only five ligand concentrations (in duplicate). Analysis by LIGAND, by using K_D = 0.9 nM for [^{3}H]U69,593, yielded estimates or lower limits for K_i.

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Analogues of the Muscarinic Agent 2′-Methylspiro[1-azabicyclo[2.2.2]octane-3,4′-[1,3]dioxolane]: Synthesis and Pharmacology

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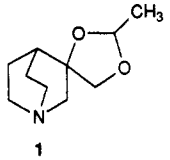
A number of tetrahydrofuran analogues of 2′-methylspiro[1-azabicyclo[2.2.2]octane-3,4′-[1,3]dioxolane] (1) have been prepared with the aim to obtain information about the relative importance of each of the oxygens in 1 for efficacy and for selectivity. In addition, the dimethyl and desmethyl analogues of 1 were prepared. The new compounds were compared to *cis*- and *trans*-1 with regard to their ability to displace (−)-[^{3}H]-3-quinuclidinyl benzilate ((−)-[^{3}H]QNB) from muscarinic receptors in cerebral cortex, heart, parotid gland, and urinary bladder from guinea pigs. Functioinal studies were made on isolated guinea pig bladder and ileum. The new compounds exhibited both lower affinity and efficacy than *cis*-1. A conformational study was performed, and the effects of steric and electronic factors on the biological activity of the compounds are discussed.

Introduction

The identification of a multitude of muscarinic receptor subtypes[1] has spurred the search for agonists and antagonists with selectivity for a given receptor subtype. Much interest has been focused on the development of selective muscarinic M1 agonists since such agents are of potential use in the therapy of Alzheimer type dementia and related disorders in which central cholinergic transmission is deficient.[2] However, little is known about the relationship between structure and subtype–receptor selectivity of agonists/antagonists. To further investigate the structural requirements for muscarinic agonist activity and subtype–receptor selectivity we have synthesized some analogues of 2′-methylspiro[1-azabicyclo[2.2.2]octane-3,4′-[1,3]dioxolane] (AF30; 1). Compound 1 was first synthesized in 1976 by Fisher et al.[3] as a rigid analogue of acetoxyquinuclidine (aceclidine; 2). The cis isomer of 1 was claimed to possess some selectivity toward M1 receptors as opposed to aceclidine. Fisher et al.[4] suggested that the selectivity was related to the low conformational flexibility of the compound.[5]



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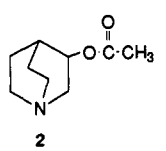
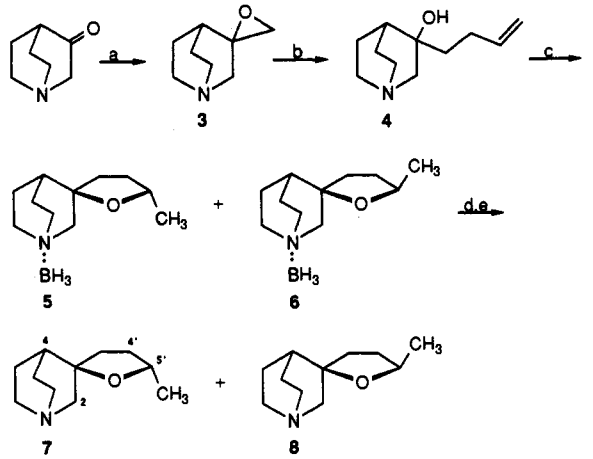


2

We have prepared a number of tetrahydrofuran analogues of 1 (7, 8, 14, and 15) with the aim to obtain information about the relative importance of each of the oxygens in 1 for efficacy and for selectivity. In addition,

Scheme I[a,b]



[a] Reagents: (a) trimethylsulfoxonium iodide, NaH, DMSO; (b) allylmagnesium bromide, CuI, ether; (c) (i) $Hg(OAc)_2$, THF/H_2O, (ii) $NaBH_4$, NaOH, benzyltriethylammonium chloride; (d) separation of diastereomers with flash chromatography; (e) HCl, MeOH. [b] Only relative stereochemistry is indicated.

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(1) See, e.g.: (a) Bonner, T. I. New Subtypes of Muscarinic Acetylcholine Receptors. Subtypes of Muscarinic Receptors IV. *Trends Pharmacol. Sci. Suppl.* **1989**, 11–15.

(2) See, e.g.: Krogsgaard-Larsen, P.; Jensen, B.; Falch, E.; Jørgensen, F. S. Heterocyclic Muscarinic Agonists: Structural and Therapeutic Aspects. *Drugs Future* **1989**, *14*, 541–561.

(3) (a) Fisher, A.; Weinstock, M.; Gitter, S.; Cohen, S. A New Probe for Heterogeneity in Muscarinic Receptors: 2-Methylspiro-(1,3-dioxolane-4,3′)-quinuclidine. *Eur. J. Pharmacol.* **1976**, *37*, 329–338. (b) Cohen, S.; Fisher, A. Ger. Offen. 2,650,845, 1977; *Chem. Abstr.* **1977**, *87*, 135298v.

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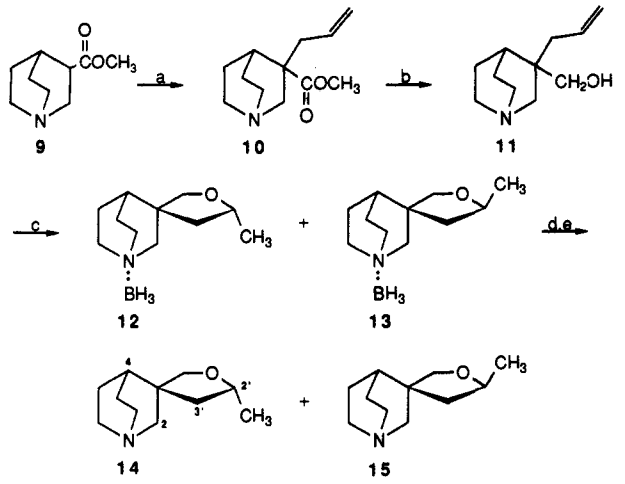
Scheme II[a,b]



[a] Reagents: (a) LDA, allyl bromide, THF; (b) $LiAlH_4$, ether; (c) (i) $Hg(OOCCF_3)_2$, THF/H_2O, (ii) $NaBH_4$, NaOH, benzyltriethylammonium chloride; (d) separation of diastereomers with flash chromatography; (e) HCl, MeOH. [b] Only relative stereochemistry is indicated.

we prepared the dimethyl (**16**) and desmethyl (**17**) analogues of **1**. The new compounds were compared to *cis*- and *trans*-**1** with regard to their ability to displace (−)-[^{3}H]-3-quinuclidinyl benzilate ((−)-[^{3}H]QNB) from muscarinic receptors in cerebral cortex, heart, parotid gland, and urinary bladder from guinea pigs. In addition, functional studies were made on isolated guinea pig bladder and ileum.

Chemistry

Synthesis. The synthetic sequences to compounds **7**, **8**, **14**, and **15** are shown in Schemes I and II. 3-Methylenequinuclidine oxide (**3**) was prepared from 3-quinuclidinone by treatment with trimethylsulfoxonium methylide in dimethyl sulfoxide according to the method of Corey and Chaykovsky[6] (Scheme I). The crude epoxide **3**, which was obtained by distillation, was treated with allylmagnesium bromide and a catalytic amount of copper(I)[7] to give the allylated **4**. Cyclization of **4** with mercuric acetate followed by reductive demercuration ($NaBH_4$/phase-transfer catalysis) produced a mixture of diastereomers **5** and **6** in which the quinuclidine nitrogen had formed a stable complex with borane.[8,9] This enabled us to separate the stereoisomers by flash chromatography without complicating tailing. The diastereomeric purity of **5** (98% de) and **6** (98% de) was estimated from ^{1}H NMR spectra by integration over the doublets due to the methyl groups. Decomplexation of **5** and **6** by treatment with strong acid in methanol gave **7** and **8**.

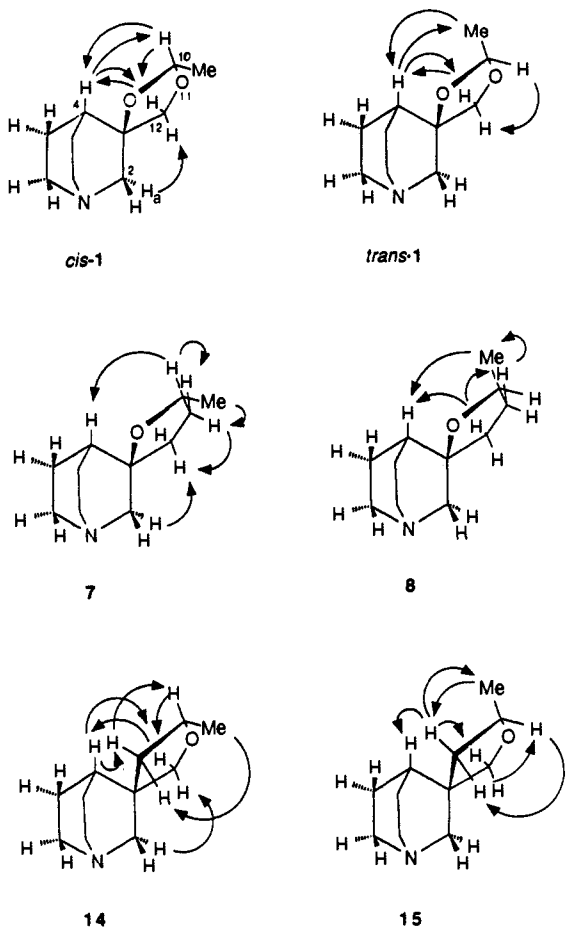
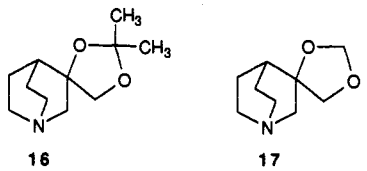


Figure 1. Observed NOEs used for stereochemical assignment.

