Cholinergic Activity of 2-Azabicyclo[2.2.2]octane Analogs of Acetylcholine

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Abstract □ The *cis*- and *trans*-isomers of 5- and 6-acetoxy-2-methyl-2-azabicyclo[2.2.2]octane and the corresponding methiodide salts were synthesized and evaluated for cholinergic activity. The stereochemistry of the intermediate tertiary aminoalcohols is discussed. In the guinea pig ileum assay, all compounds were inactive except two, one of which was an effective competitive antagonist of acetylcholine while the other demonstrated agonist properties.

Keyphrases □ Acetylcholine, 2-azabicyclo[2.2.2]octane analogs—synthesized and screened for cholinergic activity □ 2-Azabicyclo[2.2.2]octane acetylcholine analogs—synthesized and screened for cholinergic activity □ Cholinergic activity—2-azabicyclo-[2.2.2]octane analogs of acetylcholine

In continuing studies on the conformational requirements for drug–receptor interactions, some conformationally restricted analogs of acetylcholine were prepared. The 2-azabicyclo[2.2.2]octane ring system was used to restrict the conformation of the choline portion of the molecule.

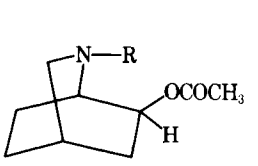
Data compiled on the conformation of acetylcholine both in the crystal form and in water solution point to a synclinal *(sc)* preferred conformation of the N—C—C—O linkage (1–5). Based on these results, Chothia (6) proposed that the nicotinic and muscarinic receptors interact with two different sides of acetylcholine when the transmitter is in a *sc*-conformation. Thus, this approach to the cholinergic receptor is to use the preferred conformation of acetylcholine to explain its action at the nicotinic and muscarinic receptors.

Beers and Reich (7) took a different approach which led to the opposite conclusion. They examined a series of agonist and antagonist molecules, looking for common dimensional features and seeking conformations of acetylcholine that suited these dimensions; they concluded that acetylcholine is present in the antiperiplanar *(ap)* conformation at both receptors. These two receptor hypotheses are cited to point out the still many unanswered questions regarding the active conformation of acetylcholine and the nature of the cholinergic receptor.

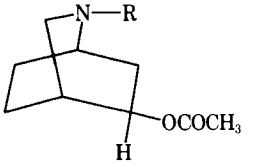
Isomers I-methiodide, II-methiodide, III-methiodide, and IV-methiodide were prepared as conformationally restricted analogs of acetylcholine. Isomers I-methiodide and II-methiodide are related to the *sc*-conformation of acetylcholine, while III-methiodide and IV-methiodide are related to the *ap*-conformation of acetylcholine. Isomers I-methiodide and III-methiodide are ethanolamine analogs, while II-methiodide and IV-methiodide are propanolamine analogs. The maintenance of equivalent stereochemical positions of the pharmacophoric groups in both ethanol and propanolamine derivatives gives this ring system a distinct advantage over other rigid systems used in conformationally restricted analog studies. Isomers I-methiodide and III-methiodide were previously prepared and tested for cholinergic activity (8) and were found to be inactive. However, in rigid analog studies, it is important to compare the relative biological activities within a given series of compounds; thus, these isomers were examined along with II-methiodide and IV-methiodide to complete the study of all isomers of this system.

The work of Cho *et al.* (9) showed some interesting relationships between tertiary amine acetates and their corresponding methiodides. Within a series of compounds in which the tertiary amine derivatives were more effective cholinergics than the methiodide analogs, the basic nitrogen was contained in a ring; in pairs of compounds in which the reverse was true, the nitrogen was attached to an aliphatic chain. These differences have been rationalized in terms of the conformations of the two types of compounds in solution and upon the assumption that a *trans*-conformation of acetylcholine is necessary for muscarinic activity.

The hypothesis of Cho *et al.* (9) was that aliphatic tertiary amines tend to form cyclic conformations *(i.e., sc)* in solution and are, therefore, inactive. Since rigid tertiary amine acetates held in an extended conformation cannot form a cyclic conformation, they are usually more active than their methiodides. Pharmacological evaluation of I, II, III, and IV was conducted to examine these ideas within the 2-azabicyclo[2.2.2]octane ring system. Isomers I and II are fixed in a rigid *cis*-relationship, while III and IV are fixed in a *trans*-relationship to the nitrogen atom. A comparison of the pharmacological activities of these isomers and their methiodides could provide some useful information regarding the nature of drug–receptor interactions at the muscarinic receptors.



