Absolute Configurations of Enantiomeric 1,2-Dihydrobenzo[*b*]fluoranthene-*trans*-1,2-diols and Diastereomeric 1,2,3,3a-Tetrahydrobenzo[*b*]fluoranthene-*trans*-1,2-diols[1]

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1,2-Dihydrobenzo[*b*]fluoranthene-*trans*-1,2-diol (1) was converted by hydrogenation to two diastereomeric 1,2,3,3a-tetrahydrobenzo[*b*]fluoranthene-*trans*-1,2-diols (**2a** and **2e**) which differed in the conformational preference of the hydroxyl groups. The diastereomeric **2a** had a shorter retention time on both reversed-phase and normal-phase HPLC than **2e**. Proton NMR spectral analyses indicated that the hydroxyl groups of **2a** and **2e** preferentially adopt quasidiaxial and quasidiequatorial conformations, respectively. The diastereomeric conformers **2a** and **2e** were interconvertible when they were dissolved in some organic solvents at elevated temperatures. The enantiomers of **1**, **2a**, and **2e** were separated by normal-phase HPLC using a chiral stationary phase (Pirkle type IA) column. The hydroxyl groups of the more strongly retained enantiomers of **1**, **2a**, and **2e** by the chiral stationary phase have identical (1*R*,2*R*) absolute stereochemistries which were established by the exciton chirality CD method.

Benzo[*b*]fluoranthene (**3**, Figure 1) is a nonalternant polycyclic aromatic hydrocarbon (PAH) and is one of the most commonly detected environmental PAHs.[2] It is a moderately potent mouse skin carcinogen.[3,4] The trans-1,2-dihydrodiol **1** is one of many metabolites of **3**[4] and its mutagenic activity toward *Salmonella typhymurium* tester strain TA100 is much weaker than that of **3**.[5] Analogous to the metabolism of alternant PAHs such as benzo[*a*]-pyrene and benz[*a*]anthracene,[6] **1** formed in the metabolism of **3** may also be enriched in one of two enantiomers. Many carcinogenic PAHs are stereoselectively metabolized to ultimate carcinogens as well as to detoxified products by mammalian drug-metabolizing enzyme systems.[6,7] The aim of this study was to establish the CD–absolute configuration relationship of enantiomeric **1**. The results will help the detailed understandings of the metabolic stereoselective pathways of **3**.

Results and Discussion

Saturation of the 3,3a-double bond of **1** produced an additional asymmetric center at C_{3a}, resulting in a total of four diastereomers (there are in fact a total of 8 diastereomers due to presence of conformers, see below). Two diastereomeric pairs (**2a** and **2e** in a 1:1 ratio) were separated by normal-phase HPLC (Table I). The diastereomeric **2a** has a shorter retention time (hence more polar) than **2e** on reversed-phase HPLC using a C18 column and a mixture of water/methanol (1:1, v/v) as the elution solvent. However, **2e** has a longer retention time than **2a** on normal-phase HPLC using a silica gel column and a mixture of hexane/ethanol/acetonitrile (17:2:1, volume ratio) as the elution solvent. The diastereomeric **2a** and **2e** were each separated by chiral stationary phase (CSP) HPLC into a pair of enantiomers (Table II and CD spectra in Figure 2A). The CD Cotton effects of **2a-2** and **2e-2**, which are the more strongly retained enantiomers of **2a** and **2e** respectively, have opposite signs (Figure 2A).

The enantiomer of **2e** more strongly retained by the CSP was converted to a bis(*p*-(dimethylamino)benzoate) by reaction with *p*-(dimethylamino)benzoyl chloride.[8,9] The CD spectrum of the dibenzoate (Figure 2B) showed a negative CD band with a maximum at 324 nm resulting from dipole–dipole interactions between the two benzoate groups.[12] It is apparent that dipole–dipole interactions between the benzoate groups and the aromatic structure

Table I. Chiral Stationary Phase, Reversed-Phase, and Normal-Phase HPLC Separations of Enantiomeric 1,2-Dihydrobenzo[*b*]fluoranthene-*trans*-1,2-diols and Diastereomeric 1,2,3,3a-Tetrahydrobenzo[*b*]fluoranthene-*trans*-1,2-diols

	retention time (min)				
			CSP-HPLC[b]		
diol[a]	RP-HPLC[b]	NP-HPLC[b]	enantiomer 1	enantiomer 2	RV[c]
1 (aa)	4.3	11.9	20.3 (1*S*,2*S*)	21.7 (1*R*,2*R*)[d]	1.4
2a (aa)	5.3	10.4	11.3 (1*S*,2*S*)	13.4 (1*R*,2*R*)	3.2
2e (ee)	6.8	14.3	13.6 (1*S*,2*S*)	15.3 (1*R*,2*R*)[e]	2.6

[a] Conformations of the hydroxyl groups are indicated by aa (quasidiaxial) and ee (quasidiequatorial) in parentheses. [b] See Experimental Section for chromatographic conditions. [c] Resolution value (RV) = $2(V_2 - V_1)/(W_2 + W_1)$, where V is retention volume and W is peak width at base. The void time was 1.2 min. [d] Hydrogenation of this enantiomer yielded a mixture of **2a-2** and **2e-2**.[11] [e] The absolute configuration of this enantiomer is determined by the exciton chirality CD method (see Figure 2B).