The lithium enolate of methyl quinuclidine-3-carboxylate (**9**)[10] was treated with allyl bromide to give the allylated **10** (Scheme II). The ester was reduced, and the resulting alcohol (**11**) was cyclized by treatment with mercuric trifluoroacetate. The diastereomeric borane complexes formed during the reductive demercuration (**12**, **13**) were separated chromatographically. The stereochemical purity of **12** (99% de) and **13** (99% de) was determined by ^{1}H NMR and capillary GC analysis after decomplexation.



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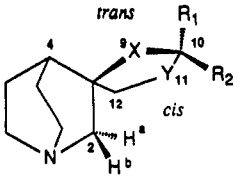
(10) Grob, C. A.; Renk, E. 196. Untersuchungen in der Chinuclidine-Reihe. 3. Mitteilung. 3-Chinuclidincarbonsäure. *Helv. Chim. Acta* **1954**, *37*, 1689–1698.

Table I. Selected ^1H and ^{13}C NMR Chemical Shifts for *cis*- and *trans*-1, 7, 8, 14, and 15[a,b]



						δ(^1H)						δ(^{13}C)		
							H-12		H-X or H-Y					
compd	X	Y	R_1	R_2	Me	H-10	cis	trans	cis	trans	Me	C-3	C-4	Me
cis-1	O	O	H	Me	cis	5.17	4.10	4.00			1.40	103.3	29.2	20.6
trans-1	O	O	Me	H	trans	5.18	3.74	4.27			1.39	103.3	30.9	20.4
7	O	CH_2	H	Me	cis	4.13	2.05	2.20	1.60	2.20	1.28	81.0	30.9	21.9
8	O	CH_2	Me	H	trans	4.18	1.94	2.26	2.10	1.58	1.25	81.0	32.2	21.7
14	CH_2	O	H	Me	cis	4.13	3.93	3.71	1.66	2.37	1.28	44.7	28.9	21.7
15	CH_2	O	Me	H	trans	4.17	3.83	3.90	2.26	1.65	1.30	45.1	29.6	21.3

[a] Solutions of 6.5–12.8 mg of compound in 0.7 mL of CD_3OD at 23 °C. [b] For convenience, the same numbering is used here for all compounds. For correct numbering, see Experimental Section or Schemes I and II.

Reference compounds *cis*- and *trans*-1 were synthesized according to literature methods.[3,5] The diastereomers were separated on aluminum oxide.[5] Compounds **16** and **17** were synthesized from the diol obtained by reduction of 3-hydroxy-3-(methoxycarbonyl)quinuclidine.[3,11]

Spectroscopy. The borane complexes were identified by their IR spectra.[12] In addition, ^1H-decoupled ^{11}B NMR spectra of the borane complexes (**5**, **6**, **12**, and **13**) consisted of a single signal. A coupled ^{11}B NMR spectrum showed a quartet with a $^1J_{H,B}$ coupling constant of 100 Hz. In ^1H NMR spectra, the borane hydrogens appeared as small, very broad peaks. The signals of the other protons were shifted upfield (0–0.4 ppm) in the borane complexes when compared to the decomplexed amine hydrochlorides.

Assignment of Relative Stereochemistry. The relative stereochemistry of diastereomers **7**/**8** and **14**/**15** was established by use of NMR spectroscopy. The distinction between the cis and trans isomers of each pair of compounds was facilitated by the observation of NOEs between protons on the cis or trans face of the five-membered ring on the one hand and C4-H or C2-H_a of the heterocyclic ring system on the other hand (cf. Figure 1). For this purpose, the signal of C4-H, which was obscured by signals due to several other protons, was identified as the only methine proton signal in the upfield part of the spectrum. Thus, in a partially relaxed inversion–recovery spectrum[13] of **15** it appears unobscured when the faster relaxing methylene protons have been nulled (cf. Figure 2b). The same signal showed an NOE upon irradiation of one of the C9-H's (Figure 2c), identifying it as C9-H_{trans}.[14] In 2D C,H

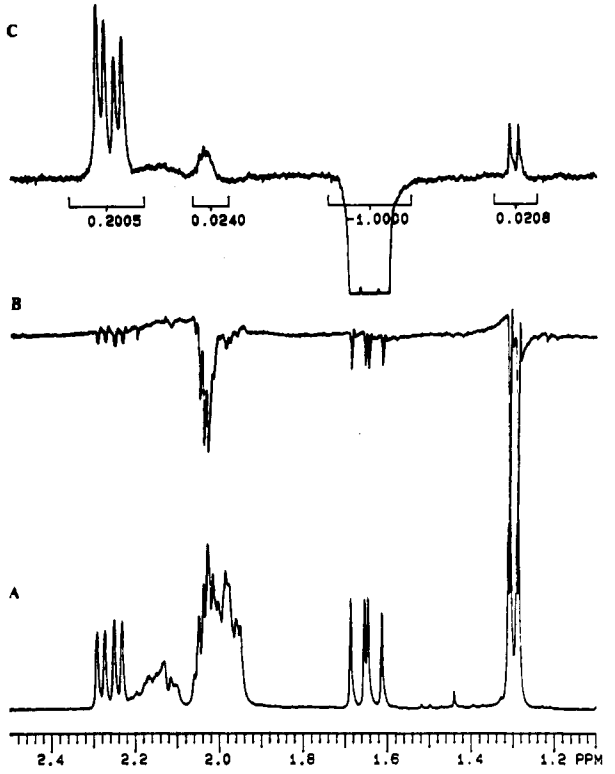


Figure 2. Partially relaxed inversion–recovery spectrum (B) and NOE difference spectrum (C) of **15**. A: reference spectrum.

shift correlation experiments (HMQC),[15] which were performed on **8** and **15**, this proton was correlated to the high-field CH carbon. Further, its multiplet was more narrow than those of the methylene protons in the same spectral region. In the spectra of all cis isomers, the signal for C2a-H appeared as a doublet of broad lines downfield of the other CH_2N protons. NOEs were observed from

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Table II. Conformations and Relative Steric Energies of the Compounds Studied

compd	conformation[a,b]							
	^3E	$^3T_2$	E_0	^0E	$^2T_3$	E_3	E_2	^2E
cis-1	2.17	2.12		0.88	0.14	0.17		0
trans-1		0	0.86				0.49	
7	1.82			0.85	0.14	0		
8	0.23	0.19	0					
14		2.96		1.49		0		2.49
15			0	2.02				
16	0.73	0.75		0.34		0		
17		0.38	0.06	0.53		0		0.60

[a] Relative energies are given in kilocalories/mole. [b] The different conformations were designated according to nomenclature defined in ref 35: *T* and *E* indicate twist and envelope conformations, respectively. The superscripts and subscripts indicate the atoms in the twist and envelope located above and below a plane defined by three or four other atoms in the five-membered ring. For convenience the atoms in the ring were numbered from 0 to 4 with the spiro carbon denoted as 0 and the methyl-substituted carbon as 2.

these doublets to protons on the cis face of the five-membered ring (Figure 1).

By applying the above procedure we were also able to assign the relative stereochemistry of *cis*- and *trans*-1. With the structural assignments in hand some interesting trends of the proton and carbon chemical shifts were observed (Table I). The chemical shift of C10-H is always larger in the trans isomer. Of the two C12-H's, the one positioned cis to the methyl group has the larger chemical shift, except in the pair 7/8. Of the two C9-H's (14, 15) or C11-H's (7, 8), the one located cis to the methyl group shows the smaller chemical shift. Throughout, the signal due to C4, which was assigned via DEPT spectra as the upfield CH carbon signal, has the smaller chemical shift in the cis isomer. The chemical shift of the methyl carbon is larger in the cis than in the trans isomers.

Molecular Mechanics Calculations. Nonrestricted force field calculations were performed with Allingers MM2-87 force field using MacMIMIC (Macintosh version of MIMIC[16]) on a Macintosh II fx. We investigated the conformational preferences of 7, 8, and 14–17 and compared them with those of *cis*- and *trans*-1. For each of the eight compounds nine starting geometries were constructed for energy minimization and geometry optimization. These geometries were constructed from the eight different envelope and twist conformations of the five-membered ring. A flat conformation of the tetrahydrofuran/dioxolane ring was also included as a starting geometry. By minimization of these 8 × 9 starting geometries, we identified a total of eight different conformations of the five-membered ring, but none of the compounds studied produced all of these eight five-membered ring conformers. Therefore, for each of the eight compounds the missing ring geometries were constructed from the appropriate minimized conformations of an analogue and these new starting geometries were energy minimized. This latter procedure provided a total of four additional low-energy conformations (in 14, 16, and 17 (2)). The identified conformers and their relative steric energies are listed in Table II and stereoscopic representations of the minimum-energy conformations are shown in Figure 3. One of the low energy conformations of *cis*-1 (^2E) is much less favored (with about 2.5 kcal/mol) in the other methyl-containing derivatives of 1. The X-ray structure of *trans*-1[5] corresponds to the minimum-energy MM2 conformer (Figure 4).

