Journal of Pharmaceutical Sciences

JUNE 1972
VOLUME 61 NUMBER 6

REVIEW ARTICLE

Monoamine Oxidase Inhibitors

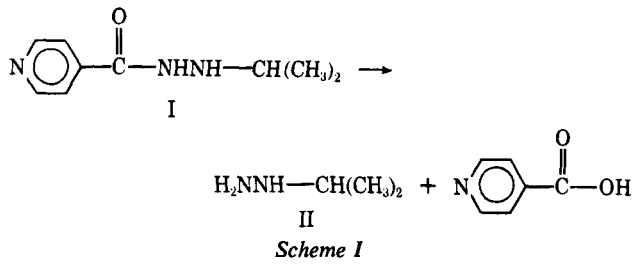
BENG T. HO

Keyphrases ☐ MAO inhibitors—review of recent research ☐ Hydrazides—as MAO inhibitors, review ☐ Hydrazines—as MAO inhibitors, review ☐ Propargylamines—as MAO inhibitors, review ☐ Cyclopropylamines—as MAO inhibitors, review ☐ Indolealkylamines—as MAO inhibitors, review ☐ Carbolines—as MAO inhibitors, review ☐ Pyridines—as MAO inhibitors, review

Monoamine oxidase [monoamine: O_2 oxidoreductase (deaminating); E.C. 1.4.3.4; MAO], having been discovered in 1928 (1), is one of the oldest classes of enzymes. Limited knowledge has been gained regarding its active site, although such possible prosthetic groups as flavin dinucleotide (2–11), copper (2, 8–10), and the sulfhydryl group (8, 9, 12–16) have been identified in mitochondrial MAO. Perhaps the difficulty in solubilizing the mitochondrial enzyme and the presence of several forms of isozymes in one organ account for this slow progress in MAO research. But despite the lack of understanding of the enzyme's properties, countless inhibitors of varying types have been discovered; and it is through the use of these inhibitors that the biochemical, pharmacological, and psychopharmacological role of the neurohormones, such as serotonin, norepinephrine, and dopamine, can be defined. Many of these inhibitors were once considered very beneficial in the treatment of mental depression.

Perhaps MAO is the enzyme with the largest number and widest variation of inhibitors. The wealth of work that has come from the study of this enzyme has resulted in several excellent review articles and monographs (17–32). It is my intention to supplement these articles and, therefore, to cover research on MAO inhibitors during the past 3–4 years. My emphasis will be on inhibitory activity toward the enzyme both *in vitro* and *in vivo*.

Confusion arises as one tries to evaluate the relative potency of MAO inhibitors of all types, because most of the inhibitory activities have been reported as the percentage of inhibition at a particular concentration of inhibitor. The use of such expressions as I_{50}, *i.e.*, the concentration of an inhibitor required to cause 50% inhibition of the enzyme activity, or pI_{50}, *i.e.*, the minus logarithm of I_{50}, lessens the confusion and seems to give adequate values. The values of I_{50} or pI_{50}, however, are comparable only within a particular assay system, since they vary depending on the concentration of the substrate. It is difficult to compare data from one article to data from another, unless the same system was employed in both cases. If the K_i value of an inhibitor is not determined, it would seem more appropriate to determine the I_{50} of a known and potent inhibitor of the same type, or even of a different type, for comparison. In addition, since the enzyme is capable of oxidizing substrates of various types, the inhibitory activity will undoubtedly vary with the substrate. The subject is further complicated by the earlier findings that the enzyme activity varies considerably according to the type of substrates, and some inhibitors either selectively inhibit only a limited fraction of the total enzyme activity or exert selectivity toward a particular tissue or organ (33–45). This, plus the recent and more direct experimental data obtained from ion-exchange chromatography (46), gel filtration (47), and electrophoresis (48–54), indicates that MAO may exist in an organ in several forms (isozymes). In comparing *in vivo* potency,



Scheme I

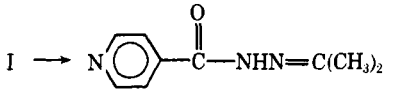
an important consideration should be the route of administration; it has been demonstrated that the extent of inhibition in an organ depends upon how the inhibitor is given (55, 56).

HYDRAZIDES

Ever since iproniazid[1] (1-isonicotinyl-2-isopropylhydrazine, I), an antituberculosis agent, was found to exert CNS stimulation (57), and later was shown to exert inhibitory effects on MAO (58, 59), tremendous numbers of hydrazines have been prepared and tested as inhibitors of the enzyme (19). To obtain maximum *in vitro* inhibition of MAO, preincubation of the enzyme with iproniazid was found to be necessary (60), indicating irreversible binding. It was proposed that iproniazid is first converted into an "active principle" before it inactivates the enzyme (61–64). Accordingly, the formation of isopropylhydrazine (II) (Scheme I) from hydrolysis prior to irreversible inhibition of MAO by iproniazid was suggested, and isopropylhydrazine was found to be a more potent inhibitor than iproniazid (65). This hypothesis was supported by the observation that iproniazid was relatively more active on brain MAO *in vivo* than *in vitro*, presumably because of the slow rate of hydrolysis to release isopropylhydrazine (42, 64). This possibility, however, was considered unlikely in view of the findings that both iproniazid and isopropylhydrazine had similar rates of inhibition and apparently similar energies of activation (65). Dehydrogenation of iproniazid to 1-isopropylidene-2-isonicotinoylhydrazine (III) (Scheme II) after the attachment of iproniazid molecules to the active site was regarded as another possibility (65).

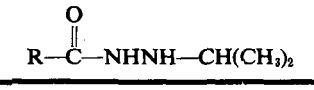
Kinetic studies showed that iproniazid was a competitive inhibitor of the enzyme from rat liver mitochondria and that the inhibitor was sensitive to pH changes and heat in a way similar to that observed in the oxidation of the substrate (tyramine), signifying its attachment to the active site of MAO (65). In contrast, McEwen *et al.* (66) reported that inhibition of purified enzyme from human mitochondria by iproniazid was noncompetitive with respect to the substrate veratrylamine.

In a medium containing cyanide (65, 67), the effect of iproniazid and alkylhydrazine on MAO was found to



Scheme II

Table I—Inhibition of Rat Liver Mitochondrial MAO by Isopropylhydrazides[a]



R	I_{50}, μM	—Relative Activity— *In Vitro*	*In Vivo*
	5.03	1.00	1.00
p-ClC_6H_4	5.59	0.89	1.34
p-HOC_6H_4	7.95	0.63	0.11
C_6H_5	15.90	0.31	0.33

[a] Wiley *et al.* (72); substrate: tyramine.

be enhanced, suggesting the cyanide either removes metal ions at the active site (65) or catalyzes the conversion of iproniazid into the active principle (68). The cyanide was also found to prevent the decrease of iproniazid-induced inhibition of mitochondrial MAO by glutathione; this finding implied the presence of a thiol group that could be responsible for binding iproniazid at the active site (65). Using 8-hydroxyquinoline, a metal-complexing agent, and *p*-chloromercuribenzoate, an agent reacting with the sulfhydryl group, Kurosawa (69) demonstrated that both the metal ions and the sulfhydryl group were situated at the same active site of the enzyme.

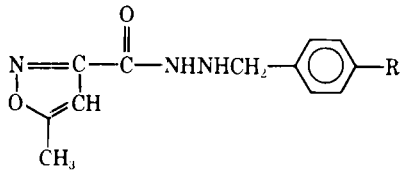
The exact mode of binding of hydrazines to MAO is still very speculative. Green (67) suggested that such metal ions as copper may be involved in the enzyme complex, causing the conversion of iproniazid into its active principle. The formation of the hydrazine–copper complex was evidenced by the change of UV absorption spectra of hydrazines when cupric ion was added (69). From the observation of Eberson and Persson (63) that the decomposition of substituted hydrazines by cupric ion produced free radicals, Green (67) deduced that the inhibition of MAO by hydrazines was the result of the formation of free radicals catalyzed by metal ions on the enzyme. Other investigators (68, 70) showed the cleavage of iproniazid and benzylhydrazine nonenzymatically to form unknown volatile substances which were powerful inhibitors of the enzyme. In summary, it is likely that the function of the cupric ion and the sulfhydryl group are both essential for the binding of iproniazid, and that the primary stage in the inhibitor's action consists of linking the inhibitor and the metal at the enzyme's active site (71).

Wiley *et al.* (72) reported the inhibitory activity of several benzoyl isopropyl hydrazides on the oxidation of tyramine by rat liver mitochondria. Substitution of a chlorine atom on the *para*-position of the benzene ring resulted in a nearly threefold increase in the *in vitro* and a fourfold increase in the *in vivo* activity; the activity of the inhibitor, *N*-*p*-chlorobenzoyl-*N'*-isopropylhydrazine, was comparable to that of iproniazid both *in vitro* and *in vivo* (Table I).

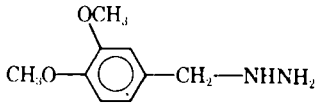
Isocarboxazid[2] (5-methyl-3-isoxazolecarboxylic acid 2-benzylhydrazide, IV) is known to be a less toxic and more potent inhibitor than iproniazid for the deami-

[1] Marsilid.

