

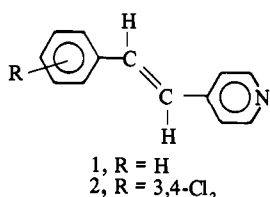
Irreversible Enzyme Inhibitors. 193.^{†,1} Inhibition of Choline Acetyltransferase. Mode of Binding by 4-Stilbazoles

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A variety of 4-stilbazole analogs were prepared and evaluated as inhibitors of choline acetyltransferase. The most potent inhibitors of the acetyltransferase have large nonpolar 3' substituents. The activity of the inhibitors does not correlate well with either the electronic or the hydrophobic nature of the 3' substituents. Substituent effects on the structurally similar azomethines were found to differ with respect to the 3' substituent. An interaction of a polarized double bond with the enzyme is proposed as one explanation for the observed results.

In an earlier report, a large variety of derivatives of 4-stilbazole (**1**) were evaluated as inhibitors of the enzyme choline acetyltransferase (ChA).² The examination of bulk tolerance adjacent to the Ph moiety of **1** showed that the Ph ring fits into a flat pocket on the enzyme which can accommodate only simple 3' and 4' substituents. Some rather remarkable increments in binding were reported for the simple substituents 3'-CH₃, 3'-Cl, and 3'-CH₃O. The 3',4'-dichloro-4-stilbazole (**2**) is the most powerful nonquater-



nized inhibitor of ChA to date. The inhibitor complexes to the enzyme 910 times more effectively than the substrate choline and 230 times more effectively than **1**.

Several rationalizations for the observed data have been presented.²⁻⁵ A common factor in the hypotheses is that a fair degree of binding can be attributed to the vinyl bridge either by direct interaction of the double bond with the enzyme or by the maintenance of a coplanar molecule which can act as both donor and acceptor in a charge-transfer complex with the enzyme.^{3,4} While the previous report presented data which indicated that a charge-transfer complex would not account for the binding of the nonquarterized compounds,² no working hypothesis was offered in its place. The preparation and evaluation of 17 additional inhibitors of ChA was undertaken to further examine the mode of binding of the stilbazole inhibitors. The results are the topic of this paper.

Enzyme Results. Choline acetyltransferase (ChA) and acetylcholine esterase (AChE) were isolated from rabbit brain acetone powder by the method of Potter, *et al.*⁶ ChA was assayed with 1 mM choline bromide and 0.05 mM [¹⁴C]acetyl-CoA by a modification of the method of McCaman and Hunt;⁷ AChE was assayed by a modification of the method of Potter⁸ using 1 mM [¹⁴C]ACh chloride as previously described.²

Previously reported values for the inhibition of ChA by **1** are $I_{50} = 470\text{--}600\ \mu\text{M}$.^{2,3} After repeated assay the consistent result of $I_{50} = 150\ \mu\text{M}$ was established (Table I). This earlier error in our results is believed to be due to a technical error. Examination of previously reported values for substituted stilbazoles rechecked favorably and **1** was the only inhibitor

in which an error greater than 25% was observed.

The bulk tolerance studies of the previous report indicated a tight steric fit of the Ph ring at the 3', 4' and 5' positions.² The 2'-Cl (**25**) and 2'-CH₃O (**24**) did not alter the binding of **1**. A study of the bulk tolerance at the 2'(6') positions was undertaken using 3'-Cl derivatives to increase the binding of the compounds.

Introduction of a 2'-EtO (**10**) on the Ph ring caused a loss in binding (Table I). The minimum I_{50} is estimated at 120 μM with a loss in binding of at least 15-fold.² This poor lateral fit of the Ph ring had been previously demonstrated by 3'-CH₃O-5'-CH₃-4-stilbazole which was less effective than **1** by 40-fold.²

Larger substituents were examined in the 2' position in the hope of forcing a conformational change in the enzyme which would result in a net increase in binding.⁹ Unfortunately binding was not increased by the large hydrophobic groups of **11** and **12**.

While a 2',3'-benzo substituent was reported by Cavalitto as a very effective inhibitor ($I_{50} = 25\ \mu\text{M}$),³ the 3'-Cl-2'-PrO (**9**) was not effective at 250 μM . The steric restrictions which allow only small substituents such as Cl and CH₃O apparently extend to the 2' and 6' positions.

The AChE is fairly insensitive to substituents on the Ph ring of **1** (Table I). The differences in inhibition are no greater than 3-fold among those compounds which do inhibit AChE.