Table II. Interconversion of Diastereomeric Conformers 2a and 2e

	interconversion of conformer (%)[a]		
solvent	**2a-1** → **2e-1**	**2a-2** → **2e-2**	**2e-2** → **2a-2**
methanol	21	74	NT
methanol/water (1:1)	10	NT	1
ethanol	12	1	NT
15% of solvent A in hexane[b]	1[c]	10	NT
acetone	12	23	10
hexane	0	0	0
acetonitrile	0	0	1
ethyl acetate	0	0	0
tetrahydrofuran	0	0	NT

[a] The samples were each kept in the indicated solvent at 60 °C for 16 h and the solvent was evaporated at room temperature under a stream of nitrogen. Each sample was analyzed by CSP-HPLC. Conversion of conformers was not observed when the samples were kept at room temperature in any one of the tested solvents for several days. NT = not tested. [b] Solvent A is ethanol/acetonitrile (2:1, v/v). [c] Under identical conditions, 9% of **2e-1** was converted to **2a-1**.

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Table III. Chemical Shifts (First Rows) and Coupling Constants (Second Rows) of Protons of 1, 2a, and 2e

	chemical shift (ppm)[a] and coupling constant (Hz)										
	carbinol protons					aromatic protons					
diol	H_1	H_2	H_3	$H_{3'}$	H_{3a}	H_4	$H_{5,6,7}$	H_8	H_9	$H_{10,11}$	H_{12}
1[b]	5.60	4.95	6.98[c]			8.03	7.57	8.32	8.12	7.64	8.41
	($J_{1,2} < 2$, $J_{2,3} = 4.3$, $J_{11,12} = 7.0$ Hz)										
2a	5.41	4.71	2.81	2.01	4.51	7.73	7.50	8.31	8.10	7.60	8.36
	($J_{1,2} < 2$, $J_{2,3} = 4.2$, $J_{2,3'} < 2$, $J_{3,3'} = 12.4$, $J_{3,3a} = 4.6$, $J_{3',3a} = 12.4$ Hz)										
2e	5.33	4.70	2.96	1.61	4.19	7.73	7.50	8.30	8.08	7.60	8.63
	($J_{1,2} = 5.8$, $J_{2,3} = 4.4$, $J_{2,3'} = 11.4$, $J_{3,3'} = 11.6$, $J_{3,3a} = 4.4$, $J_{3',3a} = 12.4$ Hz)										

[a] Chemical shifts of protons (in ppm) are relative to those of tetramethylsilane. [b] The assignments of protons are similar but not identical with those reported by Amin et al.[5] who used $CDCl_3$ as the solvent. [c] H_3 of 1 is not a carbinol proton and is listed here for convenience.

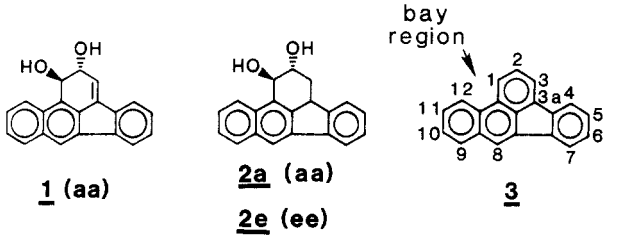

Figure 1. Structure and numbering system of 1,2-dihydrobenzo[*b*]fluoranthene-*trans*-1,2-diol (**1**), diastereomeric 1,2,3,3a-tetrahydrobenzo[*b*]fluoranthene-*trans*-1,2-diols (**2a** and **2e** which differ in conformational preferences), and benzo[*b*]fluoranthene (**3**). The abbreviations ee (quasidiequatorial) and aa (quasidiaxial) indicate the preferred conformations of the hydroxyl groups.

