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Metabolism of β-Adrenergic Antagonists. Evidence for an Arene Oxide–NIH Shift Pathway in the Aromatic Hydroxylation of Oxprenolol

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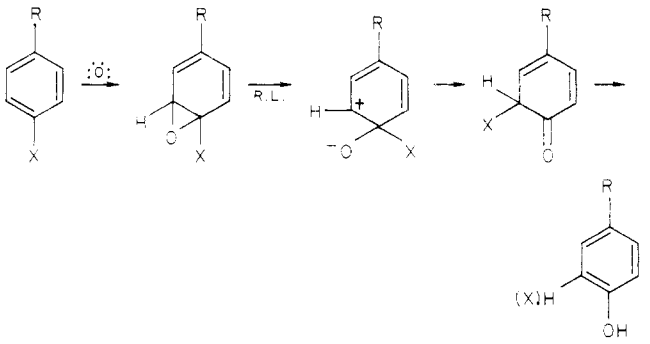
The metabolic hydroxylation of 4′-deuteriooxprenolol [1-(isopropylamino)-3-[2′-(allyloxy)-4′-deuteriophenoxy]-2-propanol] prepared from the 4′-bromo compound was examined in the rat (in vivo). GC–MS analysis of the 4′- and 5′-hydroxyoxprenolol obtained showed 65% retention of deuterium in each of the metabolites. The results indicate that an arene oxide–NIH shift pathway is operative in these hydroxylation processes. The equal magnitude of deuterium retention is supportive of a 4′,5′-arene oxide as a major contributor to their formation.

Although Boyland[1] suggested that formation of many of the metabolites of aromatic hydrocarbons in mammals could be explained by postulating arene oxide intermediates nearly 30 years ago, it was only about 10 years ago that the first arene oxide, naphthalene 1,2-oxide,[2,3] was isolated and shown to be an obligatory intermediate in the metabolism of naphthalene to 1-naphthol and other products. Evidence that the hydroxylation of 4′-tritiophenylalanine to tyrosine occurs with migration of the tritium isotope[4,5] and that 1-deuterionaphthalene is converted to 2-deuterio-1-naphthol[2,3,6] were additional key elements in development of the arene oxide–NIH shift concept (Scheme I). Subsequently, scores of substrates have been studied and shown to be hydroxylated in vivo and in vitro by pathways consistent with this mechanism.[7,8]

The arene oxide–NIH shift pathway has been studied in only a few drug molecules. However, from model benzenoid substrates, having substituents which are not readily ionized and that are hydroxylated by monooxygenases in vitro, it was found that 40–65% of deuterium (and more of tritium) migrates and is retained. In those aromatic substrates with ionizable substituents, only 0–30% deuterium is retained.[7,9,10] In vivo hydroxylation of anisole or diphenyl ether showed that about 60% of the deuterium isotope is retained.[7] In some systems, methyl or halogen atoms have been shown to migrate.[7]

The results of several mechanistic investigations are consistent with the process shown in Scheme I, with two exceptions: (1) in the hydroxylation of some compounds where very stable carbonium ion intermediates are possible, diols formed by hydration of intermediate epoxides exist[11] and oxygen walk processes have been found to occur;[11-13] and (2) in some compounds, containing highly electron-withdrawing substituents, hydroxylation by an oxygen-insertion mechanism has been suggested to account for observed deuterium isotope effects.[14]

Scheme I. Suggested Pathway for Formation of Phenolic Metabolites by the Arene Oxide–NIH Shift Pathway with Spontaneous Rearrangement of the Epoxide Shown under Neutral Conditions



Since we had demonstrated the occurrence of metabolic 4′- and 5′-hydroxylation of oxprenolol,[15] it was a logical extension to investigate the possible role of one or more arene oxides in the metabolic hydroxylation of the aromatic ring of oxprenolol (**1**). If an arene oxide is an intermediate in the aromatic hydroxylation, then the products 4′- and 5′-hydroxyoxprenolol (**2** and **3**) could arise from at least one but possibly from three different arene oxides. Possible pathways for the formation of 4′-hydroxyoxprenolol (**2**) include a 3′,4′- and/or a 4′,5′-arene oxide, and formation of 5′-hydroxyoxprenolol (**3**) could result from a 4′,5′- and/or a 5′,6′-arene oxide.

