N-Dealkylation of Oxprenolol: Formation of 3-Aryloxypropane-1,2-diol, 3-Aryloxylactic Acid, and 2-Aryloxyacetic Acid Metabolites in the Rat

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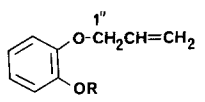
Abstract □ Oxprenolol (**1**), like related β-adrenergic antagonists, undergoes oxidative *N*-dealkylation to form the corresponding 3-aryloxypropane-1,2-diol (**2**), 3-aryloxylactic acid (**3**), and 2-aryloxyacetic acid (**4**) metabolites. Compounds **3** and **4** were synthesized by conversion of 2-allyloxyphenol (**5**) to the aryloxyacetaldehyde **6** and subsequent elaboration to the desired acids. Both acids (**3** and **4**) and glycol **2** were confirmed as metabolites formed from **1** in vivo in the rat and in vitro in the rat liver 9000×*g* supernatant fraction. Incubation of a pseudoracemate of **1**, made up of equal molar amounts of (2*S*)-**1**-d_0 and (2*R*)-**1**-d_2, showed that **2** and **3** arise principally from (2*S*)-**1** by *S*/*R* ratios of ~ 5:1 and 2:1, respectively. On the other hand, acetic acid derivative **4** arises about equally from both enantiomers of **1**.

Oxprenolol [1-(isopropylamino)-3-[2-allyloxy)phenoxy]-2-propanol] (**1**) is an important aryloxypropanolamine β-adrenergic antagonist which is metabolized by a variety of pathways.[1-6] These pathways include aromatic hydroxylation with formation of the corresponding 4′- and 5′-phenols,[5-9] *O*-dealkylation with formation of metabolites arising from loss of the *O*-alkyl group or from loss of the propanolamine side chain,[2,4] *O*-glucuronidation,[2,4] and a multistep oxidative *N*-dealkylation process.[4,8] Glucuronide conjugates of some of the oxprenolol metabolites have also been noted.[1-6]

Metabolites arising from the oxidative *N*-dealkylation of oxprenolol have been observed in the human,[2] rat,[1,10] and dog.[3,4] The de-isopropyl metabolite of oxprenolol is formed from initial *N*-dealkylation. Subsequent deamination has been reported to provide an intermediate aldehyde[10] which is reduced to a 1,2-glycol metabolite **2**[4] or oxidized to the lactic acid metabolite **3**.[2] Like structurally related propranolol, an acetic acid metabolite **4** has also been reported.[2] While these compounds have been identified by gas chromatography–mass spectrometry (GC–MS), verification of their structures by synthesis has not been reported, and stereochemical aspects of their formation have not been studied.

In this paper, we report confirmation of the structures of the two acidic metabolites (**3** and **4**) and of the glycol **2**. These compounds were synthesized, their structures confirmed, and metabolic studies were performed in the rat in vivo and in vitro (in the rat liver 9000×*g* supernatant fraction) to quantitate formation of them. We also sought to determine whether the *N*-dealkylation pathway was stereochemically similar to the analogous process in the metabolism of propranolol.[11]

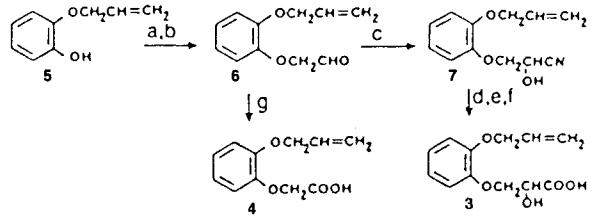


1, R = $CH_2CH(OH)CH_2NHCH(CH_3)_2$
2, R = $CH_2CH(OH)CH_2OH$
3, R = $CH_2CH(OH)COOH$
4, R = CH_2COOH

Stereoselectivity of formation of the 1,2-glycol **2** and the two acidic metabolites (**3** and **4**) was examined in the rat liver 9000×*g* supernatant fraction using pseudoracemic oxprenolol [made up of equal molar amounts of (2*S*)-oxprenolol-d_0 and (2*R*)-oxprenolol-1″,1″-d_2][6] as substrate.

