Selective Monoamine Oxidase Inhibitors. 1. Compounds Related to 4-Aminophenethylamine

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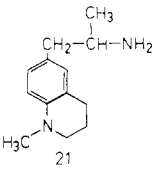
A series of derivatives of 4-aminophenethylamine was synthesized and their effect on monoamine oxidase (MAO) activity in the brain was evaluated. Several of the new compounds were potent and selective inhibitors of the A form of MAO but were poor inhibitors of the B form. The most active compounds were the 2,6-dichloro- (**9**) and the 2-halogeno-4-dimethylaminophenethylamines (**5**, **6**, and **8**). Some of the compounds also strongly antagonized aggressive behavior in isolated male mice. This effect was correlated to the MAO inhibition when tyramine was used as substrate. Significant correlations between MAO inhibition in vivo and potentiation of the syndromes produced by 5-hydroxytryptophan and tryptamine and antagonism of reserpine sedation were obtained.

The observation that the enzyme monoamine oxidase (MAO) occurs in multiple forms[1,2] which differ in efficacy in deaminating various substrates and with different sensitivity to inhibitors[3,4] has initiated a search for selective MAO inhibitors as potential antidepressants in many laboratories. According to the findings of Johnston,[1] two types of MAO may be recognized, namely the A and B forms. 5-Hydroxytryptamine (5-HT)[1] and noradrenaline (NA)[5] are almost selectively deaminated by the A form and phenethylamine by the B form.[6] Other substrates such as tyramine and tryptamine are deaminated by both A and B forms.[1,6] The liver has much higher MAO activity than other peripheral organs and contains a large amount of the B form,[7] whereas the enzyme in central monoaminergic neurones is mainly the A form.[5] An ideal MAO inhibitor for potential use as an antidepressant drug should consequently selectively inhibit the A form and be well distributed to the brain. Although almost all therapeutically used MAO inhibitors act irreversibly, a reversible inhibitor is from pharmacokinetical point of view to be preferred. Of the MAO inhibitors now known, clorgyline is a selective, irreversible inhibitor of the A form as originally reported by Johnston in his classification of MAO into A and B forms.[1] We have found α-ethyltryptamine to be a reversible, selective inhibitor of the A form (unpublished observations). Pargyline is somewhat more active on the B form than the A form[8] whereas other irreversibly acting MAO inhibitors, e.g., pheniprazine and nialamide, are equally active on both types.

In our search for new selective MAO inhibitors we observed that *p*-dialkylamino-substituted phenethylamine derivatives have properties indicating that these compounds are selective MAO inhibitors of therapeutical interest. In this report we describe the synthesis and screening of a series of 4-aminophenethylamine derivatives.

Chemistry. The 4-dialkylaminophenethylamine derivatives **2**–**6**, **8**, **9**, and **11**–**14** in Table I were prepared by reduction of the corresponding 4-dialkylamino-β-alkyl-β-nitrostyrenes with lithium aluminum hydride (Scheme I). The 1,2,3,4-tetrahydroquinoline compound **21** and the 2-dimethylaminophenethylamine compound **22** were prepared similarly. 4-Ethylamino-α-methylphenethyl-



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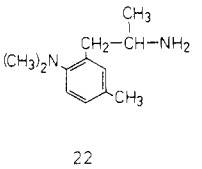

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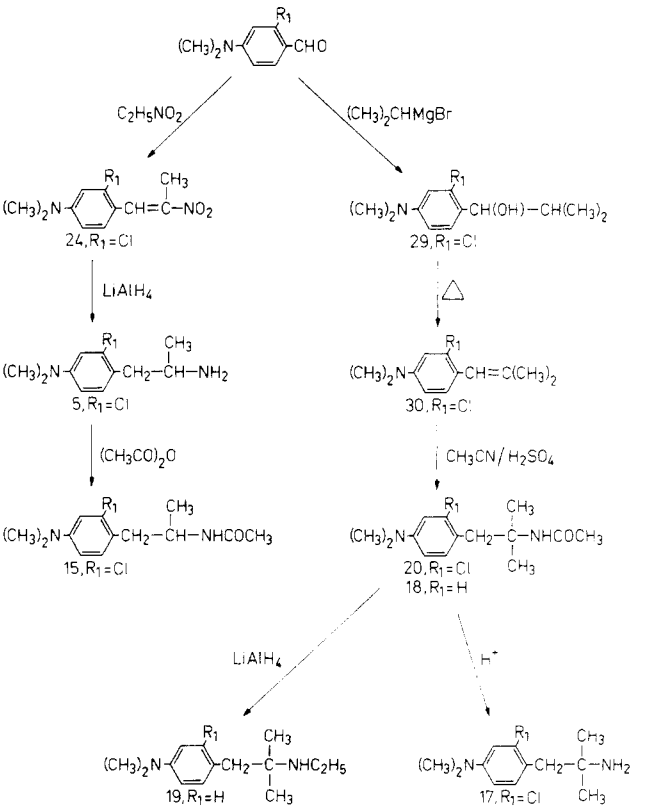
Scheme I



amine (**10**) was obtained from 4-acetamido-β-methyl-β-nitrostyrene[9] using the same reduction method.

The intermediate 4-dimethylamino-β-alkyl-β-nitrostyrenes in Table II were prepared by a procedure involving the condensation of the appropriate benzaldehydes with nitropropane or nitroethane in the presence of ammonium acetate. The β-nitrostyrenes corresponding to compounds **4**, **11**, **21**, and **22** were obtained as viscous oils which could not be crystallized or distilled without decomposition. The products were, therefore, reduced with lithium aluminum hydride without further purification. The crude amines obtained were then purified by distillation or by recrystallization of the hydrochlorides.

