Biochemical Pharmacology, Vol. 25, pp. 1013–1020. Pergamon Press, 1976. Printed in Great Britain.

NEUROAMINE-DERIVED ALKALOIDS: SUBSTRATE-PREFERRED INHIBITORS OF RAT BRAIN MONOAMINE OXIDASE *IN VITRO*

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(*Received* 7 *August* 1975; *accepted* 10 *October* 1975)

Abstract The effects of tetrahydropapaveroline (THP), salsolinol (SAL) and various hydroxylated and methoxylated tetrahydroprotoberberine (THPB) alkaloids on monoamine oxidase (MAO) forms A and B in rat brain homogenates were investigated. The substrates utilized were serotonin, a specific substrate for type A MAO; tyramine, a substrate for both type A and B MAO; and benzylamine, a preferred substrate for type B MAO. The concentrations of THP, SAL, 2,3,9,10-tetrahydroxyberbine and 2,3,10,11-tetrahydroxyberbine producing 50 per cent inhibition (I_{50}) of the oxidation of serotonin were 1.0 mM, 0.25 mM, 0.24 mM and 0.04 mM, respectively. In marked contrast, the I_{50} concentrations of these alkaloids with benzylamine as substrate were 4.4 mM, 50 mM, 5.6 mM and 13 mM, respectively. These findings indicated that SAL and the tetrahydroxyberbines were substrate-preferred inhibitors of type A MAO whereas THP was a relatively nonspecific inhibitor of rat brain MAO. Kinetic data revealed that THP, SAL and 2,3,10,11-tetrahydroxyberbine inhibited the oxidation of serotonin in a typical competitive manner with apparent K_i values of 0.82 mM, 0.11 mM and 0.05 mM, respectively. THP and SAL noncompetitively inhibited benzylamine oxidation with apparent K_i values of 5.0 mM and 52 mM, respectively, while 2,3,10,11-tetrahydroxyberbine competitively inhibited the oxidation of benzylamine with an apparent K_i of 3.8 mM. Sequential replacement of the hydroxyl groups at the 2,3,9,10 and 11 positions of the berbine ring system by methoxyl groups substantially decreased the potency and selectivity of MAO inhibition. The interaction of these alkaloids with the metabolic pathways of neurotransmitters suggests that these compounds may be of relevance in the modification of central synaptic function.

Monoamine oxidase (MAO; monoamine: oxygen oxidoreductase (deaminating) EC 1.4.3.4.) has a wide tissue distribution and is inhibited by a large number of diverse compounds. Compounds of the hydrazide, hydrazine, propargylamine, cyclopropylamine, β-carboline, indolealkylamine and pyrimidine classes have been shown to be effective inhibitors of the enzyme [1, 2].

The multiplicity of monoamine oxidase has been rigorously investigated. Early studies demonstrated the separation of monoamine oxidases capable of metabolizing either aliphatic or aromatic monoamines exclusively [3]. Liver MAO from various species was found to metabolize a homologous series of monoamines at differing maximal velocities [4]. Based on kinetic inhibition studies of the oxidative deamination of serotonin and tyramine, it was concluded that these two substrates were metabolized by enzyme variants of MAO [5]. Partial separation of two fractions from rat liver mitochondria with varying MAO activities utilizing two substrates has been reported [6].

Some investigators have also postulated the existence of multiple forms of MAO because several bands of enzyme activity were separated by polyacrylamide gel electrophoresis [7–12]. These electrophoretically separable forms of MAO were differentiated by their thermostability [10, 11], substrate specificities [7, 10, 12] and inhibitor sensitivities [10, 11]. Other studies, employing substrate-selective inhibitors, have contributed more information elucidating the multiplicity of MAO. Utilizing the substrate selective inhibitor clorgyline, Johnston [13] found that two forms of MAO (type A and type B) were present in rat brain. Clorgyline [14–16], Lilly-51641 [14] and harmaline [17] have been considered to be substrate selective inhibitors of MAO type A, while deprenyl [14, 15], imipramine [18], chlorpromazine [19], pargyline [17] and desmethylimipramine [18] were shown to be more effective inhibitors of the B form of MAO. Substrate preferences for MAO type A and B have also been described. Serotonin [13, 16], norepinephrine [20] and normetanephrine [21] were considered to be preferred substrates for MAO type A, while benzylamine [13, 16] and phenethylamine [15] were preferred substrates for MAO type B. Tyramine [13, 16], dopamine [21] and tryptamine [21] were found to be substrates common to both enzyme forms.

Yang and Neff [15] demonstrated that types A and B MAO enzyme activities could be partially separated by continuous sucrose density gradient centrifugations. Additionally, McCauley and Racker were able to distinguish between types A and B MAO on the basis of differing antigenic properties [22]. The neural distribution of types A and B MAO has also received much attention. Equal proportions of types A and B MAO were found in various regions of brain [23]. Type A MAO was shown to be the predominant form in sympathetic nerves [24, 25] and superior cervical ganglion [20, 26], while type B MAO was the major form associated with the cells of the pineal gland [26].

