Drug-Induced Modifications of the Immune Response. 1. Substituted 1-Phenylisoquinolines

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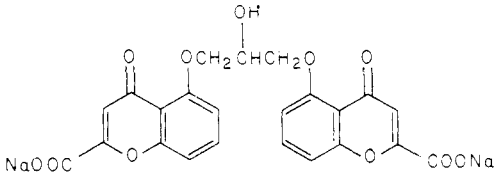
A series of 1-phenylisoquinolines and related compounds was prepared and tested for potential antiallergic activity. Several compounds of this series inhibited the antigen-induced wheal formation in rat passive cutaneous anaphylaxis (PCA) assay, a commonly used test for antiallergic activity. Many of these compounds also inhibited the antigen-induced histamine release from passively sensitized guinea pig lung slices. Furthermore, almost all of these derivatives inhibited the cyclic nucleotide phosphodiesterase, suggesting this as one of several possible mechanisms of action.

Cromolyn sodium (1) has been shown to be effective in



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the treatment of bronchial asthma[1] by inhibiting the release of the mediators of the allergic reaction.[2] However, the drug is not active orally in man, and when given by inhalation it does not have an adequate systemic response (e.g., protection against food allergies in stomach and skin). After the discovery of cromolyn sodium, much of the chemical research has centered around chemical structures resembling the chromone molecule.

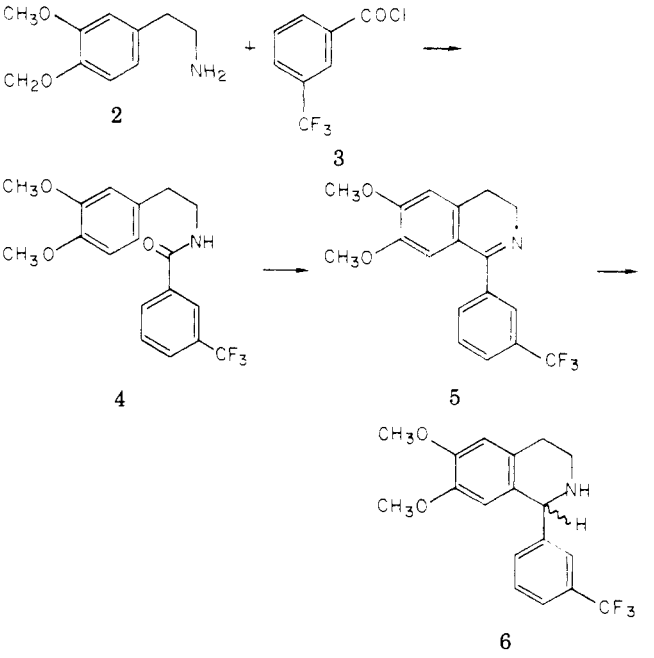
In our efforts to find antiallergic activity in compounds other than chromones, a variety of di- and tetrahydro-1-phenylisoquinolines and related compounds were prepared and tested.

Structures containing the isoquinoline moiety are well known to possess diverse biological activity.[3] For example, a number of 1-phenylisoquinolines have been found to possess antispasmodic,[4] antibacterial,[5] antidiabetic,[6] antiarrhythmic,[7] vasodilator,[8] ganglionic blocking,[9] and bronchodilator[10] activity. However, to our knowledge, the only reference to the antiallergic activity of 1-phenylisoquinolines was a Japanese patent by Kono and Sugiyama[11] in which a number of substituted 1,2,3,4-tetrahydroisoquinolines, including the 1-phenyl-6-methoxy-2,3,7-trimethyl analogue, were listed.

