Importance of *peri*-Interactions on the Stereospecificity of Rat Hydroxysteroid Sulfotransferase STa with 1-Arylethanols

Erden Banoglu and Michael W. Duffel*

Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242

Received September 25, 1998

Hydroxysteroid (alcohol) sulfotransferases catalyze the sulfation of polycyclic aromatic hydrocarbons (PAHs) that contain benzylic hydroxyl functional groups. This metabolic reaction is often a critical step in the activation of a hydroxyalkyl-substituted PAH to form an electrophilic metabolite that is capable of forming covalent bonds at nucleophilic sites on DNA, RNA, and proteins. Since hydroxyalkyl-substituted PAHs are often metabolically formed by the stereoselective enzymatic hydroxylation of a benzylic position on an alkyl-substituted PAH, we have investigated the possibility that the sulfation of hydroxyalkyl aromatic hydrocarbons is also stereoselective. Homogeneous preparations of rat hepatic hydroxysteroid (alcohol) sulfotransferase STa were utilized to investigate the stereoselectivity of its catalytic function with the enantiomers of model 1-arylethanols. While only minimal stereoselectivity was observed for the catalytic efficiency of STa with the enantiomers of 1-(2-naphthyl)ethanol and 1-acenaphthenol, the enzyme was stereospecific for (*R*)-(+)-1-(1-naphthyl)ethanol, (*R*)-(+)-1-(1-pyrenyl)ethanol, and (*R*)-(+)-1-(9-phenanthryl)ethanol as substrates. Moreover, (*S*)-(−)-1-(1-naphthyl)ethanol, (*S*)-(−)-1-(1-pyrenyl)ethanol, and (*S*)-(−)- 1-(9-phenanthryl)ethanol were competitive inhibitors of STa. Structural and conformational analyses of these 1-arylethanols indicated that steric interactions between the substituents on the benzylic carbon and the hydrogen in the *peri*-position on the aromatic ring system were important determinants of the stereospecificity of the enzyme with these molecules. The findings presented here have implications for the more accurate prediction of the ability of hydroxyalkyl-substituted PAHs to be activated via metabolic formation of electrophilic sulfuric acid esters.

Introduction

Hydroxysteroid (alcohol) sulfotransferases (HSTs)[1] catalyze the formation of sulfuric acid esters from a wide range of endogeneous and xenobiotic alcohols as well as from neutral hydroxysteroids such as dehydroepiandrosterone (DHEA) (*1*−*4*). Those sulfotransferases that can catalyze the sulfation of xenobiotic alcohols and hydroxysteroids have been organized into gene families, and various systems of nomenclature have been described elsewhere (*5*−*7*). In addition to these systems of nomenclature, the Human Genome Organization now recognizes SULT as a prefix for systematic classification of human sulfotransferases. This report involves the major hepatic sulfotransferase in female rats which is most often identified by the trivial name hydroxysteroid sulfotransferase a (STa) as assigned in the purification described by Watabe and co-workers (*8*, *9*).

Among xenobiotic alcohols that serve as substrates for HSTs, benzylic alcohols derived from alkyl-substituted polycyclic aromatic hydrocarbons (PAHs) are of significant toxicological interest. Several of these compounds are metabolized to electrophilic sulfuric acid esters that are capable of forming covalent DNA adducts (*10*−*12*). Direct evidence for the metabolic formation of electrophilic sulfuric acid esters from hydroxymethyl PAHs was reported by Watabe and co-workers (*13*), when they isolated an unstable sulfuric acid ester of 7-(hydroxymethyl)-12-methylbenz[*a*]anthracene (HMBA) from rat liver cytosol that had been fortified with 3′-phosphoadenosine 5′-phosphosulfate (PAPS), the physiological cosubstrate for sulfotransferases. Furthermore, the sulfuric acid ester of HMBA was shown to be strongly mutagenic and found to form covalent bonds with the amino groups of purine bases in calf thymus DNA (*14*). Other examples of hydroxymethyl PAHs that are metabolically activated via sulfuric acid esterification include 1-(hydroxymethyl)pyrene (*15*), 5-(hydroxymethyl)chrysene (*16*), 6-(hydroxymethyl)benzo[*a*]pyrene (*17*), and 9-(hydroxymethyl)-10-methylantharacene (*18*). Sulfation of these hydroxymethyl PAHs was significantly inhibited by DHEA, a typical substrate for hydroxysteroid (alcohol) sulfotransferases (*12*, *17*−*19*).

In addition to the hydroxymethyl PAHs that are converted to electrophilic metabolites through sulfation, a few PAHs containing secondary benzylic hydroxyl

* To whom correspondence should be addressed. Telephone: (319) 335-8840. Fax: (319) 353-5594. E-mail: michael-duffel@uiowa.edu.

[1] Abbreviations: DHEA, dehydroepiandrosterone; DIP-chloride, *B*-chlorodiisopinocampheylborane; HST, hydroxysteroid (alcohol) sulfotransferase; k_{cat}, catalytic turnover number (moles of sulfuric acid ester product formed per minute per mole of STa subunit); log *P*, logarithm of the partition coefficient; MTPA, α-methoxy-α-(trifluoromethyl)-phenylacetic acid; NOE, nuclear Overhauser effect; PAH, polycyclic aromatic hydrocarbon; PAP, adenosine 3′,5′-diphosphate; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; ROESY, rotating frame nuclear Overhauser effect NMR spectrometry; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; STa, rat hydroxysteroid (alcohol) sulfotransferase a.

10.1021/tx980219f CCC: $18.00
Published on Web 02/12/1999

group(s) have also been shown to be metabolically activated by sulfotransferases. For example, both 4-hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene and 3,4-dihydroxy-3,4-dihydrocyclopenta[*cd*]pyrene, major metabolites of the environmental carcinogen cyclopenta[*cd*]pyrene, were converted to electrophilic sulfuric acid esters in sulfotransferase-catalyzed reactions (*20*). 1-(1-Pyrenyl)ethanol, 1-(2-pyrenyl)ethanol, 10*H*-indeno[1,2,7,7a-*bcd*]pyren-10-ol, and 1-(6-benzo[*a*]pyrenyl)ethanol were metabolically activated by PAPS-fortified cytosolic fractions derived from the livers of female rats, and the level of sulfation of these PAH derivatives correlated with the greater hepatic activity of HSTs in female rats as opposed to male rats (*15*, *21*). With the exception of 1-(1-pyrenyl)ethanol, all secondary benzylic alcohols in these studies were used as racemates, and no indication of stereoselectivity was reported in these metabolic activations.

STa has been purified from hepatic cytosol derived from female Sprague-Dawley rats and shown to exhibit high catalytic activity for hydroxymethyl PAHs as well as for hydroxysteroids such as DHEA (*8*, *9*). This work clearly showed that homogeneous STa catalyzed the sulfation of four hydroxymethyl PAHs. However, since the relative activities of the enzyme with these hydroxymethyl PAHs were determined at a single concentration of substrate, the effect of structure on the catalytic efficiency of the STa with this class of substrates was not addressed.

