Molecular Modification of Anticholinergics as Probes for Muscarinic Receptors. 1. Amino Esters of α-Substituted Phenylacetic Acid and Related Analogues[1]

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Two series of compounds having the general structure of $C_6H_5CRR'COOCH_2CH_2NEt_2$ were synthesized and examined for their antispasmodic activities. These compounds were selected as structural probes for exploring the nature of muscarinic cholinergic receptor binding sites that interact with atropine-like anticholinergics. These studies indicate a rather strict size limitation for the hydrophobic region of the receptor and suggest intramolecular hydrogen bonding as a possible means to explain the observed stereoselectivity.

Systematic structure–activity relationship (SAR) studies have provided valuable information into the nature of the muscarinic receptor.[2,3] One of the most interesting facts to emerge from these studies is the high stereospecificity for most atropine-like anticholinergics (i.e., *S*-(−) isomer is the active form of atropine) and the high potency observed for compounds with a suitably placed hydroxy group.[2,4] Although, it is generally assumed that the most critical determinant of atropine-like anticholinergic activity is the presence of two large hydrophobic groups in the acyl portion of the structure, the role of the hydroxymethyl moiety and the need for at least one aromatic group for optimal antimuscarinic activity remains unclear.

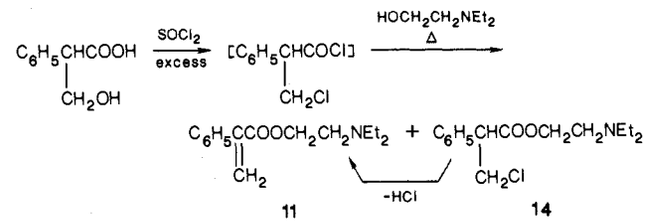
As part of a systematic investigation of the topographical areas of the muscarinic receptor, two series of compounds with the general formula of $C_6H_5CRR'COOCH_2CH_2NEt_2$ were prepared as molecular probes (Table I). In this paper we describe the synthesis and the antispasmodic activity of these compounds on isolated guinea pig and/or rat ileum. Studies with compounds **1–7** (R = C_6H_5, R′ = H, Me, Et, *n*-Pr, *i*-Pr, *n*-Bu, and *i*-Bu) should provide valuable information as to the size limitation of the hydrophobic region while compounds **8–10** [R = H, R′ = $(CH_2)_nOR''$] may provide clues for the role of the hydroxymethyl moiety of atropine molecule in its enhancement of anticholinergic potency. Compound **11** was prepared in an attempt to alkylate the possible hydrogen-bonding subsite frequently proposed.[2,5] The results obtained, along with the antispasmodic activity of isomeric 2-phenylcyclohexyl (diethylamino)ethyl ethers previously synthesized in our laboratory,[6] enable us to offer a possible alternative explanation for the role of hydroxy group and for the observed stereospecificity for many potent atropine-like anticholinergics.

Chemistry

The majority of the compounds listed in Table I were prepared either from the acid chloride and 2-(diethylamino)ethanol in the standard ester synthesis (method A) or by the reaction of the acid and (diethylamino)ethyl chloride according to the procedure of Horenstein and Pählicke[7] (method C). The latter method has proved exceedingly useful, particularly in cases where the acid chlorides are unstable or could not be prepared because of the presence of reactive functional groups (i.e., OH, etc.). Compound **6** was obtained from compound **1** by alkylation with *n*-butyl bromide in a mixture of liquid ammonia and anhydrous ether (method B).

Treatment of optically pure amino alcohols **8a** and **8b** with *p*-toluenesulfonyl chloride in pyridine provided compound **9a** and **9b**, respectively, in poor yield (method D). A possible reason for the low yield of this reaction may be the ease of detosylation due to the presence of an acidic benzylic proton in the molecule. With this notion in mind, the α,β-unsaturated analogue **11** was prepared from tropic acid as outlined in Scheme I. Thus treatment of tropic acid with excess amount of thionyl chloride followed by refluxing with 2-(diethylamino)ethanol gave a mixture of amino esters **11** and **14**, which were separated by column chromatography. The desired product **11** was obtained in 31% yield. The chloro derivative **14** was characterized by its ^1H NMR spectrum but was not tested pharmacologically due to its instability.

