Synthesis and Characterization of All Four Isomers of the Muscarinic Agonist 2′-Methylspiro[1-azabicyclo[2.2.2]octane-3,4′-[1,3]dioxolane]

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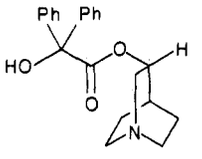
The four separated isomers of the muscarinic agonist 1, previously known as AF30, have been synthesized by a route that has allowed the absolute stereochemistry of each isomer to be assigned. With the chirality of (−)-camphanic acid known, X-ray analysis of the more crystalline intermediate diastereomeric camphanate **5A** allowed the absolute stereochemistry at the quinuclidine chiral center to be determined. Each diastereomer was separately transformed into the spirodioxolane with concomitant introduction of the second chiral center. Chromatographic separation followed by a second crystal structure determination revealed the absolute stereochemistry of all four isomers of 1. Detailed biological evaluation of each isomer indicated that while the 3(*R*),2′(*S*) isomer was the most active in binding studies, it was the 3(*R*),2′(*R*) isomer that displayed the largest functional selectivity between ganglion (M-1 site) and heart (M-2 site). With the same internal chiral standard, the absolute configuration of the more active enantiomer of **3** was shown to be *S*, confirming earlier literature reports.

There has been considerable recent interest in the development of ligands that are selective for those subtypes of muscarinic receptors classified as M-1 (pirenzepine-sensitive) and M-2 (relatively less sensitive to pirenzepine).[1] In particular, an agonist capable of acting on central muscarinic receptors without activating those in cardiac and smooth muscle and in exocrine glands (M-2) may be beneficial in alleviating the symptoms of presenile and senile dementia[2] (also known as Alzheimer's disease and senile dementia of the Alzheimer type, respectively). Since it has been proposed[3] that the cortical muscarinic receptors are similar to those present in sympathetic ganglia (M-1), many agonists have been assessed for activity in the isolated superior cervical ganglion relative to their ability to stimulate muscarinic receptors in isolated preparations of heart and small intestine. Muscarinic agents that have been described as selective ganglionic stimulants include [4-[[(*m*-chlorophenyl)carbamoyl]oxy]-2-butynyl]trimethylammonium chloride (McN-A-343),[4] *N*-benzyl-*N*-methyl-3-pyrrolidinyl acetate (AHR602),[5] and 2′-methylspiro[1-azabicyclo[2.2.2]octane-3,4′-[1,3]dioxolane] (**1**, AF30).[6,7] Of these, only the last would be expected to penetrate the blood–brain barrier and therefore be of use in senile dementia.

Although AF30 has previously[7] been separated into diastereomeric pairs (called "cis" and "trans"), complete purification of the "trans" pair has not been reported. To assist our studies in the design of new muscarinic agonists and because of the problems associated with the assessment of the pharmacological profile of the compound as a mixture of four isomers, it was important to separate **1** into its constituent isomers. Additionally, there has been considerable confusion in the literature about the absolute stereochemistry of related muscarinic ligands. Thus, while the absolute configuration of the more active isomer of the muscarinic antagonist 3-quinuclidinyl benzilate (**2**, QNB) has been assigned[8,9] as *R*, there is now general agreement[10] that it is the *S* isomer of 3-acetoxyquinuclidine (**3**) that is the active agonist. On the other hand, the most recent opinion[10] is that (*R*)-*N*-methyl-3-acetoxyquinuclidine (**4**) is the more potent isomer of the related quaternary amines.

We report on the synthesis of all four separated isomers of **1**, the determination by X-ray crystallography of the absolute stereochemistry of the most active isomer, and the results of a comparison of the activities of **1** and the separated four isomers in biochemical and pharmacological assays. Additionally, the absolute stereochemistry of the isomers of 3-acetoxyquinuclidine, resolved by using the literature procedure, has been unequivocally assigned by using the same internal stereochemical standard.



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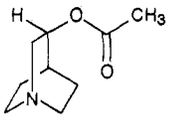

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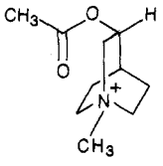

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Results

I. Chemistry and X-ray Crystallography. The synthetic route (Scheme I) to the separated isomers of **1** follows the original methodology[11] except for a diversion via the diastereomeric camphanate esters **5** derived from (−)-camphanic acid chloride **6**.[12] The isomeric purity of

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(1) For reviews, see: "Subtypes of Muscarinic Receptors" in *Trends Pharmacol. Sci.*, Suppl. 1984 and 1986.
(2) Perry, E. K. *Br. Med. Bull.* **1986**, *42*, 63.
(3) Hammer, R.; Giachetti, A. *Life Sci.* **1982**, *31*, 2991.
(4) Roszkowski, A. P. *J. Pharmacol. Exp. Ther.* **1961**, *132*, 156. Roszkowski, A. P., Yelnosky, J. *J. Pharmacol. Exp. Ther.* **1967**, *156*, 238.
(5) Franko, B. V.; Ward, J. W.; Alphin, R. S. *J. Pharmacol. Exp. Ther.* **1963**, *139*, 25.
(6) Fisher, A.; Weinstock, M.; Gitter, S.; Cohen, S. *Eur. J. Pharmacol.* **1976**, *37*, 329.
(7) Fisher, A.; Abraham, S.; Lachman, C.; Lass, Y.; Akselrod, S.; Akerman, E.; Cohen, S. *Monogr. Neural Sci.* **1980**, *7*, 41.
(8) Meyerhöffer, A. *J. Med. Chem.* **1972**, *15*, 994.
(9) Rehavi, M.; Maayani, S.; Sokolovsky, M. *Life Sci.* **1977**, *21*, 1293.
(10) Ringdahl, B.; Ehler, F. J.; Jenden, D. J. *Mol. Pharmacol.* **1982**, *21*, 594. The authors of this paper refer to (*S*)-(+)-aceclidine as the more active isomer both in terms of pharmacological potency and in vitro binding affinity. The sign of rotation must clearly refer to that of the hydrochloride.
(11) Cohen, S.; Fisher, A. U.S. Patent 4 104 397, 1978.

