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Relationship between Antihistamine and Antidepressant Activity in Hexahydroindenopyridines

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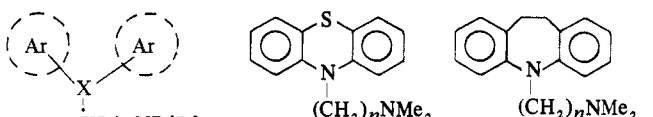
The antihistamine phenindamine 4 (R = Me) has been converted to two epimeric hexahydroindenopyridines **5b** and **6b**, and the pharmacological profile of both of these products shown to be similar to that of the antidepressant desmethylimipramine. These findings do not support the postulate that a relationship exists between antihistamine and antidepressant drugs which depends on the closeness of approach of basic and aromatic centers. Two short series of N-substituted and analogous hexahydroindenopyridines were also made but no activity of note was found. The structural requirements for antihistamine activity in phenindamine and the present compounds are discussed.

It has been recognized for some time that a degree of sedation accompanies the action of most therapeutically effective antihistaminic agents (H_1-receptor antagonists).[1] The intensity of this central effect varies appreciably among the numerous structural types of these drugs, and in the case of phenindamine, central stimulation is usually observed.[2,3] Central depression, therefore, is not necessarily a corollary of peripheral H_1-antagonist activity.

The structural pattern **1**, which can be identified in some form in most antihistaminic agents, can also be recognized in the phenothiazine tranquilizers (*e.g.*, promazine, **2a**) and in the tricyclic antidepressants (*e.g.*, imipramine, **3a**). Both promazine and imipramine exhibit H_1-antagonist properties but these are much more marked in their lower homologs, **2b** and **3b**.[4,5] Several groups of workers have observed that antihistaminic agents possess to varying degrees the pharmacological properties of antidepressant agents, *e.g.*, potentiation of the cardiovascular effects of catecholamines,[6,7] antagonism of reserpine-induced hypothermia,[8] potentiation of amphetamine-induced excitation,[9] and blockade of amine uptake in central and peripheral neurones.[10]

In an attempt to reconcile the structural similarities of the H_1-receptor antagonists of general formula **1** and the psychotherapeutic drugs **2a** and **3a** with their respective biological



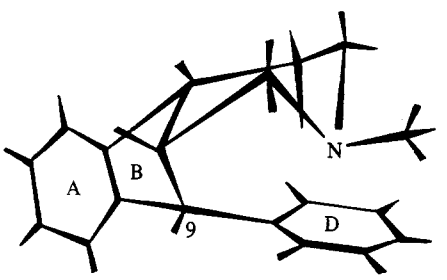
1
Ar = aromatic ring
X = CH, N or CH–O–

2
a, n = 3
b, n = 2

3
a, n = 3
b, n = 2

activities, it occurred to us that differing lengths, or preferred conformations of side chains, with consequent variations in the degree and type of interaction of the polarizable aromatic π electrons with the polar N (which will be protonated at the biological pH of 7.4), could result in affinity for different receptors or in altered transport and binding properties. If this hypothesis is correct, it follows that minor molecular modifications of the basic side chain might convert an antihistaminic drug to a substance with predominantly "antidepressant" properties as is observed in the change from **3b** to **3a**. Phenindamine **4** (R = Me) was the antihistaminic drug chosen for such an investigation since its basic center is part of a fused ring system and small structural changes should result in predictable spatial variations of the required type. Its mild central stimulant properties were also of interest in the antidepressant context.

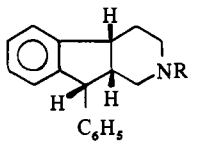
Phenindamine is a rigid molecule in which close approach of the basic center to either aromatic ring is impossible. It is already known that hydrogenation to dihydrophenindamine results in virtually complete loss of H_1-antagonist activity, but the stereochemistry of the product was not studied by the original workers.[11,12] Two of the present authors have shown that the product of hydrogenation is all cis isomer **5a** (see Table I) which is readily epimerized in alkali at C_9 to the H_{4a},H_{9a}-cis; H_9,H_{9a}-trans isomer **6a**.[13] The rings B/C trans fused isomers were not encountered. Examination of Dreiding framework and Corey-Pauling-Koltun space-filling models show that both of the B/C cis fused isomers are flexible molecules, identical with regard to the nearest possible approach of the N to ring **A**. However, if interaction with the C_9 phenyl ring is considered, there is a clearcut difference between **5a** and **6a**. In the latter, approach of the N to the aromatic center is impossible, but in the former, an N-ring D distance of 2-2.5 Å is attainable (see diagram).



