Conjugation to form sulfuric acid esters is an important reaction in the metabolism of many drugs and other xenobiotics, neurotransmitters, and hormones (1–4). These biotransformations are catalyzed by sulfotransferases in reactions that involve the transfer of a sulfuryl group from 3′-phosphoadenosine 5′-phosphosulfate (PAPS)1 to acceptor substrates, thereby producing products that are usually more water soluble and often less toxic. Hydroxysteroid (alcohol) sulfotransferases catalyze the sulfation of many steroids (1, 5, 6) as well as various other alcohols (7, 8). Benzylic alcohols are among the many diverse substrates for mammalian sulfotransferases. These alcohols are often found as intermediary metabolites obtained from cytochrome P-450 monooxygenase-catalyzed reactions involving the stereochemical oxidation of benzylic carbon atoms (9). Benzylic alcohols can also be obtained through metabolic reduction of benzylic carbonyls (10–12) and through the cytochrome P450- and epoxide hydro-lase-mediated metabolism of polycyclic aromatic hydrocarbons (13), reactions that are also stereoselective.

Although sulfation of alcohols often leads to less toxic and more readily excreted metabolites, some sulfo-oxo metabolites of benzylic and aliphatic alcohols are sufficiently electrophilic that they can covalently bind to cellular macromolecules (14). Some examples of these electrophilic metabolites include the sulfuric acid esters derived from 5-hydroxymethyl-chrysene (15), safrole (16), estragole (16), 7,12-dihydroxymethylbenz[a]anthracene (17), and 3,4-dihydroxy-3,4-dihydroxycyclopenta [cd]pyrene (18). Because the metabolic formation of benzylic alcohols is usually stereoselective, the stereochemical aspects of the subsequent sulfation of these intermediates may have important implications for the carcinogenicity of these polycyclic aromatic hydrocarbons. Furthermore, a recent report on the DNA-binding properties of the antiestrogen tamoxifen, also indicates that stereochemical considerations may be important in the formation of α-hydroxytamoxifen, an allylic secondary alcohol, and its subsequent sulfation to an electrophilic sulfuric acid ester capable of forming DNA adducts (19).

As implied by the name, hydroxysteroids also represent a class of chiral secondary alcohols that serve as substrates for hydroxysteroid (alcohol) sulfotransferases. Strott and co-workers have reported on the isolation and cloning of two distinct sulfotransferases from the guinea pig adrenal (20, 21) that catalyzed the stereospecific sulfation of 3α- and 3β-hydroxysteroids. These enzymes were the first characterized sulfotransferases that demonstrated substrate specificity based on the stereochemistry of a 3-hydroxyl group on a hydroxy steroid.

We have previously observed that one of the aryl (phenol) sulfotransferases, AST IV, displayed stereoselectivity, and in some cases absolute stereospecificity, in catalyzing the sulfation of benzylic alcohol substrates (22). Moreover, this stereoselectivity increased with the size of the alkyl substituent on the benzylic carbon. Phenol sulfotransferases have also been shown to stereoselectively catalyze the O-sulfonation of the phenolic groups in 4-hydroxypropranolol and terbutaline, molecules where the chiral center is remote from the site of sulfation (23). Although aryl sulfotransferases have shown pronounced stereoselectivity for some benzylic alcohols, these enzymes

**ABSTRACT:**

Hydroxysteroid (alcohol) sulfotransferase STa catalyzes the 3′-phosphoadenosine 5′-phosphosulfate-dependent O-sulfonation of a diverse array of alcohols including neutral hydroxysteroids. Many of the secondary alcohols that interact with this sulfotransferase are the metabolic products of stereoselective oxidation or reduction reactions. The role that the stereochemistry of secondary alcohol substrates plays in the catalytic efficiency of STa was investigated with a series of chiral benzylic alcohols and the enantiomeric 3-hydroxyl-containing steroids, androsterone and epandrosterone. In the case of STa, the degree of stereoselectivity was observed with the 3-hydroxyl-containing steroids, androsterone and epandrosterone, results with these substrates were also consistent with the conclusion that the stereochemistry of secondary alcohols is an important factor in the catalytic efficiency of STa.

