Synthesis of 4'-Hydroxypropranolol Sulfate, a Major Non-β-Blocking Propranolol Metabolite in Man

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4'-Hydroxypropranolol sulfate (8) was recently identified as a major metabolite of propranolol (Inderal). In order to confirm the structure and to further study disposition and biological activity, we have synthesized 8 with use of 1,4-naphthoquinone (1) as the starting material. Reduction and alkylation with benzyl iodide gave 4-(benzyloxy)naphthol (3). Sulfation and chlorosulfuric acid in *N,N*-dimethylaniline gave potassium 1-(benzyloxy)-4-naphthol sulfate (4). Catalytic hydrogenation, alkylation with [[[(trifluoromethyl)sulfonyl]oxy]methyl]oxirane (6), and amination in isopropylamine gave 8. Racemic 8 was found to be 100–1000 times less potent than racemic propranolol as a β-adrenergic receptor blocking agent in the dog.

Although extensive studies of the metabolism of propranolol have been in progress over 15 years,[1] only recently has a major part of the disposition of the drug been clarified. The finding of nonextractable propranolol metabolites, which are not glucuronides, can now account for the missing fraction of the dose both in laboratory animals[2] and in man.[3] A major component of these metabolites is a sulfate conjugate of 4'-hydroxypropranolol, accounting for about 20% of the dose. It was identified by enzymatic hydrolysis and various spectroscopic techniques, including fast atom bombardment mass spectrometry.[4]

As 4'-hydroxypropranolol sulfate has an intact side chain, the possibility existed that it might have β-receptor blocking properties, as previously reported for 4'-hydroxypropranolol and several other ring hydroxylated metabolites.[5,6] For confirmation of the structure of this metabolite, determination of its biological activity, and use in drug disposition studies, we have prepared 4'-hydroxypropranolol sulfate. In this paper we describe the total synthesis of this metabolite and the evaluation of its β-adrenergic blocking activity in the dog.

Chemistry. Although Idle et al.[7] reported the one-step synthesis and separation of epinephrine 3- and 4-*O*-sulfate, the yields are exceedingly poor. On the basis of this information, we feared that direct sulfation of 4'-hydroxypropranolol would likewise give poor yields due to the presence of the reactive secondary alcohol and amino functions. Synthesis via 4'-hydroxypropranolol using protecting groups for these functional groups would offer little advantage since the synthesis of 4'-hydroxypropranolol is not trivial. We, therefore, developed the method reported here, which allows the preparation of 4'-hydroxypropranolol sulfate and other conjugated 4'-hydroxypropranolol metabolites. Since the synthon used to attach the side chain has been used to prepare pure enantiomers of β-blocking drugs,[8] the method reported here is applicable to both the syntheses of the racemic compounds as well as the optically active forms.

The route chosen is depicted in Scheme I. 1,4-Naphthoquinone was reduced by dithionite to give **2a** in near-quantitative yield.[9] One of the hydroxyl groups was then protected as a benzyl ether. Although 4-(benzyloxy)-1-naphthol (**3**) has been prepared previously, the yields were poor.[9] We prepared **3** in considerably better yield by first converting 1,4-naphthohydroquinone (**2a**) to its monosodium salt (**2b**) then alkylating with benzyl iodide (0.4 equiv to minimize dialkylation) to give **3**. Sulfation[10] of **3** was effected in *N,N*-dimethylaniline–carbon disulfide with chlorosulfuric acid to give **4**, which is then debenzylated with hydrogen over palladium on carbon to give potassium 1-hydroxy-4-naphthol sulfate (**5a**). It was an-

