Enantioselective and Diastereoselective Hydroxylation of Bufuralol. Absolute Configuration of the 7-(1-Hydroxyethyl)-2-[1-hydroxy-2-(*tert*-butylamino)ethyl]benzofurans, the Benzylic Hydroxylation Metabolites

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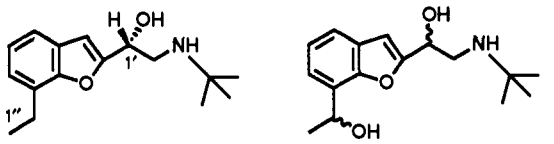
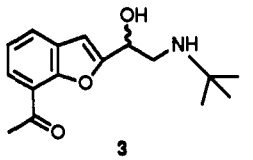
Asymmetric synthesis of the diastereomeric 7-(1-hydroxyethyl)-2-[1-hydroxy-2-(*tert*-butylamino)ethyl]benzofurans (2), the benzylic hydroxylation metabolites of bufuralol (1), is described, and the absolute configurations of these diastereomers are assigned. 1″-Oxobufuralol (3) was reduced with a complex of (2*S*)-(−)-2-amino-3-methyl-1,1-diphenylbutan-1-ol and borane, yielding 2, which had a 95:5 ratio of the possible 1″*R* and 1″*S* isomers as determined by HPLC. Separation of the resulting diastereomers was facilitated by derivatization with the enantiomers of 1-phenethyl isocyanate (PEIC). The absolute configurations 1′*S*,1″*R* and 1′*R*,1″*R* were assigned to the diastereomers formed in excess, **2c** and **2b**, on the basis of the known stereochemistry of reduction of closely related alkyl phenyl ketones to *R* alcohols by using this chiral borane reagent. The circular dichroism spectra of the four isomeric benzylic alcohols were in agreement with these assignments. In the presence of the rat liver microsomal fraction, benzylic hydroxylation of bufuralol was significantly product stereoselective favoring formation of diastereomers with the 1″*R* absolute stereochemistry at the new chiral center in products from (1′*R*)-1 by a ratio of 4.5:1 [(1′*R*,1″*R*)-2:(1′*R*,1″*S*)-2] and by nearly 8:1 [(1′*S*,1″*R*)-2:(1′*S*,1″*S*)-2] from (1′*S*)-1. (1′*R*)-Bufuralol was more rapidly hydroxylated than was (1′*S*)-1, by about 3-fold. In the presence of human liver microsomes, (1′*R*)-bufuralol was also more rapidly hydroxylated than was (1′*S*)-1, by ca. 2.5-fold. However, product stereoselectivity from the 1′*R* enantiomer was reversed from that observed in the rat liver microsomal oxidation, with more (1″*S*)-carbinol being formed than 1″*R* isomer by nearly 4-fold. From (1′*S*)-1, about equal amounts of the two possible hydroxybufuralol diastereomers were formed. The results from the human liver microsomal studies are consistent with observed enantioselectivity of hydroxylation of bufuralol in vivo in humans.

Bufuralol (1) is a widely studied, potent, nonselective β-adrenergic receptor antagonist with partial β_2-agonist properties.[1-4] It is a chiral molecule, and the 1′*S* enantiomer is significantly more active in adrenergic assays.[5] The metabolism of bufuralol is complex, affording several oxidative metabolites,[6] some of which interact with β-adrenergic receptors. The isomeric benzylic alcohols (**2a–d**) and their corresponding ketones (**3**) may contribute to the pharmacological properties observed after bufuralol administration.[7] Of the two diastereomeric alcohols derived from (1′*S*)-bufuralol, one is 1.5–3 times as potent as (1′*S*)-1 as an antagonist, and it is a more potent β_2-agonist by more than 50-fold.[5] The other diastereomer is less potent than (1′*S*)-1, and it is a more active β_2-agonist by about 2-fold. The (1′*S*)-ketone (1′*S*-**3**) is more potent than (1′*S*)-1 as an agonist by about 50-fold, but it is about equipotent in antagonist assays. Like (1′*R*)-bufuralol, the diastereomeric alcohols and the ketone with the 1′*R* configuration are very weak or inactive as β-adrenergic antagonists.[5]





The individual enantiomers of 1 appear to be metabolized at different rates,[7,8] and benzylic hydroxylation makes a different contribution to the overall profile of metabolites from each enantiomer.[4-8] Some of the oxidative pathways appear to require cytochrome P_{450DB}-catalyzed oxidation,[9-16] and a deficiency in this cytochrome P_{450} mono-

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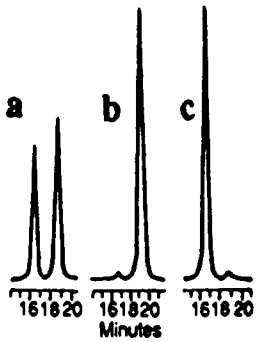


Figure 1. (a) HPLC separation of (1′*R*)-1 and (1′*S*)-1 as their (*S*)-phenylethylurea derivatives; (b) resolved (1′*S*)-1 as its (*S*)-phenylethylurea derivative, 96% ee; (c) resolved (1′*R*)-1 as its (*S*)-phenylethylurea derivative, 96% ee.

oxygenase (P_{450}IID6) in poor metabolizers accounts for the observed genetic polymorphism, an autosomal recessive trait, which occurs in ca. 3–7% of Caucasian populations.[14,15]

Although the four diastereomers of bufuralol alcohols **2a–d** have been synthesized from (1′*S*)- and (1′*R*)-bufuralol in pairs [**2a** and **2c** from (1′*S*)-**1**, and **2b** and **2d** from (1′*R*)-**1**], and members of these diastereomeric pairs have been separated as their oxazolidinone derivatives by HPLC, the absolute configurations of the four diastereomers have not been determined.[5] We sought to determine their absolute configurations in order to examine the stereoselectivity of the side-chain hydroxylation of bufuralol by liver microsomal enzymes. Preparation of pure enantiomers of bufuralol (**1**) and stereoselective synthesis of the diastereomeric side-chain-hydroxylated metabolites **2a–d** were undertaken to assign their absolute configurations.

