Allenic Amines: A New Class of Nonhydrazine MAO Inhibitors

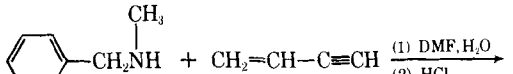
By R. P. HALLIDAY, C. S. DAVIS, J. P. HEOTIS, D. T. PALS, E. J. WATSON*, and R. K. BICKERTON

N-2,3-Butadienyl-*N*-methylbenzylamine HCl (U-1247) and *N*-2,3-butadienyl-*N*-methyl-*o*-chlorobenzylamine HCl (U-1379) have been synthesized and potent *in vivo* monoamine oxidase inhibition demonstrated. Antihypertensive activity comparable to that produced by pargyline was demonstrated in hypertensive rats with U-1247. When administered to mice, U-1247 and U-1379 produced initial stimulation of motor activity in contrast with pargyline which produced initial depression.

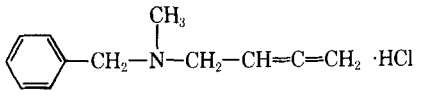
Swett *et al.* (1) in 1963 described the structural requirements necessary for monoamine oxidase inhibitory (MAOI) activity within the pargyline series. These workers indicated that a triple bond was essential for MAOI activity. It was the purpose of this study to synthesize and evaluate the activity of two allenic derivatives of pargyline which contained no triple bond.

N-2,3-Butadienyl-*N*-methylbenzylamine hydrochloride (U-1247) was prepared by a modification of the general method reported by Engelhardt (2). Engelhardt condensed water-soluble amines with vinylacetylene (I) in water to produce allenic amines. Since methylbenzylamine (II) is only slightly soluble in water, a mixture of *N*,*N*-dimethylformamide and water was used as the reaction solvent.

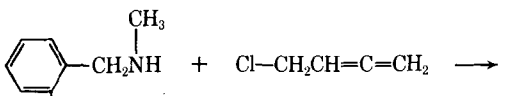
N - 2,3 - Butadienyl - *N* - methyl - *o* - chlorobenzylamine hydrochloride (U-1379) was prepared by the method of Vartanian and Badanian (3). *o*-Chloro-*N*-methylbenzylamine (II*a*) was alkylated with 4-chloro-1,2-butadiene (III) to give U-1379. U-1247 may be prepared by alkylation of *N*-methylbenzylamine (II) with III.



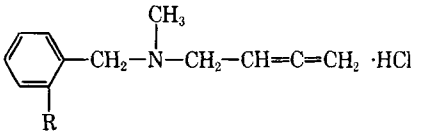
(II) (I)



U-1247



(II or II*a*) (III)



R = H (U-1247) and Cl (U-1379)

EXPERIMENTAL

N-2,3-Butadienyl-N-methylbenzylamine Hydrochloride (U-1247)—Into a 1-L. Parr bomb (capacity about 854 ml.) was placed a mixture of benzylmethylamine (121 Gm., 1.0 mole), 130 ml. of water, and 170 ml. of *N*,*N*-dimethylformamide. The homogeneous mixture was cooled by placing the bomb in a dry ice–acetone bath while nitrogen was bubbled through the mixture. After the mixture had cooled to −30°, vinylacetylene (52 Gm., 1.0 mole) was condensed into the bomb. The bomb was closed, pressurized to 150 p.s.i. with nitrogen, and heated to 70–98° for 20 hr. with shaking.

The bomb was allowed to cool for 1 day before venting and purging with nitrogen. The contents of the bomb were removed and steam distilled immediately. The steam distillate was transferred to a separator and extracted with two 1-L. portions of ether. The ethereal extracts were dried over anhydrous sodium sulfate and then concentrated under vacuum. The residue was distilled, b.p. 85–94°/2.0 mm., yield 21 Gm. (12%). The infrared spectrum indicated a strong band at 5.10 μ which is characteristic of the allene group. No absorption bands characteristic of acetylenic compounds were observed. Vapor-phase chromatography indicated the product consisted of a single material.