Scheme I

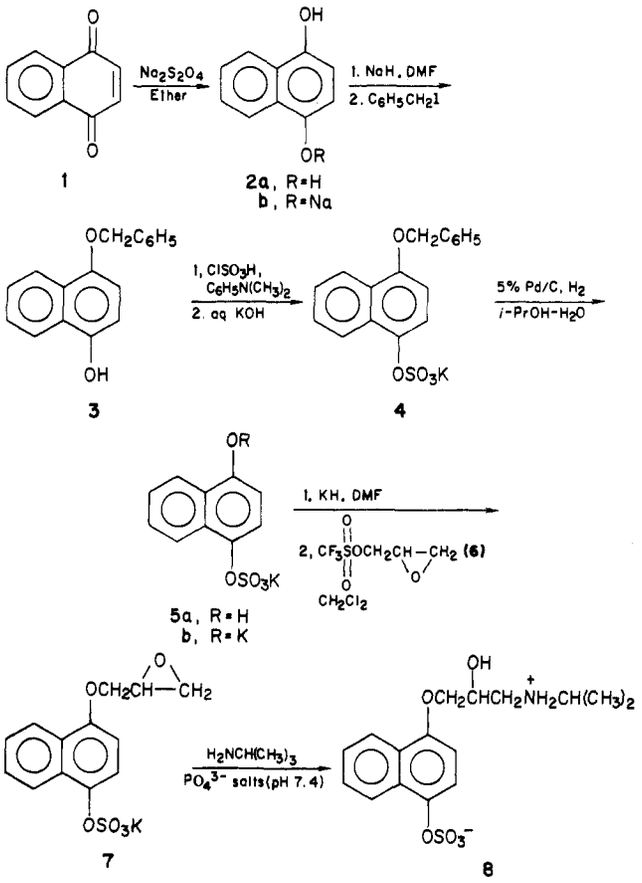

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Table I. Comparative Effects of Propranolol and 4′-Hydroxypropanolol Sulfate as β-Adrenergic Receptor Antagonists in Anesthetized Dogs: Apparent pA_2 Values (Mean ± Standard Error) for Blockade of Isoproterenol-Induced Increase in Heart Rate and Contractile Force (β_1) and Isoproterenol-Induced Vasodilation (β_2)

compd	heart rate	contractile force	vasodilation
propranolol (N = 4)	7.43 ± 0.08	7.60 ± 0.08	7.95 ± 0.06
4′-hydroxypropranolol sulfate (N = 3)	5.12 ± 0.08	4.75 ± 0.07	4.82 ± 0.15

ticipated that **5** would be a poor nucleophile and the harsh conditions[6,11] to effect alkylation, refluxing dilute or neat epichlorohydrin in the presence of a base, would cause extensive loss of the sulfate moiety. McClure et al.[12] have shown that even weakly nucleophilic phenols will under mild conditions directly displace the triflate group from [[[(trifluoromethyl)sulfonyl]oxy]methyl]oxirane[8] (**6**) to give [(aryloxy)methyl]oxiranes in excellent yields. An added benefit is that this procedure permits the use of solvent polar enough to dissolve sulfate **5a** and **5b** directly without the necessity of forming lipophilic ion pairs.[13] Therefore, **6** was added to **5b** to yield **7**. Amination of the epoxide in isopropylamine gave 4′-hydroxypropranolol sulfate (**8**). It was found that the presence of phosphate salts remaining in HPLC-purified **7** from the pH 7.4 buffer both accelerated the amination reaction and decreased side products in the amination to yield **8**.

Pharmacology. The β-adrenergic receptor antagonist potency of racemic 4′-hydroxypropranolol sulfate was determined in pentobarbital-anesthetized open-chest mongrel dogs and compared with that of racemic propranolol. Dose–response curves were generated to intravenous (iv) isoproterenol for increase in heart rate (β_1) and myocardial contractile force (β_1) and decreases in arterial blood pressure (β_2) before and 15 min after the intravenous administration of propranolol or 4′-hydroxypropranolol sulfate. Shifts in the isoproterenol dose–response curves were used to calculate apparent pA_2 values. As shown in Table I, 4′-hydroxypropranolol sulfate has little or no β-receptor blocking activity, being 100–1000 times less potent than propranolol. We conclude, therefore, that although **8** is a major metabolite of propranolol, it is not likely that it contributes significantly to the β-blocking effects of the drug.