We sought to investigate the possible role of an arene oxide–NIH shift process by analyzing the 4′- and 5′-hydroxyoxprenolol metabolites formed from in vivo metabolism of oxprenolol specifically deuterated at the 4′ position. Since, as metabolites, 4′-hydroxyoxprenolol (**2**)

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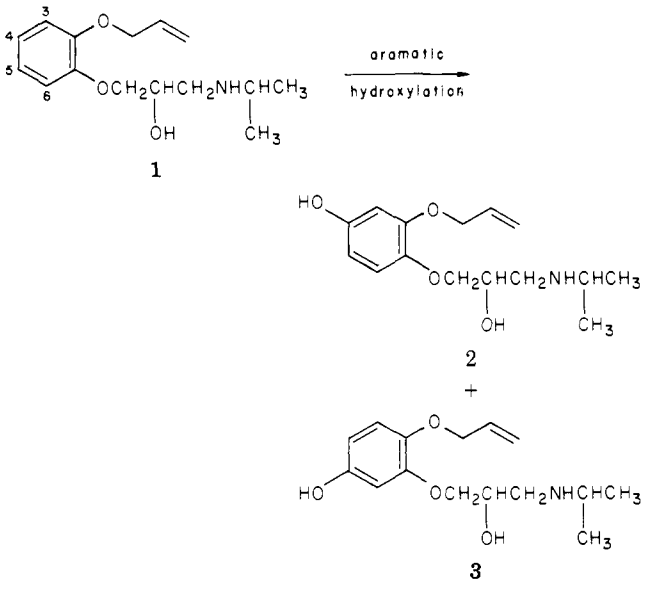


predominates over 5′-hydroxyoxprenolol (**3**), the use 4′-deuteriooxprenolol (**4**) was preferred over the corresponding 5′-deuterio compound.

Synthesis. The approach taken toward the synthesis of 4′-deuteriooxprenolol (**4**) used 4-bromo-2-hydroxyacetophenone (**6**) as starting material (Scheme II), which was obtained from 3-bromophenyl acetate (**5**)[16] by Fries rearrangement according to the method of Buckle.[17] O-Allylation of **6** with 1 equiv of allyl bromide in K_2CO_3/DMF gave 4-bromo-2-(allyloxy)acetophenone (**7**) in 88% yield.

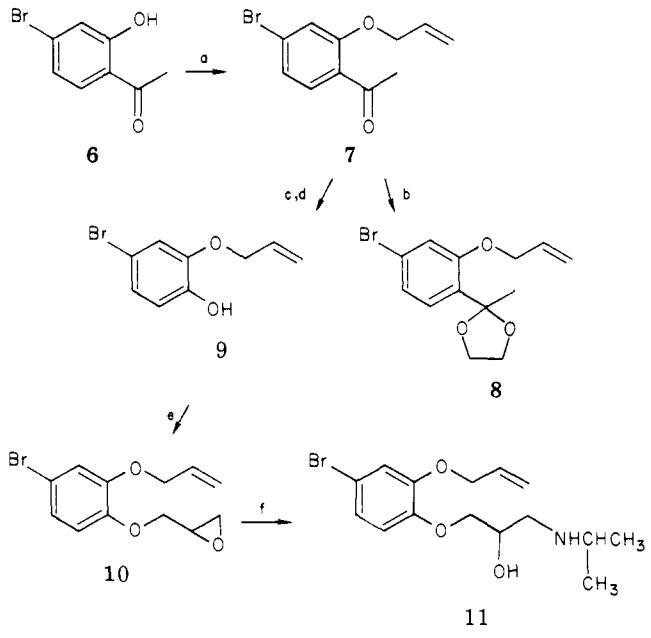
A variety of unsuccessful attempts were made at reductive dehalogenation of ketal **8**, which was obtained in 98% yield from **7** and ethylene glycol, including the use of *n*-butyllithium,[18] and Mg and Li reductions. Use of $NaBH_4$–Pd/C[19] successfully replaced the bromine atom with hydrogen but also reduced the allyl side chain and cleaved the ethylene glycol ketal.

A modification of the approach was made by preparing 4′-bromooxprenolol (**11**). Acetophenone **7** was converted to phenol **9** by Baeyer–Villager oxidation (*m*-chloroperbenzoic acid) and hydrolysis. Alkylation of **9** with epichlorohydrin (K_2CO_3, acetone) gave 3-[4-bromo-2-(allyloxy)phenoxy]-1,2-epoxypropane (**10**) in 90% yield. The epoxide was opened with isopropylamine (100 °C), affording 4′-bromooxprenolol (**11**) in 60% yield.

Since metal reductions gave very unsatisfactory reductive dehalogenation results with intermediate **8**, the prospect of the direct reduction on **11** seemed tenuous. A more satisfactory method of reductive dehalogenation had been found with $NaBH_4$–Pd/C;[19] however, quantitative hydrogenation of the allyl side chain also would occur. A logical solution seemed to be removal of the allyl side chain from 4′-bromooxprenolol, reductive debromination of the aromatic ring with $NaBH_4$–Pd/C, followed by reintroduction of the allyl side chain.

