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Diacetoxypiperidinium Analogs of Acetylcholine†

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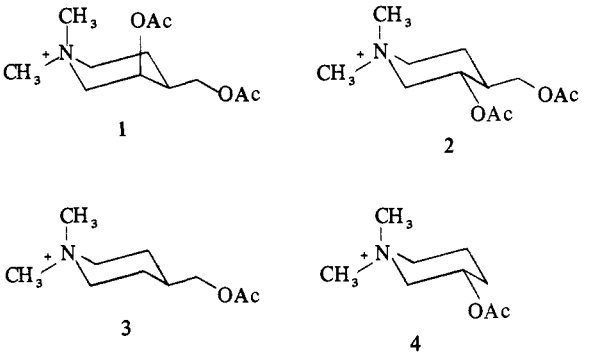
The syntheses of *cis*- and *trans*-*N,N*-dimethyl-4-acetoxymethyl-3-acetoxypiperidinium iodides (1 and 2), *N,N*-dimethyl-4-acetoxymethylpiperidinium iodide (3), and *N,N*-dimethyl-3-acetoxypiperidinium iodide (4) are described. Muscarinic action, 1/100 that of acetylcholine, was found in 1 and 4. Compounds 2 and 4 were relatively good substrates for acetylcholinesterase; compared to acetylcholine respective rates of hydrolysis of 55 and 71% were observed. Analysis of the models leads to the conclusion that the optimal torsional angle, the N–C–C–O portion of the molecule, is synclinal for agonist binding and antiperiplanar for esterase binding.

Many conformational studies have attempted to correlate acetylcholine (ACh) structure with the various biological activities. Most of these efforts have been focused on the optimal torsional angle of the N–C_α–C_β–O portion of ACh and the relationship to both muscarinic action and substrate activity for acetylcholinesterase (AChE). Approaches used to verify if this torsional angle defines the biological action have included the relative biological effects of conformational ACh analogs[1-23] (see ref 24 for a report on the muscarinic and esterase activities of **4**), X-ray crystallography of ACh analogs, and quantum mechanical calculations of preferred conformations of ACh.[25-30]

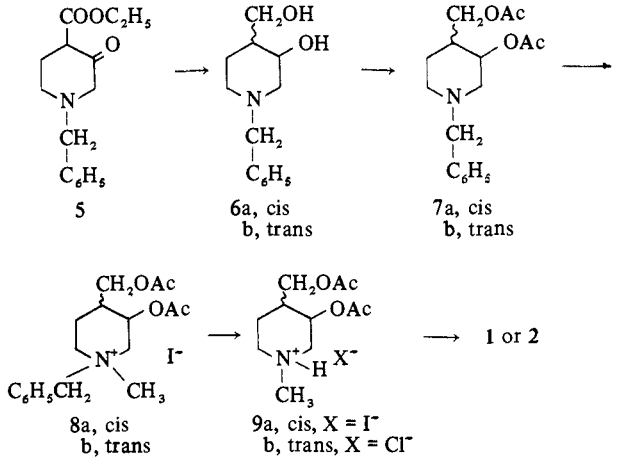
Recent conclusions defining the torsional angle with respective ACh activities are not consistent. While there is a great deal of support for muscarinic reception of the synclinal ACh structure ($\Phi \sim 60$), there is strong evidence for the antiperiplanar ($\Phi \sim 180$). Similarly, ACh as a substrate for AChE is proposed by the majority of investigators to adopt the 150° torsional angle; however, some studies suggest that the fully extended 180° angle is optimal.

In the course of our work some compounds became available that are useful models for examining the torsional angle of the N-C-C-O fragment analogous to ACh. *cis*- and *trans*-dimethyldiacetoxypiperidinium salts **1** and **2**, while not approaching the rigidity of perhydroquinoline or decalin models, have preferred conformers that can be assigned on the basis of the energy of steric interaction arising in the 3 and 4 substituents. The monoacetoxypiperidinium salts **3** and **4** were synthesized as control models to examine the biological response of the respective acetoxy groups individually.

Sodium borohydride reduction of **5** gave a 4:1 mixture of the diols **6a** and **6b** which were separated on alumina.



