Synthesis and Biologic Distribution of Radioiodinated β-Adrenergic Antagonists[1a]

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Iodinated analogues **2**, **7**, and **8** were prepared from propranolol, practolol, and acebutolol in 30–50% yields. Radioisotopic exchange between carrier-free $Na^{125}I$ and the molten iodinated β-adrenergic antagonist yielded the corresponding ^{125}I-labeled product. The biodistribution in rats, determined at 15 and 60 min postinjection, indicated that the radioiodinated analogues of the cardioselective drugs practolol and acebutolol localized to a greater degree in the liver and heart than the analogue of propranolol. Conversely, [^{125}I]iodopropranolol (**2**) was concentrated to a greater extent in the lungs than [^{125}I]iodopractolol (**7**) or [^{125}I]iodoacebutolol (**8**). Therefore, ^{123}I- or ^{131}I-labeled cardioselective β-adrenergic antagonists, such as **7** or **8**, may prove useful as radiodiagnostic agents for the external imaging of the myocardium.

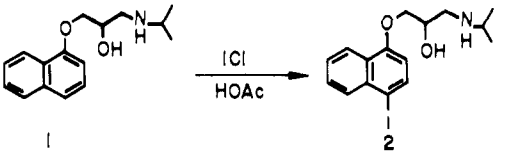
The availability of γ-emitting radiopharmaceuticals which selectively delineate normal from either ischemic or infarcted myocardium has had a significant impact on the detection and diagnosis of a variety of cardiomyopathies. The clinical usefulness of these agents has been somewhat limited by a number of physical and/or biological properties. The lack of a truly satisfactory agent has led to the examination of drugs which exert their pharmacologic effects upon the heart as potential myocardial imaging agents. The extensive literature on the pharmacologic activity of the competitive β-adrenergic antagonists upon the cardiovasculature and their strong affinity for myocardial receptors led us to examine the feasibility of using radioiodinated analogues of these compounds as cardioselective radiopharmaceuticals.

Several groups of investigators have reported the synthesis of high specific activity ^{125}I-labeled β-adrenergic antagonists[2] and have demonstrated the highly stereospecific affinity of these agents in vitro for the β-adrenergic receptors in a variety of tissues, including turkey[3] and rat erythrocytes,[4] rat cerebral cortex,[5] rat liver,[6] rat myocardium,[2b] and two rat glioma cell lines.[7] The in vivo binding in mice of [^{125}I]hydroxybenzylpindolol has been described.[8] The agents that have been radioiodinated have been prepared from drugs which are basically nonselective with respect to cardiac (β_1) vs. vascular (β_2) receptors and, therefore, they would not be expected to demonstrate a preference for myocardial tissue.[9]

Because the chemistry and cardiovascular pharmacology of propranolol,[10] practolol,[11] and acebutolol[12] have been well documented, we have chosen these drugs as the parent compounds for radioiodination. The differences in pharmacologic effects, which range from little selectivity between β_1 and β_2 receptors for propranolol to the high cardioselectivity of practolol, would allow us to explore whether a relationship exists between tissue distribution and biologic activity. Analogues of these drugs, similar to those compounds proposed for radioiodination, have already been synthesized and the preparation of the desired products appears quite feasible. In this paper we report the synthesis and the biologic distribution in rats of three ^{125}I-iodinated analogues of these β-adrenergic antagonists in which the radionuclide is situated distally to the 3-oxy-1-(isopropylamino)propan-2-ol pharmacophore.

