Synthesis and Biological Properties of 2-, 5-, and 6-Fluoronorepinephrines

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2-Fluoro-, 5-fluoro-, and 6-fluorodimethoxybenzaldehydes were prepared by photochemical decomposition of the corresponding diazonium fluoroborates. The aldehydes were converted to the cyanohydrin trimethylsilyl ethers, which, in turn, were reduced to the dimethoxyphenethanolamines. Boron tribromide demethylation afforded the racemic ring-fluorinated norepinephrines. An alternate route, using the dibenzyloxyfluoroaldehyde, was also used to prepare 6-fluoronorepinephrine. The fluorine substituent markedly increases the phenolic acidities of these analogues. The biological properties conferred upon norepinephrine by the fluorine substituents in peripheral and central adrenergically responsive systems clearly demonstrate that 2-fluoronorepinephrine is a nearly pure β-adrenergic agonist, while 6-fluoronorepinephrine is an α-adrenergic agonist. 5-Fluoronorepinephrine retains both β- and α-adrenergic agonist properties. Receptor-binding studies with specific radiolabeled ligands indicate that the specificity conferred by the site of fluorine substituents results from a change in the affinity of these analogues for the α- and β-adrenergic receptors.

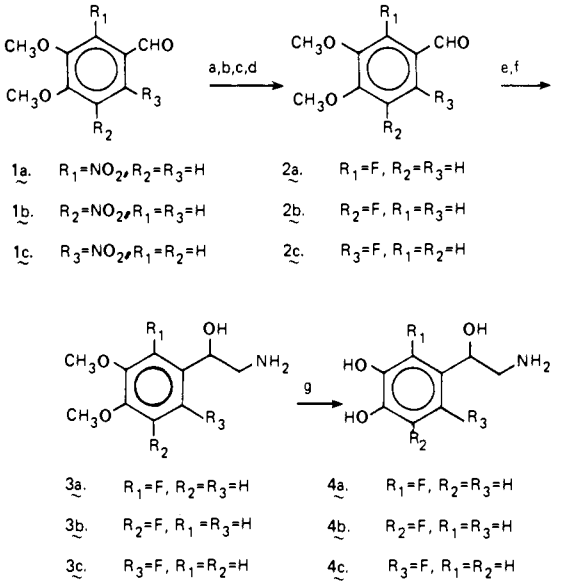
It has been more than 40 years since the term "adrenergic" was introduced by Dale[1] to describe nerve fibers that release the sympathetic transmitter and 30 years since von Euler identified the transmitter as (-)-norepinephrine[2] (NE). The term adrenergic was extended to describe the receptors on which the transmitters acted. Beginning with the work of Ahlquist,[3] the application of selective agonists in conjunction with selective antagonists has permitted a classification of the actions of catecholamines in terms of two distinct receptor groups designated α and β. Subsequent investigations utilizing a growing number of agonists and antagonists have resulted in the extension of this basic classification into several subsets of receptors.[4] The discovery and localization of catecholamine-containing neurons in the central nervous system,[5] the ability to measure both the affinity of various ligands for catecholamine receptors,[6] as well as the number of receptors[7] have contributed to a marked increase in attempts to define the molecular interactions between transmitter and receptor.

Many structural analogues of NE have been prepared which show partial agonist activity toward a variety of adrenergically responsive biological systems.[8] Extensive structure–activity studies have been performed with analogues including those derivatives with modifications of the ethanolamine side chain, of the phenolic substituents, and with removal or transposition of the ring hydroxyl alone or in combination with modification of the ethanolamine side chain.[9] These studies have resulted in the development of several useful medicinals.[10] It is noteworthy, however, that studies of the effects of ring substitution on the parent catechol itself are quite limited.[11] Because of the nearly absolute requirement for one or more phenolic groups in biogenic amines for agonist activity in many systems, there is no doubt that this functionality plays a key role in receptor–transmitter complex. For this reason, perturbation in the physical properties of this part of the NE molecule could be expected to lead to altered biological behavior. Fluorine substitution offers an attractive means to test the role of the phenolic groups in this regard, since its high electronegativity introduces electronic alterations, e.g., lowered pK_a, but no appreciable steric modifications.[12] To this end, we have prepared the isomeric, ring-fluorinated analogues of NE and have evaluated their potency in several biological systems. This work represents an extension of our present studies of ring-fluorinated biogenic amines.[13]