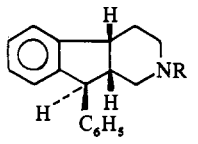
Dreiding molecular model of **5a**

Clearly, the series **4**, **5a**, and **6a** provide an excellent opportunity for testing the hypothesis that different N-aromatic separations are responsible for changes from antihistaminic to antidepressant properties. Initial encouragement was obtained when it was shown that, in contrast to phenindamine, **5a** possesses a pharmacological profile similar in some respects to that of desmethylimipramine (DMI), and this paper reports the synthesis and structure-activity relationships of a short series of indenopyridines in which

Table I



5



6

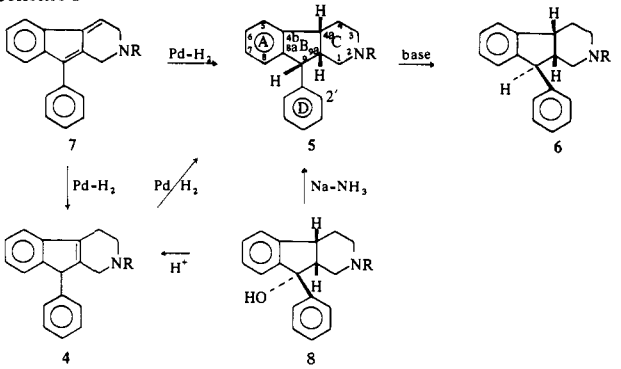
Number	R	Salt	Mp, °C	Analysis	Molecular formula	Crystn solvent	Amphet[a]	TBZ[b]	Res[c]	Nor[d]	Blood pressure[e] response (species)	pA_2 (histamine)
5a	CH_3	HBr	252-254		$C_{19}H_{21}N \cdot HBr$	EtOH	+	+++	++	+++	0 (cat dog)	5.2
5b	H	HCl	278-280	C,H,N,Cl	$C_{18}H_{19}N \cdot HCl$	EtOH-EtOAc	+	+++	++++	++	PP (dog)	5.7
5c	$COOC_2H_5$		71-73	C,H,N	$C_{21}H_{23}NO_2$		0		0	+		
5d	$C(=NH)NH_2$	HI	274-275	C,H,N	$C_{19}H_{21}N_3 \cdot HI$	*i*-PrOH-EtOH	0	0	0	0		
5e	$CH_2C{\equiv}CH$	HCl	245-246	C, H,N,Cl	$C_{21}H_{21}N \cdot HCl$	H_2O	+	+++	+	+	P (cat)	
5f	$CH_2CH{=}CH_2$	HCl	228-234	C,H,N,Cl	$C_{21}H_{23}N \cdot HCl$	H_2O	+	+++	+	++	P (cat dog)	5.5
6a	CH_3	HCl	276-278	C,H,N	$C_{19}H_{21}N \cdot HCl$	EtOH-EtOAc	0	+	++++	++	0 (dog)	7.6
6b	H	HCl	265-267	C,H,N,Cl	$C_{18}H_{19}N \cdot HCl$	*i*-PrOH	+	++	++++	+++	P (cat dog)	7.8
Phenindamine							+	0	++++	++++	0 (dog)	8.8
D.M.I.							++++	+++	++++	++++	PP (cat dog)	7.1

[a]Potentiation of amphetamine excitation. [b]Reversal of tetrabenazine sedation. [c]Antagonism of reserpine hypothermia. [d]Antagonism of norepinephrine hypothermia. [e]Effect on the blood pressure response to norepinephrine.

