1 H, pyridylhydrazine C_6 H), 8.65 (s, 1 H, NH, exchanges with deuterium oxide), 8.9 (d, $J_{2,3} = J_{5,6} = 6$ Hz, of d, $J_{2,6} = 1.5$ Hz, pyridylcarbonyl C_2 and C_6 H). Exact mass for $C_{11}H_{10}N_4O$: calcd, 214.0842. found (high-resolution MS), 214.0848.

1,2-Bis(4-pyridylcarbonyl)-1-phenylhydrazine (10c). Reaction of methyl isonicotinate (**3b**; 4.16 g, 30.38 mmol) with a mixture of sodium hydride (1.604 g, 66.84 mmol) and phenylhydrazine (30.38 mmol) as described under procedure C gave a product which was purified on a 2.5 × 26 cm neutral alumina column. Elution with ether–methanol (9:1, v/v; 300 mL) gave **10c**: 0.521 g (5.4%); mp 148–150 °C; IR ($CHCl_3$) 1680 and 1710 cm^{-1} (CO); NMR δ 7.1–7.5 (m, 7 H, phenyl, C_3 and C_5 H), 7.65 (d, $J_{2,3} = J_{5,6} = 5$ Hz, of d, $J_{3,5} = 1.75$ Hz, 2 H, C_3 and C_5 H), 8.6 (d, $J_{2,3} = J_{5,6} = 5$ Hz, 2 H, C_2 and C_6 H), 8.72 (d, $J_{2,3} = J_{5,6} = 5$ Hz, 2 H, C_2 and C_6 H), 10.3 (s, 1 H, NH, exchanges with deuterium oxide). Exact mass for $C_{18}H_{14}N_4O_2$: calcd, 318.1106; found (high-resolution MS), 318.1111.

Pharmacological Methods. Analgesic activity was evaluated by the phenylquinone writhing test.[5] Five male Swiss albino mice weighing 18–22 g were used in each group. The test compound, suspended in a solution of physiological saline and Tween 80 surfactant, was administered subcutaneously, and 30-min later each mouse received a 0.03% phenyl-*p*-benzoquinone solution in a volume of 0.1 mL/10 g of body weight intraperitoneally. The total number of writhes exhibited by each animal in the test group was recorded and compared to that of a vehicle treated control group. The percent change is calculated according to the following equation: % change = (no. of writhes in treated group/no. of writhes in control group) × 100 - 100. A compound causing a 30–50% reduction is considered to be slightly active, whereas one causing a greater than 50% reduction in the number of writhes is an active analgesic agent.

Antiinflammatory activity was measured by the method of Winter.[6] Six female Sprague–Dawley rats weighing 120–160 g were used for each group. Carrageenan (0.1 mL, 1%) in physiological saline was injected subcutaneously under the plantar skin of the hind paw following subcutaneous injection of the test compound suspended in physiological saline and Tween 80 surfactant. The volume of the injected paw was measured immediately after and at 3 and 5 h after the injection of the test compound for calculation of percent inhibition. Table I summarizes the pharmacological results in the above assays.

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Aromatic Hydroxylation of β-Adrenergic Antagonists. Formation of 4′- and 5′-Hydroxy-1-(isopropylamino)-3-[2′-(allyloxy)phenoxy]-2-propanol from Oxprenolol[1]

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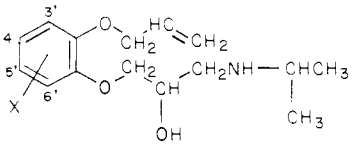
The metabolic aromatic hydroxylation of oxprenolol [1-(isopropylamino)-3-[2′-(allyloxy)phenoxy]-2-propanol] in rats was examined. Synthesis of the isomeric ring methoxyoxprenolols (**3b–6b**) was accomplished from the isomeric methoxysalicylaldehydes by O-allylation, followed by Baeyer–Villiger oxidation. The propanolamine side chain was elaborated by O-alkylation of the Bayer–Villiger product with epichlorohydrin and subsequent oxirane opening with isopropylamine. Gas chromatography–mass spectra of the trifluoroacetyl derivatives of these standards was compared with urinary metabolites obtained from the rat, after methylation with diazomethane and derivatization with trifluoroacetic anhydride. Both 4′- and 5′-hydroxyoxprenolol (**4a** and **5a**) were present in an approximate 4:1 ratio. No 3′- or 6′-hydroxyoxprenolol (**3a** and **6a**) was detected. The metabolites obtained from a human urine treated in the same manner gave similar results with both **4a** and **5a** present.

β-Adrenergic antagonists have been used in a variety of cardiovascular disorders, including cardiac arrhythmias,[2] angina pectoris, hypertrophic subaortic stenosis, and hypertension, and in other disease states, including psychiatric disorders.[3] In hypertension, they are useful alone or in combination with a variety of other drugs, such as α-adrenergic blocking agents, α-methyldihydroxyphenylalanine, diuretics, vasodilators, etc. Our interests in these agents included their metabolic fate, since in some cases parent drug molecules are converted to compounds which may have pharmacological activity, e.g., metabolites formed from propranolol,[4-7] metoprolol,[8] and alprenolol.[6]

Oxprenolol[9] [1-(isopropylamino)-3-[2-(allyloxy)phenoxy]-2-propanol] (**1**) is an important aryloxypropanolamine β-adrenergic antagonist whose metabolism has been extensively studied.[10-15] Oxprenolol is metabolized by hydroxylation of the aromatic ring, by oxidation of the propanolamine side chain, and by glucuronidation.[10,11,14] A possible glucuronide conjugate of an oxprenolol metabolite has been isolated from rats.[14] A different con-



1, X = H
2, X = OH
3a, X = 3′-OH
b, X = 3′-OMe
4a, X = 4′-OH
b, X = 4′-OMe
5a, X = 5′-OH
b, X = 5′-OMe
6a, X = 6′-OH
b, X = 6′-OMe

