Synthesis and Biological Activity of a 2-Bromoethylamine (Mustard) Derivative of Hemicholinium-3 and Hemicholinium-15

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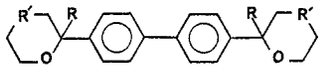
Work on the synthesis and investigation into the biological activity of a 2-bromoethylamine (mustard) analogue of hemicholinium-3 (HC-3) and hemicholinium-15 (HC-15) is reported. Hemicholinium-3 bromo mustard (HC3-BrM, 5) and hemicholinium-15 bromo mustard (HC15-BrM, 11) cyclize in aqueous solution to a biethylenimine derivative (6) and a monoethylenimine derivative (12) that are structurally almost identical with HC-3 (1) and HC-15 (8), respectively. As with HC-3 or HC-15, these mustards strongly inhibited sodium-dependent, high-affinity choline uptake (SDHACU) activity in vitro. This inhibitory activity was found to result solely from the interaction of the cyclized ethylenimine form of the mustards with the uptake system. When compared on an equivalent concentration basis, HC3-BrM effected substantially greater inhibition of SDHACU than HC-3 and is, thus, at present the most potent known synthetic inhibitor of this uptake system. Synaptosomes incubated with a low concentration of precyclized HC3-BrM (25 nM) and then treated to remove all free and reversibly bound drug exhibited a maintained reduction (~32%) in the V_{max} of SDHACU activity. This behavior is in marked contrast to the findings with HC-3, the inhibition of which was totally removed by simple washing. It is suggested that the biethylenimine form of HC3-BrM undergoes a covalent, and thus possibly irreversible, bond formation with group(s) functionally involved with the SDHACU system.

The sodium-dependent, high-affinity choline uptake (SDHACU) system of peripheral and central cholinergic nerve terminals plays a regulatory and rate-determining role in the intraneuronal synthesis of acetylcholine[1]. To date, hemicholinium-3 [2,2-(4,4-biphenylene)bis(2-hydroxy-4,4-dimethylmorpholinium bromide)] (1) and a



1 (HC-3) R=OH; R′=$\overset{+}{N}(CH_3)_2$

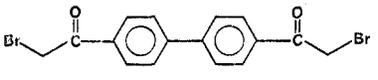
2 R=OH; R′=N−CH_2CH_2OH

3 R=C_2H_5O; R′=N−CH_2CH_2OH

4 R=C_2H_5O; R′=N−CH_2CH_2Br

5 (HC3-BrM) R=OH; R′=N−CH_2CH_2Br

6 R=OH; R′=$\overset{+}{N}$



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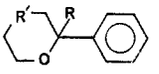
number of recently prepared homologues[2] represent the most potent known competitive inhibitors of SDHACU, exhibiting affinities for the system some 50 times greater than choline itself.[3] This report outlines details of the synthesis and some preliminary observations on the action of a bis(2-bromoethylamine) analogue of hemicholinium-3 (HC-3), subsequently named hemicholinium-3 bromo mustard (HC3-BrM, 5), upon the SDHACU activity expressed in synaptosomes prepared from rat striatum. This compound would under suitably defined aqueous conditions be expected to cyclize into a highly reactive biethylenimine intermediate[4] (6) that is structurally similar to HC-3 and, thus, may exhibit a comparable affinity for the SDHACU system. It was conceivable, by analogy with other ethylenimine-forming mustards,[5,6] that 6 might, by alkylating membrane-bound groups involved in the high-affinity binding of HC-3, effect a long-lasting inhibition of SDHACU activity.

As a model route to the synthesis of HC3-BrM, a procedure was devised for synthesizing the equivalent haloalkylamine derivative of hemicholinium-15 [2-phenyl-2-hydroxy-4,4-dimethylmorpholinium bromide](8), a compound which represents one-half of the symmetrical HC-3 structure. Details of this procedure, in addition to the effects of the cyclized form of HC15-BrM on SDHACU activity, are also included in this paper.



