Tertiary 3- and 4-Haloalkylamine Analogues of Oxotremorine as Prodrugs of Potent Muscarinic Agonists

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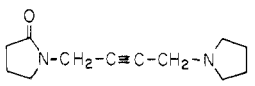
A series of tertiary 3- and 4-haloalkylamines related to the muscarinic agent oxotremorine was synthesized. The compounds cyclized in neutral aqueous solution to quaternary ammonium salts, which, in contrast to the parent haloalkylamines, were potent muscarinic agonists in vitro. When administered systemically to mice, the haloalkylamines produced central (tremor and analgesia) and peripheral (salivation) muscarinic effects. Central potency was dependent on the rate of cyclization and on the route of administration. The *N*-methyl-*N*-(4-chlorobutyl)amine derivative **7** cyclized rapidly ($t_{1/2}$ < 0.4 min at 37 °C) and elicited tremor on iv but not on ip injection, whereas the *N*-methyl-*N*-(3-chloropropyl)amine **3** cyclized slowly ($t_{1/2}$ = 436 min) and was not tremorogenic by either route of administration. The *N*-methyl-*N*-(3-bromopropyl)amine **4** ($t_{1/2}$ = 11 min) and its iodo analogue **5** ($t_{1/2}$ = 14 min) were quite potent in eliciting central muscarinic effects on both iv and ip injection to mice. It is concluded that haloalkylamine analogues of oxotremorine may serve in vivo as prodrugs for potent quaternary ammonium salts and that they are capable of circumventing the blood–brain barrier to such salts.

Previously, we have synthesized and studied a series of 2-haloalkylamines related to the muscarinic agent oxotremorine, *N*-(4-pyrrolidino-2-butynyl)-2-pyrrolidone.[1-4]

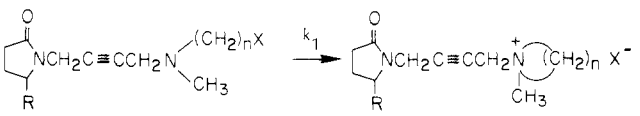


OXOTREMORINE

These compounds cyclized spontaneously at physiological pH to aziridinium ions.[1,3] When administered systemically to mice and rats, they produced profound central muscarinic effects, which were ascribed to the aziridinium ions formed by in vivo cyclization of the parent 2-haloalkylamines.[4,5] Furthermore, central potency was critically dependent on the rate of cyclization to the aziridinium ion. For example, the rapidly cyclizing 2-bromoethylamine **2** (Table I) was considerably more potent than its (2-chloroethyl)amino analogue **1** in producing tremor and analgesia.[3,4] However, these agonist effects generally were quite short-lasting and were replaced by long-lasting antimuscarinic effects because of alkylation of muscarinic receptors by the reactive aziridinium ion.[4,5] The alkylating abilities of these 2-haloalkylamines have made them valuable tools for studying muscarinic receptors.[6]

Tertiary 3- and 4-haloalkylamines cyclize to azetidinium and pyrrolidinium ions, respectively. These are known to be more stable under physiological conditions than aziridinium ions and therefore are less likely to be attacked by nucleophiles.[7] In order to study 3- and 4-haloalkylamine analogues of oxotremorine as carriers for the transport of stable (nonalkylating) ammonium ions into the central nervous system, we synthesized compounds **3–7** (Table I). Such compounds may potentially have long-lasting central muscarinic effects since, once inside the central nervous system, the ammonium ions should be removed only with difficulty, unless active transport by a carrier occurs.[8] This paper describes the synthesis, cyclization kinetics, and initial pharmacological investigations of compounds **3–7**.

Table I. Apparent First-Order Rate Constants for the Cyclization of Compounds **1–7**



no.	R	*n*	X	k_1,[a] min^{-1}	$t_{1/2}$, min
1	H	2	Cl	0.019[b]	36.5
2	H	2	Br	0.850 ± 0.035[c]	0.8
3	H	3	Cl	0.0016 ± 0.0003	436
4	H	3	Br	0.0610 ± 0.0041	11.4
5	H	3	I	0.0491 ± 0.0055	14.1
6	CH_3	3	Br	0.0555 ± 0.0045	12.5
7	H	4	Cl	>2	<0.4

[a] Rate constants were estimated by measurements of halide ion release at pH 7.4 and 37 °C. Values are means ± SE from three estimates. [b] From ref 1. [c] From ref 3.

