Experimental Section[5]

2,5-Dimethoxy-4-methyl-β-nitrostyrene.—A mixture of 5.4 g (30 mmoles) of 2,5-dimethoxy-*p*-tolualdehyde, 2.5 g of NH_4OAc, 25 ml of CH_3NO_2, and 25 ml of C_6H_6 was refluxed for 20 hr, during which time H_2O was azeotroped with a Dean–Stark tube. After cooling, the resulting solution was washed successively with H_2O (two 25-ml portions), saturated solution of $NaHSO_3$ (two 25-ml portions), and H_2O (two 25-ml portions). The C_6H_6 layer was dried (Na_2SO_4) and evaporated *in vacuo* leaving 6.0 g (90%) of yellow solid, mp 111–112°. Recrystallization from C_6H_6–C_7H_{16} (1:2) gave 5.3 g (79%), mp 118–119°. This melting point remained unchanged upon another recrystallization. *Anal.* ($C_{11}H_{13}NO_4$) C, H, N.

2,5-Dimethoxy-4-methyl-β-phenethylamine (4).—To a stirred suspension of 3.0 g (80 mmoles) of $LiAlH_4$ in 50 ml of THF was added a solution of 4.4 g (18 mmoles) of 2,5-dimethoxy-4-methyl-β-nitrostyrene in 50 ml of THF. The mixture was refluxed for 1 hr, cooled in ice, and treated with a mixture of H_2O and THF to decompose excess $LiAlH_4$. The resulting mixture was filtered and the filter cake was extracted with THF. The combined THF solution was evaporated *in vacuo* leaving 3.7 g of oily product. A solution of this oil in 25 ml of Et_2O was treated with Et_2O–HCl to precipitate 3.4 g (83%) of the hydrochloride salt, mp 200–203°. Recrystallization from EtOH gave 1.8 g, mp 212–213°. Addition of Et_2O to the filtrate yielded 750 mg, mp 211–213°. The total yield was 62%. *Anal.* ($C_{11}H_{18}ClNO_2$) C, H, N.

In a separate run distillation of free amine yielded 59% of a liquid, bp 95–105° (0.15 mm), $n^{23}D$ 1.5385.

2,5-Dimethoxy-N,N,4-trimethyl-β-phenethylamine (6).—To 14.0 g (0.3 mole) of formic acid, cooled in ice–H_2O, was added dropwise 3.0 g (0.016 mole) of 2,5-dimethoxy-4-methylphenethylamine (4), then 3.6 g (0.12 mole) of formalin in 10-ml portions. The mixture was refluxed for 5 hr. After cooling to room temperature, 7 ml of concentrated HCl was added and the resulting solution was evaporated *in vacuo* leaving an oil. This oil was dissolved in 25 ml of H_2O and extracted with $CHCl_3$ (two 25-ml portions). The aqueous layer was made basic with 2 *N* NaOH and extracted with Et_2O (three 25-ml portions). The Et_2O extracts containing the product was concentrated to about 25 ml. Addition of Et_2O–HCl to this solution precipitated the amine hydrochloride, yield 2.2 g (55%), mp 165–167°. Recrystallization from EtOH–Et_2O gave 1.7 g (42%), mp 168–169°. *Anal.* ($C_{13}H_{22}ClNO_3$) C, H, N.

2,5-Dimethoxy-N,4-dimethyl-β-phenethylamine (5).—A mixture of 5.8 g (30 mmoles) of 2,5-dimethoxy-4-methyl-β-phenethylamine (4), 4.2 g (40 mmoles) of benzaldehyde, and 15 ml of C_6H_6 was refluxed for 30 min and then subjected to distillation until the temperature reached 100°. To the remaining viscous liquid was added slowly a solution of 5.4 g (40 mmoles) of Me_2SO_4 in 20 ml of C_6H_6. The mixture was first heated until the reaction began. For several minutes no further heat was applied; then the mixture was refluxed for 30 min. Next, 20 ml of water was added and refluxing was continued for an additional 30 min. The aqueous phase was separated, extracted with C_6H_6 (three 25-ml portions), made basic with 2*N* NaOH, and again extracted with C_6H_6 (three 25-ml portions). The combined C_6H_6 extracts were dried (Na_2SO_4) then evaporated *in vacuo*. Distillation of the residue gave 4.9 g (79%) of product, bp 96–99° (0.075 mm), $n^{25}D$ 1.5278. When a solution of this product in 50 ml of Et_2O was treated with Et_2O–HCl, a hydrochloride salt precipitated, yield 5.3 g (72%), mp 150–151°. Recrystallization from EtOH gave 4.4 g (60%), mp 150–151°. *Anal.* ($C_{12}H_{20}ClNO_2$) C, H, N.