8 (HC-15) R=OH; R′=$\overset{+}{N}(CH_3)_2$

9 R=C_2H_5O; R′=N−CH_2CH_2OH

10 R=C_2H_5O; R′=N−CH_2CH_2Br

11 (HC15-BrM) R=OH; R′=N−CH_2CH_2Br

12 R=OH; R′=$\overset{+}{N}$ 

Chemistry. The ability of the N-substituted tertiary phenacylethanolamines, formed by the reaction of phenacyl bromide with secondary ethanolamines, to undergo spontaneously the formation of stable six-membered morpholine ring hemiacetals has been reported.[7] In addition, the product of the condensation between α,α-di-

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bromo-4,4-biacetophenone (7) and *N,N*-dimethylethanolamine undergoes, immediately following solution in water, a similar intramolecular cyclization to form the bis-quaternary hemiacetal morpholine derivative HC-3.[8] The initial step in the synthesis of HC3-BrM involved condensing 7, which was prepared according to the method of Long and Schueler,[9] with diethanolamine to produce 2.

Structure–activity studies indicate that a 4-position quaternary nitrogen and tertiary hydroxy group present at position 2 on the substituted morpholine ring are essential for the potent inhibitory activity of HC-3 and related compounds.[10,11] To protect the 2-position hydroxy prior to halo substitution at position 4 of the morpholine ring, I took advantage of the predisposition of the hemiacetals to undergo, in the presence of a reactive alcohol and a catalytic amount of acid, formation to the related 2-alkoxyacetals.[7,12] It proved possible in the synthesis of HC15-BrM to produce the required 2-phenyl-2-ethoxy-4-(2-hydroxyethyl)morpholine (9) in one step (see Experimental Section); however, in the synthetic route to HC3-BrM, the analogous reaction between 7 and diethanolamine, which involved using a 2-fold mole excess of the secondary amine, produced only the bishemiacetal derivative (2). Presumably, this was a result of the basic conditions that prevailed throughout the reaction. Synthesis of the acetal (3) was achieved by dissolving the hemiacetal base (2) in ethanol acidified with dry HCl.

As found for the hydrochloride salts of the N-substituted phenacylmorpholine hemiacetals,[7] the hydrochloride salt of 2 was extremely hygroscopic and as such proved difficult to handle. This characteristic was absent in the corresponding salt of the acetal (3). Similarly, whereas the hydrochloride of 2 reduced Tollens reagent immediately at room temperature, that of 3 produced appreciable darkening only after the solution had been warmed.

The acid-labile nature of the protective acetal grouping present on compounds 3 and 9 made it necessary to perform the subsequent bromination step under nonacidic conditions. The interaction of tertiary phosphines with carbon tri- or tetrabromide has been found to effect, under mild essentially neutral conditions, the conversion of primary and secondary alcohols into the corresponding alkyl bromides.[13-15] The 2-bromoethylamine derivatives 4 or 10 were thus prepared by the reaction of 3 or 9 with tris(dimethyamino)phosphine and bromoform in CH_2Cl_2 (Experimental Section). This phosphane was used in preference to others (e.g., triphenyl- or trioctylphosphine), since the water-soluble nature of the phosphine oxide produced in the reaction greatly simplified the procedure for product isolation.[16]

The final step in the synthesis involved stirring the hydrochloride salt of 4 or 10 in dilute aqueous acid to remove the protective acetal group and generate the requisite hemicholinium bromo mustards HC3-BrM (5) or HC15-BrM (11), respectively.