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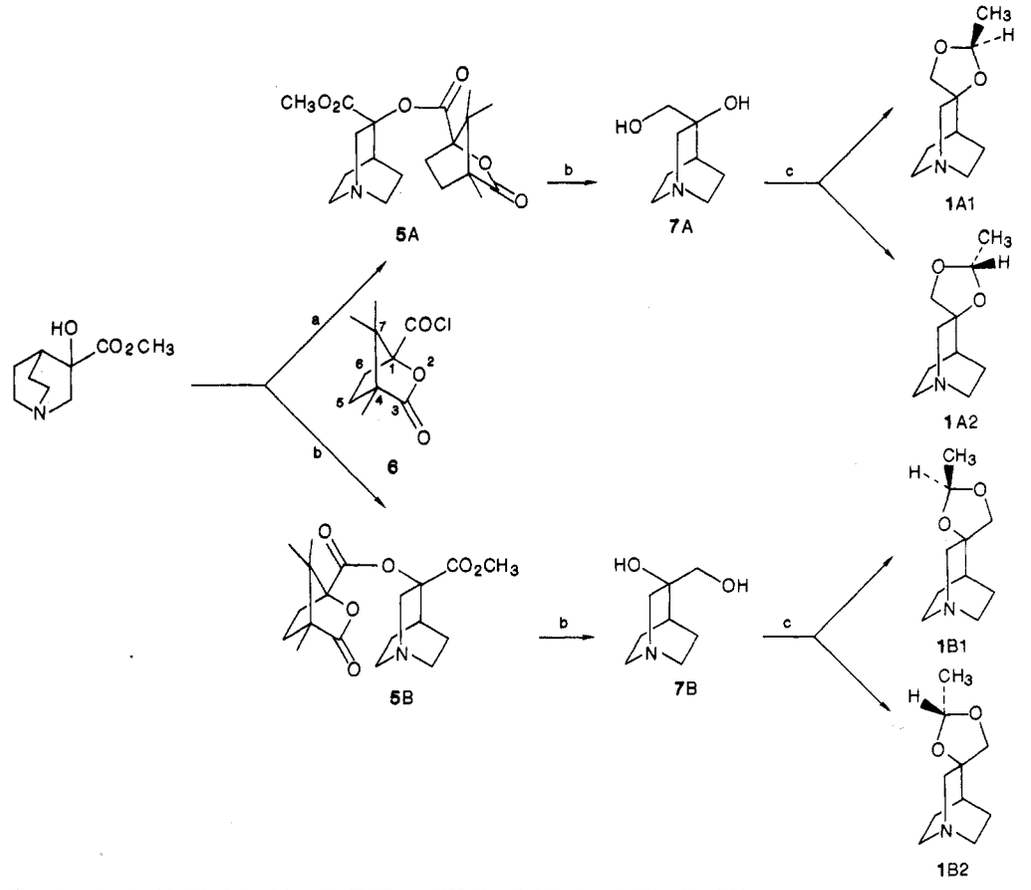
Scheme I[a]



[a] Reagents: a, CH_2Cl_2, Et_3N, 10 °C, **6**; b, $LiAlH_4$, THF; c, CH_2Cl_2, 0 °C, CH_3CHO, $BF_3{\cdot}OEt_2$.

these esters was readily confirmed by both GC and NMR. Thus the faster running isomer on TLC on alumina (diastereomer A) had a shorter retention time on capillary GC (SE30 column) and was completely free of the peak corresponding to the second isomer (diastereomer B). The NMR spectrum of the former was characterized by two low-field singlets at δ 1.05 and 1.13 (6 H and 3 H, respectively) whereas diastereomer B had three methyl signals in this region (δ 1.01, 1.11, and 1.13).

The camphanate esters **5** were separately reduced with $LiAlH_4$ to give two enantiomeric diols **7**, which were converted directly to the spiro dioxolane. In each case, the concomitant introduction of a second chiral center generated two new diastereomeric compounds, which could be separated by chromatography on alumina and assessed for isomeric purity by GC. The two dioxolanes (1A1 and

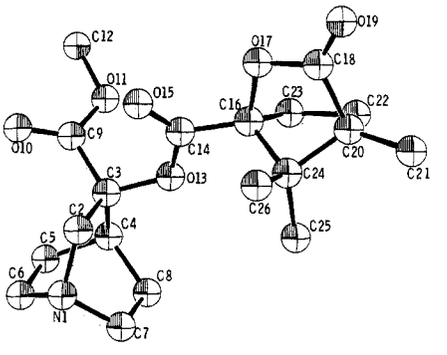


Figure 1. Computer-generated ORTEP drawing of **5A** derived from the X-ray coordinates with hydrogen atoms removed for clarity.

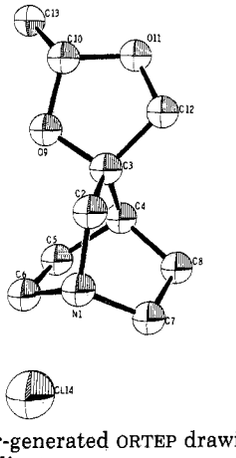
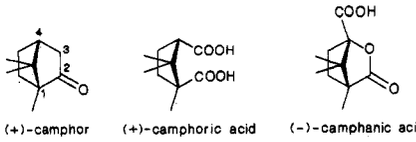


Figure 2. Computer-generated ORTEP drawing of **1B2** derived from the X-ray coordinates.

(12) There may be some confusion as to the absolute stereochemistry of (−)-camphanic acid because of the differing numbering schemes used by the major suppliers. (−)-Camphanic acid is derived from (+)-camphor:



(−)-Camphanic acid may be assigned (1*R*,4*S*)-camphanic acid (Aldrich) by using the camphor numbering scheme. However, we have chosen to number camphanic acid symmetrically so that (−)-camphanic acid can be ascribed a 1*S*,4*R* configuration (Fluka):

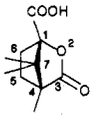
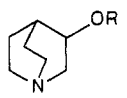


Table I.[a] Comparison of Optical Rotation Data with Literature[13] values



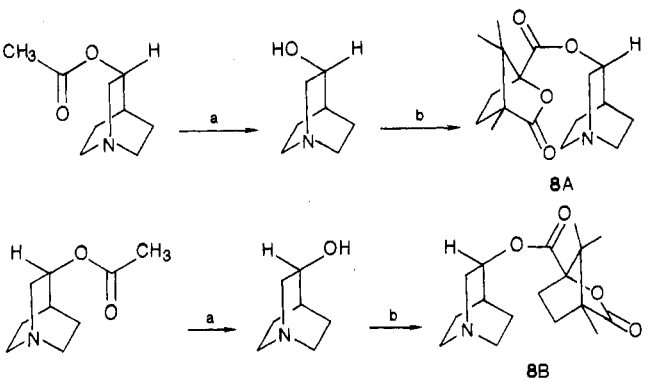
R	salt	$[\alpha]^{23}_D$, deg	lit. $[\alpha]^{22}_D$, deg
	(a) Compounds Derived from (+)-Tartaric Acid (*R* Series)		
$COCH_3$	(+)-hydrogen tartrate	+4.0 (*c* 2.2, H_2O)	+3.6 (*c* 2.2, H_2O)
$COCH_3$	free base	+26.3 (*c* 3, EtOH)	+29.8 (*c* 3, EtOH)
$COCH_3$	HCl	−16.4 (*c* 2, H_2O)	−14.8 (*c* 2, H_2O)
$COCH_3$	methiodide	−10.3 (*c* 2, H_2O)	−10.3 (*c* 2, H_2O)
H	free base	−37.0 (*c* 1, 1 M HCl)	−45.7 (*c* 2.9, 1 M HCl)
camphanate, 8A	free base	+7.4 (*c* 1, MeOH)	
	(b) Compounds Derived from (−)-Tartaric Acid (*S* Series)		
$COCH_3$	(−)-hydrogen tartrate	−3.8 (*c* 2.2, H_2O)	−3.7 (*c* 2.2, H_2O)
$COCH_3$	free base	−29.8 (*c* 3, EtOH)	−30.1 (*c* 2.8, EtOH)
$COCH_3$	HCl	+16.0 (*c* 2, H_2O)	+14.2 (*c* 2.2, H_2O)
$COCH_3$	methiodide	+10.4 (*c* 2, H_2O)	+10.5 (*c* 2, H_2O)
H	free base	+39.3 (*c* 1, 1 M HCl)	+46.5 (*c* 3, 1 M HCl)
camphanate, 8B	free base	−11.5 (*c* 1, MeOH)	

[a] Conditions (concentration and solvent) are given in parentheses.

