of doxorubicin and to Richmond Wolgemuth and Larry Hagerman of Adria Laboratories for in vivo testing.

Registry No. 1·HCl, 25316-40-9; 2, 84624-14-6; 3, 84624-15-7; 3·TFA, 84624-16-8; 4, 84624-17-9; 5, 84624-18-0; 6, 84624-19-1; 7, 84624-20-4; 8, 84624-21-5; 9, 84624-22-6; 10, 84624-23-7; 10 (free base), 84680-48-8; 12, 24210-16-0; 13, 27506-15-6; 14, 84642-33-1; 15, 84624-24-8; 16, 84624-25-9; 17, 84624-26-0; 17 (free base), 84680-49-9; N^{α}-Boc-N^{ϵ}-Fmoc-Lys, 84624-27-1; N^{ϵ}-Fmoc-Lys·TFA, 84624-29-3; Boc-Leu, 13139-15-6; Boc-Val, 13734-41-3; leucine benzyl ester *p*-toluenesulfonate, 1738-77-8; *N*-hydroxysuccinimide, 6066-82-6; plasmin, 9001-90-5.

Cardioselectivity of β-Adrenoceptor Blocking Agents. 2. Role of the Amino Group Substituent

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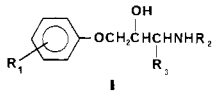
A series of 1-(aralkylamino)-3-(aryloxy)propan-2-ols were synthesized, and their apparent dissociation constants (K_{app}) were determined by using rat ventricular muscle (RVM) and rat lung membrane (RLM) preparations. Analysis of the binding studies suggests the existence of different modes of binding dependent on the presence or absence of the 4-substituent in the aryloxy ring and the nature of that ring. Without 4-substitution only one compound (4), bearing the 2-(2-methoxyphenoxy)ethyl substituent on the amino group, shows high cardioselectivity. Introduction of the 4-acylamido substituent into the phenoxy ring renders all compounds cardioselective. The cardioselective influence of 4-substitution is diminished or eliminated when the phenoxy ring is replaced by naphth-1-yloxy.

In our earlier paper[1] we defined cardioselectivity of β-adrenoceptor blocking agents at the molecular level as having a higher affinity to the β_1 than to the β_2 adrenoceptor. That definition, based on the physiological studies of the β adrenoceptors by Lands et al.,[2] requires the measurement of the apparent dissociation constants of the investigated blockers. Various researchers use different physiological testing methods, making structure–activity relationship studies difficult to compare. The use of an isolated receptor system allows a more exact look at the structural requirements for binding and the differences between the classes of receptors by eliminating obscuring factors such as blood clearance, metabolism, and distribution, which are encountered in in vivo studies.

The existing literature points to molecular alterations in the 1-amino-3-(aryloxy)propan-2-ols (I) that may lead to cardioselectivity. Those alterations are (1) 4-substitution (R_1) in the aryloxy group with a rigid substituent of at least three atoms in size;[3] (2) placement of an aralkyl,[4] aryloxyalkyl,[5] or alkyloxyalkyl[5] on the 1-amino group (R_2); or (3) stereospecific alkyl substitution on carbon 1 (R_3) in the propan-2-ol moiety.[6,7] The assumption that all three above-mentioned alterations do not change the site of binding with the receptors should permit "fine tuning", leading to a compound that incorporates the optimal groups in one molecule of superior cardioselectivity.



I

To check a part of that assumption and to complement the existing knowledge of the structural differences between the receptors, we describe herein the synthesis of 15 1-(aralkyl)- or 1-[[(aryloxy)alkyl]amino]-3-(aryloxy)-propan-2-ols (Table I) and report the apparent dissociation constants of 26 β-adrenoceptors blockers (Table II).