Results

Chemistry. The amino alcohols (**8**, **9**, and **10**) used as precursors of compounds **3–7** were synthesized in a Mannich reaction from *N*-2-propynyl-2-pyrrolidone or 5-methyl-*N*-2-propynyl-2-pyrrolidone, paraformaldehyde, and the appropriate *N*-methyl-*N*-alkylamine. The 3-halopropylamines **3**, **4**, and **6** were obtained from **8** or **9**, triphenylphosphine, and carbon tetrachloride or carbon tetrabromide. Treatment of the 3-bromopropylamine **4** with sodium iodide yielded the iodide **5**. The rapidly cyclizing 4-chlorobutylamine **7** was prepared from **10** under acidic conditions using thionyl chloride.

Rates of Cyclization. The cyclization of **3–7** in phosphate buffer was studied by measurements of halide ion release. Compound **3** cyclized very slowly. Maximal release of Cl^- (80% of the theoretical amount) was achieved only after about 30 h at 37 °C. Compounds **4–6** cyclized considerably faster (Figure 1). The cyclization reactions followed first-order kinetics. The 4-chlorobutylamine **7** released 1 equiv of Cl^- almost instantaneously on dissolution in the buffer. Apparent first-order rate constants (k_1) for halide release were estimated by nonlinear regression analysis and are summarized in Table I.

Muscarinic Activity in Vitro. When assayed as the parent haloalkylamines, **3–5** showed very weak spasmogenic effects (Figure 2). These were somewhat more pronounced with **4** and **5** than with **3** and were probably due to formation of azetidinium ion during the incubation in the tissue bath. Compound **6** was inactive under these

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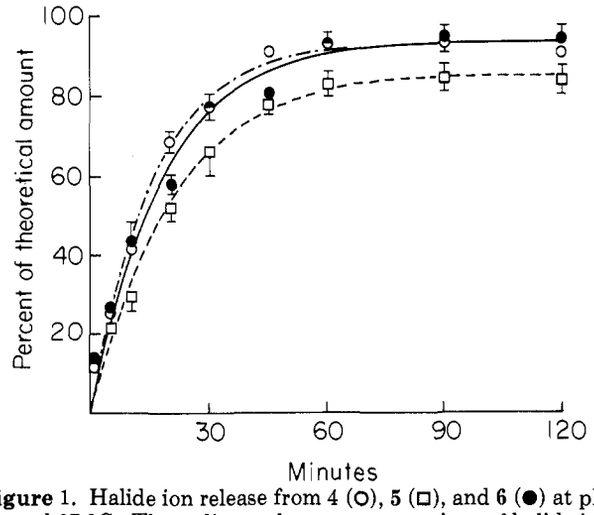

Figure 1. Halide ion release from 4 (○), 5 (□), and 6 (●) at pH 7.4 and 37 °C. The ordinate shows concentrations of halide ion as percentages of the maximum of 1 equiv/mol of the parent compounds. Values are means ± SE from three determinations. The curves represent the best fit to a first-order rate equation.

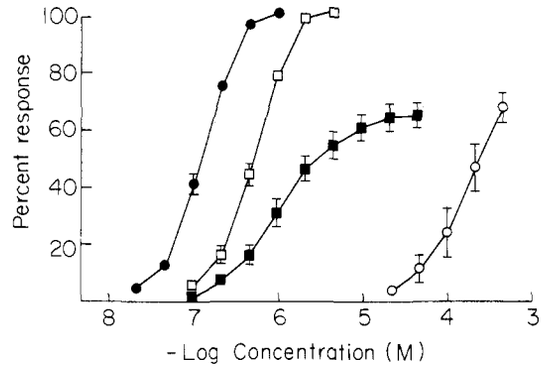

Figure 2. Concentration–response curves of precyclized 3 (●), 6 (■), 7 (□), and "noncyclized" 3 (○) in the isolated guinea pig ileum. The compounds were cyclized as described in the Experimental Section. Responses are expressed relative to the maximum response elicited by oxotremorine. Vertical bars show standard errors. Number of preparations used is given in Table II.

conditions. The potency of 7 could not be measured because of its rapid cyclization.

After cyclization, 3–5 were equipotent in causing contractions of the ileum. They had about the same potency as carbachol (Table II). The potency of precyclized 7 was similar to that of *N*-methyloxotremorine, which is the cyclization product of 7. The 5-methylpyrrolidone 6, after cyclization, appeared to be a partial agonist. The contractile responses to all compounds were prevented or antagonized by methylatropine (0.1 μM), whereas hexamethonium (0.3 mM) was without effect. These results show that the effects of 3–7 on the ileum were mediated via muscarinic receptors.

Muscarinic Activity in Vivo. Saliva production in mice was used as a measure of peripheral muscarinic activity. All of the compounds produced salivation when administered as the parent haloalkylamines (Table III). After cyclization, the potency of 3–5 increased and they became equipotent (ED_{50} ~ 0.1 μmol/kg). Tremor and analgesia served as measures of central muscarinic activity. Compounds 4 and 5 elicited tremor upon both iv and ip injection, whereas 7 was tremorogenic only after iv administration. The 3-chloropropylamine 3 produced tremor only at lethal doses. Compound 6 and the cyclized forms of 4, 5, and 7 were inactive as tremorogenic agents. All of the compounds showed analgesic activity, but were considerably less potent than oxotremorine (Table III).