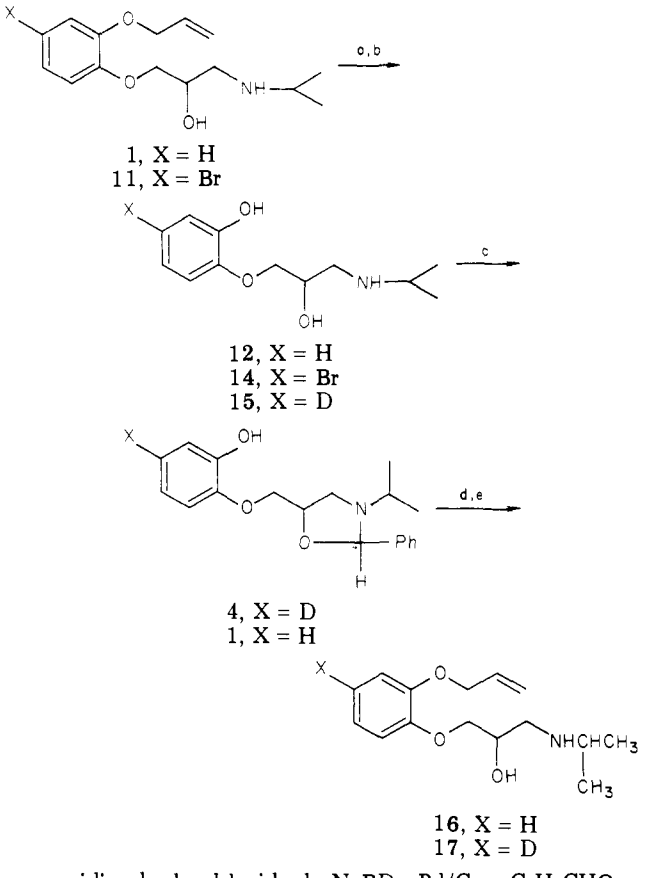
Using oxprenolol (**1**) as a model, we successfully cleaved the allyl ether to produce **12** by using 6 equiv of pyridine hydrochloride at 170 °C (2 h). With a successful method of O-deallylation, the problem of reintroduction of the allyl group remained. One difficulty faced was the potential competition of the amino group with the phenolic hydroxyl group in nucleophilic attack on allyl bromide. Attempted use of a patented method[20] which described the allylation of 1-amino-3-(2-hydroxyphenoxy)-2-propanol (**13**) with allyl bromide (NaOMe in EtOH at reflux) gave poor results.

Scheme II



a, allyl bromide, K_2CO_3 in DMF; b, $HOCH_2CH_2OH$, *p*-TsOH; c, *m*-Cl-$C_6H_5CO_3H$; d, NaOH, H_2O, e, epichlorohydrin, K_2CO_3, acetone; f, $H_2NCH(CH_3)_2$

Scheme III



a, pyridine hydrochloride; b, $NaBD_4$, Pd/C; c, C_6H_5CHO; d, allyl bromide, K_2CO_3 in DMF; e, HCl, H_2O, and acetone

To sterically hinder the amine nitrogen, a 2-phenyloxazolidine of the side-chain hydroxy group and amine nitrogen was formed with benzaldehyde (Scheme III) similar to the reported formation of the 2-methyloxazolidine of oxprenolol.[21] Deallyloxprenolol (**12**), when allowed to react with 1.4 equiv of benzaldehyde, gave a

mixture of diastereomeric 2-phenyl-3-(isopropylamino)-5-[(2′-hydroxyphenoxy)methyl]oxazolidines (**16**) in nearly quantitative yield. Both diastereomers were present, as shown by two C-2 benzylic protons (δ 5.12 and 5.20) in a 1.0:0.7 ratio. In addition, the isopropyl methyl groups showed a complex pattern, attributed to the diastereomeric differences. O-Allylation was accomplished in 85% yield with equal molar amounts of **16** and allyl bromide. The intermediate 2-phenyl-3-(isopropylamino)-5-[[2′-(allyloxy)phenoxy]methyl]oxazolidine (**18**) was readily cleaved using aqueous acid in acetone.

Having proved the model successful, 4′-bromooxprenolol (**11**) was O-deallylated to give **14** in 60% yield. The deuteration was performed using Pd/C–$NaBD_4$ in MeOD to give deuterated phenol **15** in nearly quantitative yield. High-resolution mass spectrometry showed incorporation of 83% deuterium and no trace of the parent bromophenol **14**.