The benzaldehydes used in this work were prepared in a step involving the formylation of appropriately substituted anilines according to the Vilsmeier–Haack reaction.[10–12] The low yield of 6-dimethylamino-*m*-tolu-

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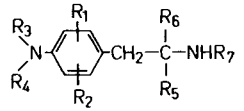
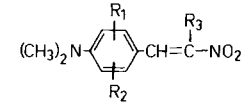
Table I. Derivatives of 4-Aminophenethylamine



Compd	R_1	R_2	R_3	R_4	R_5	R_6	R_7	Mp, °C	Yield, %	Formula	Analyses
1	H	H	H	H	H	CH_3	H	270-271	72	$C_9H_{14}N_2 \cdot 2HCl$	Cl^-
2[a]	H	H	CH_3	CH_3	H	CH_3	H	218-219	32	$C_{11}H_{18}N_2 \cdot 2HCl$	Cl^-
3	2-CH_3	H	CH_3	CH_3	H	CH_3	H	208-209	83	$C_{12}H_{20}N_2 \cdot 2HCl$	C, H, Cl, N
4	3-CH_3	H	CH_3	CH_3	H	CH_3	H	232-233	20	$C_{12}H_{20}N_2 \cdot 2HCl$	C, H, Cl, N
5	2-Cl	H	CH_3	CH_3	H	CH_3	H	199-200	80	$C_{11}H_{17}ClN_2 \cdot 2HCl$	C, H, Cl (total), Cl^-, N
6	2-Br	H	CH_3	CH_3	H	CH_3	H	195-196	70	$C_{11}H_{17}BrN_2 \cdot 2HCl$	C, H, Cl^-, N
7	3-Br	H	CH_3	CH_3	H	CH_3	H	190-191	70	$C_{11}H_{17}BrN_2 \cdot 2HCl$	C, H, Br, Cl^-, N
8	2-F	H	CH_3	CH_3	H	CH_3	H	204-205	72	$C_{11}H_{17}FN_2 \cdot 2HCl$	C, H, F, Cl^-, N
9	2-Cl	6-Cl	CH_3	CH_3	H	CH_3	H	199-200	95	$C_{11}H_{16}Cl_2N_2 \cdot 2HCl$	C, H, Cl (total), Cl^-, N
10	H	H	H	C_2H_5	H	CH_3	H	184-185	39	$C_{11}H_{18}N_2 \cdot 2HCl$	C, H, Cl^-, N
11	H	H	CH_3	C_2H_5	H	CH_3	H	209-210	32	$C_{12}H_{20}N_2 \cdot 2HCl$	C, H, Cl^-, N
12	H	H	C_2H_5	C_2H_5	H	CH_3	H	219-220	70	$C_{13}H_{22}N_2 \cdot 2HCl$	C, H, Cl^-, N
13	H	H	$C_6H_5CH_2$	$C_6H_5CH_2$	H	CH_3	H	156-157	50	$C_{23}H_{26}N_2 \cdot 2HCl$	H, Cl, N; C[b]
14	2-Cl	H	CH_3	CH_3	H	C_2H_5	H	229-230	85	$C_{12}H_{19}ClN_2 \cdot 2HCl$	C, H, Cl^-, N
15	2-Cl	H	CH_3	CH_3	H	CH_3	$COCH_3$	118-119	40	$C_{13}H_{19}ClN_2O$	C, H, Cl, N, O
16	H	H	CH_3	CH_3	CH_3	CH_3	H	239.5-240.5	28	$C_{12}H_{20}N_2 \cdot 2HCl$	C, H, Cl^-, N
17	2-Cl	H	CH_3	CH_3	CH_3	CH_3	H	216-217	20	$C_{12}H_{19}ClN_2 \cdot 2HCl \cdot H_2O$	C, H, Cl, N, O
18	H	H	CH_3	CH_3	CH_3	CH_3	$COCH_3$	156-157	40	$C_{14}H_{22}N_2O$	C, H, N, O
19	H	H	CH_3	CH_3	CH_3	CH_3	C_2H_5	232-232.5	61	$C_{14}H_{24}N_2 \cdot 2HCl$	C, H, N, O
20	2-Cl	H	CH_3	CH_3	CH_3	CH_3	$COCH_3$	131-133	35	$C_{14}H_{21}ClN_2O$	C, H, Cl, N, O
21								221-222	24	$C_{13}H_{20}N_2 \cdot 2HCl$	C, H, Cl, N
22								128-129	42	$C_{12}H_{20}N_2 \cdot 2HCl \cdot C_2H_5OH$	C, H, Cl, N, O

[a] See ref 20. [b] C: calcd, 68.48; found, 67.9.

Table II. β-Alkyl-4-dimethylamino-β-nitrostyrenes



Compd	R_1	R_2	R_3	Mp, °C	Yield, %	Formula	Analyses
23	2-CH_3	H	CH_3	75-76	34	$C_{12}H_{16}N_2O_2$	H, N, O; C[a]
24	2-Cl	H	CH_3	93-94	37	$C_{11}H_{13}ClN_2O_2$	C, H, Cl, N, O
25	2-Br	H	CH_3	102-103	43	$C_{11}H_{13}BrN_2O_2$	C, H, Br, N, O
26	2-Cl	6-Cl	CH_3	113-114	83	$C_{11}H_{12}Cl_2N_2O_2$	C, H, Cl, N, O
27	2-Cl	H	C_2H_5	90-91	10	$C_{12}H_{15}ClN_2O_2$	C, H, Cl, N
28	2-F	H	CH_3	103-104	53	$C_{11}H_{13}FN_2O_2$	C, H, F, N, O

[a] C: calcd, 65.44; found, 64.7.

aldehyde was improved by a reversed addition procedure of the reagent. The preparation of 4-dimethylamino-*o*-tolualdehyde[11] from *N,N*-dimethyl-*m*-toluidine was effected by a modified procedure using the phosphorus tribromide reagent.[13] In addition, the interaction of an excess of the dimethylformamide–phosphorus oxychloride reagent with *N,N*-dimethyl-*m*-toluidine was found to give rise to diformylation of the toluidine, yielding 4-(dimethylamino)-6-methylisophthaldehyde. Diformylation of 3,5-dimethyl-*N,N*-dimethylaniline during the Vilsmeier–Haack aldehyde synthesis has previously been reported to give 4-(dimethylamino)-2,6-dimethylisophthalaldehyde.[14] 4-Ethylmethylaminobenzaldehyde has formerly been obtained from *N*-ethyl-*N*-methylaniline by the Duff formylation procedure.[15] The preparation of 6-dimethylamino-*m*-tolualdehyde from 2-dimethylamino-5-methylbenzyl alcohol by a modified Oppenauer oxidation process has been described.[16] The acetamides **18** and **20** were prepared by the Ritter reaction of the corresponding β,β-dimethylstyrenes and acetonitrile. The obtained amides yielded on acid hydrolysis the α,α-dimethylphenethylamines **16** and **17**.

The synthesis of 4-(2,2-dimethylvinyl)-*N,N*-dimethylaniline[17] and 3-chloro-4-(2,2-dimethylvinyl)-*N,N*-dimethylaniline was achieved by a route involving the reaction of the appropriate benzaldehydes with isopropylmagnesium bromide, followed by thermal dehydration of the obtained benzyl alcohols.