Acetylation of **6a** and **6b** gave the respective diacetates **7a** and **7b**. The nmr evidence for the assignment of structure (Table I) is based on the position and half width of



the methine porton at C_3. The trans compounds **6b** and **7b** with the C_3H axial show a higher field absorption than the equatorial C_3H (**6a**, **7a**) and a half width of about 20

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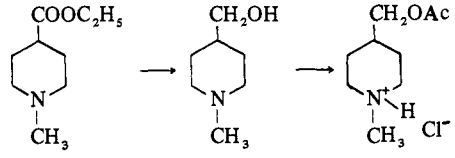
cps; this compares to a similar value of 14 cps for the *cis* isomers **6a** and **7a**.

Conversion of **7a** and **7b** to the respective methiodides **8a** and **8b**, hydrogenolysis to the amines **9a** and **9b**, and conversion to the methiodides **1** and **2** completed the synthesis.

The monoacetate **3** was synthesized from ethyl 1-methyl-4-piperidinecarboxylate (**10**) *via* lithium aluminum hydride reduction to **11**,[31] acetylation, yielding **12**, and conversion to the methiodide **3**. Compound **4** was synthesized by the method of Biel and coworkers.[32]



10 11 12

Biological Results. The muscarinic activity was tested on five different preparations of guinea pig ileum and at least five different concentrations of each compound given by the cumulative dose–response method using ACh^+Cl^- as the reference.[33] The most active muscarinic agents were the monoacetate **4** and the cis diacetate **1**, estimated to be about 1/100 that of ACh. Activity in the trans isomer **2** was not observed in concentrations up to 10^5 times that of ACh. The 4-acetoxymethyl compound **3** was a weak agonist, approximately 1/1000 as active as ACh.

Table I. Chemical Shift of the C_3 Methine Proton

Compd	C_3-H, δ	$W^{1/2}$, cps	Compd	C_3-H, δ	$W^{1/2}$, cps
6a	3.9	9	8b	5.2	20
6b	3.5		9a	5.4	8
7a	5.0	8	9b	5.1	
7b	4.9	21	1	5.4	10
8a	5.4	10	2	5.2	17

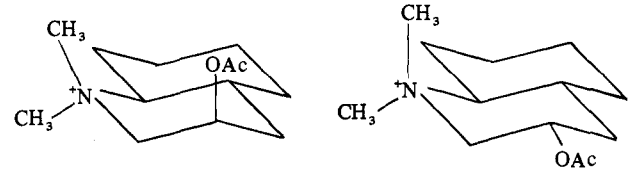
Atropine-like action was found in the benzylic derivatives **8a** and **8b**. The *cis* derivative **8a** was a better muscarinic blocking agent ($pA_2 \sim 6.33$) than the trans **8b** ($pA_2 \sim 4.33$); atropine for comparison was a pA_2 of 8.1. The effects on cholinesterase were measured in several systems. Purified enzyme from horse serum (Type IV, Sigma) was the system used to estimate inhibition results against pseudo- or butyrylcholinesterase. Purified enzyme from the electric eel (Type III, Sigma) was used to estimate inhibition of "true" AChE.

Compounds **1–4** were weak competitive inhibitors of both horse and eel cholinesterase; the best inhibitor, **2**, had a K_i of 9×10^{-5} *M*. This is not unusual since many simple quaternary ammonium compounds show inhibition in this range.[34–36]

As substrates for horse serum cholinesterase, **1**, **3**, and **4** were poor with the exception of the 3-acetoxy compound **4**, which was hydrolyzed at 28% the rate of ACh. Using eel AChE, compounds **2** (55% the rate of ACh) and **4** (71%) were relatively effective substrates (Table II).

From the studies on the agonist action it can be assumed that the muscarinic effect of **1** and **4** is through interaction of the 3-acetoxy group since compound **3** is virtually inactive. The question of muscarinic action of ACh being exerted *via* a synclinal (60°) or an antiperiplanar (180°) torsional angle for the N^+–C–C–O chain is compared in **1** and **2**. It is reasonable to assume that **2** will maintain a trans-diequatorial orientation and represent the antiperiplanar 180° structure in analogy to ACh.