Results

The two postulated acidic metabolites of oxprenolol were synthesized as analytical standards for subsequent metabolic studies. The synthesis of the lactic acid metabolite (**3**) was adapted from a similar procedure applied to synthesis of naphthoxylactic acid (Scheme I).[12,13] Reaction of 2-allyloxyphenol (**5**) with bromoacetaldehyde diethyl acetal in the presence of base afforded an acetal which was hydrolyzed to aldehyde **6** and subsequently converted to cyanohydrin **7**. Conversion of **7** to the desired acid was accomplished by addition of HBr and subsequent hydrolysis of the imido ester. Formation of the desired lactic acid **3** was complicated by the HBr-catalyzed cleavage of the allyl ether. A maximum yield of 25% was obtained. The desired acetic acid derivative **4** was obtained by Ag_2O oxidation of aldehyde **6**. Glycol **2** was available from previous work.[14]



Scheme I—*Reagents: (a)* $BrCH_2CH(OCH_2CH_3)_2$, K_2CO_3; *(b)* H_3O^+; *(c)* $NaHSO_3$, *KCN; (d) HBr, MeOH; (e)* H_2O; *(f) NaOH(EtOH), then* H_3O^+; *(g)* Ag_2O

Determination of the glycol, lactic acid, and acetic acid metabolites (**2**–**4**) arising from *N*-dealkylation of oxprenolol was accomplished by GC–MS analysis using 1-naphthaleneacetic acid as standard. From the in vivo experiments in the rat, urinary conjugate(s) of the glycol were cleaved with β-glucuronidase sulfatase and extracted at pH 1.0. Derivatization with diazomethane converted the acids to methyl esters and all hydroxyl groups were trifluoroacetylated with trifluoroacetylimidazole. Using the mass spectrometer in the selected-ion monitoring mode, three peaks were observed at the same GC retention times as derivatives of the synthetic standards. Mass spectra of these urinary metabolites were identical to the analytical standards with appropriate parent ions and fragments as noted in the *Experimental Section*. From this single-dose study in rats (20 mg/kg ip), the quantities of these metabolites

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Table I—Quantitation of Urinary Oxprenolol Metabolites Formed *In Vivo* in the Rat[a]

Product	Free, % of Dose	Free + Conjugates, % of Dose
Glycol **2**	0.2 ± 0.2	1.0 ± 0.2
Lactic acid **3**	1.6 ± 0.6	2.0 ± 0.7
Acetic acid **4**	0.1 ± 0.1	0.2 ± 0.1

[a] Values are expressed as mean ± SD of eight pair of rats given 20 mg/kg ip of pseudoracemic oxprenolol. Urine samples were collected for 24 h.

Table II—Formation of Metabolites from Pseudoracemic Oxprenolol in the Rat Liver 9000×*g* Supernatant Fraction[a]

Product	From (2*S*)-**1**-d_0, nmol	From (2*R*)-**1**-d_2, nmol	*S*/*R* Ratio
Glycol **2**	9.60 ± 1.40	1.78 ± 0.29	5.4
Lactic acid **3**	2.23 ± 0.17	1.10 ± 0.08	2.0
Acetic acid **4**	0.13 ± 0.01	0.14 ± 0.02	0.9

[a] Incubation for 2 h. Values are expressed as mean ± SD (n = 4).

total ~3% of the administered dose, demonstrating this is a relatively minor pathway for metabolism of oxprenolol in the rat (Table I), similar to results from the metabolism of propranolol in the rat.[14,15]

Information concerning the stereochemical nature of this pathway was obtained in vitro by determination of metabolites arising from incubation of a pseudoracemate of oxprenolol [made up of equal molar amounts of (2*S*)-oxprenolol-d_0 and (2*R*)-oxprenolol-1″,1″-d_2] in the presence of the rat liver 9000×*g* rat liver supernatant fraction. Quantitation was accomplished by monitoring of the parent ions for each enantiomer. Data obtained from a representative in vitro experiment is shown in Table II. Glycol **2**, the major *N*-dealkylation product under these conditions, as expected, was formed primarily from (2*S*)-oxprenolol by a ratio of ~5:1. The lactic acid metabolite **3** also arises preferentially from 2*S*-oxprenolol (~2:1), while the acetic acid metabolite **4** is formed approximately equally from both enantiomers of oxprenolol (*S*/*R* ratio = 0.9:1).

