AMPAC programm.[29] Ab initio calculations were carried out using the extended 4-31G basis set[30] in GAMESS (Generalised Atomic and Molecular Electronic Structure System, Revision A, M. F. Guest).[31] Two-dimensional potential maps were displayed on an Iris Silicon Graphics work station (Model 4D 70G) using software developed by Dr. F. E. Blaney in collaboration with Polygen Corp.

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Acknowledgment. We thank Mr. Duncan M. Smith for NMR analysis, and Miss Emma Collings for technical assistance.

Registry No. **3**, 38206-86-9; **4**, 139348-20-2; **5**, 93117-84-1; **6**, 133366-38-8; **7**, 133366-39-9; **8a**, 133366-07-1; **8b**, 139348-21-3; **8c**, 133366-10-6; **9**, 51627-76-0; **10**, 139348-22-4; **11**, 139348-23-5; **12a**, 133365-87-4; **12b**, 133365-85-2; **13**, 3731-38-2; **14**, 136681-83-9; **15**, 53242-77-6; **16**, 139348-24-6; **17**, 6238-14-8; **18**, 139348-25-7; **19**, 1619-34-7; **20**, 127424-06-0; **21a**, 139348-27-9; **21b**, 139348-28-0; **21c**, 139348-30-4; **22**, 139348-31-5; **23**, 41253-21-8; **24**, 13440-29-4; **25**, 139348-32-6; **26a**, 139348-34-8; **26b**, 139348-35-9; **27**, 40370-03-4; **28**, 139348-36-0; NH(Me)OMe, 1117-97-1; $MeNHNH_2$, 60-34-4; TMS-N_3, 4648-54-8; $Me_3COCONHNH_2$, 870-46-2; MeC(OMe)=NH, 14777-27-6; MeNHCH=N—N=CHNHMe, 139348-37-1; 3-nitrobenzoyl azide, 3532-31-8; 5-methyltetrazole, 4076-36-2; sodium azide, 26628-22-8; acetonitrile, 75-05-8.

Muscarinic Receptor Subtype Specificity of (*N*,*N*-Dialkylamino)alkyl 2-Cyclohexyl-2-phenylpropionates: Cylexphenes (Cyclohexyl-Substituted Aprophen Analogues)

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A series of aprophen [(*N*,*N*-diethylamino)ethyl 2,2-diphenylpropionate] analogues, called cylexphenes, were synthesized with alterations in (1) the chain length of the amine portion of the ester, (2) the alkyl groups on the amino alcohol, and (3) a cyclohexyl group replacing one of the phenyl rings. The antimuscarinic activities of these analogues were assessed in two pharmacological assays: the inhibition of acetylcholine-induced contraction of guinea pig ileum, and the blocking of carbachol-stimulated release of α-amylase from rat pancreatic acinar cells. These two tissues represent the M_3(ileum) and M_3(pancreas) muscarinic receptor subtypes. In addition, the analogues were also evaluated for their competitive inhibition of the binding of [^{3}H]NMS to selected cell membranes, each containing only one of the m_1, M_2, m_3, or M_4 muscarinic receptor subtypes. The m_1 and m_3 receptors were stably transfected into A9 L cells. The replacement of one phenyl group of aprophen with a cyclohexyl group increased the selectivity of all the analogues for the pancreatic acinar muscarinic receptor subtype over the ileum subtype by more than 10-fold, with the (*N*,*N*-dimethylamino)propyl analogue exhibiting the greatest selectivity for the pancreas receptor subtype, over 30-fold. The cylexphenes also showed a decrease in potency in comparison to the parent compound when examined for the binding of [^{3}H]NMS to the M_2 subtype. In agreement with the pharmacological data obtained from the pancreas, the (*N*,*N*-dimethylamino)propyl cylexphene **3** demonstrated the greatest selectivity for the m_3 subtype, and additionally showed a preference for the m_1 and M_4 receptor subtypes over the M_2 receptor subtype in the binding assay. Thus, this compound showed a potent selectivity according to the pharmacological and binding assays between the muscarinic receptor subtypes of the pancreas and ileum. In both the pharmacological and binding assays, the potency of the analogues decreased markedly when the chain length and the bond distance between the carbonyl oxygen and protonated nitrogen were increased beyond three methylene groups. When the structures of these analogues were analyzed using a molecular modeling program, the bond distance between the carbonyl oxygen and protonated nitrogen was deduced to be more important for the antagonist activity than subtype specificity.