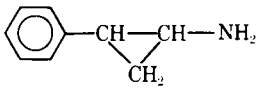
[2] Marplan.



IV: R = H
V: R = $N(CH_3)_2$



VI

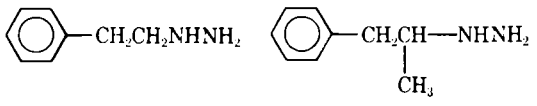


VII

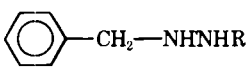
nation of tyramine (19, 73). Introduction of a dimethylamino group on the *para*-position of the benzene ring of IV yielded 1-(*p*-dimethylaminobenzyl)-2-(5-methyl-3-isoxazolylcarbonyl)hydrazine (Ro 5-1025, V), a short-acting MAO inhibitor (74–76). When tested for potentiation of 5-hydroxytryptophan stimulation, V was eight times more active than iproniazid but one-fourth as active as isocarboxazid; the duration of action of iproniazid and isocarboxazid was 25 days but that of V was less than 24 hr.

HYDRAZINES

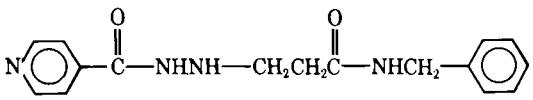
Benzylhydrazines—3,4 - Dimethoxybenzylhydrazine (vetracin, VI) is a potent MAO inhibitor (77); it exerts maximum inhibition of deamination of tyramine by rat brain and liver mitochondrial enzyme during the first 30 min. of preincubation (78). This phenomenon resembles observations in other aromatic alkylhydrazines (79). However, the effect of vetracin on the deamination of tyramine was the same when incubation was carried out in an atmosphere of oxygen or hydrogen as it was in air, and the replacement of air by nitrogen did not decrease the inhibition of vetracin (79). In this aspect, vetracin is different from iproniazid (65), but it resembles another MAO inhibitor, tranylcypromine[3] (*trans*-2-phenylcyclopropylamine, VII) (80). Kinetic studies with rat liver mitochondrial MAO showed that the inhibition by vetracin was noncompetitive with respect to such substrates as tyramine, serotonin, and benzyl-



VIII IX



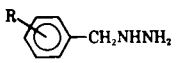
X: R = H
XI: R = $COCH_3$
XII: R = $COOCH_3$
XIII: R = $COOCH_2CH_2OH$



XIV

[3] Parnate.

Table II—*In Vitro* Inhibition of Rat Liver Mitochondrial MAO by Substituted Benzylhydrazines[a]



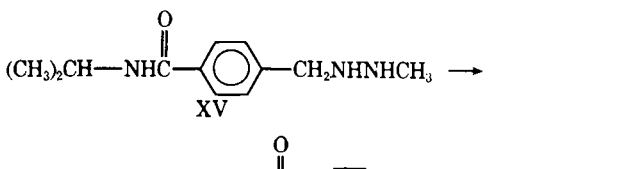
R	I_{50}, μM
3-OCH_3; 4-OCH_3	25
2-OCH_3; 5-OCH_3	9
4-OCH_3	2

[a] Verevkina *et al.* (78); substrate: tyramine.

amine and it inhibited the oxidation of dopamine by the enzyme from ox brain in a similar fashion. A monomethoxylated compound such as 4-methoxybenzylhydrazine is a more than 10-fold better inhibitor of MAO than is the 3,4-dimethoxylated derivative, vetracin (Table II).

Horita (55), in 1961, observed that in some cases the route of administration of hydrazine-type inhibitors determined selective inhibition of MAO in certain organs or tissues. Phenelzine[4] (phenethylhydrazine, VIII), for example, or pheniprazine[5] (β-phenylisopropylhydrazine hydrochloride, PIH, JB-516, IX) demonstrated much greater inhibition of MAO from brain than from liver when it was injected subcutaneously in rats; the reverse occurred when the compounds were administered orally. This selectivity was also found with benzylhydrazine (X) in mice, but it no longer occurred with some disubstituted hydrazines (XI–XIII); in mice, these disubstituted hydrazines produced similar degrees of inhibition in each of the two tissues, regardless of the route of administration (56). The lack of organ selectivity was also seen with iproniazid and nialamide[6] (isonicotinic acid 2-[2-(benzylcarbamoyl)ethyl]hydrazide, XIV) (55).

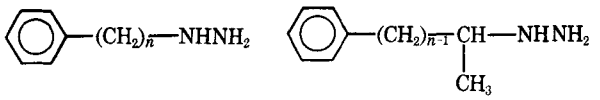
An interesting compound, procarbazine hydrochloride (*N*-isopropyl-α-(2-methylhydrazino)-*p*-toluamide monohydrochloride, MIH, Ro 4-6467/1, XV), reported by DeVita *et al.* (81), did not have any inhibitory effect on the enzyme *in vitro*. In aqueous medium or physiological fluid, it was shown to undergo autoxidation to *N*-isopropylterephthalamic acid (XVI) (82), which was also devoid of MAO inhibitory activity *in vitro* (81). In the *in vivo* study, an intraperitoneal injection of XV produced a peak of inhibition (88%) at 24 hr., which persisted significantly through 48 hr. Since the peak of inhibition by a single dose of XV was delayed and prolonged and no inhibitory effect was observed



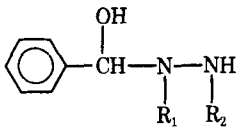
XV XVI XVII

Scheme III

[4] Nardil.
[5] Catron.
[6] Niamid.



XVIII XIX



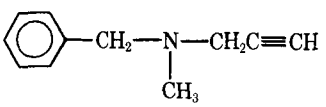
XX: $R_1 = R_2 = H$
XXI: $R_1 = H$; $R_2 = CO_2C_2H_5$
XXII: $R_1 = CO_2C_2H_5$; $R_2 = H$

in vitro, it was postulated that XV underwent metabolic conversion to methylhydrazine (XVII), which was responsible for the MAO inhibitory effect of XV *in vivo* (Scheme III).

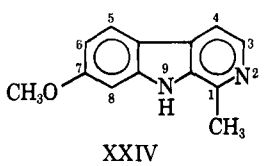
Arylalkylhydrazines—Popov *et al.* (83) studied the effect of different substrates on the inhibitory effect of some aralkylhydrazines with rat brain and liver homogenates. When the MAO activity was measured by the formation of aldehyde semicarbazone, which results from deamination of tyramine, dopamine, tryptamine, serotonin, and benzylamine, it was found that the inhibitory effect of phenylalkylhydrazine (XVIII) decreased with an increase in the methylene (CH_2) chain length up to four carbons; compounds with a five-carbon chain produced the same degree of inhibition as those with a three-carbon chain. The α-methylated analogs (XIX), however, exhibited no striking difference in inhibition. These findings agreed with those previously reported with aralkylhydrazines of one-carbon to three-carbon lengths using a manometric technique to measure enzyme activity (84, 85).

β-Hydroxyphenethylhydrazines—2-Hydroxy-2-phenethylhydrazine (XX) and its N^1- or N^2-carbethoxy derivatives (XXI and XXII) were evaluated for their *in vitro* inhibitory effect on tyramine oxidation by beef liver mitochondrial MAO (86). Substitution of either or both nitrogens of XX by a carbethoxy group resulted in decreased inhibitory activity. The parent compound, XX, was four times weaker than pargyline (XXIII) when tested in the same assay system (87). This does not agree with the finding of Biel *et al.* (88), who reported that XX was active only *in vivo* and inactive at 10^{-5} *M in vitro*.

In general, the *in vivo* activity of a hydrazide may depend upon the readiness of hydrolysis of the acyl group to free alkyl- or aralkylhydrazines (89), which is likely to be the moiety that reacts irreversibly with the enzyme. The acyl portion of hydrazide molecules should provide not only the tissue selectivity for the hydrazine to bind with MAO (31), but it may also facilitate the transport of molecules into tissues, especially of the brain.



XXIII



XXIV

PROPARGYLAMINES

Using a highly purified beef kidney MAO preparation, Hellerman and Erwin (90) reported that pargyline[7] (*N*-methyl-*N*-2-propynylbenzylamine, XXIII) became covalently bound to the active site of the enzyme *in vitro*. They extended the system *in vivo* and established the covalent nature of the binding of pargyline to MAO by exhaustive washing and treatment of proteins with trichloroacetic acid (91). The irreversibility of binding of pargyline on the enzyme was also demonstrated by preincubating the enzyme with the inhibitor, followed by dialysis for 24 hr. (92). The inhibition of purified rat liver mitochondrial enzyme by pargyline was found to be both competitive and noncompetitive (mixed type) with respect to the substrate veratrylamine and was similar to that observed with tranylcypromine (VII) (66).

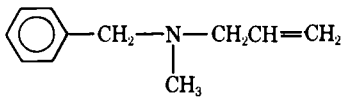
Regarding the mode of binding of pargyline on MAO, Belleau and Moran (93) hypothesized that pargyline may react with sulfhydryl groups at the active site. Vina *et al.* (92), however, found that although the enzyme was completely inhibited by pargyline during preincubation, the content of sulfhydryl groups did not change in comparison to preincubation of the enzyme in the absence of the inhibitor.