Previously, we have reported quantitative structure−activity studies on the hydrophobic and steric requirements necessary for the optimal catalytic efficiency of STa with a series of model primary benzylic alcohols (*22*). We also have observed stereoselectivity in the catalytic efficiency of STa with a series of α-alkyl-substituted chiral secondary benzylic alcohols (*23*). In the study described here, we have utilized enantiomerically pure stereoisomers of 1-arylethanols as model compounds to determine the stereochemical requirements for participation of STa in the sulfation of hydroxyalkyl PAHs.

Materials and Methods

Analysis by thin-layer chromatography (TLC) was performed on precoated, 250 μm thick, silica gel GF plates (Analtech, Newark, DE). Purifications by flash chromatography were carried out on silica gel (200−400 mesh, 60 Å) from Aldrich Chemical Co. (Milwaukee, WI). Stereoisomers of 1-(1-naphthyl)-ethanols, 1-(2-naphthyl)ethanols, (±)-1-acenaphthenol, 1-acetylpyrene, 9-acetylphenanthrene, and acenaphthene were obtained from Aldrich, and their chemical purity was confirmed by TLC. Optical purities of the benzylic alcohols were determined with a Perkin-Elmer 141 polarimeter. The specific rotations of the benzylic alcohols were as follows: (*R*)-(+)-1-(1-naphthyl)ethanol, +75.6° (*c* 0.01, CH_3OH); (*S*)-(−)-1-(1-naphthyl)ethanol, −74.8° (*c* 0.01, CH_3OH); (*R*)-(+)-1-(2-naphthyl)ethanol, +36.2° (*c* 0.015, C_2H_5OH); and (*S*)-(−)-1-(2-naphthyl)ethanol, −37.7° (*c* 0.015, C_2H_5OH). Specific rotations were determined at 589 nm and at 25 °C for all benzylic alcohols. The (+)- and (−)-isomers of DIP-chloride (*B*-chlorodiisopinocampheylborane) were used as obtained from Aldrich. 3′-PAPS was prepared according to a published procedure (*24*). All other assay components and reagents were obtained from commercial sources and were of the highest purity available. Chemical structures of the product 1-arylethanols were verified by ^{1}H NMR spectroscopy (Bruker WM-360), IR spectroscopy (Nicolet model 205), and GC/MS (Hewlett-Packard 5989 quadruple GC/MS system). **Caution:** *Polycyclic aromatic hydrocarbons and derivatives such as the polycyclic 1-arylethanols should be handled with appropriate safety precautions to avoid exposure.*

Synthesis of 1-Acenaphthenone. Acenaphthene (1.54 g in 20 mL of acetic acid) was treated at 50 °C with 3 g of chromium trioxide in 3 mL of H_2O and 8 mL of acetic acid. The mixture was stirred at this temperature for 1 h and poured into water to yield crude 1-acenaphthenone. The crude product was purified by flash chromatography (8:2 hexane/ethyl acetate) and recrystallized from an ethanol/water mixture. The resulting yellow crystals had a melting point of 119−120.5 °C [lit. (*25*) mp 120.5 °C]. The chemical structure of 1-acenaphthenone was verified by IR and ^{1}H NMR spectroscopy, and the spectral data were identical with values for 1-acenaphthenone that were previously reported (*25*).

Synthesis of Racemic 1-Arylethanols. Racemates of 1-(1-pyrenyl)ethanol and 1-(9-phenanthryl)ethanol were synthesized by the sodium borohydride reduction of 1-acetylpyrene and 9-acetylphenanthrene, respectively. To a solution of the appropriate ketone (1.2 mmol) in 20 mL of ethanol was added sodium borohydride (12 mmol). The reaction mixture was stirred at room temperature for 2 h. After the reaction mixture was evaporated to dryness, water was added to decompose the excess sodium borohydride. The solution was then extracted with ethyl acetate (2 × 50 mL). The combined organic layers were evaporated to yield the crude 1-arylethanol, which was subsequently purified by flash column chromatography with hexane/ethyl acetate (8:2) as the mobile phase. Yields of 91−96% were obtained for each of the two racemic 1-arylethanols. Chemical structures of the product racemic 1-arylethanols obtained as products were verified by ^{1}H NMR spectroscopy, IR spectroscopy, and GC/MS, and these data were identical with literature values reported for the racemic compounds (*26*).

Synthesis of Chiral 1-Arylethanols. Optically active (*R*)-(+)- and (*S*)-(−)-1-arylethanols were synthesized by asymmetric reduction of the appropriate ketone with either (+)- or (−)-DIP-chloride, respectively, according to a previously published general procedure (*27*). All glassware was oven-dried overnight, and all operations were carried out under an argon atmosphere. To a solution of 2.97 mmol of (+)- or (−)-DIP-chloride in tetrahydrofuran (5 mL) at −25 °C was added the appropriate ketone (2.97 mmol). A yellow color developed immediately. The reaction was carried out at this temperature for 11 h. The α-pinene was removed under reduced pressure (0.1 mmHg) overnight. The residue was dissolved in diethyl ether (50 mL), and diethanolamine (2.2 equiv) was added. The separated solid was filtered off after 2 h and washed twice with *n*-pentane (30 mL). The combined diethyl ether and *n*-pentane filtrates were concentrated, and the product chiral alcohol was purified by flash column chromatography with hexane/ethyl acetate (9:1) as the mobile phase. Yields of 50−75% were obtained for each of the six chiral 1-arylethanols prepared with this procedure. The IR, NMR, and GC/MS spectral data for the optically active alcohols were identical with those of authentic racemic materials (*26*).

Preparation of α-Methoxy-α-(trifluoromethyl)phenylacetic Acid (MTPA) Esters of Racemic and Chiral 1-Arylethanols. MTPA esters of enantiomers of product alcohols (both chiral and racemic) were prepared by mixing the appropriate alcohol (1 mmol), (*S*)-MTPA (1 mmol), dicyclohexylcarbodiimide (1 mmol), and 4-(dimethylamino)pyridine (0.1 mmol) in methylene chloride (10 mL) at room temperature overnight. The dicyclohexylurea that precipitated was removed by filtration. The eluate was washed successively with 50 mL portions of 0.5 N HCl, 2 N Na_2CO_3, and brine. The crude product obtained after evaporation of the organic layer was purified by flash column chromatography with hexane/ethyl acetate (8:2) as the mobile phase. The ^{1}H NMR spectrum of the MTPA ester of each racemic benzylic alcohol displayed two sets of signals due to the methoxy and benzylic methine protons, whereas each enantiomerically pure benzylic alcohol showed only a single set of signals.

1-Acenaphthenols. Stereoisomers of 1-acenaphthenol were prepared from 1-acenaphthenone.

For (*R*)-(−)-1-acenaphthenol, the ee was determined to be >99% from the ^{1}H NMR spectrum of MTPA ester: mp 126−

127 °C [lit. (*28*) 127−127.5 °C]; $[\alpha]^{25}_D$ −1.5° (*c* 0.06, $CHCl_3$) [lit. (*28*) −1.46° (*c* 1.23, $CHCl_3$)].

For (*S*)-(+)-1-acenaphthenol, the ee was determined to be >99% from the ^{1}H NMR spectrum of MTPA ester: mp 126−127 °C; $[\alpha]^{25}_D$ +1.41° (*c* 0.06, $CHCl_3$) [lit. (*28*) ee 88%, +1.34° (*c* 1.16, $CHCl_3$)].