Discussion

Synthetic methods (Scheme I) for obtaining oxprenolol metabolites utilized previously developed technology.[12,13] Other than the inconvenience of the low yield obtained in the hydrolysis of the nitrile because of ether cleavage, the synthetic scheme is satisfactory. The process should be adaptable to obtaining desired compounds for comparison with metabolites arising from other β-adrenergic antagonists of similar structure.

In contrast with humans, the *N*-dealkylation process for oxprenolol and related β-adrenergic antagonists, propranolol, alprenolol, etc., is not a major one in the rat.[11,15,16] In rats, the hydroxylation pathway accounts for a greater percentage of the known metabolites of these agents. However, a substantial amount of the parent drugs remain unaccounted for on the basis of known metabolites.[15,17] Some of these metabolites may arise as products from more than a single pathway. However, the *N*-dealkylation process of related β-adrenergic antagonists has been reported to be quantitatively more important in the dog[15] and in the human.[18,19] The availability of these standards will allow for further investigations of this pathway.

The observed stereoselectivity of the formation of these metabolites in vitro demonstrate significant differences in stereoselectivity of the processes affording glycol metabolite **2** and the lactic acid metabolite **3**. Clearly, the (2*S*)-enantiomer of oxprenolol affords the greater amount of these metabolites, but in different ratios. This difference may arise from differing stereoselectivities of the reduction and oxidation processes from the intermediate aldehyde.[10] It is also possible that these products (**2** and **3**) may be further metabolized. Thus, the observed stereoselectivities are products of stereoselectivities of formation processes as well as those of further metabolism, e.g. hydroxylation of glycol **2**[3] and oxidative decarboxylation of **3** to afford **4**. The extent of further metabolism, as well as the stereoselectivity of those processes will influence the observed stereochemical ratios.

The use of a pseudoracemate of **1** affords an excellent way to determine the stereochemical origin of acetic acid **4**, even though this metabolite is not chiral. If **4** arises from **3**, as has been suggested for the analogous metabolites of propranolol,[11,20] then the less abundant (2*R*)-**3** [(2*R*)-**3** arises from (2*S*)-**1**] affords slightly more **4** than does more abundant (2*S*)-**3**. This result is consistent with the involvement of mammalian L-lactate dehydrogenase in this multistep pathway, based on the broad specificity of the enzyme for substituted α-hydroxycarboxylic acids[21] and its known stereoselectivity.[22]

In summary, structures of metabolites **2**, **3**, and **4** have been established, and significant stereoselectivity was noted in their formation in vitro. These results probably reflect differences in stereoselectivity in metabolite formation and in their further metabolism.

Experimental Section

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Beckman IR-5A spectrophotometer. NMR spectra were recorded on the Varian EM-360 spectrometer with Me_4Si as internal standard. Notations used in the description are as follows: (s) singlet; (d) doublet; (t) triplet; (q) quartet; (m) multiplet. Mass spectra were recorded on the Hewlett-Packard 5985 GC–MS operated in the EI mode. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN.