The preparation of the parent compound 4-amino-α-methylphenethylamine (**1**) was effected by catalytic hydrogenation of 4-nitro-α-methylphenethylamine at atmospheric pressure over 5% palladium on carbon. The compound has previously been obtained from 4-aminophenyl-2-propanone by the Leuckart reaction[18] and by catalytic hydrogenation of 4-nitro-α-methylphenethylamine over Raney nickel at elevated pressure.[19]

Nuclear bromination of 4-dimethylamino-α-methylphenethylamine (**2**) in acetic acid in the presence of sodium acetate yielded 3-bromo-4-dimethylamino-α-methylphenethylamine (**7**).

The ethylation of the primary amino group of compound **16**, yielding the amine **19**, was effected by reducing the corresponding acetamide **18** with lithium aluminum hydride. The acetamide **15** was prepared in one step from 2-chloro-4-dimethylamino-β-nitro-β-methylstyrene (**24**). The intermediate compound, 2-chloro-4-dimethylamino-α-methylphenethylamine (**5**), was not isolated but reacted in situ with acetic anhydride.

Pharmacology. MAO Inhibition. The compounds were tested for their ability to inhibit the oxidative deamination of [^{3}H]phenethylamine, [^{3}H]tyramine, and [^{14}C]-5-HT in slices of the mouse brain 1 h after intraperitoneal administration. In the screening procedure the substrate concentration was low (1×10^{-7} M), since the inhibition of the active uptake of [^{14}C]-5-HT was determined simultaneously.[22] The most potent compounds were also tested at a higher substrate concentration (1×10^{-5} M).

The deamination of [^{3}H]phenethylamine was not inhibited by any of the new compounds at the highest dose tested, 31–62 μmol/kg ip. Among the reference compounds pargyline inhibited the deamination of this substrate with an ED_{50} = 41 μmol/kg ip, whereas clorgyline and α-ethyltryptamine were without any effect at 33 μmol/kg ip.

Several of the new compounds were potent in inhibiting the deamination of [^{14}C]-5-HT (Table III). The most potent inhibitors were those with a halogen at the 2 position. The importance of this substitution was further accentuated by the observation that the 2,6-dichloro derivative **9** was the most potent compound tested. It was followed in potency by the 2-bromo (**6**), 2-chloro (**5**), and 2-fluoro (**8**) derivatives. A methyl group at the 2 position (**3**) on the other hand decreased slightly the activity compared to the unsubstituted compound **2**. Changing the α-methyl group (**5**) to an ethyl group (**14**) decreased the activity and a reduced potency was also observed for the α,α-dimethyl derivative **17**. The importance of substituents on the 4-amino group was evaluated in a series of compounds (**1**, **2**, **10**–**13**, and **21**). It was found that the primary amine **1** had low activity whereas the optimal potency in this respect was obtained for the dimethylamino derivative **2**. The ethylamino derivative **10** had also rather high potency, which was reduced for compounds with larger substituents on the 4-amino group (**11**, **13**, and **21**). When the dimethylamino substituent was placed at the 2 position, the inhibitory activity was strongly reduced. The effect of substituents at the 3 position was determined by comparing the activities of compounds **4** and **7** with those of **3** and **6**. This location of the substituents decreased the potency.

The deamination of [^{3}H]tyramine, which was determined simultaneously with that of [^{14}C]-5-HT, was inhibited by the same compounds which inhibited the deamination of 5-HT. The order of activity was similar, but for most compounds higher doses had to be given in order to produce 50% inhibition. The lower inhibitory potency could be explained by the fact that the B form of MAO which contributes to the deamination of tyramine was poorly inhibited by the new compounds. The 2,6-dichloro derivative **9** was, however, about equally active on both substrates but was a poor inhibitor of the deamination of phenethylamine, whereas the halogen-free derivative **2** had the highest selectivity for 5-HT. These findings indicate that in mouse brain slices the deamination of 5-HT and tyramine differ in some respects, due either to differences in the location of the deamination process (neuronal or extraneuronal) or to differences in the enzyme forms (subgroups of the A form) taking part in the deamination.

The reversibility of the MAO inhibition was examined by recording the MAO activity 16 h after the injection of compound **5**. At this time no significant inhibition was observed, whereas clorgyline and pargyline had marked effects (Table IV).

Clorgyline, (−)-α-ethyltryptamine, and pargyline were included in the study as reference compounds. As shown in Table III the most active of the new compounds (**9**, **6**, and **5**) were more potent than, or at least as active as, clorgyline and considerably more potent than (−)-α-ethyltryptamine. Pargyline was much less active than most of the new compounds and was slightly more potent with tyramine as the substrate.

Increasing the substrate concentration 100-fold to 1×10^{-5} M did not markedly change the ED_{50} values of the new compounds in inhibiting the deamination of 5-HT (Table V). This somewhat unexpected behavior of reversible inhibitors might be due to the fact that at the low substrate concentration 5-HT is largely accumulated in the 5-HT neurons by the high affinity 5-HT uptake.[21] The high concentration of 5-HT is, on the other hand, far above the concentration at which this uptake mechanism is saturated. At this concentration the main part of 5-HT reaches the cells by diffusion and since MAO-A probably is located both intra- as well as extraneuronally, the substrate concentration might be rather similar to that in the 5-HT neurons at the low medium concentration. The deami-