1-(1-Pyrenyl)ethanols. Stereoisomers of 1-(1-pyrenyl)ethanol were prepared from 1-acetylpyrene.

For (*R*)-(+)-1-(1-pyrenyl)ethanol, the ee was determined to be 98% from the ^{1}H NMR spectrum of MTPA ester: mp 95−96 °C; $[\alpha]^{25}_D$ +53.2° (*c* 0.13, $CHCl_3$) [lit. (*26*) ee 80%, +44° (*c* 0.9, $CHCl_3$)].

For (*S*)-(−)-1-(1-pyrenyl)ethanol, the ee was determined to be 95% from the ^{1}H NMR spectrum of MTPA ester: mp 96−98 °C; $[\alpha]^{25}_D$ −55° (*c* 0.06, $CHCl_3$).

1-(9-Phenanthryl)ethanols. Stereoisomers of 1-(9-phenanthryl)ethanol were prepared from 9-acetylphenanthrene.

For (*R*)-(+)-1-(9-phenanthryl)ethanol, the ee was determined to be 96% from the ^{1}H NMR spectrum of MTPA ester: mp 125−127 °C; $[\alpha]^{25}_D$ +71.2° (*c* 0.04, $CHCl_3$) [lit. (*26*) ee 87%, +39.9° (*c* 0.6, $CHCl_3$)].

For (*S*)-(−)-1-(9-phenanthryl)ethanol, the ee was determined to be 99% from the ^{1}H NMR spectrum of MTPA ester: mp 125−127 °C; $[\alpha]^{25}_D$ −73.4° (*c* 0.03, $CHCl_3$).

NMR. One-dimensional NOE spectra of (*S*)-(−)-1-(1-naphthyl)ethanol were obtained using a Bruker WM-360 MHz spectrometer. The two-dimensional ROESY spectra were obtained using a Varian UNITY 500 pulsed Fourier transform spectrometer. Four transients were averaged to produce each 2048-point FID, with 330 complex traces collected in the F_1 dimension. Phase cycling for the F_1 dimension used the hypercomplex method (*29*). The relaxation delay was set to 6 s, and mixing times ranged from 0.025 to 0.75 s. F_2 processing was performed with a 2048-point Fourier transformation apodized by a 0.079 s Gaussian function. F_1 data were zero filled to 1024 points, followed by transformation under a 0.025 s Gaussian function. Spline baseline correction was applied in the F_2 dimension before measurement of peak intensities. Buildup curves were generated by monitoring the cross-peak intensity and volume as a function of mixing time. All data collection, processing, and analysis were performed using the Varian VNMR 4.3B package. For the calculation of the relative interatomic distances, the rotating frame Overhauser enhancements measured from ROESY cross-peak volumes (*30*) were normalized to the volume of the origin diagonal peak at each mixing time.

X-ray Crystallography. Data for (*S*)-(−)-1-(1-naphthyl)-ethanol (a colorless thin rectangular lathe, 0.08 mm × 0.24 mm × 0.62 mm) and (*S*)-(−)-1-(1-pyrenyl)ethanol (a colorless prism, 0.18 mm × 0.13 mm × 0.088 mm) were collected on an Enraf-Nonius CAD4 diffractometer (MoKα radiation, graphite monochromator) using $\theta-2\theta$ scans. Intensity standards were measured at 2 h intervals. Net intensities were obtained by profile analysis of the data. Lorentz and polarization corrections were applied. The computer programs from the MoLEN package were used for data reduction.

A preliminary model of the structure was obtained using MULTAN, a direct methods program. Least-squares refining of the model versus the data was performed with the XL computer program of the SHELXTL v5.0 package. Illustrations were made with the XP program, and tables were made with the XCIF program. Both are in the SHELXTL package. Thermal ellipsoids shown in the illustrations are at the 35% level.

Purification of Hydroxysteroid Sulfotransferase STa. Hydroxysteroid sulfotransferase STa was purified to homogeneity from female Sprague-Dawley rats, using a modification (*22*) of previously published procedures (*8*, *9*). The enzyme was homogeneous as determined by SDS−PAGE with Coomassie Blue staining. Protein concentrations were determined using a modified Lowry procedure (*31*) with bovine serum albumin as the standard.

Molecular Modeling. Molecular models of 1-arylethanols were built using the Sybyl software package (Tripos Associates, St. Louis, MO) on an Indigo workstation (Silicon Graphics, Mountain View, CA). Energy minimization for each molecule was performed using Maximin2 with the Tripos force field.

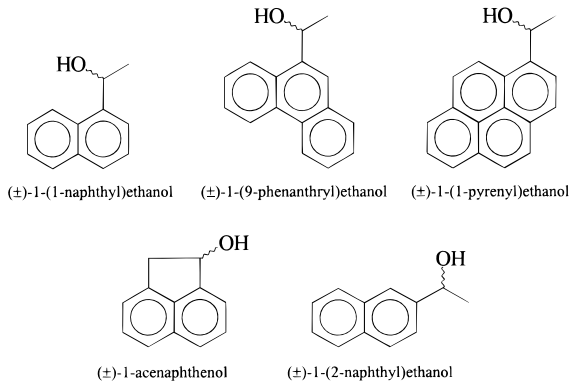

Figure 1. Structures of arylethanols investigated as substrates and inhibitors of STa.

Calculation of Hydrophobicity Constants for 1-Arylethanols. Partition coefficients for 1-arylethanols were calculated using the ACD/LogP software from Advanced Chemistry Development Inc.

Assay of Hydroxysteroid Sulfotransferase STa with 1-Arylethanols. The model 1-arylethanols were evaluated as both substrates and inhibitors of purified STa using a published HPLC procedure for determination of PAP formed in the reaction (*32*). Reaction mixtures with a 0.03 mL total volume contained 0.25 M potassium phosphate buffer (pH 7), 8.3 mM 2-mercaptoethanol, 0.3 mM PAPS, and various concentrations of the alcohols in acetone (final concentration of acetone in the assay was no more than 5% v/v). Reactions were initiated by addition of 1.0−3.0 μg of enzyme; the mixtures were incubated at 37 °C for 10−30 min, and the reactions were terminated by addition of 0.03 mL of methanol. The concentration of PAP formed in the reaction was determined by HPLC. Linear standard curves relating HPLC peak areas to concentrations of PAP were determined daily. At least six different concentrations of each alcohol were assayed, and these included concentrations both greater than and less than the apparent K_m. Apparent K_m and V_{max} values are presented as ±the standard error obtained by nonlinear least-squares curve fitting (*33*) of the velocity data to the Michaelis−Menten equation. Values for k_{cat} were calculated using a relative molecular mass of 33 124 Da for a subunit of STa as determined from the deduced amino acid sequence (*34*). Approximate values for K_m and V_{max} with (*R*)-(+)-1-(1-pyrenyl)ethanol were obtained by analyzing reaction velocities obtained in the presence of the alternative substrate *p*-butylbenzylalcohol as described by the equation

$$v_t = (V_{maxA}[A]/K_{mA} + V_{maxB}[B]/K_{mB})/(1 + [A]/K_{mA} + [B]/K_{mB})$$

where V_{maxA} and V_{maxB} are the maximal velocities with A and B as substrates, respectively (*35*). The observed velocity (v_t) of the reaction for each mixed concentration of both substrates was determined by varying the concentrations of both *p*-butylbenzylalcohol and (*R*)-(+)-1-(1-pyrenyl)ethanol. As a result, the kinetic constants K_{mB} and V_{maxB} for (*R*)-(+)-1-(1-pyrenyl)ethanol were obtained by nonlinear least-squares curve fitting of the observed velocity data to the above equation.

