ACKNOWLEDGMENTS AND ADDRESSES

Received June 4, 1971, from *Proprietaries and Toiletries Research, Consumer Products Division, Warner-Lambert Company, Morris Plains, NJ 07950*

Accepted for publication September 16, 1971.

Presented to the Pharmacology and Biochemistry Section, APhA Academy of Pharmaceutical Sciences, San Francisco meeting, March 1971.

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Relationship between MAO Inhibitory and Anticonvulsant Properties of Substituted Cinnamides

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Abstract □ Substituted cinnamides were synthesized to investigate their *in vitro* MAO inhibitory properties and their ability to protect against convulsions produced in mice by subcutaneous injections of pentylenetetrazol. The degree of protection offered by these compounds in no way paralleled their enzyme inhibitory properties.

Keyphrases □ MAO inhibitors, cinnamides—synthesis, correlation to anticonvulsant activity □ Cinnamides, substituted—synthesis, relationship between MAO inhibition and anticonvulsant activity □ Structure–activity relationships—cinnamides, MAO inhibition □ Spectrophotofluorometry—determination of MAO activity, cinnamides □ Manometry (Warburg)—determination of MAO activity, cinnamides

A large group of styrylquinoliniums (1), hydrazine derivatives (2), and semicarbazides (3, 4) are known as MAO [EC 1.4.3.4 monoamine:O_2 oxidoreductase (deaminating)] inhibitors. The high degree of inhibition by styrylquinoliniums was proposed to be related to the presence of the styryl group and, more specifically, to its ethylenic moiety (1). This is of 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 (5). Furthermore, MAO inhibitors were reported to possess antidepressant (6) and pronounced anticonvulsant properties (7, 8), presumably due to an increase in the concentration of brain amines. Certain derivatives of cinnamic acid reported to exhibit anticonvulsant activity (9, 10) and the ability of oleic acid to inhibit the enzyme MAO (11) led the authors to synthesize some cinnamides as MAO inhibitors. Attempts were made to correlate their MAO inhibitory properties with their anticonvulsant activities as a function of their chemical structure.

CHEMISTRY

The various substituted cinnamides, synthesized by the route outlined in Scheme I, are recorded in Table I.

Hippuric acid (I*a*) was synthesized by the benzoylation of glycine which, on treatment with suitable aromatic aldehydes and acetic anhydride in the presence of anhydrous sodium acetate, was converted into corresponding substituted oxazolones (I*b*). Compounds I*b* failed to react with benzocaine to give I–III in the absence of triethylamine. In the present study, it was found that the addition of two to three drops of triethylamine was sufficient to start the reaction, indicating that the presence of the slightly basic medium is essential to open the oxazolone ring. Corresponding hydrazides (IV–VI) were prepared by refluxing I–III with hydrazine hydrate (99–100%) in absolute ethanol. From these hydrazides (IV–VI), substituted semicarbazides (VII–XVIII) were prepared by refluxing them with suitable arylisocyanates in dry benzene. Substituted thiosemicarbazides (XIX–XXX) were obtained by refluxing a mixture of IV–VI and suitable arylisothiocyanates in ethanol.

EXPERIMENTAL

Melting points were taken in open capillary tube and were corrected.

Hippuric Acid (I*a*)—This was synthesized by the benzoylation of glycine according to a method reported earlier (12).

2-Phenyl-4-(*p*-substituted-benzylidene)oxazole-5-ones (I*b*)—The various oxazolones were prepared by heating a mixture of 0.96 mole of aromatic aldehyde, 1.07 moles of powdered dry hippuric acid, 0.98 mole of powdered freshly fused sodium acetate, and 2.9 moles of high grade acetic anhydride on an electric hot plate. As soon as all of the constituents melted out, the mixture was heated on a steam bath for 2 hr. The mixture was allowed to stand overnight, and the solid mass which separated out was filtered and washed with 100-ml. portions of ice-cold ethanol and finally with 100 ml. of boiling water. The various 2-phenyl-4-(*p*-substituted-benzylidene)-oxazol-5-ones were collected by filtration, dried, and used for subsequent reaction without further purification. The melting points of these compounds were found to correspond with values reported in the literature (13, 14).