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(Received April 28, 1997; accepted July 22, 1997)

1 Abbreviations used are: PAPS, 3′-phosphoadenosine 5′-phosphosulfate; PAP, adenosine 3′,5′-diphosphate; MTPA, α-methoxy-α-trifluoromethylphenoxyacetic acid; HPLC, high performance liquid chromatography.

This investigation was supported by the United States Public Health Service Grant CA38683 awarded by the National Cancer Institute, Department of Health and Human Services.

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have displayed limitations in their ability to use large polycyclic aromatic hydrocarbons as substrates (24).

In contrast to the observations with aryl sulfotransferases, several carcinogenic polycyclic aromatic hydrocarbons that bear benzyl alcohol functional groups are good substrates for both rat and human hepatic hydroxysteroid (alcohol) sulfotransferases (25, 26). The major isoform of the enzyme in rat liver was specified as sulfotransferase a (STa) (26), and it has also been designated as hSST2 (2) and hSTa (27). Our previous structure-activity studies on STa have shown that the catalytic efficiency of the enzyme increases with the hydrophobicity of the benzyl alcohol substrate (8). Steric factors may also influence the specificity of STa because quantitative studies on a series of seven-carbon alcohols as substrates for STa revealed that the catalytic efficiencies with primary alcohols were from 3-fold to 8-fold higher than those with secondary alcohols, and catalytic efficiencies with tertiary alcohols were approximately 10-fold lower than those observed for secondary alcohols (8).

In addition to hydrophobic and steric factors, the stereochemistry of chiral alcohols is a property that might influence the catalytic efficiency of STa. Indeed, the catalytic efficiency of STa with (R) or (S)-2-heptanol was 35% higher than with the (S)-(−)-enantioomer of 2-heptanol, and this relatively small difference was the first indication of stereoselectivity in the sulfonation of alcohols catalyzed by this enzyme (8). We now report on a more extensive investigation of the stereoselectivity of STa.

Materials and Methods

Thin layer chromatography (TLC) was performed on 250 µm-thick pre-coated silica gel GF plates (Anatelch, Newark, DE). Purification by flash chromatography was accomplished with silica gel (200–400 mesh, 60 Å) from Aldrich Chemical Co. (Milwaukee, WI). Androsterone (5α-androstan-3α-ol-17-one) and epiandrosterone (5α-androstan-3β-ol-17-one) were purchased from Sigma Chemical Co. (St. Louis, MO). (R)-(+)-1-phenyl-2-methyl-1-propanol, (S)-(−)-1-phenyl-2-methyl-1-propanol, (R)-(−)-1-phenyl-1-butanol, (S)-(−)-1-phenyl-1-butanol, (+)-B-chlorodiisopinocampheylborane [(+)-DIP-chloride], (−)-B-chlorodiisopinocampheylborane [(−)-DIP-chloride], and diethanolamine were obtained from Aldrich. Optical rotations of all benzyl alcohols were determined at 589 nm and at 25°C using a Perkin-Elmer model 141 polarimeter. The specific rotations of the commercially obtained benzyl alcohols were as follows: (R)-(−)-2-methyl-1-phenyl-1-propanol, +23° (c 0.02, CH3OH); (S)-(−)-2-methyl-1-phenyl-1-propanol, −25° (c 0.02, C2H5OH); (R)-(−)-1-phenyl-1-butanol, +50.6° (c 0.02, CHCl3); (S)-(−)-1-phenyl-1-butanol, −49.2° (c 0.02, CHCl3). PAPS was prepared according to a published procedure (28), and adenosine 3′,5′-diphosphate (PAP) was obtained from Sigma. Tetrahydrofuran was freshly distilled from sodium metal using benzophenone ketyl as indicator. All other chemicals, assay reagents, and buffer components were obtained from commercial sources.

Synthesis of Racemic Benzylic Alcohols. Racemates of sec benzyl alcohols were synthesized by reduction of the appropriate alkyl phenyl ketones using the general procedure described by Nystrom and Brown (29). To a solution of lithium aluminum hydride (10 ml of a 1.0 M solution in diethyl ether) was added 1 g of the appropriate alkyl phenyl ketone in 5 ml of diethyl ether at a rate that produced a gentle reflux. The reaction mixture was refluxed for 1.5 hr. After cooling the reaction mixture to 0°C, water was added to decompose the excess lithium aluminum hydride. Following addition of 20 ml of 0.5 N HCl, 2 N Na2CO3, and brine, the crude product obtained after evaporation of the organic layer was purified by flash column chromatography with n-hexane/ethyl acetate (8:2) as the mobile phase. The NMR spectrum of the MTPA esters of a variety of racemic benzylic alcohols displayed only two sets of signals due to the methoxy carbon and the benzylic methine protons (30).

