Selective Monoamine Oxidase Inhibitors. 4. 4-Aminophenethylamine Derivatives with Neuron-Selective Action

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Nine 4-aminophenethylamine derivatives were synthesized and tested for monoamine oxidase (MAO) inhibitory effects with particular attention to their selectivity for MAO within monoaminergic neurons in the rat brain. All compounds selectively inhibited the A form of MAO in vitro. Some of the compounds inhibited the MAO within the monoaminergic neurons at much lower doses than those required for inhibition of MAO within other cells in vivo. The most potent compounds in this respect were 4-amino-2-fluoro-α-methylphenethylamine (**5**) and 4-amino-2-chloro-α-methylphenethylamine (**4**).

The enzyme monoamine oxidase (MAO, EC 1.4.3.4) exists in two forms, A and B.[1] The A form is defined as that inhibited by low concentrations of the acetylenic inhibitor clorgyline, whereas the B form is inhibited by low concentrations of the acetylenic inhibitor selegiline ((−)-deprenyl).[1,2] The transmitter amine serotonin is in man a selective substrate for the A form,[3] whereas norepinephrine and dopamine are deaminated by both enzyme forms.[3,4] Although relatively little is known about the distribution of the A and B forms within the monoaminergic neurons in the human brain, MAO-A is probably involved in the regulation of the concentrations of the transmitter amines in these neurons.[5] Thus, selective and reversible inhibitors of the A form are interesting compounds with potential action as antidepressive drugs.[6] By selective inhibition of the A form it is thought to be possible to reduce serious or unpleasant side effects displayed by the nonselective inhibitors, since the B form is still active and is able to deaminate substrates for the B form and those common for both enzyme forms, e.g., tyramine. A number of selective and reversible MAO-A inhibitors have been developed and are now in clinical trials.[6]

Since MAO-A within the monoaminergic neurons is the target for MAO inhibitors aimed to interact with the transmitter amines, a further selectivity would be obtained if MAO within these neurons could be inhibited at lower doses compared with those inhibiting MAO in other cells. This could be achieved if the compound is accumulated in the aminergic neurons. The mechanism for the transport of aromatic amino acids into the neurons and the subsequent enzymatical decarboxylation has been used to concentrate irreversible MAO inhibitors in the neurons, e.g., β-(fluoromethylene)-*m*-tyrosine.[7] Other uptake mechanisms that can concentrate MAO inhibitors are the carrier mechanisms responsible for the reuptake of the transmitter amines. For instance, bretylium and amezinium have been shown to be accumulated by the noradrenergic uptake mechanism in the peripheral sympathetic nerve terminals causing MAO inhibition, although their MAO inhibitory potencies are quite low.[8,9] It was therefore of interest when we observed that amiflamine ((S)-(+)-4-(dimethylamino)-2,α-dimethylphenethylamine), which is a selective and reversible MAO-A inhibitor,[10] inhibited MAO in serotonergic and noradrenergic neurons in the rat brain at lower doses than those inhibiting MAO in other cells in the brain.[11] The *N,N*-didesmethyl derivative of amiflamine was found to be a very selective inhibitor of MAO in noradrenergic neurons with quite low activity in serotonergic neurons. The preferred inhibition of MAO in the monoaminergic neurons is most likely due to uptake and accumulation of the compounds by the amine pumps, since these preferences were antagonized by uptake inhibitors.[11,12] The structural requirements for transport by the various amine pumps are different,[13] and it might therefore be possible to develop MAO inhibitors selective for one particular system as evidenced by the potent inhibition of MAO in noradrenergic neurons by *N,N*-didesmethylamiflamine. This selectivity was not

Table I. 4-Aminophenethylamine Derivatives[a]

compd no.	mp,[b] °C	yield,[c] %	formula	anal.
2	154–155	48	$C_{10}H_{16}N_2 \cdot CH_3COOH$	C, H, N
3	134–135	33	$C_{11}H_{18}N_2 \cdot CH_3COOH$	C,[d] H, N, O
4	171–172	30	$C_9H_{13}ClN_2 \cdot CH_3COOH$	C, H, Cl, N, O
5	136–137	65	$C_9H_{13}FN_2 \cdot CH_3COOH$	C, H, F, N
6	197–198	82	$C_9H_{12}Cl_2N_2 \cdot CH_3COOH$	C, H, Cl, N, O
7	172–173	48	$C_{10}H_{15}BrN_2 \cdot CH_3COOH$	C, H, Br, N, O
8	190–191	25[e]	$C_{11}H_{18}N_2 \cdot CH_3COOH$	C, H, N, O
9	186–187	14[e]	$C_{10}H_{15}ClN_2 \cdot CH_3COOH$	C, H, Cl, N
10	197–198	37[e]	$C_{10}H_{15}FN_2 \cdot CH_3COOH$	C, H, F, N

[a] The structures are given in Table III. [b] Compounds **2**, **4**, **6**, and **8–10** are recrystallized from ethanol–isopropyl ether and **5** from ethanol–ether. Compounds **3** and **7** were obtained pure without recrystallization. [c] The yield is calculated from the corresponding aldehyde (five steps). [d] C: calcd, 65.51; found, 65.0.