Chemistry

Racemic bufuralol (**1**), prepared by our recently reported method,[17] was resolved by preparation of the diastereomeric salts with (+)- and (−)-di-*p*-toluoyltartaric acid and fractional crystallization.[1] The optical purity of resolved (1′*R*)-(+)- and (1′*S*)-(−)-bufuralol was determined by

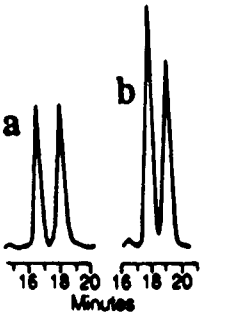
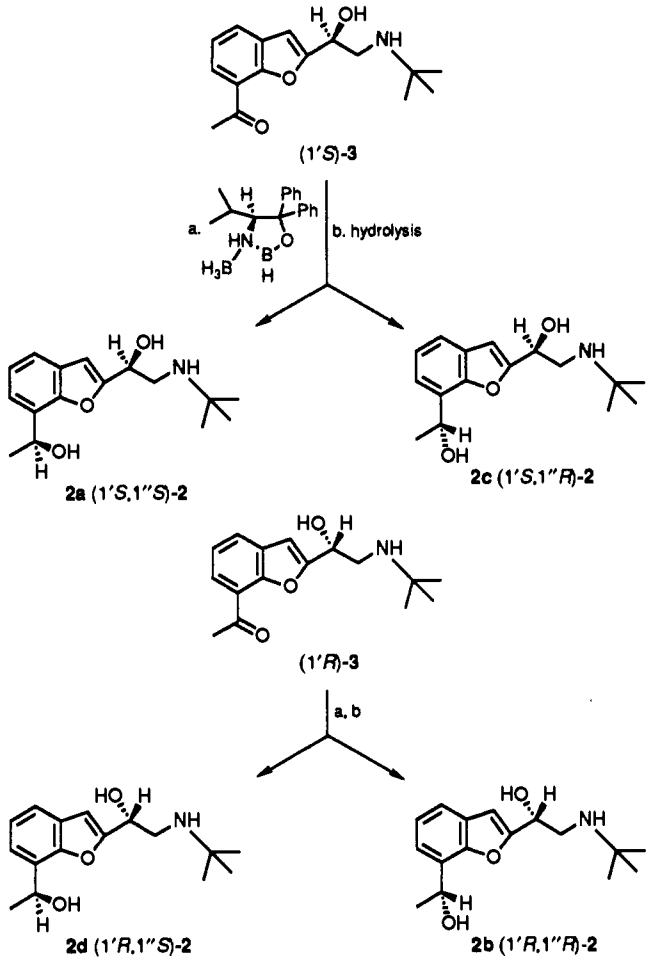


Figure 2. (a) HPLC separation (methanol–water 67:33) of the diastereomers **2a** and **2c** derivatized with (*R*)-PEIC, retention times 16.47 and 18.04 min; (b) HPLC separation of the diastereomers **2b** and **2d** derivatized with (*R*)-PEIC, retention times 17.70 and 18.98 min.

Scheme I. Chiral Borane Reduction of (1′*S*)- and (1′*R*)-3



HPLC separation of the diastereomeric ureas formed from reaction with (*S*)-(−)-1-phenylethyl isocyanate (PEIC). Base-line separation of the urea derivatives of (1′*R*)-**1** and (1′*S*)-**1** was obtained (Figure 1), with the (*S*)-(−)-1-phenylethylurea derivatives of (1′*R*)-(+)- and (1′*S*)-(−)-bufuralol being eluted with retention times of 16.2 and 18.7 min, respectively. The optical purity of the resolved enantiomers (1′*R*)-**1** and (1′*S*)-**1** was greater than 96% ee. Attempts to use HPLC columns with chiral supports prepared from acyl derivatives of cellulose bonded to macroporous silica failed to separate these enantiomers.

When the four side-chain-hydroxylated bufuralol metabolites[5] were derivatized with (*R*)-(+)-PEIC, the phenylethylureas were separated reproducibly on a 25-cm octadecylsilane (ODS) column eluting with a 67:33 meth-

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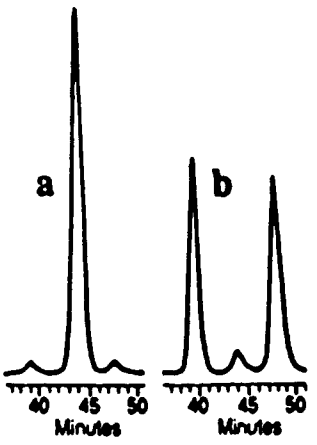

Figure 3. (a) HPLC separation (methanol–water 60:40) of the diastereomers of **2** obtained from chiral borane reduction, derivatized with (*R*)-PEIC, **2a**:(**2b** + **2c**):**2d** (2.5:95:2.5); (b) HPLC separation of the diastereomers of **2** obtained from chiral borane reduction, derivatized with (*S*)-PEIC, **2b**:(**2a** + **2d**):**2c** (47.5:47.5).

anol–water system; the four diastereomeric standards had retention times of 16.47, 17.70, 18.04, and 18.98 min for **2a**, **2b**, **2c**, and **2d**, respectively (Figure 2). This method gave base-line separation of **2a** and **2d**, but poor resolution of **2b** from **2c**. Enantiomeric pairs **2a** and **2b**, and **2c** and **2d** were readily separated from each other, but diastereomers **2b** and **2c** were not. However, as expected, when the four isomers were derivatized with (*S*)-(−)-PEIC, the order of elution changed to **2b**, **2a**, **2d**, **2c**, giving a good base-line separation of **2b** and **2c**.

Although the absolute configuration of the ethanolamine side chain is known [**2a** and **2c** have the 1′*S* chiral center and **2b** and **2d** have the 1′*R* chiral center based on synthesis from (1′*S*)-**1** and (1′*R*)-**1**, respectively],[5] the absolute configuration at the second chiral center cannot be assigned from this information. To establish the absolute configurations of the new side-chain hydroxyl groups in **2a–d**, chiral reduction of 1″-oxobufuralol (**3**) was performed.

Chiral reduction of aromatic ketones has been established as a method for obtaining chiral alcohols of known absolute configuration and in high enantiomeric excess.[18-24] In our experience, the Itsuno chiral reducing agent prepared from 2 equiv of borane and (2*S*)-(−)-2-amino-3-methyl-1,1-diphenylbutan-1-ol, derived from valine,[18-20] was stereoselective for reduction of alkyl aromatic ketones.[25]

The reduction of the racemic 1″-oxobufuralol (**3**) (Scheme I) was carried out at 23 °C, and the diastereomeric bufuralol alcohols **2a–d** were separated from the chiral auxiliary by flash chromatography. The yield of alcohols was 92%, and HPLC of (*R*)-(+)-PEIC derivatives of the reaction mixture on an ODS column using methanol–water as the mobile phase gave separation of the resulting four diastereomers into three components of approximate ratio 2.5:95:2.5 (Figure 3), the two major overlapping diastereomers having the 1″*R* configuration. Their retention times correspond to **2b** and **2c**. When (*S*)-PEIC was used as derivatizing reagent, the previous two overlapping center components became the components with the shortest and longest retention times, as expected, affording ratios of ca. 47.5:5:47.5 for the diastereomers (Figure 3). Clearly, changing the absolute stereochemistry of the derivatizing reagent results in separation of the previously unresolved diastereomers.