Preparation of α-Methoxy-α-(trifluoromethyl)phenylacetic Acid (MTPA) Esters of Chiral Secondary Benzylic Alcohols. MTPA esters of product benzyl alcohol were prepared by mixing the appropriate alcohol (1 mmol), (S)-(−)-MTPA (1 mmol), dicyclohexylcarbodiimide (1 mmol), and 4-dimethylaminopyridine (0.1 mmol) in methylene chloride (10 ml) and stirring overnight at room temperature. The dicyclohexylurea that precipitated was removed by filtration. The filtrate was washed successively with 50 ml portions of 0.5 N HCl, 2 N Na2CO3, and brine. The crude product obtained after evaporation of the organic layer was purified by flash column chromatography with n-hexane/ethyl acetate (8:2) as the mobile phase. The NMR spectrum of the MTPA esters of (S)-(−)-1-phenyl-1-pentanol showed only a single set of signals.

Synthesis of 1-Phenyl-1-pentanols. Chiral 1-phenyl-1-pentanols were prepared from valerophenone. IR (KBr) ν = 3370 cm−1 (CH-stretch), 2930–2980 cm−1 (C–H stretch, aliphatic), 1800–1500 cm−1 (C=C aromatic, 1453 cm−1 (C=O bending); 1H-NMR (CDCl3) δ 0.9 ppm (t, 3H, –CH3), δ 1.2–1.4 ppm (m, 4H, –CH2–CH2–CH3), δ 1.65–1.86 ppm (m, 2H, –CH(OH)–CH2–), δ 1.85 ppm (s, 1H, O–H), δ 4.69 ppm (q, 1H, –CH2(OH)), δ 7.2–7.32 ppm (5H, –CH=CH–). GC-MS m/z 164 (M+), 107 (M–CH3), 91 (C6H7), 77 (C5H5), 57 (C7H7). For (S)-(−)-1-phenyl-1-pentanol: [α]D = −19.2° (c 0.18, CH3OH) [lit. −20°, neat, ee 100% (31)]; ee = 100% based on 1H-NMR of MTPA ester. The MTPA ester of racemic 1-phenyl-1-pentanol displayed two sets of signals due to methoxy carbon (δ 3.41 and 3.51 ppm) and benzylic methine protons (δ 3.58 and 5.91 ppm), whereas the MTPA ester of (S)-(−)-1-phenyl-1-pentanol showed only a single set of signals at δ 3.41 and 5.91 ppm. For (R)(+)-1-phenyl-1-pentanol: [α]D = +20.2° (c 0.19, CH3OH) [lit. +20°, neat, ee 100% (32); +40.3°, c 1.0, CHCl3, ee 94% (33)]; ee = 100% based on 1H-NMR of MTPA ester. The MTPA ester of (R)(+)-1-phenyl-1-pentanol showed only a single set of signals at δ 3.51 and 5.84 ppm.