When tyramine was the substrate, interaction of pargyline with metal ions at the active site of MAO was evidenced by the finding that metal-complexing reagents such as 8-hydroxyquinoline completely protected the enzyme from irreversible inhibition by pargyline (92). A similar observation was reported for iproniazid (67, 94). The protection by 8-hydroxyquinoline was not complete with serotonin as the substrate. It has been proposed that the requirement for an irreversible inhibition of tyramine deamination is the attachment of pargyline to metal ions (94, 95).

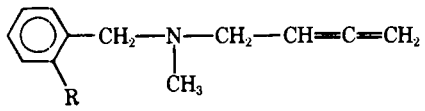
The participation of flavin in the interaction of pargyline on the active site is also possible. It has been proposed that a reversible inhibitor of MAO, such as harmine (7-methoxy-1-methyl-9*H*-pyrido[3,4-*b*]indole, XXIV), forms a charge-transfer complex with the enzyme's flavin component (93). Since pretreatment of harmine does not prevent the irreversible inhibition of tyramine deamination by pargyline (92), the involvement of flavin in binding pargyline seems unsupported. From the analogy to D-amino acid oxidase, Hellerman and Erwin (90) proposed an inhibitory mechanism for pargyline. This involves the reduction of flavin by transfer of two electrons from the methylene carbon of the propynyl group of pargyline to the flavin, which results in the formation of a flavoenzyme–product complex with the "product" having an aldimine structure.

Various modifications of this linkage were performed in investigations of the binding requirement of the propynyl group of pargyline; they all resulted in compounds of lower inhibitory activity (96). Replacement of the triple bond by a double bond, for instance, gave

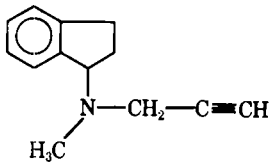
[7] Eutonyl.



XXV



XXVI: R = H
XXVII: R = Cl

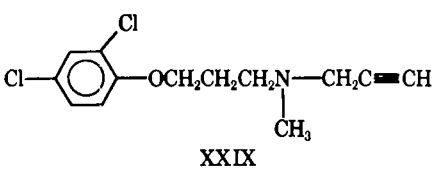


XXVIII

the alkene XXV, which not only was a 2000-times-weaker *in vitro* inhibitor than pargyline but also was devoid of activity *in vivo* (96). Two allenic derivatives of pargyline were evaluated for inhibition of tyramine deamination in rats (97). Although *N*-2,3-butadienyl-*N*-methylbenzylamine hydrochloride (U-1247, XXVI) and its *o*-chloro derivative (U-1379, XXVII) were 25 and five times less active *in vitro* than pargyline, they produced inhibition in rat liver and brain 4 hr. after oral administration to a degree comparable to that obtained by pargyline (Table III). A comparison of these allenic compounds to pargyline is not possible, since no data on MAO inhibition were given beyond 4 hr.

A new series of propargylamine-type compounds containing the indane nucleus was synthesized by Huebner *et al.* (98); one of these, *N*-methyl-*N*-2-propynyl-1-indanamine (Su-11739, XXVIII), was shown to be a very active irreversible MAO inhibitor (99). Its potency in both cerebral and cardiac MAO from rats was further demonstrated by Maitre (41), who found that XXVIII was 20 times more potent than pargyline in inhibiting serotonin and tyramine deamination in rat brains, five to eight times more potent than tranylcypromine (VII), and 30–50 times more potent than iproniazid. In rat heart, striking differences existed between XXVIII and iproniazid; the former was a 74 times better inhibitor than the latter.

The inhibition of rat brain MAO *in vitro* and *in vivo* by *N*-methyl-*N*-2-propynyl-3-(2,4-dichlorophenoxy)-propylamine hydrochloride (M&B 9302, XXIX) showed a unique but abnormal kinetic curve, different from the known inhibitors, including pargyline (44). This led Johnston (44) to hypothesize the existence of a binary system in the MAO preparation. Further study by Hall and Logan (100) demonstrated that, unlike iproniazid, the inhibition of XXIX did not require



XXIX

Table III—Inhibition of MAO with Rat Liver Homogenate by Allenic Amines[a]

Compound	*In Vitro* I_{50}, μM	*In Vivo* —ID_{50}, mg./kg.— Liver	Brain
Pargyline	0.04	0.13	3.1
XXVI	0.95	0.24	1.9
XXVII	0.23	0.51	2.2

[a] Halliday *et al.* (97); substrate: tyramine.

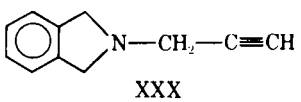
preincubation, nor was it affected by cyanide and copper ions.

A cyclic analog of pargyline, *N*-2-propynylisoindoline (XXX), was nearly as potent as pargyline in inhibiting beef liver mitochondrial MAO, with tyramine as the substrate (87).

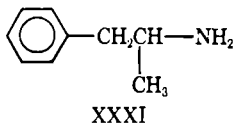
CYCLOPROPYLAMINES

Tranylcypromine (*d-trans*-2-phenylcyclopropylamine, VII) (101) is a better *in vitro* and *in vivo* inhibitor of MAO than iproniazid. Comparing the drastic difference in the inhibitory effect of tranylcypromine and amphetamine (XXXI), one concludes that the cyclopropane ring of tranylcypromine is undoubtedly a predominating factor that accounts for the inhibitor's high potency and irreversibility.

Although tranylcypromine, like pargyline and iproniazid, is capable of inhibiting MAO irreversibly, there are differences in their modes of action. Using a purified rat liver mitochondrial enzyme preparation and high concentrations of tranylcypromine, Guha (102) showed that, in contrast to other irreversible inhibitors, preincubation with tranylcypromine was not necessary to obtain the maximum inhibitory action. Earlier reports (80, 103) indicated that the incubation of tranylcypromine did not require the presence of oxygen as did iproniazid. Conflicting results were obtained by various investigators on the irreversible inhibitory nature of tranylcypromine. According to Maass and Nimmo (104), tranylcypromine inhibited rat brain MAO noncompetitively with respect to the substrate serotonin, but other groups (80, 102, 105, 106) found the inhibition to be irreversible and competitive with liver enzyme from various species when the substrate was tyramine. Kinetic data from McEwen *et al.* (66) showed that pargyline inhibited deamination of veratrylamine both competitively and noncompetitively (mixed type) when human liver mitochondrial enzyme was used. A tranylcypromine-bound enzyme preparation was subjected to prolonged dialysis by Sarkar *et al.* (105), who recovered a small fraction of the rabbit liver mitochondrial enzyme; this, however was not found by Guha



XXX



XXXI

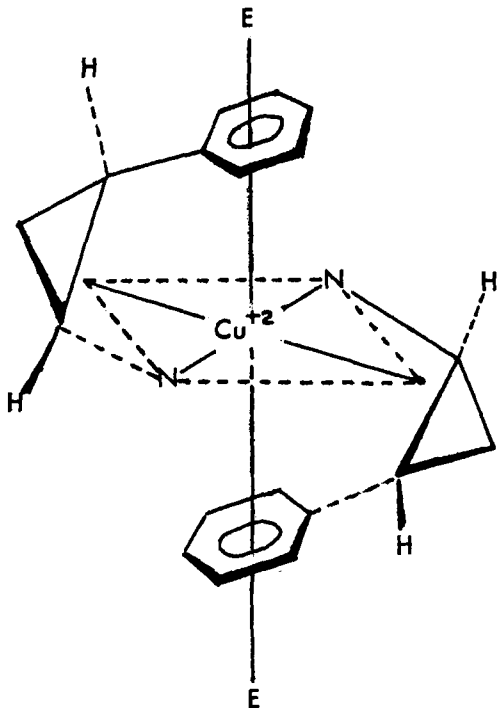
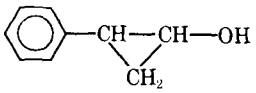


Figure 1—*MAO-Cu^{+2}-(tranylcypromine)$_2$ complex proposed by Gabay and Valcourt (108).*

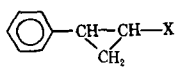
(102) with MAO from rat liver mitochondria. Reports on the ability of 4-phenylbutylamine to reverse the inhibition of tyramine deamination by tranylcypromine are contradictory (80, 107).