2,5-Dimethoxy-N,4-dimethylamphetamine (2).—The procedure was the same as described for the preparation of 2,5-dimethoxy-N,4-dimethyl-β-phenethylamine (5). The free amine was obtained in a 73% yield, bp 79° (0.075 mm) to 82° (0.05 mm), $n^{26}D$ 1.5210. When a solution of this product in Et_2O was mixed with Et_2O–HCl, the hydrochloride salt separated as an oil at first and then solidified; yield 60%, mp 122–123°. For purification, the hydrochloride salt was dissolved in a small amount of EtOH and slowly precipitated with Et_2O. In this fashion pure **2**, mp 125–126°, was obtained in 46% yield. *Anal.* ($C_{13}H_{22}ClNO_2$) C, H, N.

(5) Melting points were taken on a Mel-Temp apparatus and are corrected. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

Pharmacology. Conditioned Behavioral (VI) Test.—Adult male Sprague–Dawley rats were trained to press a lever in an operant conditioning chamber on a variable-interval 2-min (vi 2′) schedule of food reinforcement. A 45-mg Noyes pellet was delivered to the animal following each lever press on an average of every 2 min. This procedure produces a stable base line of responses from day to day. The animals were maintained on 22 hr of food deprivation and run daily for 1 hr. Immediately prior to each test session, two animals were given randomly assigned intraperitoneal doses of each compound in aqueous solution. The effect on performance was determined by calculating the per cent change in total response from the predrug session using the following formula: % change = [(predrug-drug)/predrug] × 100. Test sessions were given following 2 days of 10% or less change in performance. Dose–response relationships were obtained for each compound by averaging the results for the two animals. The dose which produced a 50% decrease in response rate (ED_{50}) was extrapolated from these curves.

Effect on Barbiturate Sleeping Time.—Mice were injected intraperitoneally with 50 μmoles/kg of compounds in 30% propylene glycol. After 5 min, sodium pentobarbital (40 mg/kg) in saline was given *via* the same route. Controls were first given 30% propylene glycol then pentobarbital in saline. The presleeping time and sleeping time (loss of righting reflex) were recorded and treated statistically.

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Substituted Quinazolonephenoxyethylhydrazines as Monoamine Oxidase Inhibitors[1a]

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The effectiveness of the chain length in inhibiting the enzyme monoamine oxidase (MAO) was reflected by the pronounced inhibition observed with phenoxyalkylhydrazines having two or four CH_2 groups as compared to those possessing three, five, or six CH_2 groups.[2] Furthermore, anticonvulsant properties exhibited by quinazolones[3] and various MAO inhibitors[4] led us to synthesize substituted quinazolonephenoxyethylhydrazines (Table I) and to determine their ability to inhibit MAO.

All quinazolonephenoxyethylhydrazines were found to inhibit MAO activity of isolated rat liver mitochondria during oxidative deamination of tyramine by rat liver homogenate using kynuramine as the substrate (Table II). The use of cyanide and semicarbazide during manometric determination of MAO activity[5] was avoided in experiments using tyramine as the substrate since O_2 uptake has been shown to reflect true enzyme activity in washed mitochondrial prepara-

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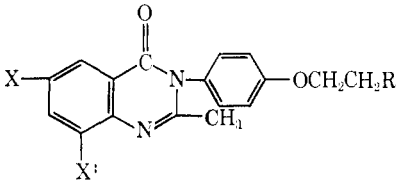
TABLE I

SUBSTITUTED QUINAZOLONES

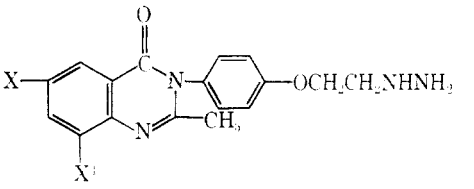


R	X	X′	Mp, °C[a]	Yield, %	Formula[b]
Br	H	H	161–163	50	$C_{17}H_{13}BrN_2O_2$
Br	Cl	H	98–100	55	$C_{17}H_{14}BrClN_2O_2$
Br	Br	H	159–160	60	$C_{17}H_{14}Br_2N_2O_2$
Br	I	H	172–175	65	$C_{17}H_{14}IBrN_2O_2$
Br	Cl	Cl	187	50	$C_{17}H_{13}BrCl_2N_2O_2$
Br	Br	Br	125–127	50	$C_{17}H_{13}Br_3N_2O_2$
$NHNH_2$	H	H	141–143	45	$C_{17}H_{18}N_4O_2$
$NHNH_2$	Cl	H	158–160	50	$C_{17}H_{17}ClN_4O_2$
$NHNH_2$	Br	H	178–180	50	$C_{17}H_{17}BrN_4O_2$
$NHNH_2$	I	H	180	60	$C_{17}H_{17}IN_4O_2$
$NHNH_2$	Cl	Cl	225	55	$C_{17}H_{16}Cl_2N_4O_2$
$NHNH_2$	Br	Br	163	52	$C_{17}H_{16}Br_2N_4O_2$

[a] Melting points were taken in open capillary tubes and are graphically corrected. [b] All compounds were analysed for C, H, and N, and analyses were found within limits.