One gram of distilled material was treated with an ethereal solution of hydrogen chloride. An oil separated which crystallized with rubbing. The solid was recrystallized from ethyl acetate to give a material with a melting point of 118–120°.

Anal.—Calcd. for $C_{13}H_{16}ClN$: C, 68.72; H, 7.69; Cl, 16.91; N, 6.68. Found: C, 68.70, 68.70; H, 7.54, 7.63, 6.12; Cl, 17.21, 17.08; N, 6.11.

N-2,3-Butadienyl-N-methyl-o-chlorobenzylamine Hydrochloride (U-1379)—*o*-Chloro-*N*-methylbenzylamine (Sapon Labs.) (122 Gm., 0.8 mole), 5 ml. of water, and 4-chloro-1,2-butadiene (3) (75 Gm., 0.85 mole) were allowed to stand for 5 days at room temperature. To the mixture was added, with vigorous shaking, 200 ml. of a saturated solution of sodium carbonate. The organic layer was extracted with two 150-ml. portions of ether. The

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ethereal extracts were dried over anhydrous sodium sulfate and then concentrated under vacuum. The residue was distilled, b.p. (1st fraction) 45–78°/1 mm.; (2nd fraction) 79–105°/1 mm. There was about 80 Gm. in the second fraction which was redistilled. The material boiling at 81–91°/0.2 mm. was taken as product, yield 45 Gm. (27%). The infrared spectrum showed a strong allenic band and no acetylenic band.

A small amount of the free base was dissolved in dry ether and treated with an ethereal solution of hydrogen chloride. An oil separated that crystallized with rubbing. The solid was recrystallized from benzene to give a material with a melting point of 121–122°.

Anal.—Calcd. for $C_{12}H_{15}Cl_2N$: C, 59.03; H, 6.19; N, 5.74. Found: C, 58.72, 58.79; H, 6.19, 6.32; N, 5.68, 5.67.

N-2,3-Butadienyl-*N*-methylbenzylamine hydrochloride (U-1247) may be prepared by the above method, yield 9%.

Monoamine Oxidase Inhibition—The colorimetric method of Stern *et al.* (4) was used to determine monoamine oxidase activity. MAO in the presence of oxygen catalyzes the conversion of tyramine to *p*-hydroxyphenylacetaldehyde (*p*HPA) which is trapped as the semicarbazone in the incubation mixture. Thus, inhibition of MAO activity by a drug results in a decrease in the production of *p*HPA. Potassium cyanide is added to the incubation mixture to inhibit competing oxidative reactions.

For *in vitro* studies, stock solutions (6×10^{-3} or 6×10^{-4} *M*) of pargyline, U-1247, and U-1379 prepared in 0.2 *M* phosphate buffer (pH 7.4) were serially diluted with buffer. Half-milliliter aliquots of the drug in buffer were added to 2.0-ml. aliquots of a 2.5% rat liver homogenate. Control mixtures were prepared by adding 0.5-ml. aliquots of phosphate buffer without drug to 2.0-ml. aliquots of the same rat liver homogenate. The mixtures were preincubated for 10 min. before the addition of 0.5 ml. of 0.06 *M* semicarbazide–0.006 *M* KCN solution containing 30 μM of tyramine. The reaction mixtures were shaken at 37° in an O_2 atmosphere for 10 min. At the end of the incubation period, 0.5 ml. of a 50% trichloroacetic (TCA) solution in water was added to each homogenate to stop the reaction.

For the *in vivo* study, rats ranging in weight from 250 to 300 Gm. were given a single oral dose of either pargyline, U-1247, or U-1379 in water. Each drug was administered to rats at various dosages using three rats per level of drug. Control rats were dosed with water. After 4 hr. the rats were sacrificed and their livers and brains removed to determine monoamine oxidase activity. Liver and brain homogenates, 2.5% (w/v), were prepared in 0.2 *M* phosphate buffer (pH 7.4); 2.0 ml. of homogenate was added to an additional 0.5 ml. of the phosphate buffer and treated as described for the *in vitro* studies.