Introduction

Aprophen [(*N*,*N*-diethylamino)ethyl 2,2-diphenylpropionate] is a potent anticholinergic and antispasmodic agent possessing a wide number of distinct pharmacological actions, including both antimuscarinic and noncompetitive nicotinic antagonist activities.[1-8] The potent antimuscarinic and, to a lesser extent, the antinicotinic effects of aprophen make it a potential drug of choice in the therapy of poisoning by organophosphate agents.[4,9] Although muscarinic receptors have been shown recently to exist in five subtypes,[10-18] the subtype specificity of aprophen and its analogues has not been determined.

Several functional groups are required in a molecule to achieve potent antimuscarinic properties.[19,20] First, a protonated nitrogen atom near one end of the molecule acts as the cationic site. Second, the center of the compound contains an electronegative ester group which is part of the anionic site. Lastly, a relatively bulky hydrophobic

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0022-2623/92/1835-1290$03.00/0

Table I. Antimuscarinic Activity of Aprophen and Cylexphenes

	compounds and substitutions[c]	biological assay values[a] ileum contraction: K_B (M)	pancreas α-amylase release: IC_{50} (M)	selectivity:[b] pancreas/ileum
I.	aprophen	3.1 (±0.8) × 10^{-9}	4.6 (±0.7) × 10^{-8}	1.5
II.	cylexphenes			
	2; n = 2, R = ethyl	4.0 ±(0.4) × 10^{-8}	5.2 (±0.2) × 10^{-8}	16.7
	3; n = 3, R = methyl	1.4 (±0.4) × 10^{-8}	1.0 (±0.7) × 10^{-8}	30.8
	4; n = 3, R = ethyl	1.8 (±0.9) × 10^{-8}	2.1 (±0.7) × 10^{-8}	19.1
	5; n = 4, R = methyl	1.5 (±0.3) × 10^{-7}	1.9 (±0.3) × 10^{-7}	17.4
	6; n = 4, R = ethyl	2.1 (±0.6) × 10^{-7}	3.8 (±0.5) × 10^{-7}	12.0
	7; n = 5, R = ethyl	1.4 (±0.5) × 10^{-6}	2.7 (±0.4) × 10^{-6}	11.5

[a] Each inhibition constant represents the mean of three to six independent experiments ± the standard deviation of the mean. [b] Selectivity ratio[17] relative to atropine is [(atropine IC_{50} value for pancreas)/(compound IC_{50} value for pancreas)]/[(atropine K_B value for ileum)/(compound K_B value for ileum)]. The inhibition constants determined for atropine are:[20] K_B = 2.0 (±0.8) × 10^{-9}, ileum assay; IC_{50} = 4.4 (±0.8 × 10^{-8}, pancreas assay. [c] For structures, see Figure 1.

and lipophilic portion is located at the opposite end of the molecule from the protonated nitrogen. The effect of altering these critical portions of the molecule on their subtype-specific antagonist properties provides information for the designing of more potent compounds and receptor subtype-selective antimuscarinics, which may maximize their protective effects.[20]

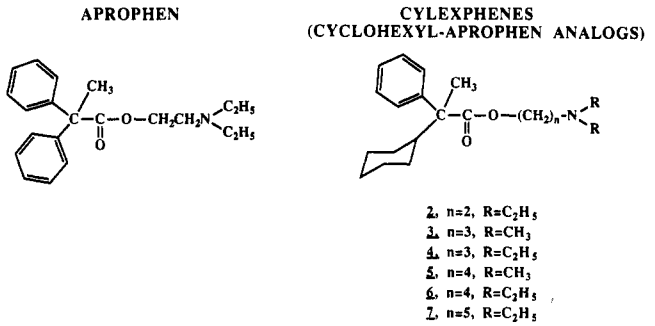

Figure 1. Structures of aprophen and cylexphenes (cyclohexyl-aprophen analogues).

In this report, aprophen analogues, called cylexphenes, were synthesized with alterations in (1) the chain length of the amine portion of the ester, (2) the alkyl groups on the amino alcohol, and (3) a cyclohexyl group replacement for one of the phenyl rings. These analogues were tested for their inhibition of acetylcholine-induced contraction of guinea pig ileum,[4,5,10-14,18] and carbachol-stimulated release of α-amylase from rat pancreatic acinar cells.[15-17,20] Currently, the ileum and pancreas muscarinic receptor subtypes are pharmacologically classified as the M_3 (smooth muscle/glandular, respectively) receptor subtype,[11] but molecular characterization has not been completed. Yet, while ileum and pancreas muscarinic receptors have similar but not identical characteristics,[21,22] there are dissimilarities in binding properties and dissociation kinetics.[20,23] To further complement and also clarify the pharmacological data, the ability of these new analogues to specifically inhibit the binding of [*N-methyl*-^{3}H]scopolamine ([^{3}H]NMS) to selected cell membranes was assessed using cell membranes containing essentially a single muscarinic receptor subtype, either m_1, M_2, m_3, or M_4.