Synthesis of 1-Phenyl-1-hexanols. Chiral 1-phenyl-1-hexanols were prepared from hexanophenone. IR (KBr) ν = 3378 cm−1 (OH-stretch), 3085–3070 cm−1 (C=H stretch, aromatic), 2951–2879 cm−1 (C–H stretch, aliphatic), 1950–1700 cm−1 (C=C aromatic, 1454 cm−1 (C=O bending); 1H-NMR (CDCl3) δ 0.8–0.9 ppm (t, 3H, –CH3), δ 1.22–1.49 ppm (m, 6H, –CH3), δ 1.58–1.88 ppm (m, 2H, –CH(OH)–CH2–), δ 1.75 ppm (s, 1H, O–H), δ 4.61 ppm (t, 1H, –CH(OH)–), δ 7.21–7.42 ppm (m, 5H, –CH=CH–). IR (KBr) ν = 3370–3380 cm−1 (OH-stretch), 3050–3060 cm−1 (C=H stretch, aromatic), 2951–2879 cm−1 (C–H stretch, aliphatic), 1950–1700 cm−1 (C=C aromatic, 1454 cm−1 (C=O bending); 1H-NMR (CDCl3) δ 0.8–0.9 ppm (t, 3H, –CH3), δ 1.22–1.49 ppm (m, 6H, –CH3), δ 1.58–1.88 ppm (m, 2H, –CH(OH)–CH2–), δ 1.75 ppm (s, 1H, O–H), δ 4.61 ppm (t, 1H, –CH(OH)–), δ 7.21–7.42 ppm (m, 5H, –CH=CH–). GC-MS m/z 178 (M+), 107 (M–C5H11), 91 (C6H7), 77 (C5H5), 57 (C7H7). For (S)-(−)-1-phenyl-1-hexanol: [α]D = +16.3° (c 0.23, CH3OH) [lit. +34.6°, c 0.9, CHCl3, ee 93% (33)]; ee = 100% based on 1H-NMR of 1-phenyl-1-hexanol. For (R)(+)-1-phenyl-1-hexanol: [α]D = +16° (c 0.23, CH3OH) [lit. +34.6°, c 0.9, CHCl3, ee 93% (33)]; ee = 100% based on 1H-NMR of 1-phenyl-1-hexanol.
MTPA ester. The MTPA ester of (R) (+)-1-phenyl-1-hexanol showed only a single set of signals at δ 3.49 and 5.56 ppm.

**Synthesis of 1-Phenyl-1-heptanols.** Chiral 1-phenyl-1-heptanols were prepared from heptanophenone. IR (KBr) ν = 3409 cm⁻¹ (O-H stretch), 3100–3030 cm⁻¹ (C-H stretch, aromatic), 2927–2850 cm⁻¹ (C-H stretch, aliphatic), 1850–1600 cm⁻¹ (C=C, aromatic), 1450 cm⁻¹ (O-H bending); ¹H-NMR (CDCl₃) δ 0.8 ppm (t, 3H, -CH₃); δ 1.22–1.49 ppm [m, 8H, -(CH₂)₃]; δ 1.58–1.88 ppm [m, 2H, -CH(OH)-CH₂-]; δ 1.71 ppm (s, 1H, OH); δ 4.61 ppm [t, 1H, -CH(OH)-], δ 7.21–7.42 ppm (m, 5H, C₆H₅); GC-MS m/z 190 (M⁺), 107 (M-C₆H₅), 91 (C₆H₅), 77 (C₇H₇). For (S) (-)-1-phenyl-1-heptanol: [α]D²⁵ = -12.2° (c 0.05, CH₃OH) [lit. -20.5, c 0.88, C₂H₅OH, ee 99.5% (34)]. ee = 100% based on ¹H-NMR of MTPA ester. The MTPA ester of racemic 1-phenyl-1-heptanol displayed two sets of signals because of methoxy (s, δ 3.41 and 3.55 ppm) and benzylic methine protons (t, δ 5.84 and 5.91 ppm), whereas the MTPA ester of (S) (-)-1-phenyl-1-heptanol showed only a single set of signals at δ 3.41 and 5.91 ppm. For (R) (+)-1-phenyl-1-heptanol: [α]D²⁵ = + 12.1° (c 0.05, CH₃OH) [lit. +19.7, c 1.7, C₂H₅OH, ee 99.5% (34)]. ee = 100% based on ¹H-NMR of MTPA ester. The MTPA ester of (R) (+)-1-phenyl-1-heptanol showed only a single set of signals at δ 3.5 and 5.84 ppm.