The present paper presents the synthesis and biological evaluation of various 1-phenyl-3,4-dihydroisoquinolines and their 1,2,3,4-tetrahydro analogues, as well as a few structurally related compounds. To determine the antiallergic activity, the compounds were tested for their effects on the passive cutaneous anaphylaxis reaction in rats,[12] their ability to inhibit the antigen-induced histamine release from guinea pig lung slices,[13] and their effects on the cyclic AMP phosphodiesterase. The first two assays represent commonly used models for this type of activity. Since a relationship between inhibition of lung anaphylaxis and inhibition of cyclic nucleotide phosphodiesterase has been suggested by other studies,[14,15] this assay was also used as a screening mechanism for antiallergic agents.

Chemistry. The title compounds (Tables I and II) were synthesized by standard methods starting with properly substituted phenethylamine **2** and acid chloride **3** and involved an initial Schotten–Baumann condensation, followed by phosphorus oxychloride cyclization of the resulting amide **4** to give the 3,4-dihydroisoquinoline base **5**, which was later reduced to the 1,2,3,4-tetrahydro derivative **6** (Scheme I).

Scheme I



Several other compounds related to the isoquinoline series were also prepared (Table III), by either following known procedures[22,23] or using the standard synthetic approach as described for the title compounds in Scheme I.

Biological Results and Discussion. The dihydroisoquinoline analogues were relatively weak inhibitors of the wheal formation in the PCA assay. Six compounds (**5**, **7**, **8**, **10**, **11**, and **15**) caused 31–46% inhibition of wheal formation when given ip at 25 mg/kg (Table IV).

All of the dihydro analogues showed moderate to potent activity as inhibitors of cAMP phosphodiesterase. In general, the 3,4-dihydroisoquinolines were more potent as inhibitors of the cyclic nucleotide phosphodiesterase than theophylline and the 1,2,3,4-tetrahydro derivatives (Table IV). Compounds **11**, **14**, and **15** caused significant inhibition of the antigen-induced histamine release from guinea pig lung slices.

When administered ip, most of the 1,2,3,4-tetrahydroisoquinolines showed significant inhibition of the passive cutaneous anaphylaxis (PCA) reaction in rats. Compounds **17**, **18**, and **24** had ED_{50} values of 24, 24, and 34 mg/kg, respectively. Furthermore, when tested orally in the PCA assay, **17** inhibited the wheal formation by 40%, while the dimethoxy analogues **18** and **24** were inactive. In terms of inhibition of the rat PCA reaction, this series appears to be more potent than the dihydro analogues (Table V).

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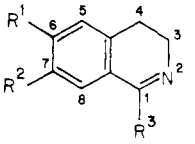
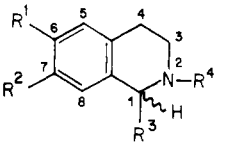
Table I. Substituted 3,4-Dihydroisoquinoline Derivatives



compd	R^1	R^2	R^3	mp, °C	formula	anal.
5	CH_3O	CH_3O	*m*-$CF_3C_6H_4$	163-165[a]	$C_{18}H_{16}NO_2F_3 \cdot HCl$	C, H, N
7	CH_3O	$C_6H_5CH_2O$	C_6H_5	207-209[b]	$C_{23}H_{21}NO_2 \cdot HCl$	C, H, N
8	CH_3O	CH_3O	*o*-$CH_3OC_6H_4$	215-217[a]	$C_{18}H_{19}NO_3 \cdot HCl$	C, H, N
9	H	CH_3	C_6H_5	236-238[a]	$C_{16}H_{15}N \cdot HCl$	C, H, N
10	H	Cl	C_6H_5	234-236[a]	$C_{15}H_{12}NCl \cdot HCl$	C, H, N
11	CH_3O	OH	C_6H_5	206-208[b]	$C_{16}H_{15}NO_2 \cdot HCl$	C, H, N
12	CH_3O	CH_3O	cyclohexyl	73-75[c]	$C_{17}H_{23}NO_2$	C, H, N
13	H	H	C_6H_5	227-229[a]	$C_{15}H_{13}N \cdot HCl$	*d*
14	CH_3O	CH_3O	C_6H_5	197-198[e]	$C_{17}H_{17}NO_2 \cdot CH_3I$	*f*
15	CH_3O	CH_3O	C_6H_5	212-214[b]	$C_{17}H_{17}NO_2$	*g*

[a] Ethanol-ether. [b] Methanol-ether. [c] Petroleum ether. [d] Ref 16 (lit. mp 223 °C). [e] Ethanol. [f] Ref 17 (lit. mp 198-199 °C). [g] Ref 18.

