Hemendra N. Bhargava, and Marion Sitt for their help in the preparation of this manuscript.

Registry No. 1, 64-95-9; 1·HCl, 50-42-0; 2, 3563-01-7; 2·HCl, 2589-00-6; 3, 905-00-0; 3·HCl, 2618-37-3; 4, 302-33-0; 4·HCl, 62-68-0; 5, 105538-97-4; 5·HCl, 25347-19-7; 6, 24452-93-5; 6·HCl, 3620-50-6; 7, 102811-91-6; 7·HCl, 102811-90-5; (±)-8, 105538-98-5; (±)-8·HCl, 63815-31-6; (+)-8a, 105539-02-4; (+)-8a·HCl, 105562-28-5; (−)-8b, 105539-04-6; (−)-8b·HCl, 105539-14-8; (+)-9a, 105538-99-6; (+)-9a·HCl, 105539-08-0; (−)-9b, 105539-05-7; (−)-9b·HCl, 105562-27-4; (±)-10, 105539-00-2; (±)-10·HCl, 105539-15-9; (+)-10a, 105562-26-3; (+)-10a·HCl, 105539-16-0; (−)-10b, 105539-17-1; (−)-10b·HCl, 105562-25-2; 11, 105539-01-3; 11·HCl, 64048-76-6; 12, 74093-49-5; 12·HCl, 74093-46-2; 13, 74093-48-4; 13·HCl, 74093-50-8; 14, 105539-13-7; (±)-15, 105539-03-5; (+)-15, 105539-09-1; (+)-15·quinine, 105539-10-4; (−)-15·quinine, 105539-12-6; (−)-15, 105539-11-5; $PhCH(CH_2Cl)COCl$, 7623-12-3; 2,2-diphenylpropanenitrile, 5558-67-8; 2,2-diphenylbutanenitrile, 5558-68-9; 2,2-diphenylpentanenitrile, 29949-16-4; 2,2-diphenylpentanoic acid, 841-32-7; 2,2-diphenylhexanenitrile, 2902-60-5; 2,2-diphenylpropanal, 22875-82-7; 2,2-diphenylbutanal, 105539-07-9; 2,2-diphenylpentanal, 101594-41-6; 2,2-diphenyl-3-methylbutanoic acid, 80743-47-1; 2,2-diphenyl-4-methylpentanoic acid, 25209-47-6; (+)-(*R*)-tropic acid, 17126-67-9; (−)-(*S*)-tropic acid, 16202-15-6; (±)-6-hydroxy-2-phenylhexanenitrile, 105539-06-8; diphenylacetonitrile, 86-29-3; methyl iodide, 74-88-4; 2,2-diphenylpropanoic acid, 5558-66-7; ethyl bromide, 74-96-4; 2,2-diphenylbutanoic acid, 4226-57-7; propyl bromide, 106-94-5; butyl bromide, 109-65-9; (diethylamino)ethanol, 100-37-8; diphenylacetyl chloride, 1871-76-7; diphenylacetic acid, 117-34-0; 2-(diethylamino)ethyl chloride, 100-35-6; tropic acid, 529-64-6; *p*-toluenesulfonyl chloride, 98-59-9.

Molecular Modification of Anticholinergics as Probes for Muscarinic Receptors. 3. Conformationally Restricted Analogues of Benactyzine[1]

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The synthesis and pharmacological evaluation of conformationally restricted analogues of certain anticholinergic agents is a powerful method for probing the topography of the muscarinic receptor. In the present study, clues as to the binding conformation of structurally flexible anticholinergics are provided by approximating certain conformations of benactyzine by synthetic analogues **1–6**, which are structurally locked into desired conformations. The pharmacological activity of each analogue is an indication of how well particular conformational models are accommodated by the receptor. The conformation of benactyzine in which an intramolecular hydrogen bond may exist between the hydroxyl group and the carbonyl oxygen of the ester group (conformation I) is approximated by the synthetic analogue 2,2-diphenyl-3-tetrahydrofuranyl (diethylamino)ethyl ether (**1**) and related analogues. Pharmacological evaluation using dose–response experiments on isolated rat ileum tissue demonstrated that these compounds noncompetitively inhibited acetylcholine-induced ileum contractions. Restriction of the conformational freedom of the amino side chain of **1** by synthesis of the hexahydro[3,4-*b*]furan derivative **3** provided a weak but competitive inhibitor at low concentration. The conformation of benactyzine in which an intramolecular hydrogen bond may exist between the hydroxyl group and the ether oxygen of the ester group (conformation II) is approximated by 2,2-diphenyl-4-[2-(diethylamino)ethyl]-3-tetrahydrofuranone (**4**). Pharmacological studies showed that this compound competitively inhibited acetylcholine-induced ileum contractions. These experiments provide evidence that receptor-bound conformation II for benactyzine is preferred over conformation I in providing competitive binding with the muscarinic receptor.

The interaction of cholinergic ligands with the muscarinic receptor has been a subject of considerable study.[2-8] A major goal in these investigations has been to determine the conformation of cholinergic agents upon binding to the muscarinic receptor. The reason for this concern is that structurally flexible muscarinic ligands may assume specific conformations upon binding in order to achieve defined physiological effects.

A variety of techniques including radioligand labeling,[9] NMR spectroscopy,[10,11] X-ray diffraction,[12] and molecular orbital calculations[13,14] have been used in attempts to elucidate the receptor-bound conformation of muscarinic agonists and antagonists. Despite these efforts, the active conformation of structurally flexible muscarinic ligands remains unclear.

Experiments with rigid analogues of acetylcholine (ACh) have provided direct and compelling evidence for the active conformation of muscarinic agents.[15,16] In this approach, the pharmacodynamically important structural features of the ligand are locked into rigid positions to give information about receptor-site topography and to provide clues as to a preferred receptor-bound ligand conformation.

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(1) This work is taken, in part, from the Ph.D. Dissertation of M.T.F., University of Illinois at Chicago, 1984; presented in part at the 186th National Meeting of the American Chemical Society, Aug 1983, Washington, D.C.
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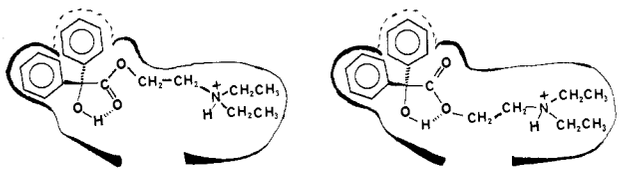
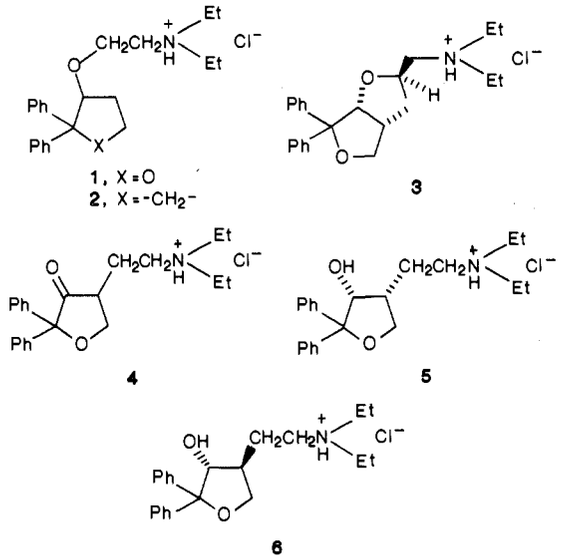


Figure 1. Conformations I and II of benactyzine and their proposed interaction with a hypothetical model of the muscarinic receptor.

Studies in our laboratory have focused on the use of antagonists as molecular probes for the muscarinic receptor.[17] In the present study, conformationally restricted analogues **1–6** of benactyzine were designed and synthesized to investigate the conformations that benzilate-derived antagonists might assume upon binding to the receptor.