α-Benzoylamino-*N*-(*p*-ethylbenzoate)-*p*-substituted-cinnamides (I–III)—These compounds were synthesized by refluxing equimolar portions of appropriate 2-phenyl-4-(*p*-substituted-benzylidene)-oxazole-5-one and benzocaine in absolute ethanol in the presence of two to three drops of triethylamine on a steam bath for 6–8 hr. On distilling the excess of ethanol, the cinnamides which separated out were filtered and recrystallized from suitable solvents. The cinnamides were characterized by their sharp melting points and elemental analyses.

α-Benzoylamino-*N*-(*p*-benzhydrazide)-*p*-substituted-cinnamides (IV–VI)—The various α-benzoylamino-*N*-(*p*-ethylbenzoate)-*p*-substituted-cinnamides were refluxed with 99–100% hydrazine hydrate (1:2 molar ratio) in absolute ethanol on a steam bath for 6–8 hr. On cooling, the solid mass which separated out was filtered and recrystallized from the appropriate solvent.

α-Benzoylamino-*N*-[*p*-(4-substituted-phenylsemicarbazides)-benzoyl]-*p*-substituted-cinnamides (VII–XVIII)—Equimolar portions of the suitable α-benzoylamino-*N*-(*p*-benzhydrazide)-*p*-substituted-cinnamide and arylisocyanate were mixed in dry benzene and re-

Table I—Physical Constants of Substituted Cinnamides

Compound	Yield, %	Melting Point	Recrystallization Solvent[a]	Analysis, % C Calc.	C Found	H Calc.	H Found	N Calc.	N Found
I	80	139°	A	72.46	72.48	5.31	5.51	6.76	7.00
II	85	145°	A	70.27	70.64	5.40	5.21	6.30	5.87
III	80	148°	A	66.88	66.75	4.68	5.01	6.24	6.41
IV	79	227°	B	69.00	68.58	5.00	5.09	14.00	14.48
V	80	230°	B	66.97	66.80	5.11	5.58	13.02	13.20
VI	85	260–261°	C	63.52	63.53	4.37	4.79	12.89	13.06
VII	75	193°	A	69.36	68.95	4.81	5.14	13.48	13.40
VIII	67	200°	A	69.79	69.70	5.06	5.36	13.13	13.50
IX	90	205–207°	B	69.79	69.97	5.06	5.46	13.13	13.54
X	78	200°	A	69.79	69.77	5.06	5.50	13.13	12.98
XI	80	195°	B	67.75	67.84	4.91	4.49	12.75	13.00
XII	70	208°	B	68.20	68.42	5.15	5.09	12.43	12.70
XIII	90	180°	A	68.20	68.43	5.15	5.43	12.43	12.92
XIV	80	219°	A	68.20	67.98	5.15	5.46	12.43	12.76
XV	84	197°	D	65.04	64.80	4.33	4.12	12.64	12.98
XVI	84	222–223°	B	65.55	65.76	4.59	5.00	12.33	12.43
XVII	89	242°	A	65.55	65.10	4.59	4.67	12.33	12.52
XVIII	85	140°	A	65.55	65.47	4.59	5.07	12.33	12.57
XIX	75	115°	E	67.28	67.52	4.67	5.00	13.08	12.10
XX	76	195°	E	67.75	67.77	4.91	5.01	12.75	13.02
XXI	90	105°	E	67.75	67.55	4.91	4.51	12.75	12.55
XXII	90	160°	F	67.75	67.69	4.91	4.72	12.75	13.00
XXIII	80	215°	A	65.84	65.62	4.77	4.25	12.38	12.58
XXIV	70	95°	E	66.30	66.72	5.00	4.62	12.08	11.71
XXV	95	155°	F	66.30	66.50	5.00	4.98	12.08	11.98
XXVI	85	111°	E	66.30	66.54	5.00	5.43	12.08	12.43
XXVII	84	100°	E	63.21	62.98	4.21	4.60	12.29	12.57
XXVIII	84	105°	E	63.75	63.52	4.45	5.00	11.99	12.01
XXIX	85	218°	E	63.75	63.52	4.45	4.76	11.99	12.00
XXX	90	235°	E	63.75	63.99	4.45	4.92	11.99	12.23

[a] A = ethanol-water, B = ethanol, C = dioxane, D = acetronitrile, E = benzene–petroleum ether (60–80°), and F = benzene-ethanol.

fluxed on a steam bath for 6 hr. The solid which separated out was filtered and washed with ether, dilute hydrochloric acid, and, finally, water. The product was dried and recrystallized with an appropriate solvent. The cinnamides thus obtained in good yields were characterized by their sharp melting points and elemental analyses.

