volume (PCV) according to the following formula.

$$TPCV = TV \times PCV$$

The effects of the nitrosoureas were determined by calculating the ratio of the tumor weight of treated animals to that of the controls (T/C, %).

$$T/C, \% = [TPCV\ (treated)/TPCV\ (control)] \times 100$$

The effect against leukemia L1210 was determined by the percent increase in life span (ILS) which was determined by the following formula.

$$ILS = [[life\ span\ (treated)/life\ span\ (control)] - 1] \times 100$$

For a single administration schedule, the blood sugar concentration was determined with groups of five male Wistar strain rats weighing about 200 g. These were not fed for 16 h prior to administration of the test compounds, which were given in a physiological saline solution. Administration was by means of intravenous injection into the femoral vein. Blood samples were taken from the caudal vein at specific time intervals and the blood sugar concentrations were determined by the glucose oxidase method.[19]

The effect of the test compounds on the blood sugar concentration after a 1-month administration period was determined with groups of five male Wistar strain rats weighing about 150 g. The test compounds were administered intraperitoneally in a physiological saline solution for 30 days, qd, days 1–30. Blood samples were taken 24 h after the last administration of the test compound and the blood sugar concentration was determined as before.

Acute toxicities were determined using groups of ten female JCL-ICR mice. The test compounds were administered intraperitoneally in a physiological saline solution. The LD$_{50}$ was calculated according to the method of Litchfield and Wilcoxon[20] from the death rate over a 7-day period.

In vitro antibacterial activities were determined by the broth dilution method. Twofold serial dilutions of each test compound were prepared in heart infusion broth (Eiken Chemical Co., Tokyo) in test tubes. A 0.1-ml volume of a cell suspension containing the test bacteria which had been cultivated for 24 h in heart infusion broth and 100-fold diluted with physiological saline solution was inoculated into the tubes aseptically and the tubes

were incubated at 30 °C for 18 h. The minimum inhibitory concentration (MIC) was expressed in μg/ml for the dosage at which the growth of test cultures was completely inhibited.

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Potential Histamine H$_2$-Receptor Antagonists.[1] 3. Methylhistamines

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Syntheses are described for all the mono- and some di- and trimethylhistamines. New methods are given for the known N^π-, N^τ-, N^α-, 2-, and 4-methylhistamines and for the novel compounds, β-methyl-, 4,N^α-dimethyl-, and 4,N^α,N^α-trimethylhistamines. Agonist activities are reported for stimulation of histamine H$_1$ (guinea-pig ileum) and H$_2$ (rat gastric acid secretion) receptors. H$_2$-Receptor agonist activities indicate that a methyl group is more readily accommodated at the 4 and N^α positions than elsewhere in the histamine molecule and that receptor binding is substantially retained with a methyl substituent in these positions. Thus, for the design of potential antagonists, two sites are identified as being worthwhile exploring for the introduction of lipophilic substituents.

Certain pharmacological actions of histamine are mediated by histamine receptors, now classified into two types, H$_1$ and H$_2$.[2,3] The effect of histamine at its H$_1$ receptor may be blocked specifically by conventional antihistaminic drugs, such as pyrilamine,[2] but H$_2$-receptor effects are not blocked by these drugs. An investigation in these laboratories into the classification and blockade of histamine H$_2$ receptors has culminated in the synthesis and characterization of H$_2$-receptor antagonist drugs

typified by burimamide,[3] metiamide,[4] and cimetidine.[5]

In this series of publications we describe various approaches used in attempts to design H$_2$-receptor antagonists. Our starting point throughout this work has been the natural agonist molecule, histamine, and we have attempted to modify it chemically in ways which, to our intuition, seemed potentially capable of providing an antagonist. One approach taken was to incorporate large nonpolar lipophilic substituents into the histamine mol-

Table I. Methylhistamines

(Structure: imidazole ring with positions labeled 4, 2; ring nitrogens N^τ, N^π; side chain β–α–N^α as C–C–N<)

