Chemical and Stereochemical Aspects of Propranolol Metabolism. Diastereomeric 1-(1-Hydroxy-2-propylamino)-3-(1-naphthoxy)-2-propanols Produced by Rat Liver Microsomal ω-Hydroxylation

H. Umesha Shetty and Wendel L. Nelson*

Department of Medicinal Chemistry, School of Pharmacy, University of Washington, Seattle, Washington 98195.
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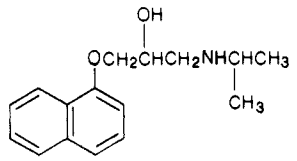
A new metabolic pathway of terminal hydroxylation (ω-hydroxylation) of the *N*-isopropyl group of propranolol (1) was established. Selected ion-monitoring GC–MS analysis, based on use of the synthesized mixture of diastereoisomers of 1-(1-hydroxy-2-propylamino)-3-(1-naphthoxy)-2-propanol (2) as a standard, established formation of both diastereoisomers of 2 as metabolites of 1. These diastereoisomers were formed in unequal amounts when 1, its hexadeuterated analogue 8 or heptadeuterated analogue 9, were incubated in the presence of the rat liver microsomal fraction. Authentic (2*R*,2″*S*)-2, obtained from the amide formed from (2*S*)-3-(1-naphthoxy)-2-hydroxypropionic acid [(2*S*)-5] and (2*S*)-alaninol by diborane reduction, facilitated examination of stereochemical aspects of this process. From incubations of the enantiomers of 1 and pseudoracemic propranolol [equimolar (2*R*)-propranolol-3,3-d_2 and (2*S*)-propranolol-d_0] in the presence of the rat liver microsomal fraction, we established that the diastereomeric products were formed in the order (2*S*,2″*S*)-2 ≅ (2*S*,2″*R*)-2 > (2*R*,2″*R*)-2 > (2*R*,2″*S*)-2. (2*S*)-1, which was metabolized to 2 to a greater extent than (2*R*)-1, showed no stereoselectivity, affording about equal amounts of (2*S*,2″*S*)-2 and (2*S*,2″*R*)-2. (2*R*)-1, which was metabolized to 2 to a lesser extent, afforded considerably more (2*R*,2″*R*)-2 than (2*R*,2″*S*)-2. ω-Hydroxylation was a minor metabolic pathway in the microsomal incubation. About 2000× less 2 than 1-amino-3-(1-naphthoxy)-2-propanol (3), the product of N-dealkylation of 1, was formed.

Propranolol (1) is an important β-adrenergic antagonist that is extensively metabolized in man and other species.[1]



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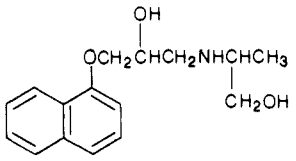

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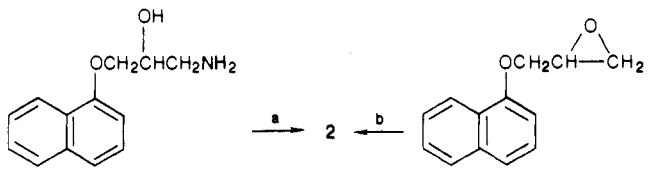
Numerous studies in vivo and in vitro in subcellular tissue fractions, e.g. mammalian liver microsomal and 9000*g* supernatant fractions, have provided considerable structural, stereochemical, and mechanistic information about intermediates and products of these multifaceted oxidative pathways. Pathways of oxidative metabolism of propranolol provide regioisomeric mono- and diphenolic products by aromatic hydroxylation[2] and lead to multiple products via N-dealkylation.[3] Products from these latter processes arise from oxidation at one of the two carbon atoms adjacent to the basic nitrogen atom. Products resulting from oxidation by both aromatic hydroxylation and N-dealkylation have been reported.[4]

Scheme I[a]



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[a] Reagents: a, $CH_3C(O)CH_2OH/NaBH_4$; b, $CH_3CH(NH_2)CH_2OH$.