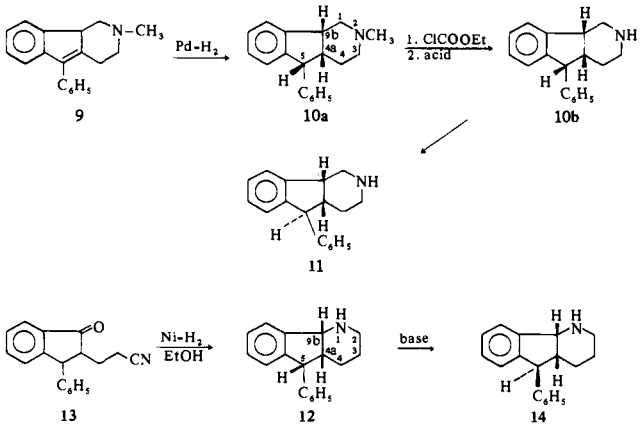
antidepressant activity reached a maximum in **5b** and **6b**.

Chemistry. The chemistry is summarized in Schemes I and II and the compounds are listed in Tables I and II. The all-cis compounds **5** were obtained by reduction of dienes **7**, phenindamine, and its analogs **4** or carbinols **8**[14] using activated Pd catalyst.[15] Carbinols **8** could also be reduced to **5** with Na–liq NH_3. In all cases only a single stereoisomer **5** was isolated, shown previously to have H_{4a}, H_9, and H_{9a} disposed cis to each other.[13] Demethylation of **5a** *via* the urethane **5c** followed by acid hydrolysis gave **5b** also prepared directly from the diene **7** (R = benzyl). Hydrolysis of **5c** under basic conditions gave a mixture of **5b** and **6b** which was separable by fractional crystallization into the pure components. Preferably **6b** was obtained from **5b** by treatment with KOH in *n*-BuOH. Both **5b** and **6b** were methylated by the method of Clarke[16] to give **5a** and **6a**, respectively. Since these are known to be epimeric at C_9, this established the interrelationship of all the compounds depicted in Scheme I. Reaction of **5b** with *S*-methylthiouronium iodide gave the amidine **5d**, and the propargyl and allyl derivatives **5e** and **5f** were obtained by direct alkylation of **5b**.

Scheme I



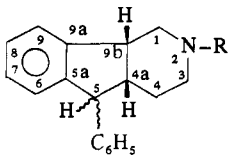
Scheme II



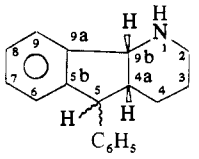
The hexahydro-2*H*-indeno[1,2-*c*]pyridine **10a** was obtained by reduction of **9** followed by demethylation to **10b**, which on treatment with strong base gave its epimer **11**.† An indication of the stereochemistry of these compounds was obtained from the nmr coupling constants of the H_5 doublet (6 Hz in **10b** and 10 Hz in **11**) which are analogous to the constants for the H_9 doublets of **5a** (6 Hz) and **6a** (8 Hz). Further evidence in support of this assignment is drawn from the relative stabilities of **10b** and **11** in strong base together with the mode of synthesis which by comparison with **5a** and **6a** would be expected to give the all-cis compound **10a**.

†After completion of this work 10a, 10b, and 11 appeared in the patent literature[17] and are claimed as analeptic agents.

Table II



H_{4a}, H_5-*cis*-10a, R = CH_3
-10b, R = H
H_{4a}, H_5-*trans*-11, R = H



H_{4a}, H_5-*cis*-12
H_{4a}, H_5-*trans*-14

No.	R	Salt	Mp, °C	Analysis	Molecular formula	Crystn solvent	Amphet[a]	TBZ[b]	Res[c]	Nor[d]	Blood pressure[e] response (cat)	pA_2 (histamine)
10a	CH_3	HCl	271-273	C,H,N,Cl	$C_{19}H_{21}N \cdot HCl$	*i*-PrOH	+	++	0	0		5.5
10b	H	HCl	234-236	C,H,N,Cl	$C_{18}H_{19}N \cdot HCl$	*i*-PrOH	+	0	0	+	0	5.4
11	H	HCl	278-280	C,H,N,Cl	$C_{18}H_{19}N \cdot HCl$		0	0	0	+++	P	
12		H-maleate	177-179	C,H,N	$C_{18}H_{19}N \cdot C_4H_4O_4$	*i*-PrOH	0	0	++++	0	P	5.7
14		H-maleate	164-165	C,H,N	$C_{18}H_{19}N \cdot C_4H_4O_4$	*i*-PrOH	0	+	++++	++++	0	5.2

[a-e] See footnotes *a-e*, Table I.

