TABLE I
ANDROGENIC–MYOTROPHIC ASSAY

Compound (total dose, mg)	Wt, mg[a]			Body wt, g	
	Ventral prostate	Seminal vesicle	Levator ani	Initial	Final
Castrate control	16.9 ± 1.07	12.0 ± 0.49	23.8 ± 2.05	54	92
Testosterone(0.3)	25.6 ± 2.47	15.1 ± 0.76	31.4 ± 2.50	55	96
p	<0.05	<0.05	<0.10–0.05		
Testosterone(0.6)	30.7 ± 3.50	17.2 ± 1.32	35.4 ± 1.71	55	94
p	<0.02	<0.05	<0.01		
Testosterone propionate(0.3)	55.5 ± 6.66	21.2 ± 1.25	34.8 ± 1.44	55	99
p	<0.01	<0.01	<0.02		
5 (3.0)	38.0 ± 8.62	12.5 ± 0.94	29.2 ± 2.65	54	91
p	ca 0.05	NS[b]	NS		
6 (3.0)	39.5 ± 6.38	13.1 ± 0.56	29.8 ± 3.31	54	92
p	<0.05	NS	NS		
7 (3.0)	39.0 ± 3.88	14.4 ± 0.59	27.6 ± 2.05	54	93
p	<0.01	<0.05	NS		
8 (0.3)	73.3 ± 4.64	26.5 ± 0.84	33.7 ± 1.93	54	98
p	<0.001	<0.001	<0.05		
9 (0.3)	111.8 ± 9.37	35.4 ± 2.84	41.4 ± 2.08	54	95
p	<0.001	<0.01	<0.01		

[a] Mean ± standard error. [b] Not significant

than testosterone (Table I). On the other hand, the $\Delta^{8(14)}$ compounds were only weakly active; in no case was potency higher than 0.2 of the corresponding testosterone activity observed. This could mean that the hypothesis of enhancement due to flattening toward the β face is incorrect. Alternatively, the presence of the double bond, or the absence of the 5β- or 14α-H may be responsible for the low order of activity.

Experimental Section[6]

5α-Androsta-8(14)-3,17-dione (2).—A solution of 2 g of 1[4] in 200 ml of Me_2CO was oxidized with Jones reagent at room temp. *i*-PrOH was added to destroy the excess Jones reagent, ice water was added, and the Me_2CO was removed under reduced pressure. The pptd powder was filtered to afford 1.8 g of product, mp 144–148°, which was recrystd from MeOH–H_2O to give a sample: mp 145–149°; nmr 0.93 (19 H_3), 1.10 (18 H_3), $[\alpha]^{20}D$ +347° (*c*, 1, $CHCl_3$). *Anal.* ($C_{19}H_{26}O_2$) C, H.

3,3-Dimethoxy-5α-androst-8(14)-en-17-one (3).—A solution of 1.5 g of **2** and 1.5 g of SeO_2 in 60 ml of MeOH was heated at 50° for 15 min. It was cooled to room temp and a solution of 2.5 g of KOH in 20 ml of MeOH was added to make the solution alkaline. It was poured into ice water, and the pptd powder was filtered to afford 1.5 g of crude product, mp 103–104°. It was recrystd from MeOH containing 1 drop of methanolic KOH to give **3**: mp 106–108°; nmr 0.70 (19 H_3), 1.07 (18 H_3), $[\alpha]^{20}D$ +222° (*c*, 1% $CHCl_3$). *Anal.* Calcd for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.10; H, 9.28.

17β-Hydroxy-5α-androst-8(14)-en-3-one (5).—To a solution of 1.2 g of **3** in 40 ml of MeOH was added slowly a solution of 1.2 g of $NaBH_4$ in 20 ml of MeOH. Ice water was added and the pptd powder was filtered to afford 1.0 g of crude **4**, mp 184–187°. A solution of 1.0 g of this **4** in 5 ml of HOAc was warmed on a steam bath for 10 min and H_2O was added dropwise. The solution was cooled and the pptd powder was filtered to afford 0.7 g of **5**, mp 180–183°. It was recrystd from MeOH–H_2O to give material, mp 182–183°, $[\alpha]^{20}D$ +65° (*c*, 1% $CHCl_3$). *Anal.* ($C_{19}H_{28}O_2$) C, H.