Scheme I



The desired 2,2-diphenylalkanoic acids, $(C_6H_5)_2CRCOOH$, were prepared either by the alkylation of diphenylacetonitrile with the required alkyl bromide or iodided followed by the hydrolysis of the nitrile according to the published procedure[8] or by the oxidation of the corresponding 2,2-diphenylalkanal utilizing the method of Bateman and Marvel.[9] The more hindered 2,2-diphenylalkanoic acid (i.e., R = *i*-Pr and *i*-Bu) were prepared by the method of Ioffe et al.[10]

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(1) This work is taken, in part, from the Ph.D. Dissertations of W.E.W., University of Illinois at the Medical Center, 1975, and L.B.S., University of Illinois at the Medical Center, 1981.
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(9) Bateman, D. E.; Marvel, C. S. *J. Am. Chem. Soc.* **1927**, *49*, 2914.
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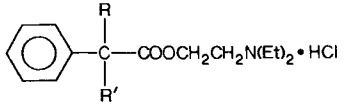
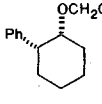
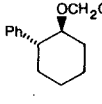
Table I. Antispasmodic Activity of Amino Esters of α-Substituted Phenylacetic Acid and Related Compounds



								pharmacological testing results[e]				
								isolated guinea pig ileum		isolated rat ileum		
compd[a]	R	R'	mp,[b] °C or $[\alpha]^{25}_D$, deg solvent	recrystn solvent	% yield	emp formula[c]	method[d] prepn	ID_{50}, M	relative potency	pA_2	ID_{50}, M	relative potency
1	C_6H_5	H	112–113[f]	EtOAc	84	$C_{20}H_{26}ClNO_2$	A	$(7.4 \pm 0.2) \times 10^{-7}$	1	7.45 ± 0.09	3.55×10^{-8}	1
2	C_6H_5	Me	157–160[g]	EtOAc	60	$C_{21}H_{28}ClNO_2$	A	$(5.3 \pm 0.1) \times 10^{-8}$	14	8.40 ± 0.08	3.96×10^{-9}	9
3	C_6H_5	Et	120–121[h]	EtOAc	78	$C_{22}H_{30}ClNO_2$[i]	A	$(3.3 \pm 0.1) \times 10^{-7}$	2.24	6.63 ± 0.09	2.34×10^{-7}	0.15
4	C_6H_5	*n*-Pr	119–121[j]	EtOAc	72	$C_{23}H_{32}ClNO_2$	A	$(1.2 \pm 0.2) \times 10^{-6}$	0.62			
5	C_6H_5	*i*-Pr	140–142[k]	EtOAc–cyclohexane	50	$C_{23}H_{32}ClNO_2$	A	$(1.3 \pm 0.1) \times 10^{-6}$	0.57			
6	C_6H_5	*n*-Bu	106–107	EtOAc	73	$C_{24}H_{34}ClNO_2$[l]	B	$(2.6 \pm 0.2) \times 10^{-6}$	0.28			
7	C_6H_5	*i*-Bu	149–151[m]	EtOAc–cyclohexane	55	$C_{24}H_{34}ClNO_2$	A	$(1.2 \pm 0.1) \times 10^{-6}$	0.62			
(±)-8	H	CH_2OH	170 (0.03 mm)		83[n]	$C_{15}H_{24}ClNO_3$[n]	C	$(7.0 \pm 0.1) \times 10^{-8}$	10.57	7.13 ± 0.03	7.40×10^{-8}	0.48
(+)-8a	H	CH_2OH	+19.5 (EtOH)		67[n]	$C_{15}H_{24}ClNO_3$[n]	C	$(2.1 \pm 0.3) \times 10^{-7}$	3.52			
(−)-8b	H	CH_2OH	−18.9 (EtOH)		52[n]	$C_{15}H_{24}ClNO_3$[n]	C	$(3.1 \pm 0.3) \times 10^{-8}$	23.87	7.40 ± 0.03	3.90×10^{-8}	0.91
(+)-9a	H	CH_2OTs	107–110	EtOAc	15	$C_{22}H_{30}ClNO_5S$	D			6.67 ± 0.13	2.13×10^{-7}	0.17
(−)-9b	H	CH_2OTS	109.5–111.5	EtOAc	18	$C_{22}H_{30}ClNO_5S$	D			6.42 ± 0.16	3.81×10^{-7}	0.09
(±)-10	H	$(CH_2)_4OH$	165–167 (0.007 mm)		84[n]	$C_{18}H_{30}ClNO_3$[n]	C	$(1.1 \pm 0.1) \times 10^{-6}$	0.67			
(+)-10a	H	$(CH_2)_4OH$	+16.6 (EtOH)			$C_{18}H_{30}ClNO_3$[n]	C	$(1.0 \pm 0.2) \times 10^{-6}$	0.74			
(−)-10b	H	$(CH_2)_4OH$	−18.3 (EtOH)			$C_{18}H_{30}ClNO_3$[n]	C	$(1.0 \pm 0.1) \times 10^{-6}$	0.74			
11		$=CH_2$	108–110	EtOAc	31	$C_{15}H_{22}ClNO_2$	E			5.86 ± 0.15	1.37×10^{-6}	0.03
12	cis			EtOAc		$C_{18}H_{30}ClNO$[o]				7.13 ± 0.16	7.48×10^{-8}	0.47
13	trans			EtOAc		$C_{18}H_{30}ClNO$[o]				6.38 ± 0.04	4.17×10^{-7}	0.09
atropine										8.73 ± 0.04	1.84×10^{-9}	19.3[p]