The novel hexahydro-1*H*-indeno[1,2-*b*]pyridine **12** was obtained by reductive cyclization of the cyanoalkylindanone **13**.[18,‡] Treatment of **12** with strong base gave the epimer **14**. The stereochemistry was again assigned on the basis of the H_5 doublet coupling constant (**12**, 6 Hz; **14**, 11 Hz) and the stability of the isomers towards base.

Pharmacology. Potential antidepressant activity was assessed by the ability of compounds to potentiate (±)-amphetamine excitation in rats,[19] reverse the sedative action of tetrabenazine in rats,[20] antagonize reserpine-[21] and norepinephrine-induced[22] hypothermia in mice, and to modify the blood pressure response to norepinephrine in cats or dogs. DMI was used as the reference substance. Brief descriptions of procedure and rating systems are given in the Experimental Section, and results on test compounds are given in Tables I and II, together with antihistaminic pA_2 values obtained by the standard procedure using guinea pig ileum.

Discussion

The pharmacological results in Table I show that conversion of phenindamine to **5a** causes a considerable change in biological properties. The antihistaminic pA_2 value falls by 3.6 log units, confirming earlier work,[11] but potent reversal of tetrabenazine sedation is introduced. This latter test is thought to be of particular significance for predicting antidepressant activity in man.[23] Demethylation of **5a** to **5b** results in the additional property of strong potentiation of the blood pressure response to norepinephrine in the chloralosed cat. N-Substituted compounds **5c-5f** were all of lower activity, though **5e** and **5f** retained good potency in the tetrabenazine test.

Except for much lower potency in the amphetamine excitation test, the overall antidepressant profiles of both **5b** and **6b** resemble that of DMI fairly closely, although **6b** also had appreciable H_1-antagonist activity. Thus no clear-cut relationship between the separation of basic and aromatic centers, and possession of antihistaminic and antidepressant properties is discernible in this series, since close approach is possible with **5b** but not with **6b**. However, striking separation of the two pharmacological types was achieved with phenindamine and **5b**.

The analogs in which the N of dihydrophenindamine is moved into 2 alternative positions in ring C are shown in Table II. Models show that the ease of approach of N to the aromatic centers in **10a**, **10b**, and **12** is about the same as with **5b** but no interaction is possible with **11** and **14**. In the event, all these compounds had very low activity both as antihistaminic and antidepressant agents.

Symptomatology studies were carried out in rats for all compounds in Tables I and II. Compounds **5a**, **5b**, **6a**, **6b**, **10a**, **10b**, **11**, **12**, and **14** at 40 mg/kg, ip, caused pronounced central stimulation, often accompanied by convulsions. Compounds **5c**, **5d**, **5e**, and **5f** caused varying degrees of ataxia, respiratory depression, and sedation. There was therefore no relationship between the type of gross CNS effects produced and antidepressant activity. Although **5b** possesses many properties in common with DMI, the excitant effects observed in rats were not encouraging, and even more pronounced central stimulation, together with fear reactions, was seen in dogs. Compound **5b** was shown to cause testicular damage in rats at doses in excess of 25 mg/kg per day given for 12 weeks.

The antihistamine activity of the present series is negligible except for **6a** and **6b** which, on the basis of pA_2-pA_{10} values, display true competitive antagonism of histamine. An attempt was made to rationalize these results using the recent theory of Casy and Ison.[24] They postulate that for maximum H_1-antagonist activity in structures of type **1** the N-containing side chain should be coplanar with one of the aromatic nuclei, with the N atom at a distance of 4.8 Å from the ortho C of that ring. Furthermore the dihedral angle between the planes of the 2 aromatic nuclei should be approximately 90°. It is of interest to note that the potent antihistamine phenindamine does not meet all of these requirements, the N-ortho-C distance being 4.3 Å as measured on Dreiding models. This finding suggests a considerable latitude in the permissible N-aryl distance. Similarly the rather less potent compounds **6a** and **6b** although conforming in all other respects, exhibit interaryl dihedral angles which are significantly greater than 90°. A study of Dreiding models of the other members of the series led to the tentative conclusion that **6a** and **6b** fit the Casy and Ison criteria more closely than do **5a**, **5b**, **10a**, **10b**, **11**, **12**, and **14**, in agreement with the pA_2 values in Tables I and II.