2-(2-Allyloxyphenoxy)acetaldehyde (6)—2-Allyloxyphenol (**5**) (5.0 g, 33 mmol) and 6.5 g (33 mmol) of $Br-CH_2CH(OCH_2CH_3)_2$ were added to a solution of 4.6 g (44 mmol) K_2CO_3 in 50 mL of dimethylformamide (DMF). This mixture was stirred at 110°C for 24 h, cooled to room temperature, added to 50 mL of 5% aqueous NaOH, and extracted with ether (5 × 50 mL). The combined ether extracts were washed with 5% aqueous NaOH (2 × 25 mL) and water (2 × 25 mL), dried ($MgSO_4$), and evaporated to yield a brown oil. Distillation afforded 3.7 g (42%) of the desired acetal as a light-yellow liquid, bp 120–123°C (0.2 torr). A 15.0-g (56-mmol) sample of the acetal was added to a solution of 30 mL of 2 M aqueous HCl (59 mmol) in 100 mL of acetone. The resulting solution was refluxed for 1 h, cooled to room temperature, and extracted with ether (4 × 100 mL). The combined ether extracts were washed with 5% aqueous K_2CO_3 (2 × 100 mL) and water (1 × 100 mL), dried ($MgSO_4$), and evaporated to yield a yellow oil. Distillation afforded 7.7 g (72%) of the desired aldehyde (**6**), bp 98–100°C (0.9 torr), which solidified, mp 53–56°C (softens at 37°C); IR (neat): 3480, 2910, 1740, 1590, 1500, 1450, 1420, 1250, 1125, 1030, 930, and 740 cm^{-1}; ^1H NMR (acetone-d_6): δ 9.83 (d, 1, *J* = 1 Hz, CH_2CHO), 6.87 (m, 4, ArH), 6.07 (m, 1, $—CH_2CHCH_2$), 5.27 (m, 2, $—CH_2CHCH_2$), and 4.53 ppm (m, 4, $—CH_2CHCH_2$ and $—CH_2CHO$).

2-(2-Allyloxyphenoxy)acetaldehyde Cyanohydrin (7)—Aldehyde **6** (5.0 g, 26 mmol) was added to a solution containing 6.7 g (35 mmol) of $NaHSO_3$ and 2.3 g (35 mmol) of KCN in 50 mL of water. The mixture was stirred for 3 h at room temperature and extracted with EtOAc (3 × 50 mL). The combined EtOAc extracts were washed with water (2 × 25 mL), dried ($MgSO_4$), and evaporated to yield a yellow oil (5.7 g). A portion of the crude product (1.3 g) was purified by column chromatography [60-g silica, eluted with $CHCl_3$:EtOAc:EtOH (95:5:2)] to yield 0.65 g (50%) of **7** as a yellow oil; IR (neat):

3430, 1590, 1500, 1450, 1420, 1255, 1200, 1125, 1015, 930, and 745 cm^{-1}; ^1H NMR (acetone-d_6): δ 4.80 [t, 1, J = 5 Hz, $-CH_2CH(CN)OH$] and 4.13 ppm [d, 2, J = 5 Hz, $-CH_2CH(CN)OH$].

3-(2-Allyloxyphenoxy)2-hydroxypropionic Acid (3)—Cyanohydrin **7** (500 mg, 2.3 mmol) was dissolved in 0.3 mL (8 mmol) of methanol, and the mixture was cooled to 0°C. HBr (20 mmol in 1.4 mL of MeOH) was added, and the resulting mixture was stirred at 0°C for 30 min. The resulting tan imido ester of **3** was hydrolyzed in 18 mL of water at room temperature for 30 min with stirring. The mixture was extracted with EtOAc (3 × 20 mL), and the combined EtOAc layers were dried ($MgSO_4$) and evaporated to yield a tan oil. Purification by column chromatography [20-g silica gel, eluted with CH_2Cl_2:EtOAc (3:1)] yielded 240 mg of the methyl ester of **3** as a colorless oil; MS: m/z 252 (M^+, 49), 151 (24), 121 (29), 109 (base peak), and 81 (21).

Anal.—Calc. for $C_{13}H_{16}O_5$ (**3** methyl ester): C, 61.90; H, 6.39. Found: C, 61.66; H, 6.50.