1A2) derived from 5A (and, of course, 1B1 and 1B2 derived from 5B) were also readily distinguished by the appearance of the AB pattern resulting from the methylene protons in the dioxolane ring. To determine the absolute stereochemistry of each isomer of 1, it was necessary to first deduce that of the enantiomeric diols 7 by reference to their precursor camphanate esters. Using the known chirality of (−)-camphanic acid (3-oxo-4(*R*),7,7-trimethyl-2-oxabicyclo[2.2.1]heptane-1(*S*)-carboxylic acid), X-ray analysis of the less polar camphanate ester (5A) showed that 5A, and hence compounds derived from this, and *S* stereochemistry at the quinuclidine chiral center (Figure 1). The crystal structure of the slower running isomer on TLC of the dioxolanes derived from 5B indicated that this isomer (1B2) had *R* configuration at the dioxolane 2-position assuming *R* stereochemistry at the quinuclidine center (Figure 2). Thus the absolute configuration of each of the four stereoisomers of 1 is as indicated in Scheme I.

Using the literature method,[13] we obtained the two enantiomers of 3-acetoxyquinuclidine hydrochloride (3) and its methiodide (4) by employing (+)-tartaric acid in order to obtain selective crystallization of 3(*R*)-acetoxyquinuclidine (+)-hydrogen tartrate (stereochemistry implied from the literature). Isolation of the free base from the filtrate followed by crystallization with (−)-tartaric acid gave the *S* isomer (Table I). Each hydrogen tartrate salt was separately basified and hydrolyzed to give (*R*)- and (*S*)-3-quinuclidinol, and these were converted to their camphanate esters (8, Scheme II). The two diastereomers thus formed were inseparable by TLC on alumina or by capillary GC (SE30 column) but were distinguishable by high-field NMR. Again, with the stereochemistry of the internal chiral standard known, the crystal structure of the (−)-isomer revealed that the quinuclidine 3-position had *S* stereochemistry (Figure 3) and hence confirmed the same assignment to the more active isomer of 3-acetoxyquinuclidine.

Scheme II[a]



[a] Reagents: a, 2 N NaOH, 20 °C; b, CH_2Cl_2, 10 °C, Et_3N, 6.

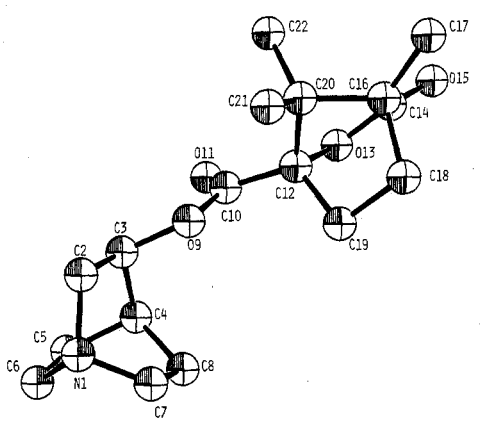


Figure 3. Computer-generated ORTEP drawing of 8B derived from the X-ray coordinates.

Table II. Comparison of Muscarinic Binding Affinities of the Isomers of 1 with Those of Carbachol and Muscarine

compound	heart[a] (M-2), [^{3}H]NMS: K_{app},[b] μM (N_H)[c]	cortex[a] (M-1), [^{3}H]pirenzepine: K_{app},[b] μM (N_H)[c]	M-2/ M-1
carbachol	1.9 (0.51)	45 (0.59)	0.042
muscarine	2.3 (0.49)	28 (0.64)	0.082
1	13 (0.76)	6.8 (0.92)	1.9
1A1 + 1B1 ("cis")	8.4 (0.72)	5.9 (0.97)	1.4
1A1	64 (0.84)	55 (0.92)	1.2
1A2	92 (0.85)	66 (0.99)	1.4
1B1	2.2 (0.70)	2.7 (0.89)	0.81
1B2	8.5 (0.78)	4.1 (0.97)	2.1

[a] Binding affinities determined in rat heart and cortex and described in Experimental Section. [b] K_{app} is the apparent affinity constant corrected from the IC_{50} by using the Cheng–Prusoff equation and concentrations of 0.2 nM [^{3}H]-*N*-methylscopolamine and 1 nM [^{3}H]pirenzepine. [c] N_H is the Hill coefficient of the displacement curve.

II. Biochemistry. The four separate isomers of 1, the "cis" pair of isomers, and 1 itself were compared to the classical agonists muscarine and carbachol in muscarinic binding assays[14] (Table II). M-1 receptor activity was estimated by using low concentrations of [^{3}H]pirenzepine to selectively label the M-1 receptor population in the rat cerebral cortex. In parallel studies, M-2 receptor affinity was measured by using [^{3}H]-*N*-methylscopolamine (NMS) binding to membranes prepared from rat heart, a tissue containing a predominance of M-2 receptors. The degree of deviation from unity of the Hill coefficient estimated from the displacement curves for each agonist was taken

(13) Ringdahl, B.; Resul, B.; Dahlbom, R. *Acta Pharm. Suec.* **1979**, *16*, 281.

(14) Watson, M.; Yamamura, H. I.; Roeske, W. R. *Life Sci.* **1983**, *32*, 3001.

Table III. Comparison of Isomers of 1 with Standards (Carbachol and Muscarine) in in Vitro Pharmacological Assays

	ganglion[a]			guinea pig ileum[a]			atria[a]		
compound	pEC_{50}[b] ± SEM[c]	FE[d]	n[e]	pED_{50} ± SEM	FE	n	pEC_{50} ± SEM	FE	n
carbachol				6.99 ± 0.04	1.0	22	6.68 ± 0.04	1.0	9
muscarine	6.77 ± 0.03	1.1	60						
1	4.83 ± 0.16	0.7	4	5.2 ± 0.08	0.8	4	4.08 ± 0.18	0.8	5
1A1 + 1B1 ("cis")	5.24 ± 0.23	0.7	4	5.48 ± 0.15	0.8	4	4.66 ± 0.09	0.8	3
1A1	<4		4	see text		4	see text		2
1A2	<4		4	<3.5		3			
1B1	5.25 ± 0.17	0.8	4	5.66 ± 0.08	0.8	5	4.83 ± 0.07	0.8	3
1B2	4.56 ± 0.09	0.7	4	4.70 ± 0.04	0.7[g]	3	~4.0[f]	0.2[g]	3

[a] Assays described in full in the Experimental Section. [b] -log EC_{50} (moles/liter). [c] Standard error of the mean. [d] Functional efficacy compared to 1 μM muscarine in the ganglion and the maximum response to carbachol in the ileum and atrium (individual efficacy determinations were usually ≤±0.1 of the indicated value). [e] Number of determinations. [f] Large errors in potency determinations due to low and/or variable efficacy. [g] Variable efficacy (±0.2–0.3).

as a measure of the ability of the agonist to distinguish between different affinity states of the receptor. Of the four individual isomers of 1, activity appeared to reside predominantly in 1B1. The compound was not selective for the M-1 site although relative to carbachol and muscarine, there is a shift in selectivity away from the M-2 receptor. The combination of a steep displacement curve (high Hill coefficient) in the M-1 assay and a moderately steep Hill coefficient in the heart is characteristic of a partial agonist profile for isomer 1B1. Other isomers behave increasingly as weaker partial agonists with correspondingly lower affinity for both receptor sites.