Chemistry. As illustrated in Scheme I, the phenol substrates II (purchased or synthesized by well-known methods) were converted to epoxide intermediates III by using the conditions described by Shtacher.[8] The epoxides were purified by crystallization from ethyl acetate or column chromatography on silica gel with 10% MeOH and CH_2Cl_2. The reaction of the epoxides III with an excess of amine (1.4-fold) in boiling methanol gave the desired product IV. The purification of the products often required repeated preparative LC of their free bases and recrystallization of their salts with oxalic acid. The synthesized compounds are listed in Table I.

Pharmacology. The apparent dissociation constants (K_{app}, μM) of the β-adrenoceptor blockers used in this study were determined by the use of a competitive binding assay with (−)-[^{3}H]dihydroalprenolol. The assay and the preparation of rat lung membranes (RLM) have been described in detail previously.[9,10] We used a different procedure for the preparation of rat ventricular muscle receptor-rich membrane fragments (RVM) than in our previous reports;[1,10] the RVM was prepared by the method of Baker and Potter[11] (see Experimental Section). K_{app} values represent the average of at least six individual measurements on three different days with receptor fractions prepared freshly for that day. Apparent affinities of several previously reported compounds were noticed to increase. Such changes upon altering the method of tissue preparation are not uncommon.[12,13] The method of Baker and Potter eliminates the contractile proteins that account for a large degree of nonreceptor binding of the β blockers. Such nonreceptor binding can increase the K_{app} value by

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Table I. Chemical Data

no.	mp,[a] °C	yield,[b] %	prep chromatr (silica gel, MeOH/CH_2Cl_2)	recrystn solvent	formula[c]
5	153–155	10	20/80	MeOH	$C_{21}H_{25}NO_8$[d]
6	169–171	3	20/80	MeOH	$C_{19}H_{23}NO_6$
7	195–197	10	20/80	MeOH	$C_{21}H_{24}ClNO_2$[e]
9	212–214	33	35/65	MeOH	$C_{22}H_{28}N_2O_9$
10	215–217	31	35/65	MeOH/H_2O	$C_{23}H_{28}N_2O_9$
11	184–186	26	35/65	MeOH	$C_{21}H_{26}N_2O_7$
12	219–220	32	35/65	MeOH/H_2O	$C_{25}H_{28}N_2O_7$
15	162–164	26	20/80	MeOH	$C_{26}H_{36}N_2O_9$
16	155–157	14	20/80	MeOH	$C_{27}H_{36}N_2O_9$
17	167–169	1	20/80	MeOH	$C_{26}H_{34}N_2O_9$
18	172–174	13	15/85	MeOH	$C_{28}H_{38}N_2O_9$
19	172–174	2	20/80	MeOH	$C_{25}H_{34}N_2O_7$
20	153–155	23	20/80	MeOH	$C_{28}H_{35}N_2O_5$[f]
23	215–217	3	20/80	MeOH/H_2O	$C_{27}H_{32}N_2O_9$
24	175–177	1	15/85	MeOH	$C_{31}H_{40}N_2O_9$

[a] Uncorrected. [b] Yield based on epoxide. [c] All compounds were analyzed for C, H, and N; analytical results were within ±0.4% of the theoretical values. [d] All compounds analyzed as oxalates unless otherwise indicated. [e] Hydrochloride. [f] Hemioxalate.

reducing the concentration of free ligand that will interact with the receptor. In addition, 20% of the rat heart β receptor is β_2.[14] If the method of tissue preparation selects differing amounts of β_1 vs. β_2 receptor, the preparation with the largest fraction of β_2 receptor will exhibit the lowest affinity for a cardioselective drug. We therefore suggest that our current method of tissue preparation provides a more accurate estimate of affinity for the investigated compounds.