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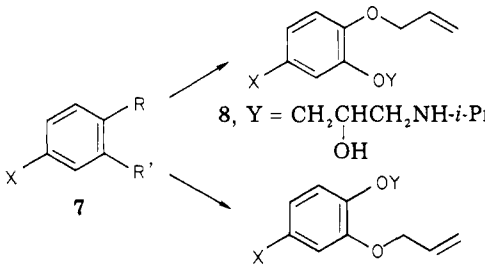
jugate, which is subject to aqueous acid hydrolysis but not to β-glucuronidase, has been identified in human urine.[11]

The metabolite (**2**) isolated from human urine (after treatment of the conjugate with aqueous 1 N HCl) was thought to be a phenol, based on mass spectral measurements on it and on the product formed by treatment of it with CH_2N_2.[11] The trifluoroacetyl (TFA) derivative of the phenolic metabolite from rats gave a single GC peak. Garteiz[14] suggested that metabolic hydroxylation in rats, and thus probably in man, would result in formation of 5′-hydroxyoxprenolol (**5a**) rather than 4′-hydroxyoxprenolol (**4a**), due to greater π-electron density of the allyloxy group[16] para to position 5′ than of the substituted propoxy group para to position 4′. Since no work had been reported on the structure of this metabolite, we set out to determine it. The structure had been inferred on chemical rationale which might not be applicable to the enzymatically mediated metabolic processes.

The high degree of symmetry of the oxprenolol molecule suggested to us that working from only limited amounts of metabolite toward a chemical proof of structure could prove very tortuous. We therefore set out to synthesize derivatives of the isometric ring hydroxylated oxprenolols, where the location of the additional oxygen-bearing substituent would be known with certainty. The plan was based on identification of the metabolite structure(s) by chromatographic comparisons of known synthetic standards with actual metabolites or derivatized metabolites.

Our initial synthetic approach to the hydroxyoxprenols was based on use of trisubstituted benzene derivatives of general structure **7**, where R and R′ are groups which could

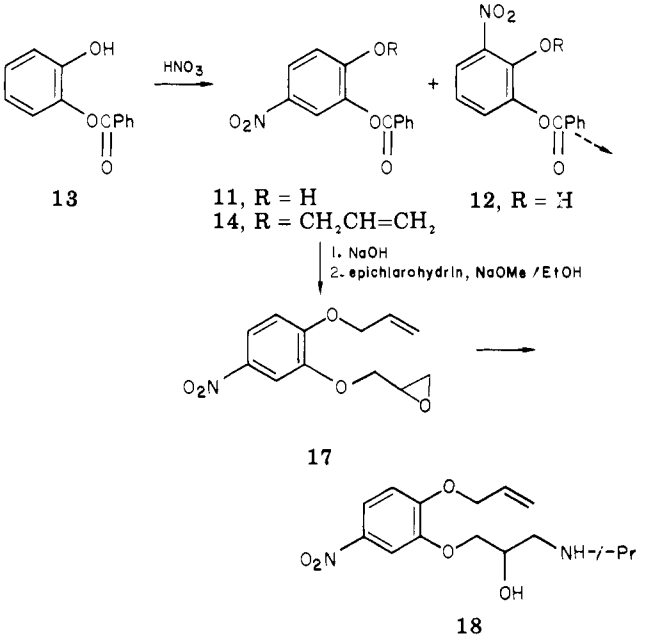


be converted into phenolic ethers, i.e., allyloxy and the three-carbon propanolamine side chain of β-adrenergic antagonists, hoping to incorporate sufficient structural flexibility in the process to convert R and R′ to either oxygen-bearing group at will. The third substituent (X) would be chosen to be readily converted to hydroxy, methoxy, or some closely related derivative. Such a scheme would allow a 1,2,4-trisubstituted benzene system (**7**) to be converted to derivatives of 4′- and 5′-hydroxyoxprenolol (**8** and **9**), and a 1,2,3-trisubstituted system could be converted to 3′- and 6′-hydroxyoxprenolol.

Because of the reported ease of separation of the mononitration products derived from catechol monobenzoate, 4-nitrocatechol 2-benzoate (**11**), and 6-nitrocatechol 2-benzoate (**12**),[17] it was thought that these compounds represented reasonable starting materials to reach all four desired nitrooxprenolols (Scheme I), which could subsequently be converted to phenolic derivatives. Since the postulated structure for the metabolite was 5′-hydroxyoxprenolol (**5a**), initial efforts were directed toward the 5′-nitro compound.

Catechol monobenzoate (**13**)[18] was nitrated to yield a mixture of 4-nitro- and 6-nitrocatechol 2-benzoate (**11** and **12**), which were separated. Alkylation of 4-nitrocatechol

Scheme I



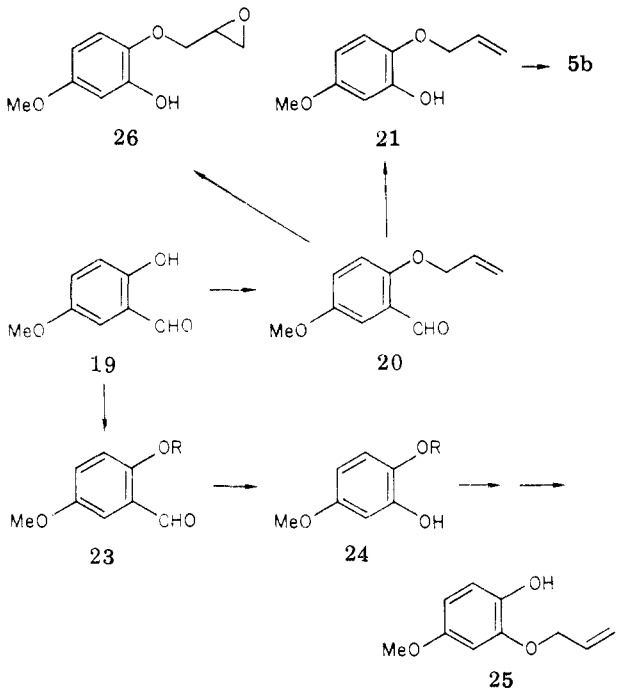
2-monobenzoate (**11**) with allyl bromide (K_2CO_3) gave the allyl ether **14** in 69% yield. Hydrolysis of **14** with alcoholic KOH gave 2-(allyloxy)-5-nitrophenol (**15**) in 88% yield. However, attempted alkylation of nitrophenol **15** with epichlorohydrin (K_2CO_3) in refluxing acetone produced a high-melting solid **16**, whose ^1H NMR spectrum lacked the characteristic pattern for the epoxide group: MS *m/e* 466. It was identified as a diaryl ether formed by epoxide ring opening with a second equivalent of nitrophenol. Isolation of diaryl ethers in the attempted alkylation of phenols with epichlorohydrin has been a continuing problem.[19,20]

