Biochemical Pharmacology, 1967, Vol. 16, pp. 79–86. Pergamon Press Ltd., Printed in Great Britain.

THE INHIBITION OF MONOAMINE OXIDASE BY STYRYLQUINOLINIUMS

R. J. TAYLOR, JR., ELIZABETH MARKLEY and LEON ELLENBOGEN

Biochemistry Research Department, Lederle Laboratories,
American Cyanamid Co., Pearl River, N.Y., U.S.A.

(*Received* 8 *June* 1966; *accepted* 2 *August* 1966)

Abstract—A large group of styrylquinoliniums was found to inhibit the oxidative deamination of dopamine and other biogenic amines by guinea pig liver monoamine oxidase. 4-(*p*-Chlorostyryl)-1-methyl-quinolinium iodide was nearly five times more potent than harmaline and 1200 times more potent than iproniazid. The position of attachment of the styryl moiety to the quinolinium affected potency, the 4-styrylquinoliniums being more potent than the 2-styrylquinoliniums. Removal of the styryl moiety decreased potency, suggesting its participation in the inhibition. The effects of other structural modifications of the quinolinium and styryl moieties on the potency of inhibition are discussed.

MONOAMINE oxidase (E.C. 1.4.3.4. monoamine: O_2 oxidoreductase (deaminating)) is an enzyme or a group of enzymes catalyzing the oxidative deamination of several biogenic amines. Monoamine oxidase (MAO) appears to be localized in the mitochondrial fraction of cells of many tissues and species.[1] Several classes of compounds have been reported to inhibit MAO. Hydrazine derivatives represent a large class of MAO inhibitors, and these were recently reviewed by Biel *et al.*[2] Harmala alkaloids, indolealkylamines, propargylamines, cyclopropylamines, and aminopyrazines represent other groups of compounds reported to inhibit MAO.[3] The present report describes the inhibition of MAO *in vitro* by a new group of compounds, styrylquinoliniums.

EXPERIMENTAL

Guinea pig liver mitochondria were used as a source of monoamine oxidase. The mitochondria were isolated by the method of Hogeboom.[4] The final preparation was suspended in 0·88 M sucrose and stored at −5°.

Monoamine oxidase activity was determined by measuring the ammonia released during the oxidative deamination of dopamine (3-hydroxytyramine). The compounds tested were dissolved in 20% dimethylsulfoxide, and 0·5 ml aliquots were mixed with 250 μmoles of phosphate buffer (pH 7·0), 0·1 ml MAO preparation, and water to give a volume of 2·5 ml. After 5 min incubation at 37°, 1·0 ml of 0·01 M substrate was added. This amount of substrate was found to be optimal for the amount of enzyme used. After 30 min incubation, 1 ml of 0·35 N sodium carbonate was added, and the vessel was immediately stoppered. A glass "spoon" containing one drop of concentrated sulfuric acid was suspended inside the vessel from its stopper, and the vessel was incubated at 37° for 22 hr. These conditions allowed for complete recovery of the ammonia as ammonium sulfate. The acid drop was rinsed from the spoon with

4 ml water; 1·0 ml Nessler's reagent was added and a colorimetric reading obtained after 10 min. The colorimetric readings were compared directly to readings for non-inhibited dimethylsulfoxide controls for the calculation of per cent inhibition of MAO, or were converted to micrograms of ammonia by the use of a standard curve. The rate of formation of ammonia was found to be linear during the 30-min incubation period.

Dopamine hydrochloride, tryptamine hydrochloride, and tyramine hydrochloride were purchased from Nutritional Biochemicals Corp., *d,l*-norepinephrine hydrochloride from Calbiochem; and serotonin creatinine sulfate from Sigma Chemical Co. Phenylcyclopropylamine (Parnate) was obtained from Smith, Kline and French Laboratories; iproniazid (Marsilid) from Hoffmann LaRoche Co.; pheniprazine (JB 516, Catron) from Lakeside Laboratories; and harmaline from Aldrich Chemical Co. All styrylquinoliniums tested were in the form of iodides or chlorides.

RESULTS

Comparison with other MAO *inhibitors*

A large group of styrylquinoliniums was found to inhibit the oxidative deamination of dopamine by guinea pig liver MAO. Many inhibited at concentrations of 10^{-6} M and 10^{-7} M, and one compound showed significant inhibition at 10^{-8} M. A comparison of the MAO inhibition by three typical styrylquinoliniums with the inhibition by iproniazid (Marsilid), pheniprazine (JB 516, Catron), 2-phenylcyclopropylamine (Parnate), and harmaline is shown in Table 1. The most potent styrylquinolinium, 4-(*p*-chlorostyryl)-1-methyl-quinolinium iodide, inhibited 30 per cent at 1×10^{-8} M, being nearly 5 times more potent than harmaline and 1200 times more potent than iproniazid.