Table II. Muscarinic Activity of the Cyclization Products of 3–7 in the Isolated Guinea Pig Ileum[a]

compd	*n*	EC_{50},[b] μM	EPMR[b,c]
3	7	0.120 ± 0.008	2.78 ± 0.24
4	7	0.114 ± 0.009	2.49 ± 0.18
5	6	0.136 ± 0.011	2.95 ± 0.22
6[d]	4	1.14 ± 0.16	23.7 ± 1.4
7	4	0.538 ± 0.047	10.2 ± 0.4
N-methyloxotremorine	4	0.401 ± 0.030	8.31 ± 0.58
carbachol	5	0.12 ± 0.012	2.60 ± 0.19
oxotremorine	14	0.044 ± 0.003	1.00

[a] The compounds were cyclized in phosphate buffer (pH 7.4) as described in the Experimental Section before being assayed on the ileum. [b] EC_{50} and EPMR are means ± SE; *n* equals number of test preparations used. [c] EPMR is the equipotent molar ratio relative to oxotremorine. [d] Compound 6 produced 69.5 ± 4.8% of the maximum response of oxotremorine. All other compounds were full agonists.

Discussion

The primary aim of the present study was to synthesize tertiary 3- and 4-haloalkylamines capable of cyclizing to quaternary ammonium salts having muscarinic actions. This goal was achieved with compounds 3–7. The 3-halopropylamines 3 and 4 cyclized 10–15-fold more slowly than the corresponding 2-haloethylamines (1 and 2, respectively), whereas the 4-chlorobutylamine cyclized more quickly than 1 and 2 (Table I). Among the 3-halopropylamines, the bromo (4) and iodo (5) derivatives cyclized about 35-fold more quickly than the chloro compound 3. These results are in good agreement with those reported for a series of *N*-(2-bromobenzyl)-*N*-ethylhaloalkylamines having adrenergic blocking actions.[9]

The cyclization products of 3–5 were equipotent as muscarinic agonists on the ileum, as might be expected if 3–5 cyclize to a common, reasonably stable *N*-methylazetidinium ion. This azetidinium ion was less potent than the corresponding aziridinium ion,[3] but more potent than *N*-methyloxotremorine (Table II). The latter appears to be formed almost quantitatively from 7, which after cyclization was equipotent with *N*-methyloxotremorine. The azetidinium ions derived from 3–6 were less potent than the corresponding azetidines,[10,11] in agreement with previous observations that N-methylation of cyclic tertiary amines decreases muscarinic activity.[12–14] In contrast to the relatively high potency of the ammonium ions, the parent haloalkylamines showed little or no muscarinic effects. Because of the rapid cyclization of 7, it was not possible to separate its effects from those of the cyclized species. However, from structure–activity studies among other analogues of oxotremorine, including *N*-methyl-*N*-propylamine and *N*-methyl-*N*-butylamine derivatives,[15] one would expect 3–7 to have antagonistic rather than agonistic properties. Although 3–7, administered as the parent compounds, induced salivation in mice, this effect

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Table III. Muscarinic Activity and Acute Toxicity of Compounds 3–7 in Mice

	ED_{50},[a] μmol/kg					
	salivation		tremor			
compd	iv	ip	iv	ip	analgesia, ip	LD_{50},[a] μmol/kg, iv
3	22.4 ± 3.1	29.3 ± 4.1	*b*	*b*	33.3 ± 4.7	245 ± 37
4	2.9 ± 0.4	6.0 ± 0.8	6.3 ± 0.9	15.6 ± 2.2	3.2 ± 0.4	46.7 ± 6.5
5	4.5 ± 0.6	10.4 ± 1.6	8.9 ± 1.2	29.7 ± 4.4	7.7 ± 1.1	>125
6	38.0 ± 5.7	80.8 ± 11.3	*c*	*c*	111 ± 19	>250
7	1.1 ± 0.1	4.2 ± 0.6	3.6 ± 0.5	*d*	10.3 ± 1.4	8.0 ± 1.1
oxotremorine	0.19 ± 0.03	0.29 ± 0.04	0.44 ± 0.06	0.59 ± 0.08	0.10 ± 0.01	6.7 ± 0.9

[a] ED_{50} and LD_{50} values are means ± SE. [b] Tremor observed only at doses near the LD_{50} value. [c] No tremor observed at doses below 250 μmol/kg. [d] Inactive.

most probably was caused by the ammonium ions. Thus sialagogic potency increased on cyclization and was roughly correlated with rate of cyclization.