Evidence has been accumulating indicating the capability of mammalian systems to elaborate alkaloids of the tetrahydroisoquinoline, benzyltetrahydroisoquinoline and tetrahydroxyberbine classes under certain circumstances. Salsolinol, the condensation product of dopamine and acetaldehyde, has been demonstrated to form in rat liver and brain homogenates during ethanol and dopamine metabolism [27]. Collins and Bigdeli [28] have also reported formation of salsolinol in brains of pyrogallol-treated rats during acute ethanol intoxication. The tetrahydroisoquinoline alkaloids formed by direct condensation of norepinephrine or epinephrine with acetaldehyde have been demonstrated in acetaldehyde perfused adrenal glands [28, 29]. Biosynthesis of tetrahydropapaveroline, a benzyltetrahydroisoquinoline alkaloid arising by condensation of dopamine with the aldehyde derivative of this parent amine, has been demonstrated *in vitro* [30–33] and *in vivo* in experimental animals [30] and in man [34]. It has been shown that tetrahydropapaveroline can be converted metabolically to tetrahydroprotoberberine alkaloids in rat liver and brain preparations by an *S*-adenosylmethionine-dependent enzyme [35, 36]. Furthermore, tetrahydroprotoberberine alkaloids have been identified as urinary excretion products in rats after administration of tetrahydropapaveroline and in parkinsonian patients receiving L-dopa therapy [37].

These neuroamine-derived alkaloids exert diverse pharmacological activities [38–57]. Because of their structural similarity to the putative neurotransmitters, the alkaloids might be expected to inhibit enzymes that metabolize biogenic amines. Initial observations in our laboratory [58] indicated inhibitory effects of salsolinol and tetrahydropapaveroline on brain MAO. Therefore, the major thrust of this present investigation was to examine the possible inhibitory effects of various tetrahydroisoquinoline, benzyltetrahydroisoquinoline and tetrahydroprotoberberine alkaloids on the multiple forms of rat brain monoamine oxidase, types A and B. To lend further insight into the nature of inhibition produced by these neuroamine-derived alkaloids, substrate-preferential inhibition and kinetic parameters were also examined.

MATERIALS AND METHODS

Reagents. Serotonin creatinine sulfate and tyramine hydrochloride were purchased from Calbiochem, La Jolla, Calif. (±) Salsolinol hydrobromide, (±) tetrahydropalmatine (2,3,9,10-tetramethoxyberbine) and benzylamine were purchased from Aldrich Chemical Company, Milwaukee, Wis. (±) Tetrahydropapaveroline hydrobromide was a gift from Wellcome Research Laboratories, Beckenham, Kent, England. Clorgyline (M and B 9302), *N*-methyl-*N*-propargyl-3-(2,4-dichlorophenoxy)-propylamine hydrochloride, was supplied by May and Baker Ltd., Dagenham, England. Deprenyl (E-250), 1-phenyl-2-(*N*-methyl-*N*-propargyl)-aminopropane hydrochloride, was supplied by Dr. J. Knoll, Semmelweiss University, Budapest, Hungary. The radioactive chemicals, 5-hydroxytryptamine binoxalate [2-^{14}C] and tyramine [1-^{14}C]hydrobromide, were purchased from New England Nuclear Corporation, Boston, Mass., and benzylamine [7-^{14}C]hydrochloride was obtained from California Bionuclear Corporation, Sun Valley, Calif. All other alkaloids (racemic mixtures) used in this study were synthesized in our laboratory. The purity and structure of these alkaloids were evaluated by liquid chromatography, gas chromatography and mass spectrometry. The structures of the inhibitor compounds used in this study are represented in Fig. 1.

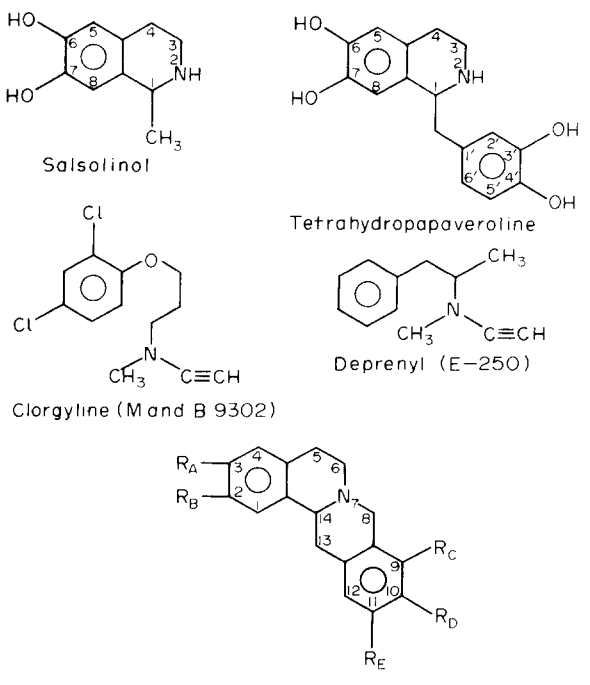

Fig. 1. Structures of inhibitors used.

Preparation of rat brain homogenates. Male albino Sprague–Dawley (TEX/SDD) rats from Texas Inbred Mice Company, Houston, Tex. were used. Rats weighing between 200–250 g were decapitated. The brains were rapidly removed and rinsed in chilled 0.1 M sodium phosphate buffer, pH 7.4. A 10% (w/v) homogenate was prepared with twelve strokes of a teflon pestle in a glass homogenizer in ice-cold 0.1 M sodium phosphate buffer, pH 7.4. The resultant homogenate was used for all subsequent enzymatic assays.

Monoamine oxidase assay. MAO activity was monitored by a radioisotopic assay as previously described [14]. Radiolabeled [^{14}C]serotonin, [^{14}C]tyramine and [^{14}C]benzylamine were used as substrates. The incubation mixtures consisted of 50 μmoles sodium phosphate (pH 7.4), enzyme preparation (3–4 mg protein), 0.5 μmoles of the particular [^{14}C]labeled substrate (sp. act. 0.1 μCi/μmole), various concentrations of selected inhibitors and distilled water in a reaction volume of 1.0 ml. Reaction blanks contained no substrate during the incubation. Reaction mixtures were preincubated for 10 min before addition of the substrate. Following a 30-min incubation at 37°, 0.3 ml of 2 N HCl was added to terminate the reaction. Substrate was then added to the blank reaction mixtures. The deaminated metabolites were extracted into 7 ml of toluene, 4-ml aliquots of the organic layer were removed and counted in 11 ml of scintillation fluid. The liquid scintillation mixture consisted of 333 ml of Triton-X-100, 667 ml of toluene, 0.2 g of 1,4-bis-[2-(5-phenyl-oxazolyl)]-benzene (POPOP) and 4 g of 2,5-diphenyloxazole (PPO). Radioactivity was determined with a Packard Tri-Carb Spectrometer. MAO activity was expressed as a percentage of the control sample that contained no inhibitor. The percentage inhibition was then calculated and plotted against the negative log of inhibitor concentration.