TABLE II

MONOAMINE OXIDASE INHIBITION BY QUINAZOLONEPHENOXYETHYLHYDRAZINES



X	X′	Tyramine[a] % inhib (3×10^{-4} M)	Tyramine[a] I_{50}, concn (1×10^{-4} M)	Kynuramine[b] % inhib (3×10^{-4} M)
H	H	25.2	7.5	46.6
Cl	H	64.3	1.5	75.5
Br	H	76.3	0.61	88.0
I	H	76.0	0.7	85.5
Cl	Cl	80.7	0.6	91.2
Br	Br	81.5	0.5	90.1

[a] Per cent inhibition was calculated from the decrease in O_2 uptake. Washed rat liver mitochondria equivalent to 250 mg of fresh tissue were used in each Warburg vessel in a total volume of 3 ml containing 66 mM phosphate buffer, pH 7.4, and 10 mM tyramine and the inhibitors were present in the side arm. The inhibitors were incubated with the enzyme preparation for 10 min before the addition of the substrate. The enzyme system was further incubated at 37° for 1 hr under O_2. [b] Per cent inhibition was calculated from the decrease in the optical density. Assay procedure and the contents of the incubation mixture are as described in the Experimental Section. Each experiment was done in duplicate and the values are the averages of three separate experiments. Figures in parentheses indicate final concentration of the inhibitors.

tions.[6] The degree of MAO inhibition was found to increase significantly by the introduction of a substituent at position 6 of the quinazolone nucleus during oxidative deamination of either tyramine or kynuramine (Table II). Further increase in the degree of MAO inhibition was observed with compounds possessing substituents at both positions 6 and 8 of the quinazolone nucleus. Such a pattern of enzyme inhibition was also reflected by the I_{50} values determined for these inhibitors using tyramine as the substrate. The relative electronegativity of the halogen substituent attached at position 6 of the quinazolone nucleus was found to have no effect on the degree of enzyme inhibition. These quinazolonephenoxyethylhydrazines were found to be weak inhibitors of MAO as compared to tranylcypromine and β-phenylisopropylhydrazine.[6]

Experimental Section

Anthranilic acids were synthesized according to the methods reported in the literature: 5-chloroanthranilic,[7] 5-bromoanthranilic,[8] 5-iodoanthranilic,[9] 3,5-dichloroanthranilic,[7] and 3,5-dibromoanthranilic acid.[8]

Acetanthranils, synthesized by refluxing a mixture of an appropriate anthranilic acid (1 mole) and 2 moles of Ac_2O,[10] were used without further purification.

Quinazolonephenoxyethyl Bromides.—Quinazolones were synthesized by heating equimolar proportions of appropriate acetanthranil and *p*-aminophenoxyethyl bromide[11] as reported earlier.[10] The various quinazolonephenoxyethyl bromides were characterized by their sharp melting point and elemental analyses (Table I).

Quinazolonephenoxyethylhydrazines.—A mixture of an appropriate quinazolonephenoxyethyl bromide (1 mole) and 2.5 moles of $NH_2NH_2 \cdot H_2O$ (99-100%) in absolute EtOH was refluxed for 15–20 hr.[10] After distilling excess EtOH the hydrazines which separated out on cooling were filtered and recrystallized (EtOH). They are recorded in Table I.

Determination of Monoamine Oxidase Activity. (a) Manometric Method.—MAO activity of isolated rat liver mitochondria was determined by the conventional Warburg manometric technique using tyramine as the substrate.[6]

(b) Spectrophotofluorometric Method.—MAO activity of rat liver homogenate (5% w/v) in 0.25 M sucrose was determined spectrophotofluorometrically using kynuramine as the substrate.[12] The reaction mixture, in final concentration, consisted of 83 mM phosphate buffer (pH 7.4) and 0.2 ml of rat liver homogenate. Quinazolonephenoxyethylhydrazines (3×10^{-4} M), kynuramine (100 μg), and H_2O were added to adjust the final volume to 3 ml. The reaction was conducted in test tubes at 37° for 30 min. After incubation 2 ml of 10% trichloroacetic acid (w/v) was added to each tube and the precipitated proteins were removed by centrifugation. Suitable aliquots of the supernatant were assayed for 4-hydroxyquinoline fluorometrically in an Aminco Bowman spectrophotofluorometer using activation light of 310 mμ and measuring fluorescence at the maximum of 380 mμ.[12] Increase in optical density provided a direct measurement of 4-hydroxyquinoline as an index of kynuramine utilization and consequently of MAO activity. Decrease in kynuramine utilization in the presence of inhibitors was used to calculate the degree of enzyme inhibition.

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