Table I. Inhibition of Sodium-Dependent High-Affinity Choline Uptake Activity (1 μM Choline) by HC-15 and HC-3 and the Mustard Analogues HC15-BrM and HC3-BrM

inhibitor	concn, M	thiosulfate, $\leq 5 \times 10^{-5}$ M	Na^+-dependent uptake % inhibn[a]
HC-15	2×10^{-5}	–	58 ± 3 (n = 3)
HC15-BrM	2×10^{-5}	–	71 ± 4 (n = 3)
	2×10^{-5}	+	9 ± 2 (n = 3)
HC-3	5×10^{-8}	–	83 ± 3 (n = 4)
	5×10^{-8}	+	85 ± 2 (n = 3)
	1×10^{-8}	–	35 ± 3 (n = 3)
HC3-BrM	1×10^{-7}	–	100 (n = 2)
	1×10^{-7}	+	0 (n = 2)
	5×10^{-8}	–	100 (n = 2)
	5×10^{-8}	+	0 (n = 2)
	1×10^{-8}	–	84 ± 3 (n = 4)

[a] All values are the means plus or minus SD for the number of separate determinations carried out (n)

Biochemistry and Inhibition Studies. Male albino Wistar rats (200–250 g) were used throughout these studies. A synaptosomal P_2 fraction of rat striatum was prepared, and the sodium-dependent, high-affinity choline uptake (SDHACU) activity was studied according to the methods of Simon, Atweh and Kuhar.[17] All inhibition studies on the bromoethylamine mustards involved the use of the precyclized hydrochloride salt of the compound (Experimental Section). A more detailed account of the biochemical actions and conclusion concerning HC3-BrM has been reported elsewhere.[18]

Table I summarizes the results obtained when HC3-BrM (2) and HC15-BrM (11) were compared with hemicholinium-3 (HC-3) and hemicholinium-15 (HC-15) as inhibitors of the SDHACU system. In both instances, the mustard compound proved a more potent inhibitor than the analogous hemicholinium. It is probable that the biethylenimine form of HC3-BrM (6) is responsible for inibition of uptake, since prior incubation with sodium thiosulfate, which did not alter the inhibitory effect of HC-3, totally abolished the inhibition produced by HC3-BrM (Table I). A similar finding and conclusion apply to the action of the monoethylenimine forming HC15-BrM (12). Since on an equivalent concentration basis HC3-BrM effected substantially greater inhibition of uptake than HC-3, it represents at present the most potent known synthetic inhibitor of the SDHACU system. The inhibition of SDHACU by HC-3 and HC3-BrM was characterized kinetically, following a 10-min incubation of the synaptosomes with drug, by means of the Woolf–Augustinsson–Hoftstee plot.[19] As reported previously,[20] HC-3 produced purely competitive inhibition, while HC3-BrM yielded mixed kinetics, both the slope ($-K_m$) and the ordinate intercept (V_{max}) of the inhibition plot being lowered in a nonparallel fashion.[18] Since the biethylenimine form of HC3-BrM (6) is structurally almost identical with HC-3 (1), the inhibition produced by the mustard might arise if subsequent to establishing a reversible equilibrium with a site(s) involved in the binding of HC-3 (K_m lowering) some of the biethylenimine ion were to alkylate a group(s) in or associated with the high-affinity site (V_{max} lowering).

The noncompetitive aspect of the inhibition produced by HC3-BrM was examined further to establish if it was

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Table II. Effect of Postwash Incubation on the Inhibition of Sodium-Dependent High-Affinity Choline Uptake by HC-3 and HC3-BrM

treatment	postwash preincubation time, min	Na-dependent high-affinity choline uptake, pmol (mg of protein)$^{-1}$ 4 min^{-1}	% inhibn[a]
control	2.5	83.7	
	7.5	85.9	
	15.0	82.2	
HC-3 (25 nM)	0	84; 85	(n = 2)
	15.0	80; 82	(n = 2)
HC3-BrM (25 nM)	2.5	58.8	31.2 ± 1.6 (n = 3)
	7.5	59.8	32.9 ± 2.0 (n = 3)
	15.0	54.9	34.0 ± 1.2 (n = 3)

[a] Values are the means plus or minus SD for the number of separate determinations carried out (n).