Since propranolol serves as an excellent substrate for oxidative metabolic processes, we examined the possiblity that hydroxylation of the *N*-isopropyl group might occur to produce 2. Although we are not aware of a precedent for ω-hydroxylation of a basic *N*-isopropyl group, related *N*-*tert*-butyl groups, which have no hydrogen atoms at the α-carbon atom, have been reported to be hydroxylated and to undergo further metabolism.[5] Lacking comparable information on aliphatic hydroxylation of small *N*-alkyl groups, comparison with *C*-alkyl groups shows that ω-hydroxylation of an isopropyl group occurs in very small amounts.[6-8]

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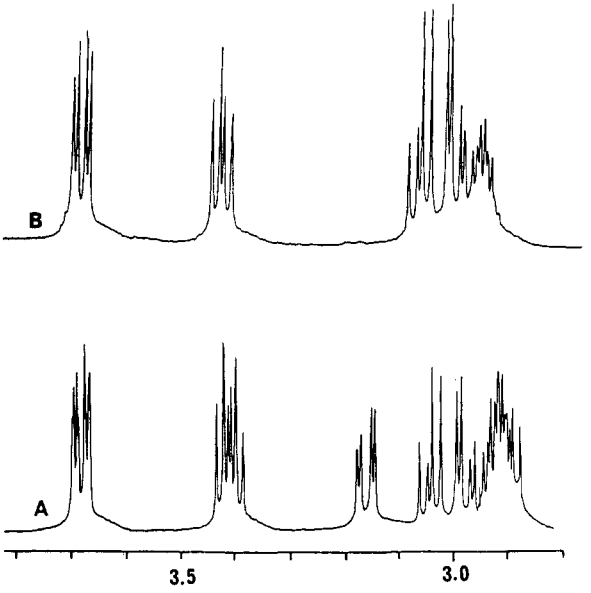


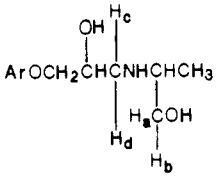
Figure 1. Partial 500 MHz ^1H NMR spectra: (A) **2**; (B) (2*R*,2″*S*)-**2**.

In order to examine this possiblity we synthesized the mixture of diastereoisomers of **2** and used it as a standard for metabolic experiments. We also report the assignment of stereochemistry of these diastereoisomers, based on synthesis of a single enantiomer of **2** of known absolute configuration. Results of metabolic experiments demonstrating the formation of these diastereoisomers in the metabolism of **1** in the presence of rat liver microsomal fraction and examination of stereochemical aspects of their formation are reported.

Results and Discussion

Chemistry. A mixture of the diastereoisomers of **2** was prepared as shown in Shceme I. Reductive amination of hydroxyacetone with **3** and sodium borohydride was less effective than opening of oxirane **4** with 2-amino-1-propanol.

The mixture of diastereomeric products of both processes showed identical, but perplexing, 500-MHz ^1H NMR spectra, due to introduction of a new chiral center and to the diastereotopic nature of geminal protons in each (Figure 1). The methylene protons H_a and H_b of **2** were observed as two overlapping doublets of doublets (δ 3.64, J_{gem} = 11.0 Hz, J_{vic} = 7.3 Hz) and as two overlapping triplets (δ 3.39, J_{app} = 7.3 Hz).



Two doublets of doublets would be expected for the diastereotopic protons in each of the diastereoisomers. Also, the chemical shift of the isopropyl methine proton in the diastereoisomers could not be fixed, although the coupled protons of the methyl group appeared as a doublet of a doublet. The signals for the other set of methylene protons, H_c and H_d, appeared as four sets of doublets of doublets near δ 3.0, as expected. A homonuclear correlation (COSY) experiment was performed. Examination of the contour plot revealed coupled geminal and vicinal protons. The methine proton (appearing at δ 2.88) is coupled to adjacent methylene protons, H_a and H_b. Thus, the unexpected splitting pattern seen in the 1D NMR can