[a] Except where indicated all compounds are racemic. [b] Uncorrected. [c] All compounds had satisfactory C, H, N, and/or Cl microanalyses and were within 0.4% of theoretical values except when noted. All compounds exhibited IR, ^1H NMR, and MS spectra consistent with the assigned structures. [d] See the Experimental Section. [e] Antispasmodic activity on isolated guinea pig ileum were tested according to the method of Magnus[13] while the method of Long and Chiou[14] was used for isolated rat ileum studies. pA_2 values were calculated according to Ariens and van Rossum[15] and mean values were obtained from linear regression analysis of two or more experiments at four or more dose levels. [f] Literature[12] 113–114 °C. [g] Literature[18] 160–161 °C. [h] Literature[8] 124–126 °C. [i] C: calcd, 70.28; found, 69.86. [j] Literature[19] 122–123 °C. [k] Literature[10] 142 °C. [l] C: calcd, 71.35; found, 70.72. [m] Literature[19] 151–152 °C. [n] Very hygroscopic as HCl salt; thus it was characterized as its free amino ester. [o] The syntheses and cardiovascular pharmacology of these compounds has been reported.[6] [p] The antispasmodic activity of adiphenine (1) had been reported to be about 5% of that of atropine.[16]

(±)-Tropic acid was successfully resolved into its optically pure isomers with quinine and quinidine as the resolving agents. Both the racemic and optically active isomers of tropic acid were then converted to the desired amino esters **8**, **8a**, and **8b**, respectively, in good yield. Similarly, 4-hydroxy-2-phenylbutanoic acid, prepared from Ivanov reagent[11] and ethylene oxide, were resolved. However, attempts to convert this acid to the desired amino ester resulted in the formation of α-phenyl-γ-butyrolactone; presumably the amino ester formed during the reaction was very unstable and readily undergoes internal lactone formation. A similar lactone formation might also be expected for 5-hydroxy-2-phenylpentanoic acid; thus it was decided to prepare 6-hydroxy-2-phenylhexanoic acid (**15**). This was successfully obtained from 6-hydroxy-2-phenylhexanenitrile prepared in accordance with the method of Anker and Cook.[12] Again this acid was resolved into its optical isomers and converted to the desired compounds **10**, **10a**, and **10b**, respectively.

Pharmacological Results and Discussion

The antispasmodic activities of compounds synthesized in the early phase of the studies were tested on isolated guinea pig ileum with methacholine as a spasmogen according to the method of Magnus.[13] However, the most active compounds among those tested were reevaluated along with the other analogues on isolated rat ileum by the procedure of Long and Chiou[14] and the activities were compared to that of atropine to ensure the validity of our early results. A dose–response experiment was carried out in which ileal tissue contractions were measured with the application of increasing concentrations of ACh to the isolated tissue bath. The ability of each compound to block ACh-induced contractions of the ileum was determined and a pA_2 value calculated. The pA_2 value represents the affinity of the compounds for the muscarinic receptor. The results of these studies are summarized in Table I. Atropine was also studied in order to provide a base line for comparison with other compounds reported in the literature.[2,3] As can be seen, adiphenine (i.e., compound **1**), the smallest member of the 2,2-diphenylalkanoate series, has a pA_2 value of 7.45, which is slightly higher than the reported value of 6.85.[15] However, the anticholinergic activity of **1**, which has been reported to be about 5% of that of atropine, agreed with our present finding.[16]

