Through these studies we have demonstrated 2 significant features: (1) the 3′ substituents exert an effect which is not electronic and is apparently not totally hydrophobic; (2) a polarized double bond is likely and this polarization is largest in the 2- and 4-stilbazoles where mesomeric or resonance contributions by the pyridine ring are largest. The lack of electronic effects or consistent hydrophobic effects suggests that the influence of the 3 substituent may be steric. A conformational change in the enzyme may occur which results in a net increase in the binding of the inhibitor, though such conformational changes are generally associated with large hydrophobic groups.

We suggested that a nucleophilic residue on the enzyme may have a strong interaction with the partial positive charge on the vinyl bridge. Even a reversible addition across the double bond is possible. The strength of this interaction would depend on the extent of the polarization and the proximity of the nucleophile. A Me quaternization is reported to increase the binding of the inhibitors.[3] The quaternization would also greatly increase the polarization of the double bond. Also, a small conformational change may influence the proximity of a nucleophilic residue. Further studies are underway to give more insight into the binding of the 4-stilbazoles and to show if such modes of binding as suggested above do contribute to the effectiveness of the stilbazoles as inhibitors of ChA.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample had an ir spectrum compatible with its structure and moved as one spot on tlc on Brinkmann silica gel GF with EtOAc and $CHCl_3$. All analytical samples gave combustion values for C, H or C, H, N within 0.4% of theoretical.

3′-Methoxy-β-methyl-4-stilbazole (22) Picrate. Method B. To a stirred THF soln of picolyl Li prepd by the addn of 6.5 ml of a 2.2 *M* Et_2O soln of PhLi (Alfa Inorganics, Inc.) to 1.3 g (13.3 mmoles) of α-picoline was added dropwise a THF soln of 2 g (13.3 mmoles) of 3′-methoxyacetophenone. The mixt was stirred overnight, then quenched with 50 ml of ice-cold 2 *N* HCl. An aqueous soln of $NaHCO_3$ was added to pH 7. The product was extd into 3 × 50 ml of EtOAc and dried (Na_2SO_4) and the solvent removed *in vacuo* leaving a light brown oil. The ir, nmr, and uv spectra were compatible with the structure of the desired alcohol.

The crude alcohol was refluxed in 50 ml of $POCl_3$ for 1 hr. The cooled reaction mixt was poured over 200 g of crushed ice and basified with 50% NaOH with addl cooling. The product was extd into 3 × 70 ml of EtOAc, washed free of base, and dried (Na_2SO_4). The solvent was removed *in vacuo* leaving a light oil which was treated with a satd EtOH–picric acid soln. The salt was collected and recrystd from EtOH–petr ether. For addl data, see Table II.

O-(3,4-Dichlorobenzyl)-4-pyridylaldoxime (18)·HCl. Method D. A mixt of 3.3 g (18 mmoles) of 3,4-dichlorobenzyloxyamine and 2.0 g (18 mmoles) of 4-pyridinecarboxaldehyde in 100 ml of PhMe was brought to reflux for 6 hr and H_2O removed by a Dean–Stark trap. The mixt was cooled and treated with a stream of dry HCl. The ppt was collected and recrystd from EtOH–petr ether (see Table II).

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Synthesis and Pharmacological Evaluation of Some β,β-Disubstituted Analogs of Acetylcholine

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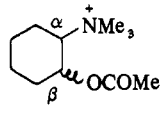
Geometrically isomeric pairs of 2-, 3-, and 4-methyl-1-acetoxy-1-dimethylaminomethylcyclohexane methiodides, the corresponding desmethyl derivative, and acetyl-β,β-dimethylcholine iodide have been prepared and evaluated for muscarinic and nicotinic activity on the guinea pig ileum or frog rectus muscle. The configuration of cis,trans pairs and certain conformational features were elucidated from spectroscopic (ir and pmr) data. Only acetyl-β,β-dimethylcholine iodide and *r*-1-acetoxy-1-dimethylaminomethyl-*c*-3-methylcyclohexane methiodide showed any muscarinic activity on the guinea pig ileum. None of the compounds possessed any antimuscarinic, nicotinic, or antinicotinic properties on this preparation in the doses studied. On the frog rectus muscle no compound possessed spasmogenic activity but all compounds were approximately 0.05 as active as gallamine in antagonizing responses to ACh. The muscarinic properties of these compounds are discussed in terms of their probable conformations and evidence that ACh agonists adopt *antiperiplanar* (or near *antiperiplanar*) $^{+}N/O$ conformations at muscarinic receptors.