freely reversible of if, as required by the above interpretation of the kinetics, it was of a more enduring nature. Following a constant 10-min incubation with either HC-3 (25 nM) or HC-3BrM (25 nM), the synaptosomes were thoroughly washed twice to remove all free and reversibly bound inhibitor (see ref 18) and then incubated for up to 15 min at 37 °C in fresh drug-free medium before assaying SDHACU activity. From Table II it is apparent that part of the inhibition effect by HC3-BrM (~32%) persists unchanged throughout this stringent wash and protracted drug-free incubation period (actual time 25 min if 10-min assay period is included). In contrast, the effect of HC-3 was totally removed by simple washing, a finding reported in studies involving far higher concentrations of the drug.[6] It is therefore suggested that these findings are in accord with the biethylenimine form of HC3-BrM undergoing the formation of a specific and covalent complex with the group(s) functionally involved with the SDHACU system.

In two alternative studies involving the use of nitrogen mustards as inhibitors of SDHACU, one of the mustards, an analogue of hexamethonium [*N,N,N',N'*-tetrakis(2-chloroethyl)-1,6-hexanediamine (C6NM)] which can exist in a biethylenimine form, has been shown to produce irreversible inhibition of SDHACU activity.[5] The other mustard an analogue of choline, produced, following a 10-min incubation period with the synaptosomes, inhibition that was diagnosed kinetically as competitive[6]. The authors of this work have subsequently shown that the choline ethylenimine ion effects a time-dependent irreversible inhibition of choline uptake, via a shift from an initially competitive to a progressively more noncompetitive interaction.[21] It will be of interest to establish if HC3-BrM, which is considerably more potent and specific than either of these mustards, will effect a total, time-dependent, irreversible inactivation of the SDHACU system.

The potent and enduring nature of the inhibition effect by HC3-BrM constitutes a potentially useful probe both in vitro for studies aimed at isolating and purifying the molecular entity responsible for SDHACU and in vivo as a means of specifically perturbing, possibly on a long-term basis, cholinergic transmission.

Experimental Section

Melting points were determined in open glass capillaries with a Mettler FP1 383 and are uncorrected. IR spectra were taken on a Perkin-Elmer 257 spectrophotometer. ^{1}H NMR spectra at 60 MHz were recorded with a Perkin-Elmer R32 NMR spectrophotometer. All of the spectra were consistent with the proposed structures. Elemental analyses were performed at the University Chemistry Department, Kings Building, Edinburgh. All results, unless noted, were within ±0.4% of the theoretical values.

α,α-Dibromo-4,4-biacetophenone (7). Anhydrous $AlCl_3$ (150 g, 1.125 mol) was added to biphenyl (61 g, 0.396 mol) in 350 mL of CS_2 at 0 °C, followed by the dropwise addition of bromoacetyl bromide (200 g, 0.992 mol). The mixture was warmed slowly and maintained under reflux until HBr evolution subsided(~3 h). The mixture was cooled to room temperature, the CS_2 was decanted, and the remaining solid was broken up and hydrolyzed in an ice-cold solution made up of 350 mL of H_2O plus 100 mL of EtOH and 10 mL of concentrated HCl. The resulting white solid was filtered from solution and, after washing with 1 L of distilled H_2O, dried thoroughly in a vacuum desiccator. This material was extracted with boiling toluene to yield 42 g of crude compound, which was then decolorized with activated charcoal and recrystallized from boiling toluene an additional 3 times to yield pure 7 (21.5 g, 0.054 mol, 14%): light yellow needles; mp 224–226 °C (lit. 226–227 °C). Anal. ($C_{16}H_{12}Br_2O_2$) C, H.