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Table II. Antimuscarinic Activity of Aprophen and Cylexphenes

	compounds and substitutions[c]	K_i values,[a] nM (±SD)				selectivity[b]					
		m_1	M_2	m_3	M_4	m_1/M_2	m_3/M_2	M_4/M_2	m_1/m_3	m_1/M_4	m_3/M_4
I.	aprophen	2.1 (0.5)	9.3 (1.3)	7.7 (1.3)	7.4 (1.8)	4.4	1.2	1.3	3.7	3.5	1.0
II.	cylexphenes										
	2; n = 2, R = ethyl	4.0 (0.6)	23.4 (4.7)	9.9 (2.1)	18.5 (4.8)	5.9	2.3	1.3	2.5	4.6	1.9
	3; n = 3, R = methyl	1.9 (0.1)	26.9 (8.8)	1.7 (0.4)	1.5 (0.6)	14.2	15.8	17.9	0.9	0.8	0.9
	4; n = 3, R = ethyl	2.9 (0.7)	19.7 (4.4)	4.0 (1.0)	4.6 (0.9)	6.8	4.9	4.3	1.4	1.6	1.2
	5; n = 4, R = methyl	40.7 (13)	258 (51)	31.2 (11)	65.6 (4.0)	6.4	8.2	3.9	0.8	1.6	2.1
	6; n = 4, R = ethyl	111 (28)	248 (54)	160 (38)	209 (76)	2.2	1.6	1.2	1.4	1.9	1.3
	7; n = 5, R = ethyl	298 (94)	246 (103)	178 (48)	173 (44)	0.8	1.4	1.4	0.6	0.6	1.0

[a] Each inhibition constant represents the mean of three to six independent experiments ± the standard deviation of the mean. [b] Selectivity ratio:[17] $m_x/m_y = (K_i\ m_y)/(K_i\ m_x)$. [c] For structures, see Figure 1.

Results

Chemistry. All the cylexphenes, except for **2** (see Figure 1 for the structures), were synthesized by standard procedures, starting with the chloride of 2-cyclohexyl-2-phenylpropionic acid **1** and the appropriate amino alcohol. The amino ester **2** was prepared by reacting an aqueous solution of *N,N*-diethylaziridinium chloride (obtained from freshly distilled (*N,N*-diethylamino)ethyl chloride) with an aqueous bicarbonate solution of the acid **1**.[24,25]

Pharmacological Assays. The cylexphenes shown in Figure 1 were tested for inhibition of guinea pig ileum contraction. IC_{50} values were obtained when the analogues were tested for the inhibition of the release of α-amylase from rat pancreatic acinar cells. The inhibition constants are shown in Table I. In the α-amylase release assay, the slopes were not significantly different from 1.0, indicating competitive inhibition of carbachol by the compounds at the muscarinic receptor active site. Competitive inhibition was also indicated for the ileum assays because the slopes of the Schild plots approached unity. The calculated selectivity values are also reported in Table I and indicate the potencies of the compounds for the functional muscarinic receptor subtype found in the pancreas or ileum.

The introduction of the cyclohexyl group increased the specificity of the analogues for the pancreatic acinar muscarinic subtype over the ileum subtype compared to the parent compound, aprophen. Aprophen's selectivity index was 1.5, implying no selectivity for either receptor subtype. All of the cylexphenes have selectivity indices greater than 10-fold for the pancreas subtype compared with the ileum subtype. The most selective cylexphene was the (*N,N*-dimethylamino)propyl ester **3**, almost 30-fold, followed by (*N,N*-diethylamino)propyl ester **4**, and two equally selective analogues, the (*N,N*-dimethylamino)butyl ester **5** and the (*N,N*-diethylamino)ethyl ester **2**. The selectivity of the esters of *N,N*-diethylbutyl **6** and *N,N*-diethylpentyl **7** were about the same, approximately 12-fold.

Inhibition of [^{3}H]NMS Binding to m_1, M_2, m_3, and M_4 Muscarinic Receptor Subtypes. The antimuscarinic potencies and subtype specificity of the compounds were characterized by their inhibition of the binding of [^{3}H]-NMS to membranes containing m_1, M_2, m_3, or M_4 subtypes. All of the compounds yielded sigmoidal competition curves with Hill coefficients not significantly different from 1.0. The K_i values and selectivity indices are listed in Table II.