α - Benzoylamino - *N* - [*p* - (4 - substituted - phenylthiosemicarbazides)-benzoyl]-*p*-substituted-cinnamides (XIX–XXX)—These cinnamides were prepared by refluxing a mixture of equimolar quantities of the appropriate α-benzoylamino-*N*-(*p*-benzhydrazide)-*p*-substituted-cinnamide and arylisothiocyanate in a minimum quantity of absolute ethanol for 48 hr. The solid mass which separated out on cooling was filtered and washed with dilute hydrochloric acid and then with water. The crude product was dried and recrystallized from the suitable solvent. The different cinnamides thus obtained were characterized by their sharp melting points and elemental analyses.

ASSAY OF MAO

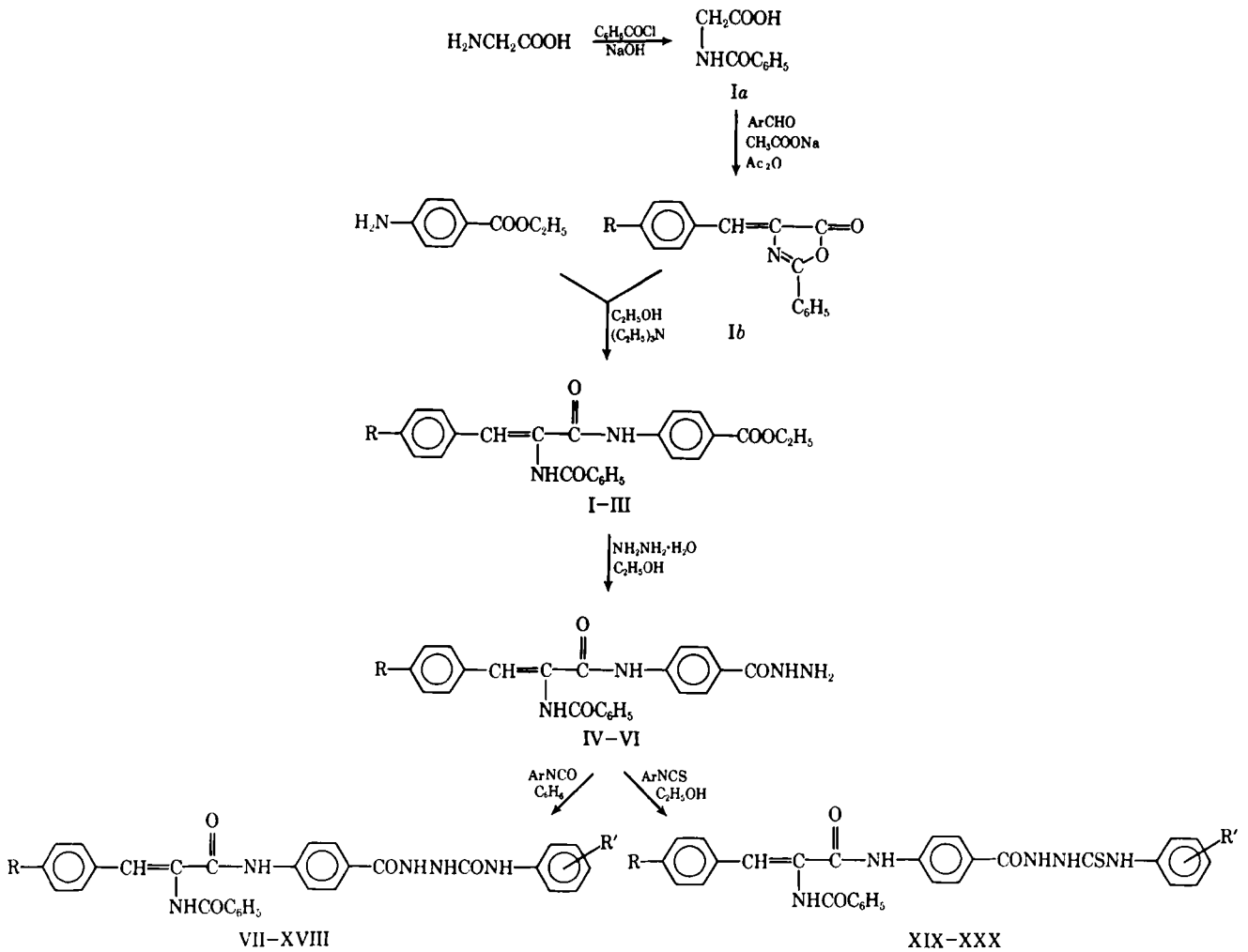
Male adult rats, weighing approximately 100–150 g., were killed by decapitation. Brains were quickly removed and homogenized in ice-cold 0.25 *M* sucrose with the help of a homogenizer (Potter-Elvehjem). The brain homogenate thus obtained was used as the source of MAO.