17β-Hydroxy-5α-androst-8(14)-en-3-one Acetate (6).—A solution of 0.1 g of **5** in 1 ml of C_5H_5N was added to 0.1 ml of Ac_2O and the mixture was kept at room temp for 24 hr. Ice water was added and the pptd powder was filtered to afford 0.95 g of product, mp 145–147°, raised to mp 148–149° after recrystallization from MeOH, $[\alpha]^{20}D$ +41° (*c*, 1% $CHCl_3$). *Anal.* ($C_{21}H_{30}O_3$) C, H.

17β-Hydroxy-5α-androst-8(14)-en-3-one Propionate (7).—To a solution of 0.1 g of **5** in 1 ml of C_5H_5N was added 0.1 ml of $(C_2H_5CO)_2O$ and the mixture was kept at room temp for 24 hr. Ice water was added and the pptd powder was filtered to afford 0.105 g of product, mp 158–163°. It was recrystd from MeOH–H_2O to give material: mp 160–162°; nmr 0.90 (19 H_3), 0.98 (18 H_3), $[\alpha]^{20}D$ +38° (*c*, 1% $CHCl_3$). *Anal.* ($C_{22}H_{32}O_3$) C, H.

(6) Melting points were determined with a Thomas-Hoover apparatus equipped with a corrected thermometer. Microanalyses were performed by the Microanalytical Department, University of California, Berkeley, Calif. Nmr spectra were obtained at a field strength of 60 MHz on samples in $CDCl_3$ solution on a Varian A 60A instrument using TMS as internal standard. Optical rotations were obtained in a 0.5-dm tube with a Rudolph photoelectric polarimeter. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

Imidazole Derivatives. Histidine Decarboxylase Inhibitors

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Histamine has been implicated in a number of physiological processes,[2,3] among them, the regulation of the microcirculation,[4] gastric secretion,[5] growth and repair processes,[6] and certain hormone actions.[7] Some clinical conditions in which histamine plays a role are anaphylaxis and allergy, wound healing, inflamation, and mastocytosis. The discovery of an inducible, specific histidine decarboxylase (HD) in mammalian tissues and the development of sensitive assays[8] has opened up new approaches to the understanding of the physiological and pathological role of histamine. In recent years, interest in histidine decarboxylase inhibitors as tools in such studies and as agents for the treatment of disorders

(1) To whom inquiries should be directed.

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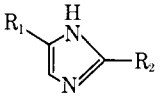
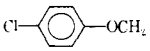
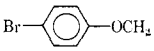
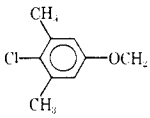
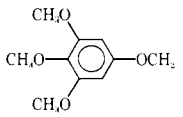
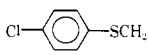
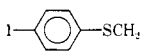
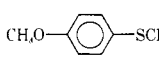
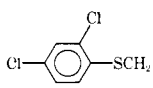
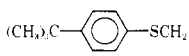
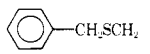
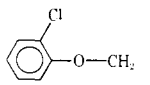
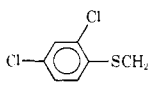
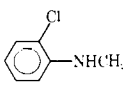
TABLE I