Since benactyzine is a structurally flexible molecule, it can assume a number of conformations in the receptor-bound state. The experiments described in this study focus on two possible conformations (I and II, see Figure 1).

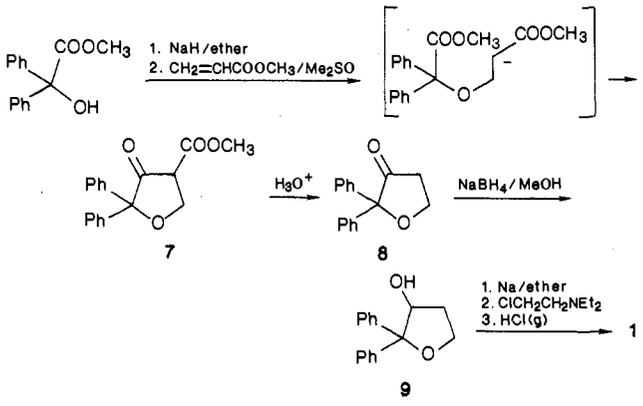
Conformation I of benactyzine allows for a possible intramolecular hydrogen bond between the hydroxyl group and the carbonyl oxygen. Compounds **1** and **2** were designed and synthesized to approximate this conformation.



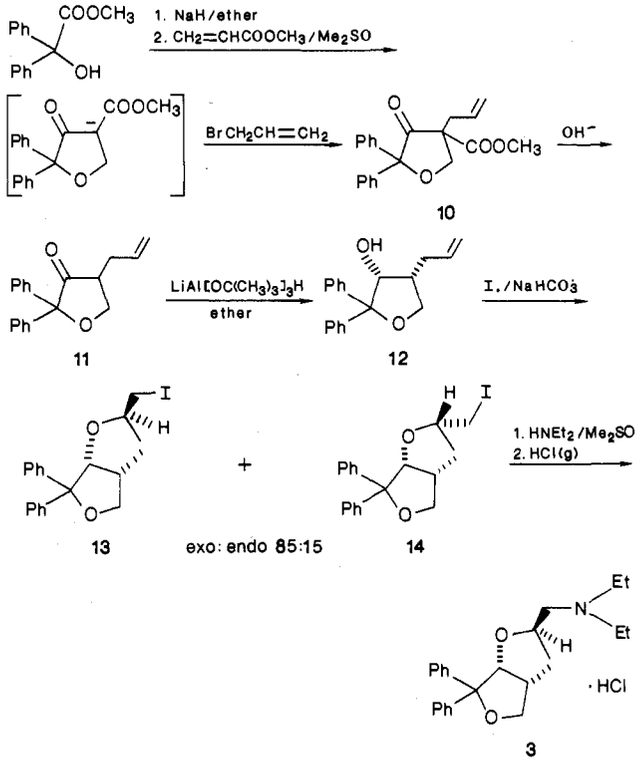
To further restrict the free rotation of the aminoethyl side chain of benactyzine, the hexahydrofuro[3,4-*b*]furan derivative **3** was prepared as a more rigid approximation of conformation I.

Conformation II of benactyzine would orient the ester group so that intramolecular hydrogen bonding between the hydroxyl group and the ether oxygen is possible.

Scheme I



Scheme II



Compound **4** was synthesized to approximate this possibility. In addition, compounds **5** and **6** should provide insight into the nature of the esteratic site of the muscarinic receptor.

In this study, we report our findings for the antispasmodic activities of compounds **1–6** on isolated rat ileum preparations. Experiments with these semirigid analogues have led us to propose a possible receptor-bound conformation for benactyzine as an example of how benzilate-derived muscarinic antagonists may interact with the receptor.

Chemistry

Compound **1** was synthesized as shown in Scheme I. Addition of methyl acrylate to methyl sodium benzilate resulted in a Michael-type addition followed by Dieckmann cyclization to form β-keto ester **7**.[18] This compound underwent acid-catalyzed hydrolysis and decarboxylation to yield 2,2-diphenyl-3-tetrahydrofuranone (**8**). Reduction of ketone **8** afforded 2,2-diphenyl-3-tetrahydrofuranol (**9**),

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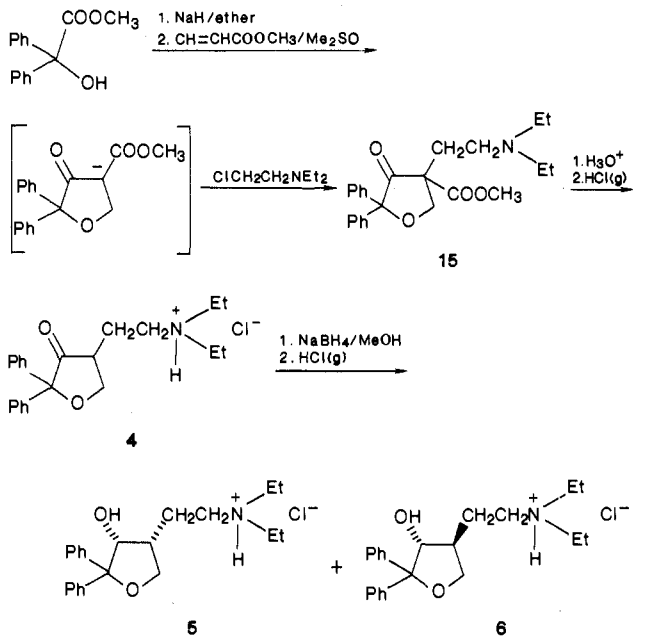
(18) Gianturco, M. A.; Friedel, P.; Giammarino, A. S. *Tetrahedron* **1964**, *20*, 1763.

Scheme III



which was converted to the desired amino ether 1 by a Williamson ether synthesis. Compound 2 was obtained from 2,2-diphenylcyclopentanone by a similar approach.

The synthesis of compound 3 required a convenient preparation of the key intermediate 4-allyl-3-tetrahydrofuranone 11. The intermediate β-keto ester produced during the synthesis of 1 was immediately converted to a β-keto ester carbanion by the methoxide anion liberated during the Dieckmann cyclization (see Scheme II). Alkylation of this carbanion by allyl bromide afforded a one-pot synthesis of the highly functionalized tetrahydrofuranone 10.[19]

Alkaline hydrolysis and decarboxylation of the alkylated β-keto ester produced the 4-allyl-3-keto 11. Stereospecific reduction of this ketone gave the *cis*-4-allyl-3-hydroxy 12 and the corresponding trans isomer in a 98:2 ratio.[20] The pure cis isomer was obtained after several recrystallizations of the crude product.

Compound 12 underwent iodoetherification in the presence of iodine and sodium bicarbonate to form a mixture of the *exo*- and *endo*-iodo ethers 13 and 14 in an 85:15 ratio.[21] The crude product was recrystallized several times to give pure samples of the exo and endo isomers. The structural assignments of each isomer have been discussed in a previous paper.[19]

The *exo*-iodo ether 13 was converted to the bicyclic amine 3 by treatment with diethylamine in Me_2SO.

The amino ketone compound 4 was prepared in similar manner to compound 3 except that 2-(diethylamino)ethyl chloride was used as the alkylating agent rather than allyl bromide (see Scheme III).

The amino ketone 4 was reduced with sodium borohydride to yield a 3:2 mixture of two isomeric alcohols (see Scheme III). On the basis of the results of reduction reactions with the related ketone 11, the major product of the reduction of 4 was expected to be the cis isomer 5.

The NMR spectrum recorded for isomer 5 had characteristics that were very similar to the spectrum of the pure *cis*-4-allyl-3-hydroxy 12. The H-3 proton (adjacent to the hydroxyl group) for both of these compounds had a chemical shift of about 4.8–5.0 ppm. The H-5 protons also showed similar chemical shifts and multiplets for 5 and 12.