The 3' substituents gave the largest increments in binding to ChA.² The 3'-CF₃ (**6**) gave a 10-fold increment in binding as compared to 3'-Cl (**3**), 3'-CH₃ (**4**), and 3'-CH₃O (**5**) which gave 19-, 12-, and 11-fold increments, respectively. The 3'-F (**8**) binds essentially the same as **1**. Since the CF₃, CH₃, Cl, and CH₃O are electronically dissimilar ($\sigma_m = 0.55, -0.17, 0.23,$ and -0.27 , respectively)¹⁰ it follows that the increase in binding by meta substituents is not attributable to electronic effects.

The 3'-I (**7**) gave an increment in binding of 170-fold which is the largest increment in binding observed for a simple meta substitution. The maximum energy of interaction in hydrophobic binding for simple substituents such as CH₃ and Cl has been calculated as 1.4 kcal/mole which amounts to a 10-fold increase in binding.¹¹ While such a maximum energy of interaction has not been calculated for the iodo substituent, one may estimate the hydrophobic contributions to binding by comparing π values for the substituents.¹² For example, one would expect greater hydrophobic contributions from substituents with large π values.

Unfortunately, a comparison of the π values for the 3' substituents does not correlate well with the observed increments in binding. For example, 3-I and 3-CF₃ have π

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Table I. Inhibition^a of Choline Acetyltransferase and Acetylcholine Esterase From Rabbit Brain by

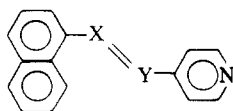
No.	R	ChA ^b				AChE ^c		
		Inhibn, μM	% inhibn	I_{50} ^d , μM	[S/I] ^e	Inhibn, μM	% inhibn	I_{50} ^d , μM
1 ^f	4-C ₆ H ₅ CH=CH			150	6.6	125	0	
3 ^f	4-(3-ClC ₆ H ₄ CH=CH)			8	130	500	33	1000 ^g
4 ^f	4-(3-CH ₃ C ₆ H ₄ CH=CH)			12	83	60	0	
5 ^f	4-(3-CH ₃ OC ₆ H ₄ CH=CH)			13	77	250	20	1000 ^g
6	4-(3-CF ₃ C ₆ H ₄ CH=CH)			15	66	500	30	1100 ^g
7	4-(3-IC ₆ H ₄ CH=CH)			0.9	1100	500	0	
8	4-(3-FC ₆ H ₄ CH=CH)			92	11			1400
9	4-(3-Cl-2-PrOC ₆ H ₃ CH=CH)	250	0			50	0	
10	4-(5-Cl-2-EtOC ₆ H ₃ CH=CH)	31 ^h	0			500	33	1500 ^g
11	4-(5-Cl-2-C ₆ H ₄ CH ₂ OC ₆ H ₃ CH=CH)	31 ^h	0			10	0	
12	4-(5-Cl-2-C ₆ H ₄ O(CH ₂) ₃ OC ₆ H ₃ CH=CH)	150	0			10	0	
13	4-C ₆ H ₅ N=CH			2800		3000	0	
14	4-(3-ClC ₆ H ₄ N=CH)			2100		500	0	
15	4-(3,4-Cl ₂ C ₆ H ₃ N=CH)			330		125	0	
16 ⁱ	4-(C ₆ H ₅ CH ₂ ON=CH)			280	3.4	500	0	
17	4-(3,4-Cl ₂ C ₆ H ₃ CH ₂ ON=CH)	250 ^h	0			250	24	600 ^g
18	4-(α -Naphthyl-CH ₂ ON=CH)	300 ^h	22	800 ^g	1.2	500	26	1500 ^g
19	3-(3-ClC ₆ H ₄ CH=CH)	500	37	1000 ^g	1	500	40	750 ^g
20	2-(3-ClC ₆ H ₄ CH=CH)			8.8	114	50	0	
21	4-(3-CH ₃ OC ₆ H ₄ C(CH ₃)=CH)	250	0	>1000		50	0	
22	4-(3-ClC ₆ H ₄ CH=CCH ₃)	500	32	1050 ^g		500	35	1600 ^g

^aThe technical assistance of Julie Beardslee, Nancy Middleton, and Pauline Minton is acknowledged. ^b Assayed with 1 mM choline bromide and 0.05 mM [¹⁴C]acetyl-CoA, as previously described. ^c Assayed with 1 mM [¹⁴C]ACh·Cl as previously described. ^d Conc'n for 50% inhibition. ^e Ratio of substrate to inhibitor giving 50% inhibition. ^f Data from ref 2. ^g Estimated from inhibition at maximum solubility. ^h Maximum solubility. ⁱ Aldrich Chemical Co.

values of 1.16 and 1.22,¹² respectively, and would be expected to bind equally well. The 3'-I (7) is more effective than the 3'-CF₃ (6) by 16-fold. Also, 3'-CH₃O (5) should not have a large hydrophobic contribution from the 3-CH₃O with $\pi = 0.04$,¹² but 5 actually complexes as well as 3'-CH₃ (4) and 3'-CF₃ (6) which have π values of 0.49 and 1.16, respectively.