Experimental Section

Chemistry. All melting points are uncorrected and were obtained on an Electrothermal capillary melting point apparatus.

‡We are indebted to Dr. A. Canas-Rodriguez for suggesting this synthesis.

Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

H_{4a},H_{9a}-cis-H_9,H_{9a}-cis-2-Methyl-9-phenyl-2,3,4,4a,9,9a-hexahydro-1*H*-indeno[2,1-*c*]pyridine (5a) was obtained from diene 7 (R = Me) by the method of Platl[12] or more conveniently by the method of Canas-Rodriguez[15] using phenindamine (4, R = Me) or 8 (R = Me) as starting material. A third procedure was by hydrogenolysis of 8 (R = Me) using Na–liq NH_3.[13]

H_{4a},H_{9a}-cis-H_9,H_{9a}-trans-2-Methyl-9-phenyl-2,3,4,4a,9,9a-hexahydro-1*H*-indeno[2,1-*c*]pyridine (6a). The all-cis compd 5a was epimerized by refluxing in KOH-*n*-BuOH (20% wt/vol) as previously described.[13]

H_{4a},H_{9a}-cis-H_9,H_{9a}-cis-9-Phenyl-2,3,4,4a,9,9a-hexahydro-1*H*-indeno[2,1-*c*]pyridine (5b) and *H_{4a},H_{9a}-cis-H_9,H_{9a}-trans*-9-Phenyl-2,3,4,4a,9,9a-hexahydro-1*H*-indeno[2,1-*c*]pyridine (6b). The 2-Me derivative 5a was demethylated by the method of Bickelhaupt[25] to give the all-*cis*-2-ethoxycarbonyl compd 5c as an off-white cryst solid (88%), mp 71–73°. Hydrolysis of 5c (21.6 g) in refluxing ethanolic KOH (20% *wv*, 90 ml) for 12 hr gave, on diln and extn with $CHCl_3$, a mixt of 5b and 6b which after pptn from Et_2O as the hydrochloride (16 g, 83%) could be sepd by fractional crystn (EtOH–EtOAc) into 5b · HCl, mp 278–280°, and 6b · HCl, mp 265–267°. Acid hydrolysis of 5c (3 g) in refluxing HBr–AcOH (50%, 20 ml) for 3 hr gave pure 5b (87%) which was converted to the hydrochloride.

H_{4a},H_{9a}-cis-H_9,H_{9a}-cis-2-Propargyl-9-phenyl-2,3,4,4a,9,9a-hexahydro-1*H*-indeno[2,1-*c*]pyridine (5e). Compd 5b (5 g) in DMF (250 ml) was treated with NaH (0.96 g) and stirred for 15 min. Propargyl bromide (2.4 g) was added slowly, followed by vigorous stirring at 60° for 4 hr. The mixt was cooled, poured onto ice, and extd (Et_2O). The ext was shaken with 0.1 *N* HCl (3 × 100 ml) and the aqueous acidic exts were bulked and evapd *in vacuo*. The crystn residue was recrystd from H_2O to give pure 5e · HCl (0.5 g), mp 245–246°.

H_{4a},H_{9a}-cis-H_9,H_{9a}-cis-2-Allyl-9-phenyl-2,3,4,4a,9,9a-hexahydro-1*H*-indeno[2,1-*c*]pyridine (5f) was prepd by the action of allyl bromide (3 ml) on 5b (5 g) as described for 5e. 5f · HCl (1.2 g) was recrystd from H_2O, mp 228–234°.