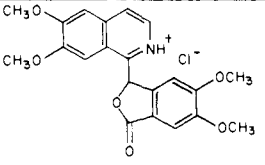
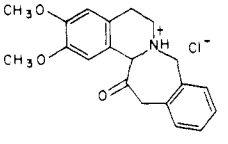
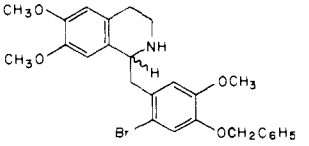
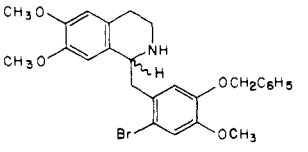
Table II. Substituted 1,2,3,4-Tetrahydroisoquinoline Derivatives



compd	R^1	R^2	R^3	R^4	mp, °C	formula	anal.
6	CH_3O	CH_3O	*m*-$CF_3C_6H_4$	H	255-257[a]	$C_{18}H_{18}NO_2F_3 \cdot HCl$	C,[b] H, N
16	CH_3O	CH_3O	*o*-$CH_3OC_6H_4$	H	235-237[a]	$C_{18}H_{21}NO_3 \cdot HCl$	C, H, N
17	H	CH_3O	C_6H_5	H	256-258[a]	$C_{16}H_{17}NO \cdot HCl$	C, H, N
18	CH_3O	CH_3O	*p*-$(CH_3)_3CC_6H_4$	H	161-163[a]	$C_{21}H_{27}NO_2 \cdot HCl$	C, H, N
19	CH_3O	CH_3O	cyclohexyl	H	237-239[a]	$C_{17}H_{25}NO_2 \cdot HCl$	C, H, N
20	CH_3O	$C_6H_5CH_2O$	C_6H_5	H	260-262[c]	$C_{23}H_{23}NO_2 \cdot HCl$	C, H, N
21	CH_3O	OH	C_6H_5	H	286-288[c]	$C_{16}H_{17}NO_2 \cdot HCl$	C, H, N
22	CH_3O	$C_6H_5CH_2O$	C_6H_5	$-C(=O)C(=O)OC_2H_5$	130-132[d]	$C_{27}H_{27}NO_5$	C, H, N
23	H	H	C_6H_5	H	225-226[a]	$C_{15}H_{15}N \cdot HCl$	*e*
24	CH_3O	CH_3O	C_6H_5	H	256-259[c]	$C_{17}H_{19}NO_2 \cdot HCl$	*f*
25	H	H	H	H	195-197[a]	$C_9H_{11}N \cdot HCl$	*g*
26	H	CH_3	C_6H_5	H	268-270[a]	$C_{16}H_{17}N \cdot HCl$	*h*

[a] Ethanol-ether. [b] C: calcd, 57.84; found, 58.32. [c] Methanol-ether. [d] Ethanol. [e] Ref 16 (lit. mp 227-229 °C). [f] Ref 19. [g] Ref 20 (lit. mp 195-197 °C). [h] Ref 21 (lit. mp 259-267 °C).

Table III. Isoquinoline Related Derivatives

no.	compd	mp, °C	formula	anal.
27		198-200[a]	$C_{21}H_{20}NO_6 \cdot HCl$	*b*
28		167-169[a]	$C_{20}H_{21}NO_3 \cdot HCl$	*c*
29		136-138[d]	$C_{26}H_{28}NO_4Br$	C, H, N
30		108-110[d]	$C_{26}H_{28}NO_4Br$	C, H, N

[a] Methanol. [b] Ref 22 (lit. mp 198-200 °C). [c] Ref 23 (lit. mp 167-169 °C). [d] Ethanol.