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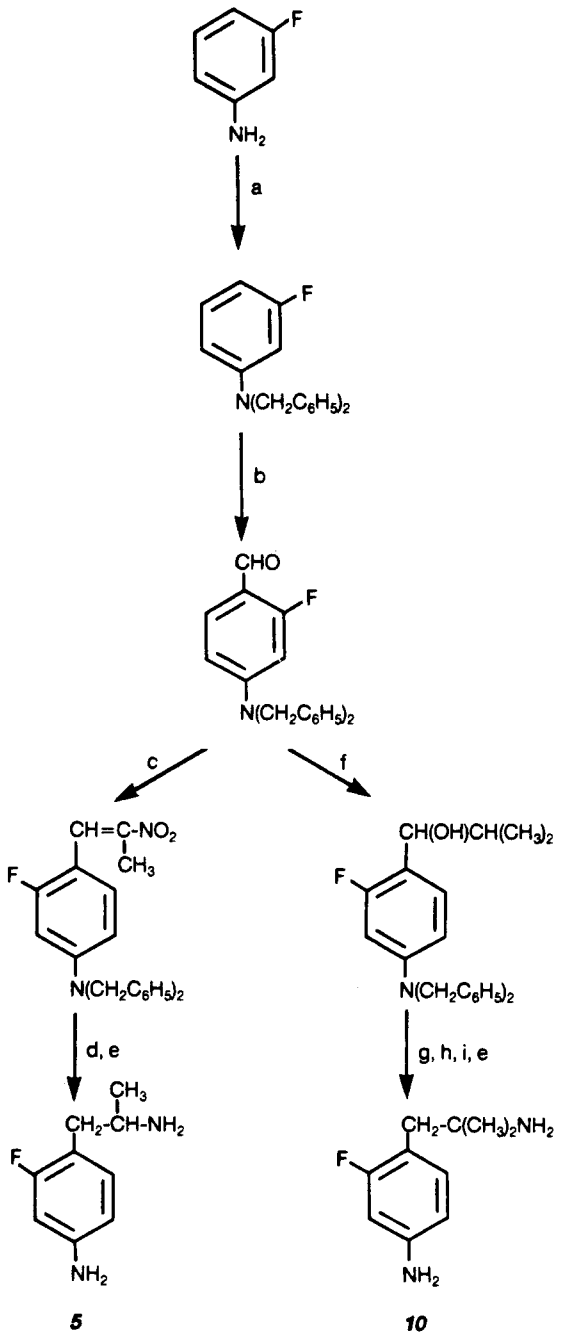
Scheme I[a]



[a] Reagents: a = $C_6H_5CH_2Cl/K_2CO_3/DMF$; b = $POCl_3/DMF$; c = $C_2H_5NO_2$; d = $LiAlH_4$; e = H_2/Pd; f = $(CH_3)_2CHMgBr$; g = Δ; h = CH_3CN/H_2SO_4; i = H^+.

observed for the (−)-enantiomer, since it was active also in serotonergic neurons.[14,15]

In order to further explore the possibility to develop selective MAO inhibitors for the various amine systems, we have now synthesized and tested a series of compounds related to 4-aminoamphetamine (1).

Chemistry. Compounds **2–6** in Table I and III were prepared as illustrated by compound **5** in Scheme I. Similar methods have previously been used in the preparation of various 4-aminophenethylamine derivatives.[16-18] The route started from the corresponding aniline, which was N,N-dibenzylated with benzyl chloride in the presence of dimethylformamide and anhydrous potassium carbonate (**11–14** in Table II). The preparation of compound **11** from *m*-toluidine and benzyl chloride in aqueous solution has been previously described.[19] The *N,N*-dibenzyl derivative of 3-ethylaniline was isolated as an oil and was used without further purification.

In the next step the *N,N*-dibenzylanilines were formylated by a standard Vilsmeier–Haack procedure to the corresponding benzaldehydes (**15–19** in Table II). The intermediate 4-(dibenzylamino)-β-methyl-β-nitrostyrenes were prepared by the condensation of the benzaldehydes with nitroethane in the presence of ammonium acetate.[16,17] All the β-nitrostyrenes, except 4-(dibenzylamino)-2-fluoro-β-methyl-β-nitrostyrene (**20**), were isolated as dark-red viscous oils, which were used directly in the next step involving lithium aluminum hydride reduction. The obtained 4-(dibenzylamino)-α-methylphenethylamines were not isolated but debenzylated in situ by catalytic hydrogenation in the presence of palladium.

The introduction of two methyl groups in the α-position of the phenethylamine side chain was achieved by a previously used route involving the reaction of the corresponding benzaldehyde with isopropylmagnesium bromide, thermal dehydration of the benzyl alcohol, and Ritter reaction of the obtained styrene.[16,17] The acetamide was hydrolyzed with hydrochloric acid and debenzylated by means of catalytic hydrogenation in the presence of palladium (**8–10**). The procedure is exemplified by the preparation of compound **10** in Scheme I. Compound **7** was prepared from *N*-[1-methyl-2-(2-methyl-4-acetamidophenyl)ethyl]acetamide (**21**) by nuclear bromination and hydrolytic deacetylation.

A different route of reactions leading to the target compound **2** has recently been published.[20]

Pharmacology. MAO Inhibition in Vitro. All the compounds examined were considerably more potent as inhibitors of the deamination of serotonin than of phenethylamine (Table III); i.e., they are selective inhibitors of the A form of MAO. The 2,6-dichloro derivative **6** had the highest potency and was 20 times more potent than the corresponding monochloro compound **4**. Substituents in the 2-position increased the inhibitory potency in the order Cl (**4**) > F (**5**) > CH_3 (**2**) > H (**1**). Enlargement of the 2-substituent to ethyl (**3**) decreased the potency considerably, which also was the case for α,α-dimethyl substitution (**8** vs. **2** and **10** vs. **5**). A bromo substituent at the 5-position did not change the inhibitory potency for MAO-A but increased that for MAO-B (cf. **7** and **2**).