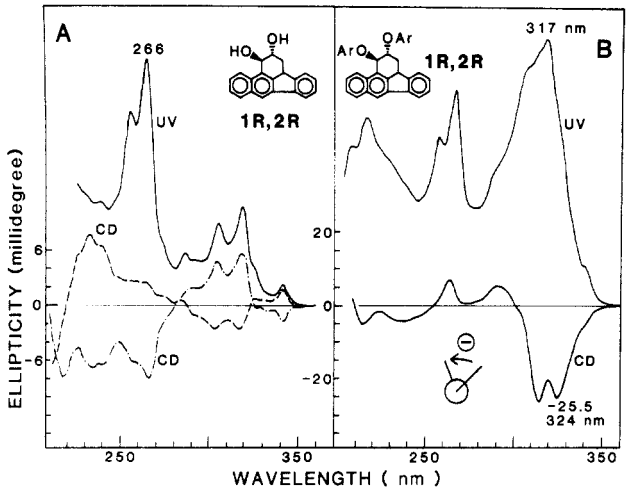

Figure 2. (A) UV absorption and CD spectra of optically pure **2a-2** (---, ϵ_{max} 266 nm; sample concn 1.0 A_{266}/mL) and **2e-2** (—, ϵ_{max} 266 nm; sample concn 1.0 A_{266}/mL). CD spectra of **2a-1** and **2e-1** (not shown) are mirror images to those of **2a-2** and **2e-2**, respectively. (B) UV absorption and CD spectra of the bis(*p*-(dimethylamino)benzoate) derivative derived from an optically pure **2e-2** (ϵ_{max} 317 nm; sample concn 1.0 A_{317}/mL).

also occurred, resulting in a negative CD band at 315 nm (Figure 2B). The negative CD band at 324 nm in the exciton chirality CD spectrum, however, provided the evidence that the enantiomer of **2e** more strongly retained by the CSP has a 1*R*,2*R* absolute stereochemistry.[10]

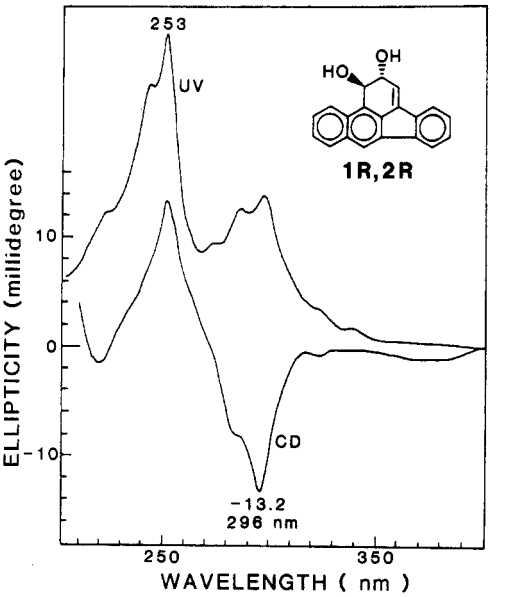

Figure 3. UV absorption and CD spectra of an optically pure enantiomer of **3** more strongly retained by the CSP (ϵ_{max} 253 nm; sample concn 1.0 A_{253}/mL).

The enantiomers of **1** were also separated by CSP-HPLC (Table I). The more strongly retained enantiomer by the CSP (see CD spectrum in Figure 3) was converted by catalytic hydrogenation to two diastereomers which, after separation by normal-phase HPLC, had identical CD spectra to those of **2a-2** and **2e-2** (both are more strongly retained enantiomers by the CSP),[11] respectively. Since **2e-2** has a 1*R*,2*R* absolute configuration (Figure 2B), the absolute configuration of the more strongly retained enantiomers of **2a** and **1** by the CSP are therefore deduced to also have 1*R*,2*R* absolute configurations.

The chemical shifts and coupling constants of protons of **1**, **2a**, and **2e** by 1D NMR analyses are shown in Table III. 1D and 2D COSY[12] analyses of **2e** in acetone-d_6 with a trace of D_2O indicate that **2a** and **2e** are a pair of conformers. The resonance signals of hydroxyl protons are not observed due to the presence of D_2O.

The assignment of resonance signals of **2e** is accomplished by 2D COSY (Figure 4) and 2D NOESY (Figure 5).[12] In 2D COSY, the coupled spins are shown as off-diagonal peaks. It is clear in 2D COSY (Figure 4) that H_1 (5.33 ppm) is coupled only to H_2; H_2 is coupled not only

(2) Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 3, International Agency for Research on Cancer, Lyon, France, pp 69–90.
(3) Wynder, E. L.; Hoffmann, D. *Cancer* **1966**, *12*, 1194–1199.
(4) Amin, A.; LaVoie, E. J.; Hecht, S. S. *Carcinogenesis* **1982**, *3*, 171–174.
(5) Amin, S.; Hussian, N.; Brielmann, H.; Hecht, S. S. *J. Org. Chem.* **1984**, *49*, 1091–1095.
(6) Yang, S. K.; Mushtaq, M.; Chiu, P.-L. In *Polycyclic Hydrocarbons and Cancer*; Harvey, R. G., Ed.; ACS Symposium Series 283, American Chemical Society; Washington, D.C., 1985; pp 19–34.
(7) Conney, A. H. *Cancer Res.* **1982**, *42*, 4875–4917.
(8) Harada, N.; Chen, S.-M.; Nakanishi, K. *J. Am. Chem. Soc.* **1975**, *97*, 5345–5352.
(9) Yang, S K.; Mushtaq, M.; Weems, H. B.; Fu, P. P. *J. Liq. Chromatogr.* **1986**, *9*, 473–492.
(10) Harada, N.; Nakanishi, K.; *Acc. Chem. Res.* **1972**, *5*, 257–263.
(11) **2a-1** (**2e-1**) and **2a-2** (**2e-2**) indicate the less (more) strongly retained enantiomer by the CSP.
(12) Bodenhausen, G.; Kogler, H.; Ernst, R. R. *J. Magn. Reson.* **1984**, *58*, 370–388.