Deuterated oxazolidine **17** was readily formed (91% yield) by stirring **15** with 1.1 equiv of benzaldehyde. O-Allylation of **17** was accomplished in 70% yield and deprotection gave 4′-deuteriooxprenolol (**4**). High-resolution mass spectrometry again showed 83% deuterium incorporation, indicating no loss of deuterium in the final two steps.

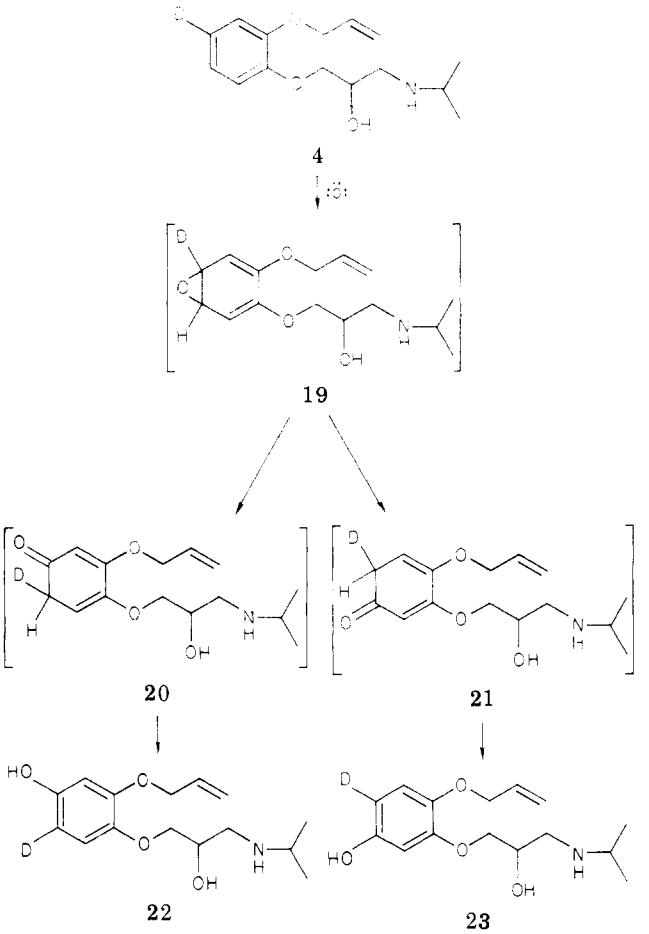
Metabolism. From rats administered 4′-deuteriooxprenolol (**4**), 20-h urines were collected, and metabolites were subjected to β-glucuronidase and extracted as previously reported.[15] Derivatization was accomplished using diazomethane followed by trifluoroacetic anhydride. Samples were subjected to GC–MS and the mass range 485–495 amu was scanned at retention times for the metabolites. Only 4′- and 5′-methoxyoxprenolol[15] (as TFA derivatives) were detected. No 3′- or 6′-methoxyoxprenolol was found. For each isomer, twin ions at 488 and 489 were present. Relative deuterium incorporation for the 4′- and 5′-OMe compounds was 0.53 and 0.54, respectively [$d_1/(d_0 + d_1)$], which corresponds to a 65% retention of deuterium based on initial deuterium incorporation of 83%. On recording the mass range from 300 to 500, a large single peak at 309 (corresponding to the propanolamine side chain) and twin ions at 374 and 375 (corresponding to loss of CF_3COOH from the parent ion), as well as the parent ions at 488 and 489, were present, corresponding to the known fragmentation of aryloxypropanolamine β-adrenergic antagonists as their TFA derivatives.[22]

Discussion

The retention of 65% deuterium in the 4′- and 5′-hydroxyoxprenolol metabolites is consistent with an arene oxide intermediate in the hydroxylation of oxprenolol. The retention of 65% deuterium is consistent with previous results of arene–NIH shift pathways in the hydroxylation of aromatic rings with nonionizable substituents, suggesting that isotopic exchange, if it occurs during β-glucuronidase treatment (pH 5.0) and/or TFA derivatization, must be very small or it does not occur.

The results of equal and substantial retention of deuterium in formation of 4′- and 5′-hydroxyoxprenolol support their formation through an arene oxide–NIH shift pathway. A simple possible explanation is that the 4′- and 5′-hydroxyoxprenolol metabolites could be derived from a common 4′,5′-oxide intermediate, **19** (Scheme IV). In formation of both metabolites from 4′-deuteriooxprenolol (**4**), migration of either hydrogen or deuterium would be expected, so that intermediate ketones **20** and **21** would possess approximately equal isotopic incorporation at the methylene groups adjacent to the ketone carbonyl. Enolization to form the corresponding deuterated phenols **22**

Scheme IV. Possible Mechanism of an Arene Oxide–NIH Shift Pathway for the Aromatic Hydroxylation of 4′-Deuteriooxprenolol to Form 4′- and 5′-Hydroxyoxprenolol, each with Deuterium Retained



and **23** would result in a retention of deuterium, which would be dependent on the isotope effect for the tautomerization. No large difference in isotope effect in the formation of these position isomers would be expected.