Results and Discussion

Chemistry. Of various procedures available for the preparation of substituted ethanolamines, manipulation of an appropriately substituted benzaldehyde seemed most applicable to our purpose. We had already prepared 2-fluoro-3,4-dimethoxybenzaldehyde (**2a**) as an intermediate in the synthesis of 2-fluorodopamine[13a] and could foresee no difficulty in preparing the remaining 5-fluoro and 6-fluoro isomers by the same method, viz., photochemical decomposition of the corresponding diazonium fluoroborates. (The "6-fluoro" isomer is correctly named 2-fluoro-4,5-dimethoxybenzaldehyde but, for simplicity throughout this discussion, will be referred to as a 6-fluoro substituted aromatic ring.) Indeed, following diazotization of the aminobenzaldehydes in fluoroboric acid, the fluoro aldehydes were readily prepared by in situ photochemical decomposition. For each of the isomeric fluoro aldehydes, elaboration of the side chain was accomplished by preparation of the cyanohydrin trimethylsilyl ether,[14] followed by in situ lithium aluminum hydride reduction to give the amino alcohols **3a–c**. Finally, boron tribromide demethylation[15] produced the desired ring-fluorinated (±)-norepinephrines **4a–c**, isolated as the hydrochlorides (Scheme I). Purification of **4b** as the hydrochloride was effected by recrystallization from methanol by addition of ether. Attempts to recrystallize **4a** and **4c** by similar

Scheme I



(a) CH_3OH, H^+; (b) H_2/Pt, EtOH; (c) $NaNO_2$, HBF_4; (d) hv, HBF_4; (e) $(CH_3)_3SiCN$; (f) $LiAlH_4$, Et_2O; (g) BBr_3, CH_2Cl_2.

Table I. Aromatic Proton and Fluorine NMR Parameters[a]

compd	^{1}H, ppm[b]	^{19}F, ppm[c]	J,[i] Hz
2a[d]	6.78 (d, H-5), 7.57 (dd, H-6)	22.2 (m, F-2)	J_{HH}^{o} = 9.0, J_{HF}^{m} = 7.1, J_{HF}^{p} = 1.4
2b[d]	7.23 (dq, H_2, H_6)	32.6 (m, F-5)	J_{HH}^{m} = 1.9, J_{HF}^{o} = 10.3, J_{HF}^{p} = −1.5[e]
2c[d]	6.65 (d, H-5), 7.29 (d, H-2)	33.4 (dd, F-6)	J_{HF}^{o} = 11.4, J_{HF}^{m} = 6.8
3a[f]	6.80 (d, H-5), 7.15 (dd, H-6)	24.4 (m, F-2)	J_{HH}^{o} = 9.0, J_{HF}^{m} = 7.1, J_{HF}^{p} = 1.4
3b[f]	6.7 (m, H-6, H-2)	31.2 (m, F-5)	[g]
3c[f]	6.58 (d, H-5), 6.98 (d, H-2)	34.5 (dd, F-6)	J_{HF}^{o} = 11.2, J_{HF}^{m} = 7.0
4a·HCl[h]	6.80 (dq, H-5, H-6)	20.7 (m, F-2)	J_{HH}^{o} = 9.0, J_{HF}^{m} = 8.0, J_{HF}^{p} = −1[e]
4b·HCl[h]	6.80 (dq, H-2, H-5)	26.9 (m, F-5)	J_{HH}^{m} = 2.1, J_{HF}^{o} = 11.0, J_{HF}^{p} = −1.6[e]
4c·HCl[h]	6.78 (d, H-5), 7.0 (d, H-2)	34.4 (dd, F-6)	J_{HF}^{o} = 11.4, J_{HF}^{m} = 7.1