Table IV. Biological Results for Substituted 3,4-Dihydroisoquinolines

compd	PCA assay,[a] ip: % inhibn of wheal form.	cAMP PDE assay: % inhibn	GPL assay: % inhibn of air
5	35[b]	95	16
7	36[b]	95	18
8	36[b]	56	
9	23	53	6
10	34[b]	44	0
11	46[b]	83	36
12	18	70	
13	0	42	17
14	22	97	63
15	31[b]	91	91
cromolyn sodium[c]	52[b]		
theophylline		51	50[d]
isoproterenol			49[e]

[a] At 25 mg/kg. [b] Treated group differed from control at $p \leq 0.05$ (unpaired Student's t test). [c] At 6 mg/kg. [d] At 1.5 mM. [e] At 0.3 μM.

Table V. Biological Results for Substituted 1,2,3,4-Tetrahydroisoquinolines

compd	PCA assay[a] ip:% inhibn of wheal form.	PCA assay[a] po:% inhibn of wheal form.	cAMP-PDE assay: % inhibn	GPL assay:% inhibn of air
6	29[b]		84	55
16	24[b]		34	46
17	41[b]	40[b]	12	60
18	37[b]	9	71	13
19	49[b]	8	19	66
20	29[b]		34	56
21	43[b]	6	19	25
22	18		0	0
23	17		7	46
24	52[b]	7	46	49
25	18		0	31
26	51[b]	17[b]	4	0
cromolyn sodium[c]	52[b]			
theophylline			51	50[d]
isoproterenol				49[e]

[a] At 25 mg/kg for both ip and po. [b] Treated group differed from control at $p \leq 0.05$ (unpaired Student's t test). [c] At 6 mg/kg. [d] At 1.5 mM. [e] At 0.3 μM.

When tested for their mediator antagonism activity in the mediator wheal assay, **20** was a weak inhibitor (29%, $p \leq 0.05$) of the serotonin-induced wheal, while **17**, **18**, and **24** were inactive.

Compounds **6**, **18**, and **24** were equipotent to, or more potent than, theophylline as inhibitors of cAMP phosphodiesterase (Table V).

Except for **18**, **22**, and **26**, all of the 1,2,3,4-tetrahydro derivatives were moderately active (i.e., more potent than theophylline but less potent than isoproterenol) as inhibitors of the antigen-induced release of histamine from guinea pig lung slices (Table V).

None of the four compounds shown in Table VI were active in the PCA assay, while all four were weak to moderate inhibitors of cAMP phosphodiesterase with less or equal activity when compared to theophylline. Derivatives **28–30** inhibited moderately the antigen-induced release of histamine from guinea pig lung slices.

In summary, the 1-phenyl-3,4-dihydroisoquinolines were less active in the PCA reaction in rats than their 1,2,-3,4-tetrahydro analogues and relatively more potent as

Table VI. Biological Results for Isoquinoline-Related Derivatives

compd	PCA assay,[a] ip: % inhibn of wheal form.	cAMP-PDE assay: % inhibn	GPL assay: % inhibn of air
27	16	33	
28	18	64	43
29	0	45	27
30	0	55	33
cromolyn sodium[b]	52[c]		
theophylline		51	50[d]
isoproterenol			49[e]

[a] At 50 mg/kg. [b] At 6 mg/kg. [c] Treated group differed from control at $p \leq 0.05$ (unpaired Student's t test). [d] At 1.5 mM. [e] At 0.3 μM.

inhibitors of cAMP phosphodiasterase, thus suggesting that inhibition of this enzyme might be involved in their effects on histamine release. Since several of these derivatives appeared to be potent inhibitors of cAMP phosphodiesterase, further work in this direction would seem desirable.