The mode of binding of tranylcypromine to MAO has been discussed (31, 108). Gabay and Valcourt (108) proposed that the amino group of tranylcypromine participates in an MAO-Cu^{+2}-(tranylcypromine)$_2$ complex which resembles the interaction of 1,10-phenanthroline or thyroxine with copper and the enzyme (Fig. 1). From their model, however, it is difficult to explain a 400- and 1500-fold decrease in MAO inhibitory activity of *trans*-2-phenylcyclopropylcarbinol (XXXII) from that of tranylcypromine with human and beef liver mitochondrial MAO (109) (Table IV), since the hydroxyl group of XXXII could participate in the formation of the complex suggested by Gabay and Valcourt (108). Our results seem to indicate that the attachment of tranylcypromine to MAO is accomplished by a protonated amino group (NH_3^+) of the tranylcypromine molecule to a carboxylate anion (COO^-) on the active site of the enzyme (Fig. 2). This may account for the marked decrease in MAO inhibition by XXXII, in which the formation of a cationic–anionic interaction is impossible and any hydrogen bonding between the hydroxyl group of XXXII and the enzyme is undoubtedly much weaker than the ionic bonding likely to exist between tranylcypromine and MAO. In comparing the tranylcypromine molecule with that of amphetamine, Biel (31) also noted that because the



XXXII

Table IV—*In Vitro* Inhibition of Liver Mitochondrial MAO by Phenylcyclopropanes[a]



X	I_{50}, μM Beef Liver	I_{50}, μM Human Liver
NH_2	0.13	3.0
OH	200	1210

[a] Ho and Tansey (109); substrate: tryptamine.

methylene group of the cyclopropane ring is "retracted," the protonated amino group (NH_3^+) of tranylcypromine is more reactive than that of amphetamine in the cationic–anionic interaction with the enzyme. Additional binding can also be anticipated from the cyclopropane ring of tranylcypromine, which is known to have properties like those of π-electron systems and, therefore, is capable of acting as a proton acceptor when interacting with a hydroxyl group of the enzyme. The intramolecular interaction between a hydroxyl group and a cyclopropane ring (O—H- - -cyclopropane) has been demonstrated (110). Furthermore, 2-phenylcyclobutylamine, which contains a cyclobutane ring of lower π-electron content than the cyclopropane of tranylcypromine, was found to be devoid of MAO inhibitory effect (111, 112).

A series of substituted 2-aryloxycyclopropylamines was synthesized (113, 114) in an attempt to alleviate the side effect of tranylcypromine discovered during its clinical evaluation (115). *trans*- and *cis*-Phenoxycyclopropylamine (XXXIII) and the *N,N*-dimethyl derivative were found to be better *in vitro* inhibitors of rat liver mitochondrial MAO than tranylcypromine (Table V). The finding that the *cis*-isomer was a more than twofold weaker inhibitor than the *trans*-isomer (114) was different from that of Zirkle *et al.* (113), who reported that in rat brain homogenate the *cis*-isomer was 10 times more active than the *trans*. Saturation of the phenyl group of the *trans*-2-phenoxycyclopropylamine gave 2-cyclohexyloxycyclopropylamine (XXXIV), which was as active as *trans*-2-phenoxycyclopropylamine in the *in vitro* inhibition of rat liver mitochondrial MAO

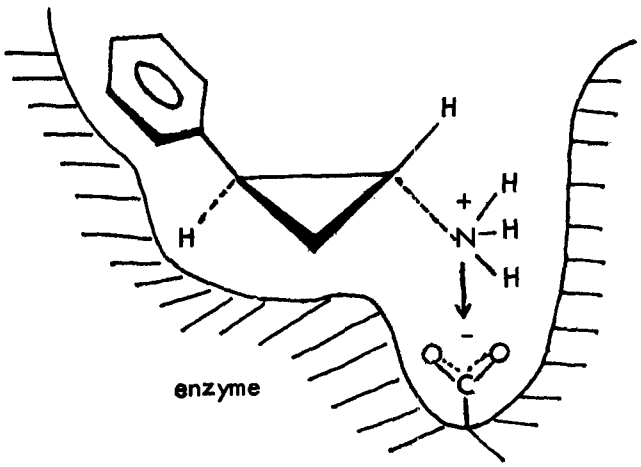
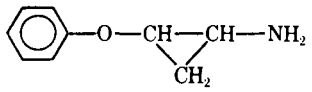
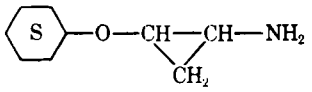


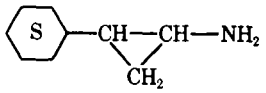
Figure 2—*Proposed cationic–anionic interaction of tranylcypromine with MAO.*



XXXIII



XXXIV



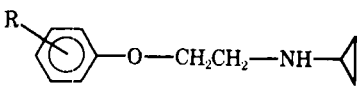
XXXV

(116). A closely related compound, 2-cyclohexylcyclopropylamine (XXXV), however, was reported to have no MAO inhibitory effect after it was tested *in vivo* for potentiating tryptamine convulsion in rats (113).

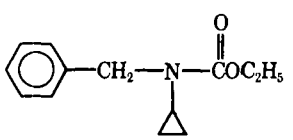
The *in vitro* and *in vivo* inhibitory activities of *N*-[2-(*o*-chlorophenoxy)ethyl]cyclopropylamine (51641, XXXV) and derivatives were studied by Fuller (117). The inhibition of rat brain mitochondrial MAO by XXXV was found to be noncompetitive with respect to the substrate serotonin or phenethylamine, and the irreversibility of this inhibition was established by dialysis (117). A preferential inhibition of deamination of indolealkylamines as compared to phenethylamines was also noted. *N*-[2-(2′,4′-Dichlorophenoxy)ethyl]cyclopropylamine (XXXVII) was shown to be as active as tranylcypromine and pheniprazine (IX) in the *in vitro* inhibition of rat liver mitochondrial MAO, using kynuramine as the substrate. By applying Hansch's substituent constants to correlate structure and activity of a series of substituted phenoxyethylcyclopropylamines, the degree of inhibition, measured by kynuramine oxidation with rat and human liver mitochondrial MAO, was found to correlate well with the value calculated with σ and π (119). Preliminary results of a clinical trial revealed that XXXV is useful in treating patients with endogenous depression (120).

Encyprate (ethyl *N*-benzyl-*N*-cyclopropylcarbamate, MO-1255, XXXVIII), regardless of the lack of MAO inhibitory property *in vitro*, is active as a dopa-potentiating agent *in vivo*. Its potential usefulness in the treatment of newly admitted depressed patients (121) has been demonstrated.

Another cyclopropylamine was shown by Huszti *et al.* (122) to inhibit preferentially the deamination of norepinephrine and serotonin, but not tyramine, with rat brain homogenate. This compound, 1-(*m*-aminophenyl) - 2 - cyclopropylaminoethanol dihydrochloride (AB-15, XXXIX), produced better *in vivo* inhibition in rat brains than did nialamide (XIV), but

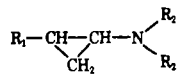


XXXVI: R = *o*-Cl
XXXVII: R = 2-Cl; 4-Cl



XXXVIII

Table V—*In Vitro* Inhibition of Rat Liver Mitochondrial MAO by Cyclopropylamines[a]



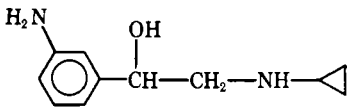
R_1	R_2	Configuration	I_{50}, μM
C_6H_5O	H	*d,l-trans*	0.16
		d,l-cis	0.40
C_6H_5O	CH_3	*cis-trans*	2.10
C_6H_5	H	*d,l-trans*	8.60
$C_6H_{11}O$	H	*d,l-trans*	0.19
		d-trans	0.20
		l-trans	0.35

[a] Finkelstein *et al.* (114, 116); substrate: tyramine.

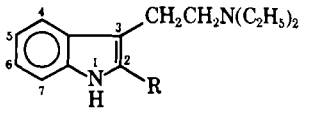
its inhibitory effect on rat brain and liver MAO was less than that of tranylcypromine.

INDOLEALKYLAMINES

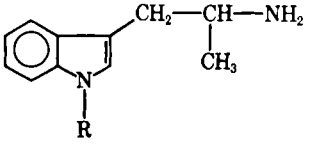
Because of their reversible nature, 3-substituted indoles are, in general, inhibitors of shorter duration. A marked change in the inhibitory activity of *N*,*N*-diethyltryptamine (DET, XL) and its 2-substituted derivatives was observed when the substrate was changed from serotonin to the structurally related tryptamine (123). Similar observations were reported by Gorkin *et al.* (124) with α-methyltryptamine (indopan, XLI), which selectively inhibits deamination of serotonin over tryptamine. After substitution of the indolic hydrogen by a methyl group, the resulting compound, *N*-methyl-α-methyltryptamine (XLII), not only showed significantly less inhibition than indopan, especially in the brain tissue, but also lost selectivity between rat liver enzyme and brain enzyme (124, 125). With rat liver mitochondrial enzyme preparation, both 3-(3′-indolyl)-



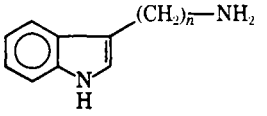
XXXIX



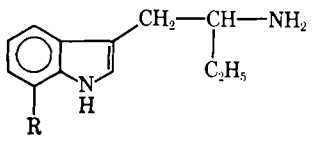
XL: R = H



XLI: R = H
XLII: R = CH_3

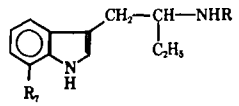


XLIII: $n = 3$
XLIV: $n = 4$



XLV: R = H
XLVI: R = CH_3

Table VI—*In Vitro* Inhibition of Guinea Pig Liver MAO by Etryptamine and Derivatives[a]



R	R_7	I_{50}, m*M*
H	H	0.26
C_2H_5	H	0.70
H	CH_3	0.03

[a] Hester *et al.* (127); substrate: serotonin.

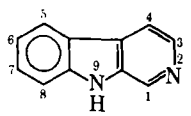
propylamine (XLIII) and 4-(3′-indolyl)butylamine (XLIV) demonstrated competitive inhibition of serotonin deamination, but the inhibition of tyramine deamination by these two compounds was uncompetitive (124).

