and **20** (90%). Dilution of the filtrate from above with additional EtOH (40 mL) and refrigeration (5 °C) of the resulting mixture for 18 h gave a second crop of **20**: yield 89 mg; mp 350 °C; HPLC showed the presence of **21** (~3%) and **20** (93%); total yield 161 mg (~81%). Anal. ($C_{21}H_{21}N_6O_7 \cdot 1.5Ca \cdot 0.5C_2H_6O \cdot 2.5H_2O$) C, H, N.

Spectral data were determined on a sample obtained in another experiment in which the calcium salt of **20** was precipitated from an aqueous solution with 3 vol of EtOH: UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 7 254 nm (21.0), 312 br sh (4.67); in 0.1 N NaOH 253 (21.2), 310 br sh (5.28); ^{1}H NMR (D_2O, 3% w/v), δ 7.67 (d of d, C_6H_4), 8.50 (CHO). Anal. ($C_{21}H_{21}N_6O_7 \cdot 1.4Ca \cdot 5H_2O$) C, H, Ca, N.

3-Amino-8-[4-[[(1,3-dicarboxypropyl)amino]carbonyl]-phenyl]-2,5,6,6a,7,8-hexahydro-1-oxo-1*H*-imidazo[1′,5′:6,1]-pyrido[3,2-*d*]pyrimidin-10-ium Chloride (8-Deaza-5,6,7,8-tetrahydro-N^5,N^{10}-methenylfolic Acid Chloride; 21). A solution of **17** (900 mg, 1.75 mmol) in 95% formic acid (30 mL) was heated with stirring at 60 °C for 1 h and evaporated to dryness in vacuo. The residue was dried in vacuo over P_2O_5 and dissolved in CF_3CO_2H (50 mL). The resulting solution was mixed with a prereduced suspension of PtO_2 (600 mg)[1] in CF_3CO_2H (40 mL) and hydrogenated at room temperature and atmospheric pressure. The catalyst was removed by filtration (Celite) and washed with CF_3CO_2H. The combined filtrate and wash was evaporated to dryness, and the residue was dissolved in 3 N HCl (90 mL). This solution was stirred for 30 min and evaporated to dryness, and the product was washed with Et_2O: yield 932 mg (89%); mp 196 °C foamed; field-desorption mass spectrum, m/e 455 (M^+); UV λ_{max} ($\epsilon \times 10^{-3}$) in 0.1 N HCl 220 nm (15.8), 331 (28.5); at pH 7 342 (26.7, unstable); in 0.1 N NaOH 253 (20.6), 300 sh (8.19); ^{1}H NMR (CF_3CO_2D, 6% w/v): δ 2.6 (br), 2.8 (br, CH_2), 5.1 (br, $CHCH_2$), 7.7 (br), 8.1 (br), (C_6H_4, methenyl CH); HPLC [pH 3.6, NH_4OAc–MeCN (9:1)] chromatograms showed that a solution of **21** in Tris buffer (pH 7.5) was slowly converted to **20**: 41% (1.5 h), 53% (3.5 h), 57% (5 h), and 100% (20 h). Anal. ($[C_{21}H_{23}N_6O_6]^+Cl^- \cdot 2HCl \cdot 2H_2O$) C, H, Cl, N.

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Synthesis and Adrenoceptor Affinity of Some Highly Polar β-Substituted Catecholamines

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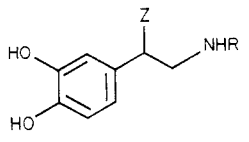
In order to assess the potential for sympathomimetic or sympatholytic activity within the series of catecholamine β-sulfonates **3a–c**, α- and β-adrenoceptor binding affinities were determined using rat brain homogenate preparations. Furthermore, their potential for indirect activity was assessed by measurement of blockade of norepinephrine uptake into rat synaptosomal preparations. Activity was uniformly low or nonexistent throughout the series. The possibility of unfavorable solution conformational distribution within the series was investigated by examination of the side chain vicinal ^{1}H NMR coupling constants, but no differences that could account for the lack of affinity were found. The observed behavior may be due to receptor intolerance of the bulky β-sulfonate substituent or an electronic mismatch in which normal H bonding is significantly altered.