2,2-(4,4-Biphenylene)bis[2-hydroxy-4-(2-hydroxyethyl)-morpholine] (2). Diethanolamine (24 g, 0.228 mol) in 180 mL of EtOH was added to a mixture of 7 (19 g, 0.048 mol) in 250 mL of 1:4 dioxane. Upon gentle warming to effect solution, the mixture was cooled and magnetically stirred for 24 h at ambient temperature. To this mixture was added 450 mL of H_2O; the solution was acidified (HCl) and extracted with Et_2O, and the extract was made alkaline (pH 10.0, NaOH) and then extracted again with CH_2Cl_2. The CH_2Cl_2 extract was dried over anhydrous $MgSO_4$, filtered through decolorizing charcoal, rotary evaporated to the point of precipitation, and placed on ice. The precipitate was vacuum filtered, washed with Et_2O, and dried in a vacuum desiccator to yield 10.5 g of 2 (0.024 mol, 50%): pale, off-white crystals; mp 152–154 °C. Anal. ($C_{24}H_{32}N_2O_6$) C, H, N.

2,2-(4,4-Biphenylene)bis[2-ethoxy-4-(2-hydroxyethyl)-morpholine] (3). Compound 2 (6 g, 0.014 mol) was dissolved in 600 mL of super-dry EtOH [refluxed and distilled over Mg-$(OC_2H_5)_2$] containing 1%, w/v, dry HCl. The tightly stoppered mixture was magnetically stirred for 48 h at ambient temperature, after which the resulting precipitate was filtered off, washed with EtOH, and dried in vacuo to yield 3.7 g of the hydrochloride salt of 3. An aqueous solution of this was made alkaline (pH 10.0, NaOH) and extracted with CH_2Cl_2 in a repeat of the procedure outlined for the isolation of 2 to yield 2.5 g of 3 (0.005 mol, 36%): mp >300 °C (with decomposition); IR of HCl salt (Nujol) ν_{max} 3320 (associated hydroxy), 2600 (tertiary amine HCl), 1610 (biphenyl), 1270, 1235 (alkyl ether), 1070, 1055 (morpholinium ether), 823 (*p*-phenyl) cm^{-1}; ^{1}H NMR of free base ($CDCl_3$) δ 7.62 (s, 8 H, aromatic), 4.3–3.85 (m, 4 H, methylene adjacent to morpholine oxygen), 3.7 (t, 4 H, J = 4.0 Hz, aminoethanol methylene adjacent to hydroxy), 3.6–2.1 (overlapping signals, 12 H, ethoxy methylene and methylenes adjacent to nitrogen), 2.58 (t, 4 H, J = 4.0 Hz, aminoethanol methylene adjacent to nitrogen), 1.23 (t, 6 H, ethoxy methyl). Anal. ($C_{28}H_{40}N_2O_6$) C, H, N.

2,2-(4,4-Biphenylene)bis[2-ethoxy-4-(2-bromoethyl)-morpholine] (4). Compound 3 (2.0 g, 0.004 mol) was dissolved in 25 mL of CH_2Cl_2 containing $CHBr_3$ (3.0 g, 0.012 mol), and the mixture was transferred to a three-neck flask. The flask was fitted with a condenser connected to a drying tube ($CaCl_2$), an inlet to permit N_2 to continuously purge the apparatus during operation, and a dropping funnel containing tris(dimethylamino)phosphine (1.6 g, 0.01 mol) in 10 mL of CH_2Cl_2. The phosphine solution was introduced slowly (~20 min) to the magnetically stirred

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contents of the flask, which had been cooled and maintained during the course of the addition in an EtOH/H_2O solution held at -15 °C. After 30 min, the cooling bath was recovered, and the contents of the flask were poured into a separating funnel and extracted 3 times with ice-cold H_2O (3 × 40 mL) to remove the phosphine oxide formed during the reaction. The CH_2Cl_2 layer was retained, dried thoroughly over anhydrous $MgSO_4$, and rotary evaporated to leave a viscous brown oil. This oil was shaken vigorously with Et_2O in which it appeared insoluble, and the resulting suspension filtered rapidly under vacuum. The Et_2O filtrate was saved and stored overnight at -30 °C to yield as a precipitate 0.62 g of **4** (0.001 mol, 25%): fine, cream-white crystals; mp >300 °C (with decomposition); IR (Nujol) ν_{max} 1610 (biphenyl), intense bands 1310, 1250 (alkyl ether), 1080, 1060 (morpholinium ether), 830 (*p*-phenyl) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.61 (s, 8 H, aromatic), 4.35–3.85 (m, 4 H, methylene adjacent to morpholine oxygen), 3.62–3.35 (m, 8 H, ethoxy methylene and methylenes adjacent to bromine), 3.31–2.15 (overlapping signals, 12 H, methylenes adjacent to nitrogen), 1.23 (t, 6 H, ethoxy methyl). Anal. ($C_{28}H_{38}Br_2N_2O_4$) C, H, N.