Colorimetric analysis of *p*HPA in the TCA treated supernatant solution was made by diluting 0.5 ml. of the solution to 3.0 ml. with water and adding 1.0 ml. of 1.5% 4-nitrophenylhydrazine hydrochloride in 1.0 *N* HCl. After extraction of the 4-nitrophenylhydrazone of *p*HPA into 2.0 ml. of chloroform, re-extraction of 1.0 ml. chloroform with 5 ml. of 0.1 *N* NaOH, and dilution of 3.0 ml. of this basic solution to 8.0 ml. with water, the absorbances were determined in a colorimeter at 420 mμ. Percent of inhibition of MAO activity by pargyline, U-1247, and U-1379 *in vivo* and *in vitro* was calculated by comparing the absorbance of the inhibited MAO samples with the appropriate control absorbance.

Comparative Acute Toxicity in Mice—LD_{50} values were determined in CFW male mice with U-1247, U-1379, and pargyline by three routes of administration. Groups of 5 mice received graded doses of drug, and the LD_{50} value was based on deaths which were observed within a 48-hr. period. These animals were also observed for gross pharmacologic effects of the compounds.

Hypotensive and Antihypertensive Activity—The hypotensive and antihypertensive activity of U-1247 and pargyline was investigated in unanesthetized rats.

Thirty rats were divided into six groups of five rats each. Three of these groups consisted of rats made hypertensive by the method of Grollman (5). Systolic blood pressures were recorded twice daily from the tail using a pneumatic pulse pickup and an electrosphygmograph (E & M instruments).

The animals were arranged so that each testing unit consisted of five normotensive and five hypertensive rats. The following procedure was adopted: Days 1 and 4. Control blood pressures were recorded from each animal twice a day. Days 5 through 11. During this, the treatment period, the animals received daily the following: Unit 1—saline 1 ml./Kg., i.p. Unit 2—pargyline 20 mg./Kg., i.p. Unit 3—U-1247 20 mg./Kg., i.p. Days 12 through 15. This was the postdrug observation period.

Effect of U-1247 and Pargyline on the Pressor Effects of Tyramine—Rats were anesthetized with urethan (1.25 Gm./Kg., i.p.) a tracheotomy performed, and polyethylene tubing inserted. A glass cannula was inserted into one of the carotid arteries for direct blood pressure recording through a Statham pressure transducer onto a Grass polygraph. A femoral vein was cannulated for injection of drugs. Two groups of five animals each were pretreated 48, 24, and 2 hr. before i.v. tyramine (0.01 mg./Kg.) challenge with either U-1247 (100 mg./Kg. *per os*) or pargyline HCl (100 mg./Kg. *per os*). Control responses to tyramine (0.01 mg./Kg., i.v.) were obtained using seven animals.

In a second study, the protocol of Tedeschi and Fellows (6) for demonstrating augmentation of peroral tyramine by monoamine oxidase inhibition was followed, with the exception that anesthesia was accomplished with intraperitoneal urethan (1.25 Gm./Kg.) instead of intravenous pentobarbital. The treated rats were given 100 mg./Kg. of U-1247 or pargyline orally 72, 48, and 24 hr. prior to challenge with orally administered tyramine.

RESULTS

MAO Inhibition—*In vitro* U-1247, U-1379, and pargyline greatly inhibited rat liver homogenate MAO activity (Table I). Fifty percent inhibition (IC_{50}) of the MAO activity in liver homogenate was elicited by these drugs at the following molar concentrations: 9.5×10^{-7} *M* for U-1247, 2.3×10^{-7} *M* for U-1379, and 4.0×10^{-8} *M* for pargyline. The IC_{50} values for liver MAO activity must be

interpreted with caution, however, since the length of the preincubation period of MAO with these inhibitors, before the addition of substrate, may affect the degree of inhibition obtained (7).

In vivo peroral administration of U-1247, U-1379, and pargyline resulted in approximately the same magnitude of inhibition of both rat liver and brain MAO (Table I). Graphs of the percent inhibition of rat liver MAO activity by U-1247, U-1379, and pargyline *versus* the logarithms of the doses resulted in the classic sigmoid curves usually obtained in this type of study. The ID_{50} values obtained from these curves were: 0.13 mg./Kg. for pargyline, 0.24 mg./Kg. for U-1247, and 0.51 mg./Kg. for U-1379. The difference between these ID_{50} values is not considered significant. Construction of the same type of graph for the *in vivo* brain data resulted in curves which were too variable to be clearly defined as sigmoid curves. U-1247, U-1379, and pargyline produced a 50% inhibition of rat brain MAO within a 1–3 mg./Kg. dose range.