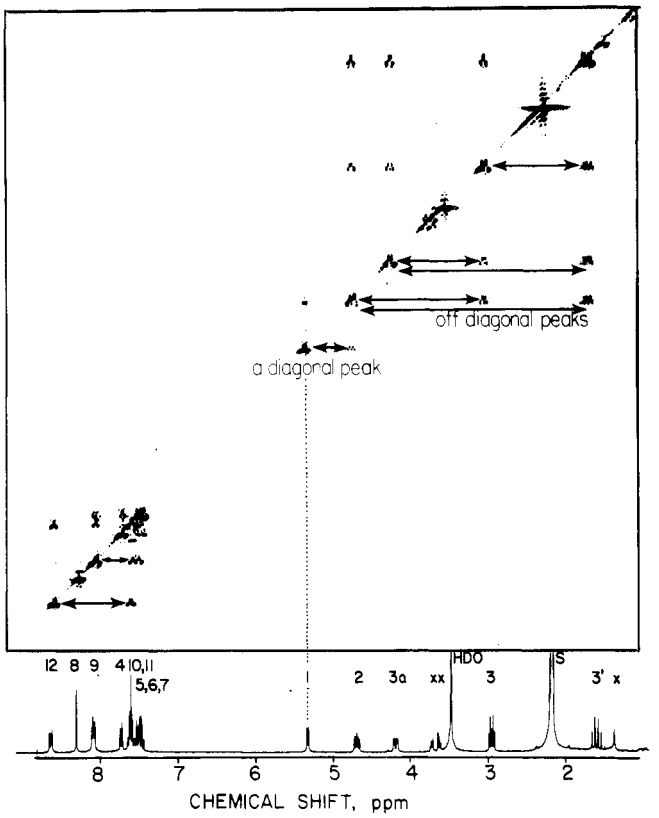

Figure 4. Contour plot of the phase-sensitive 2D COSY (upper box) and 1D (lower spectrum) NMR spectra of **2e**. The intensities of resonance signals in the 1D NMR spectrum are reflected by the intensities of dots along the diagonal line (lower left to upper right in the upper box). For example, the resonance signal of H_1 exhibits a diagonal peak and this is indicated by a connecting dotted line. Proton–proton coupling relationships are indicated by double-arrowed horizontal lines. Chemical shifts (in ppm) are relative to that of tetramethylsilane. In the 1D spectrum, HDO, S, and x indicate resonance signals due to water, solvent, and impurities, respectively.

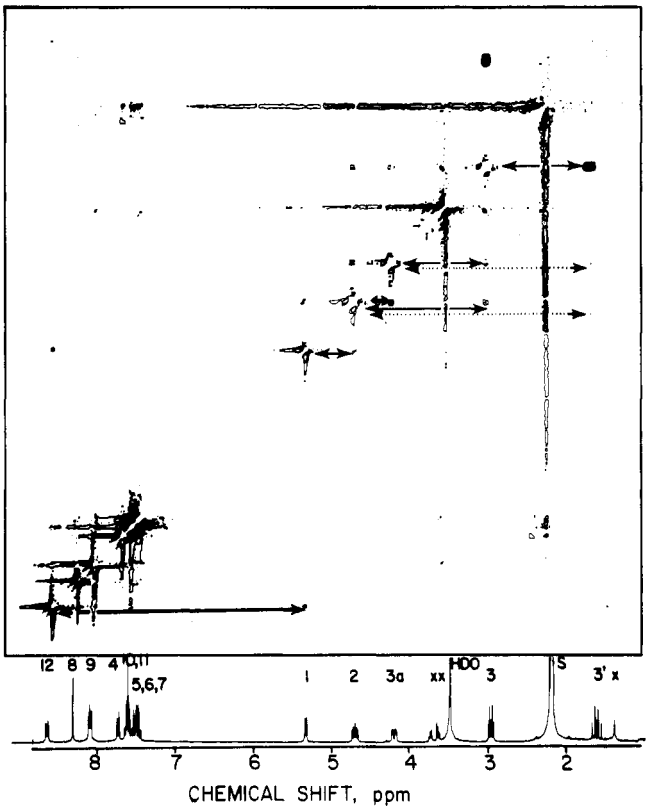

Figure 5. Contour plot of the phase-sensitive 2D NOESY (upper box) and 1D (lower spectrum) NMR spectra of **2e**. The intensities of resonance signals in the 1D NMR spectrum, shown as diagonal peaks in Figure 4, are absent in 2D NOESY because they are in negative amplitudes.[12] Protons in proximity that interact by dipole–diple relaxation are indicated by thick (H_2 & H_{3a} and H_1 and H_{12}) and thin (H_3 & $H_{3'}$, H_2 & H_3, H_1 & H_2, and H_{3a} & H_3) double-arrowed horizontal lines. Interactions betwen vincinal trans protons (H_{3a} & $H_{3'}$ and H_2 & $H_{3'}$) are indicated by double-arrowed horizontal dotted lines. Chemical shifts (in ppm) are relative to that of tetramethylsilane. In the 1D spectrum, HDO, S, and x indicate resonance signals due to water, solvent, and impurities, respectively.