Derivatives of etryptamine (α-ethyltryptamine, XLV), the compound used at one time for treating some types of depression (126), were evaluated for *in vitro* inhibitory activity with guinea pig liver MAO, using serotonin as the substrate (127). 7-Methyl-α-ethyltryptamine (XLVI) was found to be 10 times more effective as an *in vitro* inhibitor than etryptamine (Table VI); *in vivo* it was twice as effective in increasing endogenous serotonin in the rat brain.

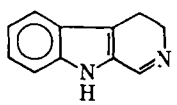
β-CARBOLINES

The inhibitory effect of harmala alkaloids is reversible *in vitro* and faster acting *in vivo*; this class of β-carbolines is more potent than the aforementioned indolealkylamines. The degree of saturation on the pyridine moiety of the β-carboline molecule (XLVII) increases their inhibitory activity in the order of tetrahydro (XLIX), dihydro (XLVIII), and the fully aromatic β-carboline (XLVII) (128, 129). The active site of beef liver MAO appears to be filled by the β-carboline, since a methyl substitution on various positions, such as C-1 and C-8 of XLVII and C-1, C-2, C-3, and C-6 of XLIX, resulted in a decrease in inhibitory activity (129, 131). Other substituents such as halogens and OCH_3 on the C-6 position of XLVII and XLIX also reduced the inhibition to varying degrees (128–131). Substituting a hydroxyl or methoxy group on either the C-6 or C-7 position of the 1-methyl derivative of the three series of β-carbolines (XLVII–XLIX) also reduced the inhibitory activity compared to the corresponding unsubstituted 1-methyl-β-carbolines (128).

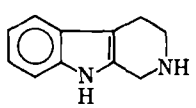
In contrast, no change in the enzyme inhibition was observed when a methyl group was placed on C-8 of



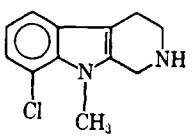
XLVII



XLVIII

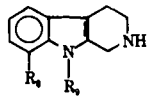


XLIX

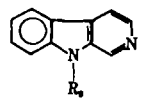


L

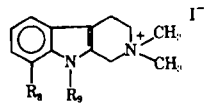
Table VII—*In Vitro* Inhibition of Beef Liver MAO by β-Carbolines[a]



R_8	R_9	I_{50}, m*M*
H	H	0.34
H	CH_3	0.01
Cl	H	0.10
Cl	CH_3	0.0038



R_9	I_{50}, m*M*
H	0.029
CH_3	0.010
$COCH_3$	0.15

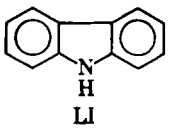


R_8	R_9	I_{50}, m*M*
H	CH_3	0.38
Cl	H	0.0033

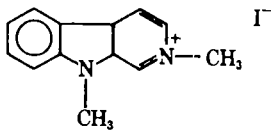
[a] Ho *et al.* (129, 130, 133, 134); substrate: tryptamine.

XLIX; but a chlorine substituted at the same position did, in fact, increase the inhibition of MAO (Table VII) (130).

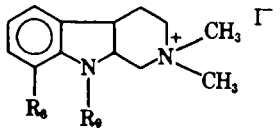
An exceptionally high, 35-fold increase in inhibitory activity of the tetrahydro-β-carboline occurred with the introduction of a methyl group on the indolic nitrogen (Table VII) (129). The increment, however, was relatively small (only threefold) when the same substitution was made at the indolic nitrogen of the aromatic β-carboline (Table VII). It is postulated that the electron-donating property of the methyl group either increased the binding capacity of the indole nucleus by formation of a charge-transfer complex with the enzyme, or bound hydrophobically to the enzyme, or both (129). That the increase in inhibitory activity of 9-methylated XLVII and L is at least partially due to the increased electron density of the β-carboline nucleus by the N-9 methyl group was further demonstrated in the aromatic series by a 15-fold loss in inhibitory activity when an electron-withdrawing group, acetyl ($COCH_3$), was introduced in place of the methyl (Table VII) (133). By taking advantage of this finding, that an increase in MAO inhibition can be achieved by both C-8 substitution of the chlorine atom and N-9 substitution of a methyl group, an inhibitor, 8-chloro-9-methyl-1,2,3,4-tetrahydro-β-carboline (L), was prepared. The increment of enzyme inhibition that resulted from this two-step substitution on the 8- and 9-positions was indeed additive (Table VII) (130); the best *in vitro*



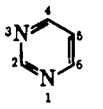
LI



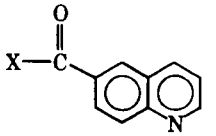
LII



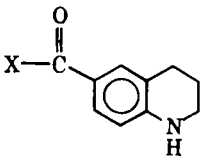
LIII: $R_8 = H$; $R_9 = CH_3$
LIV: $R_8 = Cl$; $R_9 = H$



LIX



LX: X = OH
LXI: X = $NHNH_2$
LXII: X = NH_2



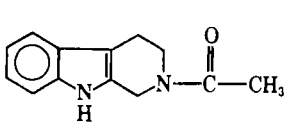
LXIII: X = OH
LXIV: X = $NHNH_2$

MAO inhibitor of the β-carboline series was more than eight times more active than iproniazid in inhibiting tryptamine oxidation by beef liver mitochondrial enzyme (129, 130).

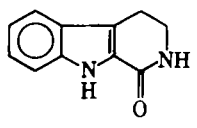
Distinctly different from the tetrahydro series, substitution of a methyl group at C-8 decreased inhibition 2.5-fold (131). This result, together with other differences between the two series of β-carbolines, such as the effects of a 9-alkyl group (132, 133) and 6-substituent (130, 131), suggests that the β-carbolines are probably bound to the enzyme in different conformations.

It appears that the binding of β-carboline involves the complexing of indole alone (XLIX), or indole plus the pyridine (XLVII) moiety of the inhibitor with the enzyme, through a possible charge–transfer interaction. This may explain the increase in inhibition that results from the substitution at the N-9 position of a methyl group, whose electron-donating property is thought to increase the electron density of the phenyl ring and to favor the attachment of an electron donor to an electron-poor locus of the enzyme. The β-carboline nitrogen is essential for its inhibitory effect, since carbazole (LI), a fully aromatic compound lacking a nitrogen, was found to be devoid of MAO inhibitory activity (128). Possibly the protonated amino group of β-carboline is responsible for interaction of the N-2 nitrogen with MAO. Evidence supporting this possibility includes: (*a*) compounds bearing a charged nitrogen such as 2,9-dimethyl-β-carbolinium iodide (LII) (see under *Peripheral MAO Inhibitors*), 2,2,9-trimethyl-1,2,3,4-tetrahydrocarbolinium iodide (LIII), and 8-chloro-2,2-dimethyl-1,2,3,4-tetrahydrocarbolinium iodide (LIV), were good enzyme inhibitors (Table VII) (134); and (*b*) the inhibitory activity of tetrahydro-β-carboline (XLIX) decreased markedly when the basicity of the amino group was altered by either acetylation (LV) or formation of a lactam linkage (LVI) (129).

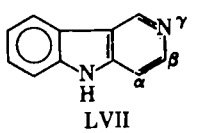
The results of *in vivo* studies (135) of several β-carbolines indicated that the methyl group on the indolic nitrogen facilitated the penetration of tetrahydro-β-carbolines into mouse brain. As found by a higher partition coefficient in heptane, this might be due to the increased lipid solubility of the compounds by the methyl group. By lowering the pKa, halogen substitution increased the amount of unchanged compound available for passage into the brain. The best *in vitro* MAO inhibitor of the series (L) was found to enter the brain to a greater extent and at a faster rate.



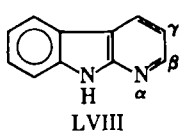
LV



LVI



LVII



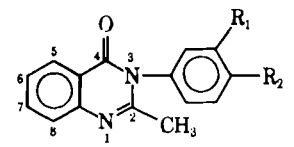
LVIII

γ- AND α-CARBOLINES

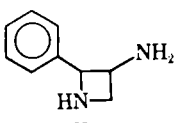
In vitro, γ-carboline (LVII) and α-carboline (LVIII) exerted a weaker effect than did the harmala alkaloids (β-carbolines) in inhibiting tyramine deamination by rat brain mitochondrial MAO (136, 137). The authors made indirect comparisons for γ- and α-carbolines with phenelzine (VIII) (Table VIII), because no harmala alkaloids were available to them and because both β-carbolines and hydrazines have been shown to attach at the same site on MAO. *In vivo*, γ-carbolines induced a decrease in 5-hydroxyindoleacetic acid in rabbits given serotonin but had a weak effect on the duration of ethanol narcosis in reserpine-treated mice and on hypothermia in reserpine-treated rabbits (136).

PYRIDINES

Pyridine derivatives were tested to determine whether or not the indole moiety of the α-, β-, and γ-carbolines is responsible for their MAO inhibitory activity (137). Compared to the carbolines (Table VIII), the pyridine derivatives showed a relatively weaker effect.