Spectrophotofluorometric Method—The method described by Krajl (15) was used for the determination of MAO activity of rat brain homogenate, using kynuramine as a substrate. The 4-hydroxyquinoline formed during oxidative deamination of kynuramine was measured fluorometrically in a spectrophotofluorometer (Aminco Bowman) using activating light of 315 nm. and measuring fluorescence at a maximum of 380 nm. The reaction mixture consisted of 0.5 ml. of phosphate buffer (pH 7.4, 0.5 *M*), 0.5 ml. of kynuramine (100 mcg.), and 0.5 ml. of brain homogenate (corresponding to 10 mg. of wet weight of the tissue). The MAO activity of the brain homogenate was determined by incubation for 30 min. at 37°. The various inhibitors used at the final concentration of 5×10^{-4} *M* were incubated with the brain homogenate for 10 min. before the addition of kynuramine. The mixture was further incubated for 30 min. The reaction was stopped by the addition of 1 ml. of 10% trichloroacetic acid and the precipitated proteins were removed by centrifugation. Suitable aliquots of the supernatant were taken in 1 *N* NaOH solution and were assayed for 4-hydroxyquinoline. An increase in the optical density provided a direct measurement of the 4-hydroxyquinoline, which was taken as an index of the enzyme activity. The percent inhibition was calculated from the decrease observed in the optical density.

Warburg Manometric Method—The MAO activity of rat brain homogenate was determined by the conventional Warburg manometric technique as described earlier (16), using tyramine as the substrate. The decrease in the oxygen uptake during oxidative deamination of tyramine in the presence of the cinnamides was used as an index of the enzyme inhibition. The reaction mixture (3 ml.) contained 66 m*M* phosphate buffer (pH 7.4), 10 m*M* tyramine, and the homogenate equivalent to 250 mg. of fresh tissue. The inhibitors were incubated with the enzyme preparation for 20 min. before the addition of the substrate. The enzyme system was further incubated at 37° using oxygen as the gas phase for an additional hour. The readings of the oxygen uptake were taken every 10 min. The inhibitors were used at a final concentration of 1×10^{-3} *M*.

Determination of Anticonvulsant Activity—Anticonvulsant property was tested against pentylenetetrazol[1]-induced seizures in albino mice of either sex weighing between 25 and 30 g. The mice were divided in groups of 10, keeping the group weights equal as far as possible. One group of 10 animals was used for one dose of the compounds. Cinnamides (100 mg./kg.), suspended in 5% aqueous gum acacia, were administered intraperitoneally. Four hours after the administration of the compounds, pentylenetetrazol was injected subcutaneously under the loose skin of the back in a dose of 80 mg./kg. This dose of pentylenetetrazol has been shown (8) to cause convulsions in almost all normal animals. The animals were observed for the next 60 min. for the occurrence of seizures. One episode of clonic spasm, which persisted for at least 5 sec., was considered a threshold convulsion. Transient intermittent jerks and tremulousness were not taken into account. Animals not exhibiting even a threshold convulsion during the 60-min. period were considered protected. The number of animals protected in each group was recorded, and percentage protection was determined for each compound.