The catecholamines norepinephrine (**1a**) and epinephrine (**1b**), in addition to being endogenous neurotransmitters, have found widespread use as therapeutic agents. A number of synthetic congeners have also been developed that exhibit enhanced or more selective sympathomimetic activity, including isoproterenol (**1c**) and, most recently, dobutamine (**1d**). A large body of knowledge has been



1a, R = H; Z = OH
b, R = CH_3; Z = OH
c, R = $CH(CH_3)_2$; Z = OH
d, R = $CH(CH_3)CH_2CH_2C_6H_4OH$; Z = H

accumulated over the years concerning the structure–activity relationships at every position of the phenethylamine nucleus, with the notable exception of the benzylic (Z) β position. Activity within the catecholamines is known to be enhanced by the presence of the β-hydroxyl group (Z = OH), but very few other substituents have been investigated. In the norepinephrine series, several sulfur analogues of **1a** were reported by Rachlin and Enemark,[1] some of which (Z = SH, SCH_3, SSC_3H, R = H) had weak pressor activity in the anesthetized cat. Larger thioethers in this series were essentially inactive, possibly indicating that bulk tolerance in this region of the receptor may be limited. No investigations of N-alkylated congeners of this series have been reported. Recently, Chavdarian et al.[2] also found very weak pressor activity in rats for the congeners of the catecholamines β-methyldopamine (1, R = H; Z = CH_3), β-methylepinine (1, R = CH_3; Z = CH_3), and β-methoxyepinine (1, R = CH_3; Z = OCH_3). Again, the pressor activities of these analogues were well below therapeutic significance.

A third set of β-substituted catecholamine derivatives is represented by the amino acids **3a–c**. The analogues have potential pharmacologic interest, since they are known to be formed as degradation products of **1a–1c** in bisulfite-stabilized parenteral dosage forms.[1,3,4] A qui-

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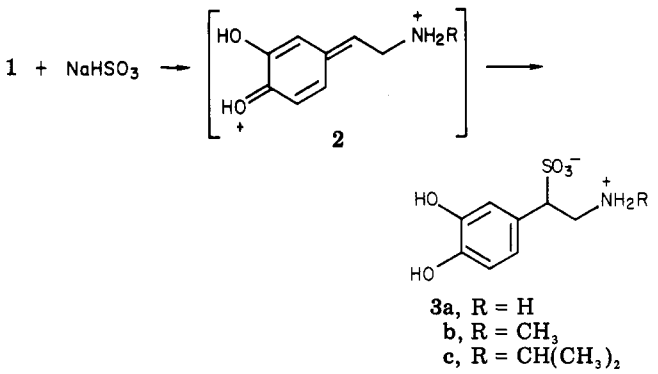

3a, R = H
b, R = CH_3
c, R = $CH(CH_3)_2$

nonemethide (2) may be an intermediate in the process, although this remains unproven. While **3a** has been shown to be ineffective for the induction of a feline pressor response[1] (an α-agonist effect), no biological activities have been reported for **3b** or **3c**. Moreover, the bioassays performed to date within the series can be considered by modern standards to be extremely narrow in scope. For example, a weak α-antagonist effect would be difficult to detect using the pressor response as an end point, as would either weak β-agonist or β-antagonist activity. In order to evaluate the potential for a broader scope of activity within the series of β-substituted congeners, we have evaluated the derivatives **3a–c** by a series of in vitro methods. We report here the results of that investigation.

Results and Discussion.

The products of interest (**3a–c**) were synthesized starting from **1a–c** by modification of the general method of Schroeter and Higuchi.[5] During the course of the synthesis we confirmed the structure of **3b** by X-ray analysis[6] and found that the compounds are true amino acids, existing in the zwitterionic form, i.e., with no counterion present in the crystal lattice. Previously, there had been some confusion regarding the structure of the compound.[7]

To detect any potential biologic activity within the series, we measured the binding affinities of **3a–c** to rat brain homogenate preparations selectively radiolabeled with dihydroergocryptine[8] or dihydroalprenolol.[9] The results are contained in Tables I and II. The binding affinities of **3a–c** to the α receptor (Table I) followed the expected trend of decreasing affinity with increasing size of the nitrogen substituent. However, the magnitudes of the binding constants are quite large, indicating a very low affinity for the series.

The most active member of the series, **3a**, had only ~1% of the affinity of NE (**1a**), when the racemic nature of the product was taken into account. This figure was based on the assumption that one enantiomer has negligible affinity for the receptor and that the observed binding is almost completely accounted for by the active enantiomer, as is the case for **1a–c**.[10] The N-alkylated derivatives **3b** and **3c** show very weak α-receptor affinities, with IC_{50}'s too large for any in vivo effect.

Table I. Absolute and Relative Binding Affinities of the Catecholamines to the α-Adrenergic Receptor

compd	IC_{50},[a] mol/L	rel affinity
(±)-3a	3.6×10^{-6} (0.7-19.4)	0.47
(±)-3b	1.0×10^{-5} (0.12-8.1)	0.17
(±)-3c	3.3×10^{-5} (0.02-436)	0.052
(−)-1a	1.6×10^{-8} (0.4-7.7)	106
(−)-1b	1.7×10^{-8} (0.6-4.6)	100

[a] Values in parentheses denote 95% confidence limits.