On the basis of the chiral borane reagent reduction, compounds **2c** and **2b** must have the 1″*R* absolute configuration, and they have the 1′*S* and 1′*R* configurations, respectively, in the ethanolamine side chain based on their synthetic origin. Thus **2c** is (1′*S*,1″*R*)-**2** and **2b** is (1′*R*,1″*R*)-**2**. Analogously, the absolute configurations of the other diastereomers were assigned; **2a** is (1′*S*,1″*S*)-**2** and **2d** is (1′*R*,1″*S*)-**2**.

The circular dichroism spectra of the bufuralol enantiomers, the four side-chain-hydroxylated metabolites, and (1″*R*)-**4** were recorded (Figure 4). Comparison of the CD spectra of (1′*S*)-bufuralol [(1′*S*)-**1**] to (1′*S*,1″*S*)-hydroxybufuralol (**2a**) showed for **2a** a significant enhancement of the negative Cotton effect of the short wavelength ^1L_a transition at 215 nm. Likewise, a positive Cotton effect is observed in the CD spectrum of (1′*R*)-**1** and an enhanced positive Cotton effect is observed for (1′*R*,1″*R*)-hydroxybufuralol (**2b**). Compared to the intensity of the Cotton effect at 215 nm of (1′*S*)-**1**, the negative Cotton effect in the CD spectrum of (1′*S*,1″*R*)-hydroxybufuralol (**2c**) is of reduced intensity, and the positive Cotton effect observed for (1′*R*,1″*S*)-hydroxybufuralol (**2d**) is less intense than that of (1-*R*)-**1**.

Model compound (1″*R*)-**4**[26] was prepared for CD comparison. Its CD curve showed a positive Cotton effect in the 215-nm region, similar to that of (1′*R*)-**1**, although of considerably lower intensity [θ_{215} = 5500 for (1″*R*)-**4** vs θ_{215} = 11 000 for (1′*R*)-**1**]. These data suggest when the two chiral centers α to the benzofuran ring system have the same absolute configuration, the observed Cotton effects are approximately additive. When the two chiral centers have opposite configurations, the observed Cotton effect

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(26) Compound (1′*R*)-**4** was prepared by chiral reduction of the corresponding ketone (**7**), as described in the Experimental Section. The optical purity of (1″*R*)-**4** was greater than 94% ee by 300-MHz ^1H NMR analysis of the MTPA esters. For comparison, the chiral center in **4** is referred to as C-1″ analogous to the C-1″ center in **2**.

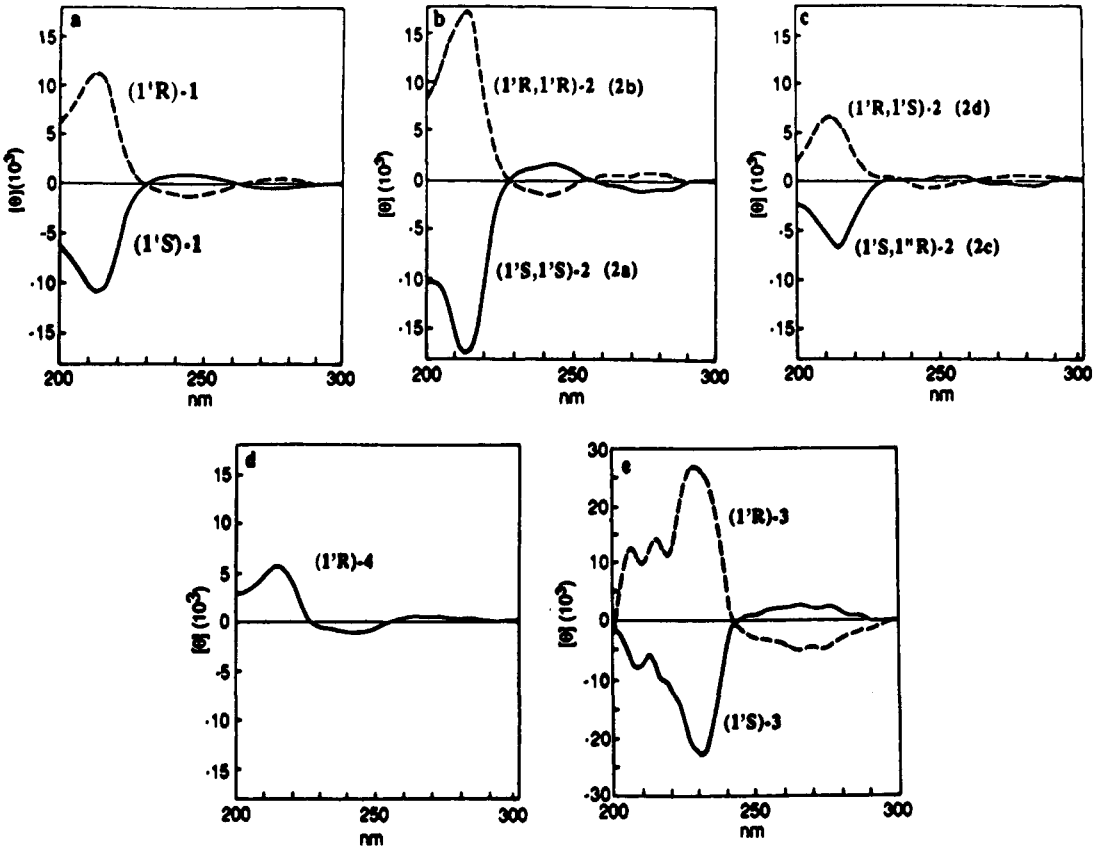


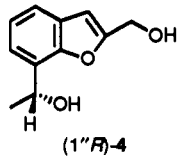
Figure 4. Circular dichroism spectra: (a) (1'*R*)-1 and (1-*S*)-1, (b) (1'*R*,1"*R*)-2 and (1'*S*,1"*S*)-2, (c) (1'*R*,1"*S*)-2 and (1'*S*,1"*R*)-2, (d) (1"*R*)-4, (e) (1'*R*)-3 and (1'*S*)-3.