Recently there has been much interest in conformationally constrained analogs of ACh in order to test the proposal that muscarinic and nicotinic effects of this agonist are mediated by distinct conformational isomers of the ACh molecule.[1,2] Cyclohexane and *trans*-decalin have served as 6-membered skeletons for such analogs, *e. g.*, **1**[3] and **2**.[4] As muscarinic agonists, these derivatives suffer from the drawback of being α-alkyl-substituted acetylcholines, for it is



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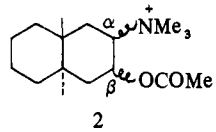

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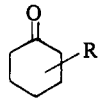
well known that the action of ACh at muscarinic sites is sharply decreased by α-methylation.[5] It was of interest, therefore, to examine the ACh analogs 8 which, while free of α substituents, would still be subject to certain conformational restraints and hence be of potential value in furthering knowledge of conformational influences upon the activity of muscarinic agonists. These 1-aminomethylcyclohexyl acetate methiodides were prepared from the appropriate cyclohexanone by the sequence 3 through 8. For



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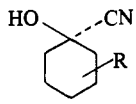

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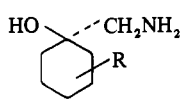

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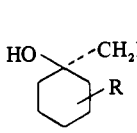

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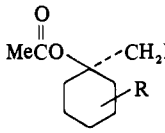
a, R = H
b, R = 2-Me



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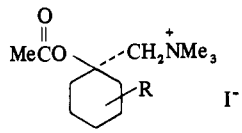

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c, R = 3-Me
d, R = 4-Me

cases where R = Me, attempts were made to separate cis-trans isomers at each stage by fractional crystallizations monitored by pmr spectroscopy; the points at which these were successful varied (see Experimental Section). When one geometrical isomer was isolated, the second was sought after further reaction of residues enriched in that component.

Experimental Section†

Cyanohydrins (4). 4a[6] and cis-trans mixts of 4b,[7] 4c,[8] and 4d[7] were prepd by reported methods. Fractional crystn of the mixt 4b (21.5 g) from a hexane mixt (bp 66–68°) gave the *t*-Me, *r*-OH isomer (13.6 g), mp 54–56° (lit.[7] 53–54°).

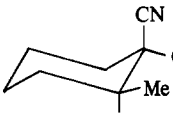
1-Aminomethylcyclohexanols (5). 4a (40 g) in Et_2O (200 ml) was added dropwise to LAH (20 g) suspended in the same solvent (800 ml) and the mixt heated under reflux for 10 hr. After cooling, excess of LAH was decompd with H_2O (50 ml) followed by 20% NaOH–H_2O (100 ml). The decanted Et_2O was dried (Na_2SO_4) and evapd to give 5a (36 g). Similarly prepd were *t*-Me, *r*-OH 5b, and *c,t*-5b, -5c, and -5d. Fractional crystn of *c,t*-5c hydrochlorides (45 g) gave the *c*-Me, *r*-OH salt (24 g).

1-Dimethylaminomethylcyclohexanols (6). A mixt of 5a (3.9 g), formalin (5 ml), HCO_2H (4.5 g), and H_2O (6 ml) was heated under reflux for 6 hr. The cool product was made alk with 20% NaOH–H_2O and extd with Et_2O. The ext was dried (Na_2SO_4) and evapd, and the residue distd to give 6a. Similarly prepd were *t*-Me, *r*-OH 6b, *c*-Me, *r*-OH 6c, and *c,t*-6b, -6c, and -6d. Fractional crystn of mixts of hydrochlorides of 6b and 6d gave *c*-Me, *r*-OH 6b and *t*-Me, *r*-OH 6d·HCl, respectively.