Experimental Section

Melting points, determined on a Thomas-Hoover capillary melting point apparatus, are uncorrected. Proton NMR spectra were recorded on a Varian EM 390 spectrometer employing Me_4Si as internal standard. Fast atom bombardment mass spectra (FAB/MS) were obtained in glycerol matrix on a Finnigan MAT-212 mass spectrometer using 8-kv argon bombardment from an Ion-Tek fast-atom gun and a stainless steel probe tip. NMR and MS are consistent with assigned structure. Preparative HPLC was done on a 5.1 × 50 cm stainless steel column packed with Whatman LPS-2 silica and on a 2.5 × 50 cm stainless steel column packed with Regis ODS, employing a Helwig chemical pump and a Waters Associates R 404 differential refractive index detector. Analytical HPLC was done on a Waters Associates M-6000A pump and a Varian variable-wavelength detector, using a 25 cm × 4.6 mm Whatman ODS-3 or an Altex 25 × 4.6 mm silica column. Elemental analyses were performed at Atlantic Micro Labs, Atlanta, GA, and are ±0.4% of the theoretical values. Analytical samples were dried in vacuo over P_2O_5 at ambient temperature and are free of impurities on HPLC.

[[[(Trifluoromethyl)sulfonyl]oxy]methyl]oxirane (6) was prepared via the procedure of Baldwin et al.[8] in 54.2% yield, bp 50.5–51 °C (0.4 mm) [lit.[8] bp 35–40 °C (0.2 mm)].

4-(Benzyloxy)-1-naphthol (3). Purified 1,4-naphthoquinone (15.81 g, 100 mmol) was placed in a separatory funnel and then $Na_2S_2O_4$ (75.1 g, 431 mmol) dissolved in 500 mL of deoxygenated H_2O was added. Ether (475 mL) was added and the reaction mixture was vigorously shaken periodically for 30 min. The aqueous phase was discarded, and the ether solution was washed once with 500 mL of saturated NaCl to which $Na_2S_2O_4$ (10 g) had been added. After drying ($MgSO_4$) and filtration under N_2, the solvent was removed on a rotovap to yield 15.37 g (97.3%) of **2** as a white solid, mp >185 °C dec.

In an oven-dried, three-neck flask equipped with a magnetic stirrer, Firestone valve, and a positive N_2 pressure, **2a** (11.16 g, 69.7 mmol) dissolved in molecular sieve dried DMF (70 mL) was added. Mineral oil free NaH (555 mg, 23.1 mmol) was added in two portions. After the mixture was stirred at room temperature for 10 min, benzyl iodide (6.04 g, 27.7 mmol) was added, and the reaction mixture was allowed to stir at room temperature overnight. The DMF was removed in vacuo at 50 °C, and the volatile impurities were removed by Kugelrohr distillation (to 150 °C). The black tar was extracted five times with 100 mL of cyclohexane and the extract concentrated on a rotovap. The residue was chromatographed on the preparative silica gel column by eluting with $CHCl_3$–MeOH (98:2) and then was recrystallized from cyclohexane (77 mL/g) to give 2.13 g of **3** (36.9%): mp 120–120.5 °C (lit.[9] mp 119 °C); ^1H NMR (Me_2SO-d_6) δ 5.19 (s, 2, OCH_2), 6.83 (m, 2, H-2, H-3), 7.2–7.6 (m, H-6, H-7, C_6H_5), 8.0–8.3 (m, 2 H-5, H-8), 9.61 (s, 1 OH).

Potassium 1-(Benzyloxy)-4-naphthol Sulfate (4). Chlorosulfuric acid (0.32 mL, 561 mg, 4.81 mmol) was added dropwise to a solution of **3** (1.0 g, 4 mmol) dissolved in *N,N*-dimethylaniline (3.5 mL, 3.3 5 g, 27.6 mmol) in CS_2 (1.2 mL) cooled in a salt ice bath. After stirring for 10 min at −10 °C, the reaction mixture was stirred at 0 °C for 1.5 h and then allowed to warm to room temperature overnight. The dark solution was cooled in an ice bath and KOH (4.5 g, 80.2 mmol) dissolved in H_2O (3 mL) was added. The precipitate was filtered, washed with ether, and dried. The crude product was suspended in H_2O, the pH was adjusted to 7.4 with 1.0 M H_2SO_4, and the product was filtered, washed with H_2O, and dried to give 1.24 g of solid. The product was recrystallized from hot MeOH (80 mL/g) after treatment with decolorizing carbon to yield 758 mg. A second crop gave 107 mg. Total yield of **4** was 865 mg (58.8%): mp >135 °C dec; ^1H NMR (Me_2SO-d_6) 5.24 (s, 2, OCH_2), 6.96 (d, J = 9 Hz, 1, H-2), 7.7–7.3 (m, 8, H-3, H-6, H-7, C_6H_5), 8.3–8.0 (m, 2, H-5, H-8). Anal. ($C_{17}H_{13}KO_5S \cdot {}^1/_2H_2O$) C, H, S.