From the SAR studies summarized in Table I, it is interesting to note that the most active analogues of the 2,2-diphenylalkanoate series is compound **2**, where the α-hydrogen of adiphenine (**1**) was replaced by a small methyl group. Further increase in the size of alkyl group drastically decreased the antispasmodic activity. These studies indicate that the hydrophobic region of the cholinergic muscarinic receptors possesses rather strict size limitation. It is noteworthy that the cis isomer of 2-phenylcyclohexyl (diethylamino)ethyl ether (**12**) has a pA_2 value of 7.13, identical with that of the (±)-tropic ester **8**. This compound and its trans-isomer **13** represent carbocyclic analogues of the atropine molecule (or the simple ester of (±)-tropic acid **8**) in which the hydroxymethyl moiety and the ester carbonyl group are linked together to form a six-membered ring through intramolecular hydrogen bonding. It is further noted that the antispasmodic activity of tropate ester **8** resides mainly in its *S*-(−) isomer similar to that of *S*-(−) hyoscyamine, the active enantiomer of atropine, while there was no difference among the racemic, *S*-(+), and *R*-(−) isomers of compound **10** (i.e., **10**, **10a**, and **10b**, respectively). These observations, along with the unsuccessful attempt to alkylate the hydrogen-bonding subsite by atropate ester, **11** via a Michael type addition, might be used to argue against the presence of a nucleophilic group in a region known to be hydrophobic in nature. Although the enantiomeric selectivity exhibited by **8** may be explained via the classical three-point interactions with the receptor, an additional role for the hydroxy group may be envisioned to account for the lack of stereoselectivity of compounds **9** and **10** and for the equipotency of carbocyclic analogue **12** to that of the tropate ester **8**. These observations, along with the inability of **11** to alkylate the hydrogen-bonding subsite, may suggest the involvement of intramolecular hydrogen bonding between the hydroxy group and the ester carbonyl group in the enhancement of atropine-like potency, i.e., to direct the essential aromatic group toward a specific location of the receptor.

Further studies with conformationally restricted analogues along resolution of the carbocyclic analogues **12** and **13** are in progress. The present studies provide valuable insight into the stereospecificity and the effective size of this presumed hydrophobic region of the muscarinic receptors.

Experimental Section

Chemistry. All melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Micro-Tech Laboratories, Skokie, IL. IR spectra were obtained with a Perkin-Elmer 337 or 700 spectrophotometer. ^1H NMR spectra were determined at 60 MHz on a Varian T-60 A spectrometer equipped with a Nicolet TT-7 Fourier transform accessory. ^1H chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane, $(CH_3)_4Si$, as the internal standard. The abbreviations s, d, t, and m refer to singlet, doublet, triplet, and multiplet, respectively. Mass spectra were obtained by Richard Dvorak, using Hitachi Perkin-Elmer RMU-6D single-focusing mass spectrometer. Spectra were recorded at 80 °C and 70 eV unless otherwise noted. Optical rotations were measured on a Perkin-Elmer polarimeter, Model 241, using a 1-dm tube.

Preparation of the 2,2-Diphenylalkanoic Acids. The general procedure employed here is suitably illustrated by the preparation of 2,2-diphenylpropanoic acid or 2,2-diphenyl-3-methylbutanoic acid.

A. From the Hydrolysis of 2,2-Diphenylpropanenitrile. A mixture of diphenylacetonitrile (19.3 g, 0.12 mol), sodamide (4.8 g, 0.12 mol), and dry benzene (60 mL) was refluxed with stirring for 2 h. The resulting mixture was cooled in a water bath and methyl iodide (28.4 g, 0.2 mol) was added dropwise. After the addition, the mixture was gently refluxed for 2 h and cooled in an ice bath. Water (300 mL) was added, and the layers were separated. The aqueous layer was extracted with ether (2 × 50 mL). The benzene layer was combined with the ether extracts and washed with water (100 mL). After removal of the solvents, the remaining residue was distilled in vacuo, yielding 17.7 g (85%) of 2,2-diphenylpropanenitrile: bp 103–105 °C (0.1 mm) [lit.[8] bp 127–130 °C (1.5 mm)]; IR (neat) 2300 cm^{-1} (C≡N); ^1H NMR ($CDCl_3$) δ 1.95 (s, 3 H, CH_3), 7.30 (s, 10 H, Ar *H*).

A solution of 11 g (0.53 mol) of the above nitrile in 70% sulfuric acid (50 mL) was heated with stirring in an oil bath at 150 °C until a test portion of this solution remained clear when diluted with water and made alkaline (about 48 h). It was cooled and poured into ice–water (500 mL) with vigorous stirring. The solid that separated was filtered and redissolved in 5% sodium hydroxide. This alkaline solution was then washed with ether, filtered, and then slowly acidified wit 10% sulfuric acid. The crude acid that precipitated was filtered, dried, and recrystallized from benzene to give 7.0 g of 2,2-diphenylpropanoic acid (61%), mp

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173–175 °C (lit.[8] mp 173–175 °C).