Compd no.	Substituent[a]	Salt	Mp, °C	Crystn solvent	Lit. mp[b]	Mol formula[c]	Agonist act. rel to histamine (=100) H_2 receptor[d]	H_1 receptor[e]
4	N^π-CH$_3$	2-Picrate	212–214	EtOH–H$_2$O	201 (10)	C$_6$H$_{11}$N$_3$·2C$_6$H$_3$N$_3$O$_7$		
		2HCl	269–271	EtOH–H$_2$O	265–266 (10)	C$_6$H$_{11}$N$_3$·2HCl	<0.2^f	<0.01^{g,h}
8	2-CH$_3$	2HCl	222–224	EtOH–Et$_2$O	217 (14)	C$_6$H$_{11}$N$_3$·2HCl	2.0^i	16.5^{j,k} (15.1–18.1)
10b	4-CH$_3$	2HCl	239–242	MeOH–Et$_2$O	231–232 (23)	C$_6$H$_{11}$N$_3$·2HCl	39^i	0.23^{j,l} (0.20–0.27)
13	N^τ-CH$_3$	2-Picrate	218–219	EtOH–H$_2$O	217 (9)	C$_6$H$_{11}$N$_3$·2C$_6$H$_3$N$_3$O$_7$		
		2HCl	205–207	EtOH	204–206 (9)	C$_6$H$_{11}$N$_3$·2HCl	<0.01^f	~0.5^{m,n}
14a	N^α-CH$_3$	2HCl	176–178	EtOH	176–177 (27)	C$_6$H$_{11}$N$_3$·2HCl	74^{h,o}	72^{j,p} (62–84)
14b	4,N^α-(CH$_3$)$_2$	2HCl	275–277	EtOH		C$_7$H$_{13}$N$_3$·2HCl	8.2^i	0.16^j (0.1–0.2)
16a	N^α,N^α-(CH$_3$)$_2$	2HCl	188–191	PrOH	183–184 (30)	C$_7$H$_{13}$N$_3$·2HCl	19^i	44^{j,q} (38–51)
16b	4,N^α,N^α-(CH$_3$)$_3$	2-Picrate	241–242	DMF–EtOH		C$_8$H$_{15}$N$_3$·2C$_6$H$_3$N$_3$O$_7$		
		2HCl	193–196	EtOH–Et$_2$O		C$_8$H$_{15}$N$_3$·2HCl	3.0^i	0.1^g
23	β-CH$_3$	2-Picrate	214–215	EtOH		C$_6$H$_{11}$N$_3$·2C$_6$H$_3$N$_3$O$_7$		
		2HCl	203	EtOH		C$_6$H$_{11}$N$_3$·2HCl	0.4^f	0.83^j (0.42–1.6)
24	β,β-(CH$_3$)$_2$	2HCl	272–275	EtOH–H$_2$O	240–245 (32)	C$_7$H$_{13}$N$_3$·2HCl	–ve^r	<0.001^g
25	α-CH$_3$	2-Picrate	202–204	H$_2$O	202–204 (33)^s	C$_6$H$_{11}$N$_3$·2C$_6$H$_3$N$_3$O$_7^s$		
		2HCl	169–170	i-PrOH		C$_6$H$_{11}$N$_3$·2HCl	0.8^f	0.36^{j,t} (0.18–0.73)
26	2,N^α,N^α-(CH$_3$)$_3$	2HCl				C$_8$H$_{15}$N$_3$·2HCl^u	1.4^i	16.8^j (15.8–17.7)

^{a} Numbering according to ref 42. ^{b} Literature reference in parentheses. ^{c} Compounds were analyzed for C, H, N, and Cl (if present). ^{d} Tested for stimulation of gastric acid secretion in anesthetized rats.[3,35] ^{e} Tested for contraction of isolated guinea-pig ileum in the presence of atropine.[3,35] ^{f} Approximate value obtained by comparison of doses required to produce equal acid secretory responses in a minimum of two preparations.[35] ^{g} Approximate value obtained by comparison of single doses required to cause equal contractile responses in a minimum of two preparations.[35] ^{h} Reported inactive by Lee and Jones.[43] ^{i} Estimation of relative activity from 3 + 3 parallel line assay.[35] ^{j} Estimate of activity relative to histamine from 2 + 2 parallel line assay;[35] 95% confidence limits in parentheses. ^{k} Lit.[43] data 30%. ^{l} Lit.[44] data ca. 1%. ^{m} Approximate value due to nonparallelism of cumulative dose–response curves. ^{n} Lit.[43] data 0.6%. ^{o} Lit.[2] data 51%. ^{p} Lit.[45] data 100%. ^{q} Lit.[46] data 80%. ^{r} No stimulation detected at 486 μmol/kg iv. ^{s} Monohydrate; Ison and Casy[34a] report mp 182–183° for the anhydrous dipicrate. ^{t} Lit.[44] data ca. 1%. ^{u} See ref 39.

ecule. Many known competitive antagonist drugs (e.g., anticholinergic agents, conventional antihistamines, and adrenergic blockers) possess such groups which may contribute to drug–receptor association through hydrophobic bonding with nonpolar areas of the receptor, either at the active site or in its immediate vicinity.[6] The problem, however, was to find appropriate groups and the correct substitution positions in the histamine molecule.