2,2-(4,4-Biphenylene)bis[2-hydroxy-4-(2-bromoethyl)-morpholine] (HC3-BrM, 5). The hydrochloride salt of **4** (100 mg, 1.5 × 10^{-4} mol) was dissolved in 20 mL of H_2SO_4 (1 M), and the solution was stirred at room temperature for 4 h. The mixture was placed on ice, neutralized with NaOH (5 M), and rapidly extracted with CH_2Cl_2. The CH_2Cl_2 extract was dried over anhydrous $MgSO_4$, rotary evaporated to a pale yellow oil, and dissolved in Na-dried benzene. The addition of dry HCl precipitated the hydrochloride salt of **5** (HC3-BrM·2HCl) (25 mg, 4.1 × 10^{-5} mol, 27%): IR (Nujol) ν_{max} 3500–3100 (associated hydroxy), 1670 (very weak, phenyl carbonyl), 1610 (biphenyl), 1070, 1060 (mopholinium ether), 815 (*p*-phenyl) cm^{-1}; ^1H NMR (Me_2CO-d_6) δ 7.9–7.78 (m, 8 H, aromatic), 4.36–3.8 (m, 4 H, methylene adjacent to morpholine oxygen), 3.76–3.5 (overlapping signals, 6 H, methylenes adjacent to bromine and including hydroxyprotons), 3.0–2.26 (overlapping signals, 12 H, methylenes adjacent to nitrogen). Anal. ($C_{24}H_{32}Br_2Cl_2N_2O_4$) C, H, N.[22]

2-Phenyl-2-ethoxy-4-(2-hydroxyethyl)morpholine (9). Phenacyl bromide (50 g, 0.185 mol) in 250 mL of EtOH was added with stirring to diethanolamine (31.5 g, 0.333 mol) dissolved in 50 mL of EtOH, and the resulting solution was refluxed for ~3 h. Following this, the acetal base **9** was obtained by a repeat of the extraction procedure for **2**. Solution of **9** in ethanolic HCl and addition of Et_2O resulted in precipitation of the hydrochloride salt: yield 24 g (0.083 mol, 33%); mp 124–126 °C. Anal. ($C_{14}H_{22}ClNO_3$) C, H, N.

2-Phenyl-2-ethoxy-4-(2-bromoethyl)morpholine (10). The procedure was similar to that outlined in the synthesis of **4**. Compound **9** (2.5 g, 0.01 mol) was dissolved with $CHBr_3$ (3.5 g, 0.014 mol) in CH_2Cl_2, and tris(dimethylamino)phosphine (1.95 g, 0.12 mol) was added. Treatment of the ethereal extract of the product with dry HCl resulted in a precipitate of **10**·HCl: yield 1.3 g (0.004 mol, 37%); mp 114–116 °C; IR of the HCl salt (Nujol) ν_{max} 2450 (tertiary amine HCl), 1250, 1165 (intense bands, alkyl ether), 1090, 1050 (morpholinium ether), 755, 700 (monosubstituted phenyl) cm^{-1}; ^1H NMR of free base ($CDCl_3$) δ 7.7–7.45 (m, 2 H, aromatic), 7.45–7.25 (m, 3 H, aromatic), 4.3–3.8 (m, 2 H, methylene adjacent to morpholine oxygen), 3.63 (t, 2 H, aminoethanol methylene adjacent to bromine), 3.6–2.08 (overlapping signals, 8 H, ethoxy methylene and methylenes adjacent to nitrogen), 1.20 (t, 3 H, ethoxy methyl). Anal. ($C_{14}H_{21}BrClNO_2$) C, H, N.