to H_1 but also to H_3 and $H_{3'}$; H_3 and $H_{3'}$ are coupled to H_{3a}. The assignments of H_1 (a doublet) and H_{3a} (a multiplet) are also consistent with the structure of **2e**. H_3 is cis to H_2 and H_{3a}, and $H_{3'}$ is trans to H_2 and H_{3a}, respectively. The aromatic protons of **2e** are also assigned. A singlet at 8.30 ppm is readily assigned to H_8. 2D COSY (Figure 4) indicates that protons with resonance signals centered at 7.72 ppm (H_4) and 7.50 ppm ($H_{5,6,7}$) are coupled to each other. Although resonance signal of H_7 should be doublets of a doublet due to ortho and meta coupling to H_6 and H_5, its exact chemical shift could not be assigned. The proton with a resonance peak at 8.63 ppm (H_{12}, a doublet) is coupled to protons with resonance peaks centered at 7.60 ppm ($H_{10,11}$, multiplet). H_9 (8.08 ppm, an apparent doublet) is also coupled to protons with resonance peaks centered at 7.60 ppm ($H_{10,11}$, multiplet). The identities of the aromatic protons were further confirmed by 2D NOESY (Figure 5). Similar to 2D COSY, protons in proximity interact with each other through dipole moments that are shown as off-diagonal peaks, but in negative amplitudes. The amplitudes of NOE is extremely sensitive to the distance between protons. In Figure 5, a pair of off-diagonal peaks is apparent which is derived from dipole–dipole interactions between H_1 and a proton with a chemical shift of 8.63 ppm. Thus the proton with a resonance peak at 8.63 ppm is readily assigned to H_{12}. In Figure 5, there are nuclear Overhauser effects between H_2 and H_{3a}, between H_1 and H_2, and between H_2 and H_3. Other aromatic protons were similarly assigned (Table III).

It is important to point out that peaks marked as solvent (S), water (HDO), and impurities (X) in 1D NMR spectra did not show any off-diagonal peaks in either 2D COSY (Figure 4) or 2D NOESY (Figure 5). The dipole–dipole interactions between H_2 and H_{3a} as well as between H_1 and H_{12} are not revealed by 2D COSY (Figure 4).

The proton resonances of **1** and **2a** were similarly assigned by comparison to those of **2e** (Table III). In comparison with H_3 of **2e** (at 2.96 ppm), H_3 of **1** (at 6.98 ppm) was shifted downfield due to the effect of the 3,3a double bond. In **2a**, the chemical shifts of both $H_{3'}$ (2.01 ppm) and H_{3a} (4.51 ppm) were shifted downfield when compared to H_3 (1.61 ppm) and H_{3a} (4.19 ppm) of **2e** (Table III).

It is clear from 2D NOESY (Figure 5) that H_{3a} of **2e** is close to H_2 and H_3, but not to $H_{3'}$. Drielding molecular model buildings indicate that C_{3a}–H bonds of both **2a** and **2e** are always in a sterically locked quasiaxial positions. The observed dipole–dipole interaction between H_{3a} and H_2 indicates that H_2 is also in a quasiaxial position. Consequently H_3 (cis to H_{3a}) is in a quasiequatorial position and $H_{3'}$ (trans to H_{3a}) is in a quasiaxial position. The trans relationships between H_2 and $H_{3'}$, and between $H_{3'}$ and H_{3a} are indicated by the coupling constants $J_{2,3'}$ (11.4 Hz) and $J_{3',3a}$ (12.4 Hz), respectively. Similarly, the cis relationships between H_2 and H_3 and between H_3 and H_{3a} are indicated by the coupling constants $J_{2,3}$ (4.4 Hz) and $J_{3,3a}$ (4.4 Hz), respectively. The coupling constants of $J_{1,2}$ of both **1** and **2a** are <2 Hz which indicate that both of the hydroxyl