Pharmacological Results and Discussion

Pharmacological testing in vitro of *cis*-1, *trans*-1, and the novel analogues comprised both functional and receptor-binding studies. The muscarinic/antimuscarinic profiles and potencies were evaluated in functional studies on the isolated guinea pig urinary bladder and ileum, respectively, using carbachol as the standard agonist. Both of these tissues were used, since a compound lacking muscarinic activity in the bladder is not necessarily completely devoid of agonistic properties in other tissues. Thus, as shown by Ringdahl (see, e.g. ref 17 and 18), compounds acting as full or nearly full agonists in the ileum may behave as antagonists in the bladder. Potential selectivity for muscarinic receptor subtypes (M_1, M_2, and M_3)[19] was investigated by means of receptor-binding studies, in which the affinity of each compound for the muscarinic receptors in the cerebral cortex (M_1), heart (M_2), parotid gland (M_3), and urinary bladder from guinea pigs was indirectly determined by competition experiments with the radioligand (−)-[^{3}H]-QNB.

Table III. Functional in Vitro Data, Determined in Guinea Pig Tissues[a]

compd	urinary bladder[b]		ileum[c]	
	K_B, μM	EC_{50}, μM	EC_{50}, μM	E_{max}, %
carbachol		1.7 ± 0.3	0.17 ± 0.02	100
cis-1	4.8 ± 0.7		6.0 ± 1.0	91 ± 6
trans-1	13 ± 0		17	24
7	93 ± 8		12	29
8	90 ± 21		30	20
14	19 ± 7		12	7
15	30 ± 11		~150	25
16	41 ± 1		22	14
17	122 ± 16		~100	~45

[a] Potency is expressed as EC_{50} (agonism) and K_B (antagonism), respectively. Values are mean ± SEM of two to eight experiments. [b] Only carbachol had muscarinic agonist activity in the urinary bladder; all other compounds were ineffective when tested in concentrations of 100–1000 μM. [c] E_{max}: maximum response relative to that elicited by carbachol in the ileum. n = 34 for carbachol and n = 9 for *cis*-1. Due to low and variable efficacy, EC_{50} and E_{max} for *trans*-1, 7, 8 and 14, 15, 16, and 17 were graphically estimated from concentration–response curves of combined data (mean values) from at least two experiments.

None of the compounds in the present study exhibited any muscarinic agonist activity in the isolated bladder, when tested in concentrations of 100–1000 μM (Table III). Antimuscarinic activity, however, was demonstrated for

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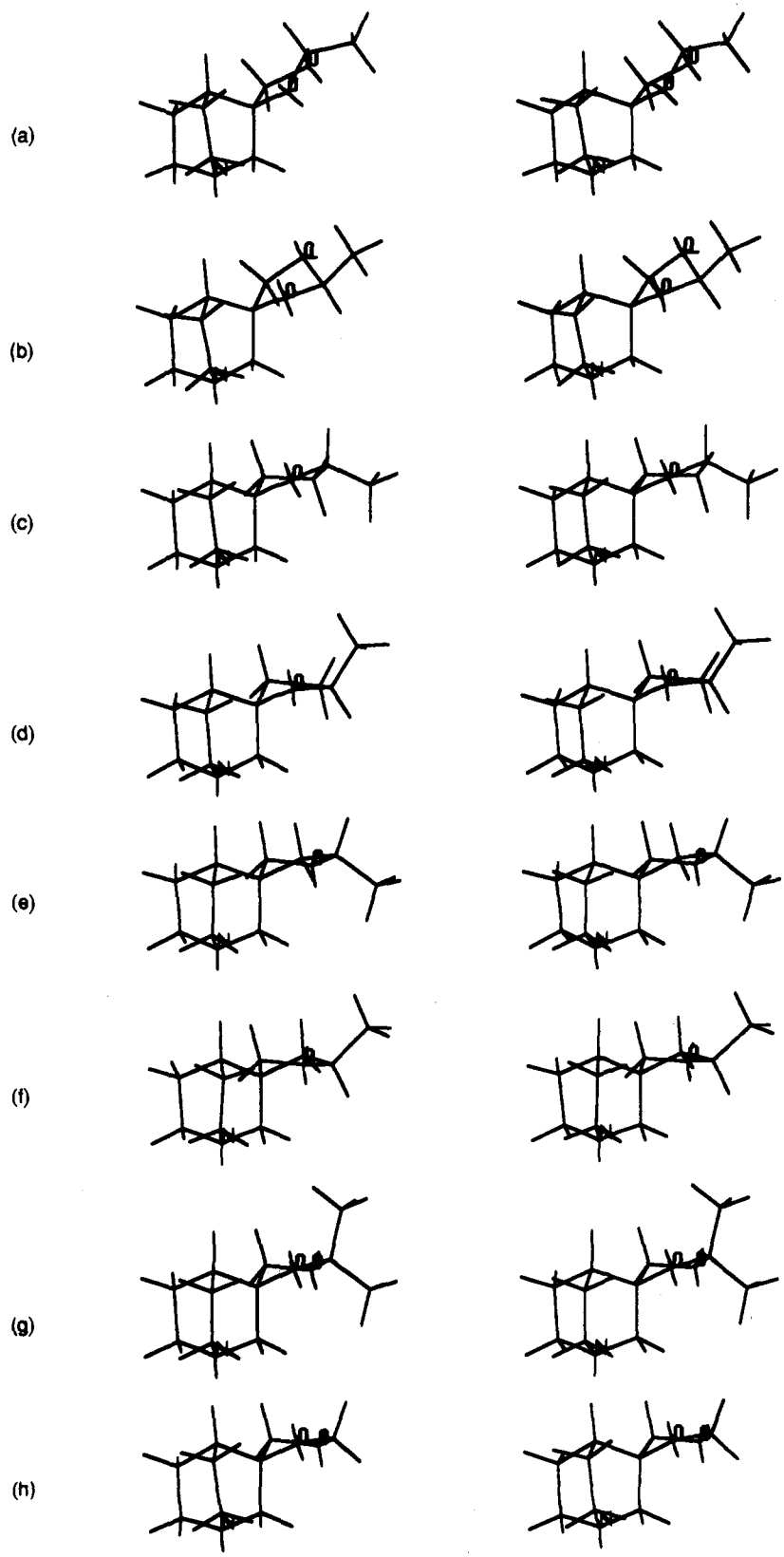

Figure 3. Stereoscopic representation of the MM2-derived minimum-energy conformations of (a) (3*R*,2'*S*)-*cis*-**1**, (b) (3*R*,2'*R*)-*trans*-**1**, (c) (3*R*,5'*S*)-**7**, (d) (3*R*, 5'*R*)-**8**, (e) (3*S*,2'*R*)-**14**, (f) (3*S*,2'*S*)-**15**, (g) (*R*)-**16**, and (h) (*R*)-**17**.

all compounds and a series of K_B values was determined versus the agonist carbachol (Table III). In the presence of antagonist, the concentration–response curves to carbachol were shifted in parallel toward higher concentrations, but the maximal responses remained unaffected. Thus, the inhibition seemed to be competitive since it could always be overcome by an increase in the carbachol concentration. The antimuscarinic potency was weak in this series of compounds, with K_B values ranging from 4.8 μM for the most potent compound, *cis*-**1**, to >100 μM (Table III). The rank order of potency was *cis*-**1** > *trans*-**1**, **14**, ≥ **15**, **16** ≥ **7**, **8**, **17**. The antimuscarinic activity of *cis*-**1** was about 2–3-fold higher than that of *trans*-**1**. A corresponding stereoselectivity was not observed for the two diastereomeric pairs of tetrahydrofuran analogues **7**/**8** and **14**/**15**, respectively. Removal of either of the two oxygens

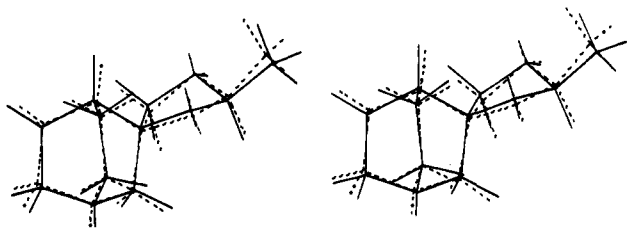

Figure 4. A computer-generated best fit of the carbon, oxygen, and nitrogen atoms of the X-ray conformation (dashed lines) and the minimized X-ray conformation (solid lines) of *trans*-1 (ref 5). The mean distance between fitted atoms was 0.11 Å.