LXV: $R_1 = H$; $R_2 = CONHNH_2$
LXVI: $R_1 = OH$; $R_2 = CONHNH_2$
LXVII: $R_1 = H$; $R_2 = OCH_2CH_2NHNH_2$



LXVIII

Table VIII—*In Vitro* Inhibition of Rat Brain Mitochondrial MAO by α- and γ-Carbolines and Pyridine Derivatives[a]

Compound	Percent Inhibiton at 10^{-4} *M* (Phenelzine = 100)
γ-Carboline	46
6-Methyl-γ-carboline	70
6-Methyl-α-carboline	80
Pyridine nitrate	66
N-Methylpyridine iodide	46
2-Aminopyridine sulfate	65
4-Aminopyridine	64

[a] Nantka-Namirski *et al.* (136, 137); substrate: tyramine.

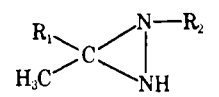
OTHER HETEROCYCLES

Pyrimidines (LIX) substituted at 2-, 4-, and/or 6-positions with the hydrazino group were shown to inhibit tyramine deamination by rat liver mitochondrial MAO (138). Tri- and dihydrazino derivatives were more active than the monohydrazino compounds.

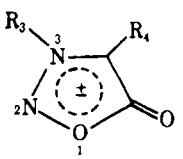
Hydrazides and benzylamides of quinoline-6-carboxylic acid (LX) were tested for inhibitory effect on rat brain MAO. The inhibitory activity of all compounds compared as follows, with phenelzine (VIII) being 100%: quinoline-6-carboxylic acid amide (LXII), 110%; quinoline-6-carboxylic acid hydrazide (LXI), inactive; and LXIV, the reduction product of LXIII, 90%.

The effects of halogen substitution at the C-6 and/or C-8 positions of *p*-[3-(2-methylquinazol-4-one)]benzhydrazide(LXV)and *p*-[3-(2-methylquinazol-4-one)]salicylhydrazide (LXVI) on the inhibition of tyramine oxidation were studied with a rat liver enzyme preparation (139, 140). Inhibitory activity increased with halogen on the C-6 position but not with halogen on both the C-6 and C-8 positions. The reverse was observed, however, with *p*-[3-(2-methylquinazol-4-one)]phenoxyethylhydrazine (LXVII), in which the inhibition favored C-6 and C-8 disubstitution (141).

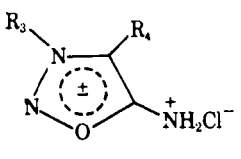
The lack of inhibitory effect of 2-phenylcyclobutylamine (111, 112) compared to the cyclopropane analog, tranylcypromine (VII), presumably was due to a loss of electronic delocalization from the three- to the four-membered ring, leading to the synthesis of the heterocyclic analog of 2-phenylcyclobutylamine (142). 3-Amino-2-phenylazetidine (LXVIII) was found to be 62% as active as iproniazid in inhibiting tyramine oxidation by rat liver homogenate.



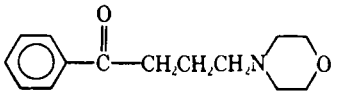
LXIX: $R_1 = CH_2C_6H_5$; $R_2 = H$
LXX: $R_1 = C_6H_5$; $R_2 = CH_3$



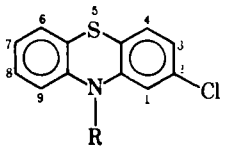
LXXI



LXXII



LXXIII



LXXIV: R = $COCH_2CH_2N(C_2H_5)_2$
LXXV: R = $CH_2CH_2CH_2N(CH_3)_2$

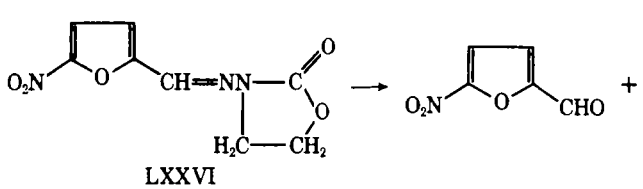
Inhibition of serotonin deamination by rat liver homogenate was investigated with diaziridines (143). 3-Phenyldiaziridine (LXIX), whose structure resembles tranylcypromine (VII) and hydrazine, was reported as active as iproniazid, whereas 3-benzyldiaziridine (LXX) was inactive, despite its structural resemblance to β-phenylisopropylhydrazine (IX).

A series of *N*-arylsydnones (LXXI) (144) and aralkylsydnonimines (LXXII) (145) inhibited MAO. The former class of compounds exhibits noncompetitive inhibition of guinea pig liver enzyme with the substrate kynuramine (144); 3-(β-phenylisopropyl)sydnonimine produced the same degree of inhibition of deamination of tyramine and serotonin by rat liver mitochondrial enzyme as iproniazid (145).

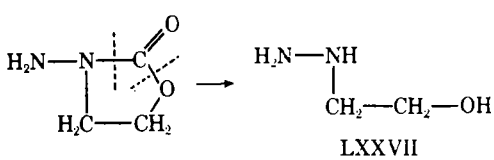
γ-Morpholinobutyrophenone (NSD 2023, LXXIII) inhibited tryptamine oxidation by mouse brain MAO more effectively *in vivo* than *in vitro* (43). A maximum of 70% enzyme inhibition was achieved at 10 mg./kg. of the inhibitor; increases in doses up to 200 mg./kg. did not increase inhibition.

Chloracizine [10-(β-diethylaminopropionyl)-2-chlorophenothiazine hydrochloride, LXXIV], like chlorpromazine (LXXV), was a weak *in vitro* inhibitor of MAO from rat liver (146). Although both showed inhibitory effects *in vivo*, their duration of action was short.

Furazolidone [3-(5-nitrofurfurylidene)amino-2-oxazolidinone, LXXVI], an irreversible inhibitor of rat liver MAO, was active only *in vivo* (147). Enzyme activity was not depressed significantly until 4 hr. after oral administration of the compound, and the maximum inhibition was found only after 16 hr., depending on the doses used. It was postulated that the furazolidone metabolite, 2-hydroxyethylhydrazine (LXXVII), was responsible for the action (Scheme IV),

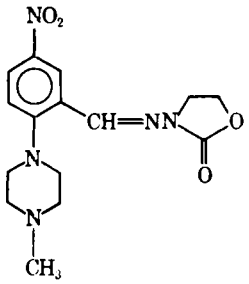


LXXVI

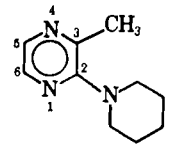


LXXVII

Scheme IV



LXXVIII



LXXIX

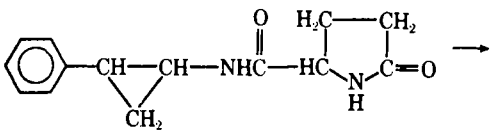
since LXXVII was shown to be highly active both *in vivo* and *in vitro* (147). Furazolidone was also found to inhibit human enzyme *in vivo* (148).

A similar example was 3-[2-(4-methyl-1-piperazinyl) - 5 - nitrobenzylidene]amino - 2 - oxazolidinone (LXXVIII), an inhibitor of rat brain MAO of about one-third the activity of pargyline when it was administered intraperitoneally; it was found to be devoid of *in vitro* activity (149).

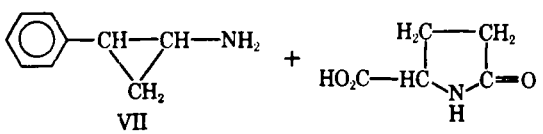
Other MAO inhibitors that require biotransformation before exerting their effect have been cited in the literature. These include modaline (2-methyl-3-piperidinopyrazine, LXXIX) (150, 151) and 5-oxo-(D-*trans*-2-phenylcyclopropyl)-L-2-pyrrolidone carboxamide (LXXX) (152). In rat liver homogenate containing the amidase, LXXX is probably hydrolyzed to yield tranylcypromine (VII), which then inhibits deamination of serotonin (Scheme V). This was further substantiated by its failure to inhibit when it was incubated with isolated liver mitochondria.

MISCELLANEOUS

N-Aralkylhydroxylamines are *in vitro* inhibitors of rabbit liver MAO with tyramine as the substrate (153). β-Phenylisopropylhydroxylamine (LXXXI), an analog of the known MAO inhibitor β-phenylisopropylhydrazine (IX), exerted 69% enzyme inhibition at 5×10^{-3} *M*, compared to 81% by tranylcypromine (VII) at the same concentration and with the same assay



LXXX

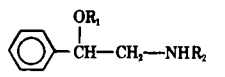


VII

pyrrolidone carboxylic acid

Scheme V

Table IX—*In Vitro* Inhibition of Rat Liver MAO by Phenylethanol Derivatives[a]



R_1	R_2	Percent Inhibition (Iproniazid = 100)
H	$CH_2CH_2C_6H_5$	36
H	$CH(CH_3)_2$	36
$CH_2C_6H_5$	$CH_2CH_2C_6H_5$	93
$CH_2C_6H_5$	$CH(CH_3)_2$	70
CH_3	$CH(CH_3)_2$	15

[a] Wells and Shields (156); substrate: tryptamine.

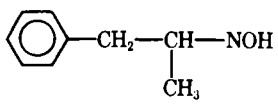
system. 1,2,3,4-Tetrahydro-β-naphthylhydroxylamine (LXXXII) was more active than tranylcypromine, whereas 1,2,3,4-tetrahydro-β-naphthylamine (LXXXIII) was devoid of MAO inhibitory activity.