By analogy with active-site-directed enzyme inhibitors designed from substrates, where both the position and nature of a lipophilic substituent can be critical,[7] we reasoned that it was essential to identify the sites in the histamine molecule where a substituent can be accommodated without loss of receptor binding. We considered that a small nonpolar substituent such as methyl would be most suitable for this part of the investigation and that if the methyl-substituted compound possessed a good level of histamine-like agonist activity we could deduce that receptor binding had been retained.

In the present paper we describe the synthesis and pharmacological activity of all the monomethyl-substituted histamines and some di- and trimethyl-substituted histamines (Table I).

Synthesis. Previous syntheses[8] of many of the methylhistamines have been unsatisfactory and it was necessary, therefore, to develop new routes to some compounds. It was also necessary to find suitable solvent

Table II. TLC Separation of Methylhistamines[a]

| Position of methyl substituent(s)[b]	System A[c]	System B[d]
Histamine	0.52	0.70
N^π-CH$_3$ (4)	0.63	0.56
N^τ-CH$_3$ (13)	0.56	0.52
4-CH$_3$ (10b)	0.56	0.70
N^α-CH$_3$ (14a)	0.46	0.68
4,N^α-(CH$_3$)$_2$ (14b)	0.51	0.70
4,N^α,N^α-(CH$_3$)$_3$ (16b)	0.72	0.57

^{a} Compounds were run alongside histamine and 4-methylhistamine on one TLC plate. ^{b} Compound number in parentheses. ^{c} MeOH–NH$_4$OH (d 0.88)–H$_2$O (6:1:1) (SiO$_2$ F$_{254}$). ^{d} EtOCH$_2$CH$_2$OH–HCl (11 N)–H$_2$O (8:1:1) (SiO$_2$ G).

systems for TLC separation of the methylhistamines. In routes where either histamine or 4-methylhistamine (10b, Table I) was used as a starting material, or might have been generated as a by-product, the final product was carefully examined by TLC for the absence of contamination by these two substances. Thus, for the compounds in Table II, by using the appropriate TLC system, histamine or 4-methylhistamine was shown to be at a level below 0.2%. It is important to establish these levels since contamination by histamine or 4-methylhistamine could give falsely high results in the pharmacological assays for agonist activity.

Scheme I

Phth:N = phthalimido
Pom = pivaloyloxymethyl

Scheme II

9a, X = OH
b, X = Cl
c, X = CN
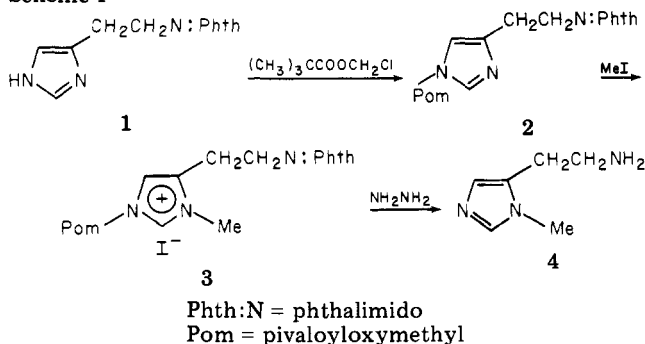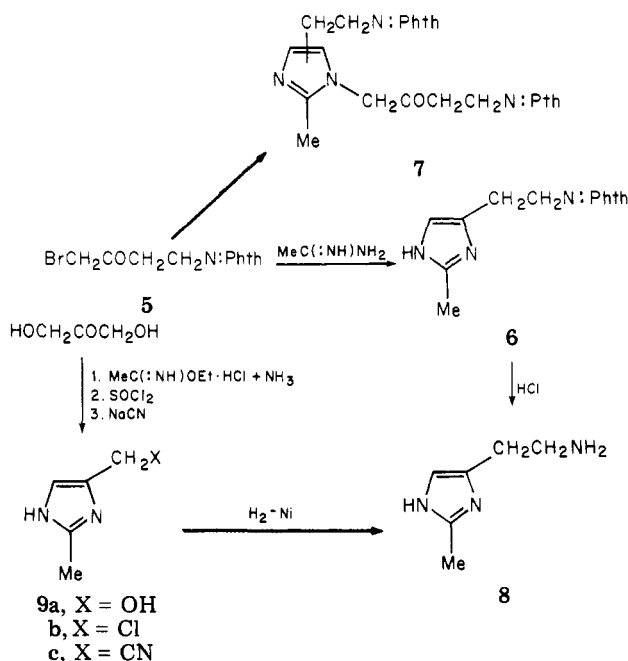
N^π-**Methylhistamine (4)** was first synthesized by Pyman[9] via a nonselective alkylation of 4(5)-imidazolyl-acetonitrile followed by reduction. An unambiguous but lengthy synthesis was described subsequently by Jones.[10] We have now developed a novel route, involving the selective methylation of histamine at the N^π position[11] (Scheme I). Reaction of N^α-phthaloylhistamine (1) with pivaloyloxymethyl chloride[12] gave the doubly protected histamine derivative 2. Treatment with an excess of MeI gave the quaternary salt 3, which was treated with hydrazine in EtOH to give 4 in good yield.