Table II. Absolute and Relative Binding Affinities of the Catecholamines to the β-Adrenergic Receptor

compd	IC_{50},[a] mol/L	rel affinity
(±)-3a	1.3×10^{-4} (0.47-33)	0.022
(±)-3b	1.1×10^{-4} (0.57-13.7)	0.026
(±)-3c	9.1×10^{-6} (6.5-12.7)	0.32
(−)-1b	2.9×10^{-8} (2.4-3.6)	100

[a] Values in parentheses denote 95% confidence limits.

Table III. Side-Chain Coupling Constants[a] and Conformational Distributions[b] for 3a-c and 1a-c

compd	J_{ab}	J_{ax}	J_{bx}	P_a	P_b	P_c
3a	−13.3	8.89	6.43	0.35[d]	0.61[d]	0.04[d]
3b	−13.0	8.83	6.15	0.32[d]	0.60[d]	0.08[d]
3c	−13.0	8.71	6.14	0.32[d]	0.59[d]	0.09[d]
1a[c]	13.2	9.05	3.35	0.14	0.76	0.10
1b[c]	12.8	9.15	3.65	0.17	0.77	0.06
1c[c]	12.8	9.65	3.15	0.11	0.83	0.06

[a] Coupling constants (hertz) were extracted from the ^1H NMR spectra and calculated as ABX spin systems. The results were then refined using a Nicolet ITRCAL program. [b] No absolute assignment of H_a and H_b is implied. [c] From ref 13. [d] Conformer ratios were calculated assuming J_t = 12.6 Hz and J_a = 3.1 Hz.

From Table II it can be seen that very similar results were obtained using the β-receptor preparation. The expected trend was again apparent: increasing affinity with increasing size of the nitrogen substituent. Just as for the above case, the binding constants were quite large. Compound **3c** was the most potent in the assay, with an IC_{50} ca. 10-fold lower than those for **3a** and **3b**. Despite this difference, the absolute magnitude of the binding constant was too high for any potential in vivo effect (<1% of **1b** affinity).

Finally, the effects of **3a–c** on synaptosomal uptake of **1a** were evaluated by established methods[11] to determine their potential for indirect adrenergic activity. The compounds were uniformly inactive at 10^{-4} M, with neither the ability to displace stored catecholamines nor the ability to block reuptake.

The extremely low activity of this series in all phases of the adrenergic process may be due to either extreme mismatches of characteristics between the highly polar zwitterionic substances and the receptor sites or, less likely, to unfavorable solution conformations of the products. Such apparent unfavorable conformational behavior has been noted in other systems.[12] To examine the possibility that unfavorable solution conformations contribute to the observed inactivity, we investigated the conformational distributions of the side chains of **3a–c** using the respective vicinal coupling constants. Similar analyses of other

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phenethylamines have been reported.[13,14] At 270 MHz in D_2O, ABX patterns result for **3a–c**, from which the coupling constants shown in Table III were extracted by standard iterative techniques. While the values for **3a–c** differ from those of **1a–c**, they reflect quantitative changes in conformational distribution that are not sufficient to account for the observed large decrease in binding affinity. These results seem to discount the possibility that strongly unfavorable conformational distributions may account for the lack of affinity of **3** for the receptor preparations.

At this point, it is unclear whether the decreased adrenoceptor and uptake affinities of **3a–c** are due to the high polarity of the molecules, the increased size of the β-substituent, or both. Based upon the relatively few reports of the β-substituted congeners of the catecholamines, it is not yet possible to separate the influence of substituent size from that of H-bonding character. It is commonly accepted that H bonding at the β position is very important to receptor affinity.[15] The naturally occurring β-hydroxyl group is both an effective proton donor and acceptor, while the highly ionic sulfonate of **3** may serve only as a proton acceptor, and then only with some difficulty. In fact, none of the congeners reported to date are nearly as effective as OH at H bonding in both a donor and an acceptor sense. Further insight may be gained into this problem if suitable functionalities that possess the necessary H-bonding characteristics at the β position are examined.

At present, we may state with certainty that the highly polar catecholamine sulfonic acids **3a–c** are ineffective at either α or β adrenoceptors and have no effect on the uptake of **1a** into synaptosomes. Thus, placement of a highly polar functional group at the β position effectively abolishes all adrenergic activity in vitro. While the reasons for this effect are not completely clear, it does not appear to be due to unfavorable conformational interactions. Whether the effect is due to either unfavorable bulk tolerance, the inability to properly hydrogen bond, or both, remains to be determined.