1-Dimethylaminomethylcyclohexyl Acetates (7). MeCOCl (4.9 g) in EtOAc (10 ml) was added to 6a (4.8 g) in the same solvent (60 ml), and the mixt was heated under reflux for 6 hr. 7a·HCl sepd from the cool product. Similarly prepd were hydrochlorides of *c*-Me, *r*-OH 7b, *t*-Me, *r*-OH 7b, *c*-Me, *r*-OH 7c, *t*-Me, *r*-OH 7d, *c–t* 7c and 7d, and 1,1-dimethyl-2-dimethylaminoethyl acetate (from the corresponding amino alcohol).[9] Fractional crystn of hydrochlorides of the mixture 7c gave the *t*-Me, *r*-OCOMe isomer.

1-Dimethylaminomethylcyclohexyl Acetate Methiodides (8). 7a·HCl (1.3 g) in chilled H_2O (10 ml) was made alk with 5% $NaHCO_3$–H_2O and extd (Et_2O). The dried (Na_2SO_4) ext was evapd and the residue in Me_2CO (5 ml) treated with excess of MeI when 8a pptd. Similarly prepd were the isomeric pairs 8b and 8c, *t*-Me, *r*-OCOMe 8d, and acetyl-β,β-dimethylcholine iodide. Fractional crystn of *c,t*-8d gave the *c*-Me, *r*-OCOMe isomer.

Stereochemistry. Assignments of configuration and preferred conformation to all isomers are based on pmr spectroscopic data (Table II). The cyanohydrins 4b, 4c, and 4d (total products) were binary mixts with major and minor components present in the ratio 3:1 as judged by intensities of the duplicate OH signals of spectra run in DMSO-d_6. OH protons are stongly H bonded to this solvent and in this condition their chemical shifts are relatively insensitive to minor concn and temp variations and hence are of value in configurational assignments.[10,11] The lower field OH resonance in each of the mixts 4 specified above is assigned to an equatorial (*eq*) OH on the grounds of (i) the deshielded nature of an *eq* as opposed to an axial (*ax*) environment in 6-membered alicyclic rings,[12,13] and (ii) the probability of the more accessible *eq*-OH being the more extensively H bonded (when a proton is so bonded it is deshielded).[14] Major isomers thus assigned are *t*-Me, *r*-OH 4b, *c*-Me, *r*-OH 4c, and *t*-Me, *r*-OH 4d of preferred conformations 9, 10, and 11 resp. Preferred conformations of *c*-Me, *r*-OH 4b and 4d, and *t*-Me,



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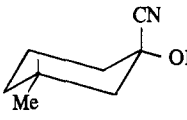

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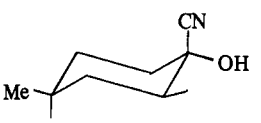

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r-OH 4c, place OH in an *ax* orientation on account of the evident larger conformational free energies of inverted forms.[15] The validity of these arguments is confirmed by their leading to the correct assignment of the isomers 4b, of known configuration.[7] Differential OH resonances also facilitated the isolation of pure *c*-Me, *r*-OH 6b (Table II, No. 5). In the 3-methylcyclohexanes, a pure isomer was not isolated until stage 5; this had an OH chemical shift which corresponded with the major OH signal of the total mixt (isomeric proportions as in 4c) and was the *c*-Me, *r*-OH isomer (Table II, No. 4). The second isomer, isolated at stage 7, was characterized by its OCOMe resonance (Table II, No. 7). In the 4-methylcyclohexanes sepn was achieved at stage 6; OH resonances could not be employed (the hydrochloride mixture was soluble only in D_2O) but the pure isomer had an *N*-CH_2 resonance that corresponded with that of the major NCH_2 singlet of the total mixt (Table II, No. 6) and was thus correlated with the major cyanohydrin of mixt 4d already assigned the *t*-Me, *r*-OH configuration. The *c*-Me, *r*-OCOMe isomer, isolated at the ultimate stage, was characterized by several resonance signals (Table II, No. 8).