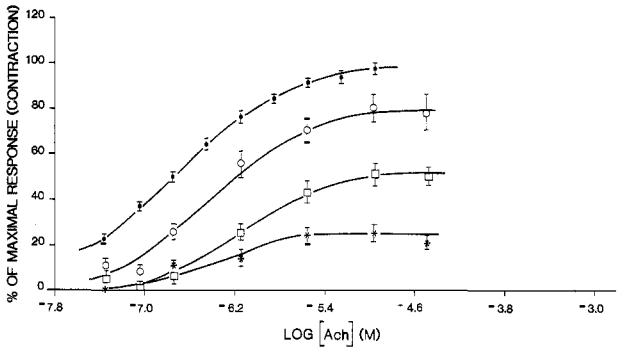


Figure 2. Effects of increasing concentrations of compound 1 on ACh-induced rat ileum contraction:control curve (●), 2.66×10^{-6} M (1.0 μg/mL) (○), 1.33×10^{-5} M (5.0 μg/mL) (□), 2.66×10^{-5} M (10.0 μg/mL) (*). Each point represents the mean value ± SEM of the response measured with eight ileal strips dissected from four animals.

The NMR spectrum obtained for isomer 6 was considerably different from that of 5. The H-3 proton of 6 had undergone a significant upfield shift to about 4.3 ppm. The H-5 protons showed two sets of multiplet signals, one centered at about 3.65 ppm and the other at 4.3 ppm. This pattern was similar to the signals due to the H-5 protons of the starting amino ketone 4. Isomer 6 was assigned as the trans alcohol because it was the minor product of the reduction and its NMR spectrum was quite different from that of the *cis*-4-allyl-3-hydroxy analogue 12.

Pharmacological Results and Discussion

Each of the compounds prepared in this study was evaluated by in vitro pharmacological techniques. A detailed method has been described by Long and Chiou.[22] Briefly, an isolated portion of rat ileum was suspended in a 10-mL tissue bath of aerated Tyrode solution. The tissue was attached in an appropriate manner to a force-displacement transducer and connected to an ink-writing physiograph for recording experimental data. A dose–response experiment was carried out in which the contractions of the ileum tissue were measured with increasing concentrations of ACh in the bath. The ability of each drug to block ACh-induced contractions of the ileum was then measured and a pA_2 value calculated. The pA_2 value represents the antimuscarinic potency of the drug being tested.[23]

When benactyzine was studied in the dose–response experiment outlined above, it was found to exhibit potent competitive antagonism toward ACh. This was concluded because a parallel shift to the right of the dose–response curves was observed as the concentration of benactyzine was increased. In addition, the blockade established by benactyzine was completely reversed by high concentrations of ACh. Benactyzine was calculated to have a pA_2 value of 7.46.

In vitro pharmacological testing demonstrated that compound 1 noncompetitively inhibited ACh-induced contractions of rat ileum tissue (see Figure 2). With increasing concentrations of compound 1, the maximal response exhibited was decreased and the blockade established by 1 could not be removed even at high ACh

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(22) Long, J. P.; Chiou, C. Y. *J. Pharm. Sci.* **1970**, *59*, 133.
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concentrations. It was concluded that compound 1 did not directly interact with the muscarinic receptor. Since this compound approximates receptor-bound conformation I for benactyzine (see Figure 1), it was concluded that this conformation is not preferred for a competitive interaction of the ligand with the receptor.

It was hoped that a comparison of the anticholinergic activities of cyclopentyl amino ether **2** with compound 1 would provide evidence for the existence of a hydrogen-bonding subsite on the receptor. However, the results of pharmacological testing with compound **2** showed that it also acted as a noncompetitive inhibitor of ACh-induced ileum contractions. This finding strengthens the conclusion that conformation I of benactyzine is not a favorable receptor-bound form, but provides no evidence for the existence of a hydrogen bonding subsite since compounds 1 and **2** do not bind directly to the muscarinic receptor.

Further support for the above conclusion was derived from the effects of compounds 1 and **2** on the binding of [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) to muscarinic receptors in rat ileum. Both compounds 1 and **2** failed to displace [^{3}H]QNB from its binding sites even at concentrations of 10^{-2} M.

One possible explanation for the noncompetitive action of compounds 1 and **2** is that the (diethylamino)ethyl side chain may encounter unfavorable steric interactions with the receptor. By further restricting the conformational freedom of this side chain as envisioned in compound **3**, it was hoped that competitive interaction with the receptor would be observed.

Pharmacological evaluation of compound **3** demonstrated that, at low concentration, this rigid analogue possessed some degree of competitive inhibition toward ACh at the muscarinic receptor. This compound was found to be a weak antagonist since it has a pA_2 value of 5.29. Moreover, as the concentration of **3** was increased, the characteristics of noncompetitive antagonism were observed since the blockade established by **3** could not be completely reversed by ACh.

This finding supports our conclusion that conformation I for benactyzine is not a preferred receptor-bound conformation. However, by restricting the conformational freedom of the (diethylamino)ethyl side chain of 1 into a bicyclic analogue **3**, some degree of competitive inhibition was observed. The noncompetitive action of 1 and **2** and weakly competitive inhibition of **3** may be due to unfavorable steric interactions between the receptor and the (diethylamino)ethyl side chain of compounds **1–3**.

An alternative receptor-bound conformation for benactyzine (conformation II) would reorient the ester group as shown in Figure 1. An intramolecular hydrogen bond between the hydroxyl group and the ether oxygen of the ester functionality may be important for establishing a semirigid five-membered ring that is characteristic of conformation II.

The amino ketone **4** was designed and synthesized as a suitable analogue of benactyzine as it would exist in conformation II. The tetrahydrofuranone ring of compound **4** approximates the semirigid ring that may be formed by a hydrogen bond in conformation II, but adds very little steric bulk when compared to the structure of benactyzine.

Compound **4** demonstrated rather potent competitive inhibition of ACh at the muscarinic receptor in the dose–response experiment on isolated rat ileum (see Figure 3). A parallel shift to the right of the dose–response curves with increasing concentrations of **4** and complete reversal of the established blockade by ACh were observed except

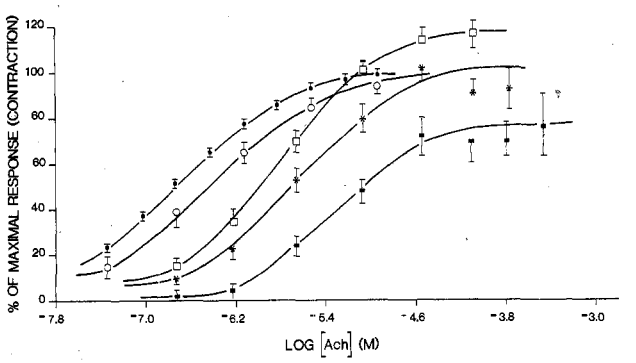

Figure 3. Effects of increasing concentrations of compound 4 on ACh-induced rat ileum contraction:control curve (●), 2.68×10^{-7} M (0.1 μg/mL) (○), 2.68×10^{-6} (1.0 μg/mL) (□), 8.03×10^{-6} M (3.0 μg/mL) (*), 1.34×10^{-5} M (5.0 μg/mL) (■). Each point represents the mean value ±SEM of the response measured with eight ileal strips dissected from four animals.

at high concentrations of the antagonist. At doses beyond 1.0×10^{-5} M of **4** some degree of noncompetitive behavior was observed. Compound **4** was calculated to have a pA_2 value of 6.35.