Determination of apparent K_m *and* K_i *values.* Five substrate concentrations (covering a 20–35-fold range and not exceeding 7.0×10^{-4} M), each in duplicate, were included for each determination of K_m with proper blanks maintained at the various concentrations. The data were plotted in single reciprocal form as described by Hofstee [59]. K_i values were calculated from averages of two inhibitor concentrations.

Protein determination. Protein concentrations relative to bovine serum albumin standards were determined by a biuret method [60].

RESULTS

Substrate selective inhibition of MAO. Plots of percentage inhibition of MAO activity with serotonin, tyramine and benzylamine as substrates vs concentration of various selected inhibitors are shown in Fig. 2. A simple sigmoidal curve characterized clorgyline's inhibition of serotonin oxidation by MAO, whereas a double sigmoidal curve (with a plateau) was obtained when tyramine was substrate. Since tyramine is assumed to be a substrate common to both

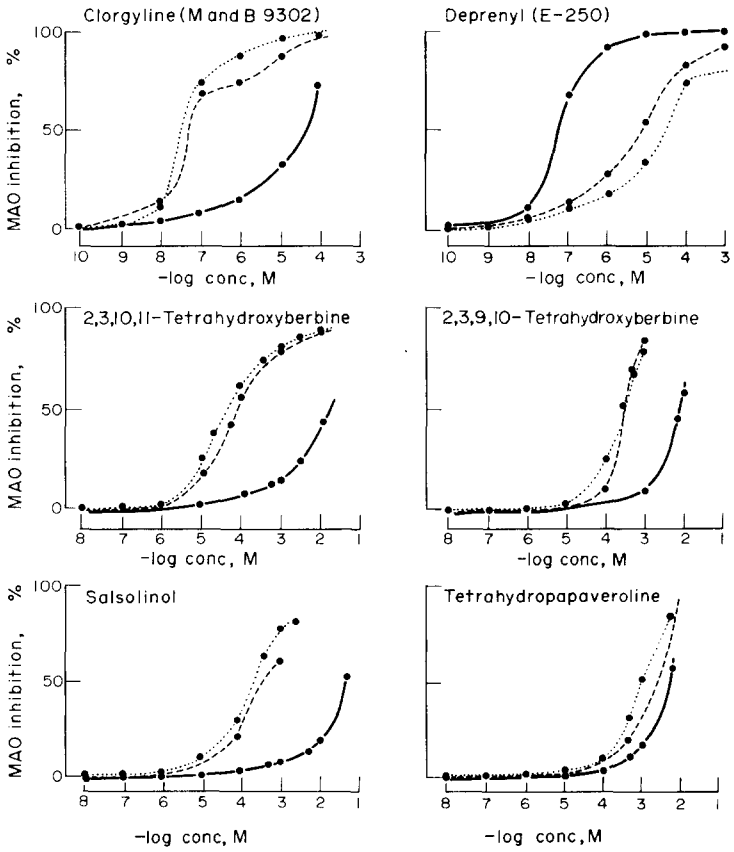

Fig. 2. Effects of various inhibitor compounds on rat brain MAO activity with 0.5 mM serotonin (· · · · · ·), tyramine ( ) or benzylamine ( ) as substrates.

forms of monoamine oxidase [13, 16], the first portion of the double sigmoidal curve presumably represents inhibition of MAO type A, and the second portion of the double sigmoidal curve indicates inhibition of MAO type B. Benzylamine oxidation was much less inhibited by clorgyline. Deprenyl, on the other hand, strongly inhibited the oxidation of benzylamine at concentrations which had little effect on the oxidation of serotonin. These results are in full agreement with the results reported by other investigators [14-16] and provide the model for characterizing the substrate selective inhibitory actions of the various neuroamine-derived alkaloids studied.

In experiments with serotonin or tyramine as substrates, the inhibition of MAO followed a simple sigmoidal curve where deamination was inhibited by a low concentration of 2,3,10,11-tetrahydroxyberbine, 2,3,9,10-tetrahydroxyberbine and salsolinol. On the other hand, the oxidation of benzylamine was relatively insensitive to inhibition by these alkaloids. The order of the degree of substrate-selective inhibition was 2,3,10,11-tetrahydroxyberbine > salsolinol > 2,3,9,10-tetrahydroxyberbine. The inhibition curves obtained with these alkaloids are comparable to the inhibition curves produced by the type A MAO substrate-selective inhibitor, clorgyline. In marked contrast, the inhibition curves for tetrahydropapaveroline with the three substrates were characteristic of a non-substrate-selective inhibitor (Fig. 2). The I_{50} values derived from these data are shown in Table 1. The I_{50}-benzylamine/I_{50} serotonin reveal that the order of preferred inhibition of type A MAO was clorgyline > 2,3,10,11-tetrahydroxyberbine > salsolinol > 2,3,9,10-tetrahydroxyberbine. Deprenyl was a selective inhibitor of type B MAO, whereas tetrahydropapaveroline had little selectivity.