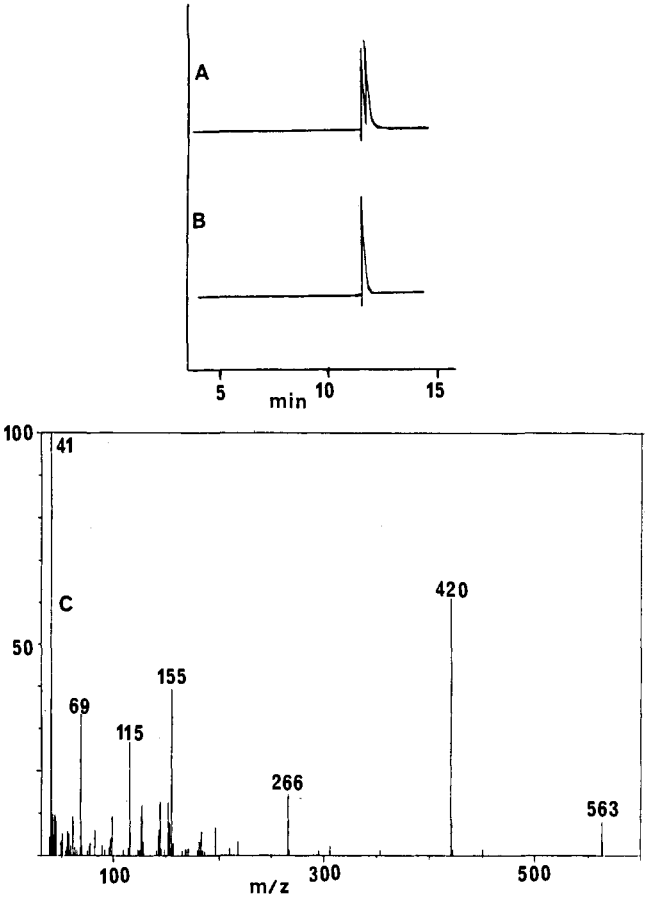


Figure 2. (A) Capillary GC trace of **2**. (B) Capillary GC trace of (2*R*,2″*S*)-**2**. (C) Electron-impact mass spectrum of **2**.

be explained in terms of partial overlapping signals of one proton in each of the diastereoisomers having nearly identical chemical shifts, J_{gem} = 11.0 Hz, J_{vic} = 7.3 Hz. The contour plot also established which sets of H_c and H_d protons were associated with each of the diastereoisomers. The ^1H NMR spectrum of single enantiomer (2*R*,2″*S*)-**2** (vide infra) confirmed these assignments (Figure 1).

Separation of the diastereoisomers of **2** as the Tris–TFA derivatives was accomplished by capillary GC. Two peaks of equal intensity were observed at retention times 11.65 and 11.81 min, respectively (Figure 2). The diastereoisomers of **2** (TFA derivatives) gave identical mass spectra. Each showed the molecular ion at m/z 563 and an intense fragment ion, derived from the side chain, at m/z 420 (61% of the base peak, Figure 2). The m/z 420 ion was monitored in the metabolic experiments. Ion m/z 266 arises from subsequent loss of the trifluoroacetoxy-substituted isopropyl residue with hydrogen atom transfer. The substituted isopropyl residue affords the ion at m/z 155.

The configurations of the diastereoisomers were assigned after synthesis of a single enantiomer of known absolute configuration. (2*R*,2″*S*)-**2** was prepared by the synthetic route shown in Scheme II. The enantiomers of **5** have been previously obtained by resolution, and their absolute configurations have been established chemically in our laboratory.[9] (2*S*)-**5** was converted to its dioxolanone **6** in refluxing 2,2-dimethoxypropane. Reaction of crude **6** with (2*S*)-2-amino-1-propanol [(2*S*)-alaninol] afforded (2*S*,2″*S*)-amide **7** (65% yield). Borane reduction of **7** afforded (2*R*,2″*S*)-**2** (reduction of the amide to the amine changes the priority sequence). The ^1H NMR spectrum

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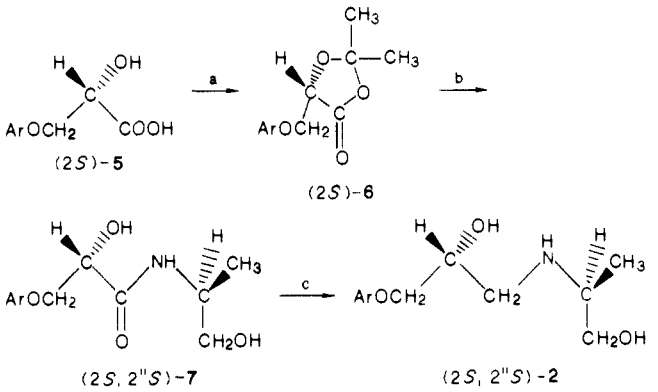
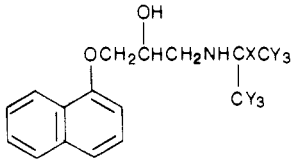
Scheme II[a]



[a] Reagents: a, $(CH_3)_2C(OCH_3)_2$, *p*-TsOH catalyst; b, (2*S*)-2-amino-1-propanol; c, BH_3, THF.

of (2*R*,2″*S*)-**2** confirmed the previous assignment (Figure 1), and capillary GC (TFA derivative) established that (2*R*,2″*S*)-**2** elutes as the earlier component of **2** (Figure 2). Enhancement of this same peak was noted when the pure enantiomer (2*R*,2″*S*)-**2** was added to the diastereoisomeric mixture. Thus, the absolute configurations are assigned as 2*R*,2″*S* and 2*S*,2″*R* for the earlier eluting diastereoisomer and as 2*R*,2″*R* and 2*S*,2″*S* for the later eluting diastereoisomer.