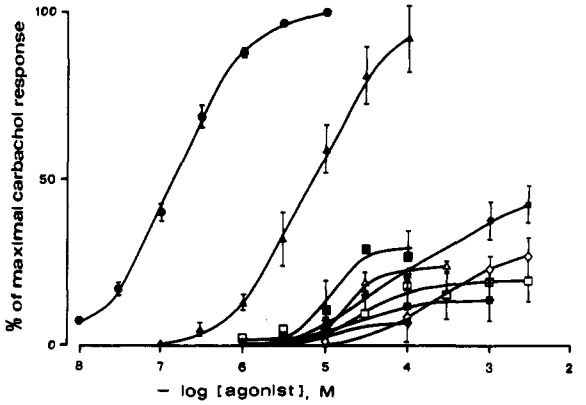

Figure 5. Concentration–response curves to carbachol (●), *cis*-1 (▲), *trans*-1 (△), 7 (■), 8 (□), 14 (◆), 15 (◇), 16 (✱) and 17 (☆) in isolated strips of guinea pig longitudinal ileal muscle. Data are expressed as percent of the maximal response induced by carbachol, and each point represents the mean ± SEM of two to nine experiments, except for carbachol, where $n = 34$.

in 1 resulted in a reduced antimuscarinic activity (Table III). The loss of activity was substantially greater in 7 and 8 (19- and 7-fold, respectively) than in 14 and 15 (4- and 2-fold, respectively), and it was more pronounced for the cis than for the trans derivatives. As compared to *cis*-1, the antimuscarinic potency was decreased in both the dimethyl-substituted analogue 16 (8–9-fold) and the desmethyl derivative 17 (25-fold; cf. Table III).

The series was further evaluated for muscarinic activity using isolated strips of the guinea pig ileum. All compounds were capable of eliciting at least some contractile response. The most potent compound, *cis*-1 (EC_{50} 6 μM), produced a maximal response comparable to that observed for carbachol (Figure 5, Table III). In contrast, *trans*-1 acted as a partial agonist in the ileum (Figure 5), since the maximal contractile response was only one-fourth of that induced by carbachol. A similar pattern was noted for the two pairs of tetrahydrofuran derivatives (7/8 and 14/15), and for the dimethyl-substituted (16) and desmethyl (17) analogues, respectively (Figure 5, Table III). These compounds were all characterized by a low and variable efficacy, and thus, it is not meaningful to make detailed comparisons. The efficacy, however, seemed to be more affected by the addition of an extra methyl group (16) than by the absence of a methyl group (17).

In order to determine the affinities for muscarinic receptors in the cerebral cortex (M_1), heart (M_2), parotid gland (M_3), and urinary bladder from guinea pig, all compounds were subjected to receptor binding studies.[20-23]

Table IV. Affinities (K_i) for Muscarinic Receptors, Determined by Competition Experiments with (−)-[^{3}H]QNB[a]

	K_i, μM			
compd	cerebral cortex (M_1)	heart (M_2)	parotid gland (M_3)	urinary bladder
cis-1	2.9 ± 0.1	9.7 ± 0.6	15 ± 0.05	14 ± 3
trans-1	6.3 ± 0.4	>21[b]	25 ± 2	40 ± 5
7	15 ± 1	15 ± 2	35 ± 3	33 ± 6
8	20 ± 3	>25[b]	78 ± 7	57 ± 10
14	6.3 ± 1.2	>20[b]	33 ± 17	>50[b]
15	6.5 ± 0.1	>20[b]	26 ± 0.1	>50[b]
16	9.3 ± 0.9	30 ± 2	39 ± 0.05	45 ± 8
17	19 ± 2	50 ± 7	86 ± 2	115 ± 8

[a] Values are mean ± SEM of two to three experiments performed in triplicate. [b] Affinity too low for determination of K_i; less than 50% inhibition was produced by the concentrations indicated.

They inhibited the receptor specific binding of the radioligand (−)-[^{3}H]-QNB in each of the tissues investigated. However, in agreement with the functional data (Table III), the affinities for muscarinic receptors were low as shown by the dissociation constants (K_i, Table IV) which ranged from 3–15 μM for *cis*-1 to 19–115 μM for 17. The rank order of potency at cortical receptors (M_1) was similar to that observed for antagonism of carbachol-induced contractions of isolated urinary bladder strips: *cis*-1 > *trans*-1, 14, 15, ≥ 16 ≥ 7, 8, 17. The general pattern in the other tissues was also similar; *cis*-1 was the most potent and the desmethyl analogue 17 was the least potent compound. Due to the low affinities it was not always possible to generate complete concentration–inhibition curves and to determine dissociation constants (cf. Table IV), and, therefore, it is not appropriate to make detailed comparisons between compounds and tissues. However, none of the compounds appeared to exhibit any selectivity for a particular receptor subtype.

The affinity of *cis*-1 was about 2 times higher than that exhibited by *trans*-1. This stereoselectivity was reduced in the diastereomeric pair 7 and 8 and it was abolished in the pair 14 and 15 (Table IV). Removal of one of the two ring oxygens resulted in a slight (approximately 2–3-fold) decrease in affinity for muscarinic receptors, except in the trans analogue 15, which was equipotent to *trans*-1. As compared to *cis*-1, the dimethyl-substituted analogue 16 exhibited approximately 3-fold lower affinity, whereas the affinity of desmethyl analogue 17 was lowered 5–8-fold.

The observation that *cis*-1 is the only derivative of the quinuclidine analogues studied here that behaves as a (nearly) full agonist in the ileum assay might indicate that the lack of a cis-positioned methyl group and/or one of the two oxygens are detrimental to efficacy.[24] Since only one oxygen is present in the tetrahydrofuran analogues, one of the potential hydrogen bond acceptor sites available in 1 is absent in these derivatives (cf. ref 25). In addition,

(20) Nilvebrant, L.; Sparf, B. Muscarinic receptor binding in the parotid gland. Different affinities of some anticholinergic drugs between the parotid gland and ileum. *Scand. J. Gastroenterol.* **1982**, *17* (Suppl. 72), 69–77.

(21) Nilvebrant, L.; Sparf, B. Muscarinic receptor binding in the guinea pig urinary bladder. *Acta Pharmacol. Toxicol.* **1983**, *52*, 30–38.

(22) Nilvebrant, L.; Sparf, B. Dicyclomine, benzhexol and oxybutynin distinguish between sub-classes of muscarinic binding-sites. *Eur. J. Pharmacol.* **1986**, *123*, 123–143.

(23) Nilvebrant, L.; Sparf, B. Differences between binding affinities of some antimuscarinic drugs in the parotid gland and those in the urinary bladder and ileum. *Acta Pharmacol. Toxicol.* **1983**, *53*, 304–313.

the methyl group is generally regarded as an important structural element for efficacy in compounds of this type.[26]

Considering that a combination of electronic, steric, and conformational factors may be of importance for efficacy, it is noteworthy that the conformational preferences vary between the various analogues (Table II). This is particularly interesting since the conformation of the five-membered ring affects the spatial position of the methyl group(s) and the oxygen(s). The efficacious *cis*-1 adopts a lowest-energy conformation (^2E) which is accessible to 14 and 17, the former lacking one of the oxygens and the latter the methyl group. However, *cis*-1 may also readily adopt a $^2T_3$ conformation, a property which it only shares with 7, a compound which differs from 14 in lacking the other oxygen atom. Compound 7 readily adopts the $^2T_3$ conformation, whereas 14 has to pay a considerable energy penalty to assume a ^2E conformation. Consequently, if the $^2T_3$ conformation would be responsible for the agonist activity of *cis*-1, the lower efficacy of 7 would have to be attributed to the lack of one of the oxygens. On the other hand, if the ^2E conformation would correspond to the agonist pharmacophore, the lower efficacy of 14 could be attributed to the conformational energy penalty and/or the absence of the other oxygen and the lower efficacy of 17 might be rationalized by the absence of a methyl group. At present we can not distinguish between these alternative rationales.

The quinuclidine analogues exhibited fairly small differences in affinity for the receptors in the various tissues. Therefore the data do not permit extensive conclusions in terms of SAR. However, the slightly higher affinity of 14 and 15 in the cerebral cortex as compared to 7 and 8 (Table IV) indicates that the O1' in 1 is the more important of the two oxygens for effective binding to the M_1 muscarinic receptors, i.e., for high affinity.

Compounds 16 and 17 were included in the present study in an attempt to determine the role of the methyl group of 1 and related compounds in the receptor interaction. Both 16 and 17 have a lower affinity for muscarinic receptors than 1. Throughout, however, the non-methyl-containing 17 has lower affinity than the dimethyl-substituted 16. The latter may be viewed as a hybrid of *cis*- and *trans*-1, and the results imply that methyl groups enhance affinity,[27] probably by providing additional possibilities for the development of stabilizing van der Waals interactions with the receptor. The lower affinity of dimethyl derivative 16 as compared to, e.g., that of *cis*-1 may, on the other hand, be the result of repulsive interactions between one of the methyl groups and the receptor.

Experimental Section

Chemistry. General Comments. Melting points (uncorrected) were determined in open glass capillaries using a Thomas-Hover apparatus. IR spectra were recorded on a Perkin-Elmer 298 infrared spectrophotometer. All spectra (including NMR) were in accordance with assigned structures. Capillary GC analysis of the compounds was performed on a Carlo-Erba 6000 Vega instrument equipped with a DB-5 fused silica column (30 m, i.d. 0.25 mm). Helium (60–80 kPa) was used as a carrier gas. The elemental analyses were preformed by Mikro Kemi AB, Uppsala, Sweden, and were within 0.4% of the calculated values. TLC was carried out on aluminum sheets precoated with aluminum oxide 60 F_{254} neutral (type E) or silica gel 60 F_{254} (0.2 mm), E. Merck.