The K_i values for aprophen were similar, about 7 nM, with respect to each of the four receptor subtypes, indicating no significant subtype selectivity. The (*N,N*-dimethylamino)propyl cyclohexyl analogue **3**, however, exhibited substantial subtype selectivity; it showed about 14- to 18-fold higher affinity for the m_1, m_3, and M_4 muscarinic receptor subtypes than the M_2. With respect to the m_3 receptor subtype, this analogue had the highest affinity of the series. This compound was also the most selective in the pancreas pharmacological assay (Table I). The cylexphenes, *N,N*-diethylpropyl **4** and *N,N*-dimethylbutyl **5**, which exhibited selectivity in the pharmacological assays (Table I), showed slight selectivity for the m_1, m_3, and M_4 muscarinic receptors over M_2 receptors in the binding assays (Table II). Notably, none of these analogues exhibited significant selectivity among the m_1, m_3, and M_4 subtypes.

Table III. Bond Distances[a] between the Amino Nitrogen and Carbonyl Oxygen

	compounds and substitutions[b]	bond distance (Å)
I.	aprophen	5.02
II.	cylexphenes	
	2; n = 2, R = ethyl	4.95
	3; n = 3, R = methyl	6.55
	4; n = 3, R = ethyl	6.78
	5; n = 4; R = methyl	7.43
	6; n = 4, R = ethyl	7.18
	7; n = 5, R = ethyl	8.96

[a] Bond distances were determined as described in the Experimental Section. [b] For structures, see Figure 1.

Distance Geometry. Table III shows the results of computer modeling of the energy-minimized bond distances between the carbonyl oxygen of the ester group and the protonated nitrogen. These structures represented only one of the energy-minimized configurations because the amino alcohol group is not rigid, but rather flexible. The compounds containing two or three methylene groups (**2**, **3**, or **4**) showed a broad range of activity over calculated bond distances of 5–6.7 Å (Tables I and II). Increasing the chain length between the carbonyl oxygen of the ester moiety and the protonated nitrogen moiety beyond three methylene groups, however, markedly decreased the potency and affinity of the analogues in all pharmacological and binding assays, respectively (Tables I and II). Since it was not feasible to decrease the chain length by synthesizing a cyclohexyl compound containing only one methylene group, the shape of the curve depicting bond distance versus activity could not be determined. Therefore, the antimuscarinic potency of a cylexphene having a smaller bond distance between the carbonyl oxygen and the protonated nitrogen than **2** remains unknown.

The calculated bond distances and antagonist potencies were similar for the paired cylexphenes: (1) (*N,N*-dimethylamino)propyl **3** or (*N,N*-diethylamino)propyl **4** analogues and (2) the (*N,N*-dimethylamino)butyl **5** or (*N,N*-diethylamino)butyl **6** analogues. In contrast, the

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selectivity for receptor subtype was different among these paired analogues because the (*N*,*N*-dimethylamino)propyl analogue **3** was the most selective (Tables I and II). This suggested that the bond distance between the carbonyl oxygen and protonated nitrogen was more important for antagonist potency, but not a deciding factor for subtype specificity.

Discussion

Aprophen, the parent compound, showed similar potency and selectivity profiles in both the ileum and pancreas pharmacological assays and the [^{3}H]NMS binding assays on membranes having only one muscarinic receptor subtype, either m_1, M_2, m_3, or M_4. Note that the K_B values reported for the ileum assay and K_i values reported for the binding assays could be directly compared. If the K_D for carbachol were not to be used in the Cheng–Prusoff equation[37] to convert the IC_{50} values determined in the α-amylase release assay to K_i or K_B values, then by calculating the relative affinities to the nonspecific antagonist atropine, a consistent comparison of receptor selectivity could be derived.[17] However, the K_i values, calculated according to the Cheng–Prusoff equation[37] for the α-amylase release assay, would be approximately 10- to 20-fold more potent than the corresponding IC_{50} values. Hence, for the nonselective antagonist aprophen, the inhibition (K_i) constants for all the assays ranged between 2 and 9 nM.

Substitution of the phenyl group in aprophen by a cyclohexyl group, resulted in about a 20-fold decrease in the antimuscarinic activity for the ileum contraction assay (Table I), without any significant change for the pancreas assay. Along with binding and dissociation kinetic data, these data support a difference between the ileum (smooth muscle) and pancreas (glandular) muscarinic receptor subtypes.[10-18,21-23] A good correlation between the pancreas α-amylase release assay and the m_3 binding assay was established because (*N*,*N*-dimethylamino)propyl **3** was the most selective cylexphene in these assays (Tables I and II). The binding assays further indicated that the (*N*,*N*-dimethylamino)propyl analogue **3** was markedly less selective for the M_2 muscarinic receptor subtype, and that this analogue showed no selectivity between m_1, m_3, or M_4 receptor subtypes. Thus, the inhibition constants determined for the cylexphenes were indicative that the ileum muscarinic receptor (smooth muscle type) was not the same as the pancreas muscarinic receptor (glandular type) and the cloned m_3 subtype. It should be noted that the cloned m_3 muscarinic receptor subtype transfected into the A9 L cells was isolated from a brain library, but so far no genetic clone has been obtained for either ileum or pancreas.[13] Therefore, the transfected m_3 gene may represent a subtype of muscarinic receptor that may share some similarities, but is not equivalent, to the pancreas muscarinic receptor.