Table I. Rates of formation of α-Hydroxybufuralol Diastereomers in Rat and Human Liver Microsomes[a]

	(1'*R*)-bufuralol			(1'*S*)-bufuralol			
	(1'*R*,1"*R*)-2 (2b)	(1'*R*',1"*S*)-2 (2d)	1"*R*/1"*S* ratio	(1'*S*,1"*R*)-2 (2c)	(1'*S*,1"*S*)-2 (2a)	1"*R*/1"*S* ratio	enantioselectivity[b]
rat liver	2270 ± 127	500 ± 79	4.5	741 ± 12	96.1 ± 2.0	7.7	3.30
human liver							
108	74.5 ± 6.4	363 ± 14.4	0.21	86.5 ± 5.6	101.0 ± 4.0	0.86	2.33
116	75.3 ± 10.8	355 ± 0.8	0.21	83.3 ± 3.2	74.9 ± 2.0	1.11	2.71
114	61.7 ± 3.2	211 ± 13.6	0.29	62.1 ± 8.4	58.8 ± 10.4	1.05	2.25
112	54.5 ± 0.2	136 ± 2.0	0.40	48.9 ± 2.8	32.0 ± 4.0	1.53	2.35
mean ± SD	66.5 ± 10.1	266 ± 111	0.28 ± 0.09	70.2 ± 17.8	66.6 ± 28.9	1.14 ± 0.28	2.41 ± 0.20

[a] Rates are expressed as pmol min^{-1} (mg of microsomal protein)$^{-1}$. Each value represents the mean of three determinations. [b] Enantioselectivity = [products from (1'*R*)-1] + [products from (1'*S*)-1]; [(1'*R*,1"*S*)-2 + (1'*R*,1"*R*)-2] + [(1'*S*,1"*R*)-2 + (1'*S*,1"*S*)-2].

represents the difference of the two effects observed independently. The effect of the 1'*R* chiral center is larger and thus is predominant.



(1"*R*)-4

The CD spectra of 1"-oxobufuralol enantiomers show more complex CD spectra with multiple Cotton effects (Figure 4). Their strongest Cotton effects are shifted to ca. 230 nm with greater intensities than observed for the enantiomers of 1, probably due to the effects of the transitions of the additional ketone carbonyl group chromophore added to the transitions due to aromatic chromophore.

Although we have not been able to compare our CD observations on these alkylbenzofuranylcarbinols to other related benzofuranyl systems due to the lack of comparable literature data, the data on these nine compounds are internally consistent[27] and in agreement with the assignments from chiral borane reduction.

Metabolism

The benzylic hydroxylation of the enantiomers of 1 was examined in the presence of rat and human liver microsomes to determine the diastereoselectivity of hydroxylation, i.e., whether formation of the new chiral center on hydroxylation occurs with some degree of enantioselectivity and/or product stereoselectivity. Metabolites were ex-

(27) No other benzofuran carbinols have been reported. A variety of sequence rules for other alkyl aryl carbinols have been generated: Verbit, L.; Pfeil, E.; Becker, W. Optically Active Aromatic Chromophores. VI. The Thiophene and Furan Chromophores. *Tetrahedron Lett.* **1967**, 2169–2172. Neilsson, D. G.; Zaker, U.; Schimgeour, C. M. The Preparation of Optically Active 3-Aryl-1,4-dioxasprio[4,5]decanes and their 2-Oxo-derivatives; a Study of the Cotton Effects Associated with these Compounds. *J. Chem. Soc.* **1971**, 898–904. Verbit, L.; Price, H. C. Optically Active Aromatic Chromophores. XI. Circular Dichroism Studies of Some 1-Substituted 2-Phenylcyclohexanes. *J. Am. Chem. Soc.* **1972**, *94*, 5143–5152. Mitscher, L. A.; Kautz, F.; LaPidus, J. Optical Rotatory Dispersion and Circular Dichroism of Diastereoisomers. I. The Ephedrines and Chloramphenicols. *Can. J. Chem.* **1969**, *47*, 1957–1963.

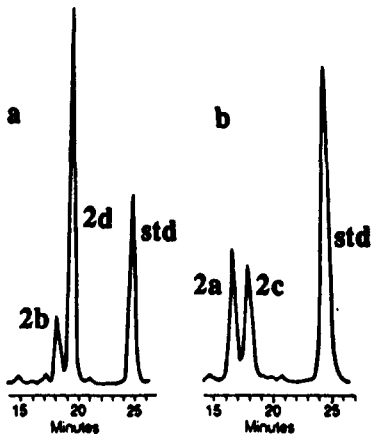


Figure 5. (a) HPLC separation of the human liver microsomal metabolite diastereomers **2b** and **2d** formed from (1′*R*)-bufuralol, derivatized with (*R*)-PEIC, retention times 18.0 and 19.2 min, internal standard (1′*R*)-**3** as its (*R*)-PEIC derivative elutes at ca. 25.0 min; (b) HPLC separation of the human liver microsomal metabolite diastereomers **2a** and **2c** formed from (1′*S*)-bufuralol, derivatized with (*R*)-PEIC, retention times 16.6 and 17.9 min; internal standard (1′*R*)-**3** as its (*R*)-PEIC derivative elutes at ca. 25.0 min.

tracted from the microsomal incubations, derivatized with (*R*)-PEIC and separated by HPLC. The hydroxylated products were quantitated using (1″*S*)-**3** as the internal standard (Figure 5). Standards of the diastereomeric alcohols **2a–d** confirmed the structural assignments. The results appear in Table I.