Data Analysis. We are interested in comparing the affinities of the compounds presented in this study for both classes of receptor and to determine which of the compounds exhibit the highest affinities within the same class of receptor; i.e., we compare the affinities of a compound to the β_1 vs. the β_2 receptor, which we express as the cardioselectivity ratio (Table II). For example, we compare the cardioselectivity ratio of practolol with that of compound 1. In addition, we compare one compound with another with respect to its affinity in the same tissue; e.g., the affinity that compound 1 exhibits for the receptor from RVM is 66-fold higher than that of practolol. The values of K_{app} are normally distributed in log space, and confidence intervals are calculated as described by Bahn.[15] The cardioselectivity ratios and 95% confidence intervals are calculated from the differences in the means of log K_{app} and the standard error in log space. The variances of the samples are not equal: the estimation of the number of degrees of freedom for obtaining t was therefore determined by using the Welch[16] approximation. Confidence intervals are reported in Table II to indicate the accuracy of each K_{app} value and ratio.

Pharmacological Results and Discussion

Hoefle et al.[4] reported a substantial increase in cardioselectivity upon the introduction of the 3,4-dimethoxyphenethyl (homoveratryl) group in place of the usual isopropyl group. Since the publication of that report, a number of β-adrenoceptor antagonists[17] and agonists[18] bearing that group were synthesized and provided pronounced cardioselectivity.

To study the implied uniqueness of the homoveratryl substituent, we have synthesized and studied four groups of compounds bearing that group or its structural variants. The investigated groups of compounds were (a) unsubstituted in the 3-phenoxy ring (**1–7**); (b) 4′-acetamido substituted (**8–12**); (c) 4′-caproamido (and homologues) substituted (**13–20**); and (d) 4′-substituted and unsubstituted in the 3-(1-naphthyloxy) ring (**21–24**). Practolol and propranolol were used as the cardioselective and nonselective reference blocker, respectively.

The variants of the homoveratryl groups were 1,4-benzodioxin-6-ylethyl, 2-methoxphenoxyethyl, phenethyl, and naphth-2-ylethyl. The first three variants should possess lipophilicities closely resembling that of the homoveratryl group,[19,20] whereas the lipophilicity of the naphth-2-ylethyl should be much greater. The findings of Makriyanis[21] led us to assume that the homoveratryl group will occupy a greater volume than the other isolipophilic groups tested and the naphth-2-ylethyl group. As a reference, each investigated group of compounds included a compound carrying the isopropyl group (**1**, practolol, **13**, and propranolol), which is far less lipophilic than the homoveratryl group. To test the reported inactivity of compound **21**[5] (inactive when tested in an anesthetized cat), we resynthesized that compound and compound **3**, both bearing the 2-methoxyethyl group, which is less lipophilic than the isopropyl group.

In the first group (**1–7**), two compounds, **1** and **4**, bearing the isopropyl and 2-methoxyphenoxyethyl, respectively, show affinities to the β_1 adrenoceptor significantly greater than the remainder. In addition, both compounds are cardioselective. The introduction of the least lipophilic amino substituents in the series, the 2-methoxyethyl group, leads to cardioselectivity and to a drastic loss of affinity to both receptors. The cardioselectivity of that compound (**3**) was also reported by Smith and Tucker[5] in the anesthetized cat model. The introduction of the homoveratryl group does not lead to the expected cardioselectivity. Also, the remaining two isolipophilic groups, 1,4-benzo-

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Scheme I

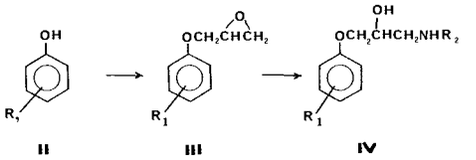
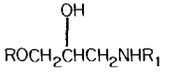