H_{4a},H_{9a}-cis-H_9,H_{9a}-cis-2-Amidino-9-phenyl-2,3,4,4a,9,9a-hexahydro-1*H*-indeno[2,1-*c*]pyridine (5d). Compd 5b (10 g) in 95% EtOH (20 ml) was treated with *S*-methylthiouronium iodide (8 g). The mixt was heated at 90° for 30 min and was then allowed to stand for 48 hr. The solid which deposited was recrystd from *i*-PrOH–EtOH and then from H_2O to give 5d · HI (2 g) mp 274–275°.

H_{4a},H_5-cis-H_{4a},H_{9b}-cis-2-Methyl-5-phenyl-1,3,4,4a,5,9b-hexahydro-2*H*-indeno[1,2-*c*]pyridine (10a). The tetrahydro derivative[26] 9 was reduced by the method of Canas-Rodriguez[15] to give 10a · HCl, mp 271–273°.

H_{4a},H_5-cis-H_{4a},H_{9b}-cis-5-Phenyl-1,3,4,4a,5,9b-hexahydro-2*H*-indeno[1,2-*c*]pyridine (10b). The 2-Me deriv 10a (7.5 g) was demethylated by the method of Bickelhaupt.[25] Acid hydrolysis (HBr–AcOH) of the crude intermediate 2-ethoxycarbonyl deriv gave 10b · HBr as a crude oil. The oil was basified and dissolved in $CHCl_3$, and 10b · HCl (7.5 g, 91%) was pptd by the addition of ethereal HCl. Recrystn from *i*-PrOH and then EtOH–EtOAc gave pure 10b · HCl, mp 234–236°.

H_{4a},H_5-trans-H_{4a},H_{9b}-cis-5-Phenyl-1,3,4,4a,5,9b-hexahydro-2*H*-indeno[1,2-*c*]pyridine (11). The hydrochloride of 10b (0.7 g) was refluxed in KOH-*n*-BuOH (20% wt/vol, 20 ml) overnight. The mixt was acidified and steam distd to remove *n*-BuOH. The residue was basified and extd (Et_2O), and the ether ext was treated with ethereal HCl to ppt 11 · HCl (0.35 g, 50%), mp 278–280°.[16]

H_{4a},H_5-cis-H_{4a},H_{9b}-cis-5-Phenyl-2,3,4,4a,5,9b-hexahydro-1*H*-indeno[1,2-*b*]pyridine (12). 2-(2-Cyanoethyl)-3-phenyl-1-indanone[18] 13 (15 g), in EtOH (150 ml), was hydrogenated over Raney nickel (3 g, damp) at 49.21 kg/cm² and 115° until uptake ceased. The catalyst was removed and the filtrate was evapd *in vacuo* to give an oil (14.5 g). The oil was dissolved in Et_2O and converted to the hydrochloride with ethereal HCl. The pptd hydrochloride was rebasified with 10% Na_2CO_3 and extd with $CHCl_3$. Evapn of the $CHCl_3$ gave a slurry which on dissolving in petr ether (bp 40–60°, 20 ml) deposited white crystals (3.5 g). Recrystn from petr ether (80–100°) gave pure 12, mp 110–113° and this was also converted to the hydrogen maleate salt, mp 177–179° (*i*-PrOH).

H_{4a},H_5-trans-H_{4a},H_{9b}-cis-5-Phenyl-2,3,4,4a,5,9b-hexahydro-1*H*-indeno[1,2-*b*]pyridine (14). Compd 12 (0.4 g) was refluxed in 20% KOH-*n*-BuOH (15 ml) for 18 hr. After the usual work-up the crude free base was dissolved in ether and converted to the maleate salt (0.1 g), mp 164–165°.

Pharmacology. (a) **Potentiation of (±)-Amphetamine Excitation.** Male albino rats (180–220 g) were dosed orally with the test compd (25 mg/kg) followed by (±)-amphetamine sulfate (5 mg/kg, ip) 1.5 hr later. The potentiation was assessed and quantified by addition of the half-hourly scores for each group of 4 treated rats and subtracting from this the total scores of the rats given amphetamine only. The results in the tables are expressed as: score of 0–25 = 0; 26–50 = +; 51–75 = ++; 76–100 = +++; >100 = ++++.