Metabolite Identification. Selected ion monitoring (SIM) mass spectrometry combined with stable-isotope methodology was employed for the determination of metabolite **2**. The side-chain fragment ion at *m*/*z* 420, which provided greater selectivity and sensitivity than the molecular ion at *m*/*z* 563, was monitored. This major fragment ion occurs only in products of hydroxylation of the side chain. Propranolol (**1**) and its deuterated analogues, propranolol-*isopropyl*-d_6 (**8**)[10] and propranolol-*isopropyl*-d_7



8, X = H, Y = D
9, X = Y = D

(**9**)[10] were incubated with the rat liver microsomal fraction. The extracted metabolites were derivatized with TFAI and subjected to GC–MS. The ions at *m*/*z* 420, 425, and 426 were monitored. Metabolites were found at retention times corresponding to the diastereoisomers of **2**. The metabolites from **8** and **9** showed ions at *m*/*z* 425 and 426, as expected for loss of one deuterium atom, confirming the assigned structure. Appropriate control experiments, incubations without substrate and incubations without the NADPH-generating system, were performed to ensure that the reaction was enzymatic. The peak areas for the diastereoisomers were determined and compared with those of the synthetic standard. Whereas the peak area ratio of the diastereoisomeric mixture of synthetic **2** was about 1:1, that of the metabolic mixture was about 2:3 [(2*R*,2″*S*)-**2** + (2*S*,2″*R*)-**2**]:[(2*R*,2″*R*)-**2** + (2*S*,2″*S*)-**2**]. These results suggested some degree of stereoselectivity in the ω-hydroxylation processes. The ratio of propranolol enantiomers did not change under the conditions of the experiment since N-dealkylation resulting in formation of **3**, the major metabolic pathway, shows no stereoselectivity.[11]

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Table I. Stereochemical Aspects of Rat Liver Microsomal ω-Hydroxylation of Propranolol (**1**)

stereoselectivity of product formation	2″*S*:2″*R* ratio, mean ± SD (*n*)
single enantiomers	
(2*S*)-**1**	1.00 ± 0.16[a] (4)
(2*R*)-**1**	0.67 ± 0.06[b] (3)
(2*S*)-**1**-d_0:(2*R*)-**1**-d_2 pseudoracemate	
from (2*S*)-**1**-d_0	1.15 ± 0.05[a] (4)
from (2*R*)-**1**-d_2	0.51 ± 0.03[b] (4)
stereoselectivity of substrate metabolized	**2*S*:2*R* ratio, mean ± SD (*n*)**
(2*S*)-**1**-d_0:(2*R*)-**1**-d_2 pseudoracemate	1.76 ± 0.04[c] (4)

[a] The ratio of (2*S*,2″*S*)-**2** to (2*S*,2″*R*)-**2** from (2*S*)-**1**. [b] The ratio of (2*R*,2″*S*)-**2** to (2*R*,2″*R*)-**2** from (2*R*)-**1**. [c] The ratio of [(2*S*,2″*S*)-**2** + (2*S*,2″*R*)-**2** from (2*S*)-**1**] to [(2*R*,2″*S*)-**2** + (2*R*,2″*R*)-**2** from (2*R*)-**1**].

Quantitatively ω-hydroxylation is a minor pathway, producing ca. 2000× less **2** than **3**, the product of N-dealkylation. While 12–14 μg (20.6 ± 0.3 nmol/mg of protein for 30 min) of **3** was produced in the metabolic incubation, 8–9 ng (10.8 ± 0.2 pmol/mg of protein for 30 min) of **2** was produced. No attempt was made to determine K_m/V_{max} parameters for this process. Microsomal incubation of synthetic **2** at up to 1 μg per incubation afforded no metabolites when products of aromatic hydroxylation or of N-dealkylation were monitored. These results indicate that **2** is not metabolized significantly under the conditions of the metabolic experiments.