The nmr of all the cis isomers (**2**, **6a**, **7a**, **8a**, and **9a**) confirmed the equatorial orientation of the C-3 methine proton. Therefore, the preferred conformation for the cis (**1**) is the axial acetate at C-3; this represents the synclinal torsional angle of ~60°. Since the 180° model (**2**) is inactive and the 60° analog (**1**) is a relatively potent agonist, the muscarinic ACh torsional angle is closer to 60°, not 180°, in agreement with the proposed 73–137° angle.[37] Similar muscarinic activities were reported for decahydroquinoline analogs **13** and **14**. Compound **13** is reported to have 1/50 the potency of ACh on the muscarinic receptor, while **14** was without detectable agonist activity.[5]



13 14

Several arguments against this interpretation are obvious. To answer the first, the 3-acetoxy group compound **4** has a preference for the equatorial acetate conformer; however, the value of ~0.5 kcal/mol suggests that approximately 20% exists in the axial conformer[16,38] which is the proposed, active synclinal form for muscarinic action. Secondly, it can be argued that the acetoxymethyl at C-4 is responsible for the muscarinic action of **1**; alternatively, the 4-acetoxymethyl group of analog **2** sterically prevents binding. Both of these points are settled by comparing the activity of **3**; no atropine-like action was detected and only weak agonist action was observed (1/100 that of **1**).

The benzylic isomers **8a** and **8b** with muscarinic blocking effects follow the predicted pattern; the cis (**8a**) is 1/100 as potent as atropine while the trans (**8b**) is 1/10,000.

The torsional angle for optimal binding of ACh to the esterase has been proposed by many investigators to be 150°. Prompted by the results of inhibition by a series of dihydro-

Table II. Substrate Activity for Cholinesterase

Compd	Enzyme	K_m, m*M*	V_{max}, μmol/ml/min	E_0, μmol (units)/ml	V_{max}/E_0	Rate of hydrolysis[a]
1	Horse serum AChE	1.39 ± 0.45	0.103 ± 0.026	3.15	0.032	0.03
3	Horse serum AChE	0.74 ± 0.09	0.052 ± 0.003	5.7	0.0091	0.01
4	Horse serum AChE	1.12 ± 0.18	0.310 ± 0.032	1.12	0.277	0.28
1	Eel AChE	1.03 ± 0.07	0.319 ± 0.015	1.75	0.182	0.16
2	Eel AChE	1.44 ± 0.24	0.736 ± 0.09	1.20	0.613	0.55
3	Eel AChE	0.35 ± 0.03	0.205 ± 0.005	1.0	0.205	0.18
4	Eel AChE	0.206 ± 0.02	0.195 ± 0.005	0.25	0.781	0.71

[a] ACh = 1.00; K_m = 0.11 ± 0.03 m*M*.

benzofurans we suggested 180° as the preferred angle for binding to the esterase.[15]

Good substrate activity has been observed in compounds 4 (71% ACh) and 2 (55% ACh). Structure 4 has the preferred equatorial conformation, yet retains flexibility to assume the axial 3-acetoxyl structure without significant 1,3-diaxial interactions. The trans (2), assumed to be frozen in the diequatorial conformer, at the extreme antiperiplanar angle represents the 180° torsional angle and is a good substrate (55%). In contrast, the cis (1), observed in nmr studies to exist in the axial 3-acetoxy conformer, represents the synclinal (60°) torsional angle and is a poor substrate (16% ACh).