In a similar manner, 2,2-diphenylbutanenitrile [79% yield, bp 145–147 °C (0.3 mm)], 2,2-diphenylbutanoic acid (64% yield, mp 170–172 °C), 2,2-diphenylpentane nitrile [66% yield, bp 126–130 °C (0.05 mm)], 2,2-diphenylpentanoic acid (18% yield, mp 153–157 °C), 2,2-diphenylhexanenitrile [64% yield, bp 127–129 °C (0.05 mm)] were obtained.

B. From the Oxidation of 2,2-Diphenylpropanal. A mixture of 2,2-diphenylpropanal (3 g, 0.014 mol), potassium permanganate (15 g), sodium bicarbonate (2 g), and water (18 mL) was heated with stirring on a steam bath until the pink color of the permanganate had disappeared. The manganese dioxide was removed by filtration and the filtrate, after being washed with ether, was acidified with 10% sulfuric acid to give the crude acid, 2.1 g (65%), which melted at 171–173 °C (recrystallized from benzene). Its IR and NMR spectra were found to be identical with that obtained from method A above.

Similarly prepared were 2,2-diphenylbutanoic acid (40% yield, mp 170–172 °C) and 2,2-diphenylpentanoic acid (16% yield, mp 151–154 °C).

C. From the Alkylation of Diphenylacetic Acid. 2,2-Diphenyl-3-methylbutanoic acid [mp 163–164 °C (lit.[8] mp 163 °C), yield 83%] and 2,2-diphenyl-4-methylpentanoic acid (mp 140–142 °C, yield 89%) were prepared by the alkylation of diphenylacetic acid in the presence of sodium amide with the appropriate alkyl halide following the method described by Ioffe et al.[10]

Preparation of 2-(Diethylamino)ethyl Esters. Method A. Reaction of an Acid Chloride with 2-(Diethylamino)ethanol. A mixture of diphenylacetic acid (8.5 g, 0.04 mol) and thionyl chloride (25 mL) was refluxed on a steam bath for 1 h. The excess thionyl chloride was azeotropically removed with dry benzene under reduced pressure and the residue redissolved in dry benzene (50 mL). To this solution was added 2-(diethylamino)ethanol (10 g, freshly distilled, 0.08 mol) with stirring. The mixture was refluxed for 20 h on a steam bath and then was allowed to cool to room temperature. It was extracted with 5% NaOH solution (60 mL) and the benzene layer was separated. The alkaline solution was extracted twice with ether (50 mL). The ether extracts werre combined with the benzene layer, washed with water, dried (anhydrous Na_2SO_4), and filtered. Removal of the solvent in vacuo gave a yellowish residue, which was distilled in vacuo to give compound **1**: 11.6 g (84%); bp 156–157 °C (0.05 mm); IR (neat), 1750 (C=O), 1150 cm^{-1} (C—O); ^1H NMR δ 1.00 (t, 6 H, CH_3), 2.64 (m, 6 H, NCH_2), 4.28 (t, 2 H, OCH_2), 5.10 (s, 1 H, α-H), 7.40 (s, 10 H, Ar H). The hydrochloride salt of compound 1 was prepared by passing anhydrous HCl gas into an ether solution of the amino ester. The white hydrochloride salt that precipitated was collected and recrystallized twice from ethyl acetate to give a white crystalline solid, mp 112–113 °C (lit.[12] mp 113–114 °C); MS, m/e 311 (M^+ – HCl). Anal. ($C_{20}H_{26}ClNO_2$) C, H, N, Cl.

Similarly prepared were compounds **2–5** and **7**, listed in Table I.

Method B. Alkylation of 2-(Diethylamino)ethyl Diphenylacetate. 2-(Diethylamino)ethyl diphenylacetate (1; 15.5 g, 0.05 mol) was added to a suspension of sodamide (2 g, 0.05 mol) in liquid ammonia. After the mixture was stirred for 30 min, a solution of n-butyl bromide (13.7 g, 0.1 mol) in anhydrous ether (20 mL) was added and the reaction mixture was stirred at room temperature for 2 h during which time liquid ammonia was added to maintain the original volume. A slight excess of ammonium chloride (3 g) was added to the mixture, and the ammonia was allowed to evaporate by stirring the mixture at room temperature. The residue remaining after the evaporation of ammonia was added to a mixture of equal volumes of ether and water (200 mL). The aqueous layer was separated and extracted twice with 50-mL portions of ether. The ether layer was combined with the ether extracts, washed with saturated sodium bicarbonate solution, and dried (anhydrous Na_2CO_3). After removal of ether, the residue was distilled in vacuo to yield 13.5 g (73%) of amino ester of **6**: bp 141–143 °C (0.001 mm); n^{25}_D 1.5389; IR (neat) 1730 (C=O), 1200 cm^{-1} (C—O); ^1H NMR ($CDCl_3$) δ 1.00 (m, 13 H, 2 NCH_2CH_3 and $CH_3CH_2CH_2CH_2$), 2.50 (m, 8 H, 3 NCH_2 and 2 β-H), 4.20 (t, 2 H, OCH_2), 7.25 (s, 10 H, Ar H).