III. Pharmacology. The activities of each isomer of 1 were compared in the three pharmacological assays described in full in the Experimental Section and summarized in Table III. In general the potency of the active compounds was greatest in the ileum and least in the atrium although their functional efficacy, compared to a full agonist, was similar in all tissues. The cis pair of isomers was more potent than 1, and the rank order of potency of the individual isomers was 1B1 > 1B2 ≫ 1A1 ≈ 1A2. The exceptions to these generalizations are more interesting. Only in the ileum did 1A1 show appreciable efficacy, but this was variable and the potency was ≤0.005 that of carbachol. 1B2 reproducibly activated the depolarizing receptors in the ganglion, but its functional efficacy was quite variable on the other tissues and rather low on the atrium.

IV. Behavioral Pharmacology. Stimulation of central muscarinic receptors in the rat has been shown[15] to induce a syndrome of increased oral activities including gaping, tongue protrusion, and a chewing-like response. The two more active isomers of 1 (1B1 and 1B2) were assessed for their ability to produce these behaviors, and dose–response curves are shown in Figure 4. Analysis of variance revealed a significant difference between the two isomers ($F(1,12) = 22.9$, $p < 0.001$), a significant dose effect ($F(2,12) = 6.25$, $p < 0.01$), and a significant drug × dose interaction ($F(2,12) = 8.16$, $p < 0.01$). These results demonstrate that over the dose range tested, 1B1 has a greater central cholinergic activity than 1B2.

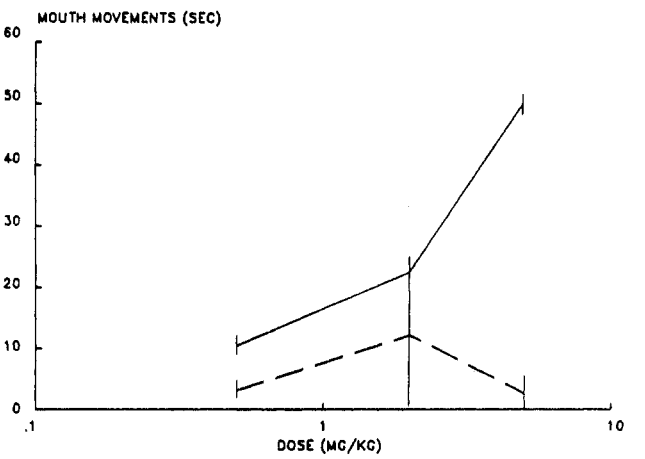


Figure 4. Dose–response curves for 1B1 (–) and 1B2 (- -) on induction of mouth movements. Each point represents the mean (±SEM) mouth movements (n = 3/dose).

Discussion

Fisher et al. have claimed[7] that 1 is a weak but selective muscarinic agonist when compared to 3-acetoxyquinuclidine and acetylcholine. The measurements, however, were performed on mixtures of isomers (either the "cis" pair or all enantiomers) in a variety of in vitro and in vivo assays. Interpretation of these results is complicated for two reasons. First, selectivity may well reside in the ability of a given receptor subtype to recognize a single stereoisomer of a chiral ligand so that reliable measurements can only be made with single isomers. Secondly, the presence of endogenous acetylcholinesterase activity will affect the potency of both 3-acetoxyquinuclidine and acetylcholine, which, in contrast to 1, are substrates for this enzyme. In this study, we have compared the potencies of the separated isomers of 1 in three in vitro pharmacological preparations relative to standards that are not substrates for acetylcholinesterase. Under these controlled conditions we have been unable to substantiate earlier findings[6,7] since, unlike the results of Fisher et al., the rank order of potency was ileum > ganglion > heart for all isomers. Ganglionic to atrial selectivity was seen in isomer 1B2, but this was reflected in a difference of efficacy rather than potency. The separated isomers also failed to show appreciable M-1 selectivity in the in vitro binding assays although each showed a marked (10–20-fold) tendency away from the M-2 site relative to the standards, carbachol and muscarine.

The most interesting feature, however, is that the absolute stereochemistry at the quinuclidine chiral center of the most active isomers 1B1 and 1B2 is *R* as opposed to *S* in **3**. The obvious comparison[16] for modeling purposes would be between (*S*)-**3** and 1A (either 1A1 or 1A2) since this would allow both the quinuclidine backbone and the "interaction pharmacophore" (comprising a protonated tertiary amine or quaternary ammonium entity, one or two hydrogen-bonding acceptors, and a small terminal alkyl group) to be topographically aligned. Studying only con-

(15) Salamone, J. D.; Lalies, M. D.; Channell, S. L.; Iversen, S. D. *Psychopharmacology (Berlin)* **1986**, *88*, 467.

(16) Schulman, J. M.; Sabio, M. L.; Disch, R. L. *J. Med. Chem.* **1983**, *26*, 817.

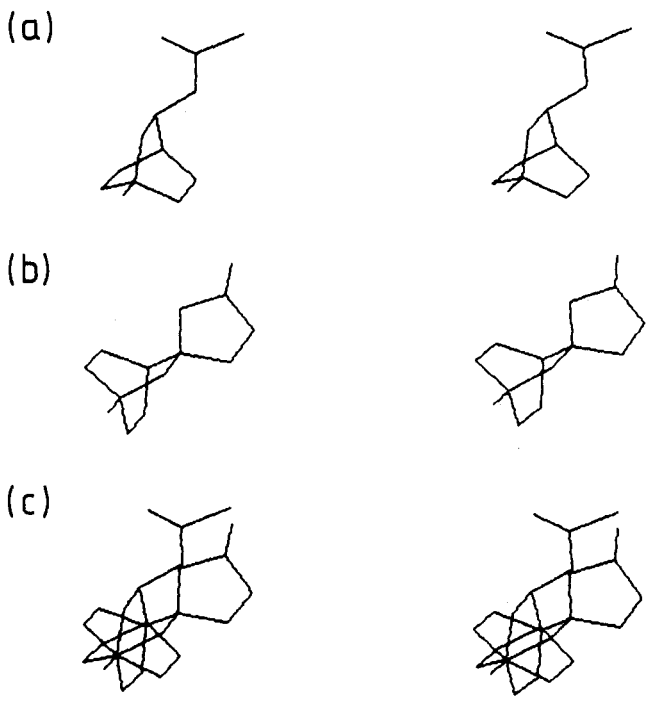


Figure 5. (a) Computer-generated stereoview of **3** (only the NH^+ hydrogen shown for clarity). (b) Stereoview of 1B1. (c) Stereoview of the superimposition of **3** and 1B1.

formations of **3** (defined by rotation about C2–C3–O–C, see Figure 5) within 2 kcal mol^{-1} of the global minimum[17] (Γ(C2–C3–O–C) = –71°), an adequate match with either 1B isomer is depicted in Figure 5. This match places the quinuclidine nucleus of both molecules in a staggered rather than superimposed orientation but allows the key elements of the pharmacophore to be correctly aligned. The molecular feature of 1B2 that confers selectivity in terms of differential efficacy in favor of the ganglion is the dioxolane methyl group held within a completely rigid framework. Acetoxyquinuclidine **3**, having an sp^2 center at this equivalent position and having some degree of conformational flexibility, is devoid of this feature and is not selective. Thus is it tempting to speculate that new muscarinic ligands with more marked selectivity will evolve from molecules such as **1**, which will uniquely satisfy the conformational and stereochemical restraints of a particular receptor subtype.