Substrate–Product Stereoselectivity. The observed difference in the ion intensity of the diastereoisomers of **2**, produced from metabolism of **1**, suggests some degree of stereoselectivity in the ω-hydroxylation process. Although base line separation of the peaks was not accomplished (Figure 2), the individual peak areas could be measured with sufficient precision to obtain reliable data on stereoselectivity, based on the known relative configuration of each of the diastereoisomers of **2**. When (2*R*)- and (2*S*)-**1** were incubated separately with the rat liver microsomal fraction, determination of these products indicated (2*R*)-**1** produces about equal amounts of (2*R*,2″*S*)- and (2*R*,2″*R*)-**2**, while (2*S*)-**1** affords more (2*S*,2″*R*)-**2** than (2*S*,2″*S*)-**2** (Table I). A pseudoracemate of propranolol, consisting of equimolar (2*R*)-**1**-*3*,*3*-d_2 and (2*S*)-**1**-d_0,[12] was also used as the substrate, and the ratio of ions *m*/*z* 420 and 422 was monitored to determine each of the diastereoisomers of **2** (Table I).

Stereoselectivity in the production of diastereoisomeric metabolites (2*R*,2″*S*)- and (2*R*,2″*R*)-**2** from (2*R*)-**1** was noted, favoring production of a new 2″*R* chiral center vs. a 2″*S* chiral center (2″*S*:2″*R* = 0.67 from the single enantiomer and 0.51 from the pseudoracemate). On the other hand, metabolism of (2*S*)-**1** showed essentially no stereoselectivity, producing about equal amounts of (2*S*,2″*S*)- and (2*S*,2″*R*)-**2**. The 2″*S*:2″*R* ratio was similar whether (2*S*)-**1** or the pseudoracemate was the substrate (2″*S*:2″*R* = 1.00 from the single enantiomer and 1.15 from the pseudoracemate).

In a related aliphatic hydroxylation process, substrate–product stereoselectivity has been observed in the hydroxylation of the enantiomers of pentobarbital.[12] The 2*S* enantiomer of pentobarbital is stereoselectively hydroxylated to give a 5:1 ratio of the two possible diastereoiso-

(11) Shetty, H. U.; Nelson, W. L., submitted for publication.
(12) Nelson, W. L.; Bartels, M. J. *J. Labelled Compd. Radiopharm.* **1983**, *20*, 1135; Nelson, W. L.; Bartels, M. J. *Drug Metab. Disp.* **1984**, *12*, 345.

meric alcohols while hydroxylation of the 2R enantiomer produces equal amounts of the two possible diastereoisomers.[13] This difference has been attributed to two separate isozymes being involved in the aliphatic hydroxylation process.[14]

The use of the pseudoracemic 1 allowed us to readily determine which enantiomer of 1 is ω-hydroxylated to the greater extent. The ratio of the ω-hydroxylated products from metabolism of each of the individual enantiomers was obtained by comparing the ratio of products from (2S)-1 [sum of (2S,2″R)- and (2R,2″S)-2] with those from (2R)-1 [sum of (2R,2″S)- and (2R,2″R)-2], or m/z 420 vs. 422, summed over both diastereomeric metabolites. The ratio of 1.76 indicated (2S)-1 is a better substrate in the ω-hydroxylation pathway. Quantitatively, the order of metabolites produced was (2S,2″S)-2 ≅ (2S,2″R)-2 > (2R,2″R)-2 > (2R,2″S)-2.

Attempts were made to determine whether 2 could be detected as an in vivo metabolite. Urine samples collected from rats administered 1 ip were treated with β-glucuronidase and subjected to extraction and derivatization in the usual manner. Examination of the extract by SIM technique revealed no detectable amount of 2. In another set of experiments, bile was collected from rats by cannulation for 6 h postadministration of propranolol. The pooled bile samples were extracted after deconjugation with β-glucuronidase. Again, no 2 was detected in any of the samples. If 2 is produced in vivo, rapid subsequent oxidation could occur, like conversion of the ω-hydroxylated primary metabolite of ibuprofen to a carboxylic acid.[7]

No literature precedent for the hydroxylation of the terminal carbon of a basic N-isopropyl moiety is available, in spite of the large number of β-adrenergic antagonists bearing this group that have been studied metabolically.[15] Since the extent of metabolism of β-adrenergic antagonists is positively correlated with lipophilicity, the possibility exists that products of this metabolic pathway may be found for other lipophilic β-adrenergic antagonists if these processes are more carefully examined. However, N-dealkylation occurs to a much greater extent than ω-hydroxylation in propranolol, and it is likely that this pathway predominates in the metabolism of other related β-adrenergic antagonists.