Conclusions reached in these studies, comparing 60 or 180° as the optimum torsional angle for the N-C-C-O fragment in acetylcholine, suggest 60-90° for action at the muscarinic receptor and 150-180° for cleavage by acetylcholinesterase. The former results do not agree with the proponents of a antiperiplanar angle for muscarinic action.[4-8,11,17,18]

Experimental Section§

cis- and *trans*-*N*-Benzyl-4-hydroxymethyl-3-hydroxypiperidine (6a,b). Ethyl *N*-benzyl-3-ketopiperidine-4-carboxylate (10 g, 40 mmol) was dissolved in dry MeOH (400 ml) and added dropwise with rapid stirring to the powdered $NaBH_4$ (27 g, 800 mmol) at room temperature. Addition was continued over 90 min to avoid vigorous reflux and foaming of the mixture. After stirring for 24 hr, H_2O (400 ml) was added dropwise over 15 min and stirring was continued for 24 hr. The MeOH was removed under reduced pressure at 40° and the remaining suspension extracted with $CHCl_3$ (3 × 500 ml). The $CHCl_3$ portions were combined, dried ($MgSO_4$), filtered, and evaporated giving 7.6 g (100%) of a colorless sweet-smelling oil which crystallized upon standing for several hours. The oil (6.8 g) was chromatographed on a 33 × 5 cm column of alumina (Woelm Grade II) eluted with 0.5% MeOH-$CHCl_3$. The cis compound 6a was the first material off the column giving 5.0 g (77%) of colorless oil which crystallized rapidly. The solid was recrystallized from Skelly B-$CHCl_3$ giving white needles, mp 77-78°. *Anal.* ($C_{13}H_{19}NO_2$) C, H, N.

The trans compound 6b was the second material giving 1.5 g (22%) of colorless oil which crystallized in the flask. Recrystallization from Skelly B-$CHCl_3$ gave white fluffy crystals, mp 104-104.5°. *Anal.* ($C_{13}H_{19}NO_2$) C, H, N.

cis-*N*-Benzyl-*N*-methyl-4-acetoxymethyl-3-acetoxypiperidinium Iodide (8a). The cis diol 6a (221 mg, 1 mmol) was dissolved in pyridine (10 ml) and Ac_2O (5 ml). The mixture was warmed on a steam bath for 1-2 min and stirred in a stoppered flask for 18 hr at room temperature. The mixture was poured into ice-H_2O (200 ml) and extracted with $CHCl_3$ (3 × 50 ml); the organic layers were combined, dried ($MgSO_4$), filtered, and evaporated *in vacuo* to give 260 mg (95%) of *cis*-*N*-benzyl-4-acetoxymethyl-3-acetoxypiperidine (7a) as a colorless oil after decolorizing with activated charcoal.

The amino diacetate 7a (305 mg, 1 mmol) was dissolved in anhydrous Et_2O (150 ml) and treated with MeI (10 ml). The mixture was warmed for several seconds in a stoppered flask and stirred at room temperature for 24 hr. The precipitate was collected in a drybox giving 320 mg (75%) of the methiodide 8a as a fine white solid. Recrystallization of 8a from anhydrous EtOH-anhydrous Et_2O gave a grannular crystalline white solid, mp 183-184°. *Anal.* ($C_{18}H_{26}INO_4$) C, H, N.

trans-*N*-Benzyl-*N*-methyl-4-acetoxymethyl-3-acetoxypiperidinium Iodide (8b). The trans diol 6b (221 mg, 1 mmol) was treated in an identical manner as the cis diol 6a for conversion to the diacetate. Work-up gave 270 mg (100%) of *trans*-*N*-benzyl-4-acetoxymethyl-3-acetoxypiperidine (7b).

The amino diacetate 7b (1.0 g, 3.3 mmol) was dissolved in anhydrous Et_2O (500 ml), treated with MeI (25 ml), and stirred at room temperature for 48 hr. Filtration yielded 650 mg (35%) of 8b as a light yellow hydroscopic solid, mp 207-208°. *Anal.* ($C_{18}H_{26}INO_4$) C, H, N.

cis-*N*-Methyl-4-acetoxymethyl-3-acetoxypiperidine Hydriodide (9a). The cis-quaternary compound 8a (3.4 g, 1.1 mmol) was dissolved in 95% EtOH (250 ml) and the benzyl group hydrogenolyzed using 10% Pd/C (1.0 g) at 50 psi of H_2 for 12 hr. The catalyst was filtered and the solvent removed *in vacuo* giving 1.50 g (95%) of a dark red oil which gave a pink solid upon addition of anhydrous Et_2O. The solid was recrystallized from absolute EtOH-anhydrous Et_2O giving 1.30 g (88%) of the hydriodide 9a, mp 145-146°. *Anal.* ($C_{11}H_{20}INO_4$) C, H, N.