Table II. Affinities and Cardioselectivity



compd	R	R_1	K_{app}, μM: RVM	K_{app}, μM: RLM	cardioselectivity: RLM/RVM
1	C_6H_5	$(CH_3)_2CH$	0.046 (0.038–0.056)[a] (0.24)[b]	0.19 (0.13–0.27)[a] (0.37)[b]	4.1 (3.0–5.6)[a] (1.5)[b]
2	C_6H_5	$3,4\text{-}(CH_3O)_2C_6H_3CH_2CH_2$	0.35 (0.29–0.42) (0.38)[b]	0.47 (0.34–0.66) (1.8)[b]	1.3 (0.99–1.8) (4.7)[b]
3[c]	C_6H_5	$CH_3OCH_2CH_2$	1.2 (1.1–1.3)	5.6 (5.0–6.0)	4.7 (4.2–5.2)
4[c]	C_6H_5	$2\text{-}(CH_3O)C_6H_4OCH_2CH_2$	0.043 (0.028–0.067)	0.99 (0.97–1.0)	23 (17–31)
5	C_6H_5	$3,4\text{-}(OCH_2CH_2O)C_6H_3CH_2CH_2$	0.16 (0.15–0.17)	0.15 (0.091–0.25)	0.94 (0.67–1.3)
6	C_6H_5	$C_6H_5CH_2CH_2$	0.24 (0.22–0.27)	0.098 (0.065–0.15)	0.41 (0.31–0.54)
7	C_6H_5	$2\text{-}C_{10}H_7CH_2CH_2$	0.39 (0.31–0.48)	0.42 (0.34–0.52)	1.3 (0.84–1.4)
practolol			2.9 (2.5–3.3)	110 (92–132)	38 (31–46)
8	$4\text{-}CH_3CONHC_6H_4$	$3,4\text{-}(CH_3O)_2C_6H_3CH_2CH_2$	1.7 (1.3–2.2)	145 (113–186)	85 (61–120)
9	$4\text{-}CH_3CONHC_6H_4$	$2\text{-}(CH_3O)C_6H_4OCH_2CH_2$	1.0 (0.67–1.5)	30 (22–41)	30 (19–46)
10	$4\text{-}CH_3CONHC_6H_4$	$3,4\text{-}(OCH_2CH_2O)C_6H_3CH_2CH_2$	3.9 (2.2–7.0)	79 (48–129)	20 (11–37)
11	$4\text{-}CH_3CONHC_6H_4$	$C_6H_5CH_2CH_2$	12 (10–13)	57 (56–58)	4.9 (4.5–5.4)
12	$4\text{-}CH_3CONHC_6H_4$	$2\text{-}C_{10}H_7CH_2CH_2$	7.0 (6.8–7.3)	22 (13–37)	3.1 (2.2–4.4)
13	$4\text{-}CH_3(CH_2)_4CONHC_6H_4$	$(CH_3)_2CH$	1.1 (0.78–1.6)	14 (11–18)	13 (8.4–19)
14	$4\text{-}CH_3(CH_2)_4CONHC_6H_4$	$(3,4\text{-}CH_3O)_2C_6H_3CH_2CH_2$	0.61 (0.54–0.68)	27 (21–35)	44 (35–56)
15	$4\text{-}CH_3(CH_2)_4CONHC_6H_4$	$2\text{-}(CH_3O)C_6H_4OCH_2CH_2$	1.0 (0.86–1.2)	13 (11–15)	13 (11–15)
16	$4\text{-}CH_3(CH_2)_4CONHC_6H_4$	$3,4\text{-}(OCH_2CH_2O)C_6H_3CH_2CH_2$	0.41 (0.27–0.61)	39 (25–61)	95 (56–160)
17	$4\text{-}CH_3(CH_2)_3CONHC_6H_4$	$3,4\text{-}(OCH_2CH_2O)C_6H_3CH_2CH_2$	0.52 (0.46–0.54)	38 (23–62)	73 (53–101)
18	$4\text{-}CH_3(CH_2)_5CONHC_6H_4$	$3,4\text{-}(OCH_2CH_2O)C_6H_3CH_2CH_2$	0.47 (0.37–0.59)	43 (39–47)	91 (70–120)
19	$4\text{-}CH_3(CH_2)_4CONHC_6H_4$	$C_6H_5CH_2CH_2$	0.78 (0.60–1.0)	86 (69–107)	110 (81–150)
20	$4\text{-}CH_3(CH_2)_4CONHC_6H_4$	$2\text{-}C_{10}H_7CH_2CH_2$	1.2 (0.94–1.5)	28 (21–37)	23 (17–33)
propranolol			0.0045 (0.0040–0.0051)	0.003 (0.0020–0.0045)	0.67 (0.48–0.92)
21	$1\text{-}C_{10}H_7$	$CH_3OCH_2CH_2$	0.098 (0.079–0.11)	0.086 (0.072–0.10)	0.90 (0.74–1.1)
22	$1\text{-}C_{10}H_7$	$3,4\text{-}(CH_3O)_2C_6H_3CH_2CH_2$	0.20 (0.16–0.26)	0.12 (0.075–0.19)	0.60 (0.41–0.87)
23	$1\text{-}(4\text{-}CH_3CONHC_{10}H_6)$	$3,4\text{-}(CH_3O)_2C_6H_3CH_2CH_2$	5.6 (4.7–6.7)	22 (18–27)	3.9 (3.1–4.9)
24	$1\text{-}[4\text{-}CH_3(CH_2)_4CONHC_{10}H_6]$	$3,4\text{-}(CH_3O)C_6H_3CH_2CH_2$	1.5 (1.2–1.9)	1.7 (1.4–2.1)	1.1 (0.88–1.4)