[a] Nonaromatic proton signals agreed, in all cases, with the assigned structures with respect to position, multiplicity, and integrated intensity. [b] Proton chemical shifts are relative to $(CH_3)_4Si$. [c] Fluorine chemical shifts were measured at 100 MHz and are recorded in parts per million downfield from hexafluorobenzene. [d] Spectra measured in $CDCl_3$. Proton spectra measured at 60 MHz. [e] The two HF coupling constants are of opposite sign. The assignment of the negative value to the para coupling constant is arbitrary. [f] Spectra measured in $CDCl_3$. Proton spectra measured at 100 MHz. [g] Spectrum was not resolved (see text). [h] Spectra measured in D_2O. Proton spectra measured at 100 MHz. [i] Superscript abbreviations used are: *o*, ortho; *m*, meta; *p*, para.

Table II. Effect of Fluorine Substitution on the α- and β-Adrenergic Potencies of Norepinephrine

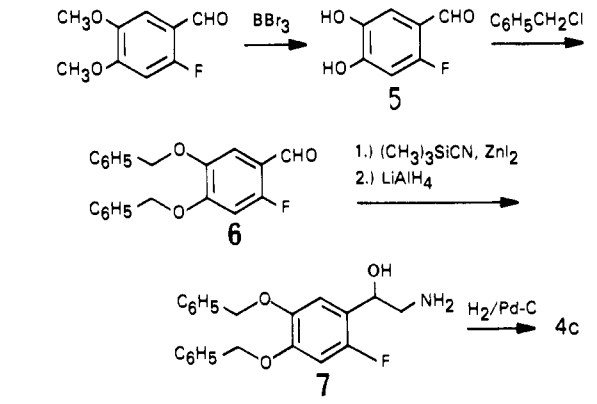
				rat cerebral cortical slices:[e] stimulation of cAMP formation; EC_{50}, μM		rat cerebral cortical membranes: displacement of α- and β-specific ligands[f]	
agonist[a]	aorta[b] (α)	atria[c] (β)	ileum[d] (β)	α	β	α	β
NE	2.6 ± 0.1	0.7 ± 0.1	15.6 ± 1.5	5	5	3	0.8
4a	110 ± 0.02	0.6 ± 0.1		>100	5	>100	2
4b	2.4 ± 0.1	0.11 ± 0.2	44 ± 2.0	5	2	15	4
4c	2.5 ± 0.1	72 ± 0.1		5	>100	3	>100

[a] In all cases the amines were racemic. [b] EC_{50} values ± SD were estimated from dose-response curves (eight) of the percent maximal contraction at doses from 0.1 to 100 μM; see Experimental Section. [c] EC_{50} values ± SD were calculated from dose-response curves (eight) of percent maximal increase in contraction rate at doses from 0.1 to 100 μM according to standard procedures; see Experimental Section. [d] EC_{50} values ± SD were calculated from dose-response curves (four) of mean percent relaxation at doses from 0.1 to 6.4 μM according to standard procedures; see Experimental Section. [e] EC_{50} values were estimated from dose-response curves (six) for accumulations of cyclic AMP at amine concentrations of 1 to 100 μM; α-adrenergic responses were measured in the presence of propranolol (10 μM) and β-adrenergic responses in the presence of phentolamine (10 μM) according to previously reported procedures; see Experimental Section. [f] EC_{50} values were estimated from dose-response curves (six) for displacement of an α-adrenergic receptor ligand (2-[[[(2',6'-dimethoxyphenoxy)ethyl]amino]methyl]benzodioxan, [^{3}H]WB-4101) or for displacement of a β-adrenergic receptor ligand, [^{3}H]-dihydroalprenolol; see Experimental Section.

procedures led to increasingly complex mixtures. This problem has been ascribed to β-alkyl ether formation brought about by a general-acid-catalyzed reaction with alcoholic solvents.[16] The presence of alcoholic reaction products was detected by mass spectrometry and high-pressure liquid chromatography. These products were not characterized further. An alternate synthetic approach to **4c** is shown in Scheme II. The fluoro aldehyde **2c** was demethylated to the fluorocatechol **5** and converted to the dibenzyl derivative **6**, followed by side-chain elaboration as before. Hydrogenolysis of the benzyl protecting groups in the presence of 1 equiv of oxalic acid produced crystalline **4c** hydrogen oxalate. Attempts to produce **4a** oxalate were thwarted by our inability to prepare the dibenzyl ether of 2-fluoro-3,4-dihydroxybenzaldehyde. Despite our inability to crystallize **4a**, purity and identity were secured by a combination of high-pressure liquid chromatography, thin-layer chromatography, mass spectroscopy, UV, and NMR (see the following discussion).