Kinetics of enzyme inhibition. Since a representation of percentage inhibition vs inhibitor concentration (Fig. 2) may not accurately represent the binding affinities of these alkaloids for the different forms of monoamine oxidase, K_m and K_i values were determined as well as inhibition type from graphic representation of kinetic data as described by Hofstee [59]. Apparent Michaelis constants of serotonin and benzylamine for rat brain MAO were determined. Under our assay conditions the amount of product formed was linear with protein concentration within the range utilized and duplicate assay systems were within 10% of one another with respect to recovered radioactivity.

The apparent K_m values for serotonin and benzylamine calculated from Hofstee plots were $1.3 \pm 1.0 \times 10^{-4}$ M and $2.3 \pm 1.2 \times 10^{-4}$ M, respectively. Michaelis constants of ^{14}C-labeled serotonin for MAO were determined in the presence of THP, SAL or 2,3,10,11-tetrahydroxyberbine and it was found that these alkaloids inhibited the oxidation of serotonin in a classical competitive manner (Fig. 3). The calculated apparent K_i values for these alkaloids with serotonin as substrate were 0.82 mM, 0.11 mM and 0.05 mM, respectively (Table 2).

Michaelis constants of ^{14}C-labeled benzylamine for MAO were also determined in the presence of THP, SAL or 2,3,10,11-tetrahydroxyberbine. THP and SAL inhibited the oxidation of benzylamine in a typical non-competitive manner, while 2,3,10,11-tetrahydroxyberbine competitively inhibited benzylamine oxidation with respective apparent K_i values of 5.0 mM, 52 mM and 3.8 mM (Fig. 4 and Table 2). The apparent K_i values and the inhibition type obtained by graphic treatment (Figs. 3 and 4) of the data on the deamination of serotonin and benzylamine in the presence of THP, SAL and 2,3,10,11-tetrahydroxyberbine are shown in Table 2.

Inhibition of serotonin, tyramine and benzylamine oxidation by selected tetrahydroprotoberberine analogs. Since 2,3,10,11-tetrahydroxyberbine and 2,3,9,10-tetrahydroxyberbine proved to be relatively potent inhibitors of rat brain MAO and exhibited substrate preferential inhibitory effects, their structural analogs were examined with respect to these parameters. The effects of the sequential replacement of ring hydroxyl groups at the 2,3,9,10 or 11 positions by methoxyl groups are shown in Table 3. Both 2,3,9,10- and 2,3,10,11-tetrahydroxyberbine exhibited substrate-preferred inhibition of serotonin oxidation (MAO type A) compared to benzylamine oxidation (MAO type B). These compounds at 1 mM concentrations inhibited serotonin oxidation approximately 80%, while only approximately 12% inhibition was observed when benzylamine was utilized as substrate. As seen with 2,3,11-trihydroxy-10-methoxyberbine and 2,9-dihydroxy-3,10-dimethoxyberbine, the potency and also the selectivity of inhibition declined when one or two methoxyl groups were substituted for hydroxyl groups. The partially methoxylated derivatives

Table 1. Substrate-preference of selected monoamine oxidase inhibitors

Inhibitor	Molar concentration producing 50% inhibition (I_{50}) of oxidation of: Serotonin	Tyramine	Benzylamine	Ratio* I_{50}-Benzylamine / I_{50}-Serotonin
Tetrahydropapaveroline	1.0×10^{-3}	2.4×10^{-3}	4.4×10^{-3}	4.4
Salsolinol	2.5×10^{-4}	5.0×10^{-4}	5.0×10^{-2}	200.0
2,3,9,10-Tetrahydroxyberbine	2.4×10^{-4}	2.5×10^{-4}	5.6×10^{-3}	23.3
2,3,10,11-Tetrahydroxyberbine	4.0×10^{-5}	6.3×10^{-5}	1.3×10^{-2}	325.0
Deprenyl	2.0×10^{-5}	7.9×10^{-1}	7.9×10^{-8}	0.00039
Clorgyline	3.5×10^{-8}	6.0×10^{-5}	5.6×10^{-5}	1600.0

Concentrations of inhibitor compound producing 50% inhibition of the oxidation of the substrate by rat brain homogenates were obtained from the curves of inhibitor concentration vs % inhibition of MAO. Assay conditions are described in text.

* Ratio represents the concentration of a particular inhibitor producing 50% inhibition of MAO utilizing benzylamine as substrate divided by concentration of the same inhibitor producing 50% inhibition of MAO utilizing serotonin as substrate.

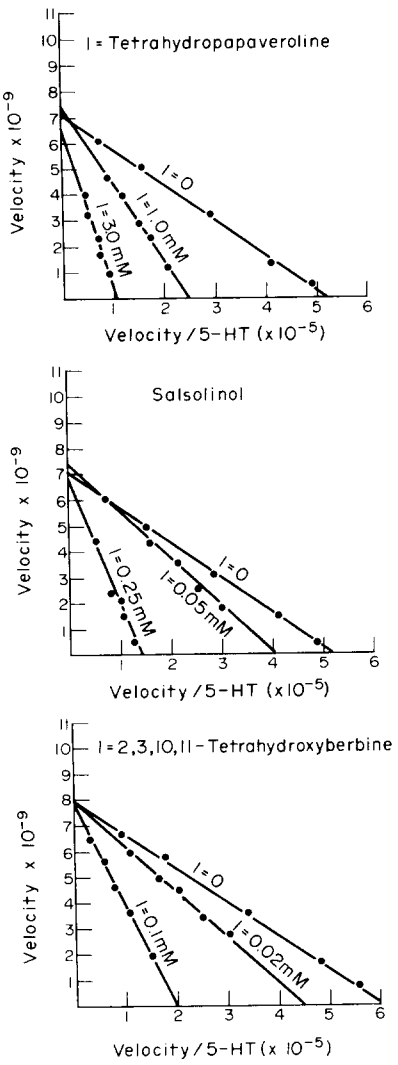

Fig. 3. Hofstee single reciprocal plots depicting inhibition of MAO type A by differing concentrations of tetrahydropapaveroline, salsolinol and 2,3,10,11-tetrahydroxyberbine with serotonin (5-HT) as the variable substrate. Velocity is expressed as nmoles serotonin oxidized/min/mg protein. The ordinate intercept is V_{max}, the abscissa intercept is V_{max}/K_m and the slope is $-K_m$.