[a] 95% confidence interval. [b] Data reported in ref 1. [c] Synthesized according to ref 5.

dioxan-6-ylethyl (5) and phenethyl (6), do not vest cardioselectivity. Within that isolipophilic selection of substituents, the highest affinity to the β_2 adrenoceptor is obtained with the phenethyl group (6), and the lowest affinity to the β_2-adrenoceptor is obtained with the 2-methoxyphenethyl group (4). The best proof that lipophilicity alone does not play an important role in affinity to either of the receptors can be illustrated (if one excludes 3 with the lowest lipophilicity) by naphth-2-ylethyl. (7), the most lipophilic amino substituent. Its affinity to the β_1 adrenoceptor is not significantly different from the less lipophilic compounds 6, 5, and 2. Its affinity to the β_2 adrenoceptor is significantly lower than the affinity of the less lipophilic compound 6 but does not differ significantly from the affinities of the other less lipophilic compounds, even that compound which bears the isopropyl group (1).

Practolol is the parent β blocker in the second group of compounds (8–12). Introduction of the 4′-acetamido group leads to a dramatic differential drop in the affinities for RVM and RLM (1 vs. practolol). The replacement of the isopropyl group in the practolol moiety with the homoveratryl and its analogues provides no trends in changes of affinity to either receptor. The range in apparent dissociation constants stretches in the case of the β_1 adrenoceptor from 1.0 (9) to 12 μM (11). In the case of the β_2 adrenoceptor, the range is from 30 (9) to 145 μM (8). All five compounds are cardioselective. The lowest cardioselectivity in that group was found in the case of 12, bearing the most lipophilic naphth-2-ylethyl group.

Although our preliminary conformational analysis using the CAMSEQ-2 program indicated high flexibility of the 1-amino-3-(aryloxy)propan-2-ols,[22,23] studies by Davies[24] suggest the opposite. The rigidity of the 4-acetamido group may cause a change in the mode of binding due to the steric obstacles present,[24] to different degrees, in both receptors in the 4-position of the 3-aryloxy ring. That may cause a translocation of the 1-amino group substituent in the 4-substituted compounds to a different point of interaction with the receptor and make a comparison between the two groups of compounds (1 to 7; practolol to 12) impossible.