b. **Reversal of Tetrabenazine Sedation.** The method was based on the observations of Sulser, *et al.*[20] Male albino rats (5 groups, 3/group) were dosed orally with the test compd at 50 mg/kg at 18 hr, and 20 mg/kg at 1.5 hr before ip injection of tetrabenazine hydrochloride (25 mg/kg). Any hyperactivity was noted on a simple presence or absence basis every hr for 5 hr. Tetrabenazine alone produced marked immobility at this dose. A maximum reversal would produce a score of 15/15 active or 100%. Results in Tables I and II are expressed as: 0–25% = 0; 26–50% = +; 51–75% = ++; 76–100% = +++.

c. **Antagonism of Reserpine Hypothermia.** The method of Askew[21] was used, the oesophageal temps being monitored with an electric thermometer. The test compds were injected ip (10 mg/kg) and the results are expressed in Tables I and II based on the net total increase in mean body temperature: 0–5° = 0; 6–10° = +; 11–15° = ++; 16–20° = +++; >20° = ++++.

d. **Antagonism of Norepinephrine Hypothermia.**[22] The ability of test compounds to reverse the hypothermic response induced by injection of norepinephrine (10 μg) directly into the lateral ventricles of mice was assessed by recording the oesophageal temperature at 15, 30, and 60 min after administration. Norepinephrine alone produces a hypothermia of 2–3°. The results are expressed as percentages of antagonism of this hypothermia: 0–25% = 0; 26–50% = +; 51–75% = ++; 76–100% = +++; >100% = ++++.

e. **Effect on the Blood Pressure Response to Norepinephrine in the Cat or Dog.** The blood pressure was measured from the femoral artery of beagle dogs anaesthetized with pentobarbital sodium (30 mg/kg, iv) or the femoral artery of cats anaesthetized with pentobarbital sodium (30 mg/kg, iv) or with chloralose (80 mg/kg, iv). The pressor response to norepinephrine (2–4 μg, iv) was measured before and after iv administration of the test substance (0.5–2.0 mg/kg). Results are expressed simply as nil (0), slight (P), or marked (PP) potentiation of this response.

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Heterocyclic O-Substituted Hydroxylamines. Antiinflammatory Activity and Possible Inhibition of Histamine Biosynthesis[1,†]

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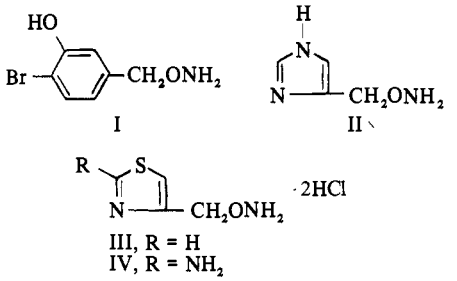
The synthesis of several heterocyclic O-substituted hydroxylamine derivatives is described. 5-[(Aminooxy)methyl]-3-phenylisoxazole · HCl possesses significant antiinflammatory activity in the carrageenin-induced rat paw edema test and also shows substantial inhibitory activity when tested *in vitro* against specific histidine decarboxylase; this lends support to the theory linking histamine to the inflammatory process.

The antiinflammatory activity of the histidine decarboxylase inhibitor, 4-bromo-3-hydroxybenzyloxyamine (I) has been demonstrated by Spector and Willoughby.[2] Furthermore, 4-[(aminooxy)methyl]imidazole (II),[3] 4-[(aminooxy)-



methyl]thiazole · 2 HCl (III),[4] and 2-amino-4-[(aminooxy)-methyl]thiazole · 2 HCl (IV)[4] inhibit specific histidine decarboxylase. We have synthesized, by literature methods,[5] aminooxymethyl derivatives of several additional heterocyclic systems.

Biological Activity and Discussion. The alkoxyamines were screened orally for antiinflammatory activity using the carrageenin-induced rat paw edema method.[6] The results are listed in Table I and represent the percentage inhibition of edema compared with controls, when doses of 200 mg/kg were given 1 hr prior to injection of carrageenin. The 5-[(aminooxy)methyl]-3-phenylisoxazole · HCl (**5**) possesses a 55% inhibition of the edema at the dose of 200 mg/kg. The other aminooxy compounds (**2, 7, 9**) show an inhibition of 29, 24, and 36%, respectively, at the same dose.