Compounds 1, **3**, and **4** were further examined for their ability to displace [^{3}H]QNB bound to rat striatal muscarinic receptors.[24] In this experiment, [^{3}H]QNB was bound to the receptor with a maximal density (B_{max}) of 1148 ± 50 fmol/mg of protein and with an apparent dissociation constant (K_d) of 43 ± 3 pM. At 160 pM concentration of [^{3}H]QNB, the specific binding of [^{3}H]QNB to striatal muscarinic receptor was 800.2 ± 30.2 fmol/mg of protein, and this was taken as 100% binding. The IC_{50} values for compounds **3** and **4** were 1.9×10^{-5} and 2×10^{-6} M, respectively, while compound 1 again failed to displace [^{3}H]QNB from striatal muscarinic receptors even at a dose of 10^{-2} M. These results were in good agreement with the dose–response experiments for these compounds.

The fact that compound **4** was found to be a competitive antagonist suggests that the receptor-bound conformation II for benactyzine is preferred over conformation I. It is interesting to note that X-ray crystallographic data also indicated a similar arrangement for benactyzine.[25]

Additional dose–response experiments demonstrated that the cis amino alcohol **5** was a competitive antagonist of ACh-induced ileum contractions and was only slightly less potent than the amino ketone **4**. Compound **5** was calculated to have a pA_2 value of 6.19. The trans amino alcohol **6** was also found to be a competitive inhibitor, but was much less potent ($pA_2 = 4.70$). The large difference in pharmacological activities of amino alcohols **5** and **6** suggests that receptor binding is affected by the stereochemical relationship between the hydroxyl group and the (diethylamino)ethyl side chain. These results are in agreement with the receptor model proposed by Gualtieri et al.[26] in which an esteratic site was characterized by a strictly oriented dipole.

It is interesting that compounds 1 and **2** were both noncompetitive antagonists of ACh-induced ileum contraction, while the six-membered carbocyclic analogues of 2-phenylcyclohexyl (diethylamino)ethyl ether previously synthesized and tested were rather potent competitive

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(26) Gualtieri, F.; Angeli, P.; Giannella, M.; Melchiorre, C.; Pigini, M. In *Recent Advances in Receptor Chemistry*; Gaultieri, F., Giannella, M., Melchiorre, C., Eds.; Elsevier North Holland: Amsterdam, 1979; pp 267–349.

antagonists.[27] These results, although not initially anticipated, suggest that tropate-derived anticholinergics such as atropine may have a different receptor-bound conformation than benzilate-derived anticholinergics such as benactyzine and QNB.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet MX-1 Fourier transform instrument. ^{1}H NMR spectra were recorded at 60 MHz on a Varian T-60A spectrometer equipped with a Nicolet TT-7 Fourier transform accessory or at 180 MHz on a Bruker CXP-180 spectrometer. ^{1}H chemical shifts are reported in part per million (δ) downfield from tetramethylsilane as the internal standard. The abbreviations s, d, t, q, dd, br, and m refer to singlet, doublet, triplet, quartet, doublet of doublets, broad signal, and multiplet, respectively.

Mass spectra were obtained with a Hitachi Perkin-Elmer RMU-6D single-focusing mass spectrometer and a Varian MAT-112S mass spectrometer. Spectra were recorded at 80 °C and 70 eV unless otherwise noted.

Gas–liquid chromatography (GLC) was performed on a Packard 7300 series chromatograph equipped with a flame ionization detector and fitted with a 6 ft × $^1/_8$ in i.d. U-shaped glass column containing either 5% XE-60 on 100/120 Chromosorb W-AW DMCS (Supelco, Inc.) or 5% Carbowax 20M on 100/120 Chromosorb W-AW DMCS with nitrogen as the carrier gas.

Thin-layer chromatographs (TLC) were developed on Merck silica gel 60, F_{254} aluminum-backed plates, and spots were visualized by UV light (254 nm). Preparative thin-layer chromatography was performed on Merck aluminum oxide F-254 plates, and bands were visualized by UV light (254 nm). Column chromatography was carried out on Merck silica gel 60 (70–230 mesh). Flash chromatography was performed according to the method of Still et al.[28] with Baker flash chromatography silica gel or Woelm aluminum oxide. Microanalyses were performed by Micro-Tech Laboratories, Skokie, IL.

The (diethylamino)ethyl chloride used was freshly prepared by treating its hydrochloride salt with 2 molar equiv of NaOH powder and distilling over direct flame with the protection of a $CaCl_2$ drying tube.

Synthesis of 2,2-Diphenyl-3-tetrahydrofuranyl (Diethylamino)ethyl Ether (1). 2,2-Diphenyl-4-carbomethoxy-3-tetrahydrofuranone (7). Into a three-necked round-bottom flask equipped with a gas regulator, a dropping funnel, and a $CaCl_2$ drying tube was added an oil suspension of NaH (1.7 g, 42 mmol). The oil was removed by rinsing the NaH three times with hexane. The NaH was covered with 150 mL of anhydrous ether and the suspension was stirred while the reaction flask was cooled in an ice bath. A solution of methyl benzilate (10 g, 41.3 mmol) in 50 mL of anhydrous ether was added dropwise over the course of 1 h. The reaction mixture was stirred at 0 °C for an additional 30 min. Vigorous stirring at 25 °C was continued overnight. The ether was then removed under reduced pressure and replaced with 80 mL of Me_2SO. With the reaction flask immersed in an ice bath, freshly distilled methyl acrylate (3.6 g, 42 mmol) was added in one lot. After 15 min the cooling bath was removed and the solution stirred for an additional 45 min at room temperature. The reaction mixture was poured into 200 mL of ice–water and the pH of the solution was adjusted to about 4.5 to 5.0 with concentrated HCl. The crude product was extracted three times with ether. The combined ether extracts were washed once with water, dried (anhydrous $MgSO_4$), and concentrated under reduced pressure to leave 11.0 g of a viscous yellow oil. Flash chromatography on silica gel with $CHCl_3$–hexane (1:1) as the solvent gave 7.9 g of **7** as a clear viscous oil (65%): TLC R_f (chloroform) 0.62; ^{1}H NMR ($CDCl_3$) δ 3.20–3.80 (m, 2 H, H-4 and H-5), 3.65 (s, 3 H, $COOCH_3$), 4.30–4.80 (m, 1 H, H-5), 7.10–7.50 (m, 10 H, Ar H); IR ($CHCl_3$, cm^{-1}) 3480 (OH), 1775 (ester C=O), 1740 (ketone C=O), 1664 (α,β-unsaturated C=O), 1480 and 1450 (aromatic C=C). The β-keto ester **7** was used without further purification.

2,2-Diphenyl-3-tetrahydrofuranone (8). Into a 250-mL round-bottom flask equipped with a magnetic stirrer were added **7** (15 g, 50.1 mmol) and 100 mL of a 10% H_2SO_4 solution. The reaction mixture was refluxed for 6 h with vigorous stirring. The mixture was then extracted three times with ether. The combined ether extracts were washed twice with cold water, dried (anhydrous $MgSO_4$), and concentrated under reduced pressure to leave 11.4 g of a light yellow solid. Recrystallization of the crude product from 95% ethanol gave 10.3 g of **8** as white needles (85%): mp 74.5–77.5 °C; homogeneous to GLC; TLC R_f (chloroform) 0.71; ^{1}H NMR ($CDCl_3$) δ 2.66 (t, 2 H, J = 7.2 Hz, H-4), 4.26 (t, 2 H, J = 7.2 Hz, H-5), 7.18–7.59 (m, 10 H, Ar *H*); IR (CCl_4, cm^{-1}) 1757 (C=O), 1598 (aromatic C=C), 1480 and 1450 (aromatic C=C); MS, m/e 238 (M^+). Anal. ($C_{16}H_{14}O_2$) C, H.