A second goal of this study was to explore the possibility of using 3- and 4-haloalkylamine analogues of oxotremorine as prodrugs for the passage of permanently charged muscarinic agonists into the central nervous system. As the ammonium ions appear to be responsible for the muscarinic actions of such haloalkylamines, their central potency should depend on the availability of the ammonium ions in the brain. This availability is determined inter alia by the rate of cyclization and route of administration.[4,8] Rapidly cyclizing compounds may undergo extensive cyclization before they can reach their central sites of action. This is especially true after ip administration because of delayed absorption into the blood stream. Accordingly, compound **7** was potent in producing tremor after iv injection to mice, but was inactive as a tremorogenic agent when administered ip. As its cyclization product, *N*-methyloxotremorine, did not elicit tremor by either route, these results suggest that the cyclization of **7**, although fast, was sufficiently slow to allow a substantial proportion of the parent 4-chlorobutylamine to enter the brain after iv administration. Slowly cyclizing compounds should reach the brain effectively after both iv and ip administration. However, metabolism and elimination of the parent haloalkylamines are likely to limit their potency. This appears to be the case with the 3-chloropropylamine **3**, which elicited tremor only at doses near its median lethal dose. In addition, with slowly cyclizing compounds, the parent haloalkylamine, whose tissue concentration initially exceeds that of the ammonium ion, may antagonize the agonist effects of the latter.

Among the compounds studied, the 3-bromo- (**4**) and 3-iodopropylamines (**5**) appeared to have the most favorable cyclization rates for producing consistent central muscarinic actions. As **4** and **5** had very low, if any, muscarinic actions by themselves, the tremorogenic and analgesic effects observed after their administration in vivo can only be explained by the presence of the corresponding azetidinium ion in the central nervous system. The inactivity of precyclized **4** and **5** in the tremor assay confirms that the azetidinium ion did not readily penetrate the blood–brain barrier and that it was in fact formed from **4** and **5** in the brain. The absence of a tremor response to **6**, which had a rate of cyclization similar to those of **4** and **5**, can be accounted for by the partial agonist properties of its azetidinium ion (Table II) and the small receptor reserve for muscarinic agonists with respect to the tremor response.[16] In agreement with this explanation, the azetidine analogue of **4** and **5** was tremorogenic,[10] whereas the azetidine analogue of **6** did not produce tremor, despite its potent partial agonist effects on the ileum.[11]

In conclusion, tertiary 3- and 4-haloalkylamine analogues of oxotremorine were able to pass the blood–brain barrier and cyclize to potent quaternary ammonium ions in sufficient quantities to produce profound muscarinic effects. However, the compounds were less active centrally than expected from their in vitro potencies. For example, **4**, after cyclization, was only 2.5-fold less active than oxotremorine on the ileum, but 15–30-fold less active in producing central effects. This apparent anomaly does not seem to be due to poor distribution of the haloalkylamines to the brain, as their basicity and lipophilicity should be similar to those of oxotremorine. In fact, the 4-chlorobutylamine **7**, after iv administration, appeared to be distributed to the brain as efficiently as oxotremorine because its potency, relative to that of oxotremorine, was similar in vivo and in vitro. We suggest that the rates of cyclization of the haloalkylamines studied were not optimal for producing high levels of the quaternary ammonium ions in the brain. Unfortunately, it is difficult to predict optimal cyclization rates, as they are likely to vary for different sites of action, animal species, and administration routes.[8] However, it appears that the rate of cyclization of the 3-haloalkylamines studied, especially **3**, was lower than the optimal rate. Consequently, analogues of **3–5** that cyclize at a greater rate are now under investigation.

Experimental Section

Melting points were determined in a heated metal block using glass capillaries and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, and, unless otherwise indicated, agreed with theoretical values within ±0.4%. Mass spectra were recorded on a Hewlett-Packard 5981A mass spectrometer at 70 eV. ^1H NMR spectra were obtained at 40 °C on a Bruker WP 200 spectrometer at 200 MHz. Chemical shifts are reported in parts per million (δ) downfield from internal $(CH_3)_4Si$ standard. Thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} analytical plates. Visualization was done with I_2 and UV.