The hydrochloride of the amino ester of **6**, after it was recrystallized from ethyl acetate, melted at 106–107 °C: MS, m/e 367 (M^+ – HCl). Anal. ($C_{24}H_{34}ClNO_2$) C, H, N, Cl.

Method C. Reaction of an Acid with 2-(Diethylamino)ethyl Chloride. A solution of (±)-6-hydroxy-2-phenylhexanoic acid (**15**; 10.4 g, 0.05 mol) in isopropyl alcohol and 2-(diethylamino)ethyl chloride (7 g, 0.05 mol) was heated on a steam bath for 6 h. The reaction mixture was cooled to room temperature and filtered to remove a small amount of precipitate. The filtrate was evaporated to dryness in vacuo to give a viscous liquid, which was washed repeatedly with ether and then dissolved in 10% NaOH (30 mL), and the aqueous solution was extracted with ether (3 × 100 mL). The ether extracts were combined, washed with water, dried (anhydrous Na_2CO_3), and decolorized with charcoal. After removal of the solvent, a nearly colorless liquid was obtained, which when purified by distillation yielded 13 g (84%) of 2-(diethylamino)ethyl 6-hydroxy-2-phenylhexanoate (**10**): bp 165–167 °C (0.007 mm); NMR as expected; MS m/e 307 (M^+). Anal. ($C_{18}H_{29}NO_3$), C, H, N.

2-(Diethylamino)ethyl (±)-tropate [**8**; 83% yield; bp 170 °C (0.03 mm)] and its optical isomers **8a** and **8b** were prepared similarly.

Method D. (+)-(*R*)-2-(Diethylamino)ethyl 2-Phenyl-3-(tosyloxy)propanoate (9a). A solution of (+)-**8a** (2.29 g, 0.009 mol) and p-toluenesulfonyl chloride (3.5 g, 0.0166 mole) in 50 mL of pyridine was stored in a refrigerator for 22 h and an additional 5 h at room temperature. After the work-up procedure, 0.5 g, (15%) of compound **9a** was obtained: TLC (ether–concentrated NH_4OH) R_f 0.28; ^1H NMR ($CDCl_3$) δ 0.94 (t, 6 H, CH_2CH_3), 2.37–2.72 (m, 9 H, NCH_2 and $ArCH_3$), 3.50 (m, 1 H, ArCH), 4.15–4.54 (m, 4 H, OCH_2 and CH_2OSO_2), 7.30 (s, 5 H, Ar H), 7.22–7.82 (4 H, AB quartet); IR (neat), 1750 cm^{-1} (C=O); MS m/e (relative intensity) 419 (1, M^+), 247 (2, $M^+ - OSO_2C_6H_4CH_3$), 103 (17, M^+ – OTs and $COOCH_2CH_2NEt_2$), 99 (12, $CH_2CH{=}N^+Et_2$), 86 (100, $CH_2{=}N^+Et_2$), 77 (9, $C_6H_5^+$), 58 (9, $CH_2{=}N^+HEt$).

The hydrochloride salt was obtained by passing anhydrous hydrochloric acid into the ether solution of the amino ester. After recrystallization from ethyl acetate, it melted at 107–110 °C. Anal. ($C_{22}H_{30}NO_5SCl$) C, H, N.

Similarly prepared was (–)-(*S*)-2-(diethylamino)ethyl 2-phenyl-3-(tosyloxy)propanate (**9b**), mp 109.5–111.5 °C.