2,2-Diphenyl-3-tetrahydrofuranol (9). A well-stirred solution of **8** (4 g, 16.8 mmol) in 180 mL of absolute methanol was cooled to 0 °C. Over a period of 1 h, $NaBH_4$ (0.95 g, 25 mmol) was added portionwise. When the addition was complete, the reaction mixture was stirred at 25 °C for an additional 1 h. The mixture was poured into 150 mL of ice–water and the pH was then adjusted to about 6 with the dropwise addition of concentrated HCl. The reaction mixture was concentrated under reduced pressure to a volume of approximately 200 mL and then extracted three times with ether. The combined ether extracts were washed once with water, dried (anhydrous $MgSO_4$), and concentrated under reduced pressure to leave 3.8 g of a white solid. Recrystallization of the crude product from hexane afforded 3.4 g of **9** as white needles (85%): mp 108.5–111.5 °C; homogeneous to GLC; TLC R_f (ethyl acetate) 0.75; ^{1}H NMR ($CDCl_3$) δ 1.52 (s, 1 H, OH), 1.91–2.22 (m, 2 H, H-4), 3.80–4.50 (m, 2 H, H-5), 4.98 (t, 1 H, J = 3.3 Hz, H-3), 7.18–7.54 (m, 10 H, Ar H); IR ($CHCl_3$, cm^{-1}) 3570 (free OH), 3450 (H-bonded OH), 1600 (aromatic C=C), 1490 and 1450 (aromatic C=C) MS, m/e 240 (M^+). Anal. ($C_{16}H_{16}O_2$) C, H.

2,2-Diphenyl-3-tetrahydrofuranyl (Diethylamino)ethyl Ether (1). Into a three-necked round-bottom flask equipped with a condenser, a dropping funnel, a nitrogen gas inlet regulator, and a magnetic stirrer was placed a well-stirred suspension of sodium (0.29 g, 12.6 mmol) in 50 mL of anhydrous ether. To this was added dropwise a solution of **9** (3 g, 12.5 mmol) in 30 mL of anhydrous ether over a period of 1 h. After the addition, the mixture was stirred at room temperature until analysis by TLC indicated that the conversion to the sodium salt was complete (ca. 3 days). Freshly prepared 2-(diethylamino)ethyl chloride (1.7 g, 12.6 mmol) was then added dropwise over a period of 30 min. After the addition was completed, the mixture was refluxed for 6 h and then cooled and allowed to stand overnight at room temperature. *tert*-Butyl alcohol was added to decompose the excess sodium and then the reaction mixture was extracted with ether. Partial purification was achieved by extracting the combined ether extracts with 5% HCl. The combined aqueous extracts were brought to a pH of 8 by the addition of Na_2CO_3 and then extracted with ether. The combined ether extracts were washed with water, dried (anhydrous $MgSO_4$), and concentrated under reduced pressure to leave 1.5 g of an oily residue (35%): homogeneous to GLC; TLC R_f (ether–ammonium hydroxide, 50:1) 0.40; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 6 H, NCH_2CH_3), 1.91–2.20 (m, 2 H, H-4), 2.23–2.62 (m, 6 H, NCH_2), 3.24–3.54 (m, 2 H, OCH_2CH_2N), 3.75–4.50 (m, 2 H, H-5), 4.52–4.65 (t, 1 H, H-3), 7.17–7.59 (m, 10 H, Ar H); IR ($CHCl_3$, cm^{-1}) 1598 (aromatic C=C), 1498 and 1447 (aromatic C=C); MS, m/e 339 (M^+).

Compound **1** was converted to a hydrochloride salt by passing dry HCl gas through a solution of **1** in anhydrous ether. The hydrochloride salt was recovered in quantitative yield and recrystallized from ethyl acetate to give a white powder: mp 156–160 °C; ^{1}H NMR (D_2O) δ 1.05 (t, 6 H, NCH_2CH_3), 2.00–2.38 (m, 2 H, H-4), 2.92 (q, 4 H, NCH_2CH_3), 3.10–3.32 (m, 2 H, OCH_2CH_2N), 3.63–4.38 (m, 4 H, OCH_2CH_2N and H-5), 4.88–5.09 (m, 1 H, H-3), 7.17–7.64 (m, 10 H, Ar H); MS (170 °C), m/e 340 (M^+ – Cl). Anal. ($C_{22}H_{30}NO_2Cl$) C, H, N.

2,2-Diphenylcyclopentyl (Diethylamino)ethyl Ether (2). To a 250-mL three-necked flask containing 100 mL of anhydrous ether was added lithium aluminum hydride (0.8 g, 21 mmol). The flask was equipped with a dropping funnel, a magnetic stirrer, and a reflux condenser and was protected from atmospheric

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moisture by a drying tube. Through the dropping funnel 2,2-diphenylcyclopentanone (4 g, 17 mmol) was added at a rate sufficient to produce gentle reflux. Stirring was continued for 30 min after which water was added slowly through the dropping funnel to decompose excess hydride. The mixture was diluted, first with 200 mL of ice–water and then with 500 mL of a 20% NaOH solution. The organic layer was separated and the aqueous layer was extracted twice with 100-mL portions of ether. The ether layers were combined, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure to leave 2.8 g of **2,2-diphenylcyclopentanol** as a yellow viscous liquid: (70%); TLC R_f (benzene) 0.23; IR (film, cm^{-1}) 3558 (free OH), 3441 (H-bonded OH), 1598 and 1489 (aromatic C=C); ^1H NMR ($CDCl_3$) δ 1.4 (br, 1 H, OH), 1.5–3.0 (m, 6 H, H-3, H-4, and H-5), 4.85 (t, 1 H, J = 4 Hz, H-1), 7.25 (s, 10 H, Ar H).

Into a 250-mL three-necked flask equipped with a condenser, a dropping funnel, a nitrogen gas inlet regulator, and a magnetic stirrer was placed a suspension of sodium (0.3 g, 13 mmol) in 40 mL of anhydrous ether. A solution of 2,2-diphenylcyclopentanol (2.5 g, 11 mmol) in 40 mL of anhydrous ether was added dropwise over a period of 30 min. After the addition, the mixture was stirred at room temperature until analysis by TLC indicated that the conversion to the sodium salt was complete. Freshly prepared (diethylamino)ethyl chloride (2.5 g, 18 mmol) was added in one portion to the reaction mixture. After the addition, the mixture was refluxed for 40 h and then cooled to room temperature. The reaction mixture was poured into 100 mL of ice-cold water and then extracted with ether several times. Partial purification was achieved by extracting the combined ether extracts with 5% HCl. The combined aqueous extracts were then brought to a pH of 8 by the addition of Na_2CO_3 and then extracted twice with ether. The combined ether extracts were washed with water, dried (anhydrous $MgSO_4$), and concentrated under reduced pressure to leave 1.1 g of an oily residue (29%). The crude product **2** was purified by preparative TLC on aluminum oxide plates with ether–ammonium hydroxide (50:1) as the solvent: TLC R_f (ether–ammonium hydroxide, 50:1), 0.60; IR (film, cm^{-1}) 1599 and 1489 (aromatic C=C); ^1H NMR ($CDCl_3$) δ 0.95 (t, 6 H, NCH_2CH_3), 1.15–2.0 (m, 6 H, H-3, H-4, and H-5), 2.25–2.7 (m, 6 H, NCH_2), 3.35–3.8 (m, 2 H, OCH_2CH_2N), 4.4 (t, 1 H, J = 4 Hz, H-1), 7.1–7.45 (m, 10 H, Ar H).

Compound **2** was converted to a hydrochloride salt by passing dry HCl gas through a solution of **2** in anhydrous ether. The hydrochloride salt was recovered in quantitative yield and recrystallized from ethyl acetate to give a white powder:mp 133–135 °C; MS (120 °C), m/e 337 (M^+ – HCl). Anal. ($C_{23}H_{32}NOCl$) H, N; C: calcd, 73.87; found, 72.96.