**Synthesis of 1-Phenyl-1-cyclohexylmethanol.** The chiral 1-phenyl-1-cyclohexylmethanols were prepared from cyclohexylphenol ketone. IR (KBr) ν = 3409 cm⁻¹ (O-H stretch), 3100–3030 cm⁻¹ (C-H stretch, aromatic), 2927–2850 cm⁻¹ (C-H stretch, aliphatic), 1850–1600 cm⁻¹ (C=C, aromatic), 1450 cm⁻¹ (O-H bending); ¹H-NMR (CDCl₃) δ 0.8–1.92 ppm [m, 12H, cyclohexyl and -CH(OH)-], δ 4.31 ppm [d, 1H, -CH(OH)-], δ 6.71–7.39 ppm (m, 5H, C₆H₅); GC-MS m/z 190 (M⁺⁺), 107 (M-C₆H₅), 91 (C₆H₅), 77 (C₇H₇). For (S) (-)-1-phenyl-1-cyclohexylmethanol: [α]D²⁵ = +19.2° (c 0.07, CH₃OH), ee = 96% based on ¹H-NMR of MTPA ester. The MTPA ester of (R) (+)-1-phenyl-1-cyclohexylmethanol showed a major single set of signals at δ 3.41 and 5.65 ppm. For (R) (+)-1-phenyl-1-cyclohexylmethanol: [α]D²⁵ = +19.2° (c 0.07, CH₃OH), ee = 96% based on ¹H-NMR of MTPA ester. The MTPA ester of (R) (+)-1-phenyl-1-cyclohexylmethanol showed a major single set of signals at δ 3.49 and 5.56 ppm.

**Purification of Hydroxysteroid (Alcohol) Sulfotransferase STα.** STα was purified to apparent homogeneity from female Sprague Dawley rats (9–10 weeks of age), using a modification (8) of previously published procedures (26, 36). The enzyme was homogeneous by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with Coomassie Blue staining. Protein concentrations were determined using a modified Lowry procedure (37), with bovine serum albumin as standard.

**Assay of Hydroxysteroid Sulfotransferase STα with Benzylic Alcohols.** The various benzylic alcohols were evaluated as both substrates and inhibitors of purified STα using a published HPLC procedure for determination of the concentration of PAP formed in the reaction (39). Reaction mixtures of 0.03 ml total volume contained 0.25 M potassium phosphate buffer at pH 7.0, 8.3 mM 2-mercaptoethanol, 0.3 mM PAPS, and various concentrations of the alcohols in acetonitrile (final concentration of acetonitrile in the assay was no more than 5% v/v). Reactions were initiated by the addition of 1.0 μg of enzyme, incubated at 37°C for 10 min., and terminated by addition of 0.03 ml of methanol. The substrate-dependent 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 K₅₀ values. The substrate concentrations used to determine the kinetic constants for the enantiomERICALLY benzylic alcohols were as follows: 1-phenyl-2-methyl-1-propanols, 0.5–7.0 mM; 1-phenyl-1-butanol, 0.4–7.0 mM; 1-phenyl-1-pentanols, 0.25–4.0 mM; 1-phenyl-1-hexanols, 0.25–2.0 mM; 1-phenyl-1-heptanols, 0.15–2.0 mM; 1-phenyl-1-cyclohexylmethanol, 0.25–3.0 mM. Apparent K₅₀ and v₅₀ values are presented as ± the standard error obtained by non-linear least squares curve fitting (39) of the velocity data to the Michaelis-Menten equation. Values for k₅₀ and kcat/K₅₀ were calculated using the relative molecular mass for a subunit of STα, 32,24, as determined from the deduced amino acid sequence (40).

**Calculation of Hydrophobicity Constants for Chiral Benzylic Alcohols.** Hydroxysteroid (alcohol) sulfotransferase STα catalyzes the sulfation of androsterone (3α-hydroxyl) and epiandrosterone (3β-hydroxyl) as model hydroxysteroid substrates. Although both epiandrosterone and androsterone showed substrate inhibition above 60 μM and 80 μM, respectively, a higher maximum velocity was demonstrated for epiandrosterone. This result indicated a small degree of stereoselectivity with epiandrosterone as the preferred substrate for STα (fig. 2).