Results

Catalytic Efficiency of STa with 1-Arylethanols. Stereoisomers of several chiral 1-arylethanols (Figure 1) were examined for their ability to interact with hydroxysteroid (alcohol) sulfotransferase STa. Kinetic constants for each STa-catalyzed sulfation reaction are presented in Table 1. The difference between the interaction of STa

Table 1. Summary of Kinetic Constants for STa-Catalyzed Sulfation of 1-Arylethanols[a]

1-arylethanol	K_m	V_{max}	k_{cat}/K_m	K_i
(*R*)-(+)-1-(1-naphthyl)ethanol	800 ± 100	52.0 ± 2.9	2.2 ± 0.3	–
(*S*)-(−)-1-(1-naphthyl)ethanol	–	–	–	250 ± 20
(*R*)-(+)-1-(9-phenanthryl)ethanol	90 ± 20	4.5 ± 0.5	1.7 ± 0.4	–
(*S*)-(−)-1-(9-phenanthryl)ethanol	–	–	–	10 ± 1
(*R*)-(+)-1-(1-pyrenyl)ethanol	7 ± 0.6	11.0 ± 1.0	49.2 ± 6.0	–
(*S*)-(−)-1-(1-pyrenyl)ethanol	–	–	–	2 ± 0.2
(*R*)-(−)-1-acenaphthenol	560 ± 60	83.0 ± 3.3	4.8 ± 0.5	–
(*S*)-(+)-1-acenaphthenol	400 ± 40	121.0 ± 3.7	9.7 ± 0.9	–
(*R*)-(+)-1-(2-naphthyl)ethanol	1000 ± 170	34.0 ± 2.2	1.1 ± 0.19	–
(*S*)-(−)-1-(2-naphthyl)ethanol	1200 ± 200	28.5 ± 1.8	0.8 ± 0.13	–

[a] Values for apparent K_m, V_{max}, k_{cat}/K_m, and K_i are expressed in μM, nmol min^{-1} (mg of STa)$^{-1}$, min^{-1} mM^{-1}, and μM, respectively. The K_i value for (*S*)-(−)-(1-naphthyl)ethanol was determined with (*R*)-(+)-(1-naphthyl)ethanol as the substrate, and the K_i values for (*S*)-(−)-1-(9-phenanthryl)ethanol and (*S*)-(−)-1-(1-pyrenyl)ethanol were determined with 4-butylbenzylalcohol as the substrate.

with the enantiomers of 1-substituted and 2-substituted naphthalene compounds was substantial. In the case of 1-(2-naphthyl)ethanol, STa catalyzed the sulfation of both enantiomers, but the effect of stereochemistry on the catalytic efficiency of STa was small, as indicated by a less than 2-fold preference for the (*R*)-(+)-enantiomer. However, with 1-(1-naphthyl)ethanol, STa clearly showed stereospecificity. As seen from Table 1, (*R*)-(+)-1-(1-naphthyl)ethanol was a substrate for the enzyme, while the (*S*)-(−)-enantiomer was a competitive inhibitor of the sulfation of the (*R*)-(+)-enantiomer catalyzed by STa.

The stereospecificity exhibited with 1-(1-naphthyl)-ethanol prompted the examination of other 1-arylethanols. A stereospecificity similar to that seen with 1-(1-naphthyl)ethanol was observed with the stereoisomers of 1-(1-pyrenyl)ethanol and 1-(9-phenanthryl)ethanol. In both cases, the (*R*)-(+)-enantiomers were substrates, while the (*S*)-(−)-isomers were not substrates for STa. However, (*S*)-(−)-1-(1-pyrenyl)ethanol and (*S*)-(−)-1-(9-phenanthryl)ethanol were competitive inhibitors of the enzyme. In the case of 1-acenaphthenol, a molecule where the conformational changes of the benzylic alcohol moiety were restricted by fixing it into the ring system, both enantiomers were substrates for STa, and STa catalyzed the sulfation of the (*S*)-(+)-enantiomer with a 2-fold difference in the catalytic efficiency.

In the case of (*R*)-(+)-1-(1-pyrenyl)ethanol, although it was shown to be a substrate for STa, it was not experimentally possible to calculate accurate K_m and V_{max} values due to limitations on the reliable determination of the concentrations of PAP formed at the lowest concentrations of substrate required. Therefore, we used an alternative approach to determine the kinetic constants for this substrate. As outlined in Materials and Methods, we utilized a kinetic analysis of the velocity of the sulfation reaction under conditions where two substrates compete for the same enzyme (*35*). As seen in Table 1, the use of *p*-butylbenzylalcohol as a competing substrate provided data that yielded an estimate of 7 μM as the apparent K_m for (*R*)-(+)-1-(1-pyrenyl)ethanol, with a maximal velocity of 11 nmol of product formed min^{-1} (mg of STa)$^{-1}$. The (*S*)-(−)-isomer was an inhibitor of STa with a K_i of 2 μM. Within the limits of detection of the assay method (i.e., 0.3 nmol of product formed min^{-1} mg^{-1}), (*S*)-(−)-1-(1-pyrenyl)ethanol did not serve as a substrate for STa.

Conformational Analysis of (*S*)-(−)-1-(1-Naphthyl)ethanol and (*S*)-(−)-1-(1-Pyrenyl)ethanol. Relative conformations of (*S*)-(−)-1-(1-naphthyl)ethanol and (*S*)-(−)-1-(1-pyrenyl)ethanol were determined by X-ray crystallography (Figures 2 and 3). As shown in Figure 2,

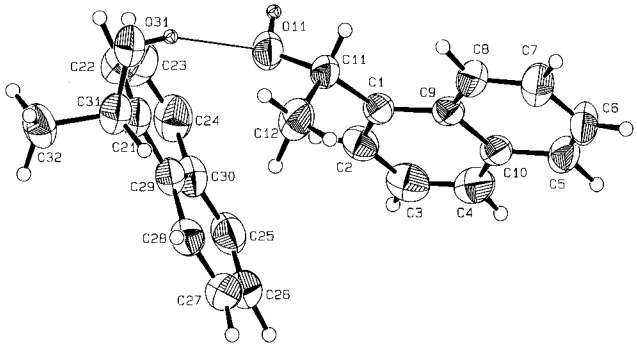

Figure 2. Conformations of (*S*)-(−)-1-(1-naphthyl)ethanol observed by X-ray diffraction of a single crystal. Hydrogen bonding between two molecules of (*S*)-(−)-1-(1-naphthyl)ethanol with differing conformations is shown (labeled as O11−H−O31).