TABLE 1. COMPARISON OF INHIBITION OF MAO BY STYRYLQUINOLINIUMS AND OTHER INHIBITORS

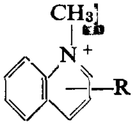
Compound	Concentration (M)	Inhibition (%)	Relative potency (iproniazid = 1)
4-(*p*-Chlorostyryl)-1-methyl-quinolinium	10^{-8}	30	1200
4-Styryl-1-methyl-quinolinium	10^{-7}	64	260
2-Styryl-1-methyl-quinolinium	10^{-6}	37	15
Harmaline	10^{-7}	71	280
2-Phenylcyclopropylamine (Parnate)	10^{-6}	59	24
Pheniprazine (JB 516, Catron)	10^{-6}	40	16
Iproniazid (Marsilid)	10^{-5}	25	1

Structure–activity relations

Four pairs of styrylquinoliniums, differing only in the position of attachment of the styryl group to the quinolinium, are compared in Table 2. In each case the 4-styryl compound was from 10 to 100 times more potent than the 2-styryl compound.

The degree of inhibition was found to be affected by halogenation of the benzene ring of the styryl group (Table 3). The *p*-chlorostyrylquinolinium was considerably more potent than the parent styrylquinolinium in both the 2-substituted and 4-substituted compounds. In the case of the 2-styrylquinoliniums, the *o*-chlorostyryl

TABLE 2. EFFECT OF POSITION OF STYRYL GROUP ON MAO INHIBITION BY STYRYLQUINOLINIUMS



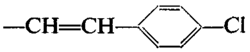
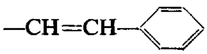
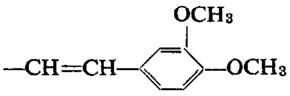
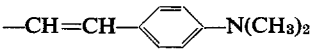
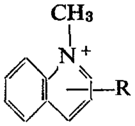
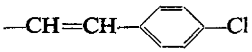
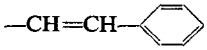
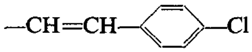
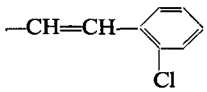
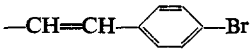
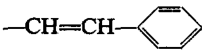
R	Position of R	Concentration (M)	Inhibition (%)
	4	10^{-7}	90
	2	10^{-6}	61
	4	10^{-7}	64
	2	10^{-6}	37
	4	10^{-7}	64
	2	10^{-5}	69
	4	10^{-7}	43
	2	10^{-6}	59

TABLE 3. EFFECT OF HALOGENATION OF STYRYL GROUP ON MAO INHIBITION

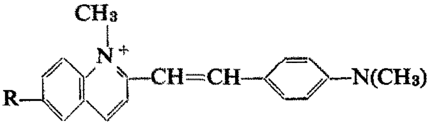


R	Position of R	Concentration (M)	Inhibition (%)
	4	10^{-8}	30
	4	10^{-7}	64
	2	10^{-6}	61
	2	10^{-6}	62
	2	10^{-6}	55
	2	10^{-6}	37

compound was as potent as the *p*-chlorostyryl compound, and the *p*-bromostyryl derivative only slightly less potent.

Some effects on MAO inhibitory activity were observed with structural modification of the quinoline group. The 6-methoxy derivative of 2-(*p*-dimethylaminostyryl)-1-methyl-quinolinium was ten times more potent than the parent compound (Table 4). However, only slight changes in potency were observed when dimethylamino- or chloro- groups were substituted at the 6-position.

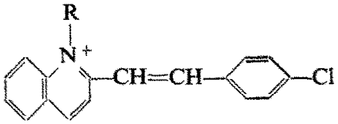
TABLE 4. EFFECT OF 6-SUBSTITUTION OF THE QUINOLINE GROUP ON MAO INHIBITION



R	Concentration (M)	Inhibition (%)
H	10^{-6}	59
CH_3O	10^{-7}	61
Cl	10^{-6}	66
$N(CH_3)_2$	10^{-6}	31

Only very slight increases in inhibition were observed with higher N-alkyl quinoliniums (Table 5). Increasing the carbon length of the N-alkyl group of 2-(*p*-chlorostyryl) quinolinium iodide from 1 to 4 increased inhibition by only 11 per cent. A larger difference in potency was observed between the 1-methyl quinolinium and the nonalkylated quinoline.