The C=O stretching mode ($\nu_{C=O}$) of all the cyclohexyl acetate methiodides 8 was near 1735 cm^{-1} and about 15 cm^{-1} wave numbers higher than that of cyclohexyl acetate itself (1.0 and 0.3% solutions in $CHCl_3$); $\nu_{C=O}$ of the tertiary base 7a coincided with that of the latter ester. These results are evidence of preferred *synclinal* +N/O conformers in the esters 8 as solutes for reasons previously given.[16]

Pharmacological Methods. (1) Guinea Pig Ileum Experiments. The ACh agonistic and antagonistic activities of the quaternary ammonium acetates reported here were evaluated on the guinea pig ileum. The guinea pigs (300–500 g) were killed and a segment of ileum (2–3 cm) was isolated and suspended in a 20-ml tissue bath filled with Kreb's soln aerated with 95% O_2 and 5% CO_2 at 37°. The compn of Kreb's soln (g/l) was: NaCl, 6.9; KCl, 0.35; $CaCl_2$, 0.28; $NaHCO_3$, 2.1; KH_2PO_4, 0.61; Mg $SO_4 \cdot 7H_2O$, 0.29, and glucose, 2.0. The agonistic activity was tested according to a reported method.[17] ACh was given in at least 3 concns of 0.005–0.01 μg/ml, and the compds were given in concns up to 200 μg/ml. The equimolar potencies of the compds and ACh are given in Table III. The antagonistic activity was tested as follows: control dose-response curves to ACh (0.005–0.01 μg/ml) and 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) (0.5–2.0 μg/ml) were first obtained, and the prepn was then exposed to the test compd by replacing Kreb's soln with Kreb's soln contg the compd (10 μg/ml). The dose-response curves of ACh and DMPP were again detd. Hexamethonium chloride (10 μg/ml) was used as a reference compd.

†Melting points (uncorrected) were taken in a Thomas-Hoover capillary apparatus. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical value. Salts were crystd from EtOH–Et_2O unless otherwise stated. Details of anal, physical constants, and yield are given in Table I. OH (or OCOMe) is the reference for all configurational assignments. Pmr spectra were recorded on a Varian A-60D spectrophotometer in $CDCl_3$ or DMSO-d_6 (TMS) or D_2O (DSS) at ambient temp; relevant data are given in Table II. Ir spectra were recorded on a Beckman IR-10 spectrophotometer.

Table I. Physical Properties etc. of Cyclohexyl ACh Analogs and Their Precursors

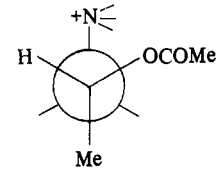
Compound	Isomer[a]	Yield,[b] %	n^{20}D, deg	Mp or bp (mm),[c] °C	Molecular formula	Analysis
5a[d]		78	1.4942	32 (0.01)	$C_7H_{15}NO$	
5b	*t*-Me	74	1.4839	60 (0.05)	$C_8H_{17}NO$	C, H, N
5b·HCl	*t*-Me	89		212-215	$C_8H_{18}ClNO$	C, H
5b	*c,t*-Me	70	1.4856	58-62 (0.05)	$C_8H_{17}NO$	C, H
5b·HCl	*c,t*-Me	90		208-210	$C_8H_{18}ClNO$	C, H
5c	*c,t*-Me	79	1.4827	68-70 (0.15)	$C_8H_{17}NO$	C, H
5c·HCl	*c,t*-Me	81		171-179	$C_8H_{18}ClNO$	
5c·HCl	*c*-Me	53		185-187	$C_8H_{18}ClNO$	C, H
5d	*c,t*-Me	81	1.4770	53-55 (0.15)	$C_8H_{17}NO$	C, H, N
6a		78	1.4645	34-36 (0.07)	$C_9H_{19}NO$	C, H
6a·HCl		92		172-174	$C_9H_{20}ClNO$	C, H, N
6b	*t*-Me	92	1.4625	56 (0.075)	$C_{10}H_{21}NO$	C, H
6b·HCl	*t*-Me	88		138-140	$C_{10}H_{22}ClNO$	C, H
6b·HCl	*c,t*-Me	85		160-172	$C_{10}H_{22}ClNO$	
6b·HCl	*c*-Me	26		188-190	$C_{10}H_{22}ClNO$	C, H
6c	*c*-Me	59	1.4603	95-96 (24)	$C_{10}H_{21}NO$	C, H, N
6c·HCl	*c*-Me	99		173-175	$C_{10}H_{22}ClNO$	C, H
6c·HCl	*c,t*-Me	82		145-150	$C_{10}H_{22}ClNO$	C, H
6d·HCl	*c,t*-Me	91		157-160	$C_{10}H_{22}ClNO$	
6d·HCl	*t*-Me	49		174-175	$C_{10}H_{22}ClNO$	C, H, N
7a·HCl		80		175-176	$C_{11}H_{22}ClNO_2$	C, H
7b·HCl	*t*-Me	74		188-190	$C_{12}H_{24}ClNO_2$	C, H
7b·HCl	*c*-Me	85		194-195	$C_{12}H_{24}ClNO_2$	C, H, N
7c·HCl	*c,t*-Me	84		202-204	$C_{12}H_{24}ClNO_2$	
7c·HCl	*t*-Me	26		205-206	$C_{12}H_{24}ClNO_2$	C, H
7c·HCl	*c*-Me	89		208-209	$C_{12}H_{24}ClNO_2$	C, H, N
7d·HCl	*c,t*-Me	97		194-196	$C_{12}H_{24}ClNO_2$	C, H
7d·HCl	*t*-Me	82		189-191	$C_{12}H_{24}ClNO_2$	
e		68		132-134	$C_8H_{18}ClNO_2 \cdot 0.5H_2O$	C, H
8a		44		156-158	$C_{12}H_{24}INO_2$	C, H
8b	*t*-Me	77		149-151	$C_{13}H_{26}INO_2$	C, H
8b	*c*-Me	62		145-147	$C_{13}H_{26}INO_2$	C, H
8c	*t*-Me	79		106-108	$C_{13}H_{26}INO_2$	C, H
8c	*c*-Me	52		150-151	$C_{13}H_{26}INO_2$	C, H
8d	*t*-Me	86		137-139	$C_{13}H_{26}INO_2$	C, H
8d	*c*-Me	62		179-181	$C_{13}H_{26}INO_2$	C, H, N
f		89		185-189[g]	$C_9H_{20}INO_2$	C, H