Analogues containing an amino alcohol longer than three methylene groups, i.e., the butyl analogues **5** or **6**, showed a significant decrease in antagonist potency and affinity in both the pharmacological and the binding assays. This was suggestive that all the receptor subtypes in this study could accomodate antagonists of roughly the same size. An optimal geometric distance between the carbonyl oxygen of the ester group and the protonated nitrogen group in a series of 2,2-diphenylpropionate analogues containing rigid amino alcohols has been determined to be around 5.2 Å.[20,26] X-ray crystallography of other 2,2-diphenylpropionate analogues also support this optimum bond distance[27] for rigid antagonists. In the present report and unlike the rigid antagonists, the cylexphenes had a flexible aliphatic chain between the ester and the protonated nitrogen, which allowed for several low energy conformations. Thus, these analogues were inherently more difficult to model than rigid antagonists. Nevertheless, the bond distances between the amino nitrogen and the carbonyl oxygen of the energy-minimized models of the propyl analogues were about 6.5 Å, and for the butyl or pentyl analogues, greater than 7 Å (Table III). These results implied that the latter distance was too large to fit optimally into the binding sites of all the muscarinic receptor subtypes. Whereas the structure–activity relationship (a parabolic curve) obtained previously with rigid antagonists[20] yields an optimum bond distance of 5.2 Å, the flexible cylexphenes exhibited, first, an optimal bond distance which was longer, and second, a broader structure–activity correlation (Tables I, II, and III). Since the muscarinic receptors studied here accomodated compounds that had a longer bond distance and were sterically flexible, an induced fit of an antagonist into the receptor site might take place, and would not represent an energy-minimized conformation. On the other hand, since there was no synthetic route to this series of antagonists containing only one methylene group, the statistical shape of the structure–activity curve could not be completely defined.

The size of the *N*-alkyl groups (*N*,*N*-dimethyl or *N*,*N*-diethyl) appeared to be more important in the [^{3}H]NMS binding assay than in the pharmacological assays. For instance, in the pharmacological assays (Table I), the bulkiness of the *N*,*N*-diethyl (**4** or **6**) decreased the selectivity of this analogue only moderately, and had little effect on antagonist potency when compared to the *N*,*N*-dimethyl analogues (**3** or **5**). Conversely, the (*N*,*N*-diethylamino)propyl analogue **4** in the [^{3}H]NMS binding assay was only marginally selective when compared with the (*N*,*N*-dimethylamino)propyl analogue **3** (Table II).

It has been proposed that the rigidity or flexibility of an antagonist can determine whether the required conformation can be achieved to interact with muscarinic receptor subtypes.[28] While atropine is a rigid amino alcohol and is not subtype specific,[17] the (*N*,*N*-dimethylamino)propyl cyclohexyl analogue **3** was apparently the only cylexphene that could best fit into the antagonist binding site of the m_1, m_3, and M_4 muscarinic receptor subtypes, but not the M_2 subtype. The greater flexibility of the cyclohexyl group compared with a phenyl ring would probably allow the antagonist to fit better into the hydrophobic binding region of the m_1, m_3, and M_4 muscarinic receptor subtypes than the M_2 subtype. Also, the pharmacological assays seemed to be more sensitive to this substitution than the binding assays, as shown by the 30-fold selectivity in the pharmacological assay and 15-fold selectivity in the binding assays.

All of the cylexphenes contain chiral centers and, thus, several stereoisomers of each compound are possible. For the present study, no attempt was made to separate these isomers. However, it has been found that optical isomers

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of the muscarinic antagonist, 3-quinuclidinyl benzilate and related antagonists show receptor subtype selectivity.[29] We are currently attempting to synthesize and isolate the stereoisomers of some of the compounds described in these studies to determine if further subtype specificity can be obtained.

The present results, along with distance geometry determination and X-ray crystallography,[7] may serve as a basis upon which to develop more potent subtype-specific analogues. The data presented here demonstrated that both the hydrophobic and amino alcohol portions of these cyclohexyl muscarinic antagonists contain modifications that impart subtype specificity and might, in part, be due to their flexibility. Lastly, binding affinities to the various muscarinic receptor subtypes studied here were markedly decreased when the bond distances between the protonated nitrogen and the carbonyl oxygen were greater than the 7 Å found in the propylamino cylexphenes.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained on a Varian XL 300 (Me_4Si). Mass spectra (MS) were obtained on a Finnigan 1015 mass spectrometer (chemical ionization, NH_3). Elemental analyses were performed by Spang Micro-Analytical Laboratory (Eagle Harbor, MI). For purity tests, TLC was performed on fluorescent silica gel plates (Polygram Sil G/UV254), and for each of the compounds, only one spot (visualized by UV light and I_2 vapors) was obtained. The amine HCl salts were prepared by adding an excess of HCl-saturated ether solution to the etheral solution of the appropriate amino ester. Recrystallizations of the salts were done in all cases from an ethyl acetate–ether mixture. All new compounds gave satisfactory microanalyses for C, H, and N within ±0.4% and/or mass spectra consistent with the assigned structures.