When the alkylation reaction was performed in *aqueous* EtOH, the desired epoxide **17** precipitated from solution, preventing further reaction which could lead to **16**. Ring opening of epoxide **17** with isopropylamine gave 5′-nitrooxprenolol (**18**). We were, however, unable to effectively reduce the nitro group to an amine, suitable for conversion to a phenol. In every case, we obtained highly colored, uncharacterized oils which rapidly darkened, thus causing us to abandon the approach.

Our alternative was to use trisubstituted benzene systems in which the desired oxygen substituent was present in the initial starting material (**7**, X = OMe), and R and R′ were substituents which could be converted to hydroxyl groups. We recognized that use of these systems in our synthetic plan had an inherent potential difficulty of requiring selective cleavage of a methyl ether in the presence of the allylic ether. However, should selective demethylation fail, the alternative possibility of methylation of the metabolite for comparison remained. Use of salicylaldehyde derivatives similar to that shown in Scheme II was envisioned where the aldehyde could, by Bayer–Villiger oxidation, be converted to a phenol and, depending on ease of allylic epoxidation under such conditions, could be converted to the epoxide in the same or a subsequent step. Thus, with suitable protecting groups, synthesis of two methoxyoxprenolols could be accomplished from one starting salicylaldehyde.

The successful synthesis of 5′-methoxyoxprenolol (**5b**) was performed starting from 5-methoxysalicylaldehyde (**19**). Allylation, using allyl bromide/K_2CO_3, in DMF produced ether **20**. Baeyer–Villiger oxidation was achieved with 2.1 equiv of *m*-chloroperoxybenzoic acid. Our intent was to both epoxidize the allyl side chain and convert the

Scheme II



aldehyde to epoxyphenol **26** suitable for the synthesis of 4′-methoxyoxprenolol (**4b**). When the Baeyer–Villiger oxidation reaction was performed at room temperature, only phenol **21** was obtained. From this intermediate, the 5′-methoxyoxprenolol (**5b**) isomer was successfully synthesized. Alkylation of phenol **21** with epichlorohydrin gave epoxide **22** in 43% yield as a colorless oil. The epoxide was opened with isopropylamine to give 5′-methoxyoxprenolol (**5**).

An attempt was made to use 5-methoxysalicylaldehyde (**19**) as an intermediate to 4′-methoxyoxprenolol (**4b**), by protecting the phenol, subsequent Baeyer–Villiger oxidation, allyl ether formation, and removal of the protecting group (**19** → **23**, **24**, **25**, etc.) (Scheme II). Our initial work with MEMCl (methoxyethoxymethyl chloride) was successful until attempted removal of the MEM group. The formaldehyde equivalent generated by $ZnBr_2$ cleavage of the MEM-protecting group rapidly reacted with the highly activated phenolic system to form a diphenylmethane derivative.

Attempts to introduce the epoxide side chain prior to the allyl group were not successful. Stephenson observed that formation of a trioxabicyclo[4.2.1]octane system readily occurs when salicylaldehyde is allowed to react with epichlorohydrin,[21] thus making use of intermediate phenol **26** impractical. Presence of a phenolic hydroxyl group ortho to the epoxide-containing side chain results in formation of 1,4-benzodioxanes.[21] These problems directed our synthetic efforts to use of the appropriate methoxysalicylaldehydes. Each, in turn, was converted to the corresponding methoxyoxprenolol by a route analogous to the conversion of **19** to **5b**. Overall yields were similar for all three, 15–19%, in four steps.

Since we had the methoxyoxprenolols **3b–6b** and the possible metabolites were **3a–6a**, it was necessary to convert one to the other to allow for comparison. Demethylation of synthesized standards would provide a convenient source for synthetic metabolite. In spite of the variety of ether cleavage methods available,[22-32] we were unable to effect selection cleavage of the methyl ether. The very mild demethylation procedures, including pyridine hydrochloride and thiolate anions, produced selective

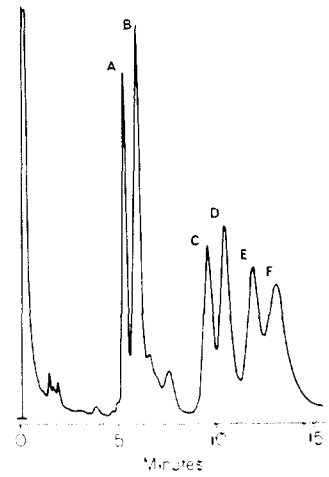
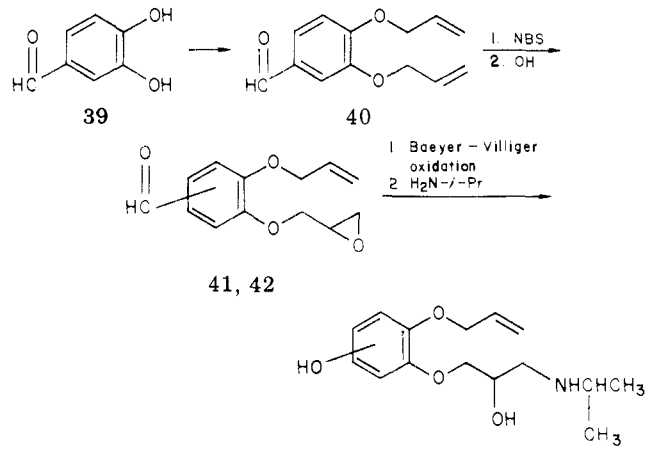


Figure 1. GC recording of oxprenolol metabolic standards as their TFA derivatives: A, oxprenolol (1); B, ring-hydroxylated oxprenolols; C, 3′-methoxyoxprenolol (**3b**); D, 6′-methoxyoxprenolol (**6b**); E, 5′-methoxyoxprenolol (**5b**); F, 4′-methoxyoxprenolol (**4b**).