It was reported that the elongation of the alkyl in the 4′-acylamido,[25] 4′-carbamoyl,[26] and 4′-ureido[27] groups increases the blocking potency of the β-adrenoceptor antagonists. The 4′-caproamido group was chosen as being of the optimal length. Compared with practolol, 13 has only slightly higher affinity to the β_1 adrenoceptor. Its affinity to the β_2 adrenoceptor is significantly higher, leading to lower cardioselectivity. Compounds bearing either a homoveratryl group or its isolipophilic variants (14–19) differ very little in their affinity to the β_1 adrenoceptor. Additionally, 20, which is more lipophilic, has similar affinity. In this group of compounds, one encounters a broader range (from 13 to 86 μM) of affinities to the β_2 adrenoceptor. The shortening (17) or lengthening (18) of the caproamido chain by one carbon has no visible influence on affinity to either receptor. That finding confirms the hypothesis of Davies[24] suggesting the existence of steric freedom at the end of the acyl chain. It is possible that in the intact animal study the observed differences in blocking potencies of compounds with altered chain length might appear due to differences in bioavailability and, therefore, to lipophilicity.

Comparison of the acetamido-substituted group with the caproamido-substituted group (8 vs. 14, etc.) permits the following observations: (a) the lengthening of the acylamido chain increases the affinity to the β_1 adrenoceptor in all compounds in group three, with the exception of 15; and (b) the increase in the affinity to the β_2 adrenoceptor is less visible, and 19 actually shows a slight decrease in the affinity with increased chain length. All compounds in the third group show a high degree of cardioselectivity at the receptor level.

The introduction of an additional benzene ring in the 3-aryloxy moiety (1 vs. propranol) leads to an increase in affinity (of at least 10-fold) to both receptors. This led us to the synthesis of compounds 22–24. To check the report of Smith and Tucker[5] indicating the lack of β-blocking activity for compound 21 in the anesthetized cat, we resynthesized and tested that compound. The propranolol analogue bearing the homoveratryl group (22) shows only a twofold improvement in affinity to the β_1 adrenoceptor when compared with 2. The improvement in the affinity to the β_2 adrenoceptor was only 4-fold (2 vs. 22), compared to 10-fold for the isopropyl compounds (1 vs. propranolol). Introduction of the 4′-acetamido substituent in the naphthyloxy part of the antagonist (23) leads to a far greater loss in affinity to the β_1 adrenoceptor (28-fold) than in the case of compounds with one benzene ring (2 vs. 8). The loss of affinity to the β_2 adrenoceptor is less pronounced. The cardioselectivity of 23 is low. Elongation of the acyl chain (24) improves the affinity to both receptors and renders the compound nonselective.

The replacement of the isopropyl (1 and propranolol) with the 2-methoxyethyl group (3 and 21) leads to 20-fold losses in affinities to both receptors in both compounds. Contrary to the in vivo tests[5] reporting 21 inactive, we have found that this compound showed affinities similar to those of other compounds.

The analysis of all four groups leads us to the conclusion that in order to affect significantly the cardioselectivity at the receptor level, the cardioselectivity-vesting amino group substituents have to be placed on a molecule of 3-(aryloxy)-1-aminopropan-2-ol bearing the unsubstituted aryloxy ring. In our studies, the homoveratryl group is not one of those substituents. Once the molecule of the β-adrenoceptor blocker is substituted in the 4′-position of the aryloxy ring, that substitution has an overriding effect on cardioselectivity. With the exception of the extremely bulky compound 24, we could not find a 4′-substituted compound that did not show some degree of cardioselectivity. The fact that the 4′-aryloxy and amino group substitution effects are not additive indicates that each of the four groups of compounds interacts with the receptors in a slightly different way depending on the nature of the aryloxy group. The role of the aralkyl substituent on the 1-amino group and its influence on the cardioselectivity is therefore secondary and changes with the mode of binding imposed by the aryloxy group, that is, of course, in adrenergic blockers containing the 3-aryloxy group.[28]

Experimental Section

Chemistry. IR spectra were recorded in KBr disks on a Perkin-Elmer spectrophotometer Model 700 and are consistent with the assigned structures. Liquid chromatography was per-

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formed on a Waters Associates ALc 202/6000 and Prep 500 high-pressure liquid chromatographs. Columns used were Waters Associates μBondapak C_{18} and C_{18}/Porasil B for ALC 202/6000 and silica gel for Prep 500. Solvent systems used were water/MeOH (ALC 202/6000) and MeOH/CH_2Cl_2 (Prep 500) of various proportions. Melting points were determined on an Electrothermal capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN., and the results obtained were within ±0.4% of the theoretical values.