In the presence of rat liver microsomes, enantioselectivity was observed with (1′*R*)-bufuralol [(1′*R*)-**1**] being more rapidly hydroxylated than the 1′*S* enantiomer by about 3.3-fold. Benzylic hydroxylation was highly product stereoselective with formation of diastereomers incorporating a new 1″*R* chiral center being preferred by more than 4.5:1 from (1′*R*)-**1** and 8:1 from (1′*S*)-**1**. The rates of microsomal hydroxylation observed were comparable to those previously reported by DuPont et al.[28]

In the presence of human liver microsomes, (1′*R*)-**1** was more rapidly hydroxylated than its 1′*S* enantiomer by about 2.5-fold, consistent with results reported by previous investigators,[10-13] and the turnover rates were comparable to previous reports from studies using liver microsomes from extensive metabolizers.[10-13,16] Benzylic hydroxylation of (1′*R*)-bufuralol is highly product stereoselective, but opposite to the diastereoselection in the presence of rat liver microsomes, favoring formation of a new 1″*S* chiral center by a ratio of nearly 4:1, averaging about 79% of the 1′*R*,1″*S* diastereomer and 21% (1′*R*,1″*R*)-**2**. Compound (1′*S*)-**1**, the less rapidly turned over enantiomer of bufuralol, afforded about equal amounts of (1″*R*)- and (1″*S*)-benzylic alcohol diastereomers. The benzylic alcohols arising from the more active 1′*S* enantiomer of bufuralol are more likely to contribute to the pharmacological properties of the parent drug.[5] Consistent with the presence of greater amounts of the diastereomeric alcohols from (1′*R*)-**1**, which is hydroxylated to a greater extent, the circulating levels of (1′*R*)-**3** are reported to be higher than for its 1′*S* enantiomer.

(1′*R*)-Bufuralol is transformed to the diastereomeric benzylic alcohols with a high degree of product stereoselectivity and significant enantioselectivity. The chiral center of the ethanolamine side chain has a significant effect on both the rate and the diastereoselection of the hydroxylation process, and thus this center appears to be an important determinant of the interaction of this substrate with the site(s) of the hydroxylation. The determination of the absolute stereochemistry of the 1″-hydroxyl group in **2** and the HPLC separation of these derivatives of **2** will facilitate more in-depth examination of stereochemical aspects of this important hydroxylation process.

Experimental Section

High-field ^{1}H NMR spectra were recorded on Varian 300 MHz spectrometer. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. HPLC analysis of bufuralol and hydroxybufuralol derivatives was performed using a LKB 2152 LC controller equipped with a LKB 2150 HPLC pump, a Waters 484 variable-wavelength UV detector (248 nm), and a Spectra Physics SP 4100 computing intergrator. Optical rotation measurements were determined on a JASCO DIP-4 digital polarimeter. Circular dichroism (CD) spectra were recorded on a JASCO 720 spectropolarimeter equipped with Genesis Systems computer and Hewlett-Packard 7475A plotter.

(1′*R*)-1-[1-Hydroxy-2-(*tert*-butylamino)ethyl]-7-ethylbenzofuran Hydrochloride [(1′*R*)-1·HCl].[1] To a solution of racemic **1** (4.0 g, 15.3 mmol) in 15 mL of hot ethyl acetate, (−)-di-*p*-toluoyltartaric acid (5.9 g, 15.3 mmol) in 15 mL of hot ethyl acetate was added. The solution was cooled, and the precipitated salt was recrystallized three times from absolute ethanol to give a salt, mp 186–188 °C. The salt was treated with 5 mL of aqueous 10% NaOH, and the free base was extracted with ethyl acetate. The combined ethyl acetate extracts were dried (Na_2SO_4), filtered, and evaporated. The residue was dissolved in absolute ethanol and acidified with HCl, and the solvent was evaporated. The residue obtained was crystallized from ethyl acetate–cyclohexane to give the HCl salt of (1′*R*)-**1** (0.621 g) as white crystals: mp 144–146 °C (lit.[27] mp 122–3 °C); $[\alpha]_{589}$ +43.5° (ethanol, c = 0.6) [lit.[1] $[\alpha]_{365}$ +135° (ethanol, *c* = 1.0)]; CD (*c* = 0.110 MeOH) $[\theta]_{300}$ 0, $[\theta]_{281}$ +326, $[\theta]_{274}$ +370, $[\theta]_{260}$ 0, $[\theta]_{247}$ −1140, $[\theta]_{230}$ 0, $[\theta]_{213}$ +11 250 deg cm^2 dmol^{-1}.

(1′*R*)-(+)-Bufuralol (1′*R*-**1**) (3.0 mg, 11.4 μmol) was derivatized by stirring with (*S*)-(−)-phenylethyl isocyanate (15 μL, 120 μmol) (Fluka) in 3 mL of $CHCl_3$ at 23 °C for 30 min. The solution was evaporated, and the residue was dissolved in methanol for HPLC analysis. HPLC of the (*S*)-PEIC-derivatized enantiomers of an Ultrasphere ODS 5-μm column, 4.6 mm × 15 cm (Altex), eluting with methanol–water (75:25) at a flow rate of 1 mL/min separated them. The phenylethylurea derivatives of (1′*R*)-(+)-bufuralol and (1′*S*)-(−)-bufuralol had retention times 16.2 and 18.7 min, respectively, indicating the presence of 1′*R*-(+) enantiomer of bufuralol [(1′*R*)-**1**] in 96% ee (Figure 1).

(1′*S*)-2-[1-(Hydroxyethyl)-2-(*tert*-butylamino)ethyl]-7-ethylbenzofuran Hydrochloride [(1′*S*)·HCl].[1] The free base (1.9 g, 7.3 mmol) enriched in the 1′*S* isomer of **1** was obtained from the mother liquors used in the resolution of the (1′*R*)-**1**. Neutralization of the salt and ethyl acetate extraction afforded the free base of **1** enriched in the 1′*S*-(−) isomer of **1**. It was dissolved in 10 mL of hot ethyl acetate and was then mixed with a solution of (+)-di-*p*-toluoyltartaric acid (2.81 g, 7.3 mmol) in 10 mL of hot ethyl acetate. The precipitate obtained when the solution was cooled in the refrigerator was crystallized three times from absolute ethanol to give a salt, mp 180–181 °C. The salt was treated with 5 mL of aqueous 10% NaOH, and (1′*S*)-**1** was extracted with ethyl acetate. The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated. The residue was treated with absolute ethanol acidified with gaseous HCl and evaporated. The hydrochloride salt obtained was crystallized from ethyl acetate–diethyl ether gave (1′*S*)-**1** (0.630 g) as white crystals: mp 145–6 °C (lit.[29] mp 123 °C); $[\alpha]_{589}$ −43.5° (ethanol, *c* = 0.6) [lit.[1]

(28) DuPont, H.; Davies, D. S.; Strolin-Benedetti, M. Inhibition of Cytochrome P-450-dependent Oxidation Reactions by MAO Inhibitors in Rat Liver Microsomes. *Biochem. Pharmacol.* **1987**, *36*, 1651–1657.