the (*S*)-(−)-1-(1-naphthyl)ethanol molecules appeared in two different conformations that were hydrogen bonded to each other in a single crystal. The only significant difference between the two conformations was in the conformation of the O11 (O31) hydroxyl groups relative to the plane of the naphthalene ring. Comparison of the corresponding torsion angles (i.e., C2−C1−C11−O11 = −27° vs C22−C21−C31−O31 = −43°) indicated the O31 group was rotated 16° from the conformation of the O11 group. This minor difference was probably an accommodation to the hydrogen bonding chains. In the case of the (*S*)-(−)-1-(1-pyrenyl)ethanol, the molecule appeared in four different conformations within a single crystal (Figure 3). Three of these conformations (conformations A−C in Figure 3) were in fact very similar, and the differences in the corresponding torsion angles for the O19A, O19B, and O19C groups relative to the plane of pyrene ring (i.e., C2A−C1A−C17A−O19A = −18.7°, C2B−C1B−C17B−O19B = −17.8°, and C2C−C1C−C17C−O19C = −8.0°) were very small. However, the fourth conformation (conformation D in Figure 3) substantially differed from the other three with respect to the conformation of O19D. The corresponding torsion angle for the O19D group (C2D−C1D−C17D−O19D = −111°) indicated that the O19D group was rotated about 90−100° from the conformations of other hydroxyl groups.

In addition, evidence for the conformation of (*S*)-(−)-1-(1-naphthyl)ethanol in solution was obtained by one-dimensional differential NOE and two-dimensional ROESY NMR in chloroform-*d*. An experiment in which one-dimensional differential NOE NMR clearly displayed through-space proximity of the *peri*-hydrogen (H at the 8-position on naphthalene ring) and CH of the hydroxymethyl substituent at the 1-position (data not shown), suggesting that the preferred conformation in

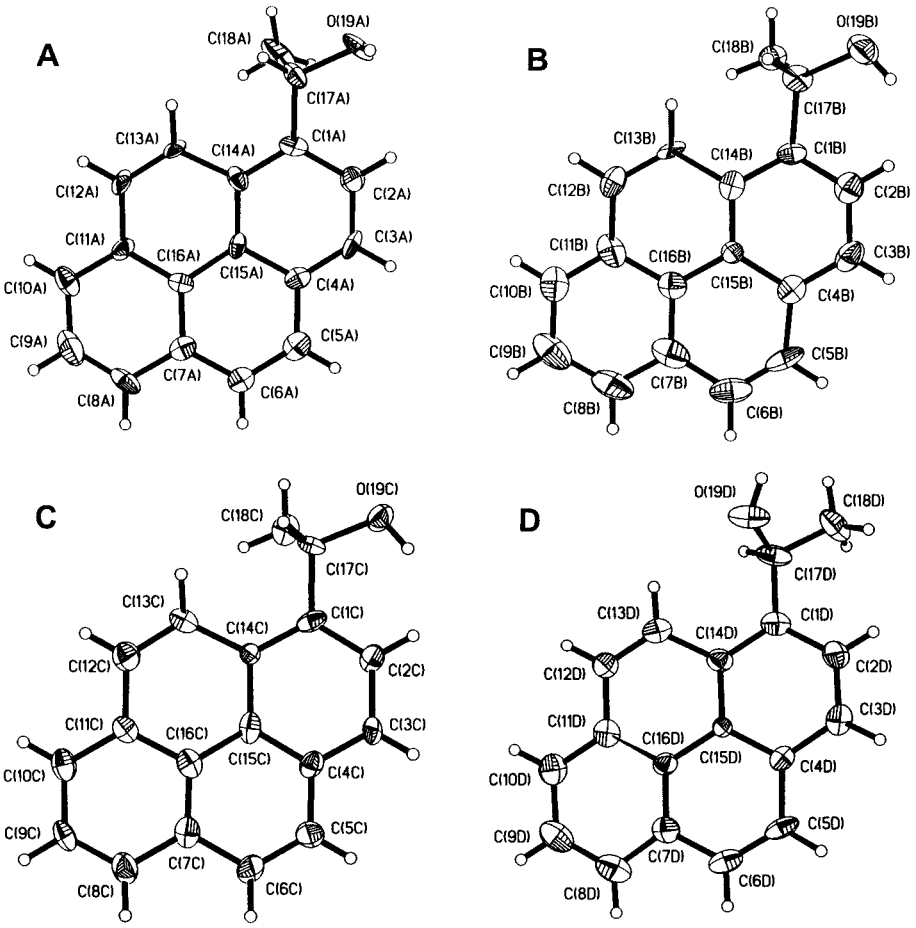

Figure 3. Conformations of (*S*)-(−)-1-(1-pyrenyl)ethanol observed by X-ray diffraction. The four conformations observed in a single crystal are shown.

the liquid was similar to the conformation in the crystal state. This was further supported by calculating the relative interatomic distances between the *peri*-hydrogen and substituents at the 1-position by ROESY (*30*). The calculated relative distances were 2.2 ± 0.2 Å for CH−H8 and 3.25 ± 0.11 Å for CH_3−H8. These relative distances were similar to those calculated from the X-ray structure: CH−H8, 2.24 Å; CH_3−H8, 3.4 Å.

Molecular Modeling Studies. Molecular modeling studies using force field calculations further revealed that conformation (a) of (*S*)-(−)-1-(1-naphthyl)ethanol determined from X-ray (Figure 2) and NMR studies was in fact the lowest-energy conformation. When the conformational energy of (*S*)-(−)-1-(1-naphthyl)ethanol was minimized, the resulting torsion angle ω (C2−C1−C11−O11) formed between the plane of the aromatic ring and the oxygen of the alcohol moiety was about −27°, as observed in the crystal state (Figure 2). Therefore, we chose this torsion angle as a starting point for investigating the similarities among these 1-arylethanols. The conformational energies of (*S*)-(−)-1-(1-pyrenyl)ethanol and (*S*)-(−)-1-(9-phenanthryl)ethanol were then minimized while keeping the torsion angle between the plane of the aromatic ring and the benzylic hydroxyl fixed at −27°. When the benzylic alcohol was at this angle, the methyl substituent was always oriented at the opposite side of the aromatic ring plane with a torsion angle of about 90° between the methyl carbon and aromatic ring. The benzylic hydroxyl assumed a conformation anti from the *peri*-hydrogen (H8) below the naphthalene ring, and this was very similar to what was observed in the crystal state. Therefore, our modeling studies revealed that, among the molecules that were inhibitors of STa, there were similarities in the orientations of the alcohol functionality and the methyl substituent with respect to the plane of the aromatic ring. Although 1-(2-naphthyl)-ethanol may assume many conformations due to possible rotation about the carbon−carbon bond between the benzylic carbon and the naphthyl ring, a similar rotation was restricted for 1-(1-naphthyl)-, 1-(1-pyrenyl)-, and 1-(9-phenanthryl)ethanol (Figure 4).

