in the analysis of the isoniazid–pyridoxine hydrochloride (10:1) mixture is clearly demonstrated, with an accuracy of 0.17–0.30%.

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Selectivity of 4-Methoxyphenethylamine Derivatives as Inhibitors of Monoamine Oxidase

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Abstract □ It has been established that the oxidative deamination of tyramine by monoamine oxidase is inhibited by (±)-4-methoxy-β-hydroxyphenethylamine and its *N*-methylated derivatives. This particular series of compounds does not inhibit the action of monoamine oxidase when tryptamine is used as the substrate. In contrast, 4-methoxyphenethylamine and its *N*-methylated homologs inhibit the monoamine oxidase-catalyzed deamination of both tyramine and tryptamine.

Keyphrases □ 4-Methoxyphenethylamine and *N*-methylated homologs—effect on monoamine oxidase-catalyzed deamination of tyramine and tryptamine □ Monoamine oxidase—deamination of tyramine and tryptamine, effect of 4-methoxyphenethylamine and *N*-methylated homologs □ Tyramine—monoamine oxidase-catalyzed oxidative deamination, effect of 4-methoxyphenethylamine and *N*-methylated homologs □ Tryptamine—monoamine oxidase-catalyzed oxidative deamination, effect of 4-methoxyphenethylamine and *N*-methylated homologs □ Enzymes—monoamine oxidase, deamination of tyramine and tryptamine, effect of 4-methoxyphenethylamine and *N*-methylated homologs □ Structure–activity relationships—4-methoxyphenethylamine and *N*-methylated homologs, effect on monoamine oxidase-catalyzed deamination of tyramine and tryptamine

4-Methoxy-β-hydroxyphenethylamine [1-(4-methoxyphenyl)-2-aminoethanol] has been detected in extracts of *Coryphantha cornifera* (DC.) Br. and R. var. *echinus* (Engelm.) L. Benson (1), while *Dolichothele longimamma* (DC.) Br. and R. was recently shown to contain *N*-methyl-4-methoxy-β-hydroxyphenethylamine [1-(4-methoxyphenyl)-2-(methylamino)ethanol] (2). These naturally occurring compounds, together with *N,N*-dimethyl-4-methoxy-β-hydroxyphenethylamine [1-(4-methoxyphenyl)-2-(dimethylamino)ethanol], have been found to inhibit the oxidative deamination of tyramine by monoamine oxidase (3). These data resulted from a screen used to correlate the pharmacological activity of cactus alkaloids and related compounds with the folkloric uses of various cacti.

Recently, it was noted that certain compounds inhibit the reaction of monoamine oxidase with tryptamine or serotonin but not with tyramine (4–7). The possibility of finding selective monoamine oxidase inhibitory activity prompted a reexamination of 4-methoxy-β-hydroxyphenethylamine and its *N*-methylated derivatives using tryptamine as the substrate.

N-Methyl-4-methoxyphenethylamine [1-(4-methoxyphenyl)-2-(methylamino)ethane] has been isolated from a number of cacti with potential psychoactivity (1, 8–12). This compound, as well as its *N*-methyl and *N*-demethyl derivatives, was tested for monoamine oxidase inhibitory activity using both tyramine and tryptamine as substrates. These studies revealed the significance of the β-hydroxy group in a series of phenethylamines with known monoamine oxidase inhibitory activity. In addition, the effects of the naturally occurring *N*-methyl-4-methoxyphenethylamine and its *N*-methyl homologs on monoamine oxidase were established.

EXPERIMENTAL

Synthesis—The synthesis of racemic 4-methoxy-β-hydroxyphenethylamine hydrochloride (I), *N*-methyl-4-methoxy-β-hy-

Table I—Inhibiting Effects of Compounds on Oxidation of Tyramine Hydrochloride and Tryptamine Hydrochloride by Rat Brain Mitochondria[a]

Compound	Tyramine Hydrochloride			Tryptamine Hydrochloride		
	Percent Inhibition	Significance	SD	Percent Inhibition	Significance	SD
I	59	+	1.6	7	—	3.4
II	70	+	1.5	17	—	3.8
III	61	+	1.4	16	—	4.3
IV	59	+	0.7	55	+	1.5
V	44	+	1.5	42	+	1.1
VI	47	+	1.2	64	+	0.9
Isocarboxazid[b]	100	+	1.5	67	+	1.0

[a] Each value represents the results obtained using three or more flasks per compound for each substrate. Percent inhibition was calculated by comparing control and experimental values for each compound with each substrate. [b] Reference.

droxyphenethylamine hydrochloride (II), and *N,N*-dimethyl-4-methoxy-β-hydroxyphenethylamine hydrochloride (III) was described previously (3).