2-Cyclohexyl-2-phenylpropionic Acid (1). This compound was synthesized with some modifications as described.[30] A solution of cyclohexyl chloride (59.0 g, 0.5 mol) in pyridine (350 mL) was added dropwise to a vigorously stirred suspension of KOH (fine powder, 175.0 g) and α-methylbenzyl cyanide (65.5 g, 0.5 mol) in pyridine (250 mL), with the reaction temperature being maintained at 5–10 °C. The reaction mixture was stirred for 24 h at 25 °C and poured onto an excess of HCl–ice mixture. The acidic aqueous mixture was extracted with ether (3 × 200 mL), the organic phase was washed with brine and dried ($MgSO_4$), and the ether was evaporated under vacuum. The crude oil was distilled under vacuum (bp 110–115 °C/1 mmHg) to afford 70.5 g (66%) of 2-cyclohexyl-2-phenylpropionitrile as a colorless oil. A mixture of 40.0 g of the above nitrile and KOH (28.0 g) in diethylene glycol (180 mL) was heated at 190 °C for 72 h, then cooled, diluted with water (500 mL), and washed with ether. The aqueous solution was acidified with dilute hydrochloric acid and extracted with ether. Concentration of the ether extracts left a pale brown solid which was recrystallized from pentane to afford 42.6 g (96%) of 1 as colorless crystals: mp 138–140 °C (lit.[30] mp 139–140 °C).

β-(*N*,*N*-Diethylamino)ethyl 2-Cyclohexyl-2-phenylpropionate (2). β-(*N*,*N*-Diethylamino)ethyl chloride (obtained from the HCl salt and freshly distilled, 2.0 g, 0.014 mol) was suspended in 15 mL of H_2O. After vigorous stirring for 2 h at room temperature, the resulting clear aqueous solution (which contained *N*,*N*-diethylaziridium chloride[24,25]) was added in one portion to a solution of 1 (1.62 g, 0.007 mol) in 90 mL of 10% $NaHCO_3$. Ethyl acetate (50 mL) was added, and the biphasic reaction mixture (pH 7.8) was stirred at room temperature for 24 h. The organic phase was separated, washed with brine (2 × 50 mL), dried ($MgSO_4$), and evaporated to leave 1.0 g (75%) of viscous colorless oil: TLC (10% MeOH–$CHCl_3$), R_f 0.6; ^{1}H NMR ($CDCl_3$) δ 7.5–7.1 (m, 5 H), 4.15 (t, 2 H, *J* = 6.5 Hz), 2.67 (t, 2 H, *J* = 6.5 Hz), 2.50 (q, 4 H, *J* = 7.1 Hz), 2.3 (m, 1 H), 1.8–1.6 (m, 4 H), 1.46 (s, 3 H), 1.40–1.0 (m, 5 H), 0.90 (t, 6 H, *J* = 7.1 Hz), 0.85–0.80 (m, 1 H); HCl salt mp 130–1 °C. Anal. ($C_{21}H_{34}NO_2Cl$) C, H, N.

γ-(*N*,*N*-Dimethylamino)propyl 2-Cyclohexyl-2-phenylpropionate (3). A solution of the acid 1 (2.32 g, 0.01 mol) and thionyl chloride (10 mL) in dry benzene (70 mL) was stirred at reflux for 4 h. After cooling to room temperature, the benzene and excess $SOCl_2$ were removed under reduced pressure, and the residue was dissolved in dry benzene (50 mL). The above solution was added dropwise to a stirred solution of 3-(*N*,*N*-dimethylamino)propan-1-ol (1.23 g, 0.012 mol) and triethylamine (1.20 g, 0.012 mol) in dry benzene (100 mL). The reaction mixture was refluxed for 4 h. After cooling, the solid was filtered, and the filtrate was evaporated to leave a viscous oil. The crude ester was dissolved in 1 N HCl (50 mL), and the acidic aqueous solution was extracted with ether (2 × 50 mL) and then was basified with solid Na_2CO_3. Extraction of the basic aqueous solution with ether (3 × 100 mL), drying ($MgSO_4$), and evaporation of the ether afforded 3 (2.8 g, 87%) as a pale yellow oil: TLC (5% MeOH–$CHCl_3$), R_f 0.55; ^{1}H NMR ($CDCl_3$) δ 7.42–7.18 (m, 5 H), 4.07 (t, 2 H, *J* = 6.5 Hz), 2.33–2.25 (m, 1 H), 2.17 (t, 2 H, *J* = 6.5 Hz), 2.14 (s, 6 H, $N(CH_3)_2$), 1.8–1.6 (m, 4 H), 1.48 (s, 3 H, CCH_3), 1.36–1.06 (m, 7 H), 0.88–0.80 (m, 1 H). HCl salt mp 144–5 °C. Anal. ($C_{20}H_{32}NO_2Cl$) C, H, N.