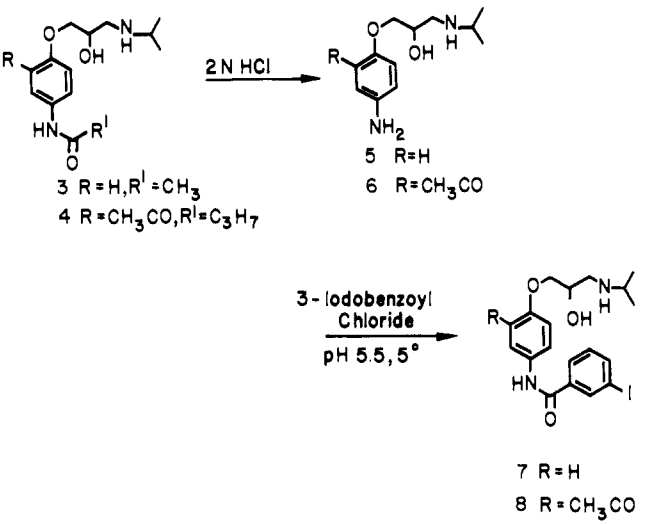
Chemistry. Although a considerable number of propranolol, practolol, and acebutolol analogues have been reported, iodinated derivatives have been notably absent. The synthesis of requisite iodinated compounds was undertaken by utilizing the parent drugs as the starting materials. Direct iodination of propranolol with iodine monochloride in acetic acid[13] (Scheme I) afforded the 4′-iodo derivative in 30–40% yields. The position of iodination was determined by the appearance in the NMR spectrum of a pair of doublets for the 2′ and 3′ protons as well as by the virtual identity of the IR and NMR spectra to those of 4′-chloropropranolol which was synthesized unambiguously from 4-chloro-1-naphthol.[10]

Scheme I



1 2

Scheme II



3 R = H, R′ = CH_3
4 R = CH_3CO, R′ = C_3H_7
5 R = H
6 R = CH_3CO
7 R = H
8 R = CH_3CO

The route by which the iodinated analogues of practolol and acebutolol were obtained is shown in Scheme II. Hydrolysis of the parent drugs gave the 4′-aminophenoxy hydrochlorides **5** and **6** which were subsequently acylated with 3-iodobenzoyl chloride.[14] Chromatography of the resulting mixture gave the pure 3-iodobenzanilides **7** and **8** in 35–50% yields.

Of the several methods for achieving radioiodine isotope exchange, the direct melt method proved to be the most convenient procedure for these compounds.[15,16] Heating the iodinated analogue at its melting point for 2 min in the presence of carrier-free $Na^{125}I$ and a small amount of $Na_2S_2O_5$ gave 35–70% exchange without significant decomposition of the compound. A chromatographic purification of the reaction mixture gave 60–80% recovery of the desired product (Table I). Thin-layer radiochromatography indicated that greater than 95% of the activity was associated with the desired product.

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Table I

compd	formula	mp, °C	recrystn solvent	% exchange[a]	radiochemical purity[b]	sp act., mCi/mmol	analyses
2	$C_{16}H_{20}NO_2I$	128.0-130.0	*i*-PrOH	33	95	33	C, H, N, I
7	$C_{19}H_{23}N_2O_3I$	168.0-170.0	*i*-PrOH	61	94	83	C, H, N, I
8	$C_{21}H_{25}N_2O_4I$	177.5-179.5	EtOAc	75	96	38	C, H, N, I

[a] Radiochromatogram of crude exchange mixture. [b] Radiochromatogram of material used for biological distribution.

Table II. Percent Injected Radioactivity in the Tissues of Rats Following Intravenous Administration of [^{125}I]Propranolol

	15 min		1 h	
	%ID/g	%ID/organ	%ID/g	%ID/organ
liver	0.74 ± 0.12[a]	7.30 ± 0.63	0.66 ± 0.15[a]	7.08 ± 0.76
spleen	1.15 ± 0.13	0.52 ± 0.11	0.60 ± 0.13	0.40 ± 0.04
lung	2.58 ± 0.85	3.90 ± 1.01	2.01 ± 0.63	2.78 ± 0.65
heart	0.35 ± 0.03	0.26 ± 0.02	0.19 ± 0.03	0.16 ± 0.02
stomach		1.99 ± 0.77		2.24 ± 0.60
kidneys	0.98 ± 0.19	1.83 ± 0.40	0.76 ± 0.21	1.58 ± 0.31
small intestine		12.98 ± 2.28		16.23 ± 4.30
large intestine		1.41 ± 0.55		0.91 ± 0.54
muscle	0.20 ± 0.02	19.67 ± 1.99	0.10 ± 0.01	10.71 ± 2.12
fat and fur		8.82 ± 2.75		9.69 ± 2.22
bones	0.16 ± 0.01	2.52 ± 0.32	0.12 ± 0.03	1.84 ± 0.35
blood	0.12 ± 0.01	1.87 ± 0.16	0.10 ± 0.02	1.44 ± 0.13
thyroid	2.76 ± 0.17	0.06 ± 0.01	6.85 ± 1.17	0.14 ± 0.02

[a] Average value and standard deviation for four rats.

