz[*a*,*h*]anthracene [(−)-2c]. (−)-**2a**[19] (11 mg, 35 μmol) was treated in the same way as described for the preparation of (−)-**1c**. Purification by HPLC afforded (−)-**2c** [2.8 mg (13%)], a vitreous, yellowish product with a chemical purity ≥98% as judged by analytical HPLC: $[\alpha]^{23}_D$ −281° (*c* 0.25, $CHCl_3$); NMR (acetone-d_6) δ 2.99 [s, 6 H, $N(CH_3)_2$], 3.01 [s, 6 H, $N(CH_3)_2$], 5.88–5.91 (m, 1 H, H_3), 6.24 (d, 2 H, ArCH=C*H*, *J* = 15.81 Hz), 6.33 (d, 2 H, ArC*H*=CH, *J* = 15.81 Hz), 6.42–6.45 (d, 1 H, H_2, $J_{2,1}$ = 10.05 Hz, $J_{2,3}$ = 3.92 Hz), 6.57 (d, 1 H, H_4, $J_{3,4}$ = 6.89 Hz), 6.68–6.73 [m, 4 H, $(CH_3)_2NArH$], 7.87 (d, 1 H, H_1, $J_{1,2}$ = 10.05 Hz); UV (EtOH) λ_{max} 367 nm, 297, 286, 278.

(+)-*trans*-5,6-Bis[[*p*-(dimethylamino)cinnamoyl]oxy]-5,6-dihydrodibenz[*a*,*h*]anthracene [(+)-3c]. (−)-**3a**[19] (11 mg, 35 μmol) was converted to (+)-**3c** as described above for (−)-**1c**. Rechromatography of the first eluting fraction afforded (+)-**3c** [7.8 mg (34%)] with a chemical purity of ≥97% as judged by analytical HPLC: $[\alpha]^{23}_D$ +361° (*c* 0.29, $CHCl_3$); NMR (CD_2Cl_2) δ 2.95 [s, 6 H, $N(CH_3)_2$], 2.97 [s, 6 H, $N(CH_3)_2$], 6.09–6.16 (dd, 2 H, ArCH=C*H*, *J* = 15.78 Hz), 6.36 (d, 1 H, H_5, $J_{5,6}$ = 5.36 Hz), 6.55 (d, 1 H, H_6, $J_{6,5}$ = 5.36 Hz), 6.57–6.61 (dd, 4 H, =CHAr*H*, *J* = 8.97 Hz), 7.30–7.35 [dd, 4 H, *H*ArN$(CH_3)_2$, *J* = 8.92 Hz], 7.56–7.61 (dd, 2 H, ArC*H*=CH, *J* = 15.84 Hz); UV ($CHCl_3$) λ_{max} 370 nm, 318, 299, 282, 243.

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