The relative position of the OH moiety with respect to the aromatic ring and the *peri*-hydrogen can be defined by the torsion angle ω (O11−C11−C1−C2); this torsion angle is −27° in the crystal state (Figure 2). The change in this torsion angle defines the rotation about the carbon−carbon bond between the benzylic carbon (C11) and the aromatic carbon (C1) and, thus, can be used to determine the conformers of 1-(1-naphthyl)ethanol at different energies by using molecular modeling tools. Figure 4 shows the changes in conformational energy as a function of torsion angle ω. The angles were measured by a clockwise rotation of an oxygen atom (O11) with respect to aromatic carbon atom C2. When O11 eclipsed C2 (O11, C11, C1, and C2 lie in the same plane), the torsion angle was defined as 0° for the purpose of the modeling experiments.

As shown in Figure 4, the calculated differences in the energy between the conformers of (*S*)-(−)-1-(1-naphthyl)-ethanol, (*S*)-(−)-1-(1-pyrenyl)ethanol, and (*S*)-(−)-1-(9-phenanthryl)ethanol are much higher than the energy difference between the conformers of (*S*)-(−)-1-(2-naph-

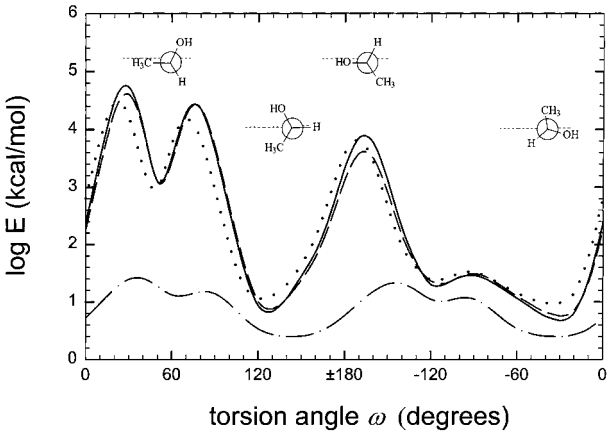

Figure 4. Conformational energies as a function of torsion angle ω. A torsion angle of 0° is defined as the conformation where the oxygen, benzylic carbon, and aromatic ring system all lie in the same plane with the oxygen at a maximum distance from the *peri*-hydrogen. Energies for the various conformations were calculated as described in Materials and Methods. Data are represented as follows: (—) (*S*)-(−)-1-(1-naphthyl)ethanol, (···) (*S*)-(−)-1-(9-phenanthryl)ethanol, (– –) (*S*)-(−)-1-(1-pyrenyl)ethanol, and (–·–) (*S*)-(−)-1-(2-naphthyl)ethanol. Representative conformations are illustrated (left to right) for ω values of 60, 120, ±180, and −30°. In these illustrations, the broken line indicates the plane of the aromatic ring system, with the longer portion of the line representing the portion of the molecule containing the *peri*-hydrogen.

thyl)ethanol. Indeed, the energy differences are very large for 1-(1-naphthyl)-, 1-(1-pyrenyl)-, and 1-(9-phenanthryl)-ethanol, where the spatial rearrangements of the benzylic hydroxyl and methyl group at the 1-position (9-position on phenanthrene ring) are obstructed by the adjacent *peri*-hydrogen. This strongly suggests that the differences in the interaction of enantiomers of these molecules at the active site of STa are conformational in origin.

Discussion

Recent reports on the activation of PAHs bearing a benzylic hydroxyl functional group have indicated that hydroxysteroid (alcohol) sulfotransferases play an important role in the metabolic activation of these molecules. In addition, since the formation of these PAHs bearing benzylic alcohol groups is often mediated stereoselectively by cytochrome P450s and epoxide hydrolases (*36*), the stereoselective formation of sulfate conjugates is likely to be an important factor in the sulfation-mediated DNA binding of chiral hydroxyalkyl polycyclic aromatic hydrocarbons. This was further supported by Glatt and his colleagues in studies with the human hydroxysteroid sulfotransferase- and rat STa-mediated metabolic activation of the stereoisomers of 1-(1-pyrenyl)-ethanol in *Salmonella typhimurium* strains (*37*). Their results with STa heterologously expressed in a strain of *S. typhimurium* showed that (*R*)-(+)-1-(1-pyrenyl)ethanol caused a mutagenic response 2-fold greater than that caused by the (*S*)-(−)-enantiomer. One of the possible reasons for such stereochemical differences is an enantiomeric selectivity by the enzyme(s) catalyzing the sulfation of 1-arylethanols. Indeed, enantioselectivity with 1-(1-pyrenyl)ethanol has been recently observed for heterologously expressed human hydroxysteroid sulfotransferase and human estrogen sulfotransferase (*38*).

Our investigations on the stereochemistry of sulfation of 1-arylethanols have centered on STa, the major hepatic isoform of sulfotransferase catalyzing sulfation of hydroxyalkyl PAHs (*9*), and a series of chiral 1-arylethanols beginning with 1-(1-naphthyl)ethanol. In naphthalene, the 1- and 8-positions are said to be *peri* to each other. In view of the geometry of naphthalene, substituents located at these positions are in much closer proximity than similar substituents located *ortho* to each other, and steric interactions should be more severe. This closer proximity has been responsible for the appearance of several unique properties of *peri*-substituted naphthalenes that have been reported by different groups over many years (*39*−*44*).

Our results with 1-(1-naphthyl)-, 1-(1-pyrenyl)-, and 1-(9-phenanthryl)ethanol show that conformational changes of the benzylic hydroxyl in these molecules are restricted to a certain range of torsion angles due to steric interactions between the *peri*-hydrogen (H8) and the methyl and hydroxyl groups of the hydroxymethyl substituent at the 1-position on naphthalene and pyrene, and the corresponding 9-position on phenanthrene. As shown in Figure 4, examination of the conformational changes of the benzylic hydroxyl group as a function of rotation about the C−C bond between the benzylic carbon and the aromatic ring carbon indicates that the differences in energy between these 1-arylethanols and 1-(2-naphthyl)ethanol are greater when the methyl and hydroxyl groups are closest to the *peri*-hydrogen. This effectively means that conformational changes of the benzylic alcohol moiety of these 1-arylethanols with *peri*-substituent interactions are restricted to a limited range of torsion angles. These results also correlate well with the torsion angles that were obtained from the crystal conformations of these molecules (Figures 2 and 3) and data obtained from two-dimensional ROESY NMR analysis of (*S*)-(−)-1-(1-naphthyl)ethanol. While it is recognized that molecular conformations within crystals may depend on solvent and crystallization conditions, our data for single crystals of 1-(1-naphthyl)ethanol and 1-(1-pyrenyl)-ethanol, each under a single crystallization condition, are consistent with the predictions obtained from our molecular modeling studies with molecules with *peri*-interactions.

In the case of the (*S*)-(−)-1-(2-naphthyl)ethanol, however, *peri*-substituent interactions are not present, and the benzylic hydroxyl may assume many conformations with a minimum of steric interactions between the methyl group and *ortho* hydrogens. Some of these conformations are very likely suitable for sulfuryl group transfer from PAPS at the active site, and this may explain the ability of the (*S*)-(−)-1-(2-naphthyl)ethanol to serve as a substrate for the enzyme, although at a reduced catalytic efficiency. Therefore, it can be assumed that in the case of the (*S*)-(−)-enantiomers of 1-(1-naphthyl)-, 1-(1- pyrenyl)-, and 1-(9-phenanthryl)ethanol where there are *peri*-substituent interactions, the preferred configuration would be the one that is unproductive with respect to transfer of the sulfuryl group transfer. Either the spatial arrangement of the methyl group on the hydroxymethyl substituent may block the approach of the sulfuryl group to the benzylic oxygen, or the hydroxyl group is too distant from the sulfuryl group of PAPS for transfer to occur. For sulfuryl group transfer to occur, conformational rotation would be required, wherein one of the substituents at the benzylic position would have to pass the *peri*-hydrogen to fulfill the conformational requirements for reaction. Such a passage

would involve a prohibitively severe energy barrier (Figure 4).