trans-*N*-Methyl-4-acetoxymethyl-3-acetoxypiperidine Hydrochloride (9b). The trans-quaternary compound 8b (1.0 g, 2.1 mmol) was dissolved in 95% EtOH (200 ml) and the benzyl group hydrogenolyzed over 10% Pd/C (0.2 g) at 50 psi of H_2 in a Parr apparatus. The catalyst was filtered and the solvent removed *in vacuo* giving 0.4 g (95%) of an orange oil which would not solidify. The oil was dissolved in H_2O (10 ml) and the solution treated with 10% Na_2CO_3. Extraction of the aqueous solution (3 x 50 ml) with $CHCl_3$, drying (Na_2SO_4), filtration, and evaporation of the solvent gave a colorless oil which was dried at 40° for 8 hr at 0.1 mm. The oil was dissolved in anhydrous Et_2O and HCl-saturated Et_2O added to the solution until no more precipitate appeared. The solution was stirred for 2 hr and the product (9b) collected by filtration in a drybox.

Alternatively, the *trans*-*N*-benzyl diacetate 7b (1.7 g, 0.6 mmol) was dissolved in dioxane (150 ml) and combined with CH_2O (0.15 g of 37% aqueous solution), 10% Pd/C (0.8 g), and H_2 at 50 psi in a Paar apparatus. After shaking 36 hr the catalyst was filtered and the solvent evaporated under reduced pressure giving 1.2 g (100%) of a colorless, fruity-smelling oil. The oil was dried at 20 mm for 4 hr and dissolved in anhydrous Et_2O. Et_2O saturated with HCl was added until no further precipitate formed and the mixture was stirred for 2 hr. The precipitate was filtered in a drybox giving 0.90 g (60%) of 9b as a white solid, mp 163-164°. *Anal.* ($C_{11}H_{20}ClNO_4$) C, H, N.

cis-*N*,*N*-Dimethyl-4-acetoxymethyl-3-acetoxypiperidinium Iodide (1). The *cis*-*N*-methyl diacetate hydriodide 9a (1.8 g, 0.5 mmol) was dissolved in H_2O (20 ml) and 10% $NaHCO_3$ added (50 ml). The solution was extracted with $CHCl_3$ (3 × 150 ml) and the $CHCl_3$ was dried (Na_2SO_4), filtered, and evaporated *in vacuo* to give the free base (1.30 g, 100%) as a colorless oil with slight fishy odor. The oil was dried for 4 hr at 20 mm, dissolved in anhydrous Et_2O (200 ml), and allowed to react with 10 ml of MeI at room temperature. The solution was stirred for 8 hr and the *cis*-dimethyl compound was collected by filtration in a drybox giving 1.6 g (77%) of analytically pure methiodide, mp 143-144°. *Anal.* ($C_{12}H_{22}INO_4$) C, H, N.

trans-*N*,*N*-Dimethyl-4-acetoxymethyl-3-acetoxypiperidinium Iodide (2). The *trans*-*N*-methyl diacetate HCl 9b (0.80 g, 0.3 mmol) was dissolved in H_2O (10 ml) and 10% $NaHCO_3$ (50 ml) was added. The solution was extracted with Et_2O (3 × 250 ml) and the Et_2O was dried (Na_2SO_4), filtered, and evaporated giving 0.65 g (100%) of free base as a colorless oil. The oil was dried for 4 hr at 20 mm, dissolved in anhydrous Et_2O (500 ml), and allowed to react with MeI (10 ml). The flask was stoppered and the mixture stirred at room temperature for 36 hr. The light yellow precipitate which formed (hydroscopic) was filtered in a drybox giving 1.0 g (83%) of compound 2 as the monohydrate, mp 176-178°. *Anal.* ($C_{12}H_{22}INO_4 \cdot H_2O$) C, H, N.