[a] With reference to OH or OCOMe. [b] Based on immediate precursor. [c] In degrees Celsius; pressure (where appropriate) in mm of Hg. [d] Lit.[6] n^{20}D 1.4940°, bp 73° (0.5). [e] 1,1-Dimethyl-2-dimethylaminoethyl acetate hydrochloride. [f] β,β-Dimethyl-ACh iodide. [g] Lit.[18] mp 185-186°.

(2) Frog Rectus Abdominis Muscle Experiments. All compds were also tested on the frog rectus muscle.[17] Frogs (*Rana pipiens*) of either sex, weighing about 20 g, were pithed. The skin of the abdomen was removed and a rectus muscle was isolated. The muscle was mounted in a 20-ml bath filled with 70% Kreb's soln (70 ml of Kreb's soln dild with distd H_2O to 100 ml) aerated with 95% O_2 and 5% CO_2 at room temp. ACh was given at concns between 0.2 and 0.5 μg/ml and the compds were administered at concns up to 100 μg/ml. Muscle contractions were recorded using an isotonic ink-writing lever. The anti-ACh activity of the compds on the rectus muscle was studied in the following manner: dose-response curves to ACh were obtained as a control, the prepn was then treated with the compd (50 μg/ml), and the dose-response curve to ACh repeated. Gallamine triethiodide (10 μg/ml) was used as a reference compound. The affinity constants of the compds and gallamine were obtd as dose ratios of ACh divided by the concn of antagonists using a 4-point assay.[17]

Results and Discussion

Details of the pharmacological results are given in Table III. In the guinea pig ileum experiments only acetyl β,β-dimethylcholine iodide and the *r*-1-acetoxy-*c*-3-methylcyclohexane methiodide **8c** demonstrated muscarinic activity since their effects were abolished by hyoscine but not by hexamethonium. Their activity was of a low order, between 0.0005 and 0.0001 that of ACh. The result for acetyl β,β-dimethylcholine iodide agrees reasonably well with that given by Cocolas, *et al.*[18] (0.001 as potent as ACh), reported while this work was in progress. Thus, β,β-dimethylation of ACh or introduction of a cyclohexyl group in the β,β position of this molecule greatly reduces its muscarinic activity on the guinea pig ileum. These findings may be interpreted in terms of an active *antiperiplanar* or near-*antiperiplanar* $^{+}$N/O conformation for ACh agonists at muscarinic sites for which there is evidence from previous studies of conformationally constrained analogs.[4,19,20] The preferred solute conformation of β-methyl-ACh is a synclinal $^{+}$N/O form (**12**),[16] and it is probable that similar conformers are favored for the β,β-dimethyl analog, *i. e.*, **13** and the equivalent form **14** (see ir evidence above). The energy barrier to interconversion of **13** (or **14**) and the antiperiplanar ("active"?) form **15** will be much higher than that involved for