I: R = CH_3



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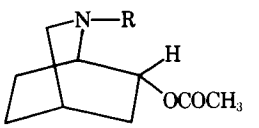
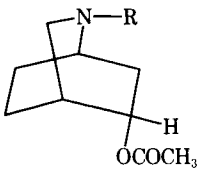
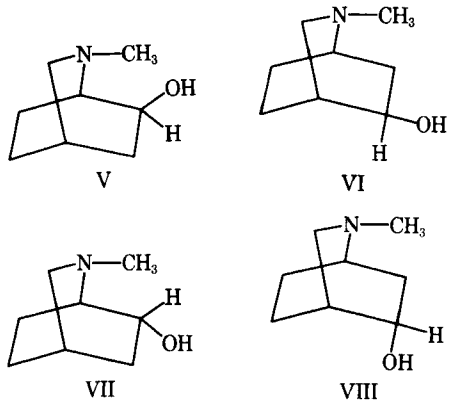


III: R = CH_3



IV: R = CH_3



DISCUSSION

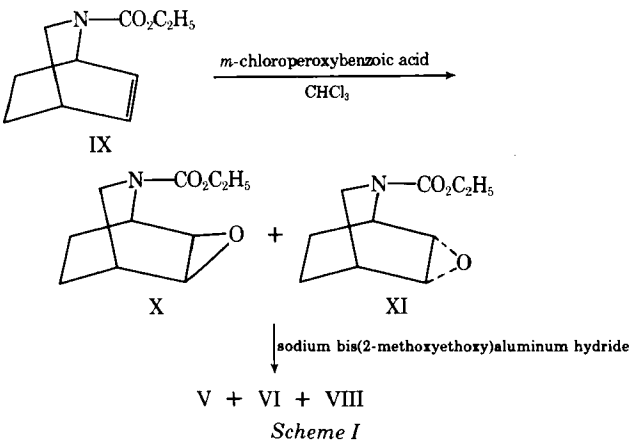
Chemistry—The necessary starting materials in this investigation were the four isomeric aminoalcohols V, VI, VII, and VIII. The stereospecific synthesis of VII, previously reported (10), was a critical step in the determination of the relative stereochemistry and the position of the hydroxy group in the four isomers.

The epoxidation of the olefin, IX (Scheme I), gave a 1:1 mixture of epoxides X and XI, which is consistent with previous work (11). Reduction of the epoxide mixture with sodium bis(2-methoxyethoxy)aluminum hydride or lithium aluminum hydride gave isolatable quantities of VI and VIII plus a small amount of V, as shown by GLC analysis of the product mixture. Since VII had been previously prepared and identified (10) as the 6-*trans*-hydroxy isomer, the *trans*-hydroxy isomer isolated in this reaction must be at the 5-position of the bicyclic ring because only hydroxy groups at the 5- or 6-position can result from normal hydride reduction of epoxides X and XI.

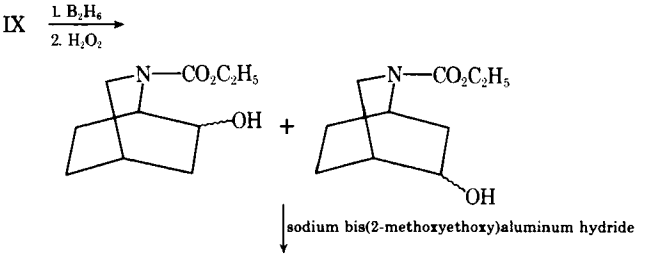
A previous study (10) reported the preparation of the 6-*cis*-hydroxy isomer, V. Therefore, the isolation of a second *cis*-hydroxy isomer from epoxide reduction must be at the 5-position (VI). Compound V was obtained as the major product of the reaction sequence outlined in Scheme II. Isomers VI and VIII can be obtained also *via* the addition of mercuric acetate to IX (12) followed by lithium aluminum hydride reduction of the intermediates.

The proof of the relative stereochemistry of the hydroxy group in these four isomers was accomplished *via* IR spectral analysis of a series of dilute solutions of each isomer. In isomers V and VI the hydroxy groups are *cis* to the ring nitrogen and are capable of intramolecular hydrogen bonding. The hydroxy groups in VII and VIII are *trans* to the nitrogen atom of the ring and intramolecular hydrogen bonding is not possible in these two isomers. The IR spectra of V, VI, VII, and VIII were taken as 1% and 0.01 and 0.002 *M* solutions in carbon tetrachloride. A 0.005 *M* solution is regarded as sufficiently dilute to exclude all intermolecular association (13). The results of the dilution studies and other physical data are shown in Table I.