Experimental Section

Synthetic reactions were carried out under an argon atmosphere. Routine ^{1}H NMR spectra were recorded on a Varian T-60 (60-MHz) spectrometer, and high-frequency ^{1}H NMR spectra were recorded on a Bruker WM-500 (500-MHz) spectrometer. Chemical shifts are reported in parts per million (ppm) relative to Me_4Si as an internal standard. Notations used in the descriptions are s = singlet, d = doublet, dd = doublet of a doublet, t = triplet, q = quartet, and m = multiplet. The homonuclear correlation experiment (COSY) required 512 spectra taken at regular increments of the 3-s delay. Each spectrum was obtained by acquiring 1 fid of 2048 points. A second Fourier transform in the other direction yielded an array of 1024 points that were unsymmetrized and plotted as a contour presentation. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Gas chromatographic analysis was performed on a Hewlett-Packard 5840A gas chromatograph equipped with a flame ionization detector (splitless) and utilizing a J & W DB-5 fused silica capillary column [30 m × 0.32 mm i.d., 0.25-μm film]. Chromatographic conditions: injector temperature 250 °C; helium flow rate, 60 mL/min; column head pressure, 15 psi; temperature program, 180 °C for 1 min and then increased to 220 °C at 3 °C/min. Electron-impact mass spectra were obtained on a VG 7070H mass spectrometer equipped with a VG 2050 data system. Gas chromatography electron-impact mass spectrometric analysis was performed on the same setup, which was interfaced with a Hewlett-Packard 5710A gas chromatograph. Gas chromatography conditions were the same as above. Mass spectral conditions: source temperature, 200 °C; ionizing voltage, 70 eV. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN. Where indicated by the symbols of the elements the analysis was within ±0.4% of theoretical values. Glassware used in the metabolic experiments was silylated with dimethyldichlorosilane.

Diastereomeric 1-(1-Hydroxy-2-propylamino)-3-(1-naphthoxy)-2-propanols (2). A. From 4. A solution of 2-[1-naphthoxymethyl]oxirane (4); 1.00 g, 5.0 mmol) and 2-amino-1-propanol (1.88 g, 25 mmol) in ethanol (10 mL) was heated at reflux for 1 h. The solvent was removed on a rotary evaporator. The oily residue was dissolved in ether (50 mL), which was washed repeatedly with water, and dried (Na_2SO_4). The ether was evaporated, and the product was purified by flash column chromatography (Kieselgel 60; $CHCl_3$–MeOH–triethylamine, 95:5:1). Recrystallization (benzene–cyclohexane, 1:1) of the chromatographed product yielded 1.04 g (76%) of 2 as white amorphous powder: mp 99–101 °C; 500-MHz ^{1}H NMR ($CDCl_3$) δ 1.13 (dd, 3, CH_3), 2.88 (m, 1, CH), 2.88, 2.95, 3.02 and 3.14 (dd, 2, CH_2NH), 3.39 and 3.64 (m, 2, CH_2OH), 4.22 (m, 3, OCH_2CH), 6.83 (m, 1, Ar H-2), 7.35–7.52 (m, 4, Ar H-3, H-4, H-6, and H-7), 7.80 (m, 1, Ar H-5), 8.23 (m, 1, Ar H-8); EIMS m/z 275 (1, $M^{+\bullet}$), 244 (90), 183 (7), 157 (5), 144 (12), 127 (8), 115 (13), 100 (20), 88 (100), 56 (26); GC–EIMS [$(TFA)_2$ derivative] m/z 563 (9, $M^{+\bullet}$), 420 (61), 266 (15), 155 (40), 144 (13), 115 (27), 99 (9), 69 (34), 41 (100). Anal. ($C_{16}H_{21}NO_3$) C, H, N.

B. From 3. A solution of 3-(1-naphthoxy)-1-amino-2-propanol (3; 435 mg, 2.0 mmol) and acetol (163 mg, 2.2 mmol) in methanol (2 mL) was stirred over molecular sieves (3A) at room temperature. After the mixture was stirred for 2 h, sodium borohydride (83 mg, 2.2 mmol) was added in small portions and the reaction mixture was stirred for additional 2 h. The methanolic solution was removed, and the molecular sieves were washed with methanol. The methanolic extracts were combined and evaporated to dryness, and the residue was partitioned between ethyl acetate (20 mL) and aqueous 5% sodium hydroxide solution (5 mL). The organic phase was separated, and the aqueous portion was extracted with ethyl acetate (10 mL). The combined organic extract was dried (Na_2SO_4) and evaporated to dryness. Upon purification as above, 330 mg of 3 (60% yield) was obtained as a white amorphous powder, mp 99–101 °C. It gave a satisfactory ^{1}H NMR spectrum.