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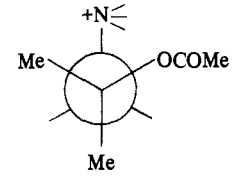

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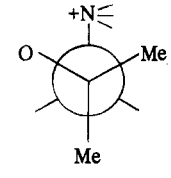

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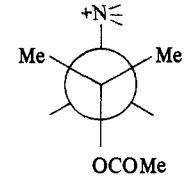

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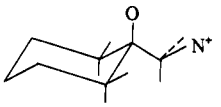
β-methylACh because the onium group in **15** is flanked by 2 Me substituents. It may be argued, therefore, that the low

Table II. Proton Magnetic Resonance Characteristics of Cyclohexyl Derivatives 4-8

No.	Structure	Isomer[a]	Solvent	Proton Group	Chemical shift[b]
1	4b (total)	c,t-Me	DMSO-d_6	OH	6.31,* 6.10
		t-Me	DMSO-d_6	OH	6.31
2	4c (total)	c,t-Me	DMSO-d_6	OH	6.45,* 6.15
3	4d (total)	c,t-Me	DMSO-d_6	OH	6.40,* 6.13
4	5c·HCl (total)	c,t-Me	DMSO-d_6	OH	5.13, 4.77*
		c-Me	DMSO-d_6	OH	5.13
5	6b·HCl (total)	c,t-Me	DMSO-d_6	OH	5.08, 4.77*
		t-Me	DMSO-d_6	OH	5.08
		c-Me	DMSO-d_6	OH	4.77
6	6d·HCl (total)	c,t-Me	D_2O	CH_2N	3.33,* 3.19
		t-Me	D_2O	CH_2N	3.33
7	7c·HCl (total)	c,t-Me	$CDCl_3$	OCOMe	2.12,* 2.08
		c-Me	$CDCl_3$	OCOMe	2.08
		t-Me	$CDCl_3$	OCOMe	2.12
8	8d	c-Me	D_2O	3-Me	0.87 (doublet)
				OCOMe	2.15
				NMe_3	3.23
				CH_2N	3.93
		t-Me	D_2O	3-Me	0.93 (doublet)
				OCOMe	2.12
				NMe_3	3.28
				CH_2N	3.98
9	8c	c-Me	$CDCl_3$	OCOMe	2.08
				NMe_3	3.65
				CH_2N	4.40
		t-Me	$CDCl_3$	OCOMe	2.12
				NMe_3	3.57
				CH_2N	4.33
10	8a		D_2O	OCOMe	2.15
				NMe_3	3.27
				CH_2N	3.93
			$CDCl_3$	OCOMe	2.13
				NMe_3	3.59
				CH_2N	4.39

[a] With reference to OH or OCOMe. [b] In ppm from TMS (δ); signals are singlets unless otherwise stated, major signal of mixtures carries asterisk.

potency of β,β-dimethylACh compared with ACh and β-MeACh is due, in part at least, to its difficulty in attaining an antiperiplanar $^+N/O$ conformation. Similar considerations apply to the cyclohexyl derivatives **8**. In both *ax* (**16**) and *eq* (**17**) acetate types synclinal are preferred to antiperi-



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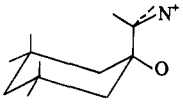

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planar $^+N/O$ conformers on the basis of minimum nonbonded interactions (see also ir evidence), and populations of antiperiplanar conformers will probably be low in all cases. The weak, although significant, activity of *c*-Me, *r*-OCOMe **8c** is in contrast to the complete inactivity of the 3-desmethyl analog **8a** and suggests than an *eq*-OCOMe group is pharmacologically advantageous in these derivatives (OCOMe will be *ax* in the preferred conformation of **8a** as a result of the greater steric requirements of $CH_2\overset{+}{N}Me_3$ compared with AcO.‡ The role of the Me substituent could then