Acute Toxicity in Mice—The comparative LD_{50} values and 95% confidence limits obtained in CFW mice with U-1247, U-1379, and pargyline by three routes of administration are presented in Table II.

These LD_{50} values were based on deaths which were observed within 48 hr. following drug administration.

The gross observable effects of these three drugs also differed. Deaths among the U-1247 treated mice occurred within 2–15 min. of drug administration and no delayed deaths were observed. The toxic signs observed were: clonic convulsions, dyspnea, cyanosis, and exophthalmus. Sublethal doses produced hyperexcitability which lasted approximately 1 hr.

Pargyline, by contrast, caused little excitability at sublethal doses while minimal lethal doses produced a 1–2 hr. depression followed in 3–4 hr. by marked hyperexcitability. When large lethal doses were given death occurred in 3–10 min. with accompanying cyanosis, dyspnea, and weak (cyanotic) clonic convulsions. Deaths following oral administration were delayed as long as 48 hr.

The gross and toxic effects of U-1379 resembled those observed following either pargyline or U-1247 treatment. Marked initial stimulation, which resembled the effects of U-1247, and latent stimulation and delayed deaths as were seen following pargyline, were observed with this compound.

Hypotensive and Antihypertensive Activity—The effect of U-1247 and pargyline on the systolic blood pressure of normotensive and hypertensive rats is summarized in Table III. The control animals, both normotensive and hypertensive, maintained a steady level of systolic blood pressure over the 16-day period. The systolic blood pressure of the hypertensive animals receiving pargyline or U-1247 decreased over the 7-day treatment period. This decrease in systolic pressure was approximately equal in the two groups and no difference in potency of the two drugs could be demonstrated. At the termination of treatment, the blood pressure rose to predrug levels in approximately 3 days. The normotensive animals which received either U-1247 or pargyline did not appear to demonstrate as marked a hypotensive effect; however, there was a

Table I—Effect of U-1247, U-1379, and Pargyline on Rat Monoamine Oxidase

	U-1247	U-1379	Pargyline
In vitro[a]			
Liver			
$IC_{50}(M)$	9.5×10^{-7}	2.3×10^{-7}	4×10^{-8}
Slope	0.30	0.68	0.58
In vivo[b]			
Liver			
ID_{50}, p.o. (mg./Kg.)	0.24	0.51	0.13
Slope	0.75	1.2	0.91
Brain			
ID_{50}, p.o. (mg./Kg.)	1.9	2.18	3.1
Slope	0.87	1.68	0.81

[a] The drugs were preincubated for 10 min. with the rat liver homogenate before addition of the substrate tyramine. [b] These results were obtained on livers or brains excised 4 hr. after peroral administration of the drugs.

Table II—Acute Toxicity of U-1247, U-1379, and Pargyline in Mice[a]

Route	Pargyline, mg./Kg.	U-1247, mg./Kg.	U-1379, mg./Kg.
p.o.	726 (613–800)	339 (318–363)	305 (289–322)
i.p.	494 (430–566)	156 (136–173)	235 (211–266)
i.v.	99 (91–106)	31.6 (29.7–35.0)	59 (54–63)

[a] LD_{50} and confidence limit values, mg./Kg.