Table III. Percent Injected Radioactivity in the Tissues of Rats Following Intravenous Administration of [^{125}I]Practolol

	15 min		1 h	
	%ID/g	%ID/organ	%ID/g	%ID/organ
liver	1.76 ± 0.37[a]	18.94 ± 1.45	1.39 ± 0.05[a]	15.97 ± 2.15
spleen	1.33 ± 0.31	0.80 ± 0.11	1.14 ± 0.12	0.60 ± 0.06
lungs	1.24 ± 0.42	1.89 ± 0.36	1.82 ± 0.37	2.19 ± 0.34
heart	0.89 ± 0.23	0.64 ± 0.09	0.51 ± 0.07	0.35 ± 0.03
stomach		0.71 ± 0.21		0.70 ± 0.22
kidneys	1.82 ± 0.50	3.45 ± 0.64	1.27 ± 0.23	2.30 ± 0.23
small intestine		2.45 ± 0.43		5.49 ± 1.85
large intestine		0.56 ± 0.04		0.94 ± 0.22
muscle	0.20 ± 0.02	18.27 ± 1.64	0.17 ± 0.03	15.96 ± 1.40
fat and fur		5.87 ± 1.47		7.19 ± 1.08
bone	0.21 ± 0.03	2.93 ± 0.25	0.18 ± 0.02	2.44 ± 0.19
blood	0.06 ± 0.01	0.79 ± 0.06	0.06 ± 0.01	0.86 ± 0.07
thyroid	4.73 ± 1.00	0.10 ± 0.02	3.58 ± 0.13	0.07 ± 0.01

[a] Average value and standard deviation for four rats.

Table IV. Percent Injected Radioactivity in the Tissues of Rats Following Intravenous Administration of [^{125}I]Acebutolol

	15 min		1 h	
	%ID/g	%ID/organ	%ID/g	%ID/organ
liver	2.40 ± 0.31[a]	20.19 ± 1.91	1.59 ± 0.21[a]	12.11 ± 2.66
spleen	0.97 ± 0.09	0.52 ± 0.09	0.60 ± 0.07	0.31 ± 0.06
lungs	1.63 ± 0.19	1.77 ± 0.23	1.27 ± 0.24	1.41 ± 0.20
heart	0.99 ± 0.13	0.66 ± 0.09	0.58 ± 0.06	0.31 ± 0.03
stomach		1.26 ± 0.39		1.19 ± 0.19
kidneys	2.80 ± 0.24	4.38 ± 0.57	1.16 ± 0.23	1.83 ± 0.15
small intestine		9.39 ± 0.69		15.40 ± 3.32
large intestine		2.42 ± 2.00		1.01 ± 0.17
muscle	0.22 ± 0.04	16.64 ± 2.23	0.20 ± 0.02	14.87 ± 0.96
fat and fur		7.66 ± 0.81		6.73 ± 0.78
bone	0.22 ± 0.03	2.49 ± 0.33	0.17 ± 0.01	1.94 ± 0.17
blood	0.09 ± 0.01	1.00 ± 0.05	0.06 ± 0.02	0.74 ± 0.12
thyroid	6.06 ± 1.25	0.12 ± 0.03		0.17 ± 0.01

[a] Average value and standard deviation for four rats.