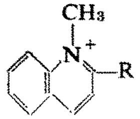
TABLE 5. EFFECT OF HIGHER N-ALKYL QUINOLINIUMS ON MAO INHIBITION

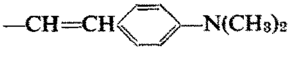


R	Concentration (M)	Inhibition (%)
None	10^{-6}	45
CH_3	10^{-6}	61
C_2H_5	10^{-6}	66
n-C_3H_7	10^{-6}	68
n-C_4H_9	10^{-6}	72

The replacement of the styryl moiety by a phenyl group reduced MAO inhibition 10-fold (Table 6). 2-(*p*-Dimethylaminostyryl)-1-methyl-quinolinium inhibited 58 per cent at 10^{-6} M, whereas 2-(*p*-dimethylaminophenyl)-1-methyl-quinolinium inhibited 55 per cent at 10^{-5} M. 2-Dimethylamino-1-methyl-quinolinium was only slightly less potent than the dimethylaminophenyl derivative.

TABLE 6. EFFECT OF REPLACEMENT OF THE STYRYL GROUP ON MAO INHIBITION



Compound	R	Concentration (M)	Inhibition (%)
2-(*p*-Dimethylaminostyryl)-1-methyl-quinolinium		10^{-6}	58
2-(*p*-Dimethylaminophenyl)-1-methyl-quinolinium		10^{-5}	55
2-Dimethylamino-1-methyl-quinolinium	$—N(CH_3)_2$	10^{-5}	36

Studies of mechanism of inhibition

4-Styryl-1-methyl-quinolinium iodide was used as a typical styrylquinolinium for studies of the mechanism of inhibition. The time course of inhibition was found to be linear over the 30-min period used in the assay, and the inhibition was constant (64 ± 3 per cent) during the same period, with 1×10^{-7} M inhibitor. No significant increase in inhibition was observed when the enzyme was preincubated with the inhibitor for 35 min instead of the 5-min preincubation in the standard procedure.

Double-reciprocal plots of dopamine concentration against rate of deamination showed the inhibition to be competitive with substrate (Fig. 1). The inhibition was found to be reversible (Fig. 2) according to the criterion of Ackermann and Potter[5] that the degree of inhibition by a reversible inhibitor is independent of enzyme concentration and depends only on the inhibitor concentration.

The styrylquinoliniums also inhibited the oxidation of other biogenic amines (Table 7). The oxidation of tyramine and serotonin was inhibited to nearly the same extent as dopamine, while inhibition of oxidation of tryptamine and norepinephrine was considerably less.

DISCUSSION

Styrylquinoliniums represent a new group of potent inhibitors of monoamine oxidase *in vitro*, some being considerably more potent than any other MAO inhibitors reported. 4-(*p*-Chlorostyryl)-1-methyl-quinolinium was nearly five times more potent than harmaline and 1200 times more potent than iproniazid. Studies of the mechanism of inhibition using 4-styryl-1-methyl-quinolinium iodide indicate the inhibition to be competitive with substrate (dopamine) and reversible.

Significant differences in the degree of inhibition were observed when tyramine, serotonin, and tryptamine were used as substrates in place of dopamine. With norepinephrine as a substrate, much less inhibition was observed. Guha[6] has similarly reported different degrees of inhibition of purified rat liver mitochondrial MAO by 2-phenylcyclopropylamine.

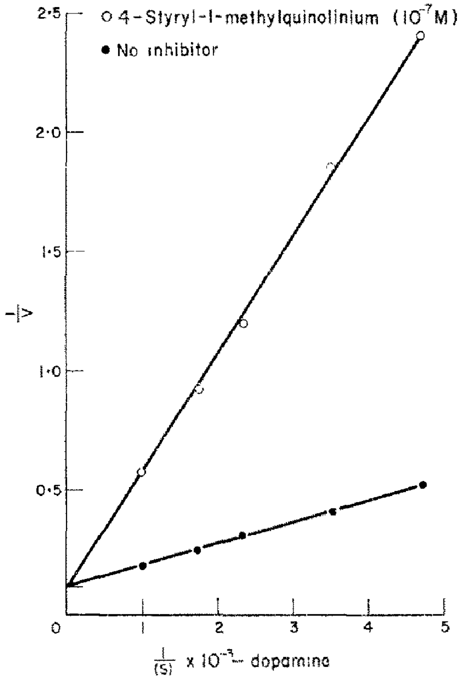


Fig. 1. Inhibition of MAO by 4-styryl-1-methyl-quinolinium iodide (1×10^{-7} M). Activity was measured in the standard incubation mixture with substrate concentrations ranging from 1×10^{-8} M to $2{\cdot}14 \times 10^{-4}$ M (V = μg NH_3/ml/30 min).