MAO Inhibition in Vivo. The MAO inhibition in vivo was examined with the phenelzine protection technique combined with the assay of the MAO activity of crude synaptosomal preparations using low concentrations of the amine substrates, i.e., [^{14}C]-5-HT (0.1 μM), [^{14}C]norepinephrine (0.25 μM), and [^{14}C]dopamine (0.25 μM) in the absence and the presence of selective amine uptake inhibitors.[12,14,15] This method allows the determination of the in vivo inhibition of MAO inside and outside the monoaminergic neurons in the rat brain.[12] The rats were given the test compound orally 1 h before the subcutaneous

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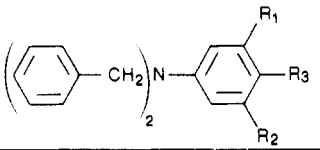
Table II. *N,N*-Dibenzylanilines and 4-Dibenzylaminobenzaldehydes



compd no.	R_3	R_2	R_1	mp, °C	yield,[b] %	formula	anal.
11	H	H	CH_3	75–76[a]	92	$C_{21}H_{21}N$	
12	H	H	Cl	67–68	90	$C_{20}H_{18}ClN$	C, H, Cl, N
13	H	H	F	47–48	76	$C_{20}H_{18}FN$	C, H, F, N
14	H	Cl	Cl	78–79	65	$C_{20}H_{17}Cl_2N$	C, H, Cl, N
15	CHO	H	CH_3	84–85	81	$C_{22}H_{21}NO$	C, H, N
16	CHO	H	Cl	113–114	83	$C_{21}H_{18}ClNO$	C, H, Cl, N, O
17	CHO	H	F	95–96	93	$C_{21}H_{18}FNO$	C, H, N
18	CHO	Cl	Cl	118–119	66	$C_{21}H_{17}Cl_2NO$	C, H, Cl,[c] N, O
19	CHO	H	C_2H_5	73–74	80	$C_{23}H_{23}NO$	C, H, N, O

[a] Reported[19] mp 78 °C. [b] Compounds **11–13** are recrystallized from ethanol, **15** and **19** from dilute ethanol, **16–18** from dimethylformamide–ethanol, **14** from methanol, and **17** from petroleum ether–ethanol. [c] Cl: calcd, 19.15; found, 19.6.

injection of phenelzine sulfate. The animals were killed 48 h later when the reversible inhibition had disappeared. Protection given by the test compound against the irreversible inhibition is then an expression of the in vivo inhibition during the initial phase after the phenelzine injection.[21]

Several of the new compounds were very potent inhibitors of MAO within the aminergic neurons with low effect outside these neurons (Table III). The 2-fluoro derivative **5** was very potent, particularly within the catecholaminergic systems. Also, the 2-chloro derivative **4** was very active and neuron selective with the highest inhibition occurring within the noradrenergic neurons. The 2,6-dichloro compound **6** also had high activity but lacked a neuron-selective effect in all three aminergic systems. The 2-methyl derivative **2** had quite high activity in the noradrenergic system but had less effect in the serotonergic and dopaminergic systems. A bromine at the 5-position (**7**) decreased the in vivo activity in all three aminergic systems, but the neuron selectivity remained in the noradrenergic neurons. The nonsubstituted *p*-aminoamphetamine **1** inhibited the dopamine MAO at lower doses than those inhibiting MAO within noradrenergic and serotonergic neurons. Enlargement of the 2-substituent to ethyl (**3**) almost abolished the in vivo MAO inhibitory effect. α,α-Dimethylation also decreased the potency and reduced the neuron selectivity (**8** vs. **2**, **9** vs. **4**, **10** vs. **5**).

Inhibition of Amine Uptake. Since the neuronal selectivity of the compounds studied most likely is due to their uptake and accumulation in the neurons by the aminergic uptake mechanisms,[11] the uptake inhibitory effects were determined as a measure of their affinities for the uptake sites. As shown in Table III all compounds had higher affinity for the noradrenergic uptake site compared with that for the serotonergic uptake site. There was no significant correlation between the inhibition of the serotonergic and noradrenergic uptake when tested with the Spearman rank test (Table V). Only one compound (**4**) was more potent in inhibiting the dopaminergic uptake than the noradrenergic uptake. However, there was a highly significant correlation between the inhibition of the dopaminergic and noradrenergic uptake in the rank test (Table V). No significance was obtained between the inhibition of the serotonergic and dopaminergic uptake.

The compounds substituted with a halogen in the 2-position (**4** and **5**) had high affinities for all three uptake systems, whereas the dichloro derivative **6** had low affinity for these uptake sites. A methyl substituent in the 2-position (**2**) decreased the affinities, and an ethyl group (**3**) did so pronouncedly.