[1] Metrazole, Knoll Pharmaceutical Co.

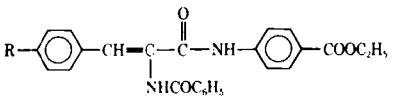


Scheme I

DISCUSSION

The present study deals with the inhibitory effects of the substituted cinnamides on MAO activity of rat brain homogenate and their ability to exhibit protection against pentylenetetrazol-induced seizures. Both spectrophotofluorometric and manometric methods were used for the determination of MAO activity. The enzyme inhibitory effects of these compounds during oxidative deamination of kynuramine and tyramine are shown in Tables II–IV. As is evident from Table IV, the degree of MAO inhibition during oxidative deamination of tyramine was greater with α-benzoylamino-*N*-[*p*-(4-substituted-phenylsemicarbazide/thiosemicarbazide)-benzoyl]-*p*-(R)-substituted-cinnamides possessing *m*-CH_3 alone than with *o*-CH_3 or *p*-CH_3 as the R′ substituent. In general, the MAO inhibitory property of α-benzoylamino-*N*-[*p*-(4-substituted-phenylthiosemicarbazide)-benzoyl]-*p*-substituted-cinnamides was found to be higher than that of corresponding semicarbazides. During the primary studies, the degree of MAO inhibition using tyramine as a

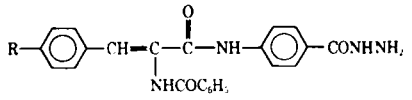
Table II—Inhibition of MAO by α-Benzoylamino-*N*-(*p*-ethylbenzoate)-*p*-substituted-cinnamides and Their Anticonvulsant Properties



Compound	R	MAO Inhibition[a], Kynuramine	MAO Inhibition[a], Tyramine	Anticonvulsant Activity, Protection, %	Anticonvulsant Activity, 24-hr. Mortality, %
I	H	70.6 ± 0.5	28.8 ± 0.9	70	20
II	OCH_3	54.6 ± 0.9	17.0 ± 0.8	60	10
III	Cl	48.2 ± 1.0	13.0 ± 1.1	10	20

[a] Vessel contents and assay procedures are as indicated in the text. Each experiment was done in duplicate. Figures indicate mean values.

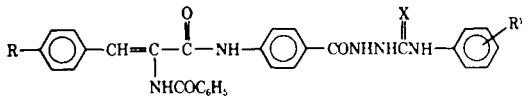
Table III—Inhibition of MAO by α-Benzoylamino-*N*-(*p*-benzhydrazide)-*p*-substituted-cinnamides and Their Anticonvulsant Properties



Compound	R	MAO Inhibition[a], % Kynuramine	MAO Inhibition[a], % Tyramine	Anticonvulsant Activity, Protection, %	Anticonvulsant Activity, 24-hr. Mortality, %
IV	H	59.3 ± 0.7	35.4 ± 0.8	50	20
V	OCH_3	48.8 ± 0.8	28.7 ± 0.8	40	10
VI	Cl	36.7 ± 0.6	41.1 ± 0.9	40	10

[a] Vessel contents and assay procedures are as indicated in the text. Each experiment was done in duplicate. Figures indicate mean values.

Table IV—Inhibition of MAO by α-Benzoylamino-*N*-[*p*-(4-substituted-phenylsemicarbazide/thiosemicarbazide)-benzoyl]-*p*-substituted-cinnamides and Their Anticonvulsant Properties