Structures of **4a–c** and the intermediates involved, particularly with regard to the position of the fluorine substituent, were confirmed by proton and fluorine NMR. Chemical shifts and coupling constants are given in Table I. The proton spectra of **2a,c**, **3a,c**, and **4c** appeared as simple AMX or ABX patterns, in which coupling constants are observed which confirm the orientation of the fluorine substituent. First-order analysis[17] of the ABX doublet of quartets observed for **2b**, **4a**, and **4b** similarly resulted in extraction of coupling constants consistent with the assigned structure. The lack of resolution of the multiplet produced by **3b** precluded unambiguous analysis.

Scheme II



The acid-strengthening effects of fluorine are seen in the following spectrophotometrically measured pK_a values: **4a**, 7.8; **4b**, 7.9; **4c**, 8.5.[18] Because of rapid oxidation at higher pH, buffer solutions above pH 8.5 were flushed thoroughly with argon immediately prior to use. Examination of the pK_a values of *o*-, *m*-, and *p*-fluorophenol make it clear that under physiological conditions the hydroxyl nearest the fluorine substituent in the fluoronorepinephrines will be the one most readily ionized.[19]

Biology. The fundamentally important biological properties conferred upon norepinephrine by the fluorine

Table III. Yields and Physical Data

compd	yield, %	mp (purifn)	formula	anal.
2a[a]				
2b	35	61-62 (sublimation)	$C_9H_9FO_3$	C, H
2c	25	94-96 (sublimation)	$C_9H_9FO_3$	C, H
3a	60[b]	98-101	$C_{10}H_{14}FNO_3$	C, H, N
3b	60[b]	100-102	$C_{10}H_{14}FNO_3$	C, H, N
3c	60[b]	109-111	$C_{10}H_{14}FNO_3$	C, H, N
4a·HCl	45	c		
4b·HCl	30	175 (dec; MeOH/ether)	$C_8H_{11}ClFNO_3$	H, N; C[d]
4c·HCl	45	c		
4c oxalate	50	180 (dec; acetonitrile/H_2O)	$C_{10}H_{12}FNO_7$	C, H, N
5	60	160 (dec; sublimation)	$C_7H_5FO_3$	C, H
6	40	134-136 (EtOAc/cyclohexane)	$C_{21}H_{17}FO_3$	C, H
7	38	83-87 (EtOAc/cyclohexane)	$C_{22}H_{22}FNO_3$	H, N; C[e]

[a] Reference 4a. [b] Yields based on aldehyde **1**. [c] Could not be crystallized; see text. [d] C: calcd, 42.96; found, 42.31. [e] C: calcd, 71.91; found, 71.30.

substituents have resulted in a rapidly expanding series of investigations on the adrenergic properties of these fluorine-substituted analogues. The results of these studies are summarized herein (see Table II). Initially, we reported the effects of fluorine substitution on the aromatic ring of NE, dopamine, and other catechols concerning their properties as substrates for the enzyme catechol *O*-methyltransferase.[20] In this regard, it was shown that fluorine substitution on the 5 or 6 position markedly shifted the preference of O methylation toward the metaphenolic hydroxyl, whereas substitution on the 2 position shifted the predominance of O methylation toward the para-phenolic hydroxyl.