Experimental Section

Melting points were determined in a Thomas-Hoover Mel-Temp apparatus. The NMR spectra were recorded on a Bruker 270-MHz spectrometer using DSS as internal standard. The infrared spectra were taken on a Beckman 620MX spectrophotometer. Microanalyses were performed by Baron Consulting Co., Orange, CT.

Dopamine β-Sulfonate (3a). A mixture of 5.00 g (24.3 mmol) of **1a**·HCl, 2.31 g (12.2 mmol) of sodium metabisulfite, and 27 mL of H_2O was heated to reflux for 1.5 h under N_2 and then stored under N_2 for 3 days at 25 °C. The solution was concentrated in vacuo to a volume of ~10 mL. After several days, white crystals had formed in the reddish-brown solution. Recrystallization four times from H_2O gave 2.845 g (55%) of **3a** as stable white crystals: mp 246–250 °C dec (lit.[1] mp >350 °C); IR (Nujol mull) 3270, 3080, 1210, 1175, 1150, 1042 cm^{-1}; NMR (D_2O) δ 6.88–7.01 (3 H, m, arom), 4.24 (1 H, m, CH), 3.79 and 3.57 (2 H, m, CH_2).

Epinine β-sulfonate (3b) was prepared as previously described,[5] using 5.00 g (27.3 mmol) of **1b** and 3.8 g (30 mmol) of anhydrous Na_2SO_3 to yield 4.28 g (68.8%) of **3b**: mp 259–260 °C dec (lit.[5] mp 263 °C dec); IR (Nujol mull) 3470, 3300, 3090, 1278, 1258, 1225, 1148, 1042 cm^{-1}; NMR (D_2O) δ 6.88–7.00 (3 H, m, arom), 4.31 (1 H, m, CH), 3.81 and 3.64 (2 H, m, CH_2) 2.76 (3 H, s, CH_3).

***N*-Isopropyldopamine β-Sulfonate (3c).** A mixture of 2.50 g (8.70 mmol) of **1c**·H_2SO_4·$3H_2O$, 1.71 g (9.03 mmol) of sodium metabisulfite, and 13 mL of H_2O was heated to reflux for 2 h under N_2. The solution was cooled and stored in the dark at room temperature for 7 days, after which it was concentrated in vacuo with heating. White crystals formed after the solution was cooled and allowed to stand for 8 days. Recrystallization from H_2O afforded 1.34 g (51%) of **3c**: mp 181–185 °C dec (lit.[4] mp 191–194 °C dec); IR (Nujol mull) 3540, 3480, 3400, 1245, 1130, 1040 cm^{-1}; NMR (D_2O) δ 6.88–7.00 (3 H, m, arom), 4.26 (1 H, dd, CH), 3.80 and 3.64 (2 H, m, CH_2), 3.51 (1 H, m, NCH), 1.32 (6 H, dd, CH_3).

Binding Studies. α-Adrenoceptor binding was measured by the method of Greenberg et al.,[8] using [^{3}H]dihydroergocryptine (DHE) (New England Nuclear) as the ligand. Rat brain was homogenized in 0.32 M sucrose and centrifuged to obtain a synaptosome-enriched fraction. After extensive washing, the pellet was suspended in 50 mM Tris-HCl buffer (pH 7.7) and incubated at 30 °C for 30 min with the ligand (0.3 nM) and the experimental compound. The incubation was terminated by rapid filtration under reduced pressure through Whatman GF/B filters. The filters were washed with 15 mL of buffer at 0 °C and placed in scintillation vials to which was added a mixture of Protosol and Econofluor (New England Nuclear), allowed to stand overnight, and counted. Specific binding was defined as the difference between binding in the presence and in the absence of 100 μM **1a**. All assays were performed in triplicate. The IC_{50} value was obtained by least-squares regression analysis.

β-Adrenoceptor binding was measured by the method of Dolphin et al.,[9] in a manner very similar to the procedure above, except that rat cerebral cortex was used. Incubation was carried out for 10 min at 35 °C using Tris buffer at pH 8 and 5 nM [^{3}H]dihydroalprenolol (New England Nuclear) as the ligand. Nonspecific binding was determined in the presence of 1 μM propranolol.

Uptake affinities were determined by a modification[16] of the method of Kuriyama et al.[11] Synaptosomal preparations from the brains of six adult rats[17] were obtained as pellets, resuspended in a Krebs modified Ringer solution containing 10 μM pargyline and 0.1% ascorbic acid, and kept on ice. The incubation mixture contained 20 μL of nonlabeled 10^{-5} M NE, 10 μL of [^{3}H]NE (New England Nuclear), and 500 μL of synaptosomal preparation. The incubation was allowed to proceed for 5 min at 30 °C, over which time the rate of uptake is linear. Blank values were taken as the amount of radioactivity taken up by synaptosomes with the temperature of the incubation mixture at 0–4 °C.

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