Table III. Biochemical and Pharmacological Screening

Compd	MAO inhibn,[a] ED_{50}, μmol/kg ip		Inhibn of active [^{14}C]-5-HT uptake,[b] ED_{50}, μmol/kg ip	Potentiation of 5-HTP,[c] ED_{50} (95% conf limits), μmol/kg ip			Potentiation of tryptamine,[d] ED_{50} ± SEM, μmol/kg		Reserpine antagonism,[e] ED_{50}, μmol/kg ip	Toxicity, LD_{50}, μmol/kg ip
	[^{14}C]-5-HT	[^{3}H]Tyramine		Head twitches	Tremor	Abduction	Tremor	Abduction		
1	>45 (8%)	>45 (22%)	>45 (0%)	52 (29-91)	48 (25-95)	131[f]	75 ± 17	64 ± 11	11	2464
2	14	80	56	2.0 (1.6-2.8)	5.2 (4.0-6.8)	4.8 (3.6-6.8)				498
3	23	75	>75 (30%)	1.4 (0.8-2.4)	3.4 (2.5-5.1)	5.2 (3.3-8.4)	14 ± 5	9 ± 2	21	415
4	>38 (10%)	>38 (20%)	>38 (0%)	1.9 (1.1-3.4)	3.4 (2.3-15)	11 (6-21)	55	>55	>75	490
5	8	12	>35 (24%)	1.1 (0.7-1.4)	1.8 (1.4-2.1)	15 (9-24)	6.3 ± 2.5	>35	7	210
6	7	13	>61 (45%)	0.20 (0.15-0.36)	0.9 (0.6-0.9)	2.1 (1.2-3.0)	8.2 ± 2.1	3.0 ± 1.5	9	152
7	22	>30 (40%)	>30 (30%)	2.7 (1.5-5.1)	1.5 (1.2-1.8)	3.0 (2.4-3.6)	36 ± 6	12 ± 8	30	318
8	10	22	74	0.28 (0.20-0.40)	0.39 (0.26-0.58)	0.6 (0.4-0.9)	1.2 ± 0.4	1.5 ± 0.4		465
9	3	3	>31 (5%)	0.3 (0.2-0.6)	0.9 (0.6-1.2)	0.6 (0.4-0.9)	2.2 ± 0.3	1.6 ± 0.3	1.6	37
10	18	20	>80 (20%)	4.8 (2.8-8.4)	14 (12-18)	14 (11-16)	22 ± 6	16 ± 3	10	517
11	>38 (40%)	>38 (41%)	>38 (7%)	1.5 (0.8-2.6)	2.3 (1.5-3.4)	1.1 (0.8-2.3)	18 ± 4	13 ± 5	15	434
12	>36 (28%)	>36 (34%)	>36 (17%)	7.9 (5.7-10.4)	13 (11-17)	12 (8-17)	>36	>36	24	403
13	>50 (0%)	>50 (0%)	>50 (0%)	>36	>36	>36	>25	>25	>50	228
14	15	42	35	1.0 (0.3-2.7)	9.0 (6.7-11)	10 (7-14)	1.7 ± 0.4	1.9 ± 0.4	14	290
16	>38 (31%)	>38 (23%)	>38 (36%)	8.3 (5.3-12.8)	55	94[f]	22 ± 7	21 ± 7	>75	471
17	18	53	>67 (29%)	3.3 (2.0-5.0)	6.3 (4.7-9.0)	12 (8-17)	9.7 ± 2.3	6.0 ± 2.0	43	500
19	>34 (11%)	>34 (8%)	>34 (18%)	>34	>34	>34	>68	>68	>68	358
21	>72 (40%)	>72 (8%)	>72 (4%)	6.4 (4.6-9.3)	10.4 (7.9-14.1)	22 (13-38)	>36	36	>72	133
22	>64 (10%)	>64 (20%)	>129 (0%)	>32	>32	>32	>32	32	>65	258
Clorgyline	10	13	>33 (0%)	1.4 (0.8-2.4)	4.5 (3.5-5.8)	5.6 (4.3-7.1)	5.2 (3.9-6.8)	2.9 (2.3-3.9)	5.5	1136
Pargyline	153	123	>255 (0%)	50 (37-66)	91 (76-107)	168 (153-188)	122 (101-147)	93 (73-119)	189	2296
(−)-α-Ethyltryptamine	24	81	>81 (33%)	3.6 (2.2-6.4)	18 (14-23)	68 (41-110)	55 (36-83)	28 (25-32)	40	706

[a] The mice were sacrificed 1 h after the administration. Brain slices were incubated with 1×10^{-7} M [^{14}C]-5-HT and [^{3}H]tyramine for 5 min at 37 °C and the deaminated products and the amines with medium and slices were determined. The ED_{50} values were calculated from dose-response curves based on at least four different doses and with four animals in each. [b] The active 5-HT uptake was defined as that sensitive to 3×10^{-4} M cocaine. The percent inhibition at the highest dose tested is given in parentheses. [c] The compounds were injected ip 1 h prior to 90 mg/kg ip of 5-HTP. [d] The compounds were injected ip 1 h before tryptamine (50 mg/kg ip). The values are means ± SEM of ED_{50}. [e] Reserpine, 2.5 mg/kg ip, was injected 1 h after the test compounds and the motor activity recorded 1 h later. [f] Extrapolated value.

Table IV. Reversibility of MAO Inhibition 16 h after the Injection

Compd	Dose, μmol/kg ip	n	MAO inhibn, % ± SEM 5-HT	Tyramine
5	17	4	0	0
Clorgyline	32	4	61 ± 1	52 ± 1
Pargyline	255	4	60 ± 1	62 ± 1

Table V. Inhibition of MAO at 1×10^{-5} M Concentration of [^{14}C]-5-HT and [^{3}H]Tyramine

Compd	ED_{50}, μmol/kg ip[a] [^{14}C]-5-HT	[^{3}H]Tyramine
5	5	28
6	8	30
8	9	16
9	2	31
Clorgyline	3	4
(−)-α-Ethyltryptamine	56	148
Pargyline	168	162

[a] The mice were killed 1 h after injection.

nation of tyramine was, on the other hand, markedly changed, since most of the compounds were not able to inhibit more than about 50–60% of the total deamination of tyramine at the high substrate concentration. The explanation of this finding is probably that tyramine at this concentration is deaminated to the same degree by both the A and B forms. The ED_{50} values given in Table V correspond accordingly to a considerably larger inhibition of the deamination by MAO-A. At the low substrate concentration the deamination by the A form dominated, since tyramine is partly accumulated in monoaminergic neurons.

Inhibition of 5-HT Uptake. All compounds examined were rather poor inhibitors of the uptake of [^{14}C]-5-HT in mouse brain slices (Table III). The α-ethyl derivative **14** was the most active, followed by the 4-dimethylamino (**2**) and 4-ethylamino (**10**) derivatives.