Experimental Section

Chemical Methods. General Directions. Except where otherwise stated, the following procedures were adopted: all ^1H NMR spectra were recorded at 360 MHz on a Bruker AM 360 instrument, mass spectra with a VG 70–250 mass spectrometer, and infrared spectra on a Perkin-Elmer 782 IR spectrometer. Optical rotations were measured by using a Thorn-NPL type 243 polarimeter, and GC was performed on a SE30 column using a Perkin-Elmer capillary gas chromatograph (8320) (injection temperature, 270 °C; detection temperature, 300 °C; start oven temperature, 100 °C; ramp rate, 6 °C/min; final oven temperature, 172 °C). Organic solvents were purified when necessary by the methods described by D. D. Perrin, W. L. F. Armarego, and D. R. Perrin (*Purification of Laboratory Chemicals*; Pergamon: Oxford, 1966). Petroleum ether refers to that fraction having a boiling point range of 60–80 °C. All solutions were dried over sodium sulfate or potassium carbonate and evaporated on a Buchi rotary evaporator with a water bath temperature set to 45 °C or below. Thin-layer chromatography and preparative chromatography were carried out on alumina using plates (Merck Art. No. 5550) and gravity columns (Merck Art. No. 1097, activity Brockman Grade II–III), respectively. Melting points are uncorrected.

Methyl 3-[[(3-Oxo-4(*R*),7,7-trimethyl-2-oxabicyclo[2.2.1]hept-1(*S*)-yl)carbonyl]oxy]-1-azabicyclo[2.2.2]octane-3(*R*)-carboxylate (5B) and Methyl 3-[[(3-Oxo-4(*R*),7,7-trimethyl-2-oxabicyclo[2.2.1]hept-1(*S*)-yl)carbonyl]oxy]-1-azabicyclo[2.2.2]octane-3(*S*)-carboxylate (5A). Methyl 3-hydroxyquinuclidine-3-carboxylate[18] (14.8 g, 80 mmol) in dry CH_2Cl_2 (200 mL) containing Et_3N (8.5 g, 84 mmol) was treated at 10 °C with (–)-camphanic acid chloride (18.2 g, 84 mmol). After 4 h at 20 °C, the mixture was poured into a saturated aqueous solution of sodium hydrogen carbonate and the layers separated. The organic layer was washed with fresh sodium hydrogen carbonate solution and then brine and finally evaporated. The resulting gum was triturated with cold ether, and after standing at 5 °C for 2 h, the solid that had precipitated was collected by filtration. This material was recrystallized from CH_2Cl_2/ether to afford the desired camphanate isomer **5B** (7.3 g): mp 155–157 °C; R_f 0.55 in EtOAc on alumina plates; t_R = 9.68 min; $[\alpha]^{22}_D$ +44.7° (MeOH, *c* 1.0); MS, *m/z* 365 (M^+), 306, 249, and 184 (base peak); ^1H NMR δ ($CDCl_3$) 1.01, 1.11, and 1.13 (each 3 H, each s, 3 × CH_3), 1.44–1.56 and 1.58–1.62 (1 H and 2 H, each m, 5-CH_2 and 8-CH), 1.70–1.75 (1 H, m, oxabicyclo 5-CH), 1.89–2.11 (3 H, m, oxabicyclo 5- and 6-CH and 8-CH), 2.21–2.23 (1 H, m, 4-CH), 2.37–2.45 (1 H, m, oxabicyclo 6-CH), 2.76–2.90 (5 H, m, 6-CH_2, 7-CH_2, and 2-CH), 3.75 (3 H, s, OCH_3), and 4.00 (1 H, d, *J* = 15.1 Hz, 2-CH). Anal. ($C_{19}H_{27}NO_6 \cdot 0.33H_2O$) C, H, N.

The filtrate and the mother liquors from above were combined and evaporated, and the oil so obtained was subjected to chromatography on alumina using a gradient elution of 1:1 EtOAc/petroleum ether to 4:1 EtOAc/petroleum ether. The pooled fractions containing only the faster running isomer were evaporated to a gum, which crystallized on standing to give the second diastereomer, **5A** (6.8 g): mp 88–89 °C; R_f 0.65 in EtOAc on alumina; t_R = 9.50 min; $[\alpha]^{22}_D$ –49.5° (MeOH, *c* 1.0); MS, *m/z* 365 (M^+), 306, 249, and 184 (base peak); ^1H NMR δ ($CDCl_3$) 1.05 and 1.13 (6 H and 3 H, each s, 3 × CH_3), 1.42–1.51 and 1.54–1.62 (1 H and 2 H, each m, 5-CH_2 and 8-CH), 1.66–1.73 (1 H, m, oxabicyclo 5-CH), 1.89–1.96 and 2.02–2.08 (1 H and 2 H, each m, 8-CH and oxabicyclo 5- and 6-CH), 2.20–2.22 (1 H, m, 4-CH), 2.41–2.49 (1 H, m, oxabicyclo 6-CH), 2.74–2.98 (5 H, m, 6-CH_2, 7-CH_2, and 2-CH), 3.75 (3 H, s, OCH_3), and 3.98 (1 H, d, *J* = 15.1 Hz, 2-CH). Anal. ($C_{19}H_{27}NO_6$) C, H, N.

3(*R*)-(Hydroxymethyl)-3-hydroxy-1-azabicyclo[2.2.2]octane (7B). The foregoing ester **5B** (7 g, 19.2 mmol) in THF (50 mL) was added to a stirred suspension of $LiAlH_4$ (2.45 g, 64.5 mmol) in THF (30 mL) at such a rate as to maintain the reaction at reflux temperature. After a further 3 h at this temperature, the mixture was cooled to 10 °C and excess reducing reagent destroyed by successive dropwise addition of EtOAc (2.8 mL), water (2.4 mL), 15% aqueous sodium hydroxide (2.4 mL), and finally water (7.1 mL). The resulting suspension was filtered, and the gum isolated from the filtrate was chromatographed on alumina in 25% MeOH in CH_2Cl_2. The product so obtained was triturated with anhydrous ether to afford the required diol (2.4 g) as a glassy solid, which was used without further purification.

3(*S*)-(Hydroxymethyl)-3-hydroxy-1-azabicyclo[2.2.2]octane (7A). Repetition of the procedure described above but starting from diastereomer **5A** of the camphanate ester afforded the title compound.

2′(*S*)-Methylspiro[1-azabicyclo[2.2.2]octane-3(*R*),4′-[1,3]dioxolane] Hydrogen Chloride (1B1) and 2′(*R*)-Methylspiro[1-azabicyclo[2.2.2]octane-3(*R*),4′-[1,3]dioxolane] Hydrogen Chloride (1B2). 3(*R*)-Hydroxymethyl-3-hydroxy-1-azabicyclo[2.2.2]octane (2.2 g) in CH_2Cl_2 (8 mL) was treated at 0 °C with acetaldehyde (8 mL) and then boron trifluoride etherate (8 mL). After 1.5 h at 5 °C, the mixture was poured onto 20% aqueous KOH solution (50 mL) stirred at 5 °C. The aqueous solution was exhaustively extracted with ether and the residue isolated from the combined organic extracts purified by chro-

(17) Using the OPTIMOL procedure (based on a molecular mechanics force field) within the Merck Molecular Modelling facility written by Dr. T. Halgren, Rahway, NJ (unpublished).