The following compounds were prepared in a similar manner starting with 2-cyclohexyl-2-phenylpropionic acid and the appropriate amino alcohol:

γ-(*N*,*N*-Diethylamino)propyl 2-cyclohexyl-2-phenylpropionate (4): pale yellow oil (81% yield); TLC (5% MeOH–$CHCl_3$), R_f 0.45; ^{1}H NMR ($CDCl_3$) δ 7.42–7.20 (m, 5 H), 4.07 (t, 2 H, *J* = 6.3 Hz), 2.47–2.25 (m, 7 H), 1.79–1.64 (m, 4 H), 1.49 (s, 3 H, CCH_3), 1.37–1.00 (m, 6 H), 0.95 (t, 6 H, *J* = 7.1 Hz), 0.85–0.80 (m, 1 H); HCl salt mp 116–7 °C. Anal. ($C_{22}H_{36}NO_2Cl$) C, H, N.

δ-(*N*,*N*-Dimethylamino)butyl 2-cyclohexyl-2-phenylpropionate (5): pale yellow oil (80% yield); TLC (5% MeOH–$CHCl_3$), R_f 0.64; ^{1}H NMR ($CDCl_3$) δ 7.40–7.18 (m, 5 H), 4.02 (symmetric m, 10 lines, 2 H), 2.31–2.18 (m, 3 H), 2.14 (s, 6 H, $N(CH_3)_2$), 1.77–1.51 (m, 6 H), 1.47 (s, 3 H, CCH_3), 1.43–1.23 (m, 3 H), 1.20–1.01 (m, 4 H), 0.83–0.78 (m, 1 H). HCl salt mp 62–4 °C. Anal. ($C_{21}H_{34}NO_2Cl$) C, H, N.

δ-(*N*,*N*-Diethylamino)butyl 2-cyclohexyl-2-phenylpropionate (6): pale yellow viscous oil (78% yield); TLC (5% MeOH–$CHCl_3$), R_f 0.46; ^{1}H NMR ($CDCl_3$) δ 7.40–7.20 (m, 5 H), 4.01 (symmetric m, 10 lines, 2 H), 2.50 (q, 4 H, *J* = 7.1 Hz), 2.45–2.25 (m, 3 H), 1.77–1.50 (m, 6 H), 1.48 (s, 3 H, CCH_3), 1.45–1.05 (m, 7 H), 1.00 (t, 6 H, *J* = 7.1 Hz), 0.83–0.78 (m, 1 H); HCl salt mp 104–5 °C. Anal. ($C_{23}H_{38}NO_2Cl$) C, H, N.

ε-(*N*,*N*-Diethylamino)pentyl 2-cyclohexyl-2-phenylpropionate (7): colorless viscous oil (34% yield after chromatography on silica with 2% MeOH–$CHCl_3$); TLC (5% MeOH–$CHCl_3$), R_f 0.4; ^{1}H NMR ($CDCl_3$) δ 7.41–7.17 (m, 5 H), 4.02 (t, 2 H, *J* = 6.5 Hz), 2.51 (q, 4 H, *J* = 7.2 Hz), 2.36 (m, 2 H), 2.28 (m, 1 H), 1.78–1.50 (m, 6 H), 1.48 (s, 3 H, CCH_3), 1.40–1.30 (m, 3 H), 1.25–1.10 (m, 6 H), 1.01 (t, 6 H, *J* = 7.2 Hz), 0.88–0.80 (m, 1 H); HCl salt mp 85–87 °C. Anal. ($C_{24}H_{40}NO_2Cl$) C, H, N.

Biological Assays. α-Amylase Secretion from Pancreatic Acinar Cells. Pancreatic acinar cells were prepared from male Sprague-Dawley rats by three successive incubations with collagenase (0.8 mg/mL).[31,32] The cells were suspended in 16 mL of Dulbecco's minimal essential medium containing 0.2% albumin, 0.01% trypsin inhibitor, and 0.09% theophylline, aerated with 100% O_2, and diluted 5-fold before use. Viability test by trypan blue exclusion was greater than 99%. The acinar cells were incubated with varied doses of each compound to be tested and 10^{-5} M carbachol in 0.5 mL. α-Amylase secreted from the acinar

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cells was determined with a Pharmacia Phadebas kit. IC_{50} values, the concentration causing a 50% decrease in α-amylase secretion, were determined using LIGAND,[33] a computer program for the analysis of inhibition curves.