***N*-[4-[(3-Hydroxypropyl)methylamino]-2-butynyl]-2-pyrrolidone (8).** *N*-Methyl-*N*-(3-hydroxypropyl)amine was synthesized from 3-hydroxypropylamine as described previously.[17] A mixture of *N*-methyl-*N*-(3-hydroxypropyl)amine (8.9 g, 0.10 mol), paraformaldehyde (3.3 g, 0.11 mol), *N*-2-propynyl-2-pyrrolidone[18] (12.3 g, 0.10 mol), and CuCl (0.2 g) in 200 mL of dioxane was heated at 80 °C for 2 h. The dioxane was evaporated in vacuum and the residue taken up in 1 N HCl. The aqueous solution was extracted twice with ether, and K_2CO_3 and NH_4OH were added to adjust to pH to 10. Extraction with CH_2Cl_2 (3 × 100 mL), drying (K_2CO_3), and evaporation yielded a brown oil, which was purified by vacuum distillation: bp 171 °C (0.03 mmHg); yield 15.5 g (69%). A sample of the distillate was purified

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further on a silica gel column using CH_2Cl_2/MeOH (4/1) as eluent: TLC R_f 0.26 in $CHCl_3$/MeOH (9/1); ^1H NMR (CD_3CN) δ 4.03 (t, 2 H, J = 2 Hz, $CONCH_2C{\equiv}$), 3.56 (t, 2 H, J = 6 Hz, CH_2OH), 3.43 (t, 2 H, J = 7 Hz, 5-CH_2), 3.30 (t, 2 H, J = 2 Hz, ${\equiv}CCH_2NCH_3$), 2.49 (t, 2 H, J = 7 Hz, $CH_3NCH_2CH_2$), 2.25 (t, 2 H, J = 8 Hz, 3-CH_2), 2.24 (s, 3 H, NCH_3), 1.9–2.1 (m, 2 H, 4-CH_2), 1.60 (quint, 2 H, J = 6 Hz, CH_2CH_2OH); MS, m/e (relative intensity) 225 (1.2) (M + 1), 179 (11.5), 136 (66.4), 126 (17.1), 108 (25.4), 98 (14.3), 94 (26.2), 88 (22.5). Anal. ($C_{12}H_{20}N_2O_2 \cdot {}^1/_2H_2O$) C, H, N.

***N*-[4-[(3-Chloropropyl)methylamino]-2-butynyl]-2-pyrrolidone Hydrochloride (3).** Compound **8** (2.0 g, 8.9 mmol) and Ph_3P (2.7 g, 10 mmol) were dissolved in dry benzene, which was then removed under vacuum. The residue was dissolved in anhydrous CH_2Cl_2 (50 mL), and CCl_4 (2.0 g, 13 mmol) in CH_2Cl_2 (10 mL) was added dropwise in a nitrogen atmosphere. The reaction mixture was left at room temperature overnight, and pentane (100 mL) was added to precipitate $Ph_3P{=}O$. After filtration, the solvents were removed and the residue was dissolved in 1 N HCl. The aqueous phase was extracted three times with $CHCl_3$, cooled by the addition of ice, and made faintly alkaline with K_2CO_3. Rapid extraction with $CHCl_3$, drying (K_2CO_3), and evaporation yielded an oil, which was immediately dissolved in anhydrous ether and converted to **3** by the addition of ethereal HCl: mp 109–110 °C (from ethanol/ether); yield 1.4 g (56%); TLC R_f 0.43 in $CHCl_3$/MeOH/NH_4OH (9/0.9/0.1); ^1H NMR (CD_3OD) δ 4.16–4.24 (m, 4 H, $CH_2C{\equiv}CCH_2$), 3.72 (t, 2 H, J = 6 Hz, CH_2Cl), 3.56 (t, 2 H, J = 7 Hz, 5-CH_2), 3.41 (t, 2 H, J = 8 Hz, $CH_3NCH_2CH_2$), 2.97 (s, 3 H, NCH_3), 2.38 (t, 2 H, J = 8 Hz, 3-CH_2), 2.25 (m, 2 H, CH_2CH_2Cl), 2.0–2.2 (m, 2 H, 4-CH_2); MS, m/e (relative intensity) 179 (21.7), 136 (100), 108 (42.3), 106 (69.1), 98 (30.7). Anal. ($C_{12}H_{20}ClN_2O$) C, H, N.

***N*-[4-[(3-Bromopropyl)methylamino]-2-butynyl]-2-pyrrolidone Hydrobromide (4).** Compound **8** (2.0 g, 8.9 mmol) and Ph_3P (2.7 g, 10 mmol) were dried as described above and dissolved in anhydrous CH_2Cl_2 (50 mL). The solution was cooled to 0 °C in a sealed bottle flushed with dry nitrogen. Then CBr_4 (4.4 g, 13 mmol) dissolved in CH_2Cl_2 (10 mL) was added dropwise through a rubber septum. The reaction mixture was allowed to warm up to room temperature, and pentane (100 mL) was added. After filtration and removal of the solvents, the free base of **4** was purified by partition as described above for **3**. The hydrobromide was precipitated from ether by the addition of ethereal HBr: mp 95–96 °C (from ethanol/ether); yield 1.9 g (52%); TLC R_f 0.44 in $CHCl_3$/MeOH/NH_4OH (9/0.9/0.1); ^1H NMR (CD_3OD) δ 3.57 (t, 4 H, J = 6 Hz, CH_2Br, 5-CH_2), 3.44 (br, 2 H, $CH_2CH_2CH_2Br$), 2.25–2.45 (m, 4 H, 3-CH_2, CH_2CH_2Br); MS, m/e (relative intensity) 179 (38.0), 152 (20.5), 150 (24.0), 136 (100), 108 (22.8), 98 (24.0). Anal. ($C_{12}H_{20}Br_2N_2O$) C, H, N. The sesquioxalate had mp 97–98 °C (from ethanol/ether). Anal. ($C_{15}H_{22}BrN_2O_7$) C, H, N.