Behavioral Changes in Reserpinized Rats. Compounds that release the biogenic amines cause typical behavioral changes in rats pretreated with reserpine.[22] Release of serotonin evokes a syndrome consisting of abduction of the hind limbs, forepaw treading, wet dog shake, and Straub tail. Release of norepinephrine and dopamine produces reversal of the reserpine-induced ptosis and the motor activity, respectively. Since transport of a compound by the aminergic uptake mechanism may cause release of this transmitter amine by accelerated exchange diffusion,[23] it was of interest to examine if the compounds examined in this study produced any behavioral changes that would indicate amine release. The results of this test are shown in Table IV. The compounds **1** and **5**, which inhibited MAO within all three aminergic systems with high selectivity, produced marked signs of amine release in these systems. The compounds **2**, **4**, and **7** produced behavioral changes that indicated release of serotonin and norepinephrine, whereas compound **10** appeared to release norepinephrine and dopamine. Compounds **6** and **9** reversed ptosis, possibly due to release of norepinephrine.

Discussion

The results in the present study confirm the conclusion in previous works that it is possible to inhibit MAO within monoaminergic neurons at doses of the inhibitors that have much less effect in other cells.[11,12,14,15] This neuron selectivity is most likely explained by the high concentrations of the inhibitors within these neurons due to accumulation via the membranous uptake mechanisms. The increased MAO inhibition by a compound within a particular aminergic system reflects accordingly the ability of the compound to be transported by the amine pump, the MAO inhibitory action being a necessary prerequisite. In the series of compounds examined, substitution with fluorine or chlorine in the 2-position (**5** and **4**) had a favorable effect on the transport of the compound into all three aminergic nerve terminals. The effect of fluorine is obviously caused by its electron-withdrawing character. Steric hindrance

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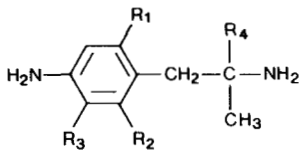
Table III. Inhibition of MAO in Vitro and Inside and Outside Monoaminergic Neurons in Vivo and Monoamine Uptake in Vitro by 4-Aminophenethylamine Derivatives



					MAO inhibn													
					in vitro[a] IC_{50}, μM			in vivo[b] ED_{50}, μmol/kg, po									inhibn of amine uptake,[c] IC_{50}, μM		
								5-HT			NE			DA					
compd no.	R_1	R_2	R_3	R_4	5-HT	PEA	PEA/ 5-HT	*N*	*EN*	*EN*/ *N*	*N*	*EN*	*EN*/ *N*	*N*	*EN*	*EN*/ *N*	5-HT	NE	DA
1	H	H	H	H	6.8	7000	1030	28*	>143 (1%)	≫5	15*	>143 (22%)	>10	8.5*	160	19	20[d]	0.07	0.16
2	CH_3	H	H	H	1.6	2500	1560	14*	>69 (13%)	≫5	5.8*	>69 (5%)	>12	35*	338	9.7	9.0	0.32	11[d]
3	C_2H_5	H	H	H	60	1200	20	>269 (0%)	>269 (0%)		>269 (17%)	>269 (0%)		>134 (6%)	>134 (0%)		10[d]	2.0	20[d]
4	Cl	H	H	H	0.27	750	2780	3.9*	78	20	1.9*	>131 (33%)	>70	6.1	20	3.3	3.0	0.19	0.07
5	F	H	H	H	0.75	800	1070	5.2*	48	9	0.8*	57	71	0.8*	18	23	1.3	0.05	0.42
6	Cl	Cl	H	H	0.012	340	28300	3.4	3.5	1	1.8	3.9	2.2	3.1	4.8	1.5	30[d]	2.2	30[d]
7	CH_3	H	Br	H	1.5	290	193	61	>106 (18%)	>2	30*	>106 (15%)	>4	33*	69	2.1	6.5	0.55	2.0
8	CH_3	H	H	CH_3	20	1500	75	105	>134 (10%)	>1	>134 (40%)	>134 (5%)		>134 (23%)	>134 (11%)		2.6	0.36	40[d]
9	Cl	H	H	CH_3	1.3	1700	1310	22*	130[d]	6	26*	116	4.5	48[d]	120	2.5	5.5	0.60	15[d]
10	F	H	H	CH_3	6.0	2800	467	83	132	1.6	66*	>132 (23%)	>2	29*	120	4.1	27[d]	0.32	1.6

[a] Determined from log concentration–inhibition curves using a rat brain mitochondrial preparation as enzyme and [^{14}C]serotonin (5-HT) (50 μM) and phenethylamine (PEA) (2.5 μM) as substrates (ref 10). [b] Determined from the protection by the test compounds against the irreversible MAO inhibition produced by phenelzine. The rats were sacrificed 48 h after the phenelzine injections, and the MAO activity remaining inside (*N*) and outside (*EN*) the synaptosomes prepared from hypothalamus (serotonin and norepinephrine (NE)) or striatum (dopamine (DA)) was determined as described in the Experimental Section. The ED_{50} values were estimated from log dose–response curves. Percent inhibition at the highest dose tested is given in parentheses. An asterisk indicates significant difference *N* vs. *EN* at three different doses ($p < 0.05$, Mann–Whitney U-test). [c] The compounds were preincubated with the synaptosomal preparations for 10 min at 37 °C and incubated for an additional 2 min with [^{14}C]-5-HT + [^{3}H]NE (cerebral cortex) or [^{14}C]DA (striatum), each at 50 nM. [d] Extrapolated values.