The methyl ester of **3**, 240 mg, was added to a solution of 5 mL of EtOH and 5 ml of 20% aqueous NaOH. The resulting mixture was stirred at room temperature for 16 h, diluted to 50 mL with water, and the EtOH was evaporated. The solution was adjusted to pH 1.0 (concentrated aqueous HCl) and extracted with EtOAc (3 × 25 mL). The combined EtOAc extracts were dried ($MgSO_4$) and evaporated to yield a yellow oil which solidified. Crystallization from H_2O yielded 130 mg (24% overall yield) of **3** as off-white needles, mp 64.5–69.0°C; IR (neat): 3050 (broad), 1730, 1500, 1250, 1220, 1015, and 750 cm^{-1}; ^1H NMR (acetone-d_6): δ 6.90 (s, 4, ArH), 6.37 (s, 2, $-CH_2CHOHCOOH$), 6.07 (m, 1, $-CH_2CHCH_2$), 5.30 (m, 2, $-CH_2CHCH_2$), 4.50 (m, 3, $-CH_2CHCH_2$ and $-CH_2CHOHCOOH$), and 4.23 ppm (d, 2, J = 4 Hz, $-CH_2CHOHCOOH$); MS: m/z 238 (M^+, 13), 151 (11), 121 (13), 109 (base peak), and 81 (22).

Anal.—Calc. for $C_{12}H_{14}O_5$ (**3**): C, 60.50; H, 5.92. Found: C, 60.62; H, 6.05.

EI–MS of **3** methyl ester–trifluoroacetic acid (TFA) derivative: m/z 348 (M^+, 40), 199 [$(CH_2CH(OTFA)COOCH_3)^+$, base peak], and 171 (50).

2-(2-Allyloxyphenoxy)acetic Acid (4)—Aldehyde **6** (197 mg, 1 mmol) in 5 mL of EtOH was added to a solution containing 346 mg (2 mmol) of $AgNO_3$ in 0.5 mL of H_2O. Aqueous KOH (0.2 g in 0.4 mL of water) was added in a dropwise manner, and the resulting mixture was stirred at room temperature for 1.5 h and filtered; the precipitate was washed with water. The basic filtrate was washed with ether (1 × 10 mL), adjusted to pH 1.0 (concentrated HCl), and extracted with $CHCl_3$ (3 × 5 mL). The combined $CHCl_2$ extracts were washed with water (1 × 10 mL), dried ($MgSO_4$), and evaporated to yield a beige solid. Recrystallization from $CHCl_3$:hexane afforded 65 mg (31%) of **5** as white needles, mp 78.0–79.5°C; IR (KBr): 3000 (broad), 1710, 1590, 1505, 1250, 1215, 1135, 1025, 745, and 740 cm^{-1}; ^1H NMR (acetone-d_6): δ 7.00 (s, 4, ArH), 6.17 (m, 1, $-CH_2CHCH_2$), 5.40 (m, 2, $-CH_2CHCH_2$), and 4.63 ppm (m, 4, $-CH_2CHCH_2$ and $-CH_2COOH$); MS: m/z 208 (M^+, 45), 150 (27), 121 (37), 109 (base peak), and 81 (25).

Anal.—Calc. for $C_{11}H_{12}O_4$ (**4**): C, 63.46; H, 5.81. Found: C, 63.36; H, 5.79.

EI–MS of **4** methyl ester: m/z 220 (M^+, 80), 181 (40), 123 (base peak), and 121 (95) (for comparison see ref. 2).

(2R)-3-(2-Allyloxyphenoxy)-1,2-propanediol(2R-2)—The (2*R*)enantiomer of **2** was prepared as previously reported,[14] mp 82–83°C. EI–MS of the **2**–diTFA derivative: m/z 416 (M^+, 20), 267 [$C_3H_5(OTFA)_2$, base peak], 153 [C_3H_4(OTFA), 95], 78 (97), and 69 (85).

Metabolism Studies—*In Vivo Experiments*—Pairs of male Sprague–Dawley rats (145–155 g) were administered racemic oxprenolol, 20 mg/kg ip in 0.5 mL of distilled water. Urine samples were collected for 24 h over 0.25 g ascorbic acid and diluted to 60 mL; aliquots (3 mL) were prepared for GC–MS analysis. Two aliquots of each urine sample were placed in 15-mL plastic centrifuge tubes and 10 μg of naphthaleneacetic acid (1 μg/μL in ethanol) and 0.5 mL of 0.2 M NaOAc were added to each sample. To one set was added 100 μL (10,000 U) of β-glucuronidase–sulfatase, and the samples were incubated at 37.5°C for 24 h. The samples were then adjusted to pH 1.0 (2 M HCl), and the acidic and neutral metabolites were extracted with ether (1 × 8 mL). Ether extracts were treated with diazomethane in ether (generated from Diazald and KOH) for 30 min and then evaporated with a nitrogen stream. All samples were derivatized with trifluoroacetylimidazole (50 μL in 150 μL of benzene) at 60°C for 15 min, just prior to GC–MS.