Experimental Section

Biological Test Procedures. (A) Rat Passive Cutaneous Anaphylaxis (PCA) Assay.[12] Male Sprague–Dawley rats (200–250 g) were each injected intradermally (50 μL, id) with two dilutions of rat serum containing ovalbumin antibodies at three separate sites. After 48 h, the four rats in each experimental group were given test compound suspended or dissolved in 1 (ip) or 3 mL (po) of 0.5% methylcellulose (100 cP). Control animals received compound vehicle. After 5 (ip) or 10 min (po), the rats were challenged iv with 4.0 mg of ovalbumin in 1.0 mL of 1.0% Evan's Blue dye in 0.9% saline. Rats were sacrificed by cervical dislocation 30 min later, the sites of antibody injection were everted, and the wheal diameters and areas were measured. Mean percent inhibition of wheal size was calculated, and significance of the drug treatment was determined using the Student's t test for unpaired data.

(B) Mediator Wheal Assay in Rats. Male Sprague–Dawley rats (four per experimental group) were given 25 mg/kg compound or vehicle, ip, 5 min before an iv injection of 1.0 mL of 1.0% Evan's Blue dye. Ten minutes later each animal was injected (id) with 0.1 μg/0.1 mL serotonin into two separate sites on the sides of the dorsal midline. On each rat, control sites were injected with physiologic saline. Five minutes after the injections of serotonin, the animals were sacrificed, and the dorsal skin was cut free to determine the size of the wheal and the effect of the test compound.

(C) Cyclic AMP Phosphodiesterase (cAMP PDE) Assay. Crude enzyme was prepared as follows. A mongrel male dog was anesthetized with Nembutal® (35 mg/kg). The heart was surgically removed and rinsed in 0.15 M NaCl (4 °C). The ventricular muscle was cut into large pieces, suspended at 4 °C in 0.25 M sucrose, 50 mM Tris-Cl (pH 7.4; 3 mL of buffer/g wet weight of tissue), and homogenized in a prechilled Waring blender for 30 s at a medium speed. The homogenate was strained through two layers of cheesecloth and then centrifuged at 150000g for 1 h. The resulting pellet was washed two times by resuspending in homogenization buffer and recentrifuging. This washed pellet was resuspended in the original volume of buffer, frozen in aliquots, and stored at −80 °C. Prior to assaying, an aliquot of the enzyme was defrosted and appropriately diluted in assay buffer.

Cyclic AMP phosphodiesterase was measured at 0.5 μM adenosine 3′:5′-monophosphate according to the "one-step" assay described by Thompson et al.[24] The reaction was carried out in 7-mL scintillation vials with a 0.2-mL assay volume containing sufficient enzyme protein reaction to maintain appropriate linear conditions and 100 μM test compound or appropriate vehicle. The assay product, adenosine, was routinely recovered at 80–90% as quantitated with [8-^{14}C]adenosine at several concentrations between 10^{-8} and 10^{-5} M. Similar recoveries (80–90%) were also obtained for hypoxanthine and inosine.

(D) Antigen-Induced Histamine Release from Passively Sensitized Guinea Pig Lung Slices (GPL Assay).[13] Lung slices (averaging 0.25 × 3 mm) were passively sensitized with guinea pig antiovalbumin serum at 37 °C for 2 h. The sensitized lung slices were washed free of excess antibodies and incubated (37 °C) with 100 μM test compound for 5 min. Antigen was then added to the appropriate tubes. The incubation was continued for 15 min. Both the histamine released into the incubation medium and the residual histamine extracted from the lung slices (0.4 N perchloric acid, 100 °C for 15 min) were measured fluorometrically with a Technicon AutoAnalyzer®. Spontaneous release of histamine in the absence of antigen (SR) and the total release of histamine in the presence of antigen (TAI) were calculated as percent of total extractable histamine in the lung slices. The effects of compounds were calculated as a percent change in the net antigen-induced histamine release (AIR) or TAI minus SR.