Method E. Preparation of 2-(Diethylamino)ethyl Atropate (11). A solution of tropic acid (6 g, 0.036 mol) in 15 mL (0.2 mol) of thionyl chloride was heated to reflux on a steam bath for 2 h. The excess thionyl chloride was removed under reduced pressure, and the trace of thionyl chloride was further removed azeotropically with dry benzene. The residue was dissolved in 50 mL of dry benzene, and 2-(diethylamino)ethanol (8.5 g, 0.072 mol) was added in one portion with stirring. The mixture was heated to reflux on a steam bath for 18 h and then cooled. It was extracted with 5% ice-cold sodium bicarbonate (2 × 50 mL) and the benzene layer was separated. The aqueous layer was extracted with ether (3 × 50 mL). The ether extracts were combined with the benzene layer, washed with ice-cold water (2 × 50 mL), and dried (anhydrous $MgSO_4$). After removal of the solvent, a yellowish oil was obtained (7.62 g, 90.1%). The product was separated by column chromatography using hexane as the initial eluent. Elution with hexane–acetone (85:15) gave the desired amine **11** as a yellowish oil (2.62 g, 31%): TLC (hexane–acetone = 85:15) R_f 0.20; ^1H NMR ($CDCl_3$) δ 1.00 (t, 6 H, CH_2CH_3), 2.57 (q, 4 H, CH_2CH_3), 2.77 (t, 2 H, J = 6.0 Hz, CH_2N), 4.30 (t, 2 H, J = 6.0 Hz, OCH_2), 5.87 (s, 1 H, vinyl proton, trans to C=O), 6.33 (s, 1 H, vinyl proton cis to C=O), 7.35 (s, 5 H, Ar H); IR (neat) 1710 (conjugate), 1640 cm^{-1} (C=C); MS m/e (relative intensity) 247 (3, M^+), 232 (2, $M^+ - CH_3$), 175 (7.5, $M^+ - NEt_2$), 131 (2, $M^+ - OCH_2CH_2NEt_2$), 103 (16, $M^+ - COOCH_2CH_2NEt_2$), 99 (12, $CH_2CH{=}N^+Et_2$), 86 (100, $CH_2{=}N^+Et_2$), 77 (10, $C_6H_5^+$), 58 (12, $CH_2{=}N^+Et(H)$).

The hydrochloride salt was obtained by passing anhydrous hydrochloric acid into the ether solution of **11**. After recrystallization from ethyl acetate, it melted at 108–110 °C. Anal. ($C_{15}H_{22}ClNO_2$) C, H, N.

(±)-6-Hydroxy-2-phenylhexanoic Acid (15). 6-Hydroxy-2-phenylhexanenitrile[12] was hydrolyzed by heating on a steam bath for 20 h in 25% sulfuric acid (120 mL). After the mixture was cooled in an ice bath, it was poured in 200 mL of cold water. An oil that separated was extracted with ether (2 × 100 mL). The ether extracts were combined, washed several times with water,

and extracted with 5% sodium hydroxide (2 × 100 mL). The alkaline solution, after being washed several times with ether, was acidified with concentrated hydrochloric acid to give an oil, which was extracted with ether (2 × 100 mL). The ether extracts were combined, washed with water, dried (anhydrous $MgSO_4$), and finally decolorized with charcoal (2 g). Upon removal of the solvent a semisolid liquid (25 g, 83%) was obtained. It decomposed on distillation in vacuo and could not be solidified. It was heated on a steam bath in vacuo for 4 h to remove any volatile impurities: IR (neat) 3600–2400 (OH, COOH), 1720 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 1.70 (m, $CH_2CH_2CH_2$), 3.70 (m, CH_2O and αH), 7.15 (s, COOH and OH), 7.40 (s, Ar H). Anal. ($C_{12}H_6O_3$) C, H.

(+)-6-Hydroxy-2-phenylhexanoic Acid (15). A mixture of (±)-15 (21 g, 0.1 mol) and quinine hydrate (38 g, 0.1 mol) was dissolved in ethanol (40 mL) by warming on a steam bath. Hot water (about 20 mL) was added until the solution became slightly turbid, which was cleared by addition of more ethanol. The resulting solution was allowed to cool to room temperature, during which time a solid precipitated. Further purification of this quinine salt by three successive recrystallizations from ethanol gave a product with constant specific rotation at $[\alpha]^{25}_D$ −74° (ethanol).

The (+)-15 was liberated from this quinine salt by the addition of 10% hydrochloric acid to yield a light yellowish viscous liquid (4.6 g, 44% recovery) with a $[\alpha]^{25}_D$ value of +48.3° in ethanol. Its IR and NMR spectra were identical with that of the racemic acid.

(−)-6-Hydroxy-2-phenylhexanoic Acid (15). The original liquor from the precipitation of the quinine salt of (+)-15 was evaporated to dryness, and the acid was liberated from the residue by addition of 10% hydrochloric acid to give a partially resolved (−)-15 having a $[\alpha]^{25}_D$ value of −37.3°.

This partially resolved acid (16 g) and quinidine hydrate (17 g) were dissolved in 60 mL of ethanol by warming on a steam bath. The resulting solution was cooled at 5 °C overnight to yield 12 g of a solid with a $[\alpha]^{25}_D$ value of +148° (ethanol). This solid was recrystallized once from ethanol (30 mL) to give 7.4 g of pure salt having a $[\alpha]^{25}_D$ value of +143.4° (ethanol) and a melting point of 155–157 °C.