NMR Spectroscopy. Routine ^1H and ^{13}C NMR spectra were recorded on a JEOL FX 90Q spectrometer at 90.0 and 22.5 MHz, respectively, and were referenced to internal tetramethylsilane. Standard high-resolution ^1H, COSY, and ^{11}B NMR spectra were recorded on a JEOL JNM-GX400 spectrometer at 400 and 128.15 MHz, respectively. NMR spectra for the structural assignment of the cis and trans isomers were obtained of solutions in CD_3OD on a Varian XL-300 spectrometer with ^1H at 300 MHz and ^{13}C at 75.4 MHz. Chemical shifts were indirectly referenced to TMS via the solvent signals (^1H, 3.35 ppm; ^{13}C, 49.0 ppm).

Inversion–recovery spectra were recorded with a $\pi-t_d-\pi/2-$acquire sequence.[13] For NOE differential spectra, the solutions were degassed by several freeze–pump–thaw cycles and sealed under argon. Enhancements were estimated as the difference between spectra for a saturated signal and one with off-resonance irradiation and were corrected for partial saturation of the target signal.[28] The carbon signal assignments originate from DEPT,[29] selective INEPT,[30] and HMQC[15] spectra.

3-(3-Butenyl)-3-hydroxy-1-azabicyclo[2.2.2]octane (4). An excess of a 1 M ether solution of allylmagnesium bromide (107.9 mmol, 100 mL) was added during 30 min to a stirred mixture of 3-methylenequinuclidine oxide (3; 1.92 g, 14.2 mmol),[6] CuI (2.05 g, 10.7 mmol), and dimethyl sulfoxide (0.98 mL, 14.2 mmol)[31] in ether (50 mL) at 0 °C under nitrogen. The mixture was allowed to stand overnight and a saturated aqueous NH_4Cl solution (10 mL) was added. The ether was decanted and the flask was rinsed with additional ether. The combined ether layers were dried (K_2CO_3), filtered, and concentrated by rotary evaporation. Gradient elution ($CHCl_3 \rightarrow CHCl_3$ + 5% MeOH) of the crude product on an Al_2O_3 column afforded 1.46 g (57%) of 4. An analytical sample was prepared by recrystallization from ether: mp 91–92 °C; IR (neat) 3360 (br), 3120; ^1H NMR ($CDCl_3$) δ 6.10–5.60 (m, 1 H, vinyl CH), 5.15–4.90 (m, 2 H, vinyl CH_2), 3.20–2.50 (m, 6 H), 2.30–1.90 (m, 3 H), 1.80–1.05 (m, 7 H); ^{13}C NMR ($CDCl_3$) δ 139.0 (vinyl CH), 114.3 (vinyl CH_2), 70.0 (C3),

(24) Recently a number of compounds closely related to those presented here have been synthesized: (a) Methyl-substituted tetrahydrofuran derivatives were synthesized but were inactive in the assays used; see: Coldham, I.; Collington, E. W.; Hallett, P.; Warren, S. Stereochemically Controlled Synthesis of Spirocyclic Lactones and Ethers From N-Methyl-4-piperidone and 3-Quinuclidinone by Phenylthio Migration. *Tetrahedron Lett.* **1988**, *29*, 5321–5324. (b) Related compounds have also appeared in patents; see: (i) Tsukamoto, S. I.; Nagaoka, H.; Usuda, S.; Harada, M.; Tamura, T. Eur. Patent 311 313, 1989. (ii) Fisher, A.; Karton, I. Eur Patent 314 444, 1989. (iii) Heldman, E.; Grunfeld, Y.; Karton, I.; Levy, A. Eur. Patent 205 247, 1986.

(25) Recently, the importance of two hydrogen-bonded acceptor sites for binding to the agonist-binding site has been discussed: Saunders, J.; Cassidy, M.; Freedman, S. B.; Harley, E. A.; Iversen, L. L.; Kneen, C.; Macleod, A. M.; Merchant, K. J.; Snow, R. J.; Baker, R. Novel Quinuclidine-Based Ligands for the Muscarinic Cholinergic Receptor. *J. Med. Chem.* **1990**, *33*, 1128–1138.

(26) See, e.g.: Ringdahl, B. Structural Determinants of Muscarinic Agonist Activity. In The Muscarinic Receptors; Brown, J. H., Ed.; Humana Press: Clifton, NJ, 1989; pp 151–218.

(27) Saunders et al. (see ref 5) have discussed the importance of the position of the methyl group for the selectivity of *cis*-1. This group also suggested that the methyl group in *cis*-1, due to its relatively well-defined position, would be responsible for the observed selectivity.

(28) Sanders, J. K. M.; Mersh, J. D. Nuclear Magnetic Double Resonance: The Use of Difference Spectroscopy. In *Prog. Nucl. Magn. Reson. Spectrosc.* **1982**, *15*, 353–400.

(29) Doddrell, D. B.; Pegg, D. T.; Bendall, M. R. Distortionless Enhancement of NMR Signals by Polarisation Transfer. *J. Magn. Reson.* **1982**, *48*, 323–327.

(30) Bax, A. Structure Determination and Spectral Assignment by Pulsed Polarization Transfer via Long-Range $^1H-^{13}C$ Couplings. *J. Magn. Reson.* **1984**, *57*, 314–318.

(31) The 3-methylenequinuclidine oxide codistilled with DMSO. The concentration of the product was determined by ^1H NMR spectroscopy. To compensate for the presence of DMSO, the epoxide was therefore reacted with an excess of Grignard reagent.

63.2, 46.6, 46.2, 39.6, 31.4, 27.4, 23.5, 21.6. Anal. ($C_{11}H_{19}NO$) C, H, N.

***cis*- and *trans*-5′-Methylspiro[1-azabicyclo[2.2.2]octane-3,2′-oxolane]-Borane Complex (5 and 6).** A solution of 4 (1.48 g, 8.2 mmol) in THF (5 mL) was added to a stirred mixture of $Hg(OAc)_2$ (3.9 g, 12.2 mmol) in H_2O–THF (20 mL, 1:1). After being stirred for 3 h at room temperature, the reaction mixture was concentrated and CH_2Cl_2 (30 mL) and a solution of benzyltriethylammonium chloride (6.0 g, 26.3 mmol) in 15 mL of 2.8 M aqueous NaOH was added. Subsequently, a solution of $NaBH_4$ (0.93 g, 24.5 mmol) in 2.8 M aqueous NaOH (5 mL) was added to the stirred mixture. After 45 min the layers were separated, and the water layer was extracted with CH_2Cl_2 (4 × 50 mL). The combined organic phases were dried (K_2CO_3), filtered, and concentrated under reduced pressure. The residue was first purified by chromatography on an Al_2O_3 column with $CHCl_3$ as eluent and then on a SiO_2 column using ether–light petroleum (1:1) as eluent. This procedure afforded 0.80 g (54%) of a 2:1 (^1H NMR) mixture of diastereomers 5 and 6. A 1.66-g amount of diastereomeric mixture (2:1, ^1H NMR), obtained from several batches, was separated by repetitive (10 times) flash chromatography on SiO_2 (ether–light petroleum (1:1) to afford 0.75 g of the cis isomer 5, 0.24 g of the trans isomer 6, and 0.66 g of mixed fractions.

Compound 5: R_f = 0.49 (SiO_2, ether–light petroleum 1:1); mp 96.0–96.5 °C; IR (KBr) 2340, 2300, 2260, 1165; ^1H NMR (CD_3OD) δ 4.11 (ddq, J = 6.0, 6.1, 8.0 Hz, 1 H, C5′-H), 3.09–2.87 (m, 6 H, C2-H's, C6-H's, C7-H's), 2.25–2.1 (m, 3 H, C3′-H, C4-H, C4′-H), 2.00–1.82 (m, 4 H, C3′-H, C5-H's, C8-H), 1.70 (m, 1 H, C8-H), 1.60 (m, 1 H, C4′-H), 1.31 (d, J = 6.1 Hz, 3 H, CH_3), BH_3 (partly obscured and not unambiguously assigned); ^{13}C NMR ($CDCl_3$) δ 80.9 (C3), 74.7 (C5′), 68.1 (C2), 53.1, 52.7 (C6, C7), 36.1, 32.6, 31.8, 23.1, 21.7, 21.0 (C4, C5, C8, C3′, C4′, CH_3). Anal. ($C_{11}H_{19}NO{\cdot}BH_3$) C, H, N.

Compound 6: R_f = 0.56 (SiO_2, ether–light petroleum 1:1); mp 87.5–88.0 °C; IR (KBr) 2340, 2300, 2260, 1165; ^1H NMR (CD_3OD) δ 4.19 (ddq, J = 6.0, 6.1, 8.2 Hz, 1 H, C5′-H), 3.09–2.88 (m, 6 H, C2-H's, C6-H's, C7-H's), 2.26 (m, 1 H, C4-H), 2.21 (ddd, J = 4.2, 8.0, 12.6 Hz, 1 H, C3′-H), 2.12 (dddd, J = 4.2, 6.0, 7.5, 12.0 Hz, 1 H, C4′-H), 1.92–1.83 (m, 4 H, C3′-H, C5-H's, C8-H), 1.70 (m, 1 H, C8-H), 1.59 (dddd, J =8.0, 8.2, 9.0, 12.0 Hz, 1 H, C4′-H), 1.29 (d, J = 6.1 Hz, 3 H, CH_3), BH_3 (partly obscured and not unambiguously assigned); ^{13}C NMR ($CDCl_3$) δ 80.8 (C3), 74.6 (C5′), 68.3, 53.1, 52.5 (C2, C6, C7), 36.5, 32.9, 30.3, 22.3, 21.7, 20.8 (C4, C5, C8, C3′, C4′, CH_3). Anal. ($C_{11}H_{19}NO{\cdot}BH_3$) C, H, N.