**Discussion** Hydroxysteroid (alcohol) sulfotransferase STα catalyzes the sulfation of various benzylic alcohols and steroids (1–4). We have shown previously that the hydrophobicity of a benzylic alcohol is a major determinant of its ability to act as a substrate for the enzyme (8). In the present study, we investigated both stereochemical and steric interac-
<table>
<thead>
<tr>
<th>Benzylic Alcohol</th>
<th>$K_m$ (app)</th>
<th>$V_{max}$</th>
<th>$k_{cat}/K_m$</th>
<th>$(R)/(S)$ Ratio&lt;sup&gt;a&lt;/sup&gt;</th>
<th>log P</th>
<th>$K_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-(+)1-phenyl-2-methyl-1-propanol</td>
<td>2.8 ± 0.8</td>
<td>27.0 ± 3.4</td>
<td>0.33 ± 0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(S)-(−)1-phenyl-2-methyl-1-propanol</td>
<td>3.0 ± 0.6</td>
<td>22.8 ± 2.0</td>
<td>0.25 ± 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(R)-(+)1-phenyl-1-butanol</td>
<td>3.3 ± 0.4</td>
<td>85.5 ± 6.0</td>
<td>0.9 ± 0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(S)-(−)1-phenyl-1-butanol</td>
<td>3.7 ± 0.3</td>
<td>58.8 ± 2.0</td>
<td>0.5 ± 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(R)-(+)1-phenyl-1-pentanol</td>
<td>1.4 ± 0.2</td>
<td>154.4 ± 7.8</td>
<td>3.6 ± 0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(S)-(−)1-phenyl-1-pentanol</td>
<td>1.9 ± 0.3</td>
<td>70.3 ± 5.8</td>
<td>1.2 ± 0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(R)-(+)1-phenyl-1-hexanol</td>
<td>0.46 ± 0.03</td>
<td>157.3 ± 4.0</td>
<td>11.3 ± 0.9</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(S)-(−)1-phenyl-1-hexanol</td>
<td>0.53 ± 0.06</td>
<td>59.2 ± 2.4</td>
<td>3.7 ± 0.4</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(R)-(+)1-phenyl-1-heptanol</td>
<td>0.38 ± 0.03</td>
<td>94.3 ± 2.0</td>
<td>8.1 ± 0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(S)-(−)1-phenyl-1-heptanol</td>
<td>0.42 ± 0.04</td>
<td>37.3 ± 1.3</td>
<td>2.9 ± 0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(R)-(+)1-phenyl-1-cyclohexylmethanol</td>
<td>1.0 ± 0.3</td>
<td>17.8 ± 2.4</td>
<td>0.6 ± 0.2</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(S)-(−)1-phenyl-1-cyclohexylmethanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.44</td>
<td>0.75 ± 0.1</td>
</tr>
</tbody>
</table>

Values for apparent $K_m$, $V_{max}$, $k_{cat}/K_m$, and $K_i$ are expressed in mM, nmol min$^{-1}$ (mg of STa)$^{-1}$, and mM, respectively. Values for log P were calculated as described under Materials and Methods.

<sup>a</sup> $k_{cat}/K_m$ for the (R)-(+) enantiomer divided by $k_{cat}/K_m$ for the (S)-(−) enantiomer.
Inhibitor are represented as follows: calculated by non-linear least squares analysis (39). Concentrations of the values for competitive inhibition of the sulfation of \( \alpha \)-alkyl-substituted chiral benzylic alcohols as model substrates. Although the difference in the configuration of the benzylic carbon will not affect the overall hydrophobic character of the two enantiomers, steric interactions with the active site of the enzyme may be affected. Thus, the enantioselectivity of STa may be one indication of the steric constraints that define the binding of substrates and inhibitors at the active site of the enzyme.

The results of our studies indicated that as the length of the \( \alpha \)-alkyl chain on a chiral benzylic alcohol was increased, the extent of stereoselectivity in the reaction catalyzed by STa also increased. Moreover, the enzyme showed absolute stereospecificity for the enantiomers of 1-phenyl-1-cyclohexylmethanol, wherein the \( \alpha \)-alkyl substituent was the sterically bulky cyclohexyl group. The \((R)(+)-\) enantiomer of 1-phenyl-1-cyclohexylmethanol was a substrate for STa, while the \((S)(-)-\) enantiomer was a competitive inhibitor of STa-catalyzed sulfation of p-butylbenzyl alcohol. In addition, the apparent \( K_m \) value for \((R)(+)-\)1-phenyl-1-cyclohexylmethanol was higher than would be expected on the basis of a comparison with either \((R)(+)-\)1-phenyl-1-hexanol or \((R)(+)-\)1-phenyl-1-heptanol. The \( V_{\text{max}} \) value for \((R)(+)-\)1-phenyl-1-cyclohexylmethanol was also significantly different from that observed with \((R)(+)-\)1-phenyl-1-hexanol and \((R)(+)-\)1-phenyl-1-heptanol. Neither this 10-fold decrease in \( V_{\text{max}} \) nor the observed increase in the apparent \( K_m \) value was consistent with the hydrophobic characteristics of these molecules. Thus, the presence of an \( \alpha \)-cyclohexyl substituent on the benzylic carbon caused steric interactions to be much more important in the reaction. Our results support the conclusion that a specific orientation of the benzylic hydroxyl on the substrate must be achieved at the active site of STa before catalysis occurs, and that steric factors may combine with the stereochemical configuration of the substrate or inhibitor to determine the catalytic efficiency of the enzyme.