(18) Grob, C. A.; Renk, E. *Helv. Chim. Acta* **1954**, *37*, 1689.

matography on alumina using a gradient elution of EtOAc up to 5% CH_3OH in EtOAc. The material isolated from the pooled fractions containing only the faster running isomer was treated in ether at 5 °C with a slight excess of ethereal HCl to yield 2′(*S*)-methylspiro[1-azabicyclo[2.2.2]octane-3(*R*),4′-[1,3]dioxolane] hydrogen chloride (1B1, 460 mg): mp 212–215 °C; R_f 0.5 on alumina in 10% MeOH in EtOAc; t_R = 5.24 min; $[\alpha]^{22}_D$ −46.8° (MeOH, *c* 0.2); MS, *m/z* 183 (M^+ of free base) 168, 139, 96 (base peak), 82, 69, and 55; ^1H NMR δ (CD_3OD) 1.35 (3 H, d, *J* = 4.7 Hz, CH_3), 1.78–2.08 (3 H, m, 5-CH_2 and 8-CH), 2.25–2.37 (2 H, m, 8-CH and 4-CH), 3.19–3.38 (5 H, m, 6-CH_2, 7-CH_2, and 2-CH), 3.56 (1 H, dd, *J* = 13.3 Hz and 2.2 Hz, 2-CH), 3.95 and 4.04 (each 1 H, each d, *J* = 8.6 Hz, CH_2O), and 5.13 (1 H, q, *J* = 4.7 Hz, C*H*CH_3). Anal. ($C_{10}H_{17}NO_2$·HCl·0.2H_2O) C, H, N.

After the mixed fractions had been eluted (480 mg), those fractions containing only the slower running isomer were collected, evaporated, and treated with ethereal HCl as described above. The solid thereby obtained was recrystallized from *i*-PrOH/Et_2O to give 2′(*R*)-methylspiro[1-azabicyclo[2.2.2]octane-3(*R*),4′-[1,3]dioxolane] hydrogen chloride (1B2, 310 mg): mp 243–244 °C; R_f 0.45 on alumina in 10% MeOH in EtOAc; t_R = 5.15 min; $[\alpha]^{22}_D$ −39.6° (MeOH, *c* 0.2); MS, *m/z* 183 (M^+ of free base), 168, 139, 96 (base peak), 82, 69, and 55; ^1H NMR δ (CD_3OD) 1.34 (3 H, d, *J* = 4.7 Hz, CH_3), 1.82–2.06 (3 H, m, 5-CH_2 and 8-CH), 2.09–2.11 (1 H, m, 4-CH), 2.25–2.36 (1 H, m, 8-CH), 3.23–3.38 (4 H, m, 6-CH_2 and 7-CH_2), 3.42 and 3.48 (each 1 H, each d, *J* = 13.7 Hz, 2-CH_2), 3.69 and 4.22 (each 1 H, each d, *J* = 9.4 Hz, CH_2O), and 5.13 (1 H, q, *J* = 4.7 Hz, C*H*CH_3). Anal. ($C_{10}H_{17}NO_2$·HCl) C, H, N.

2′(*R*)-Methylspiro[1-azabicyclo[2.2.2]octane-3(*S*),4′-[1,3]dioxolane] Hydrogen Chloride (1A1) and 2′(*S*)-Methylspiro[1-azabicyclo[2.2.2]octane-3(*S*),4′-[1,3]dioxolane] Hydrogen Chloride (1A2). Starting from 3(*S*)-hydroxymethyl-3-hydroxy-1-azabicyclo[2.2.2]octane and using the methodology described above gave 2′(*R*)-methylspiro[1-azabicyclo[2.2.2]octane-3(*S*),4′-[1,3]dioxolane] hydrogen chloride (1A1) as the faster running isomer on TLC: mp 214–215 °C; R_f 0.5 on alumina in 10% methanol in EtOAc; t_R = 5.28 min; $[\alpha]^{22}_D$ +45.3° (MeOH, *c* 0.2); MS, *m/e* 183 (M^+ of free base), 168, 139, 96 (base peak), 82, 69, 55, and 42; ^1H NMR δ (CD_3OD) 1.35 (3 H, d, *J* = 4.7 Hz, CH_3), 1.81–2.06 (3 H, m, 5-CH_2 and 8-CH), 2.25–2.36 (2 H, m, 8-CH and 4-CH), 3.21–3.37 (5 H, m, 6-CH_2, 7-CH_2, and 2-CH), 3.55 (1 H, dd, *J* = 13.3 Hz and 2.2 Hz, 2-CH), 3.95 and 4.04 (each 1 H, each d, *J* = 8.6 Hz, CH_2O), and 5.13 (1 H, q, *J* = 4.7 Hz, C*H*CH_3). Anal. ($C_{10}H_{17}NO_2$·HCl) C, H, N. The slower running material on TLC affording, after treatment with ethereal hydrogen chloride, 2′(*S*)-methylspiro[1-azabicyclo[2.2.2]octane-3(*S*),4′-[1,3]dioxolane] hydrogen chloride (1A2): mp 241–243 °C; R_f 0.45 on alumina in 10% methanol in EtOAc; t_R = 5.20 min; $[\alpha]^{22}_D$ +40.7° (MeOH, *c* 0.1); MS, *m/z* 183 (M^+ of free base), 168, 156, 139, 96 (base peak), 82, 69, and 55; ^1H NMR δ (CD_3OD) 1.34 (3 H, d, *J* = 4.7 Hz, CH_3), 1.63–2.06 (3 H, m, 5-CH_2 and 8-CH), 2.08–2.12 (1 H, m, 4-CH), 2.26–2.37 (1 H, m, 8-CH), 3.22–3.35 (4 H, m, 6-CH_2 and 7-CH_2), 3.42 and 3.48 (each 1 H, each d, *J* = 13.7 Hz, 2-CH_2), 3.69 and 4.22 (each 1 H, each d, *J* = 9.4 Hz, CH_2O), and 5.12 (1 H, q, *J* = 4.7 Hz, C*H*CH_3). Anal. ($C_{10}H_{17}NO_2$·HCl) C, H, N.