Phenol and 4-acetamidophenol were purchased from Fisher Scientific Co. and Aldrich Chemical Co., respectively. The 4-pentanamido-, 4-hexanamido-, and 4-heptanamidophenols were synthesized according to Fierz-David and Kuster.[29] The 4-acetamido-1-naphthol was obtained according to Kehrmann and Kissine.[30] The synthesis of 4-hexanamido-1-naphthalenol and a typical synthesis of an antagonist are given.

4-Hexanamido-1-naphthalenol. To a solution of sodium hydroxide (0.7 g, 18 mmol) in water (5 mL, 0 °C) was added 4-amino-1-naphthalenol (3.5 g, 18 mmol), followed by hexanoic acid (3.1 mL, 22 mmol). The mixture was heated at 100 °C, and the hexanoic anhydride (5.8 mL, 22 mmol) was added dropwise. The suspension was refluxed for 1 h, cooled, poured into 50 mL of water, and extracted with ethyl acetate (3 × 50 mL). The organic layer was washed with saturated potassium carbonate solution and water and dried over sodium sulfate. The solvent was evaporated under vacuum, and the dark oily residue was washed consecutively with petroleum ether, ether, and ethanol. The obtained dark crystals (1 g, 22% yield) decomposed at 250 °C: IR (KBr) 3500–3200, 1670, 770 cm^{-1}.

1-[[2-(2-Methoxyphenoxy)ethyl]amino]-3-(4-hexanamidophenoxy)propan-2-ol (15). A mixture of 1,2-epoxy-3-(4-hexanamidophenoxy)propane (10.0 g, 38 mmol) and 2-(2-methoxyphenoxy)ethylamine (8.8 g, 52 mmol) in 50 mL of MeOH was refluxed for 18 h. The mixture was then evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2, charged on a silica gel column, and chromatographed in 10% MeOH in CH_2Cl_2. The desired fraction was converted to the oxalate salt by using oxalic acid in MeOH. Obtained crystals were recrystallized from MeOH five times: yield 26%; mp 162–164 °C.

Tissue Preparation. Receptor-rich membrane fragments were obtained from rat ventricular muscle by the method of Baker and Potter.[11] Hearts were removed from freshly killed (by etherization) rats. The ventricular muscle was dissected free of atria, major vessels, and fat, minced with scissors, and homogenized (Brinkman Polytron PC-U) in 7 vol of ice-cold 0.25 M sucrose–Tris buffer (10 mM, pH 8.0). The crude homogenate was poured through four layers of cheesecloth. One-tenth volume of 1.5 M $NaClO_4$ was added and homogenized for 15 s. The homogenate was centrifuged at 480*g* for 10 min, and the supernatant was centrifuged at 15000*g* for 20 min. The pellet is a bilayer, and the top layer is resuspended in 10 mM Tris buffer (pH 7.4) in normal saline. Ten grams of ventricular muscle suspended in 30 mL of buffer (final volume) provides 10^{-10} M receptor.

The lungs were removed from freshly killed animals, dissected free of large bronchi, minced with scissors, and homogenized in 4 vol of buffer 1. The homogenate was centrifuged at 10000*g* for 20 min, and the supernatant was centrifuged at 100000*g* for 1 h. The pellet from 6 to 8 g of lung was suspended in 30 mL of Tris-buffered saline for the receptor assay, giving 10^{-9} M receptor. All heart and lung preparations were used on the day of preparation.