2-**Methylhistamine (8)** was first synthesized by van der Merwe[13,16b] from 1,2,4-tribenzamidobutene and acetic anhydride. Later, Tamamushi[14] described a lengthy synthesis via 2-methyl-4,5-dicarboxyimidazole. We now report two improved syntheses for this compound (Scheme II). The bromo ketone 5[15] was allowed to react with an excess of acetamidine in EtOH–DMF to give a low yield of the phthaloyl derivative 6 which was then hydrolyzed to give 8. When approximately equimolar quantities of the reactants were heated in Me$_2$SO the ring-alkylated imidazole 7 was the sole product isolated. The position of alkylation in 7 was not determined.
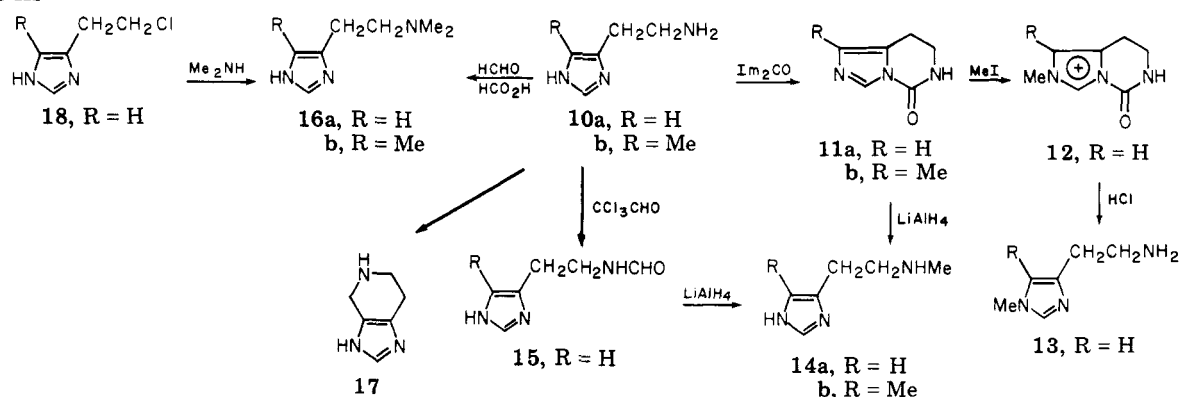
In an alternative synthesis, the carbinol 9a, prepared from 1,3-dihydroxyacetone by the method recently described by Dziuron and Schunack,[16a] was converted into 8 via the chloro compound 9b and the nitrile 9c. These workers have recently reported the synthesis of a series of 2-substituted histamines by this method.[16b]

N^τ-**Methylhistamine (13)** was first synthesized by Pyman,[9] together with N^π-methylhistamine (4), from 4(5)-imidazolylacetonitrile. It was later obtained by Rothschild and Schayer[17] by methylation of N-acetyl-histamine. An unambiguous synthesis is possible from 1-methyl-4-hydroxymethylimidazole, which may be made from the corresponding imidazoline carboxylate by dehydrogenation and reduction as described recently by Martin and co-workers.[18] However, this route would be rather lengthy. In this paper, we describe a new and unambiguous synthesis of N^τ-methylhistamine that involves the selective methylation of histamine at the N^τ position (Scheme III). The cyclic urea 11a,[19,20] prepared by reaction of histamine with carbonyl diimidazole, proved ideally suitable for the purpose of protecting the N^π- and N^α-nitrogens prior to alkylation. Methylation of 11a with an excess of MeI in DMF gave a near quantitative yield of the quaternary salt 12 which was then hydrolyzed in hot acid to give 13, also in high yield. The particular advantages of this general synthesis are (1) high overall conversion, (2) only one isomer is produced, (3) dialkylated products are absent, and (4) histamine is absent from the final product since 11a is completely alkylated.
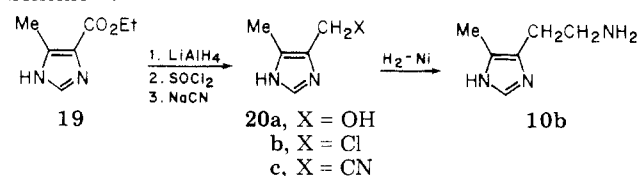
4-**Methylhistamine (10b)**[21] was prepared from the ester 19[22] by chain extension (Scheme IV). Ewins[23] previously reported the synthesis of 10b from the carbinol 20a, which he obtained by hydroxymethylation of 4(5)-methylimidazole. We find that 20a is prepared, more conveniently, by reduction of 19. The derived chloro compound 20b[23,24] was treated with NaCN in Me$_2$SO[25] to give the nitrile 20c which was then reduced catalytically[26] to give 10b.