2,3,11-trihydroxy-10-methoxyberbine and 2,9-dihydroxy-3,10-dimethoxyberbine, inhibited serotonin oxidation 45% and 43% and benzylamine oxidation by 39% and 21%, respectively, at a concentration of 1 mM. Inhibitor potency further declined and lack of substrate-preferred inhibition was evident on replacement of three or four of the hydroxyl groups by methoxyl groups as exemplified by 2-hydroxy-3,10-11-trimethoxyberbine and 2,3,10,11-tetramethoxyberbine.

DISCUSSION

Metabolism of the biogenic amines such as serotonin, norepinephrine and dopamine in brain tissue involves deamination by monoamine oxidase to the corresponding aldehydic derivatives [61, 62]. The aldehyde formed may be oxidized to the corresponding acid via NAD^+-dependent aldehyde dehydrogenases [63, 64] or reduced to the alcohol metabolite by NADPH or NADH-dependent aldehyde reductases [65, 66].

A further metabolic transformation of biogenic amines has been described by Holtz *et al.* [67]. These workers found that incubation of dopamine with guinea pig liver mitochondrial preparations of monoamine oxidase led to the formation of tetrahydropapaveroline (THP). THP is a benzyltetrahydroisoquinoline alkaloid formed by condensation of 3,4-dihydroxyphenylacetaldehyde with the unchanged parent amine.

Additional studies have shown that tetrahydropapaveroline may be metabolized by catechol-*O*-methyltransferase to its corresponding methoxylated derivatives [58] or may be converted enzymatically to various tetrahydroprotoberberine alkaloids by a benzyltetrahydroisoquinoline methyltransferase [36, 37]. Tetrahydropapaveroline, tetrahydroprotoberberines and salsolinol have been shown to be formed in mammalian systems under certain circumstances [27–37]. Various studies have shown these alkaloids to possess differing pharmacological properties. Tetrahydropapaveroline, for example, has been reported to have β-sympathomimetic properties and to produce hypotensive effects among other pharmacological actions [38–45]. The tetramethoxylated protoberberines possess sedative, tranquilizing, and analgesic properties

Table 2. Inhibition of serotonin and benzylamine oxidation by tetrahydropapaveroline, salsolinol and 2,3,10,11-tetrahydroxyberbine

Substrate	Inhibitor	Inhibition Type*	$K_i \times 10^3$ M
Serotonin	Tetrahydropapaveroline	Competitive	0.82
Serotonin	Salsolinol	Competitive	0.11
Serotonin	2,3,10,11-Tetrahydroxyberbine	Competitive	0.05
Benzylamine	Tetrahydropapaveroline	Non-competitive	5.0
Benzylamine	Salsolinol	Non-competitive	52.0
Benzylamine	2,3,10,11-Tetrahydroxyberbine	Competitive	3.8

Rat brain homogenates were used for determination of the kinetic constants which were determined from the graphic representation of the data as described in the text. All values are the average of three separate determinations performed in duplicate. Assay conditions are described in text.

* Inhibition type was determined from the graphic representation of data described by Hofstee [59].

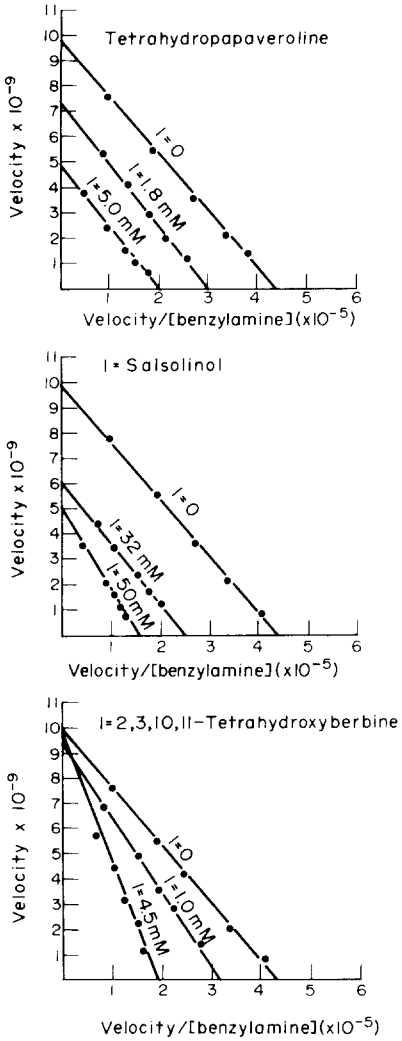

Fig. 4. Hofstee single reciprocal plots depicting inhibition of MAO type B by differing concentrations of tetrahydropapaveroline, salsolinol and 2,3,10,11-tetrahydroxyberbine utilizing benzylamine as the variable substrate. Velocity is expressed as nmoles benzylamine oxidized/min/mg protein. The ordinate intercept is V_{max}, the abscissa intercept is V_{max}/K_m and the slope is $-K_m$.