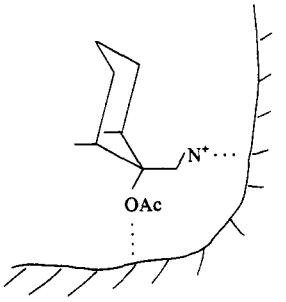
‡Comparisons of resolved pmr signals of **8a** with those of isomeric pairs of the 3- and 4-Me analogs **8c** and **8d** do not yield significant evidence on this point because only small differences in chemical shift occur between related *ax* and *eq* proton groups (Table II, No. 8-10).

Table III. ACh Agonistic and Antagonistic Activities of the Cyclohexyl Derivatives 8 and β,β-DimethylACh Iodide

Compound	Agonistic Activity (Guinea pig ileum) Equimolar Potency (ACh = 1)[a]	Antagonistic Activity (Frog rectus muscle) Affinity Constant[a]
Gallamine triethiodide		$3.6 \pm 0.1 \times 10^{-8}$
8a	Inactive	$1.2 \pm 0.05 \times 10^{-7}$
t-Me, *r*-OCOMe **8b**	Inactive	$1.4 \pm 0.04 \times 10^{-7}$
c-Me, *r*-OCOMe **8b**	Inactive	$1.7 \pm 0.2 \times 10^{-7}$
t-Me, *r*-OCOMe **8c**	Inactive	$1.3 \pm 0.1 \times 10^{-7}$
c-Me, *r*-OCOMe **8c**	$7.22 \pm 2.58 \times 10^{-5}$	$0.9 \pm 0.07 \times 10^{-7}$
t-Me, *r*-OCOMe **8d**	Inactive	$0.9 \pm 0.07 \times 10^{-7}$
c-Me, *r*-OCOMe **8d**	Inactive	$1.3 \pm 0.03 \times 10^{-7}$
β,β-Dimethyl-ACh	$4 \pm 0.8 \times 10^{-4}$ [b]	$0.8 \pm 0.04 \times 10^{-7}$

[a] ±standard error. [b] Cocolas and others[18] report 1×10^{-3}.

be that of a conformational holding group so placed that it does not impede binding to the receptor as may, for example, 2- and 4-Me in the *eq* OCOMe isomers *t*-Me, *r*-OCOMe **8b** and **8d**. An *eq*-OCOMe group is more accessible than an *ax* group and its approach to a receptor surface should be less hindered by the cyclohexyl moiety than would be the case for the *ax* isomer, as is depicted diagrammatically in **18** and **19**. Alternatively, the 3-Me group of *c*-Me, *r*-OCOMe **8c**



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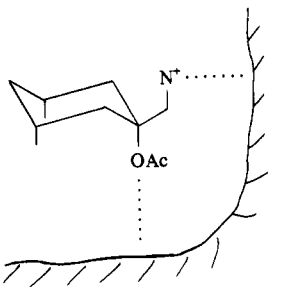

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might contribute directly to the receptor affinity of the molecule by providing an additional binding site or through an allosteric effect on the receptor. Studies on the antipodal forms of the 3-methyl isomer, now in progress, should help to clarify this problem.

None of the compounds had any spasmogenic activity on the frog rectus muscle in concn up to 100 μg/ml, a result which was not unexpected in view of knowledge that β-methylation reduces the nicotinic properties of ACh.[5] However, they antagonized the effect of ACh on this preparation. The affinity constants of the compounds were calcd as a measurement of antagonistic potency. As indicated in Table III, their affinity constants ranged from 0.8×10^{-7} to 1.7×10^{-7}, while the affinity constant of gallamine was found to be 3.6×10^{-8}. All compounds were about 0.05 as active as gallamine, and no great difference in potency was found amongst them. All compounds were found to cause a parallel shift of the dose-response curve to ACh on the frog rectus preparation, and, thus, they are classified as competitive antagonists of ACh. Their antagonistic effects were insensitive to configurational change.