Table IV. Behavioral Changes in Reserpinized Rats That Indicate Release of Serotonin (5-HT Syndrome), Norepinephrine (NE) (Reversal of Ptosis), and Dopamine (DA) (Increase in Motor Activity)

compd. no.	least dose with behavioral changes, μmol/kg, sc			compd. no.	least dose with behavioral changes, μmol/kg, sc		
	5-HT	NE	DA		5-HT	NE	DA
1	276	72	72	6	>229	115	>229
2	270	270	>270	7	211	211	>211
3	>269	>269	>269	8	>269	>269	>269
4	33	16	>131	9	>247	247	>247
5	140	17	35	10	>264	264	264

Table V. Spearman Rank Order Correlation between the Various Parameters Measured (Correlation Coefficient (r_s))

	MAO inhibn within monoaminergic neurons in vivo			inhibn of amine uptake in vitro			behavioral changes
	5-HT	NE	DA	5-HT	NE	DA	NE[a]
MAO-A inhibition in vitro	0.92[b]	0.83[b]	0.73[c]	0.10	0.01	0.25	0.44
MAO inhibition within monoaminergic neurons in vivo							
5-HT		0.95[b]	0.77[b]	0.05	0.18	0.31	0.46
NE			0.85[b]	0.13	0.40	0.44	0.61
DA				−0.04	0.48	0.58[c]	0.77[c]
inhibition of monoamine uptake in vitro							
5-HT					0.40	0.13	0.46
NE						0.79[b]	0.47
DA							0.58

[a] $n = 8$. [b] $p < 0.01$ ($n = 10$). [c] $p < 0.05$.

at the uptake carrier is probably the reason for the lack of neuron-selective action of the 2,6-dichloro derivative **6**. This substitution is, however, very favorable for the affinity for the A form of MAO and increases the potency 20 times compared with the monochloro derivative **4**.

The compounds in this series generally caused higher inhibition of MAO in the noradrenergic than in the serotonergic neurons in the rat hypothalamus. This is in contrast to the *p*-dimethylamino derivatives, reported in a previous study,[14] which caused somewhat higher inhibition within the serotonergic neurons. Thus, the larger *p*-dimethylamino derivatives appear to be better transported by the serotonin pump, while the smaller *p*-amino derivatives are preferably transported by the norepinephrine uptake mechanism.

Although several of the new compounds were very potent in inhibiting the MAO within the aminergic nerve endings, they showed only small differences between the three aminergic systems. However, it might be possible to change this by resolution of the compounds into their enantiomers. For instance, the *R*-(−) isomer of compound **2** was found to inhibit MAO in both the serotonergic and noradrenergic nerve terminals in vivo, while the S-(+) enantiomer was a very potent inhibitor of MAO within noradrenergic neurons but a weak inhibitor of the enzyme in serotonergic neurons.[14,15]

In this series of compounds the MAO-A inhibitory potencies in vitro were significantly correlated with the potencies of the inhibition of MAO within the aminergic neurons in vivo when examined with the Spearman rank order test (Table V). The MAO inhibition within the aminergic neurons was also highly significantly correlated. However, the rank order of the uptake inhibition and that of the MAO inhibition in vitro was only significant for the dopaminergic system. If the compounds that did not show selectivity for the MAO within the aminergic neurons are excluded, a significant correlation could also be found for the noradrenergic system ($r_s = 0.77$, $p < 0.05$, $n = 7$). It should be noted that the affinities for the aminergic uptake sites do not reflect the rates of transportation of the compounds into the neurons. Furthermore, the compounds tested may inhibit the amine uptake by being either transported inhibitors or nontransported inhibitors.

Transport of a compound by the aminergic uptake system may induce an outward transport of the transmitter amine by an accelerated exchange diffusion mechanism, which has been suggested as being the mechanism for the release of norepinephrine by amphetamine.[23] This may be the mode of action of the indirectly acting amines that release the transmitter amines.[24] In the reserpinized rats the release of the biogenic amines causes typical behavioral changes for each amine. In the series of compounds examined in the present study the most neuron-selective MAO inhibitors (**4** and **5**) were also the most potent ones in producing the behavioral changes. If the amine release is coupled to the inward transport of the releasing compound, the much higher doses of the compounds that were required for the release compared with those required for the neuron-selective MAO inhibition indicate that the amine pump has to be almost saturated with the compounds to induce the release. The reversal of the reserpine-induced ptosis may be a sign of norepinephrine release, although inhibition of the norepinephrine reuptake may give the same effect, which may explain the observed effect of compound **6**, which appears to lack transporting ability. The correlation between the reversal of ptosis and the MAO inhibition in the dopaminergic neurons is probably a false one. No other significant correlation between the reversal of ptosis and the other parameters was found.

Experimental Section

Melting points were determined in a Mettler FP 61 melting point recorder. ^{1}H NMR spectra were recorded on a Varian EM-360 60-MHz spectrometer. The analyses were performed by the Department of Analytical Chemistry, University of Lund, Sweden; where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within (±0.4%)*m* of the theoretical values. The absence of impurities was examined by GC with a Carlo Erba 4300 gas chromatograph equipped with a 25-m × 0.35-mm fused silica capillary column (SE52) and an FID detector.

***N*,*N*-Dibenzyl-3-fluoroaniline (13).** To a mixture of 96.0 mL (1.0 mol) of 3-fluoroaniline, 276.0 g of K_2CO_3 and 5.0 g of KI in 400 mL DMF were added dropwise in 254 mL (2.2 mol) of benzyl chloride while stirring and heating at 100 °C. The mixture was heated, stirred overnight, and poured into 3 L of an ice-water mixture. The obtained oil solidified gradually upon standing.