3-(Methoxycarbonyl)-3-prop-2-enyl-1-azabicyclo[2.2.2]-octane (10). Methyl quinuclidine-3-carboxylate hydrochloride (9)[10] (2.0 g, 9.73 mmol) was added in one portion to a stirred solution of LDA [prepared from a 1.58 M solution of *n*-BuLi in hexane (13.55 mL, 21.4 mmol) and diisopropylamine (2.26 g, 22.4 mmol)] in dry THF (90 mL) kept at −35 °C under nitrogen. The temperature was allowed to rise to −20 °C and was maintained until the salt was completely dissolved. The mixture was cooled to −50 °C and a solution of 3-bromoprop-1-ene (1.18 g, 9.73 mmol) in dry THF (10 mL) was added dropwise. The temperature was allowed to rise slowly and was kept between −40 °C and −30 °C for 3 h. The temperature was allowed to rise to 0 °C and a saturated aqueous NH_4Cl solution (0.5 mL) was added. The mixture was dried (K_2CO_3), filtered, and concentrated under reduced pressure. The residue was purified by chromatography on Al_2O_3 columns, first with $CHCl_3$ as eluent and then with gradient elution (ether → ether + 5% MeOH) to yield 1.04 g (51%) of 10: IR (neat) 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.90–5.40 (m, 1 H, vinyl CH), 5.20–4.90 (m, 2 H, vinyl CH_2), 3.67 (s, 3 H, CH_3), 3.68–3.58 (m, 1 H), 2.95–2.40 (m, 7 H), 2.15–1.30 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 176.5 (C=O), 133.4 (vinyl CH), 117.8 (vinyl CH_2), 55.8 (CH_3), 51.6 (C3), 46.9 (two coinciding peaks), 46.3, 42.6, 28.4, 25.2, 22.1. A small sample was converted into the hydrochloride and recrystallized from MeOH–acetone–ether: mp 200–201.5 °C. Anal. ($C_{12}H_{19}NO_2{\cdot}HCl$) C, H, N.

3-(Hydroxymethyl)-3-prop-2-enyl-1-azabicyclo[2.2.2]octane (11). Compound 10 (2.84 g, 13.6 mmol) was added to a stirred suspension of $LiAlH_4$ (0.75 g, 6.8 mmol) in dry ether (50 mL) under nitrogen at room temperature. After 1 h, excess reducing agent was destroyed by successive dropwise addition of EtOAc (5 mL) and 5 M aqueous HCl (25 mL) with vigorous stirring. The aqueous layer was washed with ether (50 mL), made alkaline with 5 M aqueous NaOH, and extracted with ether (4 × 200 mL). The combined ether layers were dried (K_2CO_3), filtered, and concentrated under reduced pressure to provide 2.42 g (98%) of 11 as an oil: IR (neat) 3360 (br), 3150, 1050 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.00–5.62 (m, 1 H, vinyl CH), 5.25–5.00 (m, 2 H, vinyl CH_2), 4.65 (s, OH), 3.47 (s, CH_2O), 2.90–2.65 (m, 4 H), 2.45–2.25 (m, 4 H), 2.00–1.65 (m, 3 H), 1.55–1.20 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 135.2 (vinyl CH), 117.3 (vinyl CH_2), 65.6 (CH_2O), 58.1, 47.2, 47.0, 39.5, 37.3, 25.2, 23.6, 23.0. A small sample was converted into the hydrochloride and recrystallized from MeOH–acetone–ether to provide an analytical sample: mp 155.5–156 °C. Anal. ($C_{11}H_{20}NO{\cdot}HCl$) C, H, N.

***cis*- and *trans*-2′-Methylspiro[1-azabicyclo[2.2.2]octane-3,4′-oxolane]-Borane Complex (12 and 13).** A solution of 11 (0.58 g, 3.2 mmol) in THF (1 mL) was added to a stirred solution of $Hg(O_2CCF_3)_2$ (1.50 g, 3.52 mmol) in H_2O (3 mL) and THF (1 mL). After 3 h at room temperature the reaction mixture was concentrated under reduced pressure. To the residue were added $CHCl_3$ (20 mL) and benzyltriethylammonium chloride (2.33 g, 10.24 mmol) in 5 mL of 2.8 M aqueous NaOH and then a solution of $NaBH_4$ (0.363 g, 9.6 mmol) in 2.8 M aqueous NaOH (1 mL). The layers were separated after 1 h, and the aqueous layer was extracted with $CHCl_3$ (3 × 20 mL). The combined $CHCl_3$ layers were dried (K_2CO_3), filtered, and concentrated under reduced pressure. The residue was purified by chromatography on an Al_2O_3 column with $CHCl_3$ as eluent, giving 0.43 g (71%) of a 1:1 (^1H NMR) mixture of diastereomers 12 and 13. A 1.17-g amount of the diastereomeric mixture (1:1, ^1H NMR) resulting from several batches was separated by repetitive flash chromatography (10 columns) on SiO_2 (ether–light petroleum 2:1) to provide 0.49 g of the trans isomer 13, 0.39 g of the crude cis isomer 12, and 0.23 g of mixed fractions. 12 was purified further by recrystallization from *n*-hexane to give 0.28 g of pure product.

Compound 12: R_f = 0.27 (SiO_2, ether–light petroleum 2:1); mp 109.5–110.0 °C; IR (KBr) 2340, 2300, 2260, 1165 cm^{-1}; ^1H NMR (CD_3OD) δ 4.15 (ddq, J = 6.2, 6.8, 7.6 Hz, 1 H, C2′-H), 3.86 (d, J = 8.8 Hz, 1 H, C5′-H), 3.74 (d, J = 8.8 Hz, 1 H, C5′-H), 3.14–2.93 (m, 6 H, C2-H's, C6-H's, C7-H's), 2.37 (dd, J = 7.6, 12.8 Hz, 1 H, C3′-H), 2.03 (m, 1 H, C4-H), 1.94–1.78 (m, 4 H, C5-H's, C8-H's), 1.59 (dd, J = 6.8, 12.8 Hz, 1 H, C3′-H), 1.32 (d, J = 6.2 Hz, 3 H, CH_3), BH_3 (partly obscured and not unambiguously assigned); ^{13}C NMR ($CDCl_3$) δ 78.3 (C5′), 74.6 (C2′), 67.6, 52.8, 52.3, 46.6, 43.9 (C3), 28.5, 23.0, 22.6, 21.3. Anal. ($C_{11}H_{19}NO{\cdot}BH_3$) C, H, N.

Compound 13: R_f = 0.30 (SiO_2, ether–light petroleum 2:1); mp 97.5–98 °C; IR (KBr) 2340, 2300, 2260, 1165 cm^{-1}; ^1H NMR (CD_3OD) δ 4.17 (ddq, J = 5.6, 6.0, 10.0 Hz, 1 H, C2′-H), 3.89 (d, J = 8.5 Hz, 1 H, C5′-H), 3.78 (d, J = 8.5 Hz, 1 H, C5′-H), 3.10–2.99 (m, 6 H, C2-H's, C6-H's, C7-H's), 2.18 (dd, J = 5.6, 12.2 Hz, 1 H, C3′-H), 2.02 (m, 1 H, C4-H), 1.90–1.81 (m, 4 H, C5-H's, C8-H's), 1.60 (dd, J = 10.0, 12.2 Hz, 1 H, C3′-H), 1.33 (d, J = 6.0 Hz, 3 H, CH_3), BH_3 (partly obscured and not unambiguously assigned); ^{13}C NMR ($CDCl_3$) δ 78.1 (C5′), 74.3 (C2′), 66.7, 52.2 (two coinciding peaks), 47.1, 43.9 (C3), 28.9, 22.7, 22.6, 20.6. Anal. ($C_{11}H_{19}NO{\cdot}BH_3$) C, H, N.

General Procedure for Cleavage of the Borane Complexes. To 0.065–0.200 g of borane complex (5, 6, 12, or 13) were added 5 M aqueous HCl (5 mL) and MeOH (3 mL). The mixture was heated at 50 °C for 1.5 h. The MeOH was evaporated, the residue was made basic with 5 M aqueous NaOH, and the mixture was extracted with ether (5 × 40 mL). The combined ether extracts were dried (K_2CO_3), filtered, and concentrated under reduced pressure to give a colorless oil, which was converted into the hydrochloride by addition of HCl in ether. Finally, the resulting hydrochloride was recrystallized to yield (7, 8, 14, or 15).