No	R_1	R_2	Empirical formula[a]	Mp, °C[b]	% yield	Histidine decarboxylase inhibition (%)[c]
1		H	$C_{10}H_{10}N_2O \cdot HCl$	187–188	75	20
2		H	$C_{10}H_9ClN_2O \cdot HCl$	200–201	60	47
3		H	$C_{10}H_9ClN_2O \cdot HCl$	152–154	51	34
4		H	$C_{10}H_9ClN_2O \cdot HCl$	188–190	60	28
5		H	$C_{10}H_9BrN_2O \cdot HCl$	193–195	56	50
6		H	$C_{10}H_9BrN_2O \cdot HCl$	192–193	66	45
7		H	$C_{10}H_9FN_2O \cdot HCl$	177–179	75	28
8		H	$C_{10}H_9IN_2O \cdot HCl$	176–177	51	45
9		H	$C_{11}H_{12}N_2O \cdot HCl$	187–189	56	27
10		H	$C_{12}H_{13}ClN_2O \cdot HCl$	195–196	48	27
11		H	$C_{13}H_{16}N_2O_4 \cdot HCl$	222–223	43	0
12		H	$C_{10}H_{10}N_2S \cdot HCl$	124–125	90	65
13		H	$C_{10}H_9ClN_2S \cdot HCl$	176–177	93	59
14		H	$C_{10}H_9IN_2S \cdot HCl$	200–201	82	79
15		H	$C_{11}H_{12}N_2OS \cdot HCl$	123–124	85	77
16		H	$C_{10}H_8Cl_2N_2S \cdot HCl$	184–186	46	77
17		H	$C_{14}H_{18}N_2S \cdot HCl$	164–165	52	75
18		H	$C_{11}H_{12}N_2S \cdot HCl$	142–143	45	29
19	H		$C_{10}H_9ClN_2O \cdot HCl$	199–200	53	0
20	H		$C_{10}H_8Cl_2N_2S \cdot HCl$	180–182	48	15
21		H	$C_{10}H_{10}ClN_3 \cdot 2HCl$	222–225	46	0

[a] All compounds listed in the table were analyzed for C, H, N and the results were within ±0.4% of theoretical. [b] Melting points were determined in a Thomas-Hoover capillary apparatus and are uncorrected. [c] All compounds tested at 10^{-4} *M*.

in which histamine is a factor has increased steadily.[9-15] Inhibitors which have been developed for this purpose are either weak but specific, *e.g.*, α-methylhistidine or potent but relatively nonspecific, *e.g.*, 3-hydroxy-4-bromobenzyloxyamine. We have examined a series of imidazole derivatives in a search for more potent, specific inhibitors of HD.

Chemistry.—Compounds **1-20** were prepared essentially by the method of Ruoff and Scott.[14] The method of isolation was modified so as to give analytically pure HCl salts directly. The 4(5)-chloromethylimidazole·HCl used as starting material was described by Pyman.[15] 2-Chloromethylimidazole·HCl used was described by Jones.[16]

Experimental Section

Compounds **1-20** were prepared by an identical procedure as represented by the following experiments.

4(5)-*p*-Bromophenoxymethylimidazole·HCl (6).—To a solution of 1.5 g (0.066 g-atom) of Na in 75 ml of dry EtOH was added 11.4 g (0.066 mole) of *p*-bromophenol in 25 ml of EtOH. The solution was stirred for 2.5 hr and then cooled in an ice–HCl bath. A solution of 5.0 g (0.033 mole) of 4(5)-chloromethylimidazole·HCl in 50 ml of EtOH was then added slowly over a period of about 20 min with stirring, and the mixture was then stirred an additional 6 hr at room temperature. After filtration to remove NaCl, the alcohol solvent was removed under reduced pressure. To the resulting oil was added 15 ml of H_2O and 20 ml of 6 *N* HCl. This solution was extracted with 50 ml of Et_2O to remove the unreacted phenol. The aq phase on standing deposited 4.7 g (66%) of colorless, crystalline **6**, mp 192–193°.

4(5)-(*o*-Chloroanilinomethyl)imidazole·2HCl (21).—A solution of 5.0 g (0.033 mole) of 4(5)-chloromethylimidazole·HCl and 12.8 g (0.1 mole) of *o*-chloroaniline in 100 ml of EtOH was refluxed for 6 hr. The solvent was removed *in vacuo* and the residue triturated several times with anhyd Et_2O to remove excess *o*-chloroaniline. The remaining solid, which was quite hygroscopic, was recrystallized twice from EtOH-Et_2O to give 4.3 g (46%) of **21**.