Biologic Distribution. The biologic distribution was determined in rats after intravenous administration of the radioiodinated drug (10 μCi, 0.5–1.0 mg/kg). The animals were sacrificed either at 15-min or 1-h intervals which provided an indication of early as well as delayed distribution of the radioiodinated compounds. Because external imaging of a subsequent ^{123}I- or ^{131}I-labeled analogue would reflect the presence of the radioiodine regardless of its association with the drug, no attempts were made to analyze for the presence of radioiodinated metabolites in the blood, urine, or feces. For the same reason, the distribution between free and bound drug in

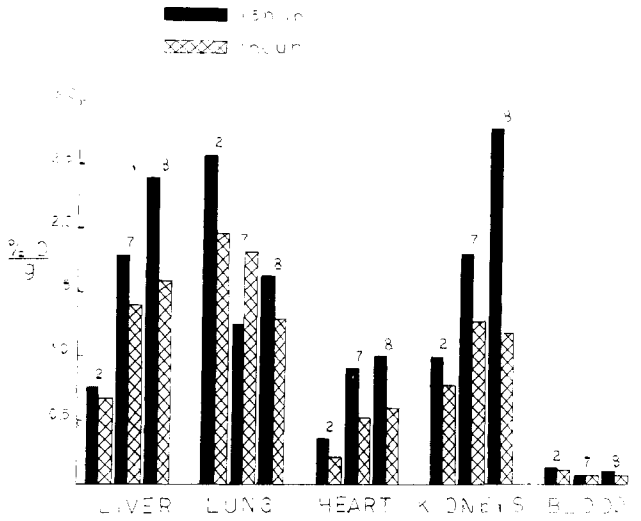

Figure 1. Selected tissue distribution (%ID/g) of ^{125}I β-antagonists at 15 min and 1 h.

the plasma was not determined.

Results and Discussion

The biologic distributions in rats of the ^{125}I-labeled analogues 2, 7, and 8 at 15 min and 1 h are shown in Tables II–IV. In general, the tissues with the highest concentrations (%ID/g) of radioiodine were the liver, spleen, lung, heart, kidney, and thyroid. Since the thyroid actively sequesters free iodide from the blood, any [^{125}I]iodide present in the injected solution or resulting from in vivo deiodination would be accumulated there, yielding an overestimated value for the concentration of the radioiodinated analogue actually present. The other organs, such as the stomach and intestines, contained lower concentrations of the agents while the lowest levels of the radioiodinated analogues were found in the muscle, fat and fur, bones, and blood. With the exception of the thyroid, the concentration of the compounds decreased in all tissues between the 15-min and 1-h time periods. The distribution by total organ content, as opposed to tissue concentration, indicated that the major sites of deposition were the liver, small intestine, muscle, and fat and fur, each having greater than 5% ID/organ.

Although there were minor differences ^{125}I-labeled practolol and acebutolol analogues 7 and 8 had similar distributional profiles which differed significantly from that of [^{125}I]iodopropranolol (2) (Figure 1). These differences are most apparent in the %ID/g of the radiopharmaceutical present in the liver, lung, heart, kidney, and blood. The concentrations of radioiodinated 7 and 8 are approximately twice that of 2 in the liver, heart, and kidney at 15 min. This relationship is still maintained at 1 h in the liver and heart, but the levels in the kidney become essentially equal. At 15 min [^{125}I]iodopropranolol is present in higher concentrations in the lungs and blood than 7 and 8 and although the blood level of 2 remains elevated with respect to 7 and 8, the difference in the lung decreases with time.

As potential myocardial imaging agents, the radioiodinated practolol and acebutolol analogues, 7 and 8, are more promising than [^{125}I]iodopropranolol (2). The greater extraction by the myocardium at 15 min, together with the lower background activity in the lungs, would permit better resolution of the cardiac silhouette. Uptake in the liver is high relative to myocardial uptake and techniques which increase the heart/liver concentration ratio, such as exercise, position, and diet preparation, might be necessary for satisfactory imaging. The more rapid blood clearance of the cardioselective radiopharmaceuticals 7 and 8 would permit earlier external visualization compared to 2. The extension of this work to the preparation of the ^{123}I- or ^{131}I-labeled analogues would provide a new class of radiopharmaceuticals for the external visualization of the myocardium in man.