Potentiation of the 5-Hydroxytryptophan Syndrome. 5-Hydroxytryptophan (5-HTP) produces a syndrome in mice characterized by head twitches, tremor, and abduction of the hind legs.[22] This syndrome is potentiated by MAO inhibitors and by inhibitors of the 5-HT uptake. This syndrome is accentuated primarily by central inhibition of MAO, since 5-HT has a low rate of penetration into the brain. Several of the new compounds were very potent in potentiating this syndrome (Table III). The head twitches were with one exception (**11**) potentiated at lower doses than the tremor and abduction of the hind legs. The doses potentiating 5-HTP induced head twitches were much lower than those producing 50% inhibition of the deamination of 5-HT in the brain slices. This discrepancy could have different explanations. One is that the in vivo–in vitro technique employed for determination of the MAO inhibition may underestimate the activity. Another is that 5-HTP is potentiated at a rather low degree of MAO inhibition. A third possibility is that the compounds are nonuniformly distributed in the brain, resulting in different degrees of inhibition in different regions. The most active of the new compounds were those with a halogen at the 2 position (**5**, **6**, and **8**). As in the test for MAO inhibition, the 2,6-dichloro derivative **9** was the most potent compound in this series. The substitution of the 4-amino group was found to be critical for high potency. The primary amine **1** had low activity whereas the dimethylamino-substituted derivative **2** had high potency. A significant relationship between the potentiation of the 5-HTP syndrome and MAO inhibition was obtained in the Spearman rank test. Thus, inhibition of the 5-HT deamination was significantly related to the potentiation of head twitches ($r_s = 0.82$, $p < 0.01$), tremor ($r_s = 0.76$, $p < 0.01$), and abduction ($r_s = 0.72$, $p < 0.01$). Inhibition of the deamination of tyramine was also significantly related to the 5-HTP syndrome: head twitches ($r_s = 0.79$, $p < 0.01$), tremor ($r_s = 0.72$, $p < 0.01$), and abduction ($r_s = 0.68$, $p < 0.01$).

Among the reference compounds α-ethyltryptamine had rather low activiity in potentiating the tremor and abduction of the hind legs, which indicates that this compound may have different distributions to the various neuron systems.

Potentiation of the Central Effects of Tryptamine. Tryptamine causes tremor and abduction of the hind legs in mice which are potentiated in combination with a MAO inhibitor. These effects are probably of central origin. Both central and peripheral MAO inhibition contributed to the effect in contrast to that in the 5-HTP test, since tryptamine is able to pass into the brain. Peripheral MAO inhibition will accordingly result in an elevated tryptamine concentration in the brain. The results of the screening of the compounds in this test are shown in Table III. The structural requirements for the potentiation of tryptamine differed from those for the 5-HTP potentiation, since a halogen or an alkyl group at the 2 position was necessary for activity within the dose ranges examined. In contrast to the 5-HTP test both the primary amine **1** and the dimethylamino derivative **2** had low activity. The most potent compounds were those with a halogen at the 2 position (**8**, **14**, **6**, and **5**) and the 2,6-dichloro derivative **9**. In the Spearman rank test there was significant correlation between inhibition of 5-HT deamination and potentiation of tryptamine (tremor $r_s = 0.85$, $p < 0.01$; abduction $r_s = 0.90$, $p < 0.01$). Similarly, good correlation was obtained for inhibition of the deamination of tyramine and potentiation of tryptamine (tremor $r_s = 0.81$, $p < 0.01$; abduction $r_s = 0.84$, $p < 0.01$).

The observation that the requirements for potentiation of tryptamine were more rigorous than for potentiation of 5-HTP is interesting. One possible explanation of this finding is that the potentiation of tryptamine is dependent on inhibition both peripherally and in the brain while for the potentiation of 5-HTP only inhibition in the brain is important. An alternative explanation is that the A form of MAO, which deaminates tryptamine, may differ from that deaminating 5-HT. Like tyramine, tryptamine is also deaminated by the B form,[6] but since the new compounds did not inhibit the deamination of phenethylamine it seems unlikely that inhibition of the B form contributed to the result. Further study will reveal if the new MAO inhibitors may differentiate between potential subgroups of the A form of MAO.

Potentiation of the Central Stimulatory Effect of Phenethylamine. By inhibition of peripheral (liver) MAO activity, pargyline and other inhibitors of MAO-B hinder the deamination of injected phenethylamine which accordingly can reach the brain. In mice phenethylamine combined with such a MAO inhibitor produces an amphetamine-like central stimulatory effect. None of the most potent new MAO inhibitors potentiated phenethylamine at 50 μmol/kg ip nor did clorgyline and α-ethyltryptamine. These findings are consistent with the view that these compounds are not B type inhibitors. Pargyline, on the other hand, caused a marked potentiation at 51 μmol/kg ip, in agreement with its effect on B type MAO.

Table VI. Effects on Aggressive Behavior and Locomotor Activity in Mice

Compd	Inhibn of aggression, ED_{50}, μmol/kg ip	Threshold dose for central stimulation, μmol/kg ip
1	<11	90
2	14	20
3	6	>75
4	7	>75
5	11	70
6	6	60
7	23	4
8	6	60
9	22	>62[a]
10	4	>80
11	>19	>75
12	21	>72
13	25	>50
14	>33	>67
16	>38	>75
17	>33	>67
19	>34	>68
21	27	36
22	>32	>65
4-Chloroamphetamine	2	6
Diazepam	8	
Clorgyline	<30	16-32
Pheniprazine	<53	13

[a] Toxic dose, convulsion and tremor.

Reserpine Antagonism. MAO inhibitors antagonize the decrease in motor activity induced by reserpine in mice. The effect of the new compounds was in this respect significantly correlated to the inhibition of the brain MAO activity (5-HT $r_s = 0.63$, $p < 0.05$; tyramine $r_s = 0.60$, $p < 0.05$). However, an exception was compound **1**, which was much more active in this test than could be expected from the MAO inhibition.

Central Stimulatory Effect. The potential central stimulatory effect of amphetamine-like type was determined by observation of the behavior of the mice during the first 2 h after the injection. The lowest doses producing a significant central stimulation are give in Table VI. The 3-bromo derivative **7** produced marked central stimulation. A few compounds (**2** and **8**) had slight stimulatory effects, but none of the new compounds showed amphetamine-like stimulation with stereotypes, excitation, etc. The new compounds had, in general, much lower ability to evoke central stimulation than pheniprazine and clorgyline.