Scheme III



deallylation. We therefore directed our efforts towards methylation of the metabolite mixture, after finding a suitable GC separation for the isomeric methoxyoxprenolols (**3b–6b**) as their TFA derivatives (Figure 1).

Suitable phenolic standards were needed. A mixture of 4′- and 5′-hydroxyoxprenolol (**4a** and **5a**) was prepared from 3,4-dihydroxybenzaldehyde (**39**) (Scheme III). Diallylation using allyl bromide produced 3,4-bis(allyloxy)benzaldehyde (**40**) in 30% yield. Allowing **40** to react with 1 equiv of NBS in aqueous dioxane produced a mixture of 3-[4′-formyl-2′-(allyloxy)phenoxy]-1,2-epoxypropane and the 5′-formyl isomers **41** and **42**, used for subsequent transformation. Baeyer–Villiger oxidation gave a mixture of phenols, 3-[4′-hydroxy-2′-(allyloxy)phenoxy]-1,2-epoxypropane and the 5′-hydroxy compounds **43** and **44**. Ring opening was accomplished with isopropylamine in a bomb to yield a mixture of 4′- and 5′-hydroxyoxprenolol (**4a** and **5a**) in 60% yield, suitable for methylation.

First attempts at methylation were done with dimethyl sulfate using a sample of the mixture of 4′- and 5′-hydroxyoxprenolol. GC–MS, after trifluoroacetylation, showed the correct parent ions for methoxyoxprenolols at the appropriate retention times. However, on repeated attempts very inconsistent results were obtained using small amounts of a mixture of phenols. Evidently, the pH, the amount of water present, and the dimethyl sulfate to

phenol ratio are important determinants for successful results. Another method was sought.

Although methyl ether formation using diazomethane usually gives poor results with some nonacidic phenols,[34,35] it has been successful with others.[36] Using the mixture of 4′- and 5′-hydroxyoxprenolol standards in ether/methanol (10:1)[34,35] and gaseous diazomethane, some methylation occurred. Although yields varied considerably from one run to the next, the 4′- and 5′-methoxyoxprenolols were consistently obtained. The procedure was considered adequate for methylation of the metabolite mixture.

Metabolites were obtained by collection of 24-h urines from male Sprague–Dawley rats administered oxprenolol 20 mg/kg ip. After treatment with β-glucuronidase, aliquots of the extracted metabolites were treated with excess CH_2N_2 (6 h) and then subjected to trifluoroacetylation.

Determination of metabolites was done by GC–CIMS monitoring, *m/e* 488 (QM) and/or 374 (QM – CF_3COOH),[15] at the appropriate retention times. By monitoring total ion current at these masses,[37] a ratio of approximately 1:4 of 5′-methoxy- to 4′-methoxyoxprenolol (**5b** to **4b**) as TFA derivatives was determined. No 3′- or 6′-methoxyoxprenolols (**3b** and **6b**) were found. From a 6-h human urine sample of one subject administered (orally) a mixture of oxprenolol-d_6 (**45**)[38] and -d_0 (1:1), which was sequentially subjected to hydrolysis with aqueous HCl and with β-glucuronidase, a ratio of 1:4.6 of 5′- and 4′-hydroxyoxprenolol (**5a** and **4a**) was found.

These data confirm the formation of 4′- and 5′-hydroxyoxprenolol as important pathways of aromatic hydroxylation of oxprenolol in the rat and in man. Under these conditions, neither 3′- nor 6′-hydroxylation was noted, although the absence of these potential metabolites cannot be completely assured. If formed, they may have been present in such small amounts that they are not detected by these analyses or possibly could be more slowly methylated by CH_2N_2 and thus not detected.

The confirmation of the presence of hydroxylated oxprenolol metabolites coupled with the observation that some of the pharmacological effects of oxprenolol in man are not well correlated with blood levels of parent oxprenolol[39] could suggest that these hydroxylated metabolites may contribute to observed effects of oxprenolol. Additional experiments to determine blood levels of such metabolites vs. time are needed. Synthesis of sufficient quantities of these hydroxylated oxprenolols is necessary to allow investigation of their pharmacological properties. This synthesis will be reported subsequently. Mechanistic aspects of this hydroxylation are reported in the following paper in this issue.

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 spectrophotometer. NMR spectra were recorded on Varian T-60 and EM-360 spectrometers using Me_4Si as internal standard. Notations used in the description are: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Microanalyses were performed by Dr. F. B. Strauss, Oxford, England. Where indicated by the symbols of the elements, analyses were within ±0.4% of theoretical values.

2-(Benzoyloxy)-4-nitrophenyl Allyl Ether (14). To 7.40 g (29.0 mmol) of 4-nitrocatechol monobenzoate[17] in 100 mL of acetone was added 3.93 g (28.0 mmol) of powdered K_2CO_3 and 5.20 g (43.0 mmol) of allyl bromide. The mixture was stirred at reflux for 18 h and then filtered, and the solvent was evaporated. Crystallization from isopropyl alcohol yielded 5.90 g (69% yield) of 14 as white needles, mp 95.0–95.5 °C. Anal. ($C_{16}H_{13}O_5N$) C, H, N.

2-(Allyloxy)-5-nitrophenol (15). To 4.90 g (16.0 mmol) of ester 14 in 100 mL of EtOH was added 1.84 g (33.0 mmol) of powdered KOH. The mixture was stirred at reflux for 2 h, and then 37% HCl was added until no further color change occurred. The solvent was then removed, yielding a yellow oil. The oil was added to 150 mL of aqueous saturated $NaHCO_3$ and extracted with 4 × 100 mL of ether. The combined ether layers were dried over Na_2SO_4, and the solvent was removed, yielding on oil which crystallized upon standing. Recrystallization from EtOH/H_2O gave 2.83 g (88% yield) of **15** as yellow prisms, mp 86–87 °C. Anal. ($C_9H_9NO_4$) C, H, N.