A series of hydroxylamine derivatives of the general formulas of LXXXIV and LXXXV [some of which are structurally related to pargyline (XXIII)] were weaker inhibitors of beef liver mitochondrial MAO than pargyline (87).

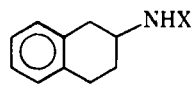
Based on the finding of Schuler and Wyss (154) that esterification of the hydroxyl group in hydrazinoalkanols increased the *in vivo* inhibitory effect on MAO, the ether derivatives of two previously reported (155) MAO inhibitors, 2-phenethylamino-1-phenylethanol (LXXXVI) and 2-isopropylamino-1-phenylethanol (LXXXVII), were prepared and evaluated for inhibitory activity on tryptamine deamination by rat liver homogenate (156). The *in vitro* activity of the benzyl ethers (LXXXVIII and LXXXIX) increased over the two parent alkanols, whereas that of the methyl ether (XC) was considerably less than LXXXVII (Table IX).

2-Bromo-2-phenylacetaldehyde (XCI) was found to be a potent inhibitor of pig brain mitochondrial MAO (157, 158).

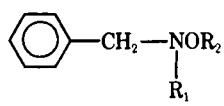
Inhibition of MAO from different tissues and species by nitroglycerin (glyceryl trinitrate, XCII) was demon-



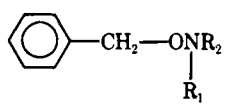
LXXXI



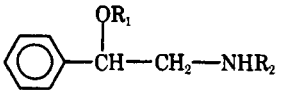
LXXXII: X = OH
LXXXIII: X = H



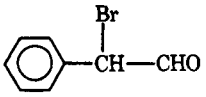
LXXXIV: R_1 = H, CH_3, or $CH_2C{\equiv}CH$; R_2 = CH_3 or $CH_2C{\equiv}CH$



LXXXV: R_1 = H or CH_3; R_2 = CH_3 or $CH_2C{\equiv}CH$



LXXXVI: $R_1 = H$; $R_2 = CH_2CH_2C_6H_5$
LXXXVII: $R_1 = H$; $R_2 = CH(CH_3)_2$
LXXXVIII: $R_1 = CH_2C_6H_5$; $R_2 = CH_2CH_2C_6H_5$
LXXXIX: $R_1 = CH_2C_6H_5$; $R_2 = CH(CH_3)_2$
XC: $R_1 = CH_3$; $R_2 = CH(CH_3)_2$



XCI

strated by Ogawa *et al.* (159). *In vivo*, a dose as low as 2.5 mcg./kg. of the compound produced 45% inhibition of rat heart MAO. Kinetic studies with purified dog heart enzyme showed that it is a competitive inhibitor with respect to the substrate tryptamine.

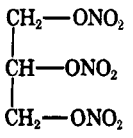
Carrano and Malone (160) reported that proadifen hydrochloride [2-(diethylamino)ethyl 2,2-diphenylvalerate hydrochloride, SK&F 525-A, XCIII], an inhibitor of the microsomal enzyme system, was as active as iproniazid in the *in vitro* inhibition of tryptamine oxidation with rat liver homogenate.

PERIPHERAL MAO INHIBITORS

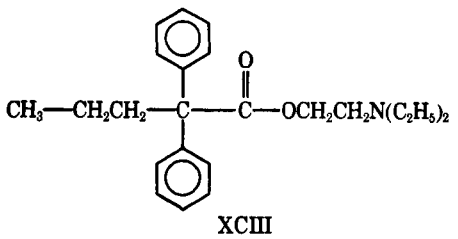
This group of inhibitors generally bears a charged moiety in the molecule and thus is not able to penetrate into the cerebral tissue; its action is restricted to the peripheral enzyme.

A group of benzylguanidines was first reported as competitive and reversible inhibitors for the deamination of serotonin by rat heart MAO (161). The most active compound of the series, *N*-*o*-chlorobenzyl-*N*,*N*'-dimethylguanidine sulfate (BW-392-C-60, XCIV), was only slightly less potent *in vitro* than pheniprazine (IX). It was later demonstrated that, being highly ionized, it exclusively inhibited peripheral (liver) MAO and caused selective blockade of liver inhibition by pheniprazine and pargyline (162).

Guanylhydrazones of aromatic carbonyl compounds were potent *in vitro* inhibitors of guinea pig liver mitochondrial MAO (163), being 2–10 times as active as iproniazid. Such substituents as methyl, methoxy, or chloro on Compound XCV increased the activity,



XCII



XCIII

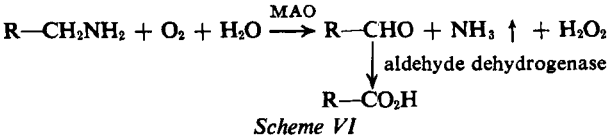
whereas a phenolic hydroxyl group reduced the inhibitory effect of XCV. A conjugated system through the benzene ring is apparently essential for the high activity of the compound. Kinetic studies showed that these inhibitors were competitive with respect to the substrate tyramine or serotonin.

Styrylquinoliniums have been demonstrated as a new group of potent inhibitors of guinea pig liver mitochondrial MAO (164). The inhibition was found to be reversible and competitive with respect to the substrate dopamine. 4-(*p*-Chlorostyryl)-1-methylquinolinium iodide (XCVI) was a nearly fivefold better inhibitor than harmaline (1-methyl-7-methoxy-3,4-dihydro-β-carboline, XCVII) and 1200-fold better than iproniazid. Both the position of the styryl group and the halogen substitution on the benzene ring were critical for maintaining the inhibitory effect, since either moving the styryl group to the C-2 position or removing the halogen from the benzene ring caused reduction of inhibition. Substitution of a methoxy group on the C-6 position resulted in a compound of greater potency than the parent compound; only a slight change in potency was observed, however, when chloro or dimethylamino was placed on the same position.

More recently, a new peripheral MAO inhibitor of the β-carboline class was developed (165). *In vitro*, 2,9-dimethyl-β-carbolinium iodide (DMCI, LII) was more potent than pargyline in inhibiting tyramine oxidation by human heart MAO and tryptamine oxidation by the enzyme from rat liver, rat heart, and human heart. The inhibition was reversible and noncompetitive (mixed type) with all enzyme preparations except rat heart, in which competitive inhibition occurred. *In vivo*, LII at 15 mg./kg. i.v. exerts greater inhibition with rat heart MAO than with the liver enzyme; a nearly 60% inhibition in the heart enzyme was maintained throughout 8 hr., which then decreased to 29% at 16 hr. In the brain of animals injected with ^{14}C- or tritium-labeled LII, radioactivity was barely detectable at all time intervals studied, indicating its selective action as a peripheral MAO inhibitor.

ASSAY OF MAO INHIBITION

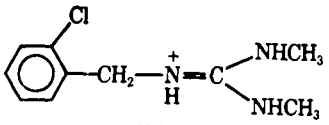
In vitro assay of MAO inhibition is based on the measurement of either the reactants or products according to Scheme VI. This includes the determination of amine



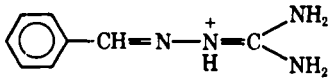
Scheme VI

consumption or oxygen uptake at the left-hand side of the reaction and of the ammonia evolution, hydrogen peroxide, aldehyde, or acid formation at the right-hand side.

Reactant—For the estimation of oxygen uptake, the manometric method using a Warburg apparatus is generally employed (166–168). This method has been widely used, in spite of its requirement for a large amount of tissue. An amine such as serotonin can be added to the enzyme preparation; at the end of the re-



XCIV



XCV

action, the unconsumed serotonin is extracted from the medium and then measured by either colorimetric (169) or fluorometric assay (170).

Product—The release of ammonia can be measured by formation of ammonium sulfate (164), by the phenol-hypochlorite method (171), or by isothermic distillation followed by reaction with the Nessler reagent (172). The hydrogen peroxide produced is either reacted with *o*-dianisidine (173) or coupled in the presence of peroxidase with homovanillic acid (174) or *p*-hydroxyphenylacetic acid (175).

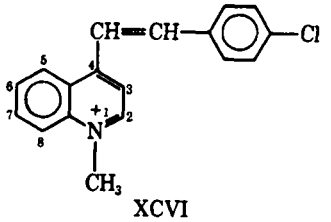
If a radioactive substrate is available, the product aldehyde and/or acid, after separation from the amine by extraction at an acidic pH, is subjected to liquid scintillation spectrometry (176, 129). The aldehyde can also be determined by measuring the absorbance of the aldehyde semicarbazone (83); an acid such as 5-hydroxyindoleacetic acid can also be estimated (177).

A unique spectrophotometric method of assaying MAO activity is based on the conversion of an amine substrate to a product which has different extinction coefficients from the substrate or absorbs at different wavelengths. Examples are 3-nitro-4-hydroxybenzaldehyde (36, 146), 3-iodobenzaldehyde (178), and 4-hydroxyquinoline from kynuramine (42, 179–181).

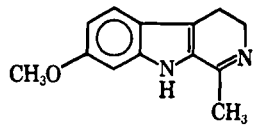
2,4,6-Trinitrobenzenesulfonic acid was employed recently in the assay of MAO activity (182); this reagent was reported to react with primary amines or amino acids, yielding a strong color derivative absorbed at 442 nm. (183).