Acetylcholine-Induced Contraction of Guinea Pig Ileum. Distal ileum was obtained from male albino guinea pigs (350–500 g), and a segment approximately 2 cm in length was suspended in each 10-mL organ bath in oxygenated Krebs-Ringer solution maintained at 37 °C.[34] Isometric contractions were recorded by means of a free-displacement transducer (Harvard Apparatus, Natick, MA) set at 1-g tension. After a stabilization period of 45 min, acetylcholine (ACh) was added to the bath, allowed to act for 1 min, and then washed out. The tissue was allowed 5 min to recover prior to the next addition. The maximal contractile response was designated as 100%, and other responses were reported as a percentage of that response. After a recovery period of 15 min, test compounds, followed 30 s later by ACh, were added to each bath, and the contractile responses were recorded. The K_B values (antilog of pA_2), measuring the affinity of an antagonist for the muscarinic receptor, were calculated using computer programs for the Schild plot.[35]

Inhibition of [*N*-*methyl*-^{3}H]Scopolamine Binding to Subtypes of Muscarinic Receptors. The antimuscarinic potencies of the compounds were determined in membranes prepared from cells or tissues containing a single muscarinic receptor subtype:[18] A9 L cells transfected with the cloned m_1 or m_3 muscarinic receptor subtype (obtained from Dr. M. Brann, NIH), M_2 subtype receptor from heart, and the M_4 muscarinic receptor subtype from NG108-15 neuroblastoma glioma cells (obtained from Dr. M. Nirenberg, NIH). The m_1 and m_3 receptor subtypes were genetic clones derived from a brain library and stably transfected into A9 L cells.[13] Membranes from these cells were obtained by lysing the washed cells in 2 mM Tris-HCl (pH 7.2) and 1 mM EDTA, and centrifuging them at 50000*g* for 20 min. The resulting pellet was resuspended in lysis buffer and recentrifuged. Membranes were stored frozen at −70 °C until needed. An aliquot of the membranes (150–300 μg of protein) was incubated for 60 min at 37 °C with 0.5 nM [^{3}H]NMS and various concentrations of the compounds in phosphate-buffered saline (pH 7.2). The reaction was terminated by rapid filtration over GF/B filters.[36] The filters were washed with ice-cold 0.9% NaCl and processed for scintillation counting. Nonspecific binding was determined by co-incubation with 1 μM atropine and was routinely subtracted from total binding. The resulting data was analysed using the LIGAND computer program[33,37] using the following experimentally determined K_D values for [^{3}H]NMS binding: 235, 426, 164, and 144 pM, for the m_1, M_2, m_3, or M_4 receptors, respectively.

Computer Modeling. The XIRIS Computer Aided Molecular Modeling System was used[20] to determine the structures of the compounds shown in Figure 1; the algorithms are based on the approach of Wipke et al.[38] By use of this system, estimations of the bond distances of the energy-minimized structures were made between the carbonyl oxygen and the quaternary nitrogen (the nitrogen was modeled in the protonated form). These estimations were then correlated with the biological potencies of the compounds.

Acknowledgment. This work was done while H.L. held a National Research Council Senior Research Associateship at Walter Reed Army Institute of Research. The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense (para 4-3, Ar 360-5). In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

Registry No. 1, 4370-98-3; 2, 47321-08-4; 2·HCl, 95000-33-2; 3, 139070-52-3; 3·HCl, 139070-53-4; 4, 139070-54-5; 4·HCl, 139070-55-6; 5, 139070-56-7; 5·HCl, 139070-57-8; 6, 139070-58-9; 6·HCl, 139070-59-0; 7, 139070-60-3; 7·HCl, 139070-61-4; cyclohexyl chloride, 542-18-7; α-methylbenzyl cyanide, 1823-91-2; 2-cyclohexyl-2-phenylpropionitrile, 4420-58-0; β-(*N*,*N*-diethylamino)ethyl chloride, 100-35-6; 3-(*N*,*N*-dimethylamino)propan-1-ol, 3179-63-3; 3-(*N*,*N*-diethylamino)-1-propanol, 622-93-5; 4-(*N*,*N*-dimethylamino)-1-butanol, 13330-96-6; 4-(*N*,*N*-diethylamino)-1-butanol, 2683-56-9; 5-(*N*,*N*-diethylamino)-1-pentanol, 2683-57-0; aprophen, 3563-01-7.

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