3-[2′-(Allyloxy)-5′-nitrophenoxy]-1,2-epoxypropane (17).

A. Dimer Formation [1,3-Bis[2′-(allyloxy)-5′-nitrophenoxy]-2-propanol; 16]. To 2.48 g (13.0 mmol) of 2-(allyloxy)-5-nitrophenol (**15**) in 10 mL of EtOH was added 1.75 g (19.0 mmol) of epichlorohydrin, 690 mg (13.0 mmol) of NaOMe, and 2 mL of H_2O. The mixture was stirred at reflux for 18 h and then the mixture was added to 100 mL of ether and washed with 3 × 100 mL of aqueous 2% NaOH and then with 3 × 100 mL of H_2O. Removal of solvent yielded 1.10 g of light-yellow solid (38% yield). A small sample was recrystallized from acetone/H_2O for elemental analysis as white needle crystals: mp >200 °C; EIMS *m/e* 446. Anal. ($C_{21}H_{22}O_9N_2$) C, H, N.

B. Formation of Epoxide 17. To 1.0 g (5.1 mmol) of 2-(allyloxy)-5-nitrophenol (**15**) in 2.0 mL of EtOH was added 700 mg (7.7 mmol) of epichlorohydrin and 410 mg (10 mmol) of NaOH dissolved in 2.0 mL of H_2O. After the addition of 15 mL of H_2O, the mixture was stirred at room temperature for 18 h. Filtration yielded a yellow solid, which was crystallized from isopropyl alcohol/H_2O to give **17** as a white solid in 56% yield: mp 75–76 °C; NMR ($CDCl_3$) δ 8.07–7.73 (m, 2, Ar H_4 and Ar H_6), 6.93 (d, 1, Ar H_3, J = 9 Hz), 6.53–5.80 (m, 1, $H_{2'}$), 5.67–5.20 (m, 2, 2 $H_{3'}$), 4.77 (m, 2, 2 $H_{1'}$), 4.63–3.90 (m, 2, 2 H_3), 3.63–3.30 (m, 1, H_2), 3.13–2.77 (m, 2, 2 H_1). Anal. ($C_{12}H_{13}O_5N$) C, H, N.

1-(Isopropylamino)-3-[2′-(allyloxy)-5′-nitrophenoxy]-2-propanol (5′-Nitrooxprenolol; 18). The epoxide was opened by placing 7.0 g (28.0 mmol) of **17** in a bomb with 30 mL (21.0 g, 0.35 mol) of isopropylamine. The bomb was flushed with N_2, sealed, and heated at 65 °C for 21 h. Removal of solvent gave a yellow oil, which crystallized upon standing. Recrystallization from benzene/cyclohexane yielded 4.50 g (52% yield) of light-yellow crystals: mp 93–94 °C; NMR ($CDCl_3$) δ 8.00–7.71 (m, 2, Ar H_4 and Ar H_6), 6.88 (d, 1, Ar H_3, J = 9 Hz), 6.45–5.57 (m, 1, $H_{2'}$), 5.65–5.18 (m, 2, $H_{3'}$), 4.66 (m, 2, 2 $H_{1'}$), 4.12 (s, 3, H_2, 2 H_3), 3.25–2.37 (m, 5, 2 H_1, H_α, NH, OH), 1.10 (d, 6, 2 CH_3, J = 6 Hz); IR (KBr) 3.40, 3.13, 3.30, 3.45, 6.39, 6.74, 7.56, 7.98, 8.23, 9.34, 9.96, 10.99, 11.74, 12.51, 13.66 μm. Anal. ($C_{15}H_{22}O_5N_2$) C, H, N.

2-(Allyloxy)-4-methoxybenzaldehyde (28). To a solution of 20.33 g (0.13 mol) of 2-hydroxy-4-methoxybenzaldehyde (**27**) in 50 mL of DMF was added 16.12 g (0.13 mol) allyl bromide and 18.46 g (0.13 mol) K_2CO_3. The mixture was stirred at room temperature for 22 h, then 200 mL of aqueous 5% NaOH was added, and the product was extracted with 6 × 100 mL of ether. The combined ether extracts were washed with 6 × 100 mL of aqueous 5% NaOH and dried over Na_2OH_4, and the solvent was evaporated to yield 24.23 g (94% yield) of **28** as a yellow oil, bp 124 °C (0.2 mm). A small sample was recrystallized from petroleum ether (bp 63–75 °C) for analysis as white flakes, mp 34.0–36.5 °C. Anal. ($C_{11}H_{12}O_3$) C, H.

2-(Allyloxy)-5-methoxybenzaldehyde (20). Compound **20** was prepared by a method analogous for the preparation of **28** starting from 2-hydroxy-5-methoxybenzaldehyde (**19**) and allyl bromide. The product was obtained in 93% yield as a yellow oil, bp 117–122 °C (0.3 mm).

2-(Allyloxy)-3-methoxybenzaldehyde (31). Compound **31** was prepared by a method analogous for the preparation of **28**, starting from 2-hydroxy-3-methoxybenzaldehyde (**30**) and allyl bromide. The product was obtained in 77% yield as a yellow oil, bp 105 °C (0.2 mm).

2-(Allyloxy)-6-methoxybenzaldehyde (36). To a solution of 2.31 g (15.4 mmol) of 2,6-dihydroxybenzaldehyde (**34**)[40] in 5 mL of DMF was added 2.30 g (18.5 mmol) of MeI and 2.13 g (15.4 mmol) of K_2CO_3. The mixture was stirred at room temperature for 3 days, then the resulting brown suspension was partitioned between 35 mL of aqueous 2 N HCl, and the solvent was evaporated to yield a yellow-reddish solid. The crude product was dissolved in warm acetone/H_2O and decanted from an immiscible black oil. On cooling, the solution yielded 730 mg (31%

yield) of 6-methoxysalicylaldehyde (**35**),[41] which was used without further purification.

Compound **36** was prepared by a method analogous for the preparation of **28**, starting from 6-methoxysalicylaldehyde (**35**) and allyl bromide. The product was obtained in 85% yield as a yellow oil, which was used without further purification.