(2S,2″S)-N-(1-Hydroxy-2-propyl)-3-(1-naphthoxy)-2-hydroxypropionamide [(2S,2″S)-7]. A mixture of (2S)-2-hydroxy-3-(1-naphthoxy)propionic acid (5;[9] 70 mg, 0.30 mmol), 2,2-dimethoxypropane (1 mL), and p-toluenesulfonic acid (5 mg) in benzene (4 mL) was heated at reflux for 1 h. The reaction mixture was cooled, diluted with benzene (10 mL), and washed with aqueous 5% sodium carbonate solution (5 mL). The organic phase was dried (Na_2SO_4) and concentrated on a rotary evaporator to yield oily dioxolanone 6. The crude product was dissolved in benzene (5 mL), (2S)-2-amino-1-propanol (113 mg, 1.5 mmol) was added, and the mixture was heated at reflux for 20 h. After cooling, ethyl acetate (10 mL) was added. The solution was washed with water (2 × 10 mL), dried (Na_2SO_4), and concentrated on a rotary evaporator. The oily product was subjected to flash column chromatography (Kieselgel 60; $CHCl_3$–MeOH, 95:5). Evaporation of the fractions containing the expected product yielded (2S,2″S)-7, a creamy white solid that was recrystallized from benzene as colorless needles: 74 mg (85% yield); mp 111–112 °C; ^{1}H NMR ($CDCl_3$) δ 1.10 (d, 3, CH_3, J = 6 Hz), 3.48 (m, 3, $CHCH_2OH$), 4.31 (m, 3, OCH_2CH), 6.71 (m, 1, Ar H-2), 6.98–7.60 (m, 4, Ar H-3, H-4, H-6, and H-7), 7.72 (m, 1, Ar H-5), 8.17 (m, 1, Ar H-8); EIMS m/z 289 (24, $M^{+\bullet}$), 258 (4), 146 (74), 144 (100), 128 (16), 127 (19), 116 (24), 115 (57), 114 (24), 59 (31).

(2R,2″S)-1-(1-Hydroxy-2-propylamino)-3-(1-naphthoxy)-2-propanol [(2R,2″S)-2]. To a cooled (0 °C) solution of (2S,2″S)-7 (58 mg, 0.20 mmol) in dry THF (1 mL) was added dropwise a solution of borane–THF complex (610 μL, 1 M), and

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the mixture was stirred overnight at room temperature. The reaction mixture was cooled in an ice bath, and 6 N hydrochloric acid (1 mL) was added dropwise. The mixture was stirred for 30 min, the THF was removed by rotary evaporation, and the aqueous phase was saturated with solid sodium carbonate and extracted with ethyl acetate (2 × 10 mL). The combined extract was dried (Na_2SO_4) and evaporated to dryness. TLC ($CHCl_3$–MeOH–triethylamine, 80:20:1) of this residue showed a single zone corresponding to the R_f of **2**. The product was purified by flash column chromatography (Kieselgel 60; $CHCl_3$–MeOH–triethylamine, 95:5:1) and crystallized (benzene–cyclohexane, 1:1) affording 36 mg of (2*S*,2″*S*)-**2**: 65%; mp 94–96 °C; 500-MHz ^1H NMR ($CDCl_3$) δ 1.12 (d, 3, CH_3, J = 6.5 Hz), 2.94 (m 1, $CHCH_3$), 2.99 (dd, 1, CH_2NH, J_{gem} = 12.1 Hz, J_{vic} = 3.6 Hz), 3.05 (dd, 1, CH_2NH, J_{gem} = 12.1 Hz, J_{vic} = 7.8 Hz), 3.42 (dd, 1, CH_2OH, J_{gem} = 11.0 Hz, J_{vic} = 7.3 Hz), 3.68 (dd, 1, CH_2OH, J_{gem} = 11.0 Hz, J_{vic} = 4.1 Hz), 4.18 (m, 2, OCH_2CH), 4.42 (m, 1, OCH_2CH), 6.79 (m, 1, Ar H-2), 7.32–7.53 (m, 4, Ar H-3, H-4, H-6, and H-7), 7.78 (m, 1, Ar H-5), 8.22 (m, 1, Ar H-8); EIMS m/z 275 (0.5, $M^{+\bullet}$), 244 (47), 183 (5), 144 (17), 127 (9), 115 (26), 100 (25), 88 (100), 70 (11), 56 (39). (2*R*,2″*S*)-**2** and **2** (10 μg each) were dissolved separately in dry benzene (100 μL), treated with trifluoroacetylimidazole (20 μL), and heated at 60 °C for 15 min. These derivatized samples were determined by GC. The diastereoisomers of **2** eluted with retention times 11.65 and 11.81 min, whereas (2*R*,2″*S*)-**2** had a retention time of 11.68 min (Figure 2).