The *in vitro* enzyme inhibition tests were performed on specific histidine decarboxylase derived from mouse mastocytoma, and depended on the release of $[^{14}C]CO_2$ from [carboxyl-^{14}C]histidine.[7] The molar concentrations of the alkoxyamines (**2, 5, 7, 9**) required to inhibit the enzyme by 50% (I_{50}) are in the range of 10^{-5}–10^{-6} *M*.§

Compounds **7** and **9** were screened against the L-1210 mouse lymphoid leukemia test system and showed no significant antitumor activity.# Preliminary results on **7** and **9** indicate a lack of activity against malaria in mice (*Plasmodium berghei*).**

It may be of interest to note that **5** shows substantial *in vitro* histidine decarboxylase inhibitory activity and is the most potent *in vivo* antiinflammatory agent of this series. Compound **5**, which possesses a 3-Ph ring, is more than twice as active in the carrageenin test as is **7**, a related isoxazole derivative possessing Me groups in positions 3 and 5. This may indicate the enhancement of antiinflammatory activity by the hydrophobic bonding of the Ph group.

Experimental Section

Melting points were detd on a Fisher-Johns melting point apparatus and are uncor. All analytical samples had ir and nmr spectra in agreement with their assigned structure. Ir spectra were detd with a Perkin-Elmer Model 137 spectrophotometer; nmr spectra were recorded on a Varian A-60 spectrometer (Me_4Si or DSS). Analyses indicated only by symbols of the elements were within the ±0.4% limit of the theoretical values and were performed by Elek Microanalytical Laboratories, Torrance, California.

Starting Materials. The requisite heterocyclic halomethyl derivatives, 2-anilino-5-chloromethyl-1,3,4-thiadiazole,[9] 5-bromomethylisoxazole,[10] 3-phenyl-5-bromomethylisoxazole,[11] 3,5-dimethyl-4-chloromethylisoxazole,[12] and 2-chloromethylpyrazine,[13] were prepd according to literature methods.

N-Substituted Phthalimides (1, 3, 4, 6, 8). The prepn of these compds is exemplified by the following procedure.

Method C. *N*-(**2-Anilino-1,3,4-thiadiazol-5-ylmethoxy)phthalimide (1).** A soln of 11.2 g (0.05 mole) of 2-anilino-5-chloromethyl-1,3,4-thiadiazole, 8.2 g (0.05 mole) of *N*-hydroxyphthalimide, and 10 g (0.1 mole) of Et_3N in 100 ml of MeCN was refluxed for 3 hr. The ppt, obtd upon cooling, was filtered and washed with H_2O. Recrystn from EtOH gave 12.6 g (72%) of the pure compd, mp 222–223°. *Anal.* ($C_{17}H_{12}N_4O_3S$) C, H, N.

Alkoxyamines (2, 5, 7, 9). The prepn described below illustrates the general method of synthesis employed.

Method D. 5-[(Aminooxy)methyl]-2-anilino-1,3,4-thiadiazole · HCl (2). To a suspension of 14 g (0.04 mole) of *N*-2-(anilino-1,3,4-thiadiazol-5-ylmethoxy)phthalimide in 250 ml of warm anhyd EtOH was added 2.0 g (0.04 mole) of hydrazine hydrate. The mixt was refluxed for 3 hr; the phthalhydrazide was removed by filtration of the hot soln. The filtrate was concd, and, upon cooling, 8.0 g (90%) of the free base sepd, mp 149–151°. *Anal.* ($C_9H_{10}N_4OS$) C, H, N.

The purified base was dissolved in anhyd EtOH, and addn of excess ethereal HCl gave the hydrochloride, mp 188–190° dec, nmr ($CDCl_3$) δ 5.00 (s, 2 H), 7.35–7.50 (m, 5 H). *Anal.* ($C_9H_{10}N_4OS$ · HCl) C, H, N.

In some cases the base was not isolated. After removal of the pptd phthalhydrazide, addn of excess ethereal HCl to the filtrate afforded the alkoxyamine in the form of the HCl salt.

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**These data were obtained from Walter Reed Army Institute of Research.