(29) In ref 1, the melting point of racemic 1·HCl is cited as 145–146 °C, and the melting points of the HCl salts of the enantiomers are cited as 122–123 °C. We find the melting points of the HCl salts of the enantiomers to be 145–146 °C and of racemic 1·HCl is 122–123 °C.

$[\alpha]_{365}$ −136° (ethanol, c = 1.0)]; CD (c = 0.1040, MeOH) $[\theta]_{300}$ 0, $[\theta]_{281}$ −475, $[\theta]_{275}$ −525, $[\theta]_{260}$ 0, $[\theta]_{243}$ +945, $[\theta]_{230}$ 0, $[\theta]_{213}$ =10870 deg cm^2 dmol^{-1}.

(1′*S*)-(−)-Bufuralol [(1′*S*)-1] (3.0 mg, 11.4 μmol) was derivatized by stirring with (*S*)-(−)-phenylethyl isocyanate (15 μL, 120 μmol) in 3 mL of $CHCl_3$ at 23 °C for 30 min. The solvent was evaporated, and the residue was dissolved in methanol for HPLC analysis. HPLC of the (*S*)-PEIC derivatized enantiomers on a Ultrasphere ODS 5-μm column, 4.6 × 15 cm (Altex), eluting with methanol–water (75:25) at a flow rate of 1 mL/min separated them. The phenylethylurea derivatives of (1′*R*)-(+)-bufuralol and (1′*S*)-(−)-bufuralol were readily separated, having retention times of 16.2 and 18.7 min, respectively. The 1′*S* enantiomer was present in 96% ee (Figure 1). For comparison, racemic bufuralol was derivatized by the same procedure (Figure 1).

Asymmetric Reduction of 7-Acetyl-2-[1-hydroxy-2-(*tert*-butylamino)ethyl]benzofuran (1″-Oxobufuralol) (3). To a solution of (2*S*)-2-amino-3-methyl-1,1-diphenylbutan-1-ol (1.10 g, 4.31 mmol), prepared according to the method of Itsuno et al.[19] in dry THF under nitrogen at −78 °C, was added dropwise with stirring a 1 M borane solution in THF (8.6 mL, 8.6 mmol). The mixture was allowed to warm to room temperature slowly and then was stirred at this temperature for 12 h. A solution of racemic **3** (0.072 g, 0.26 mmol) in 2 mL of dry THF was added dropwise, and the reaction mixture was stirred at room temperature for 4 h. The mixture was cooled to 0 °C, and after 5 mL of aqueous ammonia was added, it was stirred for 4 h. The reaction mixture was then extracted with (4 × 25 mL) of EtOAc. The organic phase was dried (Na_2SO_4), filtered, and evaporated. The residue was flash chromatographed on silica gel eluting with EtOAc–MeOH–Et_3N (95:5:1). The chiral auxiliary was recovered (0.81 g, 74%) and the diastereomers of **2** (0.066 g, 92%) were obtained from cyclohexane as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.45 (1 H, d), 7.32 (1 H, d), 7.22 (1 H, t), 6.68 (1 H, s), 5.38 (1 H, m), 4.83 (1 H, m), 3.03 (2 H, m), 1.64 (3 H, d), 1.15 (9 H, s).

The hydroxybufuralol isomer mixture obtained from asymmetric reduction (1.0 mg) was derivatized by stirring with (*R*)-(+)-phenylethyl isocyanate (5 μL, 40 μmol) in 1 mL of $CHCl_3$ at 23 °C for 30 min. The solution was evaporated and the residue was dissolved in methanol for HPLC analysis. HPLC of the PEIC-derivatized diastereomers on a Ultrasphere 5-μm, 4.6 mm × 25 cm (Beckman) ODS column eluting with methanol–water (67:33) at a flow rate of 1 mL/min separated the derivatized diastereomers. The four diastereomers had retention times of 16.47, 17.70, 18.04, and 18.98 min for **2a**, **2b**, **2c**, and **2d**, respectively, resulting in substantial overlap of two components and difficulty in quantitating amounts of the less readily formed diastereomers. Changing the solvent mixture to 60:40 methanol–water gave longer retention times and improved resolution. This derivatization and separation procedure was repeated with (*S*)-(−)-PEIC, and the mixture of isomers were separated. When the mixture was derivatized with (*R*)-(+)-PEIC, the retention times were 39.20 min (2.5% **2a**), 43.81 min (95% **2b**, **2c**), and 47.68 min (2.5% **2d**) (Figure 3). When the mixture from the chiral reduction procedure was derivatized with (*S*)-(−)-PEIC and separated by HPLC under identical conditions, three peaks were observed with retention times 38.89 min (47.5% **2b**), 43.51 min (5% **2a**, **2d**), and 47.35 min (47.5% **2c**) (Figure 3). The derivatization was also repeated with (*R*)-(+)- and (*S*)-(−)-PEIC for each hydroxybufuralol isomer standard (**2a**, **2b**, **2c**, and **2d**), and these derivatives were used as standards in the HPLC analysis.

(1′*S*,1″*S*)-7-(1-Hydroxyethyl)-2-[1-hydroxy-2-(*tert*-butylamino)ethyl]benzofuran maleic acid salt [(1′*S*,1″*S*)-2 (2a)]: CD (c = 0.1318, MeOH) $[\theta]_{290}$ 0, $[\theta]_{282}$ −1145, $[\theta]_{273}$ −1250, $[\theta]_{263}$ −615, $[\theta]_{255}$ 0, $[\theta]_{243}$ +1540, $[\theta]_{229}$ 0, $[\theta]_{214}$ = 17 400 deg cm^2 dmol^{-1}.

(1′*R*,1″*R*)-7-(1-Hydroxyethyl)-2-[1-hydroxy-2-(*tert*-butylamino)ethyl]benzofuran maleic acid salt [(1′*R*,1″*R*)-2 (2b)]: CD (c = 0.1301, MeOH) $[\theta]_{300}$ 0, $[\theta]_{282}$ +485, $[\theta]_{275}$ +690, $[\theta]_{263}$ +460, $[\theta]_{255}$ 0, $[\theta]_{243}$ −1520, $[\theta]_{229}$ 0, $[\theta]_{214}$ +17 440 deg cm^2 dmol^{-1}.