***N*-[4-[(3-Iodopropyl)methylamino]-2-butynyl]-2-pyrrolidone Sesquioxalate (5).** The sesquioxalate of **4** (2.5 g, 6.6 mmol) and NaI (5.0 g, 33 mmol) were dissolved in acetone (50 mL), and the solution was refluxed for 3 h. The acetone was evaporated and the residue dissolved in water. The aqueous solution was cooled and made alkaline by the addition of K_2CO_3. The free base of **5** was extracted rapidly into $CHCl_3$. After brief drying (K_2CO_3), the $CHCl_3$ was evaporated and the residue was immediately dissolved in anhydrous ether. Addition of oxalic acid in ether yielded **5**: mp 90–91 °C (from ethanol/ether); yield 1.9 g (61%); TLC R_f 0.48 in $CHCl_3$/MeOH/NH_4OH (9/0.9/0.1); ^1H NMR (CD_3OD) δ 3.25–3.40 (m, 4 H, CH_2I, $CH_2CH_2CH_2I$), 2.26 (m, 2 H, CH_2CH_2I); MS, m/e (relative intensity) 251 (4.3), 198 (17.2), 179 (54.7), 136 (100), 128 (17.2), 108 (22.5). Anal. ($C_{15}H_{22}IN_2O_7$) C, H, N.

***N*-[4-[(3-Hydroxypropyl)methylamino]-2-butynyl]-5-methyl-2-pyrrolidone (9).** Compound **9** was synthesized from 5-methyl-*N*-2-propynyl-2-pyrrolidone,[19] *N*-methyl-*N*-(3-hydroxypropyl)amine,[17] and paraformaldehyde as described above for **8**. The product was purified on a silica gel column using CH_2Cl_2/MeOH (4/1) as eluent. The yield of **9** was 57%: ^1H NMR (CD_3OD) δ 4.41 (d of t, 1 H, J_1 = 17.6 Hz, J_2 = 2 Hz), 3.78–4.0 (m, 2 H), 3.59 (t, 2 H, J = 6 Hz), 3.38 (t, 2 H, J = 2 Hz), 2.56 (t, 2 H, J = 7 Hz), 2.31 (s, 3 H), 2.20–2.45 (m, 3 H), 1.55–1.75 (m, 3 H), 1.30 (d, 2 H, J = 6 Hz).

***N*-[4-[(3-Bromopropyl)methylamino]-2-butynyl]-5-methyl-2-pyrrolidone Sesquioxalate (6).** Compound **6** was prepared from **9** as described above for **4** in 62% yield: mp 98–100 °C (from ethanol/ether); TLC R_f 0.52 in $CHCl_3$/MeOH/NH_4OH (9/0.9/0.1); ^1H NMR (CD_3OD) δ 4.42 (d of t, 1 H, J_1 = 17.6 Hz, J_2 = 2.0 Hz), 4.13 (t, 2 H, J = 2.0 Hz), 3.98 (d, 1 H, J = 17.6 Hz), 3.89 (m, 1 H), 3.55 (t, 2 H, J = 6.3 Hz), 3.35–3.42 (m, 2 H), 2.94 (s, 3 H), 2.20–2.45 (m, 5 H), 1.55–1.75 (m, 1 H), 1.30 (d, 3 H, J = 6.3 Hz); MS, m/e (relative intensity) 193 (24.9), 152 (22.5), 150 (100), 122 (16.4), 112 (19.3), 94 (41.0). Anal. ($C_{16}H_{24}BrN_2O_7$) H, N; C: calcd, 44.1; found, 44.6.