It was noted previously that the maximum increment in binding for substituents such as CH₃ and Cl is 10-fold. Electronic effects have been discounted and hydrophobic effects do not correlate well with the increments in binding. While some hydrophobic contributions are possible (keeping in mind 1.4 kcal/mole is the maximum binding energy), we must find another factor to explain the effects of 3' substituents.

Cavalitto reported an interesting series of compounds in which either C of the vinyl bridge or both carbons were replaced by N atoms.¹³ Of these azomethines, the isomer with N β to the pyridine ring (23a) was an inhibitor while the isomer with N α to the pyridine ring (23b) was ineffective as an inhibitor. Since the substituent effects were not examined, we prepared several analogs of 23a for evaluation against ChA.



23a, X = N, Y = CH
23b, X = CH, Y = N

The unsubstituted Ph (13) complexed to the enzyme 5% as effectively as 1. Introduction of a 3-Cl (14) did not increase binding of the inhibitor. The 3,4-Cl₂ (15) gave an 8-fold increment in binding. The increase from the 4-Cl can be attributed to hydrophobic binding.

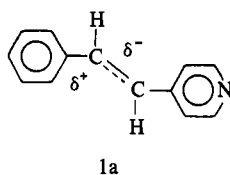
The anil 13 is believed to complex to the enzyme at the same site as 1. Cavalitto suggested that a loss in binding would be expected since the Ph ring of the anil is 30–60° out of plane with the rest of the molecule. This lack of planarity of the Ph ring cannot, however, account for the total loss in binding. For example, the nonplanar 2-CH₃O-4-stilbazole (24) and 2'-Cl-4-stilbazole (25)² (uv studies show that the ortho substituent disrupts planarity, 24, ϵ 14,000 at λ_{max} 310 m μ ; 25, ϵ 17,000 at λ_{max} 300 m μ , compared to 1, ϵ 24,000 at λ_{max} 300 m μ) bind essentially the same as 1.² The differences in binding between the stilbazoles and the anils are primarily due to the differences in the electronic nature of the bridge between the aromatic rings.

The most striking contrast between the anils and the stilbazoles is that the introduction of a 3'-Cl does not show the same effect. The 4'-Cl shows a similar effect on the anil and stilbazole which is believed to be hydrophobic in nature.² The lack of any hydrophobic contribution by the 3'-Cl of 19 suggests that the contribution by the 3'-Cl of 3 may not be hydrophobic.

An interesting variation of the bridge between the pyridine and benzene rings is embodied in the aldoxime (16). The benzyloxy function has considerable rotational freedom and need not be planar. The α -naphthyl derivative (18) is less effective by a factor of 3-fold. The 3,4-Cl₂ (17) shows no inhibition at 250 μM . These results are in accord with the bulk tolerance studies on 1.

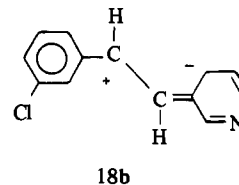
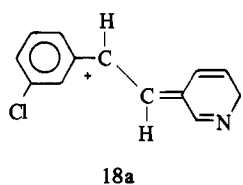
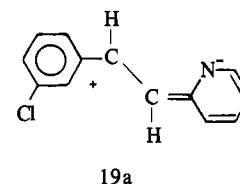
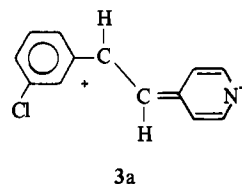
Substitution on the vinyl bridge has been reported to reduce binding of the stilbazole inhibitor.³ α -Me (22) was less effective than 2 by 120-fold. β -Me (21) showed a minimum loss of 120-fold compared to 4. The decrease in binding of 21 and 22 may be accounted for by (1) a loss in planarity of the inhibitor, and/or (2) steric interaction of the Me substituent with the enzyme.