(1′*S*,1″*R*)-7-(1-Hydroxyethyl)-2-[1-hydroxy-2-(*tert*-butylamino)ethyl]benzofuran maleic acid salt [(1′*S*,1″*R*)-2 (2c)]: CD (c = 0.1359, MeOH) $[\theta]_{290}$ 0, $[\theta]_{285}$ −465, $[\theta]_{279}$ −439, $[\theta]_{270}$ −410, $[\theta]_{263}$ 0, $[\theta]_{258}$ +355, $[\theta]_{251}$ +409, $[\theta]_{232}$ 0, $[\theta]_{214}$ −6700 deg cm^2 dmol^{-1}.

(1′*R*,1″*S*)-7-(1-Hydroxyethyl)-2-[1-hydroxy-2-(*tert*-butylamino)ethyl]benzofuran maleic acid salt [(1′*R*,1″*S*)-2 (2d)]: CD (c = 0.1380, MeOH) $[\theta]_{290}$, 0, $[\theta]_{276}$ +470, $[\theta]_{263}$ 0, $[\theta]_{247}$ −738, $[\theta]_{237}$ 0, $[\theta]_{212}$ +6530 deg cm^2 dmol^{-1}.

(1′*S*)-7-Acetyl-2-[1-hydroxy-2-(*tert*-butylamino)ethyl]benzofuran maleic acid salt [(1′*S*)-1″-Oxobufuralol maleic acid salt] [(1′*S*)-3]: CD (c = 0.1000, MeOH) $[\theta]_{300}$ 0, $[\theta]_{286}$ +875, $[\theta]_{276}$ +2340, $[\theta]_{267}$ +2616, $[\theta]_{257}$ +2080, $[\theta]_{249}$ +728, $[\theta]_{244}$ 0, $[\theta]_{231}$ −22 350, $[\theta]_{227}$ −20 810, $[\theta]_{222}$ −12 770, $[\theta]_{218}$ −9720, $[\theta]_{209}$ −7600 deg cm^2 dmol^{-1}.

(1′*R*)-7-Acetyl-2-[1-hydroxy-2-(*tert*-butylamino)ethyl]benzofuran maleic acid salt [(1′*R*)-1″-Oxobufuralol maleic acid salt] [(1′*R*)-3]: CD (c = 0.1100, MeOH) $[\theta]_{300}$ 0, $[\theta]_{286}$ −1660, $[\theta]_{273}$ −4790, $[\theta]_{265}$ −5025, $[\theta]_{259}$ −3735, $[\theta]_{251}$ −3010, $[\theta]_{244}$ 0, $[\theta]_{234}$ +22 810, $[\theta]_{228}$ +26 640, $[\theta]_{215}$ +14 240, $[\theta]_{207}$ +12 520 deg cm^2 dmol^{-1}.

7-Acetyl-2-(hydroxymethyl)benzofuran (7). A solution of 2-[5-bromo-2-(hydroxymethyl)benzofuran-7-yl]-2-methyl-1,3-dioxolane (5)[17] (250 mg, 0.80 mmol) in 100 mL of ethanol containing 5 mL of triethylamine and 100 mg of 5% Pd/C was subjected to reductive debromination by hydrogenation at 15 psi initial pressure. When the theoretical amount of hydrogen was absorbed, the catalyst was removed by filtration and the mixture was extracted with ethanol. The solution was evaporated, and the residue was extracted with 100 mL of dichloromethane. The organic extract was washed with water, dried (Na_2SO_4), and evaporated. Crystallization of the crude product from chloroform–petroleum ether (bp 30–60 °C) afforded **6**, the ethylene ketal of **7** (175 mg, 94%): mp 118–120 °C; ^{1}H NMR ($CDCl_3$) δ 7.47 (1 H, dd, J = 2.5, 7.7 Hz), 7.35 (1 H, dd, J = 2.6, 7.5 Hz), 7.16 (1 H, m), 6.64 (1 H, s), 4.79 (2 H, s), 4.07 (2 H, m), 3.86 (2 H, m), 1.86 (3 H, s).

A solution of ketal **6** (150 mg, 0.64 mmol) in a mixture of 10 mL of acetic acid and 2 mL of water was stirred under nitrogen for 10 h. The mixture was neutralized with saturated aqueous $NaHCO_3$ (cautiously) and extracted with 2 × 100 mL of dichloromethane. The combined organic extracts were washed with water, dried (Na_2SO_4), and evaporated. The residue was crystallized from chloroform–hexanes to give white crystalline ketone **7** (87 mg, 72%): mp 79–80 °C: ^{1}H NMR δ 7.75 (1 H, dd, J = 1.4, 7.8 Hz), 7.65 (1 H, dd, J = 1.4, 7.7 Hz), 7.20 (1 H, dd, J = 7.8, 7.7 Hz), 6.64 (1 H, s), 4.76 (2 H, s), 2.73 (3 H, s); ^{13}C NMR δ 196.59 (C=O), 157.68, 153.00, 129.79, 126.35, 125.21, 122.63, 121.86, 103.65, 57.72, 30.29.

(1′*R*)-2-(Hydroxymethyl)-7-(1-hydroxyethyl)benzofuran [(1′*R*)-4].[26] To a solution of (2*S*)-2-amino-3-methyl-1,1-diphenylbutan-1-ol (620 mg, 2.43 mmol),[19] in 10 mL dry THF under nitrogen at −78 °C, was added dropwise with stirring a 1 M borane solution in THF (5 mL, 5 mmol). The mixture was allowed to slowly warm to room temperature, and then it was stirred at room temperature for 12 h. A solution of **7** (50 mg, 0.26 mmol) in 2 mL of dry THF was added, and the reaction mixture was stirred at room temperature for an additional 4 h. The mixture was cooled to 0 °C, 5 mL of concentrated aqueous ammonia was added, and then stirring was continued for an additional 4 h at room temperature. The mixture was then evaporated to remove THF and the residue was extracted with 100 mL of EtOAc. The organic phase was washed with water, dried (Na_2SO_4), filtered, and evaporated. The residue was flash chromatographed on silica gel eluting with EtOAc–hexane (90:10). The chiral auxiliary (465 mg, 75%) was recovered, and the diol **7** was obtained as a white solid. Recrystallization from dichloromethane–petroleum ether (bp 30–60 °C) gave **4** as a white crystalline solid (44 mg, 88%): mp 111–112 °C; $[\alpha]_{589}$ +48.7° (MeOH, c = 0.3); ^{1}H NMR δ 7.42 (1 H, d, J = 7.6 Hz), 7.27 (1 H, d, J = 7.8 Hz), 7.19 (1 H, dd, J = 7.8, 7.6 Hz), 6.62 (1 H, s), 5.29 (1 H, q, J = 6.3 Hz), 4.70 (2 H, s), 1.61 (3 H, d, J = 6.3 Hz); ^{13}C NMR δ 156.38, 151.96, 128.94, 128.33, 122.86, 120.82, 120.25, 104.02, 66.60, 57.86, 23.40. The optical purity of **4** was 94% ee as determined by the ^{1}H NMR signals of diastereotopic methyl protons of the Mosher acid diesters [(*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid].[30,31]

(30) Dale, J. A.; Dull, D. L.; Mosher, H. S. α-Methoxy-α-trifluoromethylphenylacetic Acid, a Versatile Reagent for the Determination of Enantiomeric Composition of Alcohols and Amines. *J. Org. Chem.* 1969, *34*, 2543–2549.