Synthesis of *exo*-2-[(Diethylamino)methyl]-6,6-diphenylhexahydrofuro[3,4-*b*]furan (3). 2,2-Diphenyl-4-carbomethoxy-4-(2-propenyl)-3-tetrahydrofuranone (10). The procedure used in the preparation of this compound was similar to that of compound **7**. However, after the addition of methyl acrylate, the reaction mixture was stirred for 45 min at room temperature and freshly distilled allyl bromide (5.1 g, 42.0 mmol) was added to the reaction mixture in one lot. Stirring was continued at 25 °C for 20 h. The reaction mixture was then poured into 200 mL of ice–water. The crude product was extracted three times with ether. The combined ether extracts were washed once with water, dried (anhydrous $MgSO_4$), and concentrated under reduced pressure to leave 12.4 g of a viscous yellow oil. Purification was carried out by using flash chromatography on silica gel with benzene–petroleum ether (7:3) as the solvent to yield 8.4 g of a clear viscous oil (60%). An analytical sample was prepared by microdistillation [bp ~220 °C (0.8 mmHg)]. The pure compound **10** gave the following: homogeneous to GLC; TLC R_f (benzene) 0.51; ^1H NMR ($CDCl_3$) δ 2.16–3.09 (m, 2 H, $CH_2CH{=}CH_2$), 3.66 (s, 3 H, $COOCH_3$), 4.02–4.78 (m, 2 H, H-5), 4.82–6.09 (m, 3 H, $CH{=}CH_2$), 7.19–7.60 (m, 10 H, Ar H); IR (film, cm^{-1}) 1763 (ester C=O), 1728 (ketone C=O), 1639 (olefinic C=C), 1596, 1487, 1446 (aromatic C=C); MS, m/e 308 (M^+ – C≡O).

2,2-Diphenyl-4-(2-propenyl)-3-tetrahydrofuranone (11). Into a 250-mL round-bottom flask equipped with a magnetic stirrer were added **10** (5 g, 14.9 mmol) and 100 mL of a 5% NaOH solution. The reaction mixture was refluxed for 7 h with vigorous stirring. The reaction mixture was allowed to cool to room temperature and was then extracted with three portions of ether. The combined ether extracts were washed twice with ice-cold water, dried (anhydrous $MgSO_4$), and concentrated under reduced pressure to leave 4 g of a light yellow oil. Purification of the crude product was carried out by flash chromatography on silica gel. The solvent was benzene–petroleum ether (1:1). Further purification of a small amount was carried out for analysis by means of microdistillation. The compound distilled as a clear, viscous liquid at 205–215 °C (0.3 mmHg). The compound was distilled twice by microdistillation before submitting for elemental analysis. Compound **11** was obtained as a clear, viscous oil after purification by flash chromatography (3.7 g; 90%): homogeneous to GLC; TLC R_f (benzene) 0.70; ^1H NMR ($CDCl_3$) δ 1.54–3.01 (m, 3 H, $CH_2CH{=}CH_2$ and H-4), 3.74–4.60 (m, 2 H, H-5), 4.82–5.77 (m, 3 H, $CH{=}CH_2$), 7.17–7.60 (m, 10 H, Ar H); IR (film, cm^{-1}) 1755 (C=O), 1640 (olefinic C=C), 1598 and 1447 (aromatic C=C); MS, m/e 278 (M^+). Anal. ($C_{19}H_{18}O_2$) C, H.

***cis*-2,2-Diphenyl-4-(2-propenyl)-3-tetrahydrofuranol (12).** To a solution of **11** (1 g, 3.6 mmol) in 30 mL of dry ether was added lithium tri-*tert*-butoxyaluminum hydride (0.94 g, 3.7 mmol). The reaction mixture was stirred at room temperature until gas chromatographic analysis indicated that the reduction was complete. Wet ether (5 mL) was then added to the reaction mixture and this was followed by the dropwise addition of 10 mL of water. The organic layer was separated and the aqueous layer was extracted three times with ether. The ether layers were combined, washed twice with water, dried (anhydrous $MgSO_4$), and concentrated under reduced pressure to leave 1 g of a white solid (mp 90–92 °C), which was shown by GLC to be a 98:2 mixture of the cis and trans isomers. The pure cis alcohol **12** was obtained by multiple recrystallization from hexane to yield 0.8 g of a white crystalline powder (80%): mp 92–93.5 °C; TLC R_f (chloroform) 0.41; ^1H NMR ($CDCl_3$) δ 1.41 (s, 1 H, OH), 1.93–2.58 (m, 3 H, $CH_2CH{=}CH_2$ and H-4), 3.64–4.21 (m, 2 H, H-5), 4.64–6.05 (m, 4 H, H-3 and $CH{=}CH_2$), 7.07–7.57 (m, 10 H, Ar H); IR ($CHCl_3$, cm^{-1}) 3575 (free OH), 3440 (H-bonded OH), 1640 (olefinic C=C), 1598 and 1447 (aromatic C=C); MS, m/e 280 (M^+). Anal. ($C_{19}H_{20}O_2$) C, H.

***exo*-2-(Iodomethyl)-6,6-diphenylhexahydro[3,4-*b*]furan (13).** To a 100-mL round-bottom flask equipped with a magnetic stirrer were added 12 mL of a saturated $NaHCO_3$ solution (0.75 g, 9.0 mmol) and 40 mL of a solution of iodine (1.15 g, 4.5 mmol) in ether. This two-phase system was stirred vigorously while being cooled in an ice bath until a temperature of 0 °C in the reaction flask was achieved. A solution of **12** (0.253 g, 0.90 mmol) in 6 mL of ether was then added in one lot. Vigorous stirring at 0 °C was continued for 4 h. At the conclusion of the reaction, a solution of $Na_2S_2O_3$ was added until the excess iodine had decomposed and the ether layer was clear. The ether layer was separated, washed twice with cold water, dried (anhydrous $MgSO_4$), and concentrated under reduced pressure to leave 0.36 g of a viscous yellow liquid. This liquid crystallized upon standing to give 0.36 g of a yellow solid, mp 82–86 °C. The ^1H NMR spectrum of the crude product revealed that an 85:15 mixture of the exo and endo isomers **13** and **14** had been produced. Multiple recrystallization of the crude product from hexane afforded 0.33 g of the pure exo isomer as white needles (80%): mp 102–103.5 °C; TLC R_f (chloroform) 0.59; ^1H NMR ($CDCl_3$) δ 1.76–1.94 (m, 1 H, H-3), 2.05–2.16 (m, 1 H, H-3), 2.95–3.09 (m, 1 H, H-3a), 3.13–3.21 (m, 2 H, CH_2I), 3.76–4.09 (m, 3 H, H-2 and H-4), 5.39 (d, 1 H, J = 5.8 Hz, H-6a), 7.10–7.50 (m, 10 H, Ar H); IR (CCl_4, cm^{-1}) 1598, 1485, and 1443 (aromatic C=C); MS, m/e 406 (M^+). Anal. ($C_{19}H_{19}O_2I$) C, H.

***endo*-2-(Iodomethyl)-6,6-diphenylhexahydrofuro[3,4-*b*]-furan (14).** From the fractional recrystallization procedure to obtain the exo isomer **13**, a very small amount of the pure endo isomer **14** was isolated as a viscous oil; TLC R_f (chloroform) 0.59; ^1H NMR ($CDCl_3$) δ 1.82–1.96 (m, 1 H, H-3), 2.41–2.59 (m, 1 H, H-3), 2.96–3.11 (m, 1 H, H-3a), 3.13–3.21 (m, 2 H, CH_2I), 3.74–4.15 (m, 3 H, H-2 and H-4), 5.29 (d, 1 H, J = 6.8 Hz, H-6a), 7.10–7.52 (m, 10 H, Ar H).