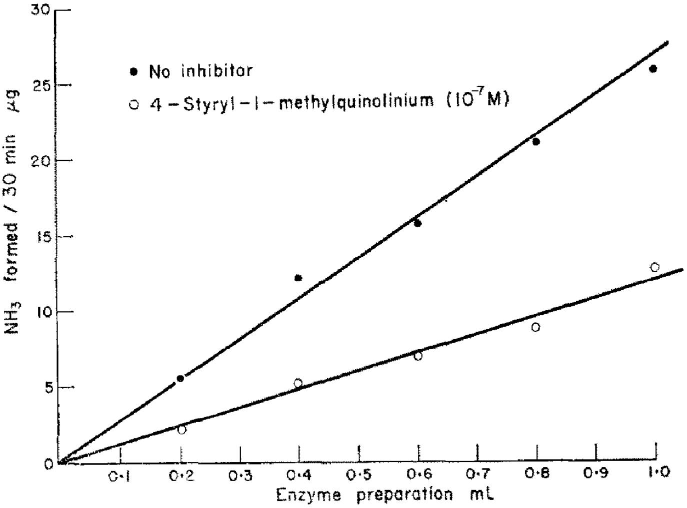


Fig. 2. Reversible inhibition of MAO by 4-styryl-1-methyl-quinolinium iodide.

Although some differences in potency were observed by structural modification of the quinoline moiety (Tables 4 and 5), greater effects were observed by modification of the styryl moiety. The position of attachment of the styryl group to the quinoline has a marked effect on potency, the 4-styrylquinoliniums being more potent than the 2-styrylquinoliniums (Table 2). Ring halogenation of the styryl moiety also enhanced potency (Table 3).

TABLE 7. INHIBITION OF THE OXIDATION OF BIOGENIC AMINES BY 4-STYRYL-1-METHYL-QUINOLINIUM IODIDE

Substrate*	Inhibition† (%)
Dopamine	46
Tyramine	66
5-Hydroxytryptamine (serotonin)	50
Tryptamine	31
Norepinephrine	8

* Substrate concentrations were 2·85 × 10^{-3}M.

† 4-Styryl-1-methyl-quinolinium iodide concentration was 1·0 × 10^{-7} M.

Replacement of the styryl group by other groups resulted in a decrease in potency. Removal of the ethylenic moiety alone reduced potency, as did complete removal of the styryl group (Table 6). This suggests that the high degree of inhibition by styrylquinoliniums may be related to the presence of the styryl group, and more specifically its ethylenic moiety. This is of significant interest because of the structural similarity between the styryl group and the phenethyl group of catecholamines, and also in view of the mechanism of inhibition of MAO suggested by Belleau and Moran.[7] They have proposed that the transition state of the MAO–substrate complex involves the formation of a double bond between the α- and β-carbons of the substrate, with the π-electrons generated contributing greatly to the binding energy in the transition state. They further suggest that the inhibition by 2-phenylcyclopropylamine (Parnate) may be due to the binding of its nucleophilic sp^2-like cyclopropane bond to an electrophilic site on the enzyme. Similarly, the presence of a double bond in the styryl group of the styrylquinoliniums may account for their high degree of inhibition. That the enhancement of inhibition by the styryl group may be due to a steric effect of chain length and not only to electronic effects has not been ruled out. Styrylquinoliniums may thus be of significant value in the elucidation of the mechanism of action of monoamine oxidase.

Acknowledgement—The authors are deeply indebted to Drs. P. H. Bell and B. L. Hutchings for their helpful discussions.

REFERENCES

1. E. O. OSWALD and C. F. STRITTMATTER, *Proc. Soc. exp. Biol. Med.* **114**, 668 (1963).
2. J. H. BIEL, A. HORITA and A. E. DRUKKER, in *Psychopharmacological Agents*, Vol. 1 (Med. Chem. Series, Vol. 4), p. 359. Academic Press, New York (1964).

3. C. L. ZIRKLE and C. KAISER, in *Psychopharmacological Agents*, vol. 1 (Med. Chem. Series, Vol. 4), p. 445. Academic Press, New York (1964).
4. G. H. HOGEBOOM, in *Methods in Enzymology*, vol. 1, p. 16 Academic Press, New York (1955).
5. W. W. ACKERMANN and V. R. POTTER, *Proc. Soc. exp. Biol. Med.* **72**, 1 (1949).
6. S. R. GUHA, *Biochem. Pharmac.* **15**, 161 (1966).
7. B. BELLEAU and J. MORAN, *Ann. N.Y. Acad. Sci.* **107**, 822 (1963).