Chemistry. Analyses for C, H, and N are within ±0.4% of the theoretical value. All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were obtained in KBr disks on a Perkin-Elmer 727 B spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian EM 360 instrument with Me_4Si as an internal standard. Mass spectra were recorded on a Varian MAT 112 instrument. Each compound had IR, NMR, and mass spectra compatible with its structure.

General Preparation of Substituted 1-Phenyl-3,4-dihydroisoquinolines. The preparation of 1-(*m*-trifluoromethylphenyl)-6,7-dimethoxy-3,4-dihydroisoquinoline (**5**) is presented as an example; the remaining analogues were prepared by essentially the same procedure.

3,4-Dimethoxyphenethylamine (**2**; 8.1 g, 0.045 mol) was dissolved in 50 mL of chloroform. Fifty milliliters of 20% aqueous potassium carbonate solution was added, and the mixture was cooled to 0 °C. Then 10.4 g (0.05 mol) of *m*-trifluoromethylbenzoyl chloride (**3**) in 20 mL of chloroform was added dropwise while stirring. After stirring the mixture for 3 h at room temperature, the two layers were separated, and the organic solution was washed sequentially with water, 10% aqueous HCl, saturated aqueous $NaHCO_3$ solution, and water, then dried, and evaporated, leaving 17.2 g of the corresponding amide **4** as a pale yellow oil which crystallized after standing, and was used in the next step without further purification.

A mixture of 12.0 g (0.034 mol) of the amide **4** and 7.65 g (0.05 mol) phosphorus oxychloride in 100 mL acetonitrile was refluxed for 2 h. The solvent was evaporated and the yellow oily residue was converted to its hydrochloride. Yield of **5** (as hydrochloride): 6.33 g; pale yellow crystals; mp 163–165 °C (ethanol–ether). Anal. ($C_{18}H_{16}NO_2F_3$·HCl) C, H, N.

Reduction of 3,4-Dihydroisoquinolines to Their 1,2,3,4-Tetrahydroisoquinoline Analogues. In a typical procedure, a mixture consisting of 3.2 g (9.5 mmol) of **5** and 0.5 g of sodium borohydride in 50 mL of ethanol was refluxed for 4 h and then worked up as usual. Yield of **6** (as hydrochloride): 2.8 g; white crystals; mp 255–257 °C (ethanol–ether). Anal. ($C_{18}H_{18}NO_2F_3$·HCl) C, H, N.

Treatment of (±)-7-(Benzyloxy)-6-methoxy-1-phenyl-1,2,3,4-tetrahydroisoquinoline (20) with Ethyl Oxalyl Chloride. The isoquinoline base **20** (3.45 g, 0.01 mol) was dissolved in 50 mL of dry pyridine. The solution was cooled to 5 °C and 1.5 g (0.011 mol) of ethyl oxalyl chloride was added dropwise, and the mixture was stirred at room temperature for 4 h. Ether was added, and the organic solution was thoroughly washed with water (some undissolved solid was filtered off), dried, and evaporated, leaving 2.75 g of **22** (after recrystallization from ethanol) as fine needles melting at 130–132 °C. Anal. ($C_{27}N_{27}NO_5$) C, H, N.

(±)-1-[2′-Bromo-4′-(benzyloxy)-5′-methoxybenzyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (29). Bromine (3.2 g, 0.04 mol) was added dropwise to a solution of 5.44 g (0.02 mol) of *O*-benzylvanillic acid in 20 mL of acetic acid at room temperature with stirring. After stirring the mixture for 30 min, water was added, and the white precipitate was filtered off and then thoroughly washed with water, leaving 3.1 g of crude 2-bromo-4-(benzyloxy)-5-methoxyphenylacetic acid, mp 188–190 °C (ethanol). Anal. ($C_{16}H_{15}O_4Br$) C, H.