Potassium 1-Hydroxy-4-naphthol Sulfate (5a). A solution of **4** (861 mg, 2.78 mmol) in 70 mL of H_2O-*i*-PrOH (1:1) was hydrogenated at 30 psi over 290 mg of 5% Pd/C. After 2 h hydrogen uptake ceased. The catalyst was removed by filtration, and the solvents were removed in vacuo to give 595 mg of a tan solid. Recrystallization from 95% EtOH gave 448 mg of **5a** as a tan solid: mp ≥160 °C dec; ^1H NMR (Me_2SO-d_6) δ 3.54 (s, ${}^3/_4H_2O$), 6.77 (d, J = 9 Hz, 1, H-2), 7.26 (d, J = 9 Hz, 1, H-3), 7.4–7.6 (m, 2, H-6, H-7), 8.0–8.2 (m, 2, H-5, H-8). Anal. ($C_{10}H_7KO_5S \cdot {}^3/_4H_2O$) C, H, S.

Potassium 1-(2,3-Epoxypropoxy)-4-naphthol Sulfate (7). To an oven-dried, three-neck flask equipped with a magnetic stirrer, addition funnel, and a Firestone valve to maintain a positive pressure of N_2, was added 694 mg of 35% KH in mineral oil (243 mg, 6.06 mmol of KH). After the mineral oil was removed by extraction with 2 × 5 mL of hexane, molecular sieve dried DMF (5 mL) was added, followed by a dropwise addition of **5a** (1.23 g, 4.2 mmol) in 10 mL of dry DMF. The reaction was stirred in a room-temperature H_2O bath for 5 min, and then **6** (984 mg, 4.77 mmol) dissolved in 10 mL of dry CH_2Cl_2 was added dropwise. The blue-green color of the solution of **5b** discharged upon the complete addition of **6**. After the mixture was stirred for 1 h, the solvents were removed in vacuo. The residue was dissolved in H_2O (25 mL), the pH was adjusted 7.5 with 1.0 M H_2SO_4, and the solution was lyophilized to yield 2.53 g of **7**. This material was used without further purification for the subsequent ami-

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nation with isopropylamine. For analysis, 7 was purified by HPlC on a Whatman ODS-2 50-cm M-9 column eluting with 0.1 M PO_4^{3-} (pH 7.4)–CH_3CN (8.5:1.5) and then desalted on a XAD-2 column. The product was eluted with MeOH and then concentrated: ^1H NMR (Me_2SO-d_6) δ 2.7–3.0 (m, 2, $CHCH_2$), 3.3–3.7 (m, 2, $OCH_2CH{\cdot}^1/_2H_2O$), 3.99, 4.48 (dd, ^2J = 12 H, ^3J = 2 Hz, 2, OCH_2), 6.88 (d, J = 9 Hz, 1, H-2′), 7.33 (d, J = 9 Hz, 1, H-3′), 7.4–7.6 (m, 2, H-6′, H-7′), 8.0–8.3 (m, 2, H-5′, H-8′). Anal. ($C_{13}H_{11}KO_6S{\cdot}^1/_2H_2O$) C, H, S.