In addition to the orientation of the benzylic hydroxyl and methyl groups, the hydrophobic characteristics of these benzylic alcohols contribute to their ability to interact with the active site of the STa. Partition coefficients (log *P*) were calculated for 1-(1-naphthyl)ethanol (log $P = 2.6$), 1-(9-phenanthryl)ethanol (log $P = 3.84$), and 1-(1-pyrenyl)ethanol (log $P = 4.33$). Values of apparent K_m [800 ± 100 μM for 1-(1-naphthyl)ethanol, 90 ± 20 μM for 1-(9-phenanthryl)ethanol, and 7 ± 0.6 M for 1-(1-pyrenyl)ethanol] decreased in relation to increasing partition coefficient values. Although these data were determined with only a few compounds, they were consistent with more extensive previous studies (*23*) on the role of hydrophobic interactions in the specificity of STa.

In conclusion, conformational restrictions due to *peri*-substituent interactions combine with hydrophobic interactions between the enzyme and substrate to determine the specificity of hydroxysteroid sulfotransferase STa for these *peri*-substituted arylethanols. These results will furnish a foundation for more extensive investigations on the molecular interactions that are required for sulfotransferase-mediated activation of chemical carcinogens and other xenobiotics that possess chiral benzylic alcohol functional groups. Such studies will undoubtedly be aided by the recently published crystallographic studies on the structure (*45*) and mechanism (*46*) of the mouse estrogen sulfotransferase. However, although one expects a relatively high correlation between the gross three-dimensional folding of an HST and an estrogen sulfotransferase, structure−activity relationships such as those defined here for 1-arylethanols provide an essential component in the development and refinement of homology models for the active sites of HSTs that are useful in predicting the specificity of the enzymes for substrates and inhibitors.

Acknowledgment. We gratefully acknowledge the assistance in crystallographic analysis provided by Dr. Dale C. Swenson of The University of Iowa X-ray Structure Facility. This investigation was supported by U.S. Public Health Service Grant CA38683 awarded by the National Cancer Institute, Department of Health and Human Services.

References

(1) Strott, C. A. (1996) Steroid sulfotransferases. *Endocr. Rev.* **17**, 670−697.

(2) Duffel, M. W. (1997) Sulfotransferases. In *Comprehensive Toxicology Volume 3, Biotransformation* (Guengerich, F. P., Ed.) pp 365−383, Elsevier, Oxford, U.K.

(3) Mulder, G. J., and Jakoby, W. B. (1990) Sulfation. In *Conjugation Reactions in Drug Metabolism* (Mulder, G. J., Ed.) pp 107−161, Taylor & Francis, New York.

(4) Luu-Thee, V., Bernier, F., and Dufort, I. (1996) Steroid sulfotransferases. *J. Endocrinol.* **150**, S87−S97.

(5) Yamazoe, Y., Nagata, K., Ozawa, S., and Kato, R. (1994) Structural similarity and diversity of sulfotransferases. *Chem.-Biol. Interact.* **92**, 107−117.

(6) Rikke, B. A., and Roy, A. K. (1996) Structural relationships among members of the mammalian sulfotransferase gene family. *Biochim. Biophys. Acta* **1307**, 331−338.

(7) Weinshilboum, R. M., Otterness, D. M., Aksoy, I. A., Wood, T. C., Her, C., and Raftogianis, R. B. (1997) Sulfation and sulfotransferases 1: Sulfotransferase molecular biology: cDNAs and genes. *FASEB J.* **11**, 3−14.

(8) Watabe, T., Ogura, K., Satsukawa, M., Okuda, H., and Hiratsuka, A. (1994) Molecular cloning and functions of rat liver hydroxysteroid sulfotransferases catalysing covalent binding of carcinogenic polycyclic arylmethanols to DNA. *Chem.-Biol. Interact.* **92**, 87−105.

(9) Ogura, K., Sohtome, T., Sugiyama, A., Okuda, H., Hiratsuka, A., and Watabe, T. (1990) Rat liver cytosolic hydroxysteroid sulfotransferase (sulfotransferase a) catalyzing the formation of reactive sulfate esters from carcinogenic polycyclic hydroxymethylarenes. *Mol. Pharmacol.* **37**, 848−854.

(10) Miller, J. A., and Surh, Y.-J. (1994) Sulfonation in chemical carcinogenesis. In *Handbook of Experimental Pharmacology* (Kauffman, F. C., Ed.) Vol. 112, pp 429−457, Springer-Verlag, Berlin.

(11) Miller, J. A. (1994) Sulfonation in chemical carcinogenesis-history and present status. *Chem. Biol. Interact.* **92**, 329−341.

(12) Chou, H.-C., Ozawa, S., Fu, P. P., Lang, N. P., and Kadlubar, F. F. (1998) Metabolic activation of methyl-hydroxylated derivatives of 7,12-dimethylbenz[*a*]anthracene by human liver dehydroepiandrosterone-steroid sulfotransferase. *Carcinogenesis* **19**, 1071−1076.

(13) Watabe, T., Ishizuka, T., Isobe, M., and Ozawa, N. (1982) A 7-hydroxymethyl sulfate ester as an active metabolite of 7,12-dimethylbenz[*a*]anthracene. *Science* **215**, 403−405.

(14) Watabe, T., Fujieda, T., Hiratsuka, A., Ishizuka, T., Hakamata, Y., and Ogura, K. (1985) The carcinogen, 7-hydroxymethyl-12-methylbanz[*a*]anthracene, is activated and covalently binds to DNA via a sulfate ester. *Biochem. Pharmacol.* **34**, 3002−3005.

(15) Glatt, H., Christoph, S., Czich, A., Pauly, K., Vierzok, A., Seidel, A., Coughtrie, M. W. H., Doehmer, J., Falany, C. N., Philips, D. H., Yamazoe, Y., and Bartsch, I. (1997) Rat and human sulfotransferases expressed in Ames *Salmonella typhimurium* strains and Chinese hamster V79 cells for the activation of mutagens. In *Control Mechanisms of Carcinogenesis* (Hengstler, J. G., and Oesch, F., Eds.) pp 98−115, Druckerei Thieme, Meissen.

(16) Okuda, H., Nojima, H., Watanabe, N., and Watabe, T. (1989) Sulphotransferase-mediated activation of the carcinogen 5-hydroxymethyl-chrysene. *Biochem. Pharmacol.* **38**, 3003−3009.

(17) Surh, Y.-J., Liem, A., Miller, E. C., and Miller, J. A. (1989) Metabolic activation of the carcinogen 6-hydroxymethylbenzo[*a*]-pyrene: formation of an electrophilic sulfuric acid ester and benzylic DNA adducts in rat liver in vivo and reactions in vitro. *Carcinogenesis* **10**, 1519−1528.