A mixture of 7.0 g (0.02 mol) of 2-bromo-4-(benzyloxy)-5-methoxyphenylacetic acid and 3.6 g (0.02 mol) of 3,4-dimethoxyphenethylamine was heated for 2 h at 185–190 °C (under N_2) and then extracted with $CHCl_3$. The organic solution was washed sequentially with dilute HCl, aqueous $NaHCO_3$ solution, and water, then dried, and evaporated, leaving 6.7 g of *N*-(3,4-dimethoxyphenethyl)-2-[4-(benzyloxy)-5-methoxy-2-bromophenyl]acetamide which was used in the next step without further purification.

Phosphorus oxychloride (0.71 g, 6.6 mmol) was added to a solution of 2.4 g (4.6 mmol) of *N*-(3,4-dimethoxyphenethyl)-2-[4-(benzyloxy)-5-methoxy-2-bromophenyl]acetamide in 30 mL of acetonitrile. The reaction mixture was refluxed under N_2 for 2 h and then the solvent was evaporated, leaving 2.2 g of 1-[2′-bromo-4′-(benzyloxy)-5′-methoxybenzyl]-6,7-dimethoxy-3,4-dihydroisoquinoline which was dissolved immediately in 30 mL of ethanol. Sodium borohydride (1 g) was added portionwise at room temperature with stirring. The reaction mixture was refluxed for 2 h and then worked up as usual, leaving 1.8 g of the corresponding tetrahydroisoquinoline base **29**, mp 136–138 °C (ethanol). Anal. ($C_{26}H_{28}NO_4Br$) C, H, N.

(±)-1-[2′-Bromo-4′-methoxy-5′-(benzyloxy)benzyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (30).[25] Bromine (24.0 g, 3 mol) was added dropwise to a solution of 40.0 g (0.147 mol) of 3-(benzyloxy)-4-methoxyphenylacetic acid[26] and 160 g (1.95 mol) of sodium acetate in 1600 mL of acetic acid at room temperature with stirring. The stirring continued for 6 h, and then the mixture was poured into water. The precipitate was filtered off, washed thoroughly with water, and crystallized from ethanol. Yield of 2-bromo-4-methoxy-5-(benzyloxy)phenylacetic acid: 43.4 g; white silky crystals; mp 142–144 °C (benzene). Anal. ($C_{16}H_{15}O_4Br$) C, H.

The next three steps were carried out using the procedures described above for **29**.

A mixture of 2-bromo-4-methoxy-5-(benzyloxy)phenylacetic acid and 3,4-dimethoxyphenethylamine was heated for 2 h at 185–190 °C (under N_2), yielding the corresponding *N*-(3,4-dimethoxyphenethyl)-2-[4-methoxy-5-(benzyloxy)-2-bromophenyl]acetamide, white crystals melting at 128–130 °C (ethanol). Anal. ($C_{26}H_{28}NO_5Br$) C, H, N.

Phosphorus oxychloride condensation of *N*-(3,4-dimethoxyphenethyl)-2-[4-methoxy-5-(benzyloxy)-2-bromophenyl]acetamide in acetonitrile led to the formation of 1-[2′-bromo-4′-methoxy-5′-(benzyloxy)benzyl]-6,7-dimethoxy-3,4-dihydroisoquinoline, which was immediately reduced with sodium borohydride in ethanol to furnish the tetrahydroisoquinoline derivative **30**, white crystals melting at 108–110 °C (ethanol). Anal. ($C_{26}H_{28}NO_4Br$) C, H, N.

Acknowledgment. The authors are thankful to Dr. C. R. Kinsolving for many helpful discussions. We gratefully acknowledge the expert technical assistance of D. Donigi, J. Collins, P. Salaman, and P. Damiani in running the biological assays, as well as L. Lee for determining the elemental analyses, and R. Biniakewitz for recording the mass spectra.