Antiaggressive Action in Isolated Male Mice. Several of the new compounds were very active in antagonizing the aggressive behavior in isolated male mice (Table VI). The most potent compounds (**10**, **8**, **6**, **4**, **3**, **5**, and **2**) were at least as active as diazepam but were less active than 4-chloroamphetamine. The low activity of compounds with two methyl groups in the α position (**16**, **17**, and **19**) indicates that the α-hydrogen atom is a requirement for potent antiaggressive effects. Among the most potent compounds **8**, **6**, **5**, and **2** caused inhibition of aggression in the dose range which blocked the deamination of 5-HT. Other potent compounds, e.g., **10**, **4**, and **3**, had an antiaggressive effect at doses lower than those producing MAO inhibition. This indicates that other factors besides MAO inhibition may contribute to the antiaggressive properties in this series of compounds. The poor effect of compound **9**, which was most potent in inhibiting MAO, is interesting, since it may indicate that MAO inhibition contributes little to the effect. However, in the Spearman rank test of the new compounds which were active in antagonizing the aggressive response, a slightly significant ($r_s = 0.42$, $p < 0.05$, $n = 13$) correlation to the inhibition of tyramine deamination was observed. No significant ($r_s = 0.234$, $p < 0.05$, $n = 13$) correlation to the inhibition of the deamination of 5-HT was obtained.

Toxicity. The most potent MAO inhibitor of the new compounds, the 2,6-dichloro derivative **9**, was much more toxic than the other compounds (Table III). The low toxicity of **1** is also noteworthy. No significant correlation between toxicity and inhibition of the deamination of 5-HT ($r_s = 0.16$, $p > 0.05$) and tyramine ($r_s = 0.05$, $p > 0.05$) was found in the Spearman rank test.

Experimental Section

Chemistry. The experimental results are presented in Tables I and II and the preparation of some of the compounds is illustrated in Scheme I. The yields quoted have generally been derived from a single experiment. The analyses were performed by the Department of Analytical Chemistry at the University of Lund, Sweden, and the melting points were determined in an electrically heated metal block, using calibrated Anschütz thermometers. Temperatures are given as °C. The chloride analyses and the equivalent weight determinations were carried out at the Astra Control Laboratories, Södertälje, Sweden.

Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

The NMR spectra were recorded with a Varian A-60A NMR spectrometer.

The 4-dialkylaminophenethylamines 2–6, 8, 9, and 11–14 and compounds 21 and 22 in Table I were prepared by the following general procedure. A solution of 0.05 mol of the corresponding 4-dialkylamino-β-alkyl-β-nitrostyrene in 100–200 mL of dry ether or tetrahydrofuran (**2**, **5**, **8**, **9**, and **12**) was added dropwise to a stirred mixture of 0.2 mol of lithium aluminum hydride in 200 mL of dry ether. The mixture was stirred and heated under reflux for 5 h. After the dropwise addition of 50 mL of a saturated sodium sulfate solution while stirring and cooling in ice–water, the mixture was filtered. The filtrate was dried over anhydrous sodium sulfate and the dihydrochloride was precipitated from the solution by the addition of ether saturated with hydrogen chloride. The salt was purified by recrystallization from ethanol–isopropyl ether or from ethanol–ethyl acetate (**13**).

The purification of compounds **2**, **4**, **11**, **12**, **21**, and **22** was effected by the filtration and evaporation of the reaction mixtures and distillation of the residual oils. The boiling points of the obtained amines were, in consecutive order, 88–91 °C (0.2 mm), 101–105 °C (0.5 mm), 120–123 °C (0.04 mm), 98–102 °C (0.3 mm), 135–140 °C (0.07 mm), and 82–86 °C (0.4 mmHg). The free amines were converted to the dihydrochlorides by dissolving the bases in ether and treating the solutions with an excess of dry hydrogen chloride. Recrystallization of the obtained precipitates yielded the pure salts.

A similar reduction of 0.05 mol of 4-acetamido-β-methyl-β-nitrostyrene in 150 mL of tetrahydrofuran with 0.3 mol of lithium aluminum hydride in 200 mL of ether yielded compound **10**. The amine was distilled [bp 97–100 °C (0.03 mmHg)] and converted to the dihydrochloride which was recrystallized from ethanol–isopropyl ether. The 4-dimethylamino-β-alkyl-β-nitrostyrenes **23–28** in Table II were prepared from the appropriate benzaldehydes as follows. A mixture of 0.2 mol of the aldehyde, 0.25 mol of nitroalkane, and 15 g of ammonium acetate in 100–200 mL of ethanol (**24**, **27**, and **28**) or 1-propanol was heated under reflux while stirring for 4, 2, 7, 24, 2, and 4 h, respectively. The mixture was then poured into 1.5 L of ice–water and the obtained precipitate was filtered off. The crude compound was purified by recrystallization from ethanol or ethanol–petroleum ether (**23**).

4-Dimethylamino-*o*-tolualdehyde. *N*,*N*-Dimethyl-*m*-toluidine (67.5 g, 0.5 mol) was dissolved in 250 mL of dimethylformamide, and 35 mL of phosphorus tribromide was added dropwise while stirring and cooling in water. The temperature was not allowed to rise over 50 °C. The mixture was heated for 1.5 h on a steam bath and poured into 1.5 L of an ice–water mixture. The solution was made alkaline with sodium hydroxide and the crude product which separated was filtered off and recrystallized from isopropyl ether: yield, 20.5 g (25%); mp 63–65 °C. A second recrystallization from the same solvent gave 14.6

g of the pure aldehyde, melting at 65–66 °C (lit.[15] mp 67 °C). The NMR spectrum of this compound was found to be identical with that of the compound prepared by the Vilsmeier reaction.[11] In addition, the melting point was undepressed on admixture of samples of the two preparations.

2-Bromo-4-dimethylaminobenzaldehyde. Phosphorus oxychloride (14.5 mL, 0.16 mol) was added dropwise while stirring and cooling to a solution of 31.6 g (0.16 mol) of *N,N*-dimethyl-3-bromoaniline in 46 mL of dimethylformamide. The mixture was heated on a steam bath for 2 h and poured into 1 L of an ice–water mixture. The solution was made alkaline with sodium hydroxide and the obtained precipitate was filtered off and washed with water. The crude product was recrystallized from aqueous ethanol: yield, 19.6 g (54%); mp 81–82 °C. Anal. ($C_9H_{10}BrNO$) C, H, Br, N, O.

Similarly prepared was **6-dimethylamino-*m*-tolualdehyde,** which was obtained by the addition of 55 mL (0.6 mol) of phosphorus oxychloride to a solution of 67.5 g (0.5 mol) of *N,N*-dimethyl-*p*-toluidine in 145 mL of dimethylformamide: yield, 29.0 g (36%); bp 128–130 °C (10 mmHg) [lit.[16] bp 138–142 °C (16 mmHg)]. Anal. ($C_{10}H_{13}NO$) C, H, N, O.