Compound (−)-15 was liberated by acidifying the quinidine salt with 10% hydrochloric acid to yield (2.2 g, 20% recovery) of a light yellowish and viscous liquid with a $[\alpha]^{25}_D$ value of −50.6° in ethanol. Its IR and NMR spectra were identical with that of the racemic acid.

(+)- and (−)-tropic acids were obtained from the resolution of (±)-tropic acid in a similar manner. (+)-(R)-Tropic acid: $[\alpha]^{20}_D$ +71.2°; mp 128–129 °C (lit.[17] $[\alpha]^{20}_D$ +71.8°; mp 128–129 °C). (−)-(S)-Tropic acid: $[\alpha]^{20}_D$ -75°; mp 128–129 °C (lit.[17] $[\alpha]^{20}_D$ −78°; mp 129–130 °C).

Pharmacology. Materials and Methods. Male guinea pigs weighing 250–300 g were purchased from Scientific Small Animal Co., Inc., Arlington Height, IL. Male Sprague–Dawley rats (250–300 g) were obtained from King Animal Laboratories, Oregon, WI. Each animal used in these experiments was fasted overnight and sacrificed by cervical dislocation. 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. Atropine sulfate was obtained from Sigma Chemical Co., St. Louis, MO. Acetylcholine chloride and methacholine chloride were obtained from Merck & Co., Inc., Rahway, NJ. Tyrode solution contained 8.0 g of NaCl, 0.2 g of KCl, 0.2 g of $CaCl_2$, 0.01 g of $MgCl_2$, 1.0 g of dextrose, 1.0 g of $NaHCO_3$, and 0.05 g of monosodium phosphate in 1 L of distilled water. The Tyrode solution and all test drug solutions were freshly prepared prior to each experiment.

Antispasmodic Activity on Isolated Guinea Pig Ileum. A 2–3-cm strip of the ileum obtained from the intestine of a previously fasted guinea pig was placed in a dish containing Tyrode solution. A thread was attached at each end of the muscle strip, and one end was tied to a fixed pin in the organ bath and the other to a transducer connected to a physiograph recorder. The assay was performed at 37 °C in a 25-mL organ bath in Tyrode solution through which a gas mixture (5% CO_2–95% O_2) was bubbled. Once the tissues were acclimated to the surroundings (about 30 min), a standard dose (4 × 10^{-6} M) of methacholine chloride was introduced into the bath to induce muscle contractions. Several such injections of this standard solution were made to insure the consistency of the response. Then an appropriate concentration of the compound to be studied was administered by means of a syringe at a constant rate, directly into the tissue bath. Fifteen minutes following the administration of the compound and without washing out the compound, another dose of methacholine chloride was repeated and the response of muscle to this agonist was recorded. The difference in muscle contractions caused by methacholine chloride before and after the administration of the test compound was recorded and calculated in terms of percent inhibition. The concentration of the compound that was required to produce a decrease of 50% of the standard response to the agonist, methacholine chloride, i.e., ID_{50}, was determined by linear regression analysis and used as an indication of antispasmodic potency of the compounds being tested.

Antispasmodic Activity on Isolated Rat Ileum. Male Sprague–Dawley rats weighing 250–300 g were used in these experiments. Each animal was 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 fixed level by means of a fine silk thread in the tissue bath. The other end of the muscle was attached to a force-displacement transducer, which in turn was connected to an ink-writing recorder (Narco-Biosystems, Table Model No. DMP-4A). The bioassay was performed at 35 °C in a 10-mL organ bath containing Tyrode solution through which a stream of a 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 later a second dose–response curve was determined in the presence of the test compound. 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: 0.05, 0.1, 0.3, and 1.0 μg/mL. These doses were added in sequence in a cumulative manner at 3-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 10 min of equilibration, higher doses of ACh in the sequence 1, 3, 10, 30, 100, and 300 μg/mL were added to the bath in a cumulative manner at 3-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 $pA_2 = pA_x + \log (x - 1)$. 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 to produce 50% response in the presence of an antagonist to that in the absence of the antagonist. By definition, 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 antagonist (ACh).

Attempted Alkylation of the Muscarinic Receptor. With longer incubation time (30 min), the ACh-induced contraction of isolated rat ileum was completely blocked by atropate ester 11 at 40 μg/mL. However, by washing the tissue with fresh Tyrode solution, 50% of the contractibility was recovered within 1 h although total recovery required approximately 5 h.

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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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