4-(Dibenzylamino)-2-fluorobenzaldehyde (17). To a stirred solution of 201.1 g (0.69 mol) of compound **13** in 230 mL of DMF was added while stirring and cooling in tap water 65 mL (0.71 mol) of $POCl_3$. The mixture was heated for 2 h in a steam bath and poured into 2 L of an ice-water mixture. The mixture was made basic with 10 M NaOH, and the product was filtered, washed with water, air-dried, and recrystallized from ethanol–petroleum ether: yield, 204.0 g.

4-(Dibenzylamino)-2-fluoro-β-methyl-β-nitrostyrene (20). A mixture of 70.2 g (0.22 mol) of compound **17**, 22.0 mL of nitroethane, and 15 g of ammonium acetate in 250 mL of EtOH was heated under reflux for 5 h. After cooling the crude product was filtered and recrystallized: yield, 59.1 g (71%); mp 96–97 °C. Anal. ($C_{23}H_{21}FN_2O_2$) C, H, F, N.

4-Amino-2-fluoro-α-methylphenethylamine Acetate (5). A solution of 58.3 g (0.15 mol) of compound **20** in 350 mL of THF was added dropwise to a stirred mixture of 20.0 g of $LiAlH_4$ in 350 mL of dry Et_2O. The mixture was stirred and heated under reflux for 2.0 h. After dropwise addition of 100 mL of saturated Na_2SO_4 solution while stirring and cooling in ice, the mixture was filtered. The filtrate was collected, and the solvent was evaporated under reduced pressure. The residual oil was dissolved in a

mixture of 100 mL of 12 M HCl and 500 mL of 50% AcOH and was hydrogenated with H_2/Pd (5%) at normal pressure and approximately 50 °C. When the uptake of hydrogen had stopped, the catalyst was filtered off and the filtrate was evaporated to about 100 mL and alkalized with 10 M NaOH. The mixture was extracted with Et_2O, and the extract was dried over NaOH and Na_2SO_4. The solution was treated with 10 mL of AcOH, and the semisolid salt was collected and recrystallized: ^{1}H NMR (D_2O) δ 7.2–6.9 (m, 1 H, Ar), 6.7–6.4 (m, 2 H, Ar), 3.7–3.2 (m, 1 H, CH), 2.9–2.7 (d, 2 H, CH_2), 1.9 (s, 3 H, CH_3), 1.3 (d, 3 H, CH_3).

4-Amino-2-fluoro-α,α-dimethylphenethylamine Acetate (10). A solution of 35 mL (0.35 mol) of isopropyl bromide in 50 mL of Et_2O was added dropwise with stirring to 8.0 g (0.33 mol) of magnesium turnings in 300 mL of Et_2O under dry nitrogen. When all the halide had been added, the solution was heated under reflux for 10 min. The mixture was cooled in an ice bath and stirred while 49.8 g (0.15 mol) of compound **17** was added in portions. The mixture was refluxed for 1 h, and 65 mL of 12 M HCl in 500 mL of water was added dropwise while stirring and cooling in ice-water. The mixture was neutralized with concentrated ammonium hydroxide and extracted with $CHCl_3$. The extract was dried with Na_2SO_4, and the solvent was evaporated. The residue was heated under nitrogen at 200 °C for 1 h, and the obtained product was dissolved in 50 mL of acetonitrile. To the stirred solution was added dropwise, while cooling in ice, 30 mL of concentrated H_2SO_4. The mixture was left overnight at room temperature and was then poured into 1 L of water and neutralized with 10 M NaOH. The semisolid product was collected and dissolved in a mixture of 150 mL of 12 M HCl, 75 mL of AcOH, and 75 mL of water. The solution was refluxed for 40 h and evaporated to half the volume. After the addition of 300 mL of EtOH the mixture was hydrogenated with H_2/Pd (5%) at normal pressure and approximately 50 °C. The catalyst was filtered off, and the filtrate was evaporated to half its volume. The solution was made basic with 10 M NaOH and extracted with Et_2O. The extract was dried over Na_2SO_4 and treated with 9.5 mL of AcOH. The obtained precipitate was filtered off and recrystallized: ^{1}H NMR (D_2O) δ 7.3–6.9 (m, 1 H, Ar), 6.8–6.5 (m, 2 H, Ar), 2.9 (s, 2 H, CH_2), 1.9 (s, 3 H, CH_3), 1.4 (s, 6 H, $2CH_3$).

***N*-[1-Methyl-2-(2-methyl-4-acetamidophenyl)ethyl]acetamide (21).** A solution of 16.0 g (0.07 mol) of compound **2** in 25 mL of AcOH and 50 mL of acetic anhydride was refluxed for 0.5 h. The mixture was diluted with water to 800 mL, neutralized with aqueous ammonia, and extracted with CH_2Cl_2. The extract was dried with Na_2SO_4, and the CH_2Cl_2 was evaporated. The solid residue was suspended in water, filtered, and dried: yield, 11.5 g (66%); mp 180–181 °C. Anal. ($C_{14}H_{20}N_2O_2$) C, H, N.