Table III—Effect of U-1247 and Pargyline on the Systolic Blood Pressure of Normotensive and Hypertensive Rats

		Systolic Blood Pressure[a] (mm. Hg)					
		Saline		Pargyline		U-1247	
Cond.	Day	Hypertensive	Normotensive	Hypertensive	Normotensive	Hypertensive	Normotensive
Pretreatment period	1	175	109	170	112	161	119
	4	165	109	174	114	155	114
Treatment period, 20	6	161	123	147[b]	109	152	123
mg./Kg. i.p. daily or	7	168	117	147[b]	114	144	113
equivalent volume of	8	168	114	142[b]	99[b]	138	106[b]
saline	11	169	110	136[b]	106	129[b]	104[b]
Posttreatment period	12	169	112	134[b]	98[b]	122[b]	98[b]
	13	163	112	150	111	143	109
	14	171	115	181	117	150	113
	15	169	121	176	118	154	116

[a] Blood pressure values are a mean of five animals tested twice each day specified. [b] Average daily blood pressure significantly lower than pretreatment period ($P < 0.05$).

TABLE IV—EFFECT OF U-1247 AND PARGYLINE ON TYRAMINE BLOOD PRESSURE RESPONSES

Treatment[a]	Tyramine Dose, mg./Kg.	ΔxBP, mm. Hg	±S.E.	No. Rats in Group
Control	Saline	12.0	2.16	6
Control	12.5	16.2	4.00	6
Control	50	38.5	5.35	6
Pargyline	12.5	37.0	11.05	6
U-1247	12.5	33.8	10.80	6

[a] Rats pretreated with U-1247 or pargyline (100 mg./Kg. p.o.) 72, 48, and 24 hr. prior to tyramine challenge (p.o.).

significant blood pressure reduction in both groups.

Effect of U-1247 and Pargyline on the Pressor Response to Tyramine—The mean blood pressure response to 0.01 mg./Kg. i.v. of tyramine in 7 rats was 7.7 (±2.5). After pretreatment with pargyline or U-1247, the mean response to tyramine in 5 rats was increased to 24.4 (±10.4) and 32.6 (±8.5), respectively. These differences from control were significant at the 0.1 level for pargyline and the 0.01 level for U-1247.

The effect of U-1247 and pargyline on perorally administered tyramine is summarized in Table IV.

In control rats there was a significant difference in the blood pressure response between 12.5 mg./Kg. and 50 mg./Kg. of tyramine. After pretreatment with either pargyline or U-1247, the response to 12.5 mg./Kg. of tyramine was increased, and no longer was significantly different from the response to the 50 mg./Kg. dose.

These data indicate that U-1247 and pargyline are capable of potentiating both intravenous and peroral tyramine.

DISCUSSION

This study indicates that within the pargyline series a triple bond is not essential for *in vitro* or *in vivo* MAOI activity. The *in vitro* data suggest that U-1247 is approximately 25 times less active than pargyline, while *in vivo* the two compounds are approximately equal in potency. The *in vivo* conversion of U-1247 to a more active form is one possible explanation of these results. The bioconversion of the allenic compounds to acetylenic derivatives, however, is unlikely as Swett (1) has indicated both the *N*-2 and *N*-3 butynyl-*N*-methylbenzylamine are inactive *in vivo*. The possibility of U-1247 and pargyline being converted to a common active form cannot be discounted from the data and is worthy of further study.

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Keyphrases

Monoamine oxidase inhibitors
Allenic amines—synthesis
LD_{50} value—allenic amines
Hypotensive, antihypertensive activity
Vapor-phase chromatography—identity
IR spectrophotometry—structure

Influence of Various Pretreatments (Carriers, Desiccation, and Relative Cleanliness) on the Destruction of *Bacillus subtilis* var. *niger* Spores with Gaseous Ethylene Oxide

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The ethylene oxide resistance of *Bacillus subtilis* var. *niger* spores inoculated on a variety of carriers was determined. After initial experiments indicated that cleanliness of the spores was important, desiccated and undesiccated spores of varying degrees of cleanliness on aluminum foil and chromatography paper were studied. Under the experimental conditions, desiccation of the spore population had no influence on susceptibility to ethylene oxide. The degree of cleanliness of the spore preparation was the significant factor in excessive resistance. The bacteriological controls used for testing ethylene oxide sterilization processes should be adequately cleaned so that they may accurately monitor the process.

GASEOUS ETHYLENE OXIDE is used for the sterilization of materials which cannot be sterilized by steam. As the utilization of heat- and moisture-sensitive materials increases, the importance of ethylene oxide sterilization increases for the hospital and pharmaceutical related fields.

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