[46–51]. Salsolinol and its structural analog, 6,7-dihydroxytetrahydroisoquinoline, have been the target of many pharmacological studies as well. Representative effects include vasoconstriction, synergistic action with epinephrine, inhibition of catecholamine uptake and enhanced release of catecholamines from storage sites [52 56]. Recent reports indicate that tetrahydropapaveroline and certain tetrahydroprotoberberines inhibit catecholamine uptake in synaptosomal preparations [53, 56, 57].

This study demonstrates that tetrahydropapaveroline, salsolinol and tetrahydroprotoberberbines exert inhibitory effects upon rat brain monoamine oxidase. Johnston [13] suggested that there are two forms of MAO in rat brain designated as type A and type B based on an *in vitro* study. Apparently MAO type A and B have specific functions in brain. Type A MAO metabolizes the neurotransmitters serotonin, norepinephrine and normetanephrine, while type B MAO metabolizes compounds such as benzylamine and phenethylamine.

The findings of experiments utilizing clorgyline as a substrate-preferred inhibitor of type A MAO and deprenyl as a substrate-preferred inhibitor of type B MAO are in accord with data obtained in other laboratories [13 16]. Additionally, our results indicate that SAL, 2,3,10,11-tetrahydroxyberbine and 2,3,9,10-tetrahydroxyberbine are substrate-preferred inhibitors of type A MAO. On the other hand, the inhibitory effects produced by THP showed very little substrate preference. Since I_{50} ratios are not a true indication of inhibitor binding affinities, apparent K_i values for these alkaloids were used in assessing potency of the corresponding alkaloids as MAO inhibitors. The calculated apparent K_i values for the various alkaloids examined closely correlated to the I_{50} values, indicating that the I_{50} inhibitor values provided a reasonable estimation of the inhibitor binding affinities (Tables 1 and 2).

The present data indicate that the alkaloids, tetrahydropapaveroline, salsolinol and 2,3,10,11-tetrahydroxyberbine, are competitive inhibitors of serotonin oxidation. Although derived from the catecholamines and retaining certain molecular characteristics of the catecholamines, the intramolecular geometry of these alkaloids closely approximates the indole structure of serotonin. The findings of Tabakoff *et al.* [68] suggest that separate interacting sites may exist in brain MAO of intact mitochondria for the binding of phenyl- or catechol-substituted amines and for those possessing an indole nucleus. Therefore, the substrate-selective inhibition of serotonin oxidation exhibited by these neuroamine-derived alkaloids may be attributable to their competition with the indoleamine metabolizing sites in brain tissue.

Serotonin is generally accepted as a preferred substrate for brain MAO type A. The existence of a specific enzyme site which readily accepts the serotonin molecule has been postulated [69]. Both the amino-nitrogen and the 5-hydroxyl moieties are evidently necessary for substrate binding. For example, 5-methoxytryptamine, is not preferentially metabolized by MAO type A but is utilized by MAO type B [70]. As a direct analogy to these reports, the presence of the free hydroxyl groups of the berbine molecule along with the close resemblance to the indole nucleus of serotonin may be responsible for the preferred inhibition of serotonin oxidation (MAO type A) compared to benzylamine oxidation (MAO type B). Thus, it was found that sequential replacement of the hydroxyl groups at the 2,3,9,10 or 11 positions of the berbine ring system by methoxyl groups markedly attenuates the substrate selective inhibition as well as the potency of inhibition of MAO type A. Indeed, the completely methoxylated berbine, 2,3,9,10-tetramethoxyberbine, exhibited modest substrate selective inhibition of MAO type B (Table 3).

It has become evident that pharmacologic activity of alkaloid enantiomers may differ markedly, presumably due to steric requirements at 'receptor sites' [71 74]. Activity may reside solely in one optical isomer, or one enantiomer may be inactive or even negate the actions of the other. Since the *in vivo* elaboration of neuroamine-derived alkaloids may prefer-

Table 3. Inhibition of serotonin, tyramine and benzylamine oxidation by selected tetrahydroprotoberberine alkaloids

Inhibitor	% Inhibition of MAO activity Substrate		
	Serotonin	Tyramine	Benzylamine
2,3,10,11-Tetrahydroxyberbine			
0.1 mM	62	55	7
1.0 mM	82	80	13
2,3,9,10-Tetrahydroxyberbine			
0.1 mM	14	8	0
1.0 mM	79	88	11
2,3,11-Trihydroxy-10-methoxyberbine			
1.0 mM	45	39	39
2.5 mM	64	54	54
2,9-Dihydroxy-3,10-dimethoxyberbine			
1.0 mM	43	39	21
2.5 mM	73	53	26
2-Hydroxy-3,10,11-trimethoxyberbine			
1.0 mM	12	21	6
2.5 mM	34	39	23
2,3,10,11-Tetramethoxyberbine			
2.5 mM	27	33	22
5.0 mM	34	36	32
2,3,9,10-Tetramethoxyberbine			
2.5 mM	29	27	55
5.0 mM	32	35	60

Reaction mixture contained 50 μmoles sodium phosphate buffer (pH 7.4), enzyme preparation (3–4 mg protein), varying amounts of inhibitor, 0.5 μmoles of particular ^{14}C-labeled substrate (sp. act. 0.1 μCi/μmole), distilled water to a total volume of 1 ml and incubated at 37° for 30 min. Deaminated metabolites were determined as described in text.

entially result in generation of one specific optical isomer, the actual binding or dissociation constants of these alkaloids for enzyme systems or 'receptor sites' may vary according to the particular stereoisomer generated. Only racemic mixtures of the representative alkaloids were available for use in these experiments. Resolution of the optical isomers of these alkaloids is now underway in our laboratory and stereoselectivity of the pharmacologic activity of these alkaloids is being evaluated.