Experimental Section

General. Melting points were determined on a Thomas-Hoover melting point apparatus and were uncorrected. NMR spectra were measured with a Varian T-60 spectrometer using Me_2SO-d_6 as the solvent and $(CH_3)_4Si$ as the internal standard. IR spectra were measured with a Perkin-Elmer Model 700 spectrophotometer. Elemental analyses were performed by the Schwarzkopf Microanalytical Laboratory, N.Y., and were within ±0.4% of the theoretical values unless otherwise noted. Thin-layer chromatography (TLC) was performed with Eastman-Kodak chromagram sheets, 13181 and 13252, with fluorescent indicator. Biologic samples containing radioactivity and radiochromatograms were counted in a NaI(Tl) γ-well counter. Propranolol hydrochloride and acebutolol hydrochloride were supplied by Ayerst Laboratories, Inc., and May and Baker, Ltd., respectively.

Chemistry. (±)-3-(4′-Iodonaphthoxy)-1-(isopropylamino)propan-2-ol (2). A solution consisting of propranolol hydrochloride (5.0 g), iodine monochloride (2.9 g), and glacial acetic acid was heated at reflux for 3 h. After removal of solvent the residue was purified by column chromatography (Al_2O_3) and crystallized from 2-propanol to give the pure product (2.7 g, 41%), mp 128.0–130.0 °C. Anal. ($C_{16}H_{20}NO_2I$) C, H, N, I.

(±)-3-[*N*-(3-Iodobenzoyl)-4-aminophenoxy]-1-(isopropylamino)propan-2-ol (7). Practolol hydrochloride (3) (1.1 g) was deacetylated via the procedure of Basil et al.[12] to yield compound 5 which was iodobenzoylated in a pH 5.5 solution at 5 °C. The solution was brought to pH 9 and extracted with $CHCl_3$, the extract was evaporated, and the residue was crystallized from 2-propanol to give the pure product (0.8 g, 45%), mp 168.0–170.0 °C. Anal. ($C_{19}H_{23}N_2O_4I$) C, H, N, I.

(±)-3-[*N*-(3-Iodobenzoyl)-2-acetyl-4-aminophenoxy]-1-(isopropylamino)propan-2-ol (8). Employing the same procedure used for 7, 1.86 g (5 mmol) of acebutolol hydrochloride (4) was hydrolyzed and then acylated with 3-iodobenzoyl chloride (6.0 mmol) and the product was isolated by column chromatography to yield, after recrystallization from ethyl acetate, 0.86 g (34%), mp 177.5–179.5 °C. Anal. ($C_{21}H_{25}N_2O_4I$) C, H, N, I.

[^{125}I]Iodine Isotope Exchange. To 10 mg of the iodinated analogue (2, 7, or 8) in a test tube fitted with a serum stopper and N_2 inlet exit needles were added 1–4 mCi of carrier-free Na^{125}I (2200 Ci/mmol) in 5 μL of 0.08 N NaOH and 4 μL of $Na_2S_2O_5$ (1.3 μg/μL). The sides of the test tube were rinsed with 0.3 mL of methanol and the tube was gradually lowered into a hot oil bath. The methanol was allowed to distill under a stream of nitrogen. The residue was then heated for 2 min at a temperature 5 °C greater than the melting point of the analogue. The residue was cooled to ambient temperature, dissolved in 0.5 mL of 2.5% MeOH–$CHCl_3$, and applied to the top of the alumina column (8.0 × 0.5 cm i.d.). The column was eluted with 2.5% MeOH–$CHCl_3$ and the fractions (0.5 mL) which contained the bulk of the product were combined and evaporated to dryness under a stream of nitrogen. The exchange ranged from 35 to 75% and the recovery from 65 to 80%. The purity of the recovered radioiodinated products was determined by TLC on alumina (2.5% MeOH–$CHCl_3$) and greater than 95% of the activity was associated with a single component having the same R_f as the unlabeled compound and being coincident with the single UV absorbent component. Approximately 3–4% of the observed activity was associated with a non-UV-active component located at the solvent front in either system.