2-(Allyloxy)-4-methoxyphenol (25). To a solution of 22.0 g (0.115 mol) of **28** in 350 mL of CH_2Cl_2 was added 29.3 g (0.143 mol) of 85% *m*-chloroperoxybenzoic acid. The solution was cooled initially with an ice bath to prevent refluxing of the CH_2Cl_2. The reaction was stirred at room temperature for 24 h, then added to 200 mL of EtOAc, and washed with 5 × 100 mL of aqueous saturated $NaHCO_3$. Evaporation of the solvent gave a purple oil, which was stirred for 2.5 h with 8.02 g (0.143 mol) of KOH in a 100 mL of aqueous solution containing 25 mL of EtOH. After adjusting to pH 6 by the dropwise addition of 37% aqueous HCl, the mixture was extracted with 4 × 100 mL of ether, and then the combined ether extracts were washed with 4 × 100 mL of saturated aqueous $NaHCO_3$. After drying over $MgSO_4$, the solvent was evaporated to give a yellow oil, which crystallized on standing. Recrystallation from isopropyl alcohol/hexane gave 15.6 g (77% yield) of **25** as white needles, mp 69–69.5 °C. Anal. ($C_{10}H_{12}O_3$) C, H.

2-(Allyloxy)-5-methoxyphenol (21). Phenol **21** was prepared from aldehyde **20** by a method analogous to that for the preparation of **25**. The phenol was isolated in quantitative yield as a crude red oil, which was distilled affording **21** as a yellow oil, bp 110 °C (0.2 mm).

2-(Allyloxy)-3-methoxyphenol (32). Phenol **32** was prepared from aldehyde **31** by a method analogous to that for the preparation of **25**. The phenol was obtained as a yellow oil, bp 95 °C (0.2 mm), in 69% yield.

2-(Allyloxy)-6-methoxyphenol (37). Phenol **37** was prepared from aldehyde **36** by a method analogous for the preparation of **25**. The phenol was obtained as a brown oil in quantitative yield and used without further purification.

3-[2′-(Allyloxy)-4′-methoxyphenoxy]-1,2-epoxypropane (29). To a solution of 15.80 g (88.0 mmol) of phenol **25** in 200 mL of acetone was added 12.11 g (0.13 mol) of epichlorohydrin and 12.11 g (88.0 mmol) of K_2CO_3. After the mixture was stirred at reflux for 46 h, the solvent was evaporated to 30 mL, combined with 100 mL of aqueous 5% NaOH, and extracted with 5 × 100 mL of ether. The combined ether extracts were washed with 5 × 100 mL of aqueous 5% NaOH. After drying ($MgSO_4$), removal of the solvent gave an orange oil, which was distilled, bp 129 °C (0.15 mm), to give **29** in 41% yield, which crystallized upon standing. Recrystallization from hexane gave white needles, mp 52–53 °C Anal. ($C_{13}H_{16}O_4$) C, H.

3-[2′-(Allyloxy)-5′-methoxyphenoxy]-1,2-epoxypropane (22). Epoxide **22** was obtained by alkylation of phenol **21** with epichlorohydrin in a manner analogous to the preparation of **29**. Epoxide **22** was isolated in 43% yield as a colorless oil, bp 156 °C (0.2 mm).

3-[2′-(Allyloxy)-3′-methoxyphenoxy]-1,2-epoxypropane (33). Epoxide **33** was obtained by alkylation of phenol **32** with epichlorohydrin in a manner analogous to the preparation of **29**. Epoxide **33** was isolated in 47% yield as a colorless oil, bp 125–130 °C (0.2 mm).

3-[2′-(Allyloxy)-6′-methoxyphenoxy]-1,2-epoxypropane (38). Epoxide **38** was obtained by alkylation of phenol **37** with epichlorohydrin in a manner analogous to the preparation of **29**. Epoxide **38** was isolated in 52% yield as a colorless oil by microdistillation at 0.45 mm, bath temperature 130–175 °C.

1-(Isopropylamino)-3-[2′-(allyloxy)-4′-methoxyphenoxy]-2-propanol (4′-Methoxyoxprenolol; 4b). A solution of 7.30 g (31.0 mmol) of epoxide **29** in 30 mL (20.7 g, 0.33 mol) of isopropylamine was heated at 80 °C in a bomb for 20 h. Evaporation of excess isopropylamine gave an orange oil, which crystallized upon standing. The crude product was dissolved in 150 mL of aqueous 2 N HCl and washed with 4 × 100 mL of ether. The resulting aqueous phase was made alkaline with NaOH pellets and then extracted with 6 × 100 mL of ether. The combined ether extracts were washed with 4 × 100 mL of aqueous 5% NaOH and dried over $MgSO_4$, and the solvent was removed to give a yellow oil, which crystallized into a white mass. Recrystallization from hexane/isopropyl alcohol gave 6.40 g (70% yield) of **4b** as white crystals: mp 64.0–65.5 °C; NMR ($CDCl_3$) δ 6.90 (d, 1, Ar H_6, J = 8 Hz), 6.53 (d, 1, Ar H_3, J = 3 Hz), 6.40 (dd, 1, Ar H_5, J = 8 and 3 Hz), 6.20–5.68 (m, 1, $H_{2'}$), 5.52–5.00 (m, 2, $H_{3'}$), 4.52–4.32 (m, 2, $H_{1'}$), 3.93 (s, 3, 2 H_3, H_2), 3.00–2.00 (m, 5, 2 H_1, H_α, OH, NH), 1.05 (d, 6, 2 CH_3, J = 6 Hz); IR (KBr) 3.92, 3.02, 3.14, 3.36, 3.48, 6.22, 7.94, 8.13, 8.57, 8.89, 9.49, 9.74, 9.93 10.68 μm. Anal. ($C_{16}H_{25}O_4N$) C, H, N.

1-(Isopropylamino)-3-[2′-(allyloxy)-5′-methoxyphenoxy]-2-propanol (5′-Methoxyoxprenolol; 5b). Epoxide **22** was opened using isopropylamine by a method analogous to that for the preparation of **4b**. The free base was isolated in 74% yield as white needles, mp 71.5–73.5 °C (hexane–isopropyl alcohol). Anal. ($C_{16}H_{25}O_4N$) C, H, N.