4-Methoxyphenethylamine [1-(4-methoxyphenyl)-2-aminoethane] hydrochloride (IV) was available commercially[1] and served as the starting point for the synthetic production of the other two members of this series. *N*-Methyl-4-methoxyphenethylamine hydrochloride (V) was synthesized by refluxing equimolar quantities of 4-methoxyphenethylamine and ethyl formate[2] to give an *N*-formyl intermediate, which was subsequently reduced with lithium aluminum hydride. The free base resulting from this reaction was converted to a fluffy white hydrochloride, mp 181–182° [lit. (10) mp 182–184°].

N,N-Dimethyl-4-methoxyphenethylamine [1-(4-methoxyphenyl)-2-(dimethylamino)ethane] hydrochloride (VI) was produced by reacting V with 36% formaldehyde[3] followed by a sodium borohydride reduction of the Schiff-base intermediate. Conversion of the resulting colorless oil to the hydrochloride salt gave short white needles, mp 173–174° [lit. (13) mp 175–176°].

In each case, the identity of the final product was verified by IR and NMR spectra in addition to the melting-point data.

Warburg Studies—As an evaluation of possible monoamine oxidase inhibition, the effects of the compounds on the oxidation of tyramine and tryptamine hydrochlorides by rat brain mitochondria were investigated. Brain mitochondria (containing monoamine oxidase) were prepared from albino Wistar female rats, 150–200 g, according to the method of Brody and Bain (14). Monoamine oxidase activity was determined in the presence and absence of the respective compounds by conventional manometric techniques[4] (15).

In the manometric determinations, the main compartment of the Warburg flasks contained 0.8 ml of a 0.1 *M* buffered solution of the compound studied (or buffer), 1 ml of mitochondrial suspension (representing 500 mg wet weight of original brain tissue), and sufficient 0.01 *M* phosphate buffer (pH 7.4) to make a total volume of 2.7 ml. All aliquots for a given run were taken from the same mitochondrial preparation. The side arm of each flask contained 0.3 ml of 0.1 *M* tyramine or tryptamine hydrochloride. After the flasks were allowed to equilibrate for 15 min at 37°, the manometer valves were closed and the side arm contents of each were tipped in.

Manometers were set at 150 mm, and readings were taken at 15-min intervals for 90 min. Values for the 90-min reaction period were obtained for each flask and were multiplied by the respective flask constants after adjusting for changes in the thermobarometer. The total microliters of oxygen uptake for each flask was thus obtained.

Reference flasks, using 0.05 *M* isocarboxazid (0.8 ml) as the monoamine oxidase inhibitor, were employed as already described and were used for comparison.

Statistical Analysis—The mean and standard error were determined for each control or treated group. Values thus obtained were compared, using the difference between the means and the standard error of the difference between the means for the respective control-treated pairs of groups. The Student *t* test was applied to the results, and the probability was determined; *p* values of less than 0.05 were considered significant.

[1] Calbiochem.
[2] Eastman Kodak Co.
[3] Fisher Scientific Co.
[4] Precision Warburg apparatus, Precision Scientific Co.

RESULTS AND DISCUSSION

Direct measurements of monoamine oxidase inhibition using Warburg studies revealed a certain degree of selectivity among a series of 4-methoxyphenethylamine derivatives (Table I). Significant ($p < 0.05$) inhibition was observed for all compounds tested when tyramine was the substrate. However, with tryptamine as the substrate, only 4-methoxyphenethylamine and its *N*-methyl homologs (IV–VI) revealed significant inhibitory effects. These positive results were validated by the activity exhibited by reference isocarboxazid.

Substrate selectivity was observed with the 4-methoxy-β-hydroxyphenethylamine series (I–III) when these substances failed to inhibit the enzymatic oxidative deamination of tryptamine. These data suggest that rat brain monoamine oxidase possesses one active site for the oxidation of tyramine and another for tryptamine. An alternative explanation would involve the presence of an isozyme specific for each of the two substrates examined. Apparently, the presence of a β-hydroxy group in the 4-methoxyphenethylamine moiety interferes with the binding of these derivatives with the active site and/or isozyme responsible for the oxidation of tryptamine.

Mescaline is a well-known hallucinogenic substance and is responsible for the effects produced upon ingesting *Lophophora williamsii* (Lem.) Coult., the famed peyote cactus. However, many reputedly psychoactive cacti do not contain mescaline. It has always been difficult to substantiate the purported actions of these cacti with the known pharmacological properties of the isolated alkaloids. Based upon evidence presented in this paper, the monoamine oxidase inhibitory effects of some phenethylamines found in alleged mind-altering cacti may provide a partial explanation. The potency and selectivity of the monoamine oxidase-inhibiting phenethylamines, together with the cooccurrence of indirectly acting amines, might help to explain the long-term and short-term pharmacological effects of the cactus in question.

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