Determination of Apparent Dissociation Constants. The apparent drug dissociation constants (K_{app}) were determined by competition with [^{3}H]dihydroalprenolol ([^{3}H]DHA, New England Nuclear Corp., 36 Ci/mmol). A 0.1-mL aliquot of the drug in 50% EtOH/H_2O was added to test tubes at 50-fold the desired final concentration. The tissue preparation containing [^{3}H]DHA at 6 to 10 nM was added in 0.5-mL aliquots, incubated at 37 °C for 15 min, and stored on ice until the extent of binding was determined. The amount of [^{3}H]DHA bound was determined by filtration on GF/C filters.[31] Portions of 0.1 mL were added to 5 mL of ice-cold saline and rapidly filtered, and the filtrate was washed with 9 mL of ice-cold saline (both operations take less than 10 s). Binding not associated with β adrenoceptors (amount bound in the presence of 10^{-5} M propranolol) is 20–25% for heart preparations and 10% for lung preparations. Results were plotted as percent specifically bound vs. log of total drug concentration.[32] Apparent dissociation constants were then calculated from the IC_{50} by the method of Chang and Prusoff.[33]

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Registry No. 1 oxalate, 70579-96-3; 2 oxalate, 70579-89-4; 3 oxalate, 64463-92-9; 4 oxalate, 64463-65-6; 5 oxalate, 84927-98-0; 6 oxalate, 84928-00-7; 7·HCl, 84928-01-8; 7, 84928-25-6; 8 oxalate, 70579-92-9; 9 oxalate, 84928-03-0; 10 oxalate, 84928-05-2; 11 oxalate, 84928-07-4; 12 oxalate, 84928-09-6; 13 oxalate, 70579-95-2; 14 oxalate, 70579-88-3; 15 oxalate, 84928-11-0; 16 oxalate, 84928-13-2; 17 oxalate, 84928-15-4; 18 oxalate, 84928-17-6; 19 oxalate, 84928-19-8; 20 0.5-oxalate, 84944-00-3; 21 oxalate, 84928-20-1; 22 oxalate, 4282-08-0; 23 oxalate, 84928-22-3; 24 oxalate, 84928-24-5; II (R_1 = H), 108-95-2; II (R_1 = 4-CH_3CONH), 103-90-2; II [R_1 = 4-$CH_3(CH_2)_3CONH$], 84928-26-7; II [R_1 = 4-$CH_3(CH_2)_4CONH$], 37795-91-8; II [R_1 = 4-$CH_3(CH_2)_5CONH$], 82568-63-6; III [R_1 = 4-$CH_3(CH_2)_4CONH$], 84928-28-9; III (R_1 = H), 122-60-1; III (R_1 = 4-CH_3CONH), 6597-75-7; III (R_1 = 4-$CH_3(CH_2)_3CONH$), 84928-29-0; III [R^1 = 4-$CH_3(CH_2)_5CONH$], 84928-30-3; 2-$(CH_3O)C_6H_4OCH_2CH_2NH_2$, 1836-62-0; 3,4-$(OCH_2CH_2O)C_6H_3CH_2CH_2NH_2$, 10554-64-0; $C_6H_5CH_2CH_2NH_2$, 64-04-0; 2-$C_{10}H_7CH_2CH_2NH_2$, 2017-68-7; 3,4-$(CH_3O)_2C_6H_3CH_2CH_2NH_2$, 120-20-7; 4-acetamido-1-naphthol, 85-12-1; 4-amino-1-naphthol, 2834-90-4; hexanoic acid, 142-62-1; 4-hexanamido-1-naphthol, 84928-27-8; 1,2-epoxy-3-(4-acetamido-1-naphthyloxy)propane, 69114-50-7; 1,2-epoxy-3-(4-*n*-hexanamido-1-naphthyloxy)propane, 84928-31-4.

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