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Acetylcholinesterase Reactivators. Pyridyl and Anilyl Trifluoromethyl Ketoximes

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The synthesis of 2-, 3-, and 4-trifluoromethyl ketoxime isomers of pyridine and *N,N*-dimethylaniline is described. The *in vitro* testing on phosphorylated AchE revealed good reactivating potency for 3-pyridinium trifluoromethyl ketoxime methochloride (1Mb). The compounds were weak reactivators *in vivo*. In protective studies, 1a, 1b, and 1c (2-, 3-, and 4-pyridyl trifluoromethyl ketoximes) were found to be quite active in protecting against several LD_{50} of either Paraoxon or Sarin.

Since the discovery of oximes[1] as potent reactivators of organophosphorus-inhibited acetylcholinesterase (AchE), 2-pyridyl aldoxime methiodide (2-PAM)[2] has been found to be be particularly effective, and most oximes assayed as potential reactivators have been modeled after this aldoxime. Many *N*-pyridinium derivatives of 2-PAM have thus been investigated.[3-6] Derivatives where the pyridine ring has been substituted or replaced by other N-heterocycles are also described as AchE reactivators.[5,7-9]

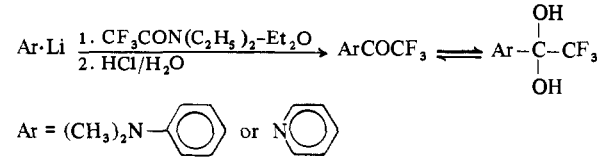
On the other hand, most oximes utilized or investigated as AchE reactivators are aldoximes, and only a few examples are known where the aldehydic hydrogen of 2-PAM has been substituted in order to obtain ketoxime analogs. The reactivating potencies of methyl[10] and phenyl[11] ketoxime derivatives of 2-PAM have been assessed. The phenyl ketoxime analogs although many times less active than the corresponding aldoximes are much better reactivators of organophosphorus-inhibited AchE than the methyl ketoxime analogs. While phenyl 3-pyridinium ketoxime methiodide is an excellent reactivator of methylsulfonylated AchE[12] most pyridinium aldoximes including 2-PAM are not. A closely related oxime, 2-pyridinium 1-acetophenone oxime methiodide shows one-sixth of the reactivating potency of 2-PAM with phosphorylated AchE.[13] Recently, 2-pyridyl 2-pyridinium ketoxime methyl bromide has been shown to be over 20 times as active as phenyl 2-pyridinium ketoxime methiodide.[14]

Since the substitution of the aldehydic hydrogen of 2-PAM analogs by an electron-rich group such as phenyl or pyridyl has led to active AChE reactivators, it appeared of interest to prepare some pyridinium and anilinium trifluoromethyl ketoximes (Table I, 1, 1M, 2, 2M). The rationale of the trifluoromethyl substituent was to obtain a pK_a value similar to that of 2-PAM while retaining an electron-rich but less bulky group in proximity of the oxime function.

The *in vitro* reactivating potencies of these new ketoximes have been evaluated with AchE inhibited by isopropylmethylphosphonofluoridate (Sarin), and correlations have been attempted with their structural and physical properties. Some *in vivo* data are also reported.

Chemistry. The new oximes were obtained in high yields (75% to quantitative) by standard techniques, *i. e.*, by refluxing the corresponding trifluoromethyl ketone or its *gem*-diol in a H_2O-MeOH solution with $NH_2OH \cdot HCl$ and sodium acetate. The trifluoromethyl ketones, some of which form very stable *gem*-diols, were prepared according to Scheme I as described previously.[15] The quaternization

Scheme I



of the basic nitrogen of the aryl moiety of the trifluoromethyl ketoximes was effected by refluxing with MeI in anhydrous EtOH or Me_2CO. The latter was found to be a better solvent giving cleaner reactions and better yields. The quaternary iodides were then converted to the chlorides by passing the former over an ion-exchange resin (IRA/400).

N,N-Dimethyl 2-trifluoromethyl ketoxime could only be quaternized by prolonged heating (15 days) with MeI and gave only a poor yield (26.5%).

Determination of the pK_a. Effects of the CF_3 Group on the Oximino Function. The pK_a's of the trifluoromethyl ketoximes were measured by a spectrophotometric method[16] and are shown in juxtaposition to those of their aldoxime analogs in Table II. Dyatkin and coworkers[17] pre-

†Taken from the Ph.D. thesis of M. Saucier, University of Montreal, 1970.