N^α-**Methylhistamine (14a)**, which was originally synthesized by Garforth and Pyman[27] from 4(5)-(2-chloroethyl)imidazole (18), and the novel 4,N^α-dimethylhistamine (14b) were most conveniently prepared
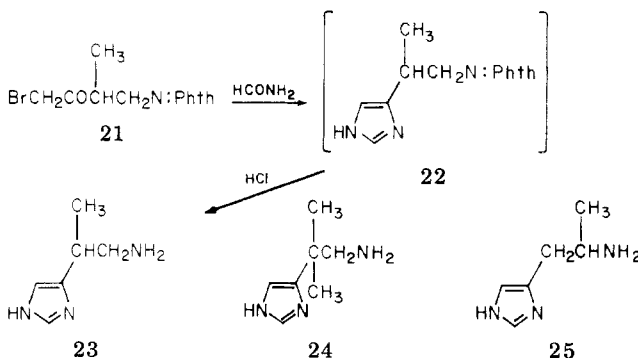
Scheme III

18, R = H

16a, R = H
b, R = Me

10a, R = H
b, R = Me

11a, R = H
b, R = Me

12, R = H

15, R = H
17

14a, R = H
b, R = Me

13, R = H

Scheme IV

Me CO₂Et → Me CH₂X → Me CH₂CH₂NH₂

1. LiAlH₄
2. SOCl₂
3. NaCN

H₂-Ni

19

20a, X = OH
b, X = Cl
c, X = CN

10b

Scheme V

CH₃
|
BrCH₂COCHCH₂N:Phth → HCONH₂ → [CH₃ | CHCH₂N:Phth]

21

22

HCl

CH₃
|
CHCH₂NH₂

23

CH₃
|
CCH₂NH₂
|
CH₃

24

CH₃
|
CH₂CHNH₂

25

by reduction of the cyclic ureas 11a,b, as described by Mechoulam and Hirshfeld[19] (Scheme III). 14a was also synthesized by reduction of the novel N^α-formylhistamine (15).[11a]

The novel 4,N^α,N^α-trimethylhistamine (16b)[11a] was readily prepared by performing an Eschweiler–Clarke reaction[28] on 4-methylhistamine (10b) (Scheme III). This method could not be used for the synthesis of N^α,N^α-dimethylhistamine (16a) since, in the absence of a 4-substituent, cyclization to spinaceamine (17) occurs;[29] compound 16a was therefore prepared from the chloro compound 18 as reported.[27,30]

β-Methylhistamine (23), previously unreported, was synthesized via a Bredereck reaction[31] involving the bromo ketone 21 and HCONH₂ (Scheme V). The intermediate phthaloyl derivative 22 was hydrolyzed without being isolated.

β,β-Dimethylhistamine (24) was prepared as reported by Jönsson.[32]

α-Methylhistamine (25) was prepared as reported by Alles et al.[33] with the exception that the dihydrochloride was characterized. Alternatively, 25 may be prepared from histidine by reduction to histidinol, conversion into α-bromomethylhistamine, and hydrogenolysis, as described recently by Ison and Casy[34a] and performed independently in these laboratories by Suschitsky.[34b]

Pharmacological Results and Discussion. The histamine derivatives, as their dihydrochlorides, were assayed for histamine H₂-receptor agonist activity, in vivo, by their ability to stimulate the secretion of acid from the lumen-perfused anesthetized rat preparation.[3] Details of the preparation used have been published previously.[35] Agonist activities were determined relative to histamine as standard in an analytical dilution assay using 3 + 3 doses in a Latin square design.[35] No compound was found to be a more active agonist than histamine. The results obtained, recorded in Table I, show that methyl substitution in histamine markedly influences the level of the H₂-receptor agonist activity and that the position of substitution is critical.[36] A methyl group is tolerated well at the ring 4 position or on the side-chain N^α atom; it is accommodated less well at the ring 2 position or on the side-chain α- or β-carbon atoms. Methyl substitution at the ring N^π or N^τ atoms appears to prevent the histamine molecule from functioning as an effective agonist at H₂

receptors. The appreciable H₂-receptor agonist activity of 4,N^α- and N^α,N^α-dimethylhistamines indicates that two substituents may be accommodated. The effect of combining methyl substituents is, however, not necessarily simply additive as may be seen by comparing the N^α,-N^α-tertiary amines with the corresponding primary amines. Thus, 4,N^α,N^α-trimethylhistamine (16b) is much less active than 4-methylhistamine but 2,N^α,N^α-trimethylhistamine (26) is similar in activity to 2-methylhistamine.