Table IV. Estimated Dihedral Angles in the Tetrahydro Ring of 2a and 2e Using Drielding Molecular Models

diol	absolute configuration[a] of protons at C_1	C_2	C_{3a}	conf[b]	approximate dihedral angle (deg) C_1-H & C_2-H (trans)	C_2-H & C_3-H (cis)	C_2-H & $C_{3'}$-H (trans)	C_3-H & C_{3a}-H (cis)	$C_{3'}$-H & C_{3a}-H (trans)
2a-1[c]	β	α	β	boat	90	60	60	60	180
	β	α	α	chair	60	30	90	30	150
2a-2[c]	α	β	β	chair	60	30	90	10	130
	α	β	α	boat	90	60	60	60	180
proton–proton coupling constant in racemic **2a**[d]					2.0	4.2	2.0	4.6	12.4
2e-1[c]	β	α	β	chair	180	30	150	10	130
	β	α	α	boat	150	60	180	60	180
2e-2[c]	α	β	β	boat	150	60	180	60	180
	α	β	α	chair	180	30	150	30	150
proton–proton coupling constant in racemic **2e**[d]					5.8	4.4	11.4	4.4	12.4

[a] β and α protons are toward and away from the viewer, respectively, in the structure shown in Figure 1. [b] Conformation of the tetrahydro ring. [c] See ref 12. [d] From Table III.

groups in each diol are in quasiaxial positions.[13] The small coupling constant $J_{2,3'}$ (<2 Hz) in **2a** is also consistent with the hydroxyl groups' quasiaxial conformations.

The coupling constant $J_{1,2}$ of **2e** is 5.8 Hz. Examination of Drielding molecular models indicates that the dihedral angle between C_1–H and C_2–H bonds is 150–180° (Table IV). Thus the hydroxyl groups of **2e** are in quasiequatorial positions. The coupling constant between the quasiaxial H_7 and H_8 of diastereomeric 7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenes was reported to be 8–9 Hz.[14] Thus the values of $J_{1,2}$ (5.8 Hz) between quasiaxial protons observed for **2e** is unusually small in comparisons with the coupling constant (8–9 Hz) reported earlier for vicinal quasiaxial protons in benzo[*a*]pyrene tetrahydro tetrols[1] and 11.4 and 12.4 Hz observed for $J_{2,3'}$ in **2e** and $J_{3',3a}$ in **2a**, respectively (Table IV).

There are four possible diastereomeric configurations among the protons at C_1, C_2, and C_{3a} of each of the two conformers **2a** and **2e** (Table IV). If α and β are used to designate the absolute configurations of protons, then the protons at C_1, C_2, and C_{3a} of each of the two conformers **2a** and **2e** may have four possible configurations ($\alpha\beta\alpha$, $\beta\alpha\alpha$, $\alpha\beta\beta$, and $\beta\alpha\beta$). In **2a** and **2e**, carbinol protons (not drawn in Figure 1) in α and β configurations are away and toward the viewer, respectively. Both **2a** and **2e** are each resolved into one pair of enantiomers (enantiomeric with respect to the protons at C_1 and C_2). The resolved enantiomers of either **2a** and **2e** are enantiomeric only with respect to the chirality of hydroxyl groups (or protons) at C_1 and C_2. Therefore the protons at C_{3a} do not contribute to chiral interactions in the CSP-HPLC separation of enantiomers. It is apparent that the additional asymmetric center at C_{3a} resulting from the hydrogenation of **1** does not play any role in the separation of enantiomeric diastereomers (e.g., **2a-2** contains both $\alpha\beta\beta$ and $\alpha\beta\alpha$, see Table IV).

Examination of Drielding molecular models also indicates that tetrahydro rings of **2a** and **2e** can adopt either a "boat" or a "chair" conformation. The estimated dihedral angles among the protons are shown in Table IV. Each of the diastereomeric **2a** and **2e** has a pair of enantiomers that are mirror images of each other. For example, both $\beta\alpha\alpha$ and $\alpha\beta\beta$ enantiomers of **2a** have "chair" conformations and both $\beta\alpha\beta$ and $\alpha\beta\alpha$ enantiomers of **2e** have "boat" conformations. Although the second enantiomeric pair of either **2a** or **2e** have the same conformation in the tetrahydro rings, the enantiomers are not exact mirror images of each other (Table IV). The reason for the nonsuperimposable enantiomeric diastereomers is apparently due to the sterically rigid C_{3a}. These structural features are very different from those of diastereomeric tetrahydro tetrols of benzo[*a*]pyrene.[14]

It is interesting to note the opposite signs of CD cotton effects in enantiomeric **2a-2** and **2e-2**[11] (Figure 2A) whose hydroxyl groups at C_1 and C_2 have identical 1*R*,2*R* absolute configurations. This is the first example of a pair of tetrahydrodiol conformers that have identical absolute configuration and opposite signs of CD Cotton effects. Upon changes in the conformational preference (quasidiequatorial vs. quasidiaxial), CD Cotton effects of a large number of enantiomeric dihydrodiols are known to change signs at certain wavelength regions.[15]

The diastereomeric conformers **2a** and **2e** are interconvertible in some organic solvents (Table II). The conversion of either **2a-1** → **2e-1** or **2a-2** ⇄ **2e-2** can be monitored either by the change in the sign of CD Cotton effects at one of a wide range of wavelengths (Figure 2A) or by CSP-HPLC analysis. The interconversions of enantiomeric **2a** and **2e** at 60 °C in some commonly used organic solvents were analyzed by CSP-HPLC (Table II). Conversion of **2a** to **2e** occurred in polar solvents containing methanol, ethanol, and acetone. Enantiomeric **2a-2** is more readily converted to its conformer than **2a-1** in methanol, acetone, and hexane containing 15% of ethanol/acetonitrile (2:1, v/v). Conversion of **2e** to **2a** also occurred in acetone. No interconversion occurred when the samples were kept at room temperature in any one of the solvents for several days. No significant interconversion of conformers was observed using hexane, acetonitrile, ethyl acetate, and tetrahydrofuran as the solvent.