Although the K_i values obtained with racemic mixtures of the various alkaloids for MAO are in the millimolar range for benzylamine oxidation (MAO B), the corresponding K_i values for serotonin oxidation (MAO A) are in the low millimolar range and in the micromolar range for 2,3,10,11-tetrahydroxyberbine. It is of interest to note that the inhibitor potency of 2,3,10,11-tetrahydroxyberbine exceeds that of the tricyclic antidepressants, imipramine, doxepin, iprindole and amitriptyline, which share with this alkaloid the property of inhibiting MAO and neuronal reuptake of biogenic amines [18, 19, 57, 75–79]. It is relevant to note that *in vitro* homogenate preparations contain the basic cellular components but in a disrupted and diluted state and direct comparison between homogenates and *in vivo* situations is not possible. Thus, data generated with homogenates offer only an indication of potential *in vivo* effects.

While only small amounts of these neuroamine-derived alkaloids have been detected in brain tissue to date, the possibility exists that these alkaloids may be elaborated, under certain circumstances, in localized discrete brain structures yielding local concentrations of alkaloids capable of eliciting pharmacologic alterations in aminergic neurons. Therefore, production of neuroamine-derived alkaloids characterized in part by their capability to preferentially inhibit monoamine oxidase and to alter neuroamine uptake and release may be implicated in some of the pharmacologic and behavioral actions related to alcohol abuse [80] or L-dopa therapy in parkinsonianism [81–83].

Acknowledgement—This work was supported by USPHS Grant 5-R01-AA-00226 and the Veterans Administration.

REFERENCES

1. B. T. Ho, *J. pharm. Sci.* **61**, 821 (1972).
2. C. L. Zirkle and C. Kaiser, in *Psychopharmacological Agents* (Ed. M. Gordon), p. 472. Academic Press, New York (1964).
3. E. Werle and F. Roewer, *Biochem. Z.* **322**, 320 (1952).
4. G. A. Alles and E. V. Heegaard, *J. biol. Chem.* **147**, 487 (1943).
5. W. Hardegg and E. Heilbronn, *Biochim. biophys. Acta* **51**, 553 (1961).
6. V. Z. Gorkin, *Nature, Lond.* **200**, 77 (1963).
7. H. C. Kim and A. D'Iorio, *Can. J. Biochem.* **46**, 295 (1967).
8. G. G. S. Collins, M. B. Youdim and M. Sandler, *F.E.B.S. Lett.* **1**, 215 (1968).
9. J. D. Shih and S. Eiduson, *Nature, Lond.* **224**, 1309 (1969).
10. M. B. Youdim, G. G. S. Collins and M. Sandler, *Nature, Lond.* **223**, 626 (1969).
11. G. G. S. Collins, M. Sandler, E. D. Williams and M. B. H. Youdim, *Nature, Lond.* **225**, 817 (1970).
12. L. A. Sierens and A. D'Iorio, *Can. J. Biochem.* **48**, 659 (1970).