Enzymology.—Histidine decarboxylase was prepared and assayed essentially as described by Snyder and Epps.[17] Female Sprague-Dawley rats (250-300 g; food and water *ad lib*) were anesthetized (Et_2O) and their stomachs removed and chilled in ice. The glandular portions of the stomachs were cleaned, weighed, homogenized in 5 vol of ice-cold 0.05 *M* KPO_4 buffer, pH 7.2, and centrifuged at 10,000*g* for 20 min at 0°. Enzyme activity was measured as CO_2-^{14}C from DL-histidine-1-^{14}C. The reaction medium (2 ml) contained 0.4 ml of 10,000*g* supernatant, 0.05 *M* KPO_4 buffer, pH 7.2, 10^{-5} *M* pyridoxal phosphate, and 10^{-3} *M* L-histidine (0.1 μCi of DL). The enzyme was preincubated for 10 min prior to starting the reaction with substrate and the incubation was continued with agitation for 2 hr at 37°. The reaction was stopped with 1 ml of 1 *M* citric acid and the evolved CO_2-^{14}C was trapped during 30 min at 37° in 0.2 ml of Hyamine contained in the center well of the reaction vessel. The radioactivity was then determined in a liquid scintillation spectrometer.

Dopa decarboxylase was obtained as an acetone powder from guinea pig kidney.[18] Assay was based on the measurement of CO_2-^{14}C from DL-3,4-dihydroxyphenylalanine-1-^{14}C (DL-DOPA). Enzyme (5–10 mg) was incubated for 20 min at 37° in a medium (2 ml) containing 0.05 *M* KPO_4 buffer, pH 6.8, 10^{-5} *M* pyridoxal phosphate, and 5×10^{-4} *M* L-DOPA (0.1 μCi of DL-DOPA). Drugs and appropriate controls were preincubated with enzyme for 10 min prior to starting the reaction with substrate. The reaction was terminated by the addition of 0.6 ml of 1 *M* H_2SO_4 and then processing was continued as for histidine decarboxylase above.

TABLE II

EFFECT OF 14 AND 16 ON DOPA DECARBOXYLASE

Compd	Concn, *M*	Enzyme inhibn, %
14	10^{-3}	59
	10^{-4}	2
16	10^{-3}	11
	10^{-4}	4
α-Methyl dopa	10^{-4}	57

Results and Discussion

The results are summarized in Table I. Activity against the enzyme was tested with the compound being tested at a concentration of 10^{-4} *M*. None of the compounds fulfills the need for potent, specific inhibitors of HD. The relatively small number of compounds and the limited variety of structural modifications in this series does not permit a sophisticated analysis of structure–activity relationships. Nevertheless, some information has been gained which could be useful in further work on imidazole derivatives.

In the O series, substitution with Cl increases activity in the order *para* < *meta* < *ortho*. Cl, Br, and I are equivalent in the *ortho* position whereas in the *para* position the bromo compound is more active than the chloro derivative. *Ortho* substitution with F or Me contributes little to activity suggesting that resonance effects are more important than inductive ones. The trimethoxy derivative was inactive.

Substitution of S for phenolic O increases activity. In this series (**12–17**) aromatic substitution results in relatively minor changes in activity. Here also, the *p*-Cl derivative **13** is similar in activity to the parent compound, but in general, substituents supplying electron density increase activity somewhat. When S is removed from conjugation with the ring (**18**) we have a compound comparable with the parent drug in the O series. The N isostere **21** is inactive. We may speculate that a high electron density about the ether linkage contributes importantly to binding on the enzyme surface. Consistent with this is the fact that the inductive effect of the ring is greatest in aniline and least in thiophenol.

Activity is drastically reduced in the 2-substituted imidazoles as can be seen by comparing **2** with **19**, and **16** with **20**.

Compounds **14** and **16** were tested against guinea pig kidney dopa decarboxylase under conditions which were optimal for the decarboxylation of DOPA in order to get some idea concerning their specificity. Table II illustrates the results. As expected these compounds are considerably less active against this enzyme in comparison to specific histidine decarboxylase (see Table I). It should be noted that although equipotent against the latter enzyme, there is a significant difference between the activity of **14** and **16** against dopa decarboxylase.

Acknowledgment. We thank Bruce B. Butler for technical assistance.

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