In Vitro Metabolic Experiments. 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 4 volumes of the same buffer with a Potter-Elvehjem Teflon pestle homogenizer. The homogenate was centrifuged at 9000*g* for 30 min, and the supernatant fraction was recentrifuged at 100000*g* for 1 h. The microsomal pellet was resuspended in the same buffer at a concentration of 20–30 mg of protein/mL. The entire operation was performed at 0–4 °C. Microsomal protein was determined by the method of Lowry et al.[16]

A typical incubation mixture contained 0.250 mg of the substrate (**1**, **8**, **9**, or pseudoracemic propranolol), $MgCl_2$ (4.76 mg, 50 μmol), glucose 6-phosphate (14.1 mg, 50 μmol), $NADP^+$ (3.83 mg, 10 μmol), glucose 6-phosphate dehydrogenase (5 units), and microsomal protein (3 mg) in 3 mL of 0.2 M pH 7.4 phosphate buffer. After incubating at 37 °C for 30 min, the mixtures were made alkaline by the addition of saturated sodium carbonate solution (0.5 mL) and then extracted with benzene (2 × 5 mL). The organic layer was separated by centrifugation, and the combined layers were dried (Na_2SO_4). The solvent was removed by a stream of N_2, the residue was dissolved in dry benzene (100 μL), TFAI (20 μL) was added, and the solution was heated at 60 °C for 15 min.

In order to quantitate **2** and **3**, 30-min incubations were performed with 1-*3,3*-d_2 as substrate. Known amounts of protio standards were added, and the mixtures were extracted and derivatized in the usual manner. The ratios of deuterated and nondeuterated compounds were determined by monitoring the ions at m/z 420 and 422 for **2** and at m/z 409 and 411 for **3**. A standard curve of known ratios of 1 and 1-*3,3*-d_2 was used for quantitation.

In Vivo Metabolic Experiments. Pairs of male Sprague–Dawley rats (approximately 150 g) were administered propranolol, 20 mg/kg ip, in distilled water. A control group of animals received water only. Urine was collected for 24 h over ascorbic acid (0.25 g), diluted to 60 mL with distilled water, and aliquots (3 mL) were extracted for the analysis. To duplicate aliquots from each urine sample was added sodium acetate solution (0.5 mL, 0.2 N), and the samples were incubated with β-glucuronidase-sulfatase (10000 units) at 37 °C for 12 h. The extraction and derivatization procedure was the same as described in the in vitro metabolic experiments.

Three male Sprague–Dawley rats (approximately 225 g) were anesthetized with urethane (1.3 g/kg ip), the abdominal cavities were opened with a U-shaped incision, and the bile ducts were cannulated with PE-10 tubing attached to a length of silastic tubing which drained into a polypropylene tube. Bile samples were collected for 30 min. Propranolol, 15 mg/kg, in distilled water added to each abdominal cavity (0.5 mL), and collection of bile samples was continued for 6 h. The samples were stored at −80 °C until analysis. The bile samples were combined, sodium acetate solution (0.5 mL, 0.2 N) was added, and the sample was incubated with β-glucuronidase-sulfatase (10000 units) at 37 °C for 12 h. The extraction and derivatization procedure was the same as described in the in vitro metabolic experiments.

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Registry No. (±)-**1**, 13013-17-7; (2*S*)-**1**, 4199-09-1; (2*R*)-**1**, 5051-22-9; (±)-**2** (isomer 1), 103478-75-7; (±)-**2** (isomer 2), 103478-76-8; (±)-**2** (isomer 1, $(TFA)_2$ deriv.), 103498-84-6; (±)-**2** (isomer 2, $(TFA)_2$ deriv.), 103478-77-9; (2*R*,2″*S*)-**2**, 103531-64-2; (2*S*,2″*S*)-**2**, 103531-65-3; (2*S*,2″*R*)-**2**, 103531-66-4; (2*R*,2″*R*)-**2**, 103531-67-5; **3**, 30200-48-7; **4**, 87144-72-7; **5**, 80789-59-9; (2*S*)-**6**, 103478-79-1; (2*S*,2″*S*)-**7**, 103478-78-0; (2*S*)-$MeCH(NH_2)CH_2OH$, 2749-11-3; $MeCOCH_2OH$, 116-09-6; $MeCH(NH_2)CH_2OH$, 78-91-1; $MeC(OMe)_2CH_3$, 77-76-9; propanolol, 525-66-6.

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