13. J. P. Johnston, *Biochem. Pharmac.* **17,** 1285 (1968).
14. A. J. Christmas, C. J. Coulson, D. R. Maxwell and D. Riddell, *Br. J. Pharmac.* **45,** 490 (1972).
15. H. Y. T. Yang and N. H. Neff, *J. Pharmac. exp. Ther.* **187,** 365 (1973).
16. D. W. R. Hall, B. W. Logan and G. H. Parsons, *Biochem. Pharmac.* **18,** 1447 (1969).
17. R. F. Squires, *Biochem. Pharmac.* **17,** 1401 (1968).
18. J. A. Roth and C. N. Gillis, *Biochem. Pharmac.* **23,** 2537 (1974).
19. J. A. Roth and C. N. Gillis, *Molec. Pharmac.* **11,** 28 (1974).
20. C. Goridis and N. H. Neff, *Br. J. Pharmac.* **43,** 814 (1974).
21. N. H. Neff and H. Y. T. Yang, *Life Sci.* **14,** 2061 (1974).
22. R. McCauley and E. Racker, *Molec. Cell. Biochem.* **1,** 73 (1973).
23. M. A. Schwartz, R. J. Wyatt, H. Y. T. Yang and N. H. Neff, *Archs gen. Psychiat.* **31,** 557 (1974).
24. C. Goridis and N. H. Neff, *Proc. Soc. exp. Biol. Med.* **140,** 573 (1972).
25. B. Jarrott, *J. Neurochem.* **18,** 7 (1971).
26. C. Goridis and N. H. Neff, *Neuropharmac.* **10,** 557 (1971).
27. Y. Yamanaka, M. J. Walsh and V. E. Davis, *Nature, Lond.* **227,** 1143 (1970).
28. M. A. Collins and M. G. Bigdeli, *Life Sci.* **16,** 585 (1975).
29. G. Cohen and M. A. Collins, *Science, N.Y.* **167,** 1749 (1970).
30. A. J. Turner, K. M. Baker, S. Algeri, A. Frigerio and S. Garattini, *Life Sci.* **14,** 2247 (1974).
31. M. J. Walsh, V. E. Davis and Y. Yamanaka, *J. Pharmac. exp. Ther.* **174,** 388 (1970).
32. V. E. Davis, M. J. Walsh and Y. Yamanaka, *J. Pharmac. exp. Ther.* **174,** 401 (1970).
33. P. Holtz, K. Stock and E. Westerman, *Nature, Lond.* **203,** 656 (1964).
34. M. Sandler, S. B. Carter, K. R. Hunter and G. M. Stern, *Nature, Lond.* **241,** 439 (1973).
35. L. R. Meyerson and V. E. Davis, *Fedn Proc.* **34,** 508 (1975).
36. L. R. Meyerson, K. D. McMurtrey, J. L. Cashaw and V. E. Davis, *Trans. Am. Soc. Neurochem.* **6,** 182 (1975).
37. V. E. Davis, J. L. Cashaw and K. D. McMurtrey, in *Advances in Experimental Biology and Medicine* (Ed. M. M. Gross), Vol. 59, p. 65. Plenum Press, New York (1975).
38. P. P. Laidlaw, *J. Physiol., Lond.* **40,** 480 (1910).
39. P. Holtz, K. Stock and E. Westerman, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **248,** 387 (1964).
40. R. Santi, A. Bruni, S. Luciani, C. E. Toth, M. Ferrari, G. Fassina and A. R. Contessa, *J. Pharm. Pharmac.* **16,** 287 (1964).
41. R. Santi, M. Ferrari, C. E. Toth, A. R. Contessa, G. Fassina, A. Bruni and S. Luciani, *J. Pharm. Pharmac.* **19,** 45 (1967).
42. W. R. Kukovetz and G. Pöch, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **256,** 301 (1967).
43. W. R. Kukovetz and G. Pöch, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **256,** 310 (1967).
44. J. L. McNay and L. I. Goldberg, *J. Pharmac. exp. Ther.* **151,** 23 (1966).
45. G. Fassina, C. E. Tóth and R. Santi, *Prog. Biochem. Pharmac.* **3,** 277 (1967).
46. H. Nakanishi, *Jap. J. Pharmac.* **12,** 208 (1962).
47. H. Nakanishi, *Jap. J. Pharmac.* **14,** 317 (1964).
48. H. Yamamoto, *Jap. J. Pharmac.* **13,** 230 (1963).
49. H. Higuchi, T. Matsuo, G. Nakatani and K. Shimamoto, *Jap. J. Pharmac.* **13,** 36 (1963).
50. B. Hsu and K. C. Kin, *Archs. int. Pharmacodyn. Ther.* **139,** 318 (1962).
51. B. Hsu and K. C. Kin, *Biochem. Pharmac.* **12,** (Suppl.), 101 (1963).
52. R. S. Greenberg and G. Cohen, *Eur. J. Pharmac.* **18,** 291 (1972).
53. R. Heikkila, G. Cohen and D. Dembiec, *J. Pharmac. exp. Ther.* **179,** 250 (1971).
54. G. Cohen, C. Mytilineou and R. E. Barrett, *Science, N.Y.* **175,** 1269 (1972).
55. V. M. Tennyson, G. Cohen, C. Mytilineou and R. Heikkila, *Brain Res.* **51,** 161 (1973).
56. S. Locke, G. Cohen and D. Dembiec, *J. Pharmac. exp. Ther.* **187,** 56 (1973).
57. H. S. Alpers, B. R. McLaughlin, W. M. Nix and V. E. Davis, *Biochem. Pharmac.* **24,** 1391 (1975).
58. A. C. Collins, J. L. Cashaw and V. E. Davis, *Biochem. Pharmac.* **22,** 2337 (1973).
59. B. H. J. Hofstee, *Nature, Lond.* **184,** 1296 (1959).
60. K. F. Tipton, *Biochim. biophys. Acta* **135,** 910 (1967).
61. H. Blaschko, D. Richter and H. Schlossmann, *Biochem. J.* **31,** 2187 (1937).
62. H. Blaschko, *Pharmac. Rev.* **4,** 415 (1952).
63. R. A. Deitrich, *Biochem. Pharmac.* **15,** 1911 (1966).
64. E. Racker, *J. biol. Chem.* **177,** 883 (1949).
65. B. Tabakoff and V. G. Erwin, *J. biol. Chem.* **245,** 3263 (1970).
66. R. A. Anderson, L. R. Meyerson and B. Tabakoff, *Brain Res.* in press.
67. P. Holtz, K. Stock and E. Westerman, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **246,** 133 (1963).
68. B. Tabakoff, L. R. Meyerson and S. G. A. Alivisatos, *Brain Res.* **66,** 491 (1974).
69. C. M. McEwen, F. Sasaki and W. R. Lenz, *J. biol. Chem.* **243,** 5217 (1968).
70. M. D. Houslay and K. F. Tipton, *Biochem. J.* **139,** 645 (1974).
71. H. Sheppard and C. R. Burghardt, *Res. Commun. chem. Path. Pharmac.* **8,** 527 (1974).
72. D. R. Feller, R. Venkatraman and D. D. Miller, *Biochem. Pharmac.* **24,** 1357 (1975).
73. R. F. Shonk, D. D. Miller and D. R. Feller, *Biochem. Pharmac.* **20,** 3403 (1971).
74. G. Cohen, R. E. Heikkila, D. Dembiec, D. Sang, S. Teitel and A. Brossi, *Eur. J. Pharmac.* **29,** 292 (1974).
75. J. A. Roth and C. N. Gillis, *Biochem. Pharmac.* **24,** 151 (1975).
76. J. A. Roth, *Life Sci.* **16,** 1309 (1975).
77. G. Hertting, J. Axelrod and L. G. Whitby, *J. Pharmac. exp. Ther.* **134,** 146 (1961).
78. J. Glowinski and J. Axelrod, *J. Pharmac. exp. Ther.* **149,** 43 (1965).
79. A. Carlsson, H. Corrodi, K. Fuxe and T. Hokfelt, *Eur. J. Pharmac.* **5,** 367 (1969).
80. S. W. French, in *The Biology of Alcoholism* (Eds. B. Kissin and H. Begleiter), Vol. 1, p. 437. Plenum Press, New York (1971).
81. A. Barbeau, *A. Rev. Pharmac.* **14,** 91 (1974).
82. S. Fahn, *Neurology* **24,** 431 (1974).
83. L. E. Claveria, D. B. Calne and J. G. Allen, *Br. med. J.* **2,** 641 (1973).