CD (c = 0.1098, MeOH) $[\theta]_{290}$ 0, $[\theta]_{283}$ +318, $[\theta]_{271}$ +450, $[\theta]_{266}$ +425, $[\theta]_{258}$ 0, $[\theta]_{243}$ −1125, $[\theta]_{233}$ −735, $[\theta]_{228}$ 0, $[\theta]_{215}$ +5510 deg cm^2 dmol^{-1}.

Metabolic Studies. Rat and Human Liver Microsomes. Male Sprague-Dawley rats (approximately 150 g) were decapitated, and the livers were removed and rinsed with 0.01 M (pH 7.4) sodium phosphate buffer containing 1.15% KCl. The livers were then homogenized in three volumes of the same buffer with a Potter-Elvehjem Teflon pestle homogenizer. The homogenate was centrifuged at 9000g for 30 min, and the supernatant fraction was recentrifuged at 100000g for 1 h. The microsomal pellet was suspended in 0.01 M phosphate buffer containing 0.25 M sucrose and 1 mM EDTA. The protein concentration was 20–30 mg/mL. The entire operation was performed at 0–4 °C. Microsomal protein was determined by the method of Lowry et al.,[32] using bovine serum albumin as standard.

Human liver microsomes were prepared from tissue kept in the human liver bank of the Department.[33] Frozen liver (stored at −70 °C), 10 g, was thawed in distilled water at room temperature and then homogenized with three volumes of 50 mM potassium phosphate buffer, pH 7.4, containing 0.25 M sucrose and 1 mM EDTA, using a Potter-Elvehjem Teflon pestle homogenizer. The homogenate was centrifuged at 9000g for 30 min, and the supernatant fraction was centrifuged at 105000g for 1 h. The microsomal pellet was resuspended in the buffer and centrifuged again. The washed microsomal pellet was resuspended 8 mL of buffer containing 100 mM potassium phosphate, 20% glycerol and 1 mM EDTA, pH 7.4 All steps were performed at 0–4 °C. Protein concentration was determined by the method of Lowry et al.[32]

Microsomal Metabolism. The incubations mixtures (3 mL) contained NADP$^+$ (2.3 mg, 1 mM), glucose 6-phosphate (16.92 mg, 20 mM), magnesium chloride (2.85 mg, 10 mM), microsomal protein (3 mg), (1′R)- or (1′S)-bufuralol (0.15 mg, 200 μM) and glucose-6-phosphate dehydrogenase (5 units) in phosphate buffer (0.20 M, pH 7.4). Incubations were carried out at 37 °C for 30 min and then terminated by immersing the mixtures in ice.

Extraction and Derivatization of Metabolites. The microsomal incubation mixtures were made alkaline by the addition of 0.5 mL of saturated sodium carbonate solution after the addition of (1′S)-1″-oxobufuralol [(1′S)-**3**] as the internal standard. The metabolites were then extracted using 5 mL of a 3:2 (v/v) mixture of ethyl ether and dichloromethane. After centrifugation, the organic layer was transfered to a React-vial and evaporated to dryness using a stream of dry nitrogen. The residue was dissolved in 200 μL of dichloromethane (dried over 3-Å molecular sieves) and allowed to react with 5 μL of (+)-(R)-PEIC for 1 h. Dichloromethane was removed by evaporation, and the residue was reconstituted in 500 μL of methanol. The sample was filtered through a membrane filter (0.45 μm) before injecting (10 μL) into the HPLC.

HPLC Analysis. The HPLC system consisted of a LKB 2150 pump, equipped with a Waters 484 tuneable absorbance detector (248 nm, 0.075 AUFC) and a Spectra-Physics 4100 computing integrator. The diastereomeric metabolites were resolved using an Ultrasphere ODS 5 μm column, 4.6 mm × 250 mm (Beckman), with a guard column (Altech Hypersil, C_{18}). The components were eluted isocratically with a mixture of methanol–water (69:31), at a flow rate of 1 mL/min. The retention times were 18.01 and 19.18 min, respectively for the (R)-PEIC-derivatized diastereomers **2b** and **2d**, formed from (1′R)-bufuralol. The retention times for the (R)-PEIC-derivatized diastereomers **2a** and **2c**, formed from (1′S)-bufuralol, were 16.61 and 17.89 min, respectively. The internal standard (1′R)-**3** eluted at 24.51 min. After each run of a sample from the metabolism study, the column was washed by eluting with a mixture of methanol–water (90:10) for 15 min. The column was then reequilibrated with the mobile phase for 15 min before injecting the next sample.

Calibration curves were constructed separately over the concentration ranges 2–10 and 10–60 μg for the assay of the diastereomers of **2** formed on incubation with human and rat liver microsomes, respectively. The peak area ratios of the metabolite to the internal standard was plotted against the analyte amount. The detection limit was 0.1 μg per incubation, or 2 ng on the column with a signal to noise ratio of 4. A regression coefficient of >0.996 was obtained for both calibration curves.

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Registry No. (1′R)-**1**, 64100-61-4; (1′R)-**1**·HCl, 57704-11-7; (1′S)-**1**, 64100-62-5; (1′S)-**1**·HCl, 57704-10-6; **2a**, 97805-55-5; **2a** maleate, 97805-57-7; **2b**, 97805-58-8; **2b** maleate, 97805-60-2; **2c**, 97805-54-4; **2c** maleate, 97805-56-6; **2d**, 97805-59-9; **2d** maleate, 97805-61-3; (1′R)-**3**, 84952-27-2; (1′R)-**3** maleate, 97858-39-4; (1′S)-**3**, 84952-24-9; (1′S)-**3** maleate, 97858-38-3; **4**, 135455-49-1; **5**, 135455-50-4; **6**, 135455-51-5; **7**, 135455-52-6.

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