These results positively identify two positions, 4 and N^α, in the histamine molecule where methyl substitution does not result in appreciable loss in H₂-receptor binding. They indicate two sites which are worthwhile exploring for the introduction of lipophilic substituents in the design of potential antagonists. The results with the disubstituted compounds suggest that substituents should also be introduced in combination at both sites.

The weakly active agonists bearing methyl groups on ring nitrogens (4, 13) or side-chain carbon atoms (23–25) were subsequently tested for H₂-receptor antagonist activity against histamine-stimulated gastric acid secretion in the anesthetized rat.[3] The compounds were injected intravenously after a plateau of secretion had been established to an intravenous infusion of histamine. However, no significant antagonism was detected up to 256 μmol/kg (minimum of three preparations except for 23, which was tested only once due to insufficiency of compound).

The methylhistamines were also tested for H₁-receptor agonist activity measured on guinea-pig ileum in vitro (Table I).[37,38] Compounds were compared with histamine as standard using a 2 + 2 assay based on cumulative dose–response curves.[3,35] The results show that for H₁-receptor agonism, as for H₂-receptor stimulation, a methyl group is tolerated well on the N^α atom but not on the ring N^π or N^τ atoms nor on the α- or β-carbon atoms. However, substitution at the 4 position results in a much greater loss of H₁ activity than does substitution at the 2 position. The selectivity of action of the 2- and 4-ring methylated primary amines 8 and 10b has previously provided important evidence for the differentiation of H₁ and H₂ receptors.[3] It is evident from Table I that the selectivity of action of these two compounds is not much affected by additional substitution on the N^α atom, thus providing additional examples (14b, 16b, and 26) of substances which differentiate the two receptor populations.

Experimental Section

2-Methylhistamine (8). Route A. A solution of NaOEt, from Na (13.8 g) in dry EtOH (1200 ml), was added to a solution of acetamidine hydrochloride (56.8 g, 0.6 mol) in EtOH (400 ml). The stirred mixture was heated to reflux and a solution of 1-bromo-4-phthalimidobutan-2-one[15] (59.2 g, 0.2 mol) in a mixture of EtOH (1 l.) and DMF (200 ml) was added over 3 h. The mixture was heated for a further 2 h, cooled, and filtered and the filtrate evaporated to dryness. The residual oil was dissolved in EtOH and the solution charcoal-filtered, and the filtrate was acidified with ethanolic HCl. Concentration and addition of EtOAc gave a buff-colored precipitate which was purified by precipitating the base from H₂O and reacidifying with ethanolic HCl to give 6 as the dihydrochloride (8.7 g, 15%), mp 291–294° dec. A further recrystallization (EtOH) gave a sample, mp 294–296° dec. Anal. ($C_{14}H_{14}ClN_3O_2$) C, H, N, Cl.

A solution of 6 (8.25 g) in 5 N HCl (250 ml) was heated under reflux overnight, cooled to 0°, and filtered. Evaporation of the

filtrate followed by addition of dry EtOH and then Et_2O gave **8** as the dihydrochloride (5.4 g, 97%).

Reaction of **5** (5 g, 0.017 mol) with acetamidine (from the dihydrochloride, 2.0 g, 0.021 mol) in Me_2SO (120 ml) on a steam bath for 2 h, evaporation, and trituration of the residue with *i*-PrOH and then hot MeOH gave **7** as the hydrobromide (1.8 g): mp 280–283° (H_2O); δ (TFA) 2.7 (s, CH_3), 3.3 (t, CH_2Im + CH_2CO), 4.25 (t, CH_2N), 5.35 (s, CH_2), 7.26 (m, Im 4-H), 8.0 (s, Ar). Anal. ($C_{16}H_{23}BrN_4O_5$) C, H, N, Br. The base (mp 215–216°, EtOH) was obtained by treatment of the hydrobromide with $NaHCO_3$.

Route B. Ethyl acetimidate hydrochloride (100.0 g, 0.81 mol) was added gradually to liquid NH_3 (750 ml) in a precooled autoclave followed by 1,3-dihydroxyacetone (72.8 g, 0.80 mol). The contents were heated at 60–70° (45–50 atm) for 6 h, allowed to cool, filtered, and concentrated. The residue was extracted with Me_2CO and crystallized twice from *i*-PrOH–Et_2O to give **9a** as the base (62 g, 68%), mp 120–122° (*i*-PrOH–Et_2O). The hydrochloride (*i*-PrOH–EtOH) had mp 149–151° (lit.[40] 151–152°). This carbinol was converted (69%) into the HCl of **9b**, mp 145–148° (lit.[40] 149–150°), which was then allowed to react with NaCN as described for the 4-methyl isomer **20b** to give **9c** (61%), mp 168–170° (*i*-PrOH). Anal. ($C_6H_7N_3$) C, H, N. Reduction as for **20c** gave **8** as the dihydrochloride (53%).