1-(Isopropylamino)-3-[2′-(allyloxy)-3′-methoxyphenoxy]-2-propanol (3′-Methoxyoxprenolol; 3b). Epoxide **33** was opened using excess isopropylamine by a method analogous to that for the preparation of **4b**. The free base was obtained in 60% yield as white needles, mp 67–68 °C (hexane–isopropyl alcohol). Anal. ($C_{15}H_{23}O_4N$) N; C: calcd, 65.06; found, 65.47; H: calcd, 8.53; found, 8.12.

1-(Isopropylamino)-3-[2′-(allyloxy)-6′-methoxyphenoxy]-2-propanol (6′-Methoxyoxprenolol; 6b). Epoxide **38** was opened using excess isopropylamine by a method analogous to that for the preparation of **4b**. The free base was obtained in 42% yield as white needles, mp 43–44 °C (hexane–isopropyl alcohol). Anal. ($C_{16}H_{25}O_4N$) C, H, N.

3,4-Bis(allyloxy)benzaldehyde (40). To 10.0 g (72 mmol) of 3,4-dihydroxybenzaldehyde (**39**) in 30 mL of DMF was added 10.0 g (72 mmol) of K_2CO_3 and 19.3 g (0.16 mol) of allyl bromide. After the mixture was stirred at room temperature for 16 h, it was added to 200 mL of aqueous 5% NaOH and shaken with 300 mL of ether. The resulting emulsion was broken by centrifugation and the aqueous layer reextracted with an additional 300 mL of ether. The combined ether layers were washed with 3 × 100 mL of aqueous 5% NaOH and dried over Na_2SO_4, and the solvent was evaporated, yielding 4.70 g (30% yield) of **40** as a brown oil, which when distilled afforded a clear, yellow oil, bp 124–127 °C (0.3 mm).

Hypobromous Acid Addition to 3,4-Bis(allyloxy)benzaldehyde (40). To 2.00 g (9.2 mmol) of aldehyde **39** in a mixture of 15 mL of dioxane and 8 mL of H_2O was added 1.60 g (9.2 mmol) of *N*-bromosuccinimide. The mixture was stirred at room temperature for 24 h, then 100 mL aqueous 5% NaOH was added, and the product was extracted with 3 × 100 mL of ether. The ether was washed with 3 × 50 mL of aqueous 5% NaOH and dried over Na_2SO_4, and the solvent was evaporated to give 2.85 g of colorless oil. An NMR spectrum indicated an approximate 35% conversion to epoxides **41** and **42**, representing 740 mg (34% yield) based upon comparison of integration of epoxide H_1 protons and the aldehyde proton.

Baeyer–Villiger Oxidation of 41 and 42. To a solution of 2.80 g of crude epoxides **41** and **42**, calculated to contain 740 mg (3.2 mmol) of epoxide, in 25 mL of CH_2Cl_2 was added 1.86 g (9.2 mmol) of 85% *m*-chloroperoxybenzoic acid. The mixture was stirred at room temperature for 20 h, then dissolved in 5 mL of MeOH, and stirred with 370 mg (9.2 mmol) of NaOH dissolved in 15 mL of H_2O. After 45 min, the pH was adjusted to 6, and then the mixture was added to 100 mL of saturated $NaHCO_3$ and extracted with 3 × 100 mL of ether. The ether extracts were washed with 2 × 50 mL of aqueous saturated $NaHCO_3$ and dried over Na_2SO_4, and the solvent was removed to yield 1.91 g of reddish oil. A comparison of the integration of epoxide H_1 proton and allyl H_3 protons indicated the presence of 44% of a mixture of 3-(allyloxy)-4-(2,3-epoxypropyl)phenol (**43**) and 4-(allyloxy)-3-(2,3-epoxypropyl)phenol (**44**), representing a quantitative yield based upon starting aldehydes **41** and **42**.

1-(Isopropylamino)-3-[4′-hydroxy-2′-(allyloxy)phenoxy]-2-propanol (4a) and 1-(Isopropylamino)-3-[5′-hydroxy-2′-(allyloxy)phenoxy]-2-propanol (5a) from 43 and 44. A solution of crude oil containing 840 mg (3.8 mmol) of phenols **43** and **44** in 20 mL (13.8 g, 0.23 mol) of isopropylamine was added to a bomb and heated at 90 °C for 20 h. The solvent was evaporated under N_2, and the residue was dissolved in 100 mL aqueous 5% NaOH and washed with 3 × 100 mL of ether. The pH was made acidic with aqueous 37% HCl and then washed with 3 × 100 mL of ether. The pH was then adjusted to 9 and extracted

with 4 × 100 mL of ether. The combined ether extracts were dried over Na_2SO_4 and the solvent was evaporated to give 474 mg of a brown oil (60% yield): NMR ($CDCl_3$) δ 6.77–5.73 (m, 4, Ar H, $H_{2'}$), 5.47–5.10 (m, 2, 2 $H_{3'}$), 4.45 (s, 5, 2 $H_{1'}$, exchangeable), 3.90 (s, 3, 3 H_3, H_2), 2.93–2.57 (m, 3, 2 H_1, H_α), 1.08 (d, 6, 2 CH_3, J = 6 Hz).

The isomeric mixture of 4′- and 5′-hydroxyoxprenolols (**4a** and **5a**) formed were used as a standard for working out the methylation conditions to be used for methylating ring-hydroxylated metabolites of oxprenolol.

Analytical Aspects. Methylation of Hydroxoxprenolol Standards. A. Methylation with Dimethyl Sulfate. A 30-μg sample of synthetic 4′- and 5′-hydroxoxprenolol (**4a** and **5a**) dissolved in 30 μL of EtOH was placed in a small test tube along with 10 μL of 5% NaOH. To this was added 10 μL of a solution of dimethyl sulfate in EtOH (1:10). After 5 min, the sample was washed into a reaction vial with a little EtOH, the solvent was blown off, and the residue was TFA derivatized. A sample injected on GC gave peaks with retention times identical with both 4′- and 5′-methoxyoxprenolols (**4b** and **5b**). The correct parent ions for methoxyoxprenolols were also found.