Assay of the *in vivo* MAO inhibition can be performed by using the above-mentioned *in vitro* techniques on tissues or organs of animals pretreated with an MAO inhibitor. In drug-treated animals, other methods include direct measurement of biogenic amines such as norepinephrine and serotonin and/or metabolic products such as 5-hydroxyindoleacetic acid and normetanephrine against a control value. Such precursor amino acids as L-tryptophan or 5-hydroxytryptophan were injected into animals prior to administration of an MAO inhibitor, and the serotonin level in tissue was then compared to the control values (81, 127).

Various methods have been developed for the pharmacological evaluation of MAO inhibitors. These include reversal of reserpine-induced sedation and ptosis (44, 73, 184), potentiation of excitatory activity of 5-hydroxytryptophan (184–186) and dopa (184–187), potentiation of 5-hydroxytryptophan-induced hyperthermia (122), potentiation of tryptamine toxicity (188), potentiation of cardiovascular action of tyramine (44, 122), and potentiation of the central effect of intracerebrally administered serotonin (189). The results of



XCVI



XCVII

these tests, in general, correlate well with the direct measurement of MAO activity, yet the exceptions indicate that some actions may not be caused by enzyme inhibition and that they may involve receptors other than the enzyme receptor. 4*H*-3-Methylcarboxamide-1,3-benzoxazine-2-one (FI 6654), for instance, a compound active in various pharmacological measurements, lacks MAO inhibitory activity; its mode of action appears to interfere with cerebral serotonin in aspects other than its metabolism (190).

THERAPEUTIC VALUE

The application of an MAO inhibitor in the treatment of depression began as early as 1952, when iproniazid[1] (I) was observed to induce mood elevation and euphoria in tuberculous patients. The antidepressive effect of iproniazid coincides with the finding by Zeller *et al.* (58) that it is an inhibitor of MAO and with the theory proposed by Woolley and Shaw (191) that some mental disorders might be related to a change in the brain serotonin content. Today, the relationship between the antidepressive action of MAO inhibitors and their effect on amine metabolism has still not been substantiated, although in animal studies the behavioral changes have been correlated with changes in amine concentration (192). Iproniazid was later used in depressed patients with angina pectoris and was demonstrated to relieve the symptoms of this disorder (193, 194). It was postulated that this relief of angina by iproniazid was mediated through CNS stimulation, blockade of pain transmission, coronary dilitation, and reduction of the myocardial requirement for oxygen and therefore a decrease in cardiac work (195). The incidence of fatal hepatitis was the main reason for the withdrawal of iproniazid from clinical use in 1961.

Later, hydrazines such as pheniprazine[5] (IX), phenelzine[4] (VIII), nialamide[6] (XIV), and isocarboxazid[2] (IV) were marketed, and some of these were synthesized specifically in hopes of reducing hepatotoxicity. No significant incidence of liver damage was, in fact, observed with isocarboxazid and pargyline[7] (XXIII), which later became valuable agents in treating depressed, hypertensive patients suffering from angina pectoris; however, special care is necessary because of the drugs' prolonged hypotensive actions (195). Pheniprazine was removed from the market in 1960 after reported incidences of hepatitis and optical nerve atrophy accompanied by visual loss and color blindness. The nonhydrazine type of MAO inhibitors, such as etryptamine acetate[8] (XLV), shared the same fate in 1962 when deaths from agranulocytosis were reported. The ob-

[8] Monase.

servation of hypertension in patients with or without a cerebral vascular accident precipitated the removal of tranylcypromine[3] (VII) in 1964, but it was later put back on the market.

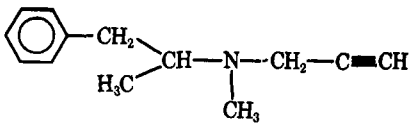
Antihypertensive therapy with an MAO inhibitor alone is seldom satisfactory. Isocarboxazid and pargyline, however, have been useful adjuncts to treatment with sulfonamide diuretics, together with reserpine or without (196).

In general, the most severe adverse side effects of MAO inhibitors have been orthostatic hypotension, usually as a consequence of their antihypertensive effect in depressed patients, and the occasional intracranial or subarachnoid hemorrhage which results from a marked rise in blood pressure. In patients treated with MAO inhibitors (197–199), the latter is associated with ingestion of such substances as ripened cheese, yeast extracts, broad beans, or even certain beers, wines, and yogurt which contain substantial quantities of tyramine. This seems to result from the prolongation and potentiation of tyramine's sympathomimetic effects by blockade of its oxidative deamination. Other drugs potentiated by MAO inhibitors include such CNS stimulants and depressants as imipramine, amitriptyline, narcotics, barbiturates, tranquilizers, anesthetics, and alcohol. The incidences of adverse action of these agents in patients receiving MAO inhibitors are possibly due to the inhibition of enzymes that detoxify these drugs. Iproniazid, for example, has been reported to interfere with the metabolism of hexobarbital by liver microsomal enzymes (200).

The hypotensive effect of MAO inhibitors, as stated, is not the result of enzyme inhibition but rather of a bretylium-like action on the postganglionic adrenergic neuron, which prevents the release of norepinephrine by nerve impulses (201). Pargyline blocks sympathetic ganglionic transmission (202, 203). Tranylcypromine, which has even greater MAO inhibitory activity, exerts little or no hypotensive effect; moreover, some incidences of a pressor effect associated with temporary sympathomimetic activity have been reported.

In view of all the above-cited adverse reactions, it is easy to understand why psychiatrists today are less than enthusiastic in prescribing MAO inhibitors for patients with depressive illness, preferring imipramine[9] and related compounds. A number of psychiatrists, however, believe that significant subgroups of depressed patients respond specifically and only to one or another of the MAO inhibitors (204). Klerman (204) suggested that MAO inhibitors be used for patients who have a history of responding to these compounds and whose family members have also responded positively. It was also suggested that the treatment be gradual and that initial doses be smaller than those recommended (195). For the sake of obtaining symptomatic improvement, treatment with MAO inhibitors merits special attention and careful planning.

Unfortunately, MAO inhibitors do not offer conclusive improvement for treating chronic, schizophrenic patients. In combined therapy with chlorpromazine[10]



XCVIII

and an MAO inhibitor, however, the latter appears to potentiate some of the pharmacological effects of the former and to prevent the occurrence of extrapyramidal symptoms. Chlorpromazine also prevents the hypertensive reaction induced by some MAO inhibitors (205, 206). Bucci (205, 206) indicated that this combination is relatively safe and clinically useful, especially in treatment of schizophrenic patients who do not respond to phenothiazine therapy alone.

Severely morbid symptoms such as hypertension, hyperpyrexia, convulsions, and even mortality have been reported with the combination of two classes of antidepressants, the MAO inhibitors and tricyclic compounds such as imipramine (207, 208) or amitriptyline (209). However, Schuckit *et al.* (210) recently suggested a reevaluation of combined therapy. They observed no significant side effects in their patients subjected to the combined therapy and reported that the medications were not discontinued as a result of adverse reactions.

CONCLUSION

Perhaps the most pressing problem in the field of MAO research at present is the determination of isozymes in various tissues and species. With the aid of isolated and purified isozymes, one may find out why the inhibitory activity of MAO inhibitors depends on the substrate used, why some inhibitors selectively block oxidation of one substrate but not of others, and why the enzyme in a particular tissue or organ is preferentially inhibited. Furthermore, work with these purified isozymes may resolve the question of whether there is an isozyme that is specific to a biogenic amine or whether an isozyme has more than one active site for oxidation of different types of substrates. With this information, inhibitors can then be designed to affect the metabolism of one biogenic amine without affecting that of another. The specific inhibition of MAO from one tissue or organ without separating all possible isozymes might be achieved by active-site-directed irreversible inhibitors, which should provide tissue specificity by exoalkylation (211).

For clinical use, a safe and effective MAO inhibitor with the following characteristics is desired. It should act specifically on brain MAO and have only weak or no effect on the peripheral enzyme; it should have no hepatotoxicity and should not potentiate the sympathomimetic effects of tyramine. Obtaining such an inhibitor is not impossible, in view of the reports that: (*a*) hepatotoxicity seems to be related to a particular structure, such as hydrazine and its derivatives, rather than to effects common to all MAO inhibitors; and (*b*) phenylisopropylmethylpropynylamine (E-250, XCVIII), in contrast to the known MAO inhibitors, not only does not potentiate but inhibits the pressor effect of tyramine in animals (212).

[9] Tofranil.
[10] Thorazine.

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ACKNOWLEDGMENTS AND ADDRESSES

Received from the *Texas Research Institute of Mental Sciences, Houston, TX 77025*

This review article is dedicated to the memory of Professor B. R. Baker, to whom I am indebted.

The author thanks Mrs. Lore Feldman and Mr. L. Wayne Tansey for assistance in editing, and Miss Gerry Musgrove and Mrs. Cathy Mandell for gathering references and typing. He also is grateful to Dr. James L. Claghorn of this institution and Dr. Vincent E. Friedewald, Jr., of The Methodist Hospital, Houston, for discussions on therapeutic use of MAO inhibitors.