In addition to these studies, we have evaluated the ability of the fluoronorepinephrines to elicit α- and β-adrenergic mediated responses in several systems. The systems tested included the α-adrenergic response in the isolated aortic strip preparation from guinea pig,[21] the β-adrenergic response of the guinea pig atria,[22] the response of isolated ileum of guinea pig,[23a,b] the amine-sensitive cyclic AMP generating system in slices of rat cerebral cortex,[24] and the displacement of α- and β-specific radioligands from isolated membrane preparations from rat brain.[24] The results of these studies are summarized in Table II.

As shown by the response of the aortic strip (Table II), the α-adrenergic potencies of **4b** and **4c** were equipotent with NE, while the potency of **4a** was only 0.03 times that of NE. As shown by the atrial response (Table II), the β-adrenergic potencies of **4a** were equipotent with NE, while **4b** showed significantly greater potency than NE. **4c** showed only a very weak β-agonist effect estimated to be 0.009 times the potency of NE. The α-adrenergic responses were completely blocked by the α antagonist, phentolamine, and unaffected by the β antagonist, propranolol, while the reverse was true for the responses in the β-adrenergic system. In the guinea pig ileum preparation, the ability of **4b** to induce ileum relaxation is approximately fourfold greater than NE. From these studies it is clear that fluorine substitution exerts a strong influence on the specificity of the interaction of NE with peripheral adrenergic receptors.[25]

We have also shown that the adrenergic properties of **4a–c** in central nervous systems are similar to those in the periphery. As indicated in Table II, **4b** was more potent than NE in stimulating the generation of cyclic AMP, while the responses of **4a** and **4c** were proportional to the β- and α-adrenergic receptor distribution, respectively, found in rat cerebral cortical slices.[24] As in the periphery, propranolol blocked the response to **4a** and not that to **4c**, while phentolamine blocked the response to **4c** and had no effect on the response to **4a**.

The ability of **4a** and **4b** to inhibit the binding of the β-adrenergic receptor specific ligand, [^{3}H]dihydroalprenolol, to receptors in rat cerebral cortex was of the same order of magnitude as NE, while **4c** was nearly inactive. On the other hand, the inhibition of the binding of the α-adrenergic receptor specific ligand, [^{3}H]WB-4101, by NE and **4b** was only slightly greater than inhibition by **4c**, while **4a** was nearly inactive. Thus, the relative specificity of the receptor-binding studies is consistent with the activity of the fluoronorepinephrines in stimulating cyclic AMP in brain slices and suggests that the specificity resides in differences in the binding affinity of the receptors for the amines.

An understanding of the mechanism responsible for these differences in the biological properties of the isomeric fluoro derivatives of NE will require further study. However, in view of these significant alterations in biological properties it may be possible to relate the changes in phenolic pK_a, altered hydrogen-bonding properties, variations in lipophilicity, and other physicochemical properties to the biological behavior in a predictable manner.

Experimental Section

Microanalysis, NMR spectra, and mass spectra were provided by the Microanalytical Services and Instrumentation Section of the Laboratory of Chemistry, NIAMDD, under the direction of Dr. David F. Johnson. High-pressure liquid chromatography was performed on a Waters Model 440 using a ^{18}C reverse-phase analytical column, eluting with 2.5% aqueous acetic acid. Identities of all compounds were authenticated by chemical ionization mass spectrometry on a Finnigan mass spectrometer, Model 1015D. Silica gel GF plates (Analtech) were used for thin-layer chromatography. Physical constants and yields are shown in Table III.

Preparation of 3,4-Dimethoxyfluorobenzaldehydes 2a–c. Preparation and isolation of the dimethyl acetal of 5-nitro-3,4-dimethoxybenzaldehyde was carried out as described for the 2-nitro derivative.[13a] The dimethyl acetal of 6-nitro-3,4-dimethoxybenzaldehyde is commercially available (Aldrich). Catalytic reduction was carried out as described[13a] but proceeded at a considerably more rapid rate for the 5- and 6-nitro aldehyde acetals as compared to the 2-nitro analogues. As before, the amino-2,4-dimethoxybenzaldehyde dimethyl acetals without purification were used directly in the fluorination procedure. In all three series, it has been found beneficial to prepare the diazonium fluoroborate as described and to store these solutions overnight at 0 °C before subjecting them to photochemical decomposition. **3,4-Dimethoxy-5-fluorobenzaldehyde (2b)** so prepared was purified by silica gel column chromatography (pentane–ether, 60:40), followed by sublimation. **3,4-Dimethoxy-6-fluorobenzaldehyde (2c)** similarly obtained was purified by silica gel column chromatography (pentane–ether, 60:40) and sublimation.