3(*S*)-[[(3-Oxo-4(*R*),7,7-trimethyl-2-oxabicyclo[2.2.1]-hept-1(*S*)-yl)carbonyl]oxy]-1-azabicyclo[2.2.2]octane (8B). 3(*S*)-Quinuclidinol[13] (1.10 g, 8.6 mmol; $[\alpha]^{23}_D$ +39.3° (*c* 1.0, 1 M HCl)) in dry CH_2Cl_2 (10 mL) containing Et_3N (0.91 g, 9.0 mmol) was treated at 10 °C with (−)-camphanic acid chloride (1.95 g, 9.0 mmol). After 4 h at ambient temperature, the mixture was poured into a saturated aqueous sodium hydrogen carbonate solution and the layers separated. The organic layer was washed with fresh sodium hydrogen carbonate solution and then brine, dried, and finally evaporated to give a cream colored solid (2.20 g). Three recrystallizations from EtOAc gave 8B as colorless crystals (1.00 g): mp 148–149 °C; R_f 0.80 in CH_2Cl_2/MeOH (9:1) on alumina plates; $[\alpha]^{23}_D$ −11.5° (MeOH, *c* 1.0); MS, *m/z* 307 (M^+), 126 (base peak), 110, 98, 83, 67; ^1H NMR δ ($CDCl_3$) 0.98, 1.08, 1.13 (each 3 H, each s, 3 × CH_3), 1.38–1.48 (1 H, m, 8-CH), 1.52–1.62 (1 H, m, 5-CH), 1.66–1.76 (2 H, m, 8-CH and oxabicyclo 5-CH), 1.78–1.98 (2 H, m, 5-CH and oxabicyclo 5-CH), 2.02–2.10 (2 H, m, 4-CH and oxabicyclo 6-CH), 2.43 (1 H, ddd, *J* = 4, 11, 15 Hz, oxabicyclo 6-CH), 2.68–2.94 (5 H, m, 6-CH_2, 7-CH_2, and 2-CH), 3.26 (1 H, dd, *J* = 8, 15 Hz, 2-CH), 4.97 (1 H, 3-CH); IR ν_{max} (Nujol) 1785 and 1735 cm^{-1}. Anal. ($C_{17}H_{25}NO_4$) C, H, N.

3(*R*)-[[(3-Oxo-4(*R*),7,7-trimethyl-2-oxabicyclo[2.2.1]-hept-1(*S*)-yl)carbonyl]oxy]-1-azabicyclo[2.2.2]octane (8A). Using 3(*R*)-quinuclidinol[13] (0.82 g, 6.4 mmol; $[\alpha]^{23}_D$ −37.0° (*c* 1.0, 1 M HCl)) and following the same methodology as above gave 8A as colorless crystals (0.33 g): mp 129–131 °C; R_f 0.80 in CH_2Cl_2/MeOH (9:1) on alumina plates; $[\alpha]^{23}_D$ +7.4° (MeOH, *c* 1.0); MS, *m/z* 307 (M^+), 126 (base peak), 110, 98, 83; ^1H NMR δ ($CDCl_3$) 1.00, 1.07, 1.13 (each 3 H, each s, 3 × CH_3), 1.40–1.50 (1 H, m, 8-CH), 1.52–1.64 (1 H, m, 5-CH), 1.66–1.78 (2 H, m, 8-CH and oxabicyclo 5-CH), 1.84–2.10 (4 H, m, 4-CH, 5-CH, oxabicyclo 5-CH, and oxabicyclo 6-CH), 2.44 (1 H, ddd, *J* = 4, 11, 15 Hz, oxabicyclo 6-CH), 2.66–2.92 (5 H, m, 6-CH_2, 7-CH_2, and 2-CH), 3.27 (1 H, dd, *J* = 8, 15 Hz, 2-CH), 4.95 (1 H, m, 3-CH); IR ν_{max} (Nujol) 1790 and 1720 cm^{-1}. Anal. ($C_{17}H_{25}NO_4$) C, H, N.

Biochemical Methods. (a) Brain Membrane Preparation. Crude synaptosomal-mitochondrial membranes were prepared by homogenizing rat cerebral cortices in 0.32 M ice-cold sucrose. The homogenate was centrifuged at 1000*g* for 15 min and the resulting supernatant spun at 17000*g* for 20 min. The yielded the crude synaptosomal-mitochondrial pellet (P_2). Prior to assay, membranes were resuspended to a final volume of 1 g of starting material to 100 mL of ice-cold Krebs–HEPES buffer pH 7.4 (NaCl, 118 mM; KCl, 4.7 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 5 mM; KH_2PO_4, 1.2 mM; $CaCl_2$, 2.5 mM; glucose, 11 mM; and HEPES, 20 mM).

(b) Heart Membrane Preparation. Rat hearts were chopped and homogenized (Polytron for 2 × 20 s). The resulting suspension was homogenized again by using a motor-driven Teflon/glass homogenizer, filtered through cheesecloth, and then centrifuged at 30000*g* for 30 min. The resulting pellet was resuspended in 10 mL of buffer, rehomogenized, and then centrifuged once more at 30000*g* for 30 min. Prior to assay, heart membranes were resuspended to a final volume of 1 g of starting material to 80 mL of Krebs–HEPES buffer.

(c) [^{3}H]Pirenzepine Assay (M-1). Binding to the M-1 receptor population of the rat cerebral cortex was determined by using 0.1–20 nM [^{3}H]pirenzepine with nonspecific binding defined with 1 μM atropine. Incubations were allowed to proceed for 60 min at 30 °C. Assays were terminated by centrifugation at 13000–15000*g* for 3 min in a bench-top Microfuge. The surface of the pellet was washed twice with ice-cold saline. The pellet was subsequently dissolved in 100 μL of soluene-350 and radioactivity estimated by using liquid scintillation spectrometry.

(d) [^{3}H]-*N*-Methylscopolamine Binding Assay (M-2). Binding to the M-2 receptor population of rat heart was estimated by using 0.01–10 nM [^{3}H]-*N*-methylscopolamine. Assays were performed by using a microcentrifugation assay as described above.

The apparent affinity constants (K_{app}) were determined from IC_{50} values (concentration required to displace 50% of the specific binding) by using the Cheng–Prusoff equation.[19]

Pharmacological Methods. (a) Superior Cervical Ganglion.[20,21] Ganglia were excised from male rats (150–200 g) killed by a blow to the thorax and then desheathed. The ganglia were placed in a bath (volume about 0.5 mL) and continuously superfused at 1.5–2.5 mL/min with an artificial cerebrospinal fluid medium at 25 °C containing 2.5 mM Ca^{2+} and 6 mM K^+ inter alia. Ganglionic potentials were recorded by using a "grease-gap" technique between the ganglion body and the internal carotid nerve. Semicumulative concentration–response relationships were determined by using 1-min agonist superfusions every 10 min. The functional efficacy of the compounds was expressed as a ratio of the maximum response to 1 μM (±)-muscarine chloride (Sigma).

(b) Guinea Pig Ileum. The actions of AF30, the cis isomers, and the fully resolved isomers were compared to that of carbachol in individual preparations of longitudinal muscle, myenteric plexus (MPLM) (Rang, 1964) from the ileum of 250–400-g male guinea pigs (Dunkin–Hartley strain) mounted in 3-mL glass organ baths

(19) Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
(20) Brown, D. A.; Fatherazi, S.; Garthwaite, J.; White, R. D. *Br. J. Pharmacol.* **1980**, *70*, 577.
(21) Newberry, N. R.; Priestley, T.; Woodruff, G. N. *Eur. J. Pharmacol.* **1985**, *116*, 191.

containing a modified Krebs–Henseleit solution at 37 °C. The concentration–response relationship of the agonist-evoked contractions was used to obtain values for EC_{50}, the concentration producing 50% of the individual maximum response, and a measure of the functional efficacy (FE) of the compounds as the maximum response relative to that obtained with carbachol in the same preparation.