2-Phenyl-2-hydroxy-4-(2-bromoethyl)morpholine (HC15-BrM, 11). **10**·HCl (0.35 g, 0.001 mol) was treated with 20 mL of H_2SO_4 (1 M) in a procedure identical with that outlined for the preparation of HC3-BrM·2HCl. The yield of **11**·HCl was 0.16 g (5 × 10^{-4} mol, 50%): mp 127–128 °C; IR (Nujol) ν_{max} 3450 (associated hydroxy), 1680 (weak, phenyl carbonyl), 1070 (morpholinium ether), 760, 710 (monosubstituted phenyl) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.7–7.45 (m, 2 H, aromatic), 7.43–7.20 (m, 3 H, aromatic), 4.4–3.45 (overlapping signals, 5 H, hydroxy, methylene adjacent to morpholine oxygen, methylene adjacent to bromine), 2.9–2.1 (overlapping signals, 6 H, methylenes adjacent to nitrogen). Anal. ($C_{12}H_{17}BrClNO_2$) C, H, N.

Storage and Cyclization Titer Values of HC3-BrM (5) and HC15-BrM (11). Hemicholinium-3 bromo mustard (**5**) was made up as a 1 mM solution of the dihydrochloride salt in 0.1 M HCl and stored frozen in 1-mL aliquots at -20 °C. The rate and extent of ethylenimine ion formation by HC3-BrM was determined by the procedure of Gill and Rang.[23] Under these conditions, a 0.25 mM solution of HC3-BrM at pH 7.4 in 10 mM phosphate buffer yielded a maximal titration equivalent of 130% ethylenimine ion after 10 min at room temperature. For simplicity this can be interpreted as 65% of the molecules existing in the biethylenimine form (**6**), although in practice the true concentration of biethylenimine ion must lie between 30 and 65% of the initial mustard concentration. Under analagous circumstances, the monoethylenimine-forming HC15-BrM (**11**) exhibited a maximal ethylenimine ion (**12**) concentration of 75%.

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Registry No. **1**, 312-45-8; **2**, 83291-87-6; **3**, 83291-89-8; **3**·HCl, 83291-88-7; **4**, 83291-90-1; **5**, 83291-91-2; **5**·2HCl, 79868-97-6; **6**, 79868-96-5; **7**, 4072-67-7; **8**, 4303-88-2; **9**·HCl, 83291-92-3; **10**·HCl, 83291-93-4; **11**, 83291-95-6; **11**·HCl, 83291-94-5; **12**, 83291-96-7; biphenyl, 92-52-4; bromoacetyl bromide, 598-21-0; diethanolamine, 111-42-2; phenacyl bromide, 70-11-1; choline, 62-49-7.

(22) N: calcd, 4.35 found, 3.86.

(23) E. W. Gill and H. P. Rang, *Mol. Pharmacol.*, **2**, 284 (1966).

Synthesis and Antiallergy Activity of 5-Oxo-5*H*-thiazolo[2,3-*b*]quinazolinecarboxylic Acids

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A series of substituted 5-oxo-5*H*-thiazolo[2,3-*b*]quinazolinecarboxylic acids was prepared and evaluated in the rat PCA test for antiallergic activity. The analogues that exhibited the highest oral activity were the 7-methoxy, 7-methylthio, and 7-isopropyl in the 2-carboxylic acid series and the 2-isopropyl in the 7-carboxylic acid series.

Although numerous compounds that inhibit the release of mediators of anaphylaxis from sensitized mast cells have been described in the recent literature, disodium cromoglycate (DSCG) is the only drug of this type currently available for the prophylactic treatment of bronchial asthma. The major disadvantage of DSCG is that it is not active orally and must be taken by insufflation. Recently, DSCG has also become available in an aerosol formulation for inhalation.

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