Preparation of 3,4-Dimethoxyfluorophenethanolamines 3a–c. The preparation of the 5-fluoro isomer illustrates the general procedure. To **2b** (200 mg, 1.08 mmol) in a carefully dried flask

under an argon atmosphere were added 0.15 mL of trimethylsilyl cyanide and approximately 10 mg of anhydrous zinc iodide.[14] The mixture was stirred at room temperature for 1 h, after which time an infrared spectrum (neat) demonstrated the absence of carbonyl adsorption. The excess trimethylsilyl cyanide was then removed in vacuo and 2 mL of anhydrous ether was added to the residue. This solution was added to a suspension of 100 mg of lithium aluminum hydride in 20 mL of ether. After refluxing for 2 h, the reaction mixture was cooled in ice and the excess hydride was decomposed by the addition of 0.1 mL of H_2O, followed by 0.1 mL of 15% NaOH, and 0.3 mL of H_2O.[26] The suspension of salts in ether was stirred for 15 min and filtered, and the salts were washed several times with ether. The ether was dried (Na_2SO_4) and evaporated to give after trituration with cold ether 133 mg (60%) of analytically pure **3b**. By identical procedures were prepared pure **3a** and **3c**.

Preparation of Fluoronorepinephrines 4a–c. In each case, boron tribromide demethylation was carried out by the usual procedure.[15] Thus, 100 mg (0.46 mmol) of **3b** was suspended in 2 mL of methylene chloride under an argon atmosphere, the flask was chilled in a dry ice/acetone bath, and, while stirring, 0.3 mL (3 mmol) of boron tribromide was added in one portion. The mixture was stirred at room temperature for 24 h, then cooled to 0 °C, and treated with 4 mL of H_2O. The methylene chloride was removed by rotary evaporation and the aqueous solution added to a column of Dowex 50-W-X4 (50 mL wet volume, prewashed with water). The column was eluted with water until the eluant was neutral, then with 100 mL of 0.5 N HCl, and then with 200 mL of 1 N HCl. By elution with 250 mL of 3 N HCl there was obtained 35 mg of **4b**, recovered by lyophilization and purified by recrystallization from methanol/ether. By the same procedure from 100 mg (0.46 mmol) of **3a** there was obtained 40 mg of **4a** (0.18 mmol). From 100 mg of **3c** there was obtained 38 mg of **4c**. **4a** and **4c** were obtained as hygroscopic air-sensitive off-white solids, which gave extensive decomposition during recrystallization attempts (see Discussion). TLC (butanol–ethyl acetate–acetic acid–water, 1:1:1:1) of **4a–c** gave one spot with R_f values of 0.67, 0.69, and 0.69 for **4a–c**, respectively. High-pressure LC similarly gave a single peak (>98%) for each compound, with retention times of 3.6, 3.6, and 3.8 min for **4a–c**, respectively. Frozen solutions (10 mM) of **4a–c** in 10^{-3} N HCl showed no decomposition over a period of 2 months, as demonstrated by TLC and high-pressure LC.

3,4-Bis(benzyloxy)-6-fluorobenzaldehyde (6). Boron tribromide demethylation of **2c** was carried out as described for **3a–c**. After treating with water, the solution was extracted four times with 25-mL portions of ether, and the organic layer was separated and extracted four times with 25 mL of 2 N NaOH. This alkaline extract was neutralized with dilute HCl and extracted with ether, and the ether extract was dried over sodium sulfate. From 200 mg of **2c** (1.08 mmol) there was obtained 100 mg of 3,4-dihydroxy-6-fluorobenzaldehyde (**5**; 0.64 mmol), recrystallized from cyclohexane/ethyl acetate. To 156 mg (1 mmol) of **5** dissolved in 1.5 mL of 95% ethanol containing 300 mg of K_2CO_3 was added 300 mg (2.3 mmol) of benzyl chloride and 10 mg of sodium iodide. After refluxing for 6.5 h, the mixture was cooled and 1 mL of water was added. The solution was then evaporated under high vacuum, and the solid residue was treated with 2 mL of 1 N NaOH and 0.5 g of ice and filtered to give, after drying, 150 mg (0.45 mmol) of **6**, purified by recrystallization from cyclohexane/ethyl acetate.