4′-Hydroxypropranolol Sulfate (8). A suspension of unpurified **7** (2.33 g) and phosphate salts from the pH 7.4 buffer (10.5 g) was stirred in isopropylamine (60 mL, 53.3 g, 902 mmol) for 19.5 h. The excess amine was removed on a rotary evaporator to yield 13.32 g of a tan solid. The residue was stirred with 100 mL of H_2O, filtered, and dried to yield 595 mg of a tan solid. The filtrate, which contained appreciable amounts of 8 in addition to several impurities, was lyophilized and chromatographed on the preparative ODS column eluting with pH 7.4 phosphate buffer–CH_3CN (92.5:7.5). The product fraction was collected, concentrated, and then desalted on the preparative ODS column eluting with MeOH to yield 230 mg of 8. The products were combined and recrystallized from H_2O (maximum H_2O temperature 80 °C) to yield 338 mg. The filtrates were concentrated to yield another 45 mg. Total yield was 383 mg (24.4% yield from **5a**) HPLC on an ODS-3 column [0.1 M PO_4^{3-} (pH 7.4)–CH_3CN (85:15) and 0.01 M NH_4OAc–CH_3CN (85:15)] showed the product to be free of impurities. 8: mp >210 °C dec; NMR (Me_2SO-d_6) δ 1.27 (d, J = 6 Hz, 6, $CH(CH_3)_2$, 3.7–2.9 (m, 3, CH_2NCH), 4.4–4.0 (m, 3, OCH_2CHO), 3.25, 5.89, 8.20 (br s, 5, OH, NH_2, H_2O), 6.83 (d, J = 9 Hz, 1, H-2′), 7.38 (d, J = 9 Hz, 1, H-3′), 7.3–7.6 (m, 2, H-6′, H-7′), 8.3–7.9 (m, 2, H-5′, H-6′); FAB/MS, m/z 356 (MH^+). Anal. ($C_{16}H_{21}NO_6S{\cdot}H_2O$) C, H, N, S.

Hydrolysis of 8 with aryl sulfatase from *Aerobacter aerogenes*[4] produced 4′-hydroxypropranolol in equimolar quantities. In addition, the HPLC retention volume for 8, using two mobile phases on a Spherisorb ODS column, was identical to the retention volume of the human and dog metabolite, demonstrating the metabolite to be 4′-hydroxypropranolol sulfate. Quantitative measurements of 8 in urine was also accomplished by HPLC. After single 80-mg oral doses of propranolol in normal healthy human subjects, 8 accounted for 21.8 ± 2.4% (mean ± SE, n = 6) of the dose.[3] After chronic oral doses, 80 mg every 6 h, 8 accounted for 24.8 ± 2.6% (n = 6) of the dose.

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Registry No. 1, 130-15-4; **2a**, 571-60-8; **3**, 73661-06-0; 4, 95648-09-2; **5a**, 95648-10-5; **6**, 95648-11-6; **7**, 95648-12-7; 8, 95673-91-9; 4′-hydroxypropranolol, 10476-53-6; propranolol, 525-66-6.

3-Amino-β-carboline Derivatives and the Benzodiazepine Receptor. Synthesis of a Selective Antagonist of the Sedative Action of Diazepam

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Seven 3-N-substituted derivatives of 3-amino-β-carboline were synthesized and their affinities for the benzodiazepine receptor were assessed in vitro. Two compounds, 3-(ethylamino)-β-carboline and 3-[(methoxycarbonyl)amino]-β-carboline (β-CMC), showing IC_{50} values of 460 and 71 nM, respectively, were selected for in vivo studies. The former compound showed long-lasting proconvulsant activity in *Papio papio* baboons while β-CMC was shown in mice to selectively antagonize the sedative effects of diazepam without exhibiting convulsant, proconvulsant, or anxiogenic activity by itself.

The discovery of specific, high-affinity receptors in the central nervous system that apparently mediate the anticonvulsant, anxiolytic, and sedative actions of benzodiazepines has greatly facilitated the process of finding new agonists and antagonists structurally unrelated to this class of compounds.[1,2] Thus, binding assays using a radiolabeled benzodiazepine enabled Braestrup and co-workers[3] to isolate a potent benzodiazepine receptor antagonist, ethyl β-carboline-3-carboxylate (β-CCE, 1), from human urine.

Although now considered to be an artifact arising from the isolation procedure rather than a true endogenous ligand of the benzodiazepine receptor,[4] β-CCE and its methyl ester analogue β-CCM (2) are of great interest since not only do they block most of the pharmacological actions of benzodiazepines but they actually exhibit effects opposite to those of benzodiazepines in various animal behavior models: β-CCE and β-CCM are, respectively, proconvulsant and convulsant in photosensitive *Papio papio* baboons[5,6] and in mice,[9] both compounds are anxiogenic in mice,[7-9] and β-CCE significantly increases the wakefulness of cats.[10] The term "inverse agonist" has been applied to these compounds to distinguish them from classical antagonists.[11]

Inspired by these results, several recent reports deal with the synthesis of new β-carboline derivatives[12-19] and the

† ICSN/CNRS.
‡ LPN.

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