5-Oxo-5,6,7,8-tetrahydroimidazo[1,5-c]pyrimidines (11a,b). Histamine base (24.4 g, 0.22 mol), obtained by treatment of the dihydrochloride with NaOEt in EtOH, and carbonyl diimidazole (55 g, 0.34 mol) were heated together with stirring to 100° over 1 h and then at 110–130° for 30 min. The solid base was ground to a fine powder under EtOH to give **11a** (27 g, 90%), mp 219–220° (lit.[19] 221–222°). Similarly, 4-methylhistamine base gave (61%) **11b**, mp 232–234°. Anal. ($C_7H_9N_3O$) C, H, N.

N^τ-Methylhistamine (13). A stirred solution of **11a** (12 g, 0.087 mol) and MeI (75 g, 0.52 mol) in dry DMF (100 ml) was heated under reflux overnight. Cooling and addition of Et_2O gave **2-methyl-5-oxo-5,6,7,8-tetrahydroimidazo[1,5-c]pyrimidinium iodide (12,** 22 g, 90%), mp 219–223° dec. Recrystallization from MeOH–Et_2O gave a sample, mp 225–227°. Anal. ($C_7H_{10}N_3IO$) C, H, N, I.

A solution of **12** (22.8 g, 0.082 mol) in 5 N HCl (200 ml) was heated under reflux overnight. Evaporation and addition of a solution of picric acid in EtOH gave **13** as the dipicrate (42.5 g, 89%), which was converted into the dihydrochloride (13.5 g).

4-Methylhistamine (10b). 4-Carboethoxy-5-methylimidazole (**19,** 540 g, 3.5 mol) was reduced with $LiAlH_4$ (189 g, 4.96 mol) in THF (14.1 l.) at room temperature and acidified with HCl to give **20a** hydrochloride (381 g, 74%), mp 243–244° (EtOH; lit.[23] 240–242°). This carbinol (272 g) was added portionwise with stirring to $SOCl_2$ (520 ml) at room temperature, heated on a steam bath for 30 min and evaporated to dryness in vacuo. Addition of EtOH–Et_2O gave **20b** hydrochloride (298 g, 74%), mp 222–225° (lit.[23] 222°). This chloro compound (135 g, 0.81 mol) was added portionwise over 10–15 min to a stirred mixture of NaCN (194 g, 4 mol) in dry Me_2SO (1.4 l.) at 40–45°. The mixture was stirred at 40° for 1 h, the solvent removed in vacuo, and the residue dissolved in H_2O (500 ml). Continuous extraction with *i*-PrOAc, evaporation, and recrystallization of the residue from *i*-PrOAc–EtOH gave **20c** (46 g, 47%), mp 166–168° (lit.[23] 163–164°). A solution of this nitrile (20 g, 0.165 mol) in EtOH (600 ml), saturated with NH_3 at –10°, was hydrogenated over Raney nickel at 145–150° (140–150 atm) for 3 h. The mixture was filtered, evaporated, and acidified with ethanolic HCl, and H_2O was added to give **10b** as the dihydrochloride (30 g, 92%).

N^α-Methylhistamine (14a). Route A. The cyclic urea (**11a,** 6.0 g, 0.044 mol) was added to a suspension of $LiAlH_4$ (5.7 g, 0.15 mol) in dry THF (350 ml) and the mixture heated under reflux for 3 h. Sufficient H_2O was added to destroy the excess of reagent; the mixture was filtered (washing with EtOH) and the filtrate acidified (HCl). Evaporation and recrystallization from EtOH gave **14a** as the dihydrochloride (6.8 g, 78%).

Route B. Chloral (6.6 g, 0.04 mol) was added dropwise to a solution of histamine base (4.4 g, 0.04 mol) in $CHCl_3$ and stirred at room temperature for 1 h. The solid formed was collected, dried, and recrystallized from MeOH–Et_2O, yielding initially histamine monohydrochloride (0.52 g, mp 192–195°) and, after concentration, N^α-formylhistamine (**15,** 4.0 g, mp 99–102°) (*i*-

PrOH–Et_2O). Anal. ($C_6H_9N_3O$) C, H, N.

Reduction of **15** (2.8 g) with $LiAlH_4$ in THF and treatment with HCl afforded **14a** (2.1 g, 53%).