Compound	R	R'	X	MAO Inhibition[a], % Kynuramine	MAO Inhibition[a], % Tyramine	Anticonvulsant Activity Protection, %	Anticonvulsant Activity 24-hr. Mortality, %
VII	H	H	O	59.2 ± 0.9	17.0 ± 1.5	10	40
VIII	H	*o*-CH_3	O	59.5 ± 0.5	39.0 ± 1.4	30	20
IX	H	*m*-CH_3	O	64.1 ± 0.6	28.1 ± 1.3	70	10
X	H	*p*-CH_3	O	56.2 ± 0.5	19.2 ± 1.0	40	30
XI	OCH_3	H	O	42.2 ± 0.5	60.6 ± 1.3	40	50
XII	OCH_3	*o*-CH_3	O	51.5 ± 0.5	30.9 ± 1.3	50	50
XIII	OCH_3	*m*-CH_3	O	59.2 ± 1.1	40.5 ± 1.4	40	30
XIV	OCH_3	*p*-CH_3	O	63.7 ± 0.6	19.2 ± 1.2	40	10
XV	Cl	H	O	46.6 ± 1.0	39.8 ± 0.9	50	10
XVI	Cl	*o*-CH_3	O	54.4 ± 0.5	28.8 ± 1.1	50	Nil
XVII	Cl	*m*-CH_3	O	40.9 ± 0.8	44.0 ± 1.4	20	10
XVIII	Cl	*p*-CH_3	O	61.7 ± 0.7	18.0 ± 0.9	30	20
XIX	H	H	S	76.3 ± 0.5	35.0 ± 0.9	30	50
XX	H	*o*-CH_3	S	72.8 ± 0.7	21.8 ± 1.1	30	60
XXI	H	*m*-CH_3	S	76.4 ± 0.4	31.0 ± 1.1	10	70
XXII	H	*p*-CH_3	S	73.2 ± 0.9	29.2 ± 0.8	10	40
XXIII	OCH_3	H	S	87.4 ± 0.5	19.8 ± 0.8	50	40
XXIV	OCH_3	*o*-CH_3	S	75.6 ± 0.6	32.0 ± 0.9	60	10
XXV	OCH_3	*m*-CH_3	S	59.6 ± 0.6	66.7 ± 1.4	30	10
XXVI	OCH_3	*p*-CH_3	S	72.0 ± 0.5	23.9 ± 0.9	Nil	70
XXVII	Cl	H	S	74.6 ± 0.6	49.0 ± 1.3	50	30
XXVIII	Cl	*o*-CH_3	S	64.8 ± 0.8	29.2 ± 0.9	60	100
XXIX	Cl	*m*-CH_3	S	67.7 ± 0.6	45.2 ± 1.2	20	50
XXX	Cl	*p*-CH_3	S	50.9 ± 0.6	38.6 ± 1.2	Nil	50

[a] Vessel contents and assay procedures are as indicated in the text. Each experiment was done in duplicate. Figures indicate mean values.

substrate was found to be very low; therefore, a final concentration of 1×10^{-3} *M* of various inhibitors was used. On the other hand, the use of these inhibitors at a final concentration of 5×10^{-4} *M* produced significant inhibition when kynuramine was used as the substrate. The degree of MAO inhibition thus observed, reflected a significant difference with the choice of the substrate used in these *in vitro* experiments.

Different degrees of enzyme inhibition also were reported with purified rat liver mitochondrial MAO by 2-phenylcyclopropylamine, using different biogenic amines as substrates (17). Similar differences in the degrees of inhibition were observed when tyramine, serotonin, tryptamine, and dopamine were used as the substrates (1). The anticonvulsant property of these inhibitors, as indicated by protection from pentylenetetrazol-induced seizures, is given in Tables II–IV. It was found that the degree of protection offered by these compounds in no way paralleled their enzyme inhibitory properties. These results provided evidence that MAO inhibition is presumably not the only mechanism responsible for the anticonvulsant properties of these substituted cinnamides. Further studies dealing with the synthesis of other related structures carrying different substituents and the determination of their nature of enzyme inhibition could prove useful toward elucidation of the chemical requirements of the active center(s) of MAO.

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 18, 1971, from the *Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow University, Lucknow, India.*

Accepted for publication September 3, 1971.

Supported by the Council of Scientific and Industrial Research, New Delhi, and the State Council of Scientific and Industrial Research, Lucknow, in the form of Junior Research Fellowships to A. K. Chaturvedi and to R. S. Misra and A. Chaudhari, respectively.

The authors thank Dr. M. L. Dhar and Dr. Nitya Nand of the Central Drug Research Institute, Lucknow, for providing microanalysis facilities, and Professor Frank S. LaBella, University of Manitoba, Winnipeg, Canada, for the supply of kynuramine. The generous gifts of the Aminco Bowman spectrophotofluorometer by the Rockefeller Foundation, New York, N. Y., and the research chemicals by the Riker Laboratories, Northridge, Calif., are gratefully acknowledged.

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