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

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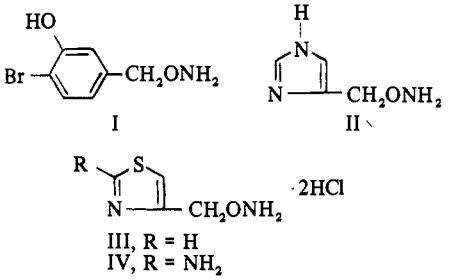
The synthesis of several heterocyclic O-substituted hydroxylamine derivatives is described. 5-[(Aminooxy)methyl]-3-phenylisoxazole · HCl possesses significant antiinflammatory activity in the carrageenin-induced rat paw edema test and also shows substantial inhibitory activity when tested *in vitro* 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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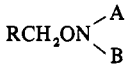
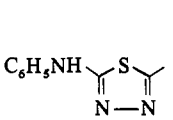
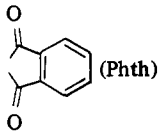
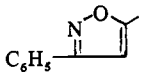
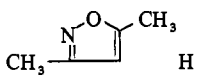
§G. H. Hamor, R. Hudgins, D. Aures, and W. G. Clark, unpublished results.

#These data were received from Cancer Chemotherapy National Service Center. Protocols for screening chemical agents and natural products against animal tumors and other biological systems are described in ref 8.

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

Table I



No.	R	A	B	Salt	Method	% yield[a]	Recrystn[b] solvent	Mp, °C	Formula[g]	Antiinflam act, % inhib of edema
1		 (Phth)			C	72	E	222–223	$C_{17}H_{12}N_4O_3S$	
2		H	H	HCl[c]	D	90	E	188–190 dec	$C_9H_{10}N_4OS \cdot HCl$	29
3		Phth			C	60	E	132–133	$C_{12}H_8N_2O_4$	
4		Phth			C	66	E	206–208	$C_{18}H_{12}N_2O_4$	
5		H	H	HCl	D	86	F	192–194 dec	$C_{10}H_{10}N_2O_2 \cdot HCl$[d]	55
6		Phth			C	70	E	143–145	$C_{14}H_{12}N_2O_4$	
7		H	H	HCl	D	70	F	161–163 dec	$C_6H_{10}N_2O_2 \cdot HCl$[e]	24
8		Phth			C	50	E	146–148	$C_{13}H_9N_3O_3$	
9		H	H	2HCl	D	90	F	140–142 dec	$C_5H_7N_3O \cdot 2HCl$[f]	36

[a] Yield of once recrystallized material. [b] E, EtOH; F, MeOH-Et_2O. [c] Free base, mp 149–151°. [d] C: calcd, 52.99; found, 52.27. Nmr (DMF) δ 5.56 (s, 2 H), 7.32 (s, 1 H), 7.44–7.50 (m, 5 H). [e] Nmr (D_2O) δ 2.16 (s, 3 H), 2.31 (s, 3 H), 4.86 (s, 2 H). [f] C: Calcd, 30.32; found, 30.82. H: Calcd, 4.58; found, 5.18. Nmr (D_2O) δ 5.32 (s, 2 H), 8.75–8.82 (m, 3 H). [g] All compds were analyzed for C, H, N.

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Pyrimidine Derivatives and Related Compounds. 15.[1] Synthesis and Analgetic and Antiinflammatory Activities of 1,3-Substituted 5-Amino-6-methyluracil Derivatives

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3-Alkyl-5-dimethylamino-6-methyl-1-phenyluracils (A), 1-alkyl-5-dimethylamino-6-methyl-3-phenyluracils (B) and their related compounds were synthesized and their acute toxicities and analgetic, antipyretic, and antiinflammatory activities were investigated. In the synthesis, substituted ureas were treated with diketene or ethyl acetoacetate, the 5 position of the resulting 1-substituted 6-methyluracils or 3-substituted 6-methyluracils was halogenated, and then the intermediate was refluxed in DMF with various amines to give 47 new 1,3-substituted 5-amino-6-methyluracil derivatives. The analgetic activities of group A (where 3-alkyl is Me or allyl) and group B (where 1-alkyl is Me, Et, or allyl) were of the same or higher order than that of aminopyrine combined with lower toxicity (0.5–0.25). Antiinflammatory activities of many of them were also comparable to or higher than that of benzydamine.

Senda, *et al.*, had previously synthesized[2] 3-cyclohexyl-5-dimethylamino-1,6-dimethyluracil (1) in which the pyrazolone ring of aminopyrine was expanded to a uracil ring. However, some difficulties were encountered in the synthesis of the uracil derivatives which have now been overcome. We have also investigated a relation between the pharmacological actions (analgetic, antipyretic, and antiinflammatory actions and acute toxicities) and chemical structures with particu-