4-Amino-5-bromo-2,α-dimethylphenethylamine Acetate (7). To a mixture of 10.0 g (0.04 mol) of compound **21** in 50 mL of AcOH was added dropwise 2.3 mL (0.044 mol) of Br_2 in 20 mL of AcOH. The mixture was stirred at room temperature for 0.5 h and poured into 500 mL of an ice-water mixture. The precipitated product was collected and recrystallized twice from aqueous EtOH yielding 6.7 g of the brominated amide, melting at 180–185 °C. The product was dissolved in 50 mL of 12 M HCl and 30 mL of water and was refluxed overnight. The solution was alkalinized with NaOH solution and extracted with Et_2O. The extract was dried over Na_2SO_4, and 2.4 mL of AcOH was added. The obtained precipitate was filtered off and washed with ether: yield, 5.8 g; ^{1}H NMR (D_2O) δ 7.2 (s, 1 H, Ar), 6.8 (s, 1 H, Ar), 3.7–3.2 (m, 1 H, CH), 3.0–2.6 (m, 2 H, CH_2), 2.2 (s, 3 H, CH_3), 2.0 (s, 3 H, CH_3), 1.4 (d, 3 H, CH_3).

Monoamine oxidase inhibition in vitro was determined with a mitochondrial preparation of rat brain as described by Ask et al.[10] [^{14}C]-5-HT (50 μM) and [^{14}C]phenethylamine (2.5 μM) were used as substrates. IC_{50} values were estimated from log concentration curves based on at least five different concentrations of the inhibitor.

Monoamine Oxidase Inhibition in Vivo. The phenelzine protection method combined with the assay of the deaminating activities in crude synaptosomal preparations of hypothalamus (serotonin and norepinephrine) or striatum (dopamine) was used for the determination of the MAO inhibition in vivo inside and outside the monoaminergic neurons.[11] In this assay low concentrations of [^{14}C]serotonin (0.1 μM), [^{14}C]-1-norepinephrine (0.25 μM), or [^{14}C]dopamine (0.25 μM) were incubated with the synaptosomal preparation in the absence and presence of selective uptake inhibitors (citalopram, maprotiline, and amfonelic acid).

Sprague-Dawley rats weighing 160-200 g were given the test compound orally 1 h before the injection of phenelzine sulfate (4 mg/kg sc in the hypothalamus experiments; 2.5 mg/kg sc in the striatal experiments). The rats were killed 48 h later when the effects of the test compound were presumed to have disappeared. The hypothalami or striata were rapidly dissected out, homogenized in 20 vol of 0.25 M sucrose, and centrifuged at 800*g* for 10 min. After a 10-min preincubation of 50 μL of the synaptosome-rich supernatant in 925 μL of Krebs–Henseleit's buffer, pH 7.4, containing 5.6 mM glucose, 1.1 mM ascorbic acid, and 0.13 mM disodium edetate, the incubation was continued for a further 10 min at 37 °C with [^{14}C]serotonin (0.1 μM) or [^{14}C]-dopamine (0.25 μM) in the absence or presence of 0.12 μM citalopram (5-HT) or 0.1 μM amfonelic acid (dopamine). In the [^{14}C]norepinephrine (0.25 μM) experiments 200 μL of the hypothalamic supernatants was used in the absence and presence of maprotiline (3 μM). The deaminated products were extracted into ethyl acetate, and the monoamine oxidase activities were calculated from the radioactivities.

The MAO inhibition inside and outside the aminergic neurons was estimated from the protection against the irreversible action of phenelzine as described by Green and El Hait.[21] The percent inhibition produced by the reversible inhibitors was calculated according to the formula

$$\left(1 - \frac{\ln (100/t)}{\ln (100/p)}\right) \times 100$$

in which t is the MAO activity in the synaptosomes from the animals treated with the test compound + phenelzine, and p is that from the rats treated with saline + phenelzine, the activity expressed in percentage of that in the control animals. The ED_{50} values were estimated from log dose–response curves based on at least three doses with four rats in each dose group.

Inhibition of the Synaptosomal Uptake of Monoamines. Crude synaptosomal preparations from rat cerebral cortex and striatum were made by homogenizing the tissues in 10 vol of ice-cold 0.32 M sucrose with all-glass Potter-Elvehjem's homogenizers. The homogenates were centrifuged at 800*g* at +2 °C for 10 min. The supernatants were centrifuged at 12000*g* at +2 °C for 10 min, and the pellets were rehomogenized in 0.32 M sucrose to the original volume. The incubation of the preparations with [^{14}C]serotonin + [^{3}H]norepinephrine (cerebral cortex) or [^{14}C]dopamine (striatum) with final concentrations of 50 nM of each amine was performed in a Micronic PPN Storage-Block-96 (Flow Laboratories) with 8 × 12 wells using two rows at each incubation. Four different concentrations of two test compounds in duplicate were examined at each incubation. Fifty microliters of the synaptosomal preparation, 400 μL of the Krebs–Henseleit's buffer, pH 7.4, containing 5.6 mM glucose, 1.1 mL ascorbic acid, 0.13 mL disodium edetate, and 50 μM pargyline, and 25 μL of the inhibitor or distilled water were added to the wells. The solutions were mixed by vortexing the block for 10 s. After 10-min preincubation at 37 °C in a water bath 25 μL of the solutions of the radioactively labeled amines was added to the two rows with a Titrertec multicannel pipette, type 12-Channel (Flow Laboratories). The reaction was immediately started by vortexing the block for 10 s on a Super-Mixer, and the incubation was continued for 2 min at 37 °C. The uptake reaction was stopped by filtration and washing for 15 s with ice-cold 0.15 M NaCl through a Whatman GF/B glass filter paper in a 24-channel cell harvester (Brandel) using the standard harvesting probe. The filters were left to dry at room temperature for about 1 h. The punched filters were transformed to counting vials; 10 mL of the scintillation liquid (Aquasol, NEN) was added, and the vials were shaken and allowed to stand for 1 h before counting. The radioactivity was measured in a Packard Tri Carb liquid scintillation photometer. The active uptake of the amines was defined as the difference between the accumulation of the radioactivity in the absence (quadruplicates) and the presence (quadruplicates) of selective uptake inhibitors, determined at each incubation. These inhibitors were citalopram (0.3 μM) for the uptake of serotonin, maprotiline (1 μM) for the uptake of norepinephrine, and amfonelic acid (0.3 μM) for the uptake of dopamine in striatal synaptosomes. The

inhibition was calculated in percent of the active uptake. The IC_{50} values were obtained from log concentration–response curves.