As argued above, any 5′-hydroxy metabolite derived from a 4′,5′-arene oxide would likely provide deuterium retention nearly identical with that seen in the 4′-hydroxy metabolite **2**. Any 5′-hydroxy metabolite derived from a 5′,6′-arene oxide would then result in deuterium retention in the 5′-hydroxy metabolite greater than that for the 4′-hydroxy isomer, since the 4′-deuterium would be retained. Therefore, if metabolism of 4′-deuteriooxprenolol (**4**) results in a 5′-hydroxy metabolite with deuterium retention greater than that found with the 4′-hydroxy isomer, then a 5′,6′-arene oxide could be a contributor. Since that is not observed, it follows that a 4′,5′-arene oxide is the more logical path to 5′-hydroxyoxprenolol.

In the case of the 4′-hydroxy metabolite, the observed NIH shift gives no evidence to determine whether a 3′,4′- or a 4′,5′-arene oxide is involved in its formation. For a 3′,4′-arene oxide pathway to be a major contributor, an additional arene oxide pathway must also contribute to formation of the 5′-hydroxy compound and the result of equal deuterium retention in both 4′- and 5′-hydroxyoxprenolol to be a coincidence. A more attractive explanation is that a single 4′,5′-arene oxide leads to both products. On the basis of these experiments, we cannot discard the possibility of contributions of multiple arene oxides, formed separately or as part of an oxygen walk process, to the formation of these metabolites. The simplest and most attractive explanation is the one shown

in Scheme IV, a single 4′,5′-arene oxide leading to both metabolites.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Beckman IR-5A spectrometer. NMR spectra were recorded on a Varian EM-360, T-60, or CFT-20 spectrometer using Me_4Si as internal standard, unless otherwise noted. Notations used in the descriptions are: s, singlet; d, doublet; dd, doublet of doublets; q, quartet; m, multiplet. CIMS data were obtained on a Biospect mass spectrometer (methane) and EIMS data on AEI-MS-902, 70 eV. Microanalysis where indicated by the symbols of the elements are within ±0.4% of the theoretical values.

4-Bromo-2-(allyloxy)acetophenone (7). To a solution of 19.0 g (88 mmol) of 4-bromo-2-hydroxyacetophenone (**6**)[17] in 30 mL of DMF was added 10.7 g (88 mmol) of allyl bromide and 12.2 g (88 mmol) of K_2CO_3. After the mixture was stirred at room temperature for 21 h, it was added to 500 mL of stirring aqueous 5% NaOH, and the resulting white solid was removed by filtration and washed with H_2O. Recrystallization from EtOH–H_2O gave 19.7 g (88% yield) of **7** as a white solid, mp 69.5–70.5 °C. Anal. ($C_{11}H_{11}O_2Br$) C, H.

2-Methyl-2-[2′-(allyloxy)-4′-bromophenyl]-1,3-dioxolane (8). A solution of 12.5 g (49 mmol) of acetophenone **7** in 100 mL of benzene with 100 mg of *p*-TsOH and 20 mL of ethylene glycol was refluxed for 9 h in the presence of a Dean–Stark trap to remove the H_2O formed. The benzene solution was cooled and added to 100 mL of 5% NaOH, the layers were separated, and the alkaline layer was extracted with 100 mL of ether. The combined organic extracts were washed with 2 × 100 mL of aqueous 5% NaOH and dried over Na_2SO_4, and the solvent was removed to yield 14.4 g (98% yield) of **8** as a yellow oil: NMR ($CDCl_3$) δ 4.18–3.80 (m, 4, 2 H_4, 2 H_5), 1.75 (s, 3, CH_3); IR no carbonyl band.

2-(Allyloxy)-4-bromophenol (9). To a solution of 13.5 g (51.6 mmol) of acetophenone **7** in 70 mL of CH_2Cl_2 was added 11.4 g (51.6 mmol) of 85% *m*-chloroperbenzoic acid. After the mixture was stirred at room temperature for 20 h, the solvent was evaporated, and the resulting semisolid was dissolved in 30 mL of EtOH and stirred with 50 mL of aqueous 5% NaOH for 20 min. The resulting solution was washed with 2 × 100 mL of ether, then acidified with aqueous 37% HCl, and extracted with 2 × 100 mL of ether. The combined ether extracts were washed with 2 × 100 mL of aqueous saturated $NaHCO_3$ and dried over Na_2SO_4, and the solvent was removed to give 4.70 g (40% yield) of crude **9** as a black oil, which was used without further purification.