We suggested earlier that the vinyl bridge may contribute



to the binding of 4-stilbazole by a direct interaction of the double bond with the enzyme. One possibility is that the double bond is polarized by a mesomeric interaction with the Ph ring and the pyridyl ring causing a partial positive charge on C adjacent to the benzene ring and a partial negative charge on C next to the pyridine ring. In this manner a nucleophilic residue on the enzyme surface would have a strong interaction with the partial positive charge on the β -carbon.

The order of binding of 4-, 3- and 2-stilbazoles was reported and confirmed as $4 > 2 > 3$.^{2,3} The reason for this order of binding is not readily apparent even using a structure such as 1a. In the limiting case when full formal charges exist on the bridge, the resonance structures which place the negative charge in the pyridine ring provide a reasonable rationalization. The resonance structures 2a and 18a would place the negative charge in the pyridine ring with added stabilization by the N. On the other hand, the resonance structures 18a and 18b offer no extra stabilization by N. We would then expect that the 2- and 4-stilbazoles would bind similarly and the 3-stilbazole would be much less effective.



In order to examine this hypothesis, 4-, 3-, and 2-stilbazoles were prepared in which a 3'-Cl was introduced to increase binding. As expected, 3 and 19 were found to complex equally well to the enzyme, $I_{50} = 8 \mu M$ and $12 \mu M$, respectively. The 3'-Cl-3-stilbazole (18) was 110-fold less effective.

The loss in binding of 18 compared to 3 and 19 can be explained by invoking the resonance arguments presented above. Alternatively, the positioning of the pyridine of 3-stilbazole may cause an unfavorable interaction between the N and the enzyme. Since the N atom of the pyridine can point in 2 directions by rotation of the pyridine ring around the pyridyl-vinyl bond, it would have to be repulsed in both positions.

Table II. Physical Properties of

No.	R	HX	Method ^a	% yield ^b	Mp, °C	Formula ^c
6	4-(3-CF ₃ C ₆ H ₄ CH=CH)	HCl	A ^d	58 ^e	231-234	C ₁₄ H ₁₁ ClF ₃ N
7	4-(3-ICl ₂ C ₆ H ₄ CH=CH)	HCl	A ^d	43 ^e	211 subl	C ₁₃ H ₁₁ ClIN
8	4-(3-FC ₆ H ₄ CH=CH)	TsOH	C	63 ^f	226-228	C ₂₀ H ₁₈ ClFNSO ₃
9	4-(3-Cl,2-PrOC ₆ H ₃ CH=CH)	Picrate	A ^d	16 ^g	190-192	C ₂₂ H ₁₉ ClN ₂ O ₈
10	4-(5-Cl,2-EtOC ₆ H ₃ CH=CH)	HCl	C ^h	53	210 subl	C ₁₈ H ₁₅ Cl ₂ NO
11	4-(5-Cl,2-C ₆ H ₄ CH ₂ OC ₆ H ₃ CH=CH)	HCl	C ⁱ	60 ^e	202-205	C ₂₇ H ₁₇ Cl ₂ NO
12	4-(5-Cl,2-C ₆ H ₅ O(CH ₂) ₃ OC ₆ H ₃ CH=CH)	Picrate	C ⁱ	12 ^j	178-179	C ₂₈ H ₂₃ ClN ₂ O ₉
13	4-C ₆ H ₄ N=CH		D	72 ^k	72-73	C ₁₂ H ₁₀ O
14	4-(3-ClC ₆ H ₄ N=CH)		D	90 ^k	77-78	C ₁₂ H ₉ ClN ₂
15	4-(3,4-Cl ₂ C ₆ H ₃ N=CH)	OH	D	62 ^l	93-94	C ₁₂ H ₈ Cl ₂ N ₂
17	4-(3,4-Cl ₂ C ₆ H ₃ CH ₂ ON=CH)	HCl	D ^m	26 ^e	193-194	C ₁₃ H ₁₁ Cl ₂ N ₂ O
18	4-(α -Naphthyl-CH ₂ ON=CH)	HCl	D ⁿ	27 ^e	200 dec	C ₁₇ H ₁₅ ClN ₂ O
19	3-(3-ClC ₆ H ₄ CH=CH)	HCl	A	3 ^e	194-196	C ₁₃ H ₁₁ Cl ₂ N
20	2-(3-ClC ₆ H ₄ CH=CH)	Picrate	A	24 ^e	227-228	C ₁₃ H ₁₃ ClN ₂ O ₇
21	4-(3-CH ₃ OC ₆ H ₄ C(CH ₃)=CH)	Picrate	B	63 ^e	180-181	C ₂₁ H ₁₈ N ₄ O ₈
22	4-(3-ClC ₆ H ₄ CH=C(CH ₃)	HCl	C	20 ^e	189-191	C ₁₄ H ₁₃ Cl ₂ N