***N*-[4-[(4-Hydroxybutyl)methylamino]-2-butynyl]-2-pyrrolidone (10).** *N*-Methyl-*N*-(4-hydroxybutyl)amine, obtained from 4-hydroxybutylamine according to a published method,[17] was converted to **10** as described above for the preparation of **8**: bp 185 °C (0.04 mmHg); yield 80%; TLC R_f 0.29 in $CHCl_3$/MeOH (9/1); ^1H NMR (CD_3OD) δ 4.12 (t, 2 H, J = 2 Hz), 3.55 (t, 2 H, J = 6 Hz), 3.54 (t, 2 H, J = 7 Hz), 3.35 (t, 2 H, J = 2 Hz), 2.20–2.50 (m, 6 H), 2.30 (s, 3 H), 2.06 (quint, 2 H, J = 8 Hz), 1.45–1.60 (m, 4 H); MS, m/e (relative intensity) 239 (4.1) (M + 1), 179 (16.7), 140 (14.9), 136 (87.7), 108 (30.7), 102 (17.1), 98 (15.4), 96 (18.3), 94 (27.3).

***N*-[4-[(4-Chlorobutyl)methylamino]-2-butynyl]-2-pyrrolidone Oxalate (7).** Compound **10** (3.5 g, 15 mmol) was dissolved in anhydrous CH_2Cl_2 (30 mL), and $SOCl_2$ (2.3 g, 19 mmol) in CH_2Cl_2 (10 mL) was added dropwise at 0 °C. The solution was refluxed for 1 h and concentrated under vacuum. The residue was dissolved in ice-cold water, and ether was added. K_2CO_3 was added to the vigorously stirred mixture until the aqueous phase became alkaline. The organic phase was separated and filtered through Na_2SO_4 into a solution of oxalic acid in ether. The oxalate salt was crystallized from ethanol/ether: mp 80–81 °C; yield 1.7 g (33%); ^1H NMR (CD_3OD) δ 4.20 (t, 2 H, J = 2 Hz), 4.12 (t, 2 H, J = 2 Hz), 3.64 (t, 2 H, J = 6 Hz), 3.54 (t, 2 H, J = 7 Hz), 3.23 (m, 2 H), 2.93 (s, 3 H), 2.38 (t, 2 H, J = 8 Hz), 2.08 (quint, 2 H, J = 8 Hz), 1.8–2.0 (m, 4 H); MS, m/e (relative intensity) 179 (54.4), 136 (100), 122 (10.6), 120 (25.6), 108 (23.0), 98 (11.9), 94 (18.5). Anal. ($C_{15}H_{23}ClN_2O_5$) C, H, N.

Measurements of Cyclization Rates. The reaction mixture (100 mL), kept at 37 °C, contained the haloalkylamine (1.5–2 mM) in 50 mM sodium phosphate buffer (pH 7.4). Halide ion released during the cyclization was measured by argentometric titration as described previously.[1] Rate constants were estimated by nonlinear regression analysis. Halide release data were fitted to the equation $\theta = 1 - e^{-k_1 t}$, where θ is the molar proportion of halide released, k_1 is the apparent first-order rate constant for the cyclization reaction, and t is time.

Guinea Pig Ileum. A standard guinea pig ileum preparation was set up in Tyrode solution (pH 7.4) at 37 °C as described previously.[20] The Tyrode solution contained hexamethonium (0.3 mM). Contractions were recorded isotonically at 1 g of tension by using an electromechanical displacement transducer and a potentiometric recorder. Concentration–response curves were constructed cumulatively by increasing stepwise the concentration of agonist by a factor of 2.15. Oxotremorine was used as the reference compound.

The muscarinic activity of the parent haloalkylamines was estimated after dissolution of **3**–**7** in ice-cold water. The potency of the ammonium ions was estimated after cyclization of the parent compounds in 50 mM phosphate buffer (pH 7.4) at 37 °C. Compound **3** was cyclized for 30 h, **4**–**6** were cyclized for 2 h, and **7** was cyclized for 1 min.

Muscarinic Activity in Mice. Male Swiss–Webster mice weighing 24–30 g were used. All compounds were dissolved in ice-cold saline and kept on ice until required but for no more than 1 h. Threshold doses for salivation and tremor were estimated by the up-and-down method[21] as described previously.[4] Drugs were administered ip or iv in the tail. The presence or absence of salivation was determined by pressing lightly the mouth of the

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animal to an absorbent paper tissue at intervals during the first 10 min after drug administration. The presence or absence of intermittent spontaneous (grade 2) tremor[22] was determined by visual inspection.

Analgesic activity was estimated in the tail-flick assay[23] by the up-and-down method.[21] After determination of control reaction times, drugs were administered ip, and posttreatment reaction times were recorded 10 min later. Animals that had posttreatment reaction times at least two times greater than the control reaction time were regarded as responders.

Acute Toxicity. LD_{50} values were determined by iv injection and the up-and-down method.[21] Mortality counts were taken at 1 h.