Experimental Section

1,2-Dihydrobenzo[*b*]fluoranthene-*trans*-1,2-diol (**1**), synthesized by Amin et al.,[5] was obtained from the Chemical Repository of the National Cancer Institute. The bis(*p*-(dimethylamino)-benzoate of **2a-2** was prepared by reaction with *p*-(dimethyl-

(13) Zacharias, D. E.; Glusker, J. P.; Fu, P. P.; Harvey, R. G. *J. Chem. Soc.*, **1979**, *101*, 4043–4051.

(14) Yagi, H.; Thakker, D. R.; Hernandez, O.; Koreeda, M.; Jerina, D. M. *J. Am. Chem. Soc.* **1977**, *99*, 1604–1611.

(15) (a) Armstrong, R. N.; Kedzierski, B.; Levin, W.; Jerina, D. M. *J. Biol. Chem.* **1981**, *256*, 4726–4733. (b) Fu, P. P.; Yang, S. K. *Biochem. Biophys. Res. Commun.* **1982**, *109*, 927–934. (c) Fu, P. P.; Yang, S. K.; *Carcinogenesis* **1983**, *4*, 979–984. (d) Chiu, P.-L.; Fu, P. P.; Yang, S. K. *Cancer Res.* **1984**, *44*, 562–570. (e) Yang, S. K.; Fu, P. P. *Biochem. J.* **1984**, 775–782. (f) Yang, S. K.; Fu, P. P. *Chem.-Biol. Interact.* **1984**, *49*, 71–88. (g) Fu, P. P.; Von Tungeln, L. S.; Chou, M. W. *Mol. Pharmacol.* **1985**, *28*, 62–71. (h) Buhler, D. R.; Unlu, F.; Thakker, D. R.; Slaga, T. J.; Conney, A. H.; Wood, A. W.; Chang, R. L.; Levin, W.; Jerina, D. M. *Cancer Res.* **1983**, 43, 1541–1549. (i) Yang, S. K., Mushtaq, M.; Weems, H. B.; Fu, P. P. *Tetrahedron Lett.* **1986**, *27*, 433–436.

amino)benzoyl chloride.[8,9] Platinum(IV) oxide ($PtO_2 \cdot H_2O$, Adams catalyst) was purchased from Alfa Products (Thiokol/Ventron Division, Danvers, MA). All solvents were HPLC grade (Mallinckrodt, Inc., Paris, KY).

HPLC was performed on 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 (254 nm) detector. Samples were injected via a Valco Model N60 loop injector (Valco, Houston, TX). Reversed-phase HPLC was performed by using a DuPont Zorbax ODS column (25 cm × 4.6 mm i.d.) and water/methanol (1:3, v/v) as the elution solvent at 1.2 mL/min. Normal-phase HPLC was carried out on a DuPont Golden Series Zorbax SIL column (6.2 mm i.d. × 8 cm) with ethyl acetate/hexane (1:3, v/v) containing 0.4% (v/v) of methanol as the elution solvent at 2 mL/min. The enantiomers of **1**, **2a**, and **2e** were separated with an HPLC column (4.6 mm i.d. × 25 cm; Regis Chemical Co., Morton Grove, IL) packed with an (*R*)-*N*-(3,5-dinitrobenzoyl)-phenylglycine ionically bonded to spherical particles of 5-μm diameter of γ-aminopropylsilanized silica gel. This chiral column is commonly known as Pirkle type IA column. Separation of enantiomeric diols was achieved isocratically with a flow rate of 2 mL/min by using premixed solvents of ethanol/acetonitrile/hexane (2:1:17, volume ratio) at ambient temperature.

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. Ultraviolet–visible absorption spectra of samples in methanol were determined on a 1-cm path-length quartz cuvette with a Varian Model Cary 118C spectrophotometer.

The proton NMR spectra were obtained on a Bruker WM-300 spectrometer equipped with an ASPECT 3000 minicomputer. The conventional 1D Fourier transform NMR spectra were collected with 32K data points. This yielded a digital resolution of 0.368 Hz/point. The spectral width was 6000 Hz. Each spectrum was obtained by an accumulation of 256 scans.