2-[3,4-Bis(benzyloxy)-6-fluorophenyl]-2-hydroxyethylamine (7). A 312-mg (0.93 mmol) sample of **6** was treated with trimethylsilyl cyanide as above, followed by reduction with lithium aluminum hydride. After hydrolysis and the usual workup, 150 mg (0.41 mmol) of **7** was obtained, purified by recrystallization from cyclohexane/ethyl acetate.

6-Fluoronorepinephrine Hydrogen Oxalate (4c Oxalate). A solution of 150 mg (0.41 mmol) of **7**, dissolved in a minimum volume of ethanol, was added to a solution of 55 mg (0.43 mmol) of oxalic acid dihydrate. The resulting suspension was dissolved in 150 mL of methanol and reduced over 50 mg of 10% Pd/C for 1.5 h. Removal of catalyst by filtration and rotary evaporation of solvents gave a solid residue. This was dissolved in a minimum volume of methanol, and ether was added to give 60 mg (0.19 mmol) of a white powder. **4c** oxalate was purified by recrystallization from acetonitrile/water.

Table IV. Ultraviolet Spectral Data for Fluorinated Norepinephrines[a]

no.	solvent[b]	λ_{max} (ϵ)	solvent[b]	λ_{max} (ϵ)
4a	A	269 (960)	B	278 (1560)
4b	A	269 (1140)	B	278 (2430)
4c	A	284 (4200)	C	293 (5100)

[a] Spectra were measured on a Cary Model 15 recording spectrophotometer. [b] Solvent A, 0.05 N HCl; B, 0.05 M phosphate buffer, pH 9; C, 0.05 M phosphate buffer, pH 9.4.

pK_a Determination. Phenol acidities were determined spectrophotometrically by measuring absorption intensity as a function of pH in phosphate buffer. The spectral data of the neutral and ionized species are given in Table IV. Because of rapid oxidation of **4a–c** at high pH, buffer solutions above pH 8.5 were thoroughly flushed with deoxygenated argon prior to use.

Biological Test Procedures. The atria, aortic strip, and ileum (1.5-cm lengths) were obtained from 250–300-g male, Hartley strain guinea pigs.[21] Each preparation was individually bathed in 10-mL chambers containing guinea pig Tyrode's solution maintained at 37 °C and continuously aerated with 95% O_2/5% CO_2. Tension was monitored on paper by a Grass Model 70D polygraph via a Grass FT03C force-displacement transducers. In practice, batteries of four separate preparations were monitored simultaneously. Stock solutions, 1–10 μM, of (±)-NE or the fluorinated derivatives freshly prepared in distilled water were introduced into the bath in volumes no greater than 100 μL. Each preparation was exposed to a full range of amine concentrations. In the atrial preparation, potency is quantified as the percent of maximal rate increase (PMRI): PMRI = $I_{60}/I_{60,max}$ where I_{60} is calculated by subtracting 1 from the quotient of the rate of contractions at 60 s following dose administration divided by the preexperiment rate of contraction, and $I_{60,max}$ is the value of I_{60} for (±)-NE. In the aortic strip preparation, potency is quantified as the percent of maximal contraction (PMC): PMC = C_{dose}/C_{max}, where C_{dose} is the largest contraction for a particular dose and C_{max} is the maximal value of C_{dose} for (±)-NE. In the ileum, results were expressed as percentages of maximal relation. Maximal relaxation was measured from the peak contracted level observed prior to drug administration to the maximal level of pen deflection recorded during drug administration. Potencies at each dose were compared using a two-tailed Student's t test with a critical probability level of 0.05. Standard deviations in the range of the EC_{50} were not greater than 0.07% in the aortic strip, 0.08% in the atrial preparation, and 0.1% in the isolated ileum.