Biologic Distribution. The radioiodinated analogues 2, 7, and 8 were dissolved in 0.2 mL of 0.1 N HCl, buffered to pH 6.5 with 0.1 M phosphate, and diluted to 2 mL with water. The solutions were filtered through a millipore filter (0.45 μ) and stored in the dark at 4 °C.

The analogues (0.05–0.10 mL) were injected iv into Sprague–Dawley rats (200–250 g) and the animals were sacrificed

either 15 min or 1 h after the injection. The organs of interest were removed, weighed, and counted in a NaI(Tl) γ-well counter. Care was exercised to avoid cross contamination of the samples. Blood (3 mL) was obtained from a vein in the thoracic cavity. The activity that was observed in each organ was converted to percent of the injected dose per organ (%ID/organ) and/or per gram of organ (%ID/g).

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Diethyl (4bα,4cα,9aα,9bα)-3,6-Dichlorocyclobuta[1,2-*b*:3,4-*b*']bisbenzofuran-9a,9b(4b*H*,4c*H*)-dicarboxylate: the Cis,syn Photodimer of Ethyl 5-Chlorobenzofuran-2-carboxylate, an Analogue Related to the Antilipidemic Drug Clofibrate[1,2]

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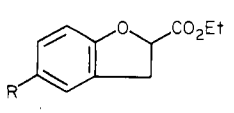
The antilipidemic properties of diethyl (4bα,4cα,9aα,4bα)-3,6-dichlorocyclobuta[1,2-*b*:3,4-*b*']bisbenzofuran-9a,9b(4b*H*,4c*H*)-dicarboxylate, herein termed dimer 8, were studied in sucrose-fed and in Triton-induced hyperlipidemic rats. Whereas clofibrate (0.4 mmol/kg) exhibited both anticholesterolemic and antitriglyceridemic activity, dimer 8 showed only antitriglyceridemic properties at the lower dose (0.2 mmol/kg) in sucrose-fed rats. Dimer 8 only lowered serum triglycerides levels in Triton WR-1339 hyperlipidemic rats, whereas clofibrate lowered both cholesterol and triglyceride levels. In the chronic sucrose-fed model, dimer 8 and clofibrate lowered hepatic HMG-CoA reductase activity and produced significant elevations in several parameters of hepatic drug metabolism. No positive relationship between serum cholesterol lowering and reduction of hepatic HMG-CoA reductase activity was observed by these agents in sucrose-fed rats.

Previous reports from these laboratories have established that the ethyl 5-chloro- and 5-phenyl-2,3-dihydrobenzofuran-2-carboxylate analogues (1 and 2) of clofibrate (3) lowered elevated serum cholesterol levels but had no effect on elevated serum triglyceride concentrations in Triton WR-1339 induced hyperlipidemic rats.[3,4] The converse was true for the unsaturated 5-chlorobenzofuran analogue 4.[4]



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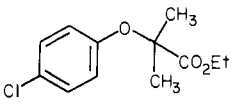
2, R = Ph



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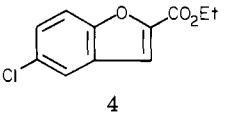

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Since each half of the cyclobutane "2 + 2" photodimer of 4 is structurally similar to either 1 or 4, it was of interest to examine the antilipidemic properties of this compound. In this study we discuss the characterization and pharmacological properties of the photodimer compared with clofibrate in both the acute Triton hyperlipidemic[5] and chronic sucrose-fed rat[6] models. This study is part of an extensive program designed to utilize clofibrate-related analogues as enzyme and lipid interactive probes in a variety of animal models.[7] The Triton hypertriglyceridemic model presumably reflects inhibition of triglyceride catabolism,[8] whereas the sucrose-fed rat model is a reflection of precursor-produced hypertriglyceridemia.[9] Further, chronic administration of aryloxy analogues is required to probe the observable effects on the modification of hepatic microsomal enzymes involved in cholesterol biosynthesis and drug oxidation.[10,11] Investigations of such parameters should provide insight into structural requirements relative to metabolic actions and antilipidemic effects.

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