Phase-sensitive 2D NMR spectroscopy (both COSY and NOESY)[12] was performed. The spectral window for 2D spectra was 2500 Hz and data points were 1K for both frequency dimensions (F1 and F2). However, 256 points were taken in the F1 domain. Up to 64 scans were accumulated in each F1 domain. Sine bell function was applied on the Fourier transform in F1 domain to eliminate the spin–lattice relaxation noice.[16] The mixing time of 2D NOESY was 1 s. All samples were dissolved in 99.8% acetone-d_6 with a trace of D_2O to exchange the hydroxyl protons and all spectra were taken at 25 °C. Chemical shifts (in ppm) are relative to that of tetramethylsilane.

CD spectra of samples (dissolved in methanol) in quartz cell of 1-cm path length at room temperature were measured on a JASCO Model 500A spectropolarimeter equipped with a Model DP-500 data processor. The concentration of the sample is indicated by $A_{\lambda 2}$/mL (absorbance units at wavelength $\lambda 2$ per mL of solvent). CD spectra are expressed by ellipticity ($\Phi_{\lambda 1}/A_{\lambda 2}$, in millidegrees) for solutions that have an absorbance of $A_{\lambda 2}$ unit (usually ≤ 1.5) per mL of solvent at wavelength $\lambda 2$ (usually the wavelength of maximal absorption). Under conditions of measurements indicated above, the molecular ellipticity ($[\Theta]_{\lambda 1}$, in deg·cm^2·dmol^{-1}) and ellipticity ($\Phi_{\lambda 1}/A_{\lambda 2}$, in millidegrees) are related to the extinction coefficient ($\epsilon_{\lambda 2}$, in cm^{-1} M^{-1}) as follows:

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

1,2-Dihydrobenzo[*b*]fluoranthene-*trans*-1,2-diol (1). Compound 1 obtained from the Chemical Repository of the National Cancer Institute was purified by normal-phase HPLC on a DuPont Golden Series SIL column. MS: *m/z* (relative intensity) 286 (M^+, 23), 268 (100).

1,2,3,3a-Tetrahydrobenzo[*b*]fluoranthene-*trans*-1,2-diols (2a and 2e). Diastereomeric conformers **2a** and **2e** were obtained by catalytic hydrogenation (THF, PtO_2, 1 atm, 30 min) of 1 and were separated by normal-phase HPLC on a DuPont Golden Series SIL column. The enantiomers of **2a** and **2e** were separated with a Pirkle type IA chiral column as described above. **2a**: MS, *m/z* (relative intensity) 288 (M^+, 23), 270 (18), 255 (6), 253 (8), 252 (7), 244 (33), 228 (36), and 215 (100). **2e**: MS, *m/z* (relative intensity) 288 (M^+, 26), 270 (10), 255 (3), 253 (5), 252 (5), 244 (39), 228 (51), and 215 (100).

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(16) Bax, A. *Two Dimensional Nuclear Magnetic Resonance in Liquids*; Delft University Press, D. Reidel Publishing Co.: Dordrecht, Holland, 1982.

On the Regioselectivity of Metal Hydride Reductions of 3-Substituted Phthalic Anhydrides

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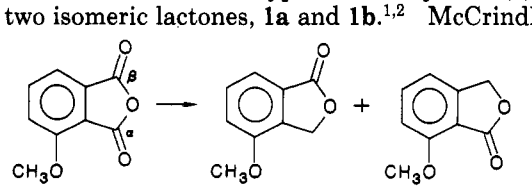
A problem of 3-methoxyphthalide reduction by metal hydrides was reinvestigated. Various effects controlling selectivity of reductions in 3-substituted phthalides were studied, and a qualitative interpretation of the results is now proposed. Methods for obtaining enhanced yields of one or the other lactonic product were developed.

The reduction of 3-methoxyphthalic anhydride (1) can yield two isomeric lactones, **1a** and **1b**.[1,2] McCrindle et



1 **1a** **1b**

al.[3] have reported highly regioselective formation of lactone **1a** (**1a**:**1b** = 87:13) in the reduction of 3-methoxyphthalic anhydride (1) by sodium borohydride. A similar pattern of regioselectivity (preferred reduction at the β-carbonyl group) was reported for the borohydride reduction of 3-(dimethylamino)phthalic anhydride, while the reduction of 3-methylphthalic anhydride was found to be nonselective.[3]

The above findings suggest that the regioselectivity of these reactions could be due to the preferential reduction of chelates formed by complexation of an appropriate

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(1) McAlees, A. J.; McCrindle, R.; Sneddon, D. W. *J. Chem. Soc., Perkin Trans 1* **1977**, 2030–2036.
(2) Brewster, J. H.; Fusco, A. M. *J. Org. Chem.* **1963**, *28*, 501.
(3) McAlees, A. J.; McCrindle, R.; Sneddon, D. W. *J. Chem. Soc., Perkin Trans 1* **1977**, 2037–2038.

0022-3263/87/1952-0129$01.50/0