Cyclic AMP generation in rat cerebral cortical slices was carried out according to the procedure of Shimizu et al.[24c] Male Sprague–Dawley rats were sacrificed by decapitation, and the brain was quickly removed, chilled in ice-cold oxygenated Krebs–Ringer bicarbonate glucose buffer, and placed on a chilled glass plate. Strips of cerebral cortical gray matter were dissected free and placed in another beaker of chilled buffer. The slices were cut on a cooled McIlwain tissue slicer set at 260 μm, the axis of the slicing block was rotated 180°, and the tissue was again sliced at 260 μm. The tissue slices were then transferred to a beaker containing 15 mL of buffer gassed with 95% O_2/5% CO_2 for 15 min. The preincubated brain slices were decanted onto a piece of nylon mesh and transferred to a beaker containing 10 mL of buffer containing adenine (30 μM) and [^{3}H]adenine (20–60 μCi) and incubated for 40 min at 37 °C while gently gassed with 95% O_2/5% CO_2. The buffer was decanted, and the slices were collected on nylon mesh, transferred in approximately equal portions with a stainless-steel spatula to 30-mL beakers containing 10 mL of buffer, and allowed to equilibrate for 5 min. Amines and other agents were added in appropriate concentrations from solutions in prewarmed, gassed buffer and the incubation continued for 10 min. The tissue was then collected on nylon mesh, transferred to conical homogenizing tubes containing 1 mL of 6% trichloroacetic acid and unlabeled cyclic AMP (0.25 μmol), and homogenized with a glass pestle. The homogenates were centrifuged for 10 min at 2000 rpm. An aliquot of the trichloroacetic acid supernatant fraction (0.05 mL) was removed and radioactivity measured by liquid scintillation spectroscopy. The cyclic [^{3}H]-

AMP formed was determined in the remaining supernatant fraction by the double-column method of Salomon et al.[27] and corrected for recovery of the carrier cyclic AMP. Protein was determined by the method of Lowry.[28] Results are expressed as percent of total radioactive nucleotide present as cyclic AMP (percent conversion).

The binding of [^{3}H]dihydroalprenolol to β-adrenergic receptors and displacement by the fluorinated amines were measured by a modification of the methods of Bylund and Snyder[29] and Alexander,[30] as previously described by Nimitkitpaisan and Skolnick,[24b] except that the final tissue pellet was resuspended in 60 volumes of Tris-HCl buffer (50 mM, pH 8). Under these conditions, the binding of [^{3}H]dihydroalprenolol was linear with respect to protein concentrations; K_d values for dihydroalprenolol were estimated to be about 1 nM by Scatchard analysis, and specific binding represented 55% of total binding. Measurement of [^{3}H]WB-4101 to α-adrenergic receptors and displacement by the fluorinated amines were determined by the method of U'Prichard et al.[6a] Under these conditions, the K_d value for [^{3}H]WB-1401 was 1.4 nM, and specific binding represented 60% of total binding.

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Synthesis and Antihypertensive Activity of 2-Benzamido-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]quinolizines

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The synthesis and antihypertensive activity of a series of 2-benzamido-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]-quinolizines are reported. A number of these compounds exhibit extremely potent hypotensive properties (e.g., *N*-methylbenzamides **42**, **48**, and **50** and *N*-ethylbenzamide **53** cause drops of 110, 103, 79, and 83 mmHg, respectively, in systolic blood pressure in the spontaneous hypertensive rat at the screening dose of 50 mg/kg po). Structure–activity relationships for the entire series are discussed.

Some years ago, the synthesis and hypotensive properties of a series of benzamidopiperidylethylindoles were reported.[1] From this group of compounds, the 4-benzamido derivative **1** (indoramin) was shown to possess potent antihypertensive activity[2–4] and was chosen as a candidate for further investigation.

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