***cis*-5′-Methylspiro[1-azabicyclo[2.2.2]octane-3,2′-oxolane] hydrochloride (7):** recrystallized from acetone–ether; yield 65%; R_f (base) = 0.5 (aluminum oxide, $CHCl_3$ + 5% MeOH); mp 183–184 °C; ^1H NMR δ 4.13 (m, 1 H, C5′-H), 3.45 (dd, J = 2.0, 13.4 Hz, 1 H), 3.36–3.26 (m, 5 H), 2.34 (m, 1 H), 2.23–2.10 (m, 2 H), 2.13 (m, 1 H, C4-H), 2.07–1.95 (m, 3 H), 1.85 (m, 1 H), 1.60 (m, 1 H, C4′-H_{cis}), 1.28 (d, J = 6.1 Hz, 3 H, CH_3); ^{13}C NMR δ 81.0 (C3), 76.9 (C5′), 61.6 (CH_2), 47.6 (CH_2), 46.9 (CH_2), 37.0 (CH_2), 33.7 (CH_2), 30.9 (C4), 21.9 (CH_3), 20.8 (CH_2), 19.6 (CH_2). Anal. ($C_{11}H_{19}NO{\cdot}HCl{\cdot}0.25H_2O$) C, H, N.

***trans*-5′-Methylspiro[1-azabicyclo[2.2.2]octane-3,2′-oxolane] hydrochloride (8):** recrystallized from acetone–ether;

yield 69%; R_f (base) = 0.5 (aluminum oxide $CHCl_3$ + 5% MeOH); mp 218–219 °C; ^1H NMR δ 4.18 (m, 1 H, C5'-H), 3.36–3.25 (m, 6 H), 2.37 (m, 1 H), 2.26 (ddd, J = 3.5, 7.5, 12.0 Hz, 1 H, C3'-H_{trans}), 2.10 (m, 1 H, C4'-H_{cis}), 2.03 (m, 1 H, C4-H), 2.02–1.90 (m, 2 H), 1.94 (m, 1 H, C3'-H_{cis}), 1.85 (m, 1 H), 1.58 (m, 1 H, C4'-H_{trans}), 1.25 (d, J = 6.0 Hz, 3 H, CH_3); ^{13}C NMR δ 81.0 (C3), 76.9 (C5'), 61.3 (C2), 47.6, 47.0 (C6 and C7), 36.5 (C3'), 33.5 (C4'), 32.2 (C4), 21.7 (CH_3), 21.5 (CH_2), 19.6 (CH_2). Anal. ($C_{11}H_{19}NO$·HCl) C, H, N.

***cis*-2'-Methylspiro[1-azabicyclo[2.2.2]octane-3,4'-oxolane] hydrochloride** (14): recrystallized from MeOH–acetone–ether; yield 80%; R_f (base) = 0.5 (aluminum oxide, $CHCl_3$ + 5% MeOH); mp 211.5–212.0 °C; ^1H NMR δ 4.13 (m, 1 H, C2'-H), 3.93 (dd, J = 0.8, 9.0 Hz, 1 H, C5'-H_{cis}), 3.71 (dd, J = 0.8, 9.0 Hz, 1 H, C5'-H_{trans}), 3.44 (d, J = 12.5 Hz, 1 H, C2-H_a), 3.40–3.29 (m, 5 H), 2.37 (dd, J = 7.7, 12.8 Hz, 1 H, C3'-H_{trans}), 2.14 (m, 1 H), 2.06–1.93 (m, 4 H), 1.66 (ddd, J = 0.8, 6.8, 12.8 Hz, 1 H, C3'-H_{cis}), 1.28 (d, J = 6.2 Hz, 3 H, CH_3); ^{13}C NMR δ 78.9 (C5'), 76.3 (C2'), 60.6 (C2), 47.3 (CH_2), 47.1 (CH_2), 46.8 (CH_2), 44.7 (C3), 28.9 (C4), 22.1 (CH_2), 21.7 (CH_3), 21.6 (CH_2). Anal. ($C_{11}H_{19}NO$·HCl) C, H, N.

***trans*-2'-Methylspiro[1-azabicyclo[2.2.2]octane-3,4'-oxolane] hydrochloride** (15): recrystallized from MeOH–acetone–ether; yield 83%; R_f (base) = 0.5 (aluminum oxide, $CHCl_3$ + 5% MeOH); mp 212.5–213.0 °C; ^1H NMR δ 4.17 (ddq, J = 5.5, 6.5, 9.6 Hz, 1 H, C2'-H), 3.90 (d, J = 8.7 Hz, 1 H, C5'-H_{trans}), 3.83 (dd, J = 0.8, 8.7 Hz, 1 H, C5'-H_{cis}), 3.41–3.32 (m, 6 H), 2.26 (ddd, J = 0.8, 5.5, 12.3 Hz, 1 H, C3'-H_{cis}), 2.15 (m, 1 H), 2.05–1.95 (m, 4 H), 1.65 (dd, J = 9.6, 12.3 Hz, 1 H, C3'-H_{trans}), 1.30 (d, J = 6.0 Hz, 3 H, CH_3); ^{13}C NMR δ 78.7 (C5'), 76.4 (C2'), 59.9 (C2), 47.9 (C3'), 47.1 (CH_2), 47.0 (CH_2), 45.1 (C3), 29.6 (C4), 22.2 (CH_2), 22.0 (CH_2), 21.3 (CH_3). Anal. ($C_{11}H_{19}NO$·HCl) C, H, N.

2',2'-Dimethylspiro[1-azabicyclo[2.2.2]octane-3,4'-[1,3]dioxolane] Hydrochloride (16). Concentrated H_2SO_4 (2 mL) was added to a solution of 3-hydroxy-3-(hydroxymethyl)-1-azabicyclo[2.2.2]octane[5] (1.0 g, 6.37 mmol) and 2,2-dimethoxypropane (1.3 g, 12.7 mmol) in acetone (10 mL). The mixture was stirred overnight. The acetone was evaporated and the resulting mixture was made alkaline with 5 M NaOH and was extracted with $CHCl_3$ (4 × 50 mL). The combined $CHCl_3$ layers were dried (K_2CO_3), filtered, and concentrated under reduced pressure. The residue was purified by chromatography on an Al_2O_3 column by use of $CHCl_3$ as eluent to provide 0.45 g (36% yield) of pure **16**. The product was converted into the hydrochloride by addition of HCl dissolved in ether and was recrystallized from acetonitrile: R_f (base) = 0.67 (Al_2O_3; $CHCl_3$ + 5% MeOH); mp 235–240 °C (lit.[2] 245.4–245.6 °C); ^1H NMR (CD_3OD) δ 4.19 (d, J = 9.2 Hz, 1 H, C5'-H), 3.92 (d, J = 9.2 Hz, 1 H, C5'-H), 3.60–3.13 (m, 6 H), 2.48–1.70 (m, 5 H), 1.39 (s, 6 H, $(CH_3)_2$); ^{13}C NMR δ 111.7 (C2'), 79.4 (C3), 72.6 (C5'), 59.0 (C2), 47.7, 46.6, 30.5, 27.2 (CH_3), 26.7 (CH_3), 20.8, 19.4. Anal. ($C_{11}H_{19}NO_2$·HCl) C, H, N.

Spiro[1-azabicyclo[2.2.2]octane-3,4'-[1,3]dioxolane] Hydrochloride (17). Concentrated H_2SO_4 (2 mL) was added to a mixture of 3-hydroxy-3-(hydroxymethyl)-1-azabicyclo[2.2.2]octane (1.0 g, 6.37 mmol) and paraformaldehyde (0.57 g, 19.1 mmol) in glacial acetic acid (10 mL).[11] The mixture was allowed to stand overnight and was then made alkaline with 5 M NaOH and extracted with $CHCl_3$ (4 × 100 mL). The combined $CHCl_3$ phases were dried (K_2CO_3), filtered, and concentrated under reduced pressure. The residue was purified by chromatography on an Al_2O_3 column with $CHCl_3$ as eluent to provide 0.73 g (68%) of pure **17**. The product was converted into the hydrochloride by addition of HCl dissolved in ether and was recrystallized from acetonitrile: R_f (base) = 0.67 (Al_2O_3, $CHCl_3$ + 5% MeOH); mp 211–212 °C; IR (neat, base) 2930, 2860, 1080 cm^{-1}; ^1H NMR (CD_3OD) δ 4.99 (m, 2 H), 4.08 (d, J = 9.0 Hz, 1 H, C5'-H), 3.82 (d, J = 9.0 Hz, 1 H, C5'-H), 3.53–3.05 (m, 6 H), 2.50–1.60 (m, 5 H); ^{13}C NMR δ 96.2 (C2'), 78.8 (C3), 73.8 (C5'), 58.3 (C2), 47.6, 46.8, 29.6, 20.7, 19.4. Anal. ($C_9H_{15}NO_2$·HCl) C, H, N.

cis*-2'-Methylspiro[1-azabicyclo[2.2.2]octane-3,4'-[1,3]dioxolane]** (cis*-1**) **and *trans*-2'-Methylspiro[1-azabicyclo[2.2.2]octane-3,4'-[1,3]dioxolane]** (***trans*-1**). These derivatives were prepared according to literature procedures.[3,5]

cis-1: R_f (base) = 0.63 (Al_2O_3, $CHCl_3$ + 5% MeOH); mp 229–230 °C (lit.[3] mp 233.8 °C); ^1H NMR δ 5.17 (q, J = 4.9 Hz, 1 H, C2'-H), 4.10 (d, J = 9.0 Hz, 1 H, C5'-H_{cis}), 4.00 (d, J = 9.0 Hz, 1 H, C5'-H_{trans}), 3.61 (dd, J = 2.3, 13.2 Hz, 1 H, C2-H_a), 3.42–3.28 (m, 5 H), 2.36 (br m, 1 H), 2.31 (m, 1 H, C4-H), 2.13–1.83 (m, 3 H), 1.40 (d, J = 4.9 Hz, 3 H, CH_3); ^{13}C NMR δ 103.3 (C2'), 79.2 (C3), 75.4 (CH_2), 59.8 (CH_2), 47.6 (CH_2), 46.7 (CH_2), 29.2 (C4), 20.6 (CH_3), 20.1 (CH_2), 19.5 (CH_2).