References and Notes

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Inhibition of Uptake of Catecholamines by Benzylamine Derivatives[1]

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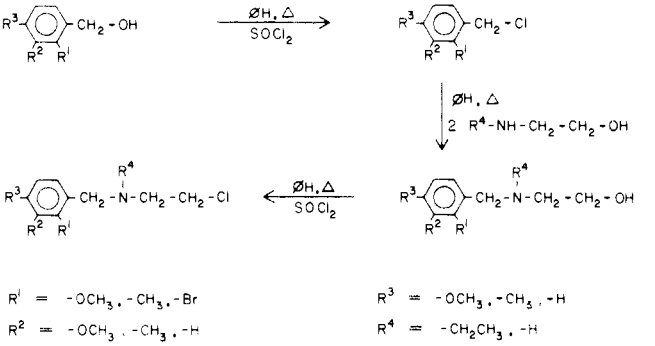
Eight benzylamine analogues of bretylium were synthesized, including *N*-(2-chloroethyl)-*N*-ethyl-2-methylbenzylamine (5), and evaluated as inhibitors of accumulation of norepinephrine and dopamine in rat brain homogenates. All compounds gave an I_{50} value (concentration of inhibitor that causes 50% reduction in control accumulation) considerably lower against norepinephrine in cortex than against dopamine in striatum. High potency (low I_{50}) and high specificity (preference for inhibition of norepinephrine transport compared to dopamine transport) are associated with a (2-chloroethyl) moiety, tertiary amino center, and ortho substitution of the aromatic function in the benzylamino group. **5** also inhibited the uptake of norepinephrine in rabbit aorta, indicating its effect against the uptake process in general. Cocaine protects against the effects of **5** in coincubation studies when compared to the appropriate controls, indicating that **5** acts at or close to the site of action of cocaine which is thought to be the uptake carrier site.

The catecholamines, norepinephrine and dopamine, are neurotransmitters whose action at the postsynaptic receptor is thought to be terminated by return to the presynaptic terminal.[2] This movement from extracellular to intracellular compartments, or uptake, is achieved by a specific carrier transport system.[2] Inhibition of this uptake process would be expected to have profound effects on neuronal activity, and this is the proposed mechanism of action of central nervous system stimulants such as cocaine,[3] amphetamine,[4] and tricyclic antidepressants.[5] A specific irreversible inhibitor would be a potent tool in the study of the transport system, since it would allow labeling of the carrier. This report describes the synthesis, structure–activity relations, and preliminary pharmacology of a series of benzylamine derivatives that inhibit norepinephrine uptake into brain and vascular tissue, probably irreversibly.

The compounds studied here are methyl- and methoxy-substituted (β-haloethyl)- and (β-hydroxyethyl)-benzylamines and are related to the (β-haloethyl)-benzylamines described by Ross and his co-workers.[6] These investigators found *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine to be a long-lasting, irreversible inhibitor of norepinephrine uptake in intact animals.[7,8] In the studies described here, the inhibitory activity was investigated by in vitro procedures, primarily in rat brain preparations enriched in synaptosomes or nerve-ending particles. The results show that some of these compounds are potent inhibitors that might be useful in studies of neuronal uptake.

Synthesis. The synthesis of all compounds followed the general scheme illustrated in Scheme I. The appropriate benzyl alcohol was converted to the chloride with thionyl chloride, followed by reaction with the necessary amino alcohol to yield the corresponding (2-hydroxyethyl)benzylamine. This amino alcohol was treated with thionyl chloride to yield the appropriate (2-chloroethyl)benzylamine, which could be isolated as the hydrochloride or oxalate salts. The structures of the compounds and their physical properties are summarized in Table I.

Scheme I. Synthesis of Uptake Inhibitors



Results

A summary of the I_{50} values determined as described in the biochemical procedures of the Experimental Section is found in Table II for rat brain. Bretylium (1) and three other *o*-bromo compounds were examined to allow comparison with compounds described by Ross et al.[6] In these initial experiments the proposed inhibitor was coincubated with the substrate catecholamine so that the observed

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