^aMethods: A, see ref 2; C, see ref 2; B and D, see Experimental Section. ^bYield of analytically pure material and is minimum. ^cAnalyzed for C, H, and N within 0.4% of theoretical. ^dFor starting Wittig reagent, see Table III. ^eRecrystd from EtOH-petr ether. ^fRecrystd from EtOH-petr ether followed by digestion in hot EtOAc. ^gRecrystd from EtOH-H₂O. ^hStarting aldehyde prep'd by alkylation of 5-Cl-salicylaldehyde with EtI was an oil used without purification. ⁱFor starting aldehyde, see Table III. ^jRecrystd from EtOH. ^kRecrystd from hexane. ^lRecrystd from EtOAc-hexane. ^mFor starting 3,4-dichlorobenzoyloxamine, see Table III. ⁿThe 1-naphthylmethoxyamine, see ref 14, was prepared by hydrazinolysis of *N*-(1-naphthylmethoxy)phthalimide by the method of Drain, *et al.*¹⁵

Table III. Physical Constants of Intermediates

No.	R	Method ^a	% yield ^b	Mp, °C	Formula ^c
26	3-CF ₃ C ₆ H ₄ CH ₂ P(C ₆ H ₅) ₃ Cl	E	66 ^d	286-288	C ₂₆ H ₂₁ ClF ₃ P
27	3-ICl ₂ C ₆ H ₄ CH ₂ P(C ₆ H ₅) ₃ Br	E ^e	23 ^f	296-298	C ₂₅ H ₂₁ BrIP
28	3-Cl,2-PrOC ₆ H ₃ CH ₂ P(C ₆ H ₅) ₃ Br	E ^{e,g}	32 ^h	164-166	C ₂₈ H ₂₇ BrClOP
29	5-Cl,2-C ₆ H ₃ CH ₂ OC ₆ H ₃ CHO	F	82 ⁱ	79-80	C ₁₄ H ₁₁ ClO ₂
30	5-Cl,2-C ₆ H ₃ O(CH ₂) ₃ OC ₆ H ₃ CHO	F	74 ^d	76-77	C ₁₆ H ₁₅ ClO ₃
31	3,4-Cl ₂ C ₆ H ₃ CH ₂ ONH ₂ ·HCl	G	53 ^j	120-122	C ₇ H ₈ Cl ₂ NO
32	C ₁₀ H ₇ CH ₂ ON(CO) ₂ C ₆ H ₄	H	78 ^k	140-142	C ₁₉ H ₁₃ NO ₃

^aMethods: E, prep'd by treating the appropriate α -halotoluene with PPh₃, see ref 2; F, alkylation of 5-Cl-salicylaldehyde with an alkyl halide by the general method of Baker and Erickson;¹⁶ G, prep'd by the hydrazinolysis of *N*-(3,4-dichlorobenzoyloxy)phthalimide, see McKay, *et al.*¹⁷ ^bExplained in Table II. ^cRecrystd from EtOH. ^dPrep'd by treating substd toluene with NBS in CCl₄. ^eRecrystd from Me₂CO-EtOH-hexane. ^fSubstd toluene prep'd by alkylation of 3-Cl-O-cresol, see Method F. 3-Cl-2-PrO-toluene and 2-bromo-3-Cl-2-PrO-toluene were oils used without purification. ^gWittig reagent was an oil which solidified slowly on trituration with EtOAc. ^hRecrystd from EtOH-H₂O. ⁱEtOH-petr ether. ^kMe₂CO-H₂O.

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 CHCl₃. 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 prep'd by the addn of 6.5 ml of a 2.2 M Et₂O 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 NaHCO₃ was added to pH 7. The product was extd into 3 x 50 ml of EtOAc and dried (Na₂SO₄) 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 POCl₃ 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 x 70 ml of EtOAc, washed free of base, and dried (Na₂SO₄). The solvent was removed *in vacuo* leaving a light oil which was treated with a sat'd 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-pyridylalldoxime (18)·HCl Method D. A mixt of 3.3 g (18 mmoles) of 3,4-dichlorobenzoyloxamine and 2.0 g (18 mmoles) of 4-pyridinecarboxaldehyde in 100 ml of PhMe was brought to reflux for 6 hr and H₂O 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*) ¹⁴N/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.*, 1³ and 2.⁴ As muscarinic agonists, these derivatives suffer from the drawback of being α -alkyl-substituted acetylcholines, for it is