The addition of *N,N*-dimethyl-*p*-toluidine (0.5 mol) to a mixture of phosphorus oxychloride and dimethylformamide yielded 12.3 g (15%) of the product.

4-Ethylmethylaminobenzaldehyde was prepared by the addition of 67.5 g (0.5 mol) of *N*-ethyl-*N*-methylaniline in 150 mL of dimethylformamide to a mixture of 140 mL of phosphorus oxychloride (1.5 mol) and 220 mL of dimethylformamide: yield, 50.0 g (61%); bp 165–170 °C (10 mmHg) [lit.[15] bp 180–185 °C (20 mmHg)]; mp 37–38.5 °C (lit.[15] mp 44 °C). Anal. ($C_{10}H_{13}NO$) C, H, N, O.

4-(Dimethylamino)-6-methylisophthalaldehyde. This compound was prepared analogously to 4-ethylmethylaminobenzaldehyde: yield, 42.0 g (44%); mp 62–66 °C. The crude product was recrystallized from aqueous ethanol: yield, 29.2 g; mp 67–68 °C. Anal. ($C_{11}H_{13}NO_2$) C, H, N, O. The NMR spectrum of the compound shows the absence of ortho coupling, thus indicating that the two aromatic protons are in the para position to one another.

***N*-[α,α-Dimethyl-β-(2-chloro-4-dimethylaminophenyl)ethyl]acetamide (20).** Concentrated sulfuric acid (30 mL) was added dropwise to a stirred ice-cooled solution of 48.4 g (0.23 mol) of 3-chloro-4-(2,2-dimethylvinyl)-*N,N*-dimethylaniline in 50 mL of acetonitrile. The mixture was left overnight at room temperature and was then poured into 1 L of crushed ice and neutralized with concentrated ammonium hydroxide (pH 6). The crude compound was filtered off and washed with water: yield, 38.5 g; mp 110–120 °C. Recrystallization from ethyl acetate–ligroine yielded 21.6 g of of the pure compound.

***N*-[α,α-Dimethyl-β-(4-dimethylaminophenyl)ethyl]acetamide (18)** was similarly obtained from 30.6 g (0.18 mol) of 4-(2,2-dimethylvinyl)-*N,N*-dimethylaniline, 9.2 mL of acetonitrile (0.18 mol), 18.5 mL of concentrated sulfuric acid, and 140 mL of acetic acid. The mixture was heated at 70 °C for 1 h and poured into ice. The compound was recrystallized from ethanol–ligroine.

2-Chloro-4-dimethylamino-α,α-dimethylphenethylamine Dihydrochloride (17). A solution of 21.6 g of compound **20** in 100 mL of concentrated hydrochloric acid and 50 mL of water was refluxed for 16 h. The solution was evaporated under reduced pressure and the residue was recrystallized from ethanol–isopropyl ether: yield, 5.0 g.

A similar hydrolysis of amide **18** yielded 4-dimethylamino-α,α-dimethylphenethylamine dihydrochloride (**16**).

2-Chloro-4-dimethylamino-α-isopropylbenzyl Alcohol (29). A solution of 104 mL (1.1 mol) of isopropyl bromide in 100 mL of ether was added dropwise with stirring to 24.0 g (1.0 mol) of magnesium turnings in 500 mL of ether 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 86.0 g (0.47 mol) of 2-chloro-4-dimethylaminobenzaldehyde was added in portions. The reaction mixture was heated under reflux for 1 h and left overnight at room temperature. Then 200 mL of concentrated hydrochloric acid in 100 mL of water was added dropwise with vigorous stirring and cooling in ice–water. The mixture was diluted with 2 L of water, neutralized with concentrated ammonium hydroxide (pH 7), and extracted with ether. The combined extracts were washed with water and dried over sodium sulfate. The ether was removed and the residue was recrystallized from petroleum ether: yield, 86.6 g (81%); mp 51–52.5 °C; neutral equivalent (determined by potentiometric titration with perchloric acid in acetic acid) calculated for $C_{12}H_{18}ClNO$ 227.74, found 228. Anal. ($C_{12}H_{18}ClNO$) C, H, N, O.

3-Chloro-4-(2,2-dimethylvinyl)-*N,N*-dimethylaniline (30). The compound was prepared by heating 60.0 g (0.26 mol) of 2-chloro-4-dimethylamino-α-isopropylbenzyl alcohol at 160 °C for 2 h. The crude product obtained (45.6 g, 84%) melted at 30–32 °C; neutral equivalent (determined by potentiometric titration with perchloric acid in acetic acid) calculated for $C_{12}H_{16}ClN$ 209.72, found 213.

4-Amino-α-methylphenethylamine Dihydrochloride (1). 4-Nitro-α-methylphenethylamine (4.35 g, 0.02 mol) was hydrogenated over 5% palladium on charcoal in 100 mL of water and 10 mL of concentrated hydrochloric acid at room temperature and atmospheric pressure. The catalyst was filtered off and the filtrate was evaporated. The residue was recrystallized from ethanol–isopropyl ether: yield, 3.2 g (72%); mp 270–271 °C (lit.[19] mp 270 °C).

3-Bromo-4-dimethylamino-α-methylphenethylamine Dihydrochloride (7). To a mixture of 2.51 g (0.01 mol) of 4-dimethylamino-α-methylphenethylamine dihydrochloride and 5.0 g of anhydrous sodium acetate in 50 mL of acetic acid, a solution of 0.51 mL (0.01 mol) of bromine in 50 mL of acetic acid was added dropwise with stirring. The mixture was stirred at room temperature for 2 h. The solvent was evaporated and the residue dissolved in 200 mL of water. The solution was made alkaline with sodium hydroxide and extracted with ether. The ether extract was dried over anhydrous sodium sulfate and acidified with hydrogen chloride in ether. The precipitated hydrochloride salt was filtered off and recrystallized from ethanol–ethyl acetate.

***N*-[α-Methyl-β-(2-chloro-4-dimethylaminophenyl)ethyl]acetamide (15).** A solution of 24.0 g (0.1 mol) of 2-chloro-4-dimethylamino-β-nitro-β-methylstyrene in 100 mL of dry tetrahydrofuran was added dropwise while stirring to 15.0 g (0.4 mol) of lithium aluminum hydride in 300 mL of dry ether. The mixture was stirred and refluxed for 2.5 h and 80 mL of saturated sodium sulfate solution was then added while stirring and cooling. The mixture was filtered, the filtrate dried with anhydrous sodium sulfate, and the solvent evaporated. To the residue 75 mL of acetic anhydride was added. The mixture was heated on a steam bath for 30 min. Excess acetic anhydride was evaporated and 500 mL of water was added to the residue. The mixture was neutralized with sodium hydroxide (pH 7) and the obtained crude acetamide was collected, dried, and recrystallized from ethanol–petroleum ether.