In Vitro Metabolism—Male Sprague-Dawley rats (130–150 g) were killed by cervical dislocation, and their livers were removed and homogenized at 0°C in 4 volumes of buffer containing 1.15% KCl in 0.01 M potassium phosphate (pH 7.4) in a Potter–Elvehjem homogenizer with a Teflon pestle. Homogenates were centrifuged at 9000×g for 30 min, and the supernatant fractions were removed and diluted to a protein concentration of 15.6 mg/mL. Suspensions containing 2 mL of the 9000×g supernatant fraction, 2 mL of 0.1 M potassium phosphate buffer (pH 7.4), $MgCl_2$ (20.4 mg, 100 μmol), glucose-6-phosphate (11.3 mg, 40 μmol), $NADP^+$ (0.5 mg, 0.6 μmol), and 0.5 mg of pseudoracemic oxprenolol [made up of equimolar amounts of (2*R*)-oxprenolol-1″,1″-d_2 and (2*S*)-oxprenolol-d_0][6] were incubated in air for 2 h at 37°C. Following the incubation, naphthaleneacetic acid (2 μg of an ethanol solution of 100 μg/mL) was added to each sample. The pH was adjusted to 1.0 (4 M HCl), and the mixture was extracted with 10 mL of ether. The ether extracts were evaporated and treated with diazomethane and trifluoroacetylimidazole as described above.

Gas Chromatographic–Mass Spectral Analysis—GC–MS analysis of the in vivo and in vitro samples was performed using Hewlett-Packard 5985 GC–MS. Separation of metabolites was accomplished using a J&W DB-5 silica capillary column (30 m × 0.25 μm film). Representative chromatographic conditions were: injector, 175°C; helium flow rate, 60 mL/min; column head pressure, 20 psi; temperature programmed from 140–250°C at 20°C/min. The ion source of the MS was maintained at 200°C. The MS was operated in the EI-selected-ion monitoring mode, ionizing voltage 70 eV. Groups of 2–4 ions were scanned at any one time with a dwell time of 20–50 ms. Ions scanned for quantitative analysis were: **2**–diTFA glycol (m/z 416 and 418), **3**–TFA methyl ester (m/z 348 and 350), **4** methyl ester (m/z 222 and 224), and naphthaleneacetic acid methyl ester (m/z 141). Retention times were: **2**–diTFA, 7.1 min; **3**–TFA methyl ester, 7.3 min; **4** methyl ester, 8.5 min; naphthaleneacetic acid methyl ester, 8.3 min.

Quantitation of the oxprenolol metabolites by GC–MS was achieved by obtaining ratios of metabolite to internal standard. Quantities were then obtained by comparison of the experimental ratios to standard curves of ratios of known amounts of metabolites to internal standards. Slopes of the standard curves were determined by linear least-squares analysis. The quantities of metabolites arising from the two enantiomers of oxprenolol were determined from in vitro experiments. Peak area ratios were obtained for each metabolite versus the internal standard. Corrected peak ion ratios were calculated according to the following equations: PS = d_0 and PR = $[d_2-(S_2 \times PS)]/0.94$, where PS and PR are the corrected peak area ratios for metabolite arising from (2*R*)- and (2*S*)-oxprenolol, respectively; d_0 and d_2 are the peak area ratios of nondeuterated and deuterated metabolites versus the internal standard; and S_2 is the ion abundance ratio (M + 2)/(M) for the metabolite arising from a separate incubation of (2*S*)-oxprenolol-d_0. The value of 0.94 arises from the isotopic abundance (deuterium incorporation) of (2*R*)-oxprenolol-1″,1″-d_2.

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