***exo*-2-[(Diethylamino)methyl]-6,6-diphenylhexahydrofuro[3,4-*b*]furan (3).** To a solution of the exo iodo ether **13** (0.3 g, 0.7 mmol) in 20 mL of Me_2SO was added 5 mL of diethylamine. The reaction mixture was heated at 45 °C for 24 h. The mixture was then added to 50 mL of ice–water and the pH was lowered to 5 by the dropwise addition of concentrated HCl. The aqueous

layer was washed with ether and then solid Na_2CO_3 was added portionwise until the solution was saturated with Na_2CO_3. The aqueous solution was extracted three times with ether, and the combined ether layers were washed once with water, dried (anhydrous $MgSO_4$), and concentrated under reduced pressure to yield 0.32 g of yellow oily residue. The crude product was purified by preparative TLC on aluminum oxide plates with ether–ammonium hydroxide (50:1) as the solvent. The pure exo amine **3** was converted to the hydrochloride salt to give 0.15 g of a white powder, which was recrystallized from ethyl acetate (60%): mp 131–133 °C; ^1H NMR (D_2O) δ 0.70–1.15 (t, 6 H, NCH_2CH_3), 1.72–2.20 (m, 2 H, H-3), 2.50–3.15 (m, 7 H, H-3a and NCH_2), 3.56–4.20 (m, 3 H, H-2 and H-4), 5.37 (d, 1 H, J = 5.8 Hz, H-6a), 7.03–7.50 (m, 10 H, Ar H); MS (200 °C), m/e 351 (M^+ – HCl). Anal. ($C_{23}H_{30}NO_2Cl \cdot H_2O$) C, H, N.

Synthesis of 2,2-Diphenyl-4-[2-(diethylamino)ethyl]-3-tetrahydrofuranone (4). 2,2-Diphenyl-4-carbomethoxy-4-[2-(diethylamino)ethyl]-3-tetrahydrofuranone (15). The procedure used in the synthesis of this compound was similar to that of compound **7**. However, after the addition of methyl acrylate, the reaction mixture was stirred for 45 min at room temperature and freshly prepared 2-(diethylamino)ethyl chloride (5.7 g, 42 mmol) was added to the reaction mixture in one lot. Stirring was continued at 25 °C for 20 h. The reaction mixture was poured into 200 mL of ice–water and the pH was adjusted to about 4 by the dropwise addition of concentrated HCl. The aqueous mixture was then washed twice with ether. Solid Na_2CO_3 was added portionwise to the aqueous extract until the solution was saturated with Na_2CO_3. The aqueous solution was then extracted three times with ether. The combined ether layers were washed once with water, dried (anhydrous $MgSO_4$), and concentrated under reduced pressure to leave 10.6 g of a dark orange viscous oil. Column chromatography on aluminum oxide using ether–ammonium hydroxide (50:1) as the solvent gave 3.5 g of **15** as a light yellow oil (22%): TLC R_f (ether–ammonium hydroxide, 50:1) 0.76; ^1H NMR ($CDCl_3$) δ 0.70–1.10 (t, 6 H, NCH_2CH_3), 1.10–1.80 (m, 2 H, CH_2CH_2N), 2.20–2.65 (m, 6 H, NCH_2), 3.65 (s, 3 H, $COOCH_3$), 3.80–4.82 (m, 2 H, H-5), 7.15–7.42 (m, 10 H, Ar H); MS, m/e 395 (M^+).

2,2-Diphenyl-4-[2-(diethylamino)ethyl]-3-tetrahydrofuranone (4). Into a 100-mL round-bottom flask equipped with a magnetic stirrer were added **15** (3 g, 7.5 mmol) and 50 mL of a 10% H_2SO_4 solution. The reaction mixture was refluxed for 18 h with vigorous stirring. Upon cooling to room temperature, the mixture was extracted twice with ether. The aqueous layer was then treated with solid Na_2CO_3 portionwise until the solution was saturated with Na_2CO_3. The aqueous solution was extracted with three portions of ether. The ether layers were combined and washed once with water, dried (anhydrous $MgSO_4$), and concentrated under reduced pressure to leave 2.3 g of a yellow viscous oil. Column chromatography on aluminum oxide with ether–ammonium hydroxide (50:1) as the solvent yielded 1.9 g of **4** as a light yellow oil (75%): TLC R_f (ether–ammonium hydroxide, 50:1) 0.65; ^1H NMR ($CDCl_3$) δ 0.79–1.05 (t, 6 H, NCH_2CH_3), 1.40–2.10 (m, 2 H, CH_2CH_2N), 2.23–2.65 (m, 7 H, NCH_2 and H-4), 3.63–4.61 (m, 2 H, H-5), 7.18–7.55 (m, 10 H, Ar H); IR (film, cm^{-1}) 1736 (C=O), 1485 and 1443 (aromatic C=C); MS, m/e 337 (M^+).

Compound **4** was converted to a hydrochloride salt by passing dry HCl gas through a solution of anhydrous ether and **4**. The hydrochloride salt was recovered in quantitative yield and recrystallized from ethyl acetate to give a white powder: mp 169.5–171.5 °C; MS (150 °C), m/e 337 (M^+ – HCl). Anal. ($C_{22}H_{28}NO_2Cl$) C, H, N.

Synthesis of *cis*- and *trans*-2,2-Diphenyl-4-[2-(diethylamino)ethyl]-3-tetrahydrofuranol (5 and 6). *cis*-2,2-Diphenyl-4-[2-(diethylamino)ethyl]-3-tetrahydrofuranol (5). To a solution of $NaBH_4$ (1 g, 26 mmol) in 40 mL of absolute ethanol was added dropwise a solution of **4** (0.5 g, 1.5 mmol) in 20 mL of absolute ethanol. The mixture was stirred at room temperature for 2 h and then poured into 100 mL of ice–water. The pH was adjusted to about 8 with concentrated HCl. The mixture was concentrated under reduced pressure to approximately 70 mL. The products were then extracted into ether and the combined ether extracts were washed with water, dried (anhydrous $MgSO_4$), and concentrated under reduced pressure. A light yellow oily residue was obtained (0.4 g; 80%). The crude product contained a mixture of the cis and trans amino alcohols **5** and **6** in a 3:2 ratio. The two isomers were separated by preparative TLC on aluminum oxide plates with ether–ammonium hydroxide (50:1) as the solvent. The cis isomer **5** was obtained as a light yellow oil: TLC R_f (ether–ammonium hydroxide, 50:1) 0.16; ^1H NMR ($CDCl_3$) δ 1.00–1.35 (t, 6 H, NCH_2CH_3), 1.42–2.03 (m, 2 H, CH_2CH_2N), 2.10–2.95 (m, 7 H, NCH_2 and H-4), 3.86–4.44 (m, 2 H, H-5), 4.98 (d, 1 H, J = 4 Hz, H-3), 7.20–7.82 (m, 10 H, Ar H).

Compound **5** was converted to a hydrochloride salt by means of the procedure given for compound **1**: mp 224–227 °C; MS, m/e (relative intensity) 339 (1, M^+), 324 (1), 183 (1), 167 (3), 165 (5), 157 (2), 152 (2), 144 (7), 143 (54), 142 (7), 128 (12), 105 (23), 87 (25), 86 (100), 77 (15), 72 (17), 58 (39). Anal. ($C_{22}H_{30}NO_2Cl$) C, H, N.

***trans*-2,2-Diphenyl-4-[2-(diethylamino)ethyl]-3-tetrahydrofuranol (6).** From the preparative TLC separation discussed in the synthesis of **5**, the trans isomer **6** was also isolated: TLC R_f (ether–ammonium hydroxide, 50:1) 0.48; ^1H NMR ($CDCl_3$) δ 1.00–1.32 (m, 6 H, NCH_2CH_3), 1.41–1.96 (m, 2 H, CH_2CH_2N), 2.08–3.01 (m, 7 H, NCH_2 and H-4), 3.63–4.61 (m, 3 H, H-3 and H-5), 7.25–7.91 (m, 10 H, Ar H).