4-Dimethylamino-*N*-ethyl-α,α-dimethylphenethylamine Dihydrochloride (19). A solution of 13.2 g (0.056 mol) of *N*-[α,α-dimethyl-β-(4-dimethylaminophenyl)ethyl]acetamide (**18**) in 150 mL of dry tetrahydrofuran was added dropwise while stirring to 3.0 g (0.08 mol) of lithium aluminum hydride in 100 mL of ether. The mixture was refluxed for 5 h. After the addition of 15 mL of saturated sodium sulfate solution, the mixture was filtered. The filtrate was dried over sodium sulfate and acidified with hydrogen chloride in ether. The crude salt was filtered off and recrystallized from ethanol–isopropyl ether.

Pharmacology. MAO Inhibition. The deamination of [^{3}H]phenethylamine, [^{3}H]tyramine, and [^{14}C]-5-hydroxytryptamine by brain slices was determined according to a method which will be described in detail elsewhere. Slices (100 mg) of the central part of the mouse brain including hypothalamus, thalamus, and midbrain were incubated for 5 min with 1×10^{-7} M of the labeled substrate in 2.0 mL of Krebs–Henseleit's buffer, pH 7.4, containing 11.2 μmol of glucose. The slices were then homogenized in an all-glass homogenizer with 1.0 mL of 1 N HCl containing carrier amounts (1 μg/mL) of the nonlabeled amine and its deaminated acid product. An aliquot of 1.1 mL of a brain homogenate in 10 vol of 1 N HCl containing the substrate and product carriers was added to 0.5 mL of the incubation medium. The brain homogenate was added in order to facilitate the extraction of tyramine and 5-HT. The acid and neutral products were extracted into 6.0 mL of ethyl acetate by vigorous shaking for 10 s. After centrifugation, 4.0 mL of the organic phase was taken for de-

termination of radioactivity. The remaining ethyl acetate was removed; the water phase was made alkaline with solid sodium carbonate and 1.0 mL of 0.5 M borate buffer, pH 10. The amine was then extracted into 6.0 mL of ethyl acetate and 4.0 mL of the organic phase was taken for the assay. The deamination of [^{3}H]tyramine and [^{14}C]-5-HT was determined simultaneously by utilizing the double-labeling technique.[21] Inhibition of the MAO activity in the brain slices was determined 1 h after the intraperitoneal injection of the test compound. The inhibition was expressed in percent and calculated on the total formation of acid and neutral products formed in the brain slices of animals treated with the test compounds or with saline.

Inhibition of the active uptake of [^{14}C]-5-HT in the brain slices was determined simultaneously from the values obtained in the MAO determination. The active uptake was defined as the uptake sensitive to 3×10^{-4} M cocaine and the inhibition was expressed in percent of the corresponding uptake of [^{14}C]-5-HT in brain slices of the control animals.[22]

Potentiation of the 5-HTP syndrome in mice was determined as previously described.[23] The test compounds were injected 1 h prior to *dl*-5-HTP, 90 mg/kg iv.

Potentiation of tryptamine tremor and abduction of hind legs was recorded with the same technique as used for the 5-HTP syndrome. The test compounds were injected ip 1 h before tryptamine, 50 mg/kg iv, and the number of animals with tremor or abduction were noted within 0.5 h after the tryptamine injection. The dose producing this effect in 50% of the animals (ED_{50}) was determined by probit analysis based on at least four dose levels including five animals per dose level.

Potentiation of Phenethylamine. Phenethylamine (10 mg/kg ip) was given to mice pretreated with reserpine, 2.5 mg/kg ip, 16 h before the experiment. In combination with inhibitors of the B form of MAO, phenethylamine causes a rapid reversal of the reserpine sedation. The test compound was injected 1 h prior to phenethylamine and the animals were observed for reserpine reversal for a period of 30 min.

Motor Activity. The threshhold dose producing central stimulation within 2 h after ip injection was determined by observation in groups of four mice.

Reserpine Antagonism. The decrease in motor activity in mice 1 h after the injection of 2.5 mg/kg ip of reserpine was determined in a locomotion cage. The test compounds were injected ip 1 h prior to reserpine. The dose preventing the decrease in motor activity with 50% (ED_{50}) 1 h after reserpine was estimated from dose–response curves based on at least four doses with 10–12 animals per dose level.

Antiaggressive effect in isolated male mice was determined as described previously.[24] The ED_{50} values are based on at least three doses with five groups per dose level and determined from log dose–response curves.

Acute toxicity was assessed in mice observed for 24 h after ip injection. The LD_{50} values were determined from log dose–response curves based on at least five doses with five animals per dose level.

Statistical Methods. Correlations between MAO inhibition and the various behavioral tests employed were calculated by the Spearman rank correlation test.[25]

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Conformational Analysis of the Molecule Luteinizing Hormone-Releasing Hormone. 3. Analogue Inhibitors and Antagonists

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Conformational energy calculations have been carried out on analogues of luteinizing hormone-releasing hormone which have been shown to be potent inhibitors of the release of luteinizing hormone and follicle-stimulating hormone. The analogues included in this study have D-amino acid substitutions in the 2 and/or 3 positions, such as [D-X^2]-LH-RH, [D-X^2,D-Y$_3$]-LH-RH, [D-X^2,Pro^3]-LH-RH, and [D-X^2,Leu^3]-LH-RH. A configurational property which was common to the low-energy conformers of all the analogues is the directional change of the *cis*-peptide bond of the pyroglutamate ring. Further, there was no overall structural change in the analogues relative to the conformation of native LH-RH, and the orientation of the aromatic side chains relative to one another remained the same throughout this series of analogues.

In two previous papers[1,2] (referred to as papers 1 and 2) of this series, low-energy conformations of the molecule luteinizing hormone-releasing hormone (LH-RH), ∠Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, were obtained using "empirical" energy calculations. The low-energy structures found[1,2] were compared to available experimental data, and the effects of various analogues on the molecular conformation were discussed.[2] In this paper,

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