A 0.002 *M* solution of VII and VIII, each in carbon tetrachloride, showed unassociated hydroxy absorption at 3600–3650 cm^{-1}. The *cis*-alcohols (V and VI) showed some unexpected differences in the



Scheme I



Scheme II

hydroxy region of the IR spectrum. A 0.002 *M* solution of V showed broad hydroxy absorption below 3500 cm^{-1}, indicating intramolecular hydrogen bonding and, therefore, a *cis*-relationship of hydroxy and nitrogen. A solution of VI at 0.002 *M* in carbon tetrachloride showed absorption for both types of hydroxy groups: a peak at 3600–3650 cm^{-1} for free hydroxy and a peak at 3500 cm^{-1} for associated hydroxy.

The unexpected results obtained for VI can be rationalized by comparing the structures of V and VI. Although the bicyclic ring is rigid, a small amount of twisting about the bonds of the ring is possible and this twisting affects V and VI in different ways. The twisting in V produces little change in the intramolecular distance from nitrogen to oxygen as determined from Dreiding models. A distance change of only 0.1 Å was observed for two conformations of V. However, larger distance changes are possible for VI. The interatomic distances can vary from 3.1 to 3.9 Å, a difference of 0.8 Å. It has been shown that the average distance from nitrogen to oxygen in a nitrogen to hydroxy group hydrogen bond is 2.8 ± 0.1 Å (14). The conformation of VI that permits a nitrogen to oxygen distance of 3.1 Å approximates the distance required for intramolecular hydrogen bonding and, thus, the hydroxy absorption at 3500 cm^{-1}. However, the conformation in which the nitrogen to oxygen distance is 3.9 Å does not permit intramolecular hydrogen bonding; thus a peak is observed at 3640 cm^{-1}, indicating free hydroxy absorption.

All cholinergic agents (I–IV and the corresponding methiodides) were prepared by the same method, using a previously stereochemically defined aminoalcohol (V, VI, VII, or VIII). The synthesis of III and III-methiodide as outlined in Scheme III is illustrative of the approach followed. The acetates I–IV were prepared using acetic anhydride and isolated by column chromatography on silica gel. The corresponding methiodides of I–IV were obtained from the acetates using methyl iodide.

Nelson and Wilson (8) noted that the NMR spectra of I-methiodide indicated that the methyl groups attached to nitrogen were not equivalent. They observed two singlets corresponding to the two methyl groups for I-methiodide and a singlet for the two methyl groups in III-methiodide. Since all four of the isomeric acetate methiodides were obtained in the present study, this unexpected observation was investigated. The signal for the two methyl groups attached to nitrogen appeared as a singlet only in III-methiodide. The methyl signals for I-methiodide, II-methiodide, and IV-methiodide appeared as two singlets, one for each of the methyl groups attached to nitrogen. The chemical shift difference ($\Delta\delta$) between the two signals varied with each isomer as shown in Table II.

Nelson and Wilson (8) postulated that the nonequivalent environment was produced by a long-range effect of the carbonyl oxygen of the acetate group. In some preliminary studies on the methiodides of the free alcohols, this nonequivalence of methyl groups also was observed; thus, the carbonyl oxygen is not the only factor involved. The oxygen attached directly to the bicyclic ring appears to contribute greatly to this effect. However, it is difficult

Table I—Physical Properties of V–VIII

Isomer	Boiling Point (20 mm)	Picrate Melting Point	OH_ν cm^{-1} (0.002 *M*)
V	106–110°	259–260°	3450
VI	96–99°	241–243°	3640 and 3500
VII	124–127°	230–231°	3640
VIII	131–133°	264–266°	3640

to determine if this effect is due to shielding or deshielding.

Pharmacology—Compounds I–IV and I-methiodide–IV-methiodide were tested for cholinergic activity as the racemic mixtures on the guinea pig ileum. All tertiary amine acetates tested were inactive at a concentration of 1×10^{-3} *M*. The inactivity of these derivatives is not consistent with the postulation of Cho *et al.* (9). The methiodide salts of the 6-acetoxy derivatives (I and