trans-1: R_f (base) = 0.59 (Al_2O_3, $CHCl_3$ + 5% MeOH); mp 230–230.5 °C; ^1H NMR δ 5.18 (q, J = 5.0 Hz, 1 H, C2'-H), 4.27 (d, J = 9.0 Hz, 1 H, C5'-H_{trans}), 3.74 (d, J = 9.0 Hz, 1 H, C5'-H_{cis}), 3.52–3.28 (m, 6 H), 2.36 (m, 1 H), 2.15 (m, 1 H, C4-H), 2.05 (m, 1 H), 1.99–1.86 (m, 2 H), 1.39 (d, J = 5.0 Hz, 3 H, CH_3); ^{13}C NMR δ 103.3 (C2'), 78.9 (C3), 73.5 (CH_2), 58.3 (CH_2), 47.7 (CH_2), 46.9 (CH_2), 30.9 (C4), 21.0 (CH_2), 20.4 (CH_3), 19.2 (CH_2). Anal. ($C_{10}H_{17}NO_2$·HCl) C, H, N.

Muscarinic Receptor Binding Studies. The tissue preparations and the general methods used have been described in detail elsewhere for the parotid gland,[20] urinary bladder,[21] heart,[22] and cerebral cortex,[22] respectively. Male guinea pigs (250–400 g of body weight) were killed by a blow on the neck and exsanguinated. The brain was placed on ice for dissection of the cerebral cortex (gray matter only). Urinary bladders, hearts, and parotid glands were dissected in a Krebs–Henseleit buffer (pH 7.4) containing 1 mM phenylmethanesulfonyl fluoride (PMSF, a protease inhibitor). Dissected tissues were homogenized in an ice-cold sodium–potassium phosphate buffer (50 mM, pH 7.4) containing 1 mM PMSF, using a Polytron PT-10 instrument (bladder, heart, parotid) and a Potter-Elvehjem Teflon homogenizer (cortex). All homogenates were diluted with ice-cold phosphate/PMSF buffer to a final protein concentration of ≤0.3 mg/mL and were immediately used in the receptor-binding assays. Protein was determined by the method of Lowry et al.,[32] using bovine serum albumin as the standard.

The muscarinic receptor affinities of the unlabeled compounds were derived from competition experiments in which the ability to inhibit the receptor specific binding of (–)-[^3H]QNB (3-quinuclidinyl [*phenyl*-4-^3H]benzilate, 32.9 Ci/mmol) was monitored as previously described.[22,23] Each sample contained 10 μL of (–)-[^3H]QNB solution (final concentration 2 nM), 10 μL of a solution of test compound, and 1.0 mL of tissue homogenate. Triplicate samples were incubated under conditions of equilibrium, i.e., at 25 °C for 60 (urinary bladder), 80 (heart and cerebral cortex), or 210 (parotid gland) min. Nonspecific binding was determined in the presence of 10 μM unlabeled atropine. Incubations were terminated by centrifugation,[21] and the radioactivity in the pellets was determined by liquid scintillation spectrometry.[21]

IC_{50} values (concentration of unlabeled compound producing 50% inhibition of the receptor specific (–)-[^3H]QNB binding) were graphically determined from the experimental concentration–inhibition curves. Affinities, expressed as the dissociation constants K_i, were calculated by correcting the IC_{50} for the radioligand-induced parallel shift and differences in receptor concentration, using the method of Jacobs et al.[33] The binding parameters for (–)-[^3H]QNB (K_D and receptor densities) used in these calculations have been determined in separate series of experiments.[20–22]

Functional in Vitro Studies. Male guinea pigs, weighing about 300 g, were killed by a blow on the neck and exsanguinated. Smooth muscle strips of the urinary bladder and ileum (longitudinal muscle only) were dissected in a Krebs–Henseleit solution (pH 7.4). The strip preparations were vertically mounted between two hooks in thermostatically controlled (37 °C) organ baths (5 mL). One of the hooks was adjustable and connected to a force transducer (FT 03, Grass Instruments). The Krebs–Henseleit solution was continuously bubbled with carbogen gas (93.5% O_2/6.5% CO_2) to maintain the pH at 7.4. Isometric tension was recorded by a Grass Polygraph (Model 79D). A resting tension of approximately 5 mN was initially applied on each muscle strip, and the preparations were allowed to stabilize for at least 45 min. The resting tension was adjusted, and the preparations were washed several times during the stabilization.

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The urinary bladder strips were used for evaluation of antimuscarinic and muscarinic activity and the ileal preparations for studies of muscarinic activity (see Pharmacological Results and Discussion). Carbachol (carbamylcholine chloride) was used as the standard agonist. Concentration-response curves to agonists were generated either by the cumulative dose-response technique (bladder strip) or by the addition of single agonist concentrations (ileal preparations). In the latter case, the preparations were washed and allowed to rest between each concentration of agonist. EC_{50} values were graphically determined.

In studies of antagonism, a control concentration-response curve to carbachol was generated by cumulative addition of carbachol to the bladder strip (i.e., stepwise increase of the agonist concentration until the maximal contractile response was reached), followed by washing out and a resting period of at least 15 min prior to addition of a fixed concentration of the test compound (antagonist) to the organ bath. After 60 min of incubation, a second cumulative concentration-response curve to carbachol was generated. Responses were expressed as percent of the maximal response to carbachol. EC_{50} values for carbachol in the absence (control) and presence of antagonist were graphically derived and dose ratios (r) were calculated.

Dissociation constants, K_B, for the antagonists were then calculated by $K_B = [A]/r - 1$, where [A] is the concentration of the test compound.[34]

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Registry No. *cis*-1 free base, 64168-64-5; *trans*-1 HCl, 64168-67-8; *trans*-1 free base, 64168-65-6; **3**, 41353-91-7; **4**, 139689-94-4; **5**, 139689-95-5; **6**, 139757-87-2; **7**·HCl, 139689-96-6; **7** free base, 139689-97-7; **8**·HCl, 139689-98-8; **8** free base, 139689-99-9; **9**, 54954-73-3; **10** free base, 139690-00-9; **10**·HCl, 139690-01-0; **11** free base, 139690-02-1; **11**·HCl, 139690-03-2; **12**, 139690-04-3; **13**, 139757-88-3; **14**·HCl, 139690-05-4; **14** free base, 139690-06-5; **15**·HCl, 139690-07-6; **15** free base, 139690-08-7; **16**·HCl, 60171-85-9; **16** free base, 60211-59-8; **17**·HCl, 139690-09-8; **17** free base, 60394-35-6; 3-bromoprop-1-ene, 106-95-6; 3-hydroxy-3-(hydroxymethyl)-1-azabicyclo[2.2.2]octane, 61573-79-3.

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Synthesis and in Vitro Characterization of Novel Amino Terminally Modified Oxotremorine Derivatives for Brain Muscarinic Receptors

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A series of novel 2-substituted acetylenic pyrrolidines and piperidines related to oxotremorine (1) were prepared and evaluated in vitro as muscarinic cholinergic agents at brain M_1 and M_2 receptors. One analogue, 3-(2-oxo-1-pyrrolidinyl)-1-[2(*R*)-pyrrolidinyl]-1-propyne hydrogen oxalate (**6a**), was found to be a partial agonist producing a PI hydrolysis response at cortical M_1 receptors approximately 3-fold larger than that produced by 1. The intrinsic activity profile of **6a** at brain muscarinic receptors is similar to those of azetidine oxo analogue **2** and dimethylamino oxo analogue (**3**). All three compounds are partial M_1 agonists and full M_2 agonists; however, the profile of **6a** in binding studies is significantly different. While **2** and **3** exhibit large M_2 selectivities ranging between 8-fold to several hundred-fold, the binding profile of **6a** shows almost no subtype selectivity.

Introduction

Alzheimer's disease (AD) is a central nervous system (CNS) neurodegenerative disorder which leads to dramatic personality changes as well as a profound dementia in individuals who are afflicted. Although AD affects a number of brain neurochemical systems, a pronounced and consistent deficiency in cholinergic markers in the neocortex and hippocampus has led to the cholinergic hypothesis of AD-related memory loss.[1-3] Decreases in markers of cholinergic activity such as choline acetyltransferase (ChAT), high affinity choline uptake, and acetylcholine esterase activity have been found to occur in AD brains.[4,5] These decreases in presynaptic cholinergic markers, particularly that of ChAT, have been correlated with the severity of dementia. It is this cortical/hippocampal cholinergic dysfunction attributed to presynaptic cholinergic neuron loss which is considered to be a major factor responsible for the memory loss that characterizes AD.[6-8] It has been suggested that muscarinic cholinergic receptors, the majority of which are postulated to reside postsynaptically to the degenerating cholinergic neurons,

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