Behavioral Studies in Reserpinized Rats. Reserpine, 5 mg/kg sc, was injected 18 h before the oral administration of the test compound. Behavioral changes (abduction of hind legs, wet dog shake, forepaw treading, Straub tail, and ptosis) were observed for 1 h after the administration of the test compound.[22] The lowest doses causing these changes were noted.

Registry No. (±)-**1**, 103882-44-6; (±)-**2**, 103818-17-3; (±)-**3**, 103818-19-5; (±)-**4**, 103818-21-9; (±)-**5**, 103834-90-8; (±)-**6**, 103818-23-1; (±)-**7**, 103882-46-8; (±)-**7** (*N*,*N*'-diacetate), 103818-35-5; **8**, 103818-25-3; **9**, 103834-92-0; **10**, 103818-27-5; **11**, 55197-79-0; **12**, 89170-76-3; **13**, 103818-28-6; **14**, 103818-29-7; **15**, 1424-65-3; **16**, 89115-16-2; **17**, 103818-30-0; **18**, 103818-31-1; **19**, 103818-32-2; **20**, 103818-33-3; (±)-**21**, 103818-34-4; MAO, 9001-66-5; F-*m*-$C_6H_4NH_2$, 372-19-0; $EtNO_2$, 79-24-3; *i*-PrBr, 75-26-3.

Syntheses of 5,6,7- and 5,7,8-Trioxygenated 3',4'-Dihydroxyflavones Having Alkoxy Groups and Their Inhibitory Activities against Arachidonate 5-Lipoxygenase

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Arachidonate 5-lipoxygenase plays a pivotal role in the biosynthesis of leukotrienes. Cirsiliol (3',4',5-trihydroxy-6,7-dimethoxyflavone), a selective inhibitor of the enzyme, was derivatized by introducing alkyl groups of various chain lengths at positions 5, 6, 7, and 8 of the A ring of the flavone skeleton. Modification of the positions 5 and 6 with an alkyl group of 5–10 carbons markedly decreased the IC_{50} values for 5-lipoxygenase inhibition to the order of 10 nM. As tested with 5- or 6-hexyloxy derivatives, a relatively selective inhibition of 5-lipoxygenase was shown. Inhibition of 12-lipoxygenase required much higher concentrations of these compounds, and cyclooxygenase was not inhibited. Modification of positions 7 and 8 did not increase the inhibitory effect of most flavone compounds.

Arachidonate 5-lipoxygenase catalyzes the oxygenation of arachidonic acid at the 5-position to produce 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid, which is further transformed to various leukotrienes.[1] In view of the important role of leukotrienes as mediators of anaphylactic reactions,[1] various synthetic compounds have been reported as specific inhibitors of the 5-lipoxygenase.[2-10] Previously we reported that cirsiliol (3',4',5-trihydroxy-6,7-dimethoxyflavone)[11] (**1a**) and pedalitin (3',4',5,6-tetrahydroxy-7-methoxyflavone)[12] were the most potent 5-lipoxygenase inhibitors (IC_{50} 0.1 μM) among about 80 flavones tested.[13,14] The vicinal diol on the 2-phenyl substituent (the B ring) was necessary for the inhibition of 5-lipoxygenase. On the basis of this finding we derivatized cirsiliol in a variety of ways to find out the structure–activity relationship and to develop a more potent and selective inhibitor. The results suggest that the activity of the 3',4'-dihydroxyflavones such as cirsiliol was enhanced by modifying the oxygenated functions in the A ring with lipophilic alkyl groups. In this paper we report the synthesis of these flavones with alkoxy groups and their structure–activity relationship as inhibitors of 5-lipoxygenase.

Chemistry. The synthetic routes employed for the preparation of the 5,6,7- and 5,7,8-trioxygenated 3',4'-dihydroxyflavones are illustrated in Schemes I and II. As shown in Scheme I, the crude 3-alkyl ethers **13a–k** derived from 3,6-dihydroxy-2,4-dimethoxyacetophenone (**12**)[15] by the partial alkylation were condensed with 3,4-bis(benzyloxy)benzoyl chloride in pyridine, and the resultant benzoates were converted into the diketone derivatives **14a–k** by the Baker–Venkataraman transformation. Cyclization of **14** with anhydrous sodium acetate afforded 6-alkoxy-3',4'-bis(benzyloxy)-5,7-dimethoxyflavones **15a–k**. The flavones **15** were also synthesized from 3',4'-bis(benzyloxy)-6-hydroxy-5,7-dimethoxyflavone (**16**)[16,17] by the alkylation with alkyl iodides. The hydrogenolysis of compounds **15** with palladium on charcoal afforded 6-alkoxy-3',4'-dihydroxy-5,7-dimethoxyflavones (**6a–k**), which were

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