TFA derivatization was accomplished by placing the sample in a 0.3-mL reaction vial along with 50 μL of trifluoroacetic anhydride. The reaction vial was sealed and heated at 60 °C for 15 min, then the solvent was evaporated under a N_2 stream, and the product was dissolved in 10 μL of EtOAc to be used for GC analysis.

B. Methylation with Diazomethane. For methylation with diazomethane, 100-μg samples of 4′- and 5′-hydroxyoxprenolols (**4a** and **5a**) were dissolved in ether/methanol (10:1) and added to a 1.0-mL reaction vial. A micro diazomethane generator was set up and diazomethane [generated from Diazald (Aldrich) in basic methanol/ether] was bubbled into the vial with N_2 until a permanent yellow color persisted. The vial was then capped and allowed to stand at room temperature for 6–12 h. Solvent was removed under N_2, and the residue was TFA derivatized as above and dissolved in 10 μL of EtOAc for GC analysis. Although yields varied considerably from one run to the next, 4′- and 5′-methoxyoxprenolols (**4b** and **5b**) were consistently obtained.

In Vivo Metabolism. A. Collection of Metabolites from Rats. To six male Sprague–Dawley rats, 120 g each, was administered ip 20 mg/kg (2.4 mg each) of a solution of oxprenolol hydrochloride (4 mg/mL in sterile isotonic H_2O). The rats were then housed overnight in metabolic cages. Twenty-four hour urines were collected and pooled (40 mL) and then incubated with 9200 units of β-glucuronidase (Sigma) at 37 °C overnight in pH 5.0 acetate buffer, which was prepared by adding 340 mg of sodium acetate to the pooled urines and then glacial acetic acid was added to reach pH 5.0. After twenty-four hour incubation, the pH was adjusted to 9.2 with solid Na_2CO_3 and extracted with 2 × 50 mL of EtOAc. The solvent was evaporated and the residue was dissolved in 200 μL of EtOAc. Samples were then either trifluoroacetylated directly or methylated with diazomethane as indicated above and then derivatized with trifluoracetic anhydride.

B. GC–MS Analysis of Metabolic Standards. Metabolic standards were analyzed by means of a Biospec mass spectrometer in the CI mode, interfaced GC containing a $^1/_8$ in. × 6 ft glass column with 10% OV-7 on Chromosorb W. At 190 °C, the following retention times were recorded (all compounds run as their TFA derivatives): oxprenolol, 467 s; ring-hydroxylated oxprenolol, 610 s; 3′-OMe, 810 s; 6′-OMe, 910 s; 5′-OMe, 1030 s; 4′-OMe, 1140 s. Retention times were recorded as the time at which maximum height of the appearance of the QM ion for each compound.

C. GC–MS Analysis of Metabolites. The methylated metabolite was analyzed by GC–MS. Initially, only the mass range 485–595 was monitored as a function of time. In so doing, peaks at 488 (QM) were recorded with retention times corresponding to the 5′- and 4′-OMe isomers. No peaks in the 485–495 mass range were noted with the retention time of the 3′- or 6′-OMe isomers. The 4′- and 5′-OMe isomers were present in an approximate 4:1 ratio, as determined by weighing the appropriate tracings of the total ion current as a function of time for each isomer.

Samples were rerun and mass spectra recorded over the range 250–500. Peaks at 488 (QM) and 378 (QM – CF_3COOH) were recorded for both isomers. This evidence suggests the presence of 4′- and 5′-hydroxyoxprenolol in the original metabolic mixture, with the 4′-hydroxy isomer predominating.

Nonmethylated metabolite was run as a 2-μL injection of a 10-μL TFA derivatized sample, which represented one-fourth of the total original sample. At the appropriate retention time, ring-hydroxylated oxprenolol was detected, as evidenced by the appearance of 458 (QM) and 348 (QM – CF_3COOH) peaks for ring-hydroxylated oxprenolol.

D. Human Metabolites of Oxprenolol. A 63-mg sample of oxprenolol composed of oxprenolol-d_6 (**45**)[38] and oxprenolol-d_0 (**1**) was given orally to one subject, and the resulting 6-h urine (265 mL) was combined with 26 mL of aqueous 37% HCl. After standing for 22 h at 37 °C, the pH was adjusted to 5.0 and the mixture incubated with 18000 units of β-glucuronidase (Sigma) at 37 °C for 26 h and then adjusted to pH 9 and the mixture was extracted with 100 mL of ether. The residue resulting after evaporation of the solvent was dissolved in 200 μL of EtOAc and methylated as described previously.

GC–MS spectral analysis in a manner identical with that for the rat metabolites showed the presence of both 4′- and 5′-OMe isomers, with the 4′-OMe isomer predominating by a 4.6:1.0 ratio, as evidenced by the appearance of QM (488) and QM + 6 (494) ions at the correct retention times. No 3′- or 6′-OMe isomers were detected.

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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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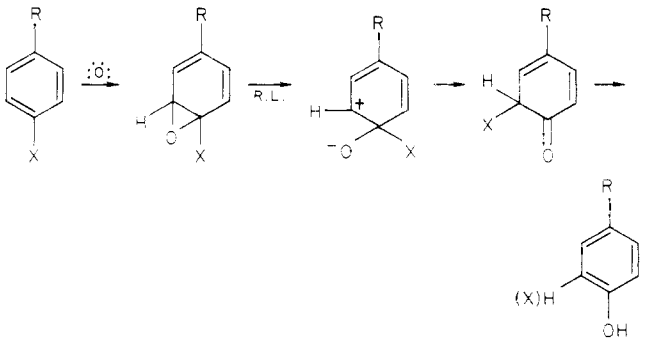
The metabolic hydroxylation of 4′-deuteriooxprenolol [1-(isopropylamino)-3-[2′-(allyloxy)-4′-deuteriophenoxy]-2-propanol] prepared from the 4′-bromo compound was examined in the rat (in vivo). GC–MS analysis of the 4′- and 5′-hydroxyoxprenolol obtained showed 65% retention of deuterium in each of the 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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