The stereoselectivity observed with benzylic alcohols as substrates for STa was also seen with the hydroxysteroid androsterone and epiandrosterone. The structure of androsterone differs from epiandrosterone only in the configuration of the 3-hydroxyl group, that is, an \( \alpha \)-orientation for androsterone and a \( \beta \)-orientation for epiandrosterone. Although these hydroxysteroids exhibit a stereoselectivity that is similar to some of the enantiomeric benzylic alcohols, they show a substrate inhibition at higher concentrations that has not yet been observed with the \( \alpha \)-substituted benzylic alcohols. This substrate inhibition resembles that previously seen with dehydroepiandrosterone as substrate for STa (8). In the case of \( \alpha \)-alkyl-substituted benzylic alcohols, concentrations approaching the limit of solubility for each substrate did not yield any substrate inhibition.

These observations on substrate inhibition, as well as the relatively low degree of stereoselectivity for androsterone and epiandrosterone with rat hepatic STa, contrast with reports on two other 3-hydroxysteroid sulfotransferases that have been characterized in the guinea pig (20, 21). Although a significant sequence identity of approximately 65% was reported between the enzymes from guinea pig and the rat hydroxysteroid sulfotransferase (20, 21), it is apparent that the differences in protein sequence are sufficient to affect the kinetics and specificity of the enzymes from the two species. Although substrate inhibition was seen for 3-hydroxysteroid substrates of STa, no such interactions were reported for either of the 3-hydroxysteroid sulfotransferases from guinea pig (20, 21). Moreover, results obtained by cloning and expressing the guinea pig \( \alpha \)-3-hydroxysteroid sulfotransferase in CHO-K1 cells indicated that this enzyme did not act on 3\( \beta \)-hydroxyl groups (21). Thus, there are distinct differences in specificity and kinetics between the hydroxysteroid sulfotransferases present in guinea pig and rat.

Additional documentation of species differences in the degree of stereoselectivity of hydroxysteroid sulfotransferases has been recently provided by Glatt et al., who have reported that 1-(1-pyrenyl)ethanol was activated to a mutagen with higher enantioselectivity by Salmon-
nella strains that contained human hydroxysteroid sulfotransferase than those that contained rat STa (27). In both strains, the (R)-(+)-enantiomer was more mutagenic than the (S)-(−)-enantiomer, and this difference in mutagenicity was attributed to higher rates of sulfation (27). Thus, although there is substantial homology between the amino acid sequences (i.e., 76% overall similarity between human hydroxysteroid sulfotransferase and rat STa), there are differences in the magnitude of stereoselectivity displayed by the hydroxysteroid sulfotransferases from these two species. Such differences are likely to be based on relatively subtle differences in steric interactions between enzyme and substrate at the active site that give rise to selectivity for the binding of one enantiomer in the proper orientation for sulfuryl transfer. Our findings with (S)-(−)-1-phenyl-1-cyclohexylethanol indicate that an enantiomer that is not properly oriented for sulfuryl transfer in the active site is nevertheless bound due to its hydrophobic characteristics and can thus serve as a competitive inhibitor. A more complete understanding of the reasons why interactions with specific amino acids at the active site would give rise to the observed stereospecificity will await further elucidation of the structures of the active sites of these sulfotransferases.

In summary, our results clearly indicate that the stereochimistry of a chiral benzylic alcohol is an important factor in its interaction with STa. In molecules with a large bulky substituent on the chiral benzylic carbon, STa shows a marked preference for one enantiomer as substrate. Therefore, both the effect of hydrophobicity of the molecule and the steric effects that are related to the substituents on the chiral benzylic carbon atom are important determinants of the type and efficiency of interactions that occur between STa and chiral benzylic alcohols. Such relationships between chemical structure and activity as substrates and inhibitors for STa may prove useful in explaining and predicting the interactions of this sulfotransferase with other xenobiotics, as well as with molecules of endogenous origin.

References