Compound **6** was converted to a hydrochloride salt by means of the procedure given for compound **1**: MS, m/e (relative intensity) 340 (1, M^+ – Cl), 324 (1), 195 (3), 183 (7), 165 (20), 157 (7), 152 (5), 143 (28), 128 (42), 105 (80), 87 (20), 86 (100), 77 (44), 72 (22), 58 (55).

Pharmacology. Materials and Methods. Male Sprague–Dawley rats weighing 250–300 g were purchased from King Animal Laboratories in Oregon, WI. A Narco-Biosystems four-channel ink-writing physiograph (Table Model No. DMP-4A) and a force-displacement transducer (Narco-Biosystems F-1000) were used to record the smooth muscle contractions. Acetylcholine bromide was obtained from Sigma Chemical Co. [^3H]-Quinuclidinyl benzilate ([^3H]QNB, specific activity of 33.1 Ci/mmol) was purchased from Du Pont NEN Research Products, Boston, MA.

Antispasmodic Activity on Isolated Rat Ileum Preparations. Each rat used in these experiments was fasted overnight and sacrificed by cervical dislocation. By means of an abdominal incision, the terminal portion of the intestine was removed and washed in oxygenated Tyrode solution at room temperature. Several strips of the ileum about 1–2-cm long were prepared. One end of the strip was attached to a fine silk thread and tied to a fixed level in the tissue bath. The other end of the muscle was attached to a force-displacement transducer, which was in turn connected to an ink-writing recorder. The bioassay was performed at 35 °C in a 10-mL organ bath containing Tyrode solution through which a stream of 95% O_2 and 5% CO_2 gas mixture was bubbled. Ileal contractions were monitored and recorded. A minimum of 30 min was allowed for tissue equilibration before testing was begun.

A dose–response curve was first determined for ACh alone and then the tissue was washed several times with adequate amounts of Tyrode solution and allowed to equilibrate. A second dose–response curve was determined in the presence of the test compound 30 min after its application.

Acetylcholine, used as a spasmogen because of its direct agonist effect, was freshly prepared since low concentrations are easily hydrolyzed. The following doses of ACh were used: 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, and 1.28 μg/mL. These doses were added in sequence in a cumulative manner at 2-min intervals. During the application of ACh, care was taken not to introduce the ACh directly onto the tissue. The response of the tissue to each dose of ACh was measured as millimeters of contraction. At the end of the ACh series, the tissue was washed several times with Tyrode solution and allowed 30 min for equilibration. A given dose of the test compound was then added to the bath. After 30 min of incubation, higher doses of ACh in the sequence 0.04, 0.1, 0.44, 1.72, 6.72, 16.72, 36.72, 76.72, and 156.72 μg/mL were added to the bath in a cumulative manner at 2-min intervals. The response to each dose of ACh was measured as millimeters of contraction.

The pA_2 value was determined on the basis of the equation $pA_2 = pA_x + \log (x - 1)$. pA_2 is the negative logarithm to base 10 of the molar concentration of an antagonistic drug that will produce 50% inhibition of the response to the agonist (ACh). The

symbol pA_x is the negative logarithm to base 10 of the molar concentration of an antagonist that will reduce the effect of a multiple dose x of ACh to that of a single dose. The x value is also the ratio of the concentration of ACh needed to produce 50% response in the presence of an antagonist to that in the absence of the antagonist.

Measurement of Specific Binding of [^{3}H]QNB to Cholinergic Muscarinic Receptors in the Rat Ileum. The binding of [^{3}H]QNB to cholinergic muscarinic receptors in the rat ileum was performed by a method analogous to that described for guinea pig longitudinal muscle.[29] Briefly, the ileum was removed from the rat as described previously for ACh-induced contraction experiments. The tissue was homogenized in 10–20 volumes of ice-cold 50 mM sodium–potassium phosphate buffer (pH 7.4) in a glass homogenizer fitted with a glass pestle. The homogenate was rehomogenized with a Polytron instrument (setting 5 for 60 s) and used for [^{3}H]QNB binding studies. To determine the specific binding, an aliquot (0.2 mL) of the homogenate containing about 400 μg of protein was incubated for 60 min at 25 °C with 50 mM sodium–potassium phosphate buffer (pH 7.4) (1.7 mL) containing [^{3}H]QNB (0.1 mL). The total volume of the incubation mixture was 2 mL. At the end of the incubation period, the contents of the tube were rapidly filtered under partial vacuum through a glass fiber filter (GF/F) with a Millipore filtration manifold. The filters were washed twice with 3-mL aliquots of the buffer. The incubations were performed in duplicates. The filters were transfered to liquid scintillation vials containing 10 mL of a cocktail (consisting of PPO (10 g), POPOP (0.5 g), toluene (2 L), and triton X-100 (1 L)). After an overnight equilibration period, the radioactivity in the samples was determined in a Packard liquid scintillation counter (Model 4640) with a counting efficiency of 54%. The specific binding of [^{3}H]QNB was defined as the difference in binding carried out in parallel in the presence and absence of 10 μM atropine sulfate. The concentration of protein in the homogenates was determined by the method of Lowry et al.[30] The amount of [^{3}H]QNB specifically bound was expressed as fmol bound per mg of protein. The receptor density (B_{max} values) and the apparent dissociation constant (K_d values) were determined from the saturation curves. The resulting data were subjected to the Scatchard analyses, and the binding constant was determined after linear regression analyses.

For competition studies, the concentration of [^{3}H]QNB selected was 0.5 nM and that of the test compounds ranged from 10^{-11} to 10^{-5} M. The IC_{50} values were determined from the competition curves.

Determination of Binding of [^{3}H]QNB to Rat Striatal Muscarinic Receptors. Rats were decapitated and the brains were rapidly removed on ice. The striata were dissected out and homogenized in 100 volumes of ice-cold 0.32 M sucrose with a Polytron homogenizer (setting 5 for 20 s). The homogenates were incubated for 1 h at 37 °C and then centrifuged at 1000g for 10 min. The pellet (crude nuclear fraction) was discarded and the resultant supernatant was homgenized with a Polytron homogenizer (setting 5 for 20 s) and used for [^{3}H]QNB binding studies.

The binding assay was carried out as reported by Das et al.[25] The specific binding of [^{3}H]QNB was defined as the difference in binding carried out in parallel in the presence and absence of 1 μM atropine sulfate. The values of B_{max} and K_d for the binding of [^{3}H]QNB to the striatal membranes were determined from the saturation curves generated by using a concentration range of 25–1000 pM. The resulting data were subjected to Scatchard analysis and the binding constants were determined after linear regression analysis. To determine the effect of test compounds on the binding of [^{3}H]QNB to striatal muscarinic receptors, the final concentration of [^{3}H]QNB was 160 pM. The concentration of the test compounds used was 10^{-9} to 10^{-2} M. The concentration needed to inhibit the binding of [^{3}H]QNB at 50% of the control (IC_{50}) was calculated for each compound.

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Registry No. 1, 105401-14-7; 1·HCl, 105401-22-7; 2, 105401-15-8; 2·HCl, 105430-47-5; 3, 105497-77-6; 3·HCl, 87223-66-3; 4, 105401-16-9; 4·HCl, 105401-25-0; 5, 105401-17-0; 5·HCl, 105401-23-8; 6, 105401-18-1; 6·HCl, 105401-24-9; 7, 105401-19-2; 8, 101110-06-9; 9, 105401-20-5; 10, 87223-62-9; 11, 87223-63-0; 12, 87223-64-1; 13, 87223-65-2; 14, 87248-84-8; 15, 105401-21-6; methyl benzilate, 76-89-1; methyl acrylate, 96-33-3; 2-(diethylamino)ethyl chloride, 100-35-6; 2,2-diphenylcyclopentanone, 15324-42-2; 2,2-diphenylcyclopentanol, 35115-45-8.

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