N-Methyl-4-hydroxymethylpiperidine (11). Ethyl *N*-methylpiperidine-4-carboxylate (10, 8.9 g, 5.2 mmol) in anhydrous Et_2O was added dropwise with stirring to $LiAlH_4$ (4.6 g, 120 mmol) in anhydrous Et_2O (300 ml) at 25°. The mixture was refluxed for 2 hr and stirred at 25° for 8 hr at which time H_2O (10.8 g, 600 mmol) was added cautiously through a septum. The reaction mixture was stirred for 2 hr, the inorganic salts were filtered, and the Et_2O was evaporated giving 6.3 g (90%) of a colorless oil which was distilled at reduced pressure to give amino alcohol 11, bp 52° (0.05 mm) [lit.[31] 115.5° (6.5 mm)].

N-Methyl-4-acetoxymethylpiperidine Hydrochloride (12). To the amino alcohol 11 (4.0 g, 3.2 mmol) in pyridine (100 ml) was added Ac_2O (25 ml). The mixture was warmed on a steam bath for

§ Melting points were obtained on a calibrated Thomas-Hoover Unimelt and are corrected. Infrared data were recorded on Beckman IR 8 and IR 10 spectrophotometers. Nuclear magnetic resonance spectra were recorded on Varian Associates Model A-60A and T-60 spectrometers using tetramethylsilane as internal standard and were as expected. Microanalyses were conducted on an F & M Model 185 C H N analyzer at the University of Kansas and, where reported, are within ±0.4% of the theoretical values.

1 min and stirred at 25° for 24 hr. The solution had become light orange and 2–3 g of activated charcoal was added to the flask and stirred for 12 hr. The solution was filtered and the Ac_2O–pyridine removed by distillation at reduced pressure giving 4.0 g (76%) of a brown oil. The oil was distilled under high vacuum to give 2.0 g (39%) of the amino ester **12**, bp 42° (0.1 mm). The amine was converted to the HCl salt for purification, mp 134–135°. *Anal.* ($C_9H_{18}ClNO_2 \cdot H_2O$) C, H, N.

***N,N*-Dimethyl-4-acetoxymethylpiperidinium Iodide (3).** To the free base of the amino ester **12** (0.85 g, 5.0 mmol) in anhydrous Et_2O (300 ml) was added MeI (5 ml) and the mixture heated in a stoppered flask on a steam bath for 0.5 min. The reaction was allowed to stir at room temperature for 14 hr and the white solid which formed was collected in a drybox by filtration. The product was dried at 40° (0.1 mm) for 12 hr giving 1.3 g (87%) of compound **12**, mp 162–163°. *Anal.* ($C_{10}H_{20}INO_2$) C, H, N.

***N,N*-Dimethyl-3-acetoxypiperidinium Iodide[32] (4).** *N*-Methyl-3-acetoxypiperidine[39] (3.5 g, 1.6 mmol) in anhydrous Et_2O (250 ml) was treated with MeI (10 ml) for 12 hr at room temperature to afford a white precipitate which was filtered in a drybox to give 4.0 g (77%) of **4** as a hydroscopic white solid, mp 148–150°. *Anal.* ($C_9H_{18}INO_2$) C, H, N.

Cholinesterase Assays. Electric eel Type III cholinesterase and horse serum Type IV cholinesterase (Sigma) were assayed by the standard titrimetric method[18] using a Radiometer pH Stat. The recorded titration was run in a constant temperature (25°), stirred, anaerobic assay cell excluding CO_2. The assay solution containing either the horse serum enzyme (2.23 mg) or the eel enzyme (0.67 mg) in 10 ml of 0.1 *M* $MgCl_2$, 0.01 *M* NaCl, and inhibitor was adjusted to pH 7.2 and treated with concentrations of ACh^+Cl^- ranging from 0.025 to 10 μmol/ml. The consumption of 0.01 *N* NaOH to maintain pH 7.2 was recorded against time and the data were analyzed using plots of $1/v$ *vs.* $1/s$, s/v *vs.* s, and v *vs.* v/s. The K_m for acetylcholine in the horse serum enzyme was 4×10^{-4} *M* and the eel gave $K_m = 1 \times 10^{-4}$ *M*.

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