(18) Surh, Y.-J., Blomquist, J. C., Liem, A., and Miller, J. A. (1990) Metabolic activation of 9-hydroxymethyl-10-methylbenzanthracene and 1-hydroxymethylpyrene to electrophilic, mutagenic, and tumorigenic sulfuric acid esters by rat hepatic sulfotransferase activity. *Carcinogenesis* **11**, 1451−1460.

(19) Surh, Y.-J., Lai, C.-C., Miller, J. A., and Miller, E. C. (1987) Hepatic DNA and RNA adduct formation from 7-hydroxymethyl-12-methylbenz[*a*]anthracene and its electrophilic sulfuric acid ester metabolite in preweanling rats and mice. *Biochem. Biophys. Res. Commun.* **144**, 576−582.

(20) Surh, Y.-J., Kwon, H., and Tannenbaum, S. R. (1993) Sulfotransferase-mediated activation of 4-hydroxy- and 3,4-dihydroxy-3,4-dihydrocyclopenta[*c,d*]pyrene, major metabolites of cyclopenta-[*c,d*]pyrene. *Cancer Res.* **53**, 1017−1022.

(21) Glatt, H., Pauly, K., Frank, H., Seidel, A., Oesch, F., Harvey, R. G., and Werle-Schneider, G. (1994) Substance-dependent sex differences in the activation of benzylic alcohols to mutagens by hepatic sulfotransferases of the rat. *Carcinogenesis* **15**, 2605−2611.

(22) Chen, G., Banoglu, E., and Duffel, M. W. (1996) Influence of substrate structure on the catalytic efficiency of hydroxysteroid sulfotransferase STa in the sulfation of alcohols. *Chem. Res. Toxicol.* **9**, 67−74.

(23) Banoglu, E., and Duffel, M. W. (1997) Studies on the interactions of chiral secondary alcohols with hydroxysteroid sulfotransferase STa. *Drug Metab. Dispos.* **25**, 1304−1310.

(24) Sekura, R. D. (1981) Adenosine 3′-phosphate 5′-phosphosulfate. *Methods Enzymol.* **77**, 413−415.

(25) Urbanski, T., and Wolff, M. (1965) 1-Acenaphthenone and its derivatives. I. A selective oxidation of acenaphthene and its nitro derivatives. *Rocz. Chem.* **39**, 1447−1453.

(26) Kato, K., Katayama, M., Fujii, S., and Kimoto, H. (1995) Optical resolution of 1-arylethanols with a condensed aromatic ring by lipase from *Pseudomonas aeruginosa*. *Biosci., Biotechnol., Biochem.* **59**, 2178−2180.

(27) Chandrasekharan, J., Ramachandran, P. V., and Brown, H. C. (1985) Diisopinocampheylchloroborane, a remarkably efficient chiral reducing agent for aromatic prochiral ketones. *J. Org. Chem.* **50**, 5446−5448.

(28) Hu, Y., Ziffer, H., and Silverton, J. V. (1989) Preparation and absolute stereochemistry of (−)-acenaphthenol. *Can. J. Chem.* **67**, 60−62.

(29) States, D. J., Haberkorn, R. A., and Ruben, D. J. (1982) Hypercomplex phase cycling. *J. Magn. Reson.* **48**, 286−292.
(30) Kessler, H. (1987) Separation of cross-relaxation and J cross-peaks in 2D rotating-frame NMR spectroscopy. *J. Am. Chem. Soc.* **109**, 607−609.
(31) Bensadoun, A., and Weinstein, D. (1976) Assay of proteins in the presence of interfering materials. *Anal. Biochem.* **70**, 241−250.
(32) Duffel, M. W., Binder, T. P., and Rao, S. I. (1989) Assay of purified aryl sulfotransferase suitable for reactions yielding unstable sulfuric acid esters. *Anal. Biochem.* **183**, 320−324.
(33) Perella, F. W. (1988) EZ-FIT: A practical curve-fitting microcomputer program for the analysis of enzyme kinetic data on IBM-PC compatible computers. *Anal. Biochem.* **174**, 437−447.
(34) Ogura, K., Kajita, J., Narihata, H., Watabe, T., Ozawa, S., Nagata, K., Yamazoe, Y., and Kato, R. (1990) cDNA cloning of the hydroxysteroid sulfotransferase STa sharing a strong homology in amino acid sequence with the senescence marker protein SMP-2 in rat livers. *Biochem. Biophys. Res. Commun.* **166**, 1494−1500.
(35) Segel, I. H. (1975) Competitive inhibition and total velocity with mixed alternative substrates. In *Enzyme Kinetics*, pp 113−118, Wiley-Interscience, New York.
(36) Yang, S. K. (1988) Stereoselectivity of cytochrome P-450 isozymes and epoxide hydrolase in the metabolism of polycyclic aromatic hydrocarbons. *Biochem. Pharmacol.* **37**, 61−70.
(37) Glatt, H. R. (1997) Bioactivation of mutagens via sulfation. *FASEB J.* **11**, 314−321.
(38) Landsiedel, R., Pabel, U., Engst, W., Ploschke, J., Seidel, A., and Glatt, H. (1998) Chiral inversion of 1-hydroxyethylpyrene enantiomers mediated by enantioselective sulfotransferases. *Biochem. Biophys. Res. Commun.* **247**, 181−185.
(39) Balasubramaniyan, V. (1966) *peri* Interaction in naphthalene derivatives. *Chem. Rev.* **66**, 567−633.
(40) Bentley, M. D., and Dewar, M. J. S. (1970) Proton nuclear magnetic resonance spectra of arylmethyl systems. *J. Org. Chem.* **35**, 2707−2710.
(41) Chen, P. H., and Kleinfelter, D. C. (1973) Peri effects in the mass spectra of some 8-substituted 1-naphthoic acids and 1-naphthylcarbinols. *J. Org. Chem.* **38**, 3015−3019.
(42) Kleinfelter, D. C., and Chen, P. H. (1969) Proximity effects in *peri*-substituted naphthalenes. I. Some 8-substituted 1-hydroxymethyl- and 1-chloromethylnaphthalenes. *J. Org. Chem.* **34**, 1741−1745.
(43) Ouellette, R. J., Marks, D. L., and Miller, D. (1967) Conformational analysis. IX. Conformational and substituent dependence of the hydroxyl proton magnetic resonance of arylcarbinols. *J. Am. Chem. Soc.* **89**, 913−917.
(44) Ouellette, R. J., and VanLeuwen, B. G. (1969) Conformational analysis-XIII polycyclic arylmethanols. *Tetrahedron* **25**, 819−825.
(45) Kakuta, Y., Pedersen, L. G., Carter, C. W., Negishi, M., and Pedersen, L. C. (1997) Crystal structure of estrogen sulfotransferase. *Nat. Struct. Biol.* **4**, 904−908.
(46) Kakuta, Y., Petrotchenko, E. V., Pedersen, L. C., and Negishi, M. (1998) The sulfuryl transfer mechanism. Crystal structure of a vanadate complex of estrogen sulfotransferase and mutational analysis. *J. Biol. Chem.* **273**, 27325−27330.

TX980219F