4,N^α-Dimethylhistamine (14b) was obtained as the dihydrochloride (82%) from **11b** by the method (route A) used for **14a.**

N^α,N^α-Dimethylhistamine (16a). Reaction of 4(5)-(2-chloroethyl)imidazole hydrochloride[30] (90 g, 0.54 mol) with excess Me_2NH (446 ml) in EtOH (890 ml) at 100° for 16 h in an autoclave gave **16a** which was isolated as the dihydrochloride (90.8 g, 79%).

4,N^α,N^α-Trimethylhistamine (16b). A mixture of **10b** as the base (prepared from 2.0 g of the dihydrochloride), HCO_2H (2.3 g), and HCHO (1.7 ml) was gently refluxed for 24 h. After evaporation to dryness a solution of picric acid in EtOH was added to the residual oil to give the crude dipicrate of **16b** (5.8 g, 95%). This dipicrate, after recrystallization, was converted into a hygroscopic dihydrochloride.

β-Methylhistamine (23). A solution of crude 2-methyl-3-phthalimidopropionyl chloride[41] (21.2 g, 0.084 mol) in dry C_6H_6 (180 ml) was added dropwise, with stirring, to a solution of diazomethane in Et_2O (600 ml) at 0°. After standing at 0° overnight, the mixture was vigorously stirred and treated with 48% HBr (20 ml). Evaporation and recrystallization from EtOH gave **21** (22.6 g, 86%), mp 97–98°. Anal. ($C_{13}H_{12}BrNO_3$) C, H, N.

A solution of this bromo ketone (10 g) in $HCONH_2$ (60 ml) was heated at 180–185° for 2 h. After evaporation in vacuo the residue was heated with H_2O (150 ml), cooled, and filtered to remove phthalimide, and the filtrate was heated under reflux with concentrated HCl (150 ml). The solution was concentrated, cooled, and filtered and the filtrate evaporated to dryness. The residue was extracted with EtOH and evaporated and the crude product purified by ion-exchange chromatography (Amberlite IRA 401, OH⁻). Concentration and addition of a solution of picric acid in EtOH gave **23** as the dipicrate (5.7 g, 30%), which was converted into the dihydrochloride.

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Quaternary Pilocarpine Derivatives as Potential Acetylcholine Antagonists. 2. Alterations in the Lactone and Imidazole Moieties[1]

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In order to investigate the chemical behavior of pilocarpine, as well as the factors which determine its pharmacological activity, systematic and specific structural changes involving the lactone and imidazole moieties have been performed. Series of model compounds with cyclic or open-chain structures and a variety of N-3 bonded chains obtained from previously prepared anticholinergic derivatives of pilocarpine have been synthesized. The changes included N-3 chains of different lengths with an acetylcholine-like structure, the introduction of nucleophilic groups such as ketoxime, hydroxamic, or both at the side chain, or following hydroxylaminolysis of the lactone, respectively. Specific structural alterations could be obtained by reacting with free hydroxylamine under carefully controlled conditions, and the existence of syn and anti isomers was disclosed in certain cases. The new groups in the pilocarpine derivatives influenced their degree of antagonism to acetylcholine. Several compounds displayed some antidotal activity.

The action of pilocarpine (1) on the parasympathetic nervous system has been extensively investigated; however, its use is presently still limited to the treatment of glaucoma. This alkaloid, isolated from the leaves of the South American shrubs *Pilocarpus jaborandi* and *Pilocarpus microphyllus* Stapf., has been the subject of structure–activity studies.[2–4] Pilocarpine occurs naturally as the cis isomer which is much more potent than the *trans*-isopilocarpine.[5] It is assumed to interact with the muscarinic receptor, and molecular configurations indicating possible modes of receptor binding were presented.[6] Several binding sites in the lactone and imidazole rings have been assumed,[7] and the lactone appears to be essential for the cholinergic activity[8,9] whereas cleavage of the imidazole ring does not completely suppress this activity.[9] In certain systems *d*-pilocarpine, known as a typical parasympathomimetic drug, may act as an anti-

cholinergic.[2] We have previously reported the synthesis of a series of quaternary derivatives of pilocarpine, most

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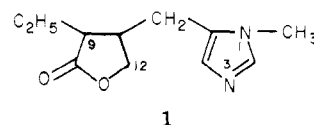
of which antagonized to a different degree the effect of acetylcholine (AcCh).[1] As a continuation, we wish to report in the present paper a systematic study involving the synthesis of new derivatives, in which characteristic groups have been added (oxime, hydroxamic, or both) in a search for a synergistic effect to that of atropine against organophosphate intoxication, as well as cholinolytics.

Chemistry. Derivatives **4** and **5** were designed to have the N-3 side chain structurally similar to acetylcholine, the