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Registry No. **3**, 110797-76-7; **4**, 110797-77-8; **5**, 110797-78-9; **6**, 110825-65-5; **7**, 110797-79-0; **8**, 110797-80-3; **9**, 110797-81-4; **10**, 110797-82-5; $HO(CH_2)_3NHMe$, 42055-15-2; $HO(CH_2)_4NHMe$, 42042-68-2; *N*-(2-propynyl)-2-pyrrolidone, 766-61-0; *N*-(2-propynyl)-5-methyl-2-pyrrolidone, 18327-34-9.

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Dimethylsulfonium and Thiolanium Analogues of the Muscarinic Agent Oxotremorine

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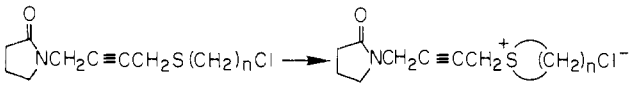
Dimethylsulfonium (**6a** and **6b**) and thiolanium analogues (**7a** and **7b**) of oxotremorine were synthesized and found to be potent muscarinic agents in vivo and in vitro. Compound **6a** exceeded oxotremorine in potency. Their affinities for muscarinic receptors in the guinea pig ileum and urinary bladder, estimated pharmacologically, were higher than those of the corresponding trimethylammonium (**8a** and **8b**) and *N*-methylpyrrolidinium compounds (**9a** and **9b**). However, the new compounds had lower intrinsic efficacies than their quaternary ammonium analogues. The compounds also had high affinity for central muscarinic receptors as measured by displacement of specifically bound (−)-[^{3}H]-*N*-methylscopolamine from homogenates of the rat cerebral cortex. Half-maximal occupation of cortical muscarinic receptors by **6a**, **6b**, **7a**, and **7b** was achieved at concentrations of 0.8, 5.4, 0.3, and 3.3 μM, respectively. The competition curves of **6a**, **6b**, and **7a** were adequately described by a two-site binding equation. The ratio of low- and high-affinity dissociation constants agreed with relative efficacy estimated on the ileum. The thiolanium salt **7a** was a fairly potent nicotinic agent on the frog rectus abdominis.

Recently, we have synthesized a series of 2-haloalkylamines related to the muscarinic agent oxotremorine, *N*-(4-pyrrolidino-2-butynyl)-2-pyrrolidone.[1,2] When administered in vivo, these compounds penetrate readily into the central nervous system and produce profound muscarinic effects, e.g., tremor and analgesia.[3,4] These effects and the persistent antimuscarinic actions that follow the initial stimulation may be ascribed to the aziridinium ions formed by in vivo cyclization of the parent 2-haloalkylamines.

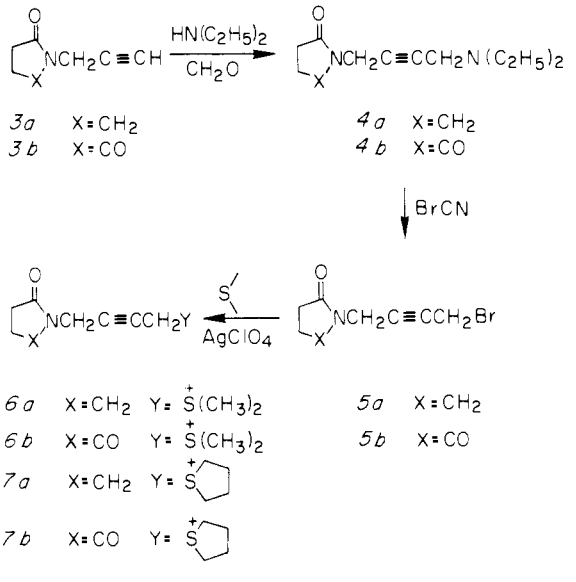
Chloroalkyl sulfides are known to cyclize to sulfonium ions.[5] It seemed possible that chloroalkyl sulfide analogues **1** of oxotremorine, like the corresponding 2-haloalkylamines, would be useful carriers for the passage of charged muscarinic compounds into the central nervous system. Before extensive studies of such chloroalkyl



1 **2**

sulfides were initiated, it was desirable to characterize the pharmacological properties of sulfonium analogues of oxotremorine. This report describes the muscarinic and nicotinic actions of dimethylsulfonium (**6a** and **6b**) and thiolanium analogues (**7a** and **7b**) of oxotremorine (Scheme I). Compound **6a** closely resembles the episulfonium ion **2** (n = 2) derived from the 2-chloroethyl sulfide **1** (n = 2), whereas **7a** is the cyclization product of the 4-chlorobutyl sulfide **1** (n = 4).

Scheme I



Chemistry. The synthesis of compounds **6a**, **6b**, **7a**, and **7b** is outlined in Scheme I. *N*-2-Propynyl-2-pyrrolidone (**3a**) and *N*-2-propynylsuccinimide (**3b**) were converted to the Mannich bases **4a** and **4b**, respectively. Treatment of

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