(c) Guinea Pig Isolated Atria. Male guinea pigs (200–400 g) were killed by cervical dislocation and the paired atria dissected out. These were mounted in a 3-mL glass organ bath containing double-glucose Krebs–Henseleit solution and maintained at 30 °C under an initial load of 1 g. The tissue was stimulated (3 Hz, 1 mA, 30 V) via two platinum electrodes mounted at either end of the organ bath and the force of contraction measured by using an isometric force transducer. Concentration–response relationships were constructed for each compound and ED_{50} values obtained by determining the concentration producing 50% of its maximum inhibitory response. Functional efficacy was determined by comparing the maximum response of a compound to that of carbachol (usually 95–100% inhibition).

(d) Induction of Mouth Movements. Eighteen male albino rats (300–400 g) (Sprague–Dawley, Bantin, and Kingman) received ip injections of either **1B1** or **1B2** in doses of 0.5, 2.0, or 5.0 mg/kg (n = 3/group). Both drugs were dissolved in sterile physiological saline. Rats were placed in perspex boxes (30 × 20 × 30 cm), and behavioral observations were conducted in three 2-min periods beginning at 10, 20, and 30 min after injection. During these periods the observer recorded the time (in seconds) in which the rat showed either the gaping, tongue-protrusion, or chewing responses. For data analysis, the total time spent performing these mouth movements was summed across the three observation periods for each rat. This data was analyzed with a two-way analysis of variance. Dose–response curves were calculated from the arithmetic mean of the summed duration scores at each drug dose.

X-ray Crystallography. (a) X-ray Crystal Structure Analysis of 5A. Suitable crystals of **5A** ($C_{19}H_{27}NO_6$) for X-ray diffraction studies formed from an oil with space group symmetry of $P2_1$ and cell constants of a = 10.856 (2) Å, b = 6.772 (1) Å, c = 13.625 (4) Å, and β = 105.61 (2)° for Z = 2 and a calculated density of 1.258 g/cm^3. Of the 1448 reflections measured with an automatic four-circle diffractometer equipped with Cu radiation, 1301 were observed ($I > 3\sigma(I)$). The structure was solved with a multisolution tangent formula approach and difference Fourier analysis and refined by using full-matrix least-squares techniques.[22] Hydrogens were assigned isotropic temperature factors corresponding to their attached atoms. The function $\Sigma w(|F_o| - |F_c|)^2$ with $w = 1/(\sigma F_o)^2$ was minimized to give an unweighted residual of 0.039. No abnormally short intermolecular contacts were noted. Since **5A** was prepared from (−)-camphanic acid, the absolute configuration of C3 can be assigned to be S. Table I–III containing the final fractional coordinates, temperature parameters, bond distances, and bond angles are available as supplementary material. Figure 1 is a computer-generated perspective drawing of **5A** from the final X-ray coordinates showing the correct absolute stereochemistry.

(b) X-ray Crystal Structure Analysis of 1B2. Suitable crystals of **1B2** ($C_{10}H_{18}NO_2Cl$) for X-ray diffraction studies formed from 2-propanol with space group symmetry of $P2_12_12_1$ and cell constants of a = 7.468 (2) Å, b = 23.942 (10) Å, and c = 6.406 (2) Å for Z = 4 and a calculated density of 1.274 g/cm^3. Of the 950 reflections measured with an automatic four-circle diffractometer equipped with Cu radiation, 832 were observed ($I > 3\sigma(I)$). The structure was solved with a multisolution tangent formula approach and difference Fourier analysis and refined by using full-matrix least-squares techniques.[22] Hydrogens were assigned isotropic temperature factors corresponding to their attached atoms. The function $\Sigma w(|F_o| - |F_c|)^2$ with $w = 1/(\sigma F_o)^2$ was minimized to give an unweighted residual of 0.061. The only short intermolecular contact was a hydrogen bond between N1 and Cl14 of total length 2.99 Å. Since previous arguments have shown the configuration of C3 to be R, position C10 of **1B2** was assigned an R configuration. Tables IV–VI containing the final fractional coordinates, temperature parameters, bond distances, and bond angles are available as supplementary material. Figure 2 is a computer-generated perspective drawing of **1B2** from the final X-ray coordinates showing the absolute stereochemistry.

(c) X-ray Crystal Structure Analysis of 8B. Suitable crystals of **8B** ($C_{17}H_{25}NO_4$) for X-ray diffraction studies formed from ethyl acetate with space group symmetry of $P2_1$ and cell constants of a = 6.267 (1) Å, b = 10.561 (2) Å, c = 12.249 (4) Å, and β = 98.32 (2)° for Z = 2 and a calculated density of 1.273 g/cm^3. Of the 1162 reflections measured with an automatic four-circle diffractometer equipped with Cu radiation, 1133 were observed ($I > 3\sigma(I)$). The structure was solved with a multisolution tangent formula approach and difference Fourier analysis and refined by using full-matrix least-squares techniques.[22] Hydrogens were assigned isotropic temperature factors corresponding to their attached atoms. The functional $\Sigma w(|F_o| - |F_c|)^2$ with w = 1 was minimized to give an unweighted residual of 0.033. No abnormally short intermolecular contacts were noted. Tables VII–IX containing the final fractional coordinates, temperature parameters, bond distances, and bond angles were available as supplementary material. Since the absolute configuration of (−)-camphanic acid is known, the absolute stereochemistry of C3 in **8B** is S. Figure 3 is a computer-generated perspective drawing of **8B** from the final X-ray coordinates showing the correct absolute stereostructure.

Registry No. **1A1**, 107869-78-3; **1A1**·HCl, 107910-79-2; **1A2**, 107869-79-4; **1A2**·HCl, 107910-80-5; **1B1**, 107869-76-1; **1B1**·HCl, 107910-77-0; **1B2**, 107869-77-2; **1B2**·HCl, 107910-78-1; **3**·(−)-hydrogen tartrate, 107770-20-7; **3**·CH_3I, 22753-06-6; **4**, 57292-21-4; **5A**, 107869-74-9; **5B**, 107770-15-0; **7A**, 107770-17-2; **7B**, 107770-16-1; **6**, 39637-74-6; **8A**, 107869-75-0; **8B**, 107770-18-3; methyl (±)-3-hydroxyquinuclidine-3-carboxylate, 107770-14-9; 3(S)-quinuclidinol, 34583-34-1; 3(R)-quinuclidinol, 25333-42-0; 3-(R)-acetoxyquinuclidine, 59653-40-6; 3(R)-3-acetoxyquinuclidine (+)-hydrogen tartrate, 107770-19-4; 3(R)-acetoxyquinuclidine hydrochloride, 57539-24-9; 3(≥)-acetoxyquinuclidine hydrochloride, 57539-22-7; acetaldehyde, 75-07-0.

Supplementary Material Available: Tables of the atomic positional and thermal parameters, bond distances, and bond angles for **5A**, **1B2**, and **8B** (11 pages). Ordering information is given on any current masthead page.

(22) The following library of crystallographic programs was used: Main, P., et al. MULTAN 80; University of York, York, England, 1980; Johnson, C. K. ORTEP-II; Oak Ridge National Laboratory, Oak Ridge, TN, 1970; Okaya, Y., et al. SDP Plus VI.1, B. A. Frenz and Associates, College Station, TX, 1984.