3-[2′-(Allyloxy)-4′-bromophenoxy]-1,2-epoxypropane (10). To a solution of 4.50 g (19.6 mmol) of phenol **9** in 30 mL of acetone was added 4.50 g (49.1 mmol) of epichlorohydrin and 2.70 g (19.6 mmol) of K_2CO_3. The mixture was stirred at reflux for 27 h, then added to 150 mL of aqueous 5% NaOH, and extracted with 2 × 200 mL of ether. The combined ether extracts were washed with 1 × 200 mL of aqueous 5% NaOH and dried over Na_2SO_4, and the solvent was evaporated to give 5.00 g (90% yield) of **10** as a clear brown oil, which was used without further purification.

1-(Isopropylamino)-3-[2′-(allyloxy)-4′-bromophenoxy]-2-propanol (4′-Bromooxprenolol; 11). A solution of 5.00 g (17.0 mmol) of epoxide **10** in 60 mL (41.4 g, 0.70 mol) of isopropylamine was added to a bomb and heated at 110 C for 18 h. On cooling, the excess isopropylamine was evaporated to give a brown oil, which was crystallized twice from hexane–isopropyl alcohol to give 3.50 g (60% yield) of **11** as white needles: mp 94.0–94.5 °C. NMR ($CDCl_3$) δ 7.18–6.58 (m, 3, Ar H), 6.35–5.67 (m, 1, $H_{2''}$), 5.48–5.07 (m, 2, 2 $H_{3''}$), 4.53–5.37 (m, 2, 2 $H_{1''}$), 3.90 (s, 3, 2 H_3, H_2), 2.92–2.50 (m, 5, 2 H_1, H_α, OH, NH), 0.98 (d, 6, 2 CH_3); IR (KBr) 3.45, 6.31, 6.67, 6.39, 79.4, 8.23 μm. Anal. ($C_{15}H_{22}O_2NBr$) C, H, N.

Deallylation of Oxprenolol (1) with Pyridine Hydrochloride. A mixture of 1.00 g (3.8 mmol) of oxprenolol (1) and 2.50 g (21.7 mmol) of pyridine hydrochloride was heated at 170 °C under a N_2 atmosphere. After 2 h, the clear, light golden syrup was dissolved in 50 mL of H_2O and made alkaline with KOH pellets. The mixture was then washed with 2 × 100 mL of ether, made acidic by the addition of aqueous 37% HCl, and washed with 100 mL of ether. The solution was adjusted to pH 9 with solid K_2CO_3 and the mixture extracted with 2 × 100 mL of ether. The combined ether extracts were dried over Na_2SO_4 and the solvent was removed to give 400 mg (47% yield) of phenol **12** as a clear, light tan syrup. The NMR and IR spectral data were identical with those obtained from **12** prepared by a less direct route.[23]

2-Phenyl-3-(isopropylamino)-5-[(2′-hydroxyphenoxy)-methyl]oxazolidine (16). To 400 mg (1.8 mmol) of phenol **12** in 10 mL of ether was added 210 mg (2.0 mmol) of benzaldehyde. The mixture was stirred at room temperature for 24 h and then the solvent was removed, yielding 560 mg (99% yield) of crude oxazolidine **16** as a clear yellow oil, which was used without further purification: NMR ($CDCl_3$) δ 7.60–6.63 (m, 9, Ar H), 5.09 and 5.19 (2 s, for H_2 in a ratio of 1.0:0.7), 4.63–3.80 (m, 3, C_5CH_2O, H_5), 4.47–2.53 (m, 3, H_α, 2 H_4), 1.22–0.93 (m, 6, 2 CH_3).

1-(Isopropylamino)-3-[2′-(allyloxy)phenoxy]-2-propanol (Oxprenolol; 1) from 16. To a solution of 560 mg (1.8 mmol) of oxazolidine **16** in 2 mL of DMF was added 217 mg (1.8 mmol) of allyl bromide and 248 mg (1.8 mmol) of K_2CO_3. The mixture was stirred at room temperature for 9 h, then 50 mL of aqueous 5% NaOH was added, and the product was extracted with 2 × 100 mL of ether. The combined ether extracts were washed with 2 × 50 mL of aqueous 5% NaOH and dried over Na_2SO_4, and the solvent was evaporated to yield 510 mg (80% yield) of **18** as a crude yellow oil. The spectral data were identical with data obtained from the oxazolidine of oxprenolol derived by reacting oxprenolol with benzaldehyde.

To a solution of 500 mg (1.4 mmol) of oxazolidine **18** in 5 mL of acetone was added 2.5 mL of aqueous 4 N HCl. The mixture was stirred at room temperature for 2 h, 50 mL of aqueous 5% NaOH was added, and the mixture was then extracted with 2 × 100 mL of ether. Removal of the solvent gave 200 mg (59% yield) of **1** as a white solid, mp 73–74.5 °C (lit.[20] 76–78 °C). An NMR spectrum was identical with the reference, oxprenolol.

1-(Isopropylamino)-3-(4′-bromo-2′-hydroxyphenoxy)-2-propanol (14). To 1.00 g (2.90 mmol) of 4′-bromooxprenolol (**11**) was added 3.00 g (2.6 mmol) of pyridine hydrochloride. The flask was heated for 4 h at 170 °C under a nitrogen atmosphere. The resulting golden syrup was added to 50 mL of H_2O and made alkaline with KOH pellets. The alkaline solution was washed with 2 × 100 mL of ether, acidified (aqueous 37% HCl), and washed with 2 × 100 mL of ether. The solution was adjusted to pH 9 with solid Na_2CO_3 and extracted with 2 × 100 mL of ether. Evaporation of the solvent afforded 520 mg (59% yield) of **14** as an impure cream-colored solid, which was used without further purification.

1-(Isopropylamino)-3-(4′-deuterio-2′-hydroxyphenoxy)-2-propanol (15). A two-necked round-bottom flask was set up with a balloon attached to one neck and a vial attached to the other by means of a flexible rubber tube. Methanol-d_1, 20 mL (99% Stohler), was added to 520 mg (1.70 mmol) of **14** and 150 mg of 5% Pd/C. To the side-arm vial was added 400 mg (9.50 mmol) of $NaBD_4$ (99% Stohler), and the entire apparatus was flushed with nitrogen. The $NaBD_4$ was added in several portions to the stirred solution over a 5-min period. The mixture was stirred for 30 min after completion of the addition of the $NaBD_4$. The mixture was then filtered (Celite) and the pad washed with hot MeOH. The MeOH solution was reduced in volume to 5 mL, and the residue was added to 60 mL of 0.1 M carbonate buffer, pH 9, and extracted with 2 × 100 mL of ether. The combined ether extracts were dried over Na_2SO_4 and evaporated to yield 370 mg (96% yield) of **15** as a clear yellow oil: EIMS (70 eV) *m/e* 226 (100, M), 211 (24, M – CH_3), 116 (12, C_6H_4NO; propanolamine side chain), 111 (23, $C_6H_5O_2D$; catechol ring), 100 (21, $C_5H_{10}NO$; propanolamine side chain minus CH_3). Deuterium incorporation [$d_1/(d_0 + d_1)$] was calculated from the 225, 226, and 227 peaks (M, M + 1, M + 2), which were in a 19:96:13 ratio by the following method: $d_1/(d_0 + d_1) = [(M + 1) - M[(\text{no. carbons})(0.011) + (\text{no. nitrogens})(0.0036)] + (M + 2)]/[M + (M + 1) + (M + 2)]$. The calculated incorporation of deuterium was 83%: NMR ($CDCl_3$) δ 6.91–6.67 (m, 3, Ar H), 4.99 (s, exchangeable), 3.98 (s, 3, 2 H_3, H_2), 2.95–2.63 (m, 3, 2 H_1, H_α), 1.07 (d, 6, 2 CH_3, J = 6 Hz); IR 3.06, 3.22, 2.46, 3.52, 6.28, 6.65, 7.86, 8.21 μm.

2-Phenyl-3-(isopropylamino)-5-[(4′-deuterio-2′-hydroxyphenoxy)methyl]oxazolidine (17). A solution of 340 mg (1.50

mmol) of deuterated phenol **15** and 175 mg (1.70 mmol) of benzaldehyde in 10 mL of ether was stirred at room temperature for 16 h. Evaporation of the solvent gave 430 mg (91% yield) of **17** as a brown oil, which was used without further purification: NMR ($CDCl_3$) δ 7.60–6.37 (m, 8, Ar H), 5.20 and 5.12 (2 s, H_2 in a ratio of 0.7:1.0), 4.60–3.80 (m, 3, H_5, C_5 CH_2O), 3.47–2.50 (m, 3, 2 H_4, H_α), 1.23–0.93 (m, 6, 2 CH_3).

1-(Isopropylamino)-3-[4′-deuterio-2′-(allyloxy)phenoxy]-2-propanol (4′-Deuteriooxprenolol; 4) from 17. To a solution of 430 mg (1.40 mmol) of oxazolidine **17** in 2 mL of DMF was added 165 mg (1.40 mmol) of allyl bromide and 190 mg (1.40 mmol) of K_2CO_3. After the mixture was stirred at room temperature for 9 h, 50 mL of aqueous 5% NaOH was added and the product extracted with 2 × 100 mL of ether. The combined ether extracts were washed with 100 mL of aqueous 5% NaOH and dried over Na_2SO_4, and the solvent was removed to give 340 mg (70% yield) of crude **24** as a yellow oil, which was used without further purification.

To a solution of 340 mg (0.96 mmol) of oxazolidine **24** in 5 mL of acetone was added 2.5 mL of aqueous 4 N HCl. The mixture was stirred at room temperature for 30 min, then added to 50 mL of aqueous 2 N HCl, and washed with 2 × 100 mL of ether. The mixture was made alkaline with KOH pellets and extracted with 2 × 100 mL of ether. The combined ether extracts were dried ($MgSO_4$) and evaporated to yield 163 mg (64% yield) of **4** as a yellow oil. Two crystallizations from hexane gave 40 mg of white solid: mp 74.0–74.5 °C; high-resolution EIMS (70 eV) *m/e* 266 (12, M), 251 (21, M – CH_3), 222 (100, M – C_2H_4O), 151 (30, $C_9H_9DO_2$, catechol monoallyl ether). Incorporation of deuterium was determined from the 222, 223, and 224 peaks in a ratio of 3.0:15.6:2.0 by the method previously outlined. A deuterium incorporation of 83% was calculated, indicating no loss of deuterium during the synthetic scheme: NMR ($CDCl_3$) δ 6.90 (s, 3, Ar H), 6.53–5.73 (m, 1, $H_{2'}$), 5.66–5.06 (m, 2, 2 $H_{3'}$), 4.58 (m, 2, 2 $H_{1'}$), 4.05 (s, 3, H_2, 2 H_3), 3.13–2.19 (m, 5, 2 H_1, H_α, NH, OH), 1.09 (d, 6, 2 CH_3, J = 6 Hz); IR (KBr) 3.51, 6.31, 6.69, 7.79, 8.23 μm.

NIH Shift. Metabolism and Mass Spectral Evaluation. Six male Sprague–Dawley rats, 125 g, were injected ip each with 2.5 mg of 4′-deuteriooxprenolol (**4**; 20 mg/kg) and 20-h combined urines (40 mL) were worked up as described in the previous paper in this issue.[15] Combined urine samples were incubated with β-glucuronidase (pH 5.0) at 37 °C, overnight. The pH was adjusted to 9.2 with sodium carbonate, and then the mixture was extracted with 50 mL of EtOAc. Solvent was evaporated and the residue dissolved in 200 μL of EtOAc. Samples (50 μL) were methylated by dissolving in ether–methanol, 10:1. Gaseous diazomethane, generated from Diazald, was bubbled into the vials with a nitrogen stream until a permanent yellow color persisted. Vials were capped and allowed to stand at room temperature for 6–12 h. The solvent was removed with a N_2 stream, and the residue was dissolved in a mixture of 50 μL of EtOAc and 50 μL of trifluoroacetic anhydride. After heating the mixture at 60 °C for 15 min, the solvent was evaporated and the residue dissolved in 10 μL of EtOAc and subjected to GC–MS analysis.[15]

CIMS (methane) analysis was first done by continuously monitoring the mass range 485–495 as a function of time. No 6′- or 3′-OMe metabolites were observed. Both 4′- and 5′-OMe isomers were observed, with the 4′-OMe being the predominant isomer. The relative ratio of deuterated to nondeuterated metabolite (measured by ion current for the appropriate masses) for the 4′- and 5′-OMe compounds was 0.53 and 0.54, respectively, as evidenced by the 488 (QM) and 489 (QM + 1) peaks. The amount of deuterated material was calculated from the areas of the QM, QM + 1, and QM + 2 peaks, where QM is the ion of the nondeuterated material and QM + 1 is the ion of the deuterated material. The equation used to calculate deuterium incorporation is described under synthesis of **16**. Taking into account the percent deuterium incorporation of the parent drug (83%), a retention of 65% (0.54/0.83 × 100%) was calculated for both isomers. The sample was rerun, and mass spectra were recorded over the mass range 300–500. A large single peak at 309 (corresponding to the propanolamine side chain) and twin ions at 374 and 375 (QM – CF_3COOH), in addition to the parent 488 and 489 (QM + 1) ions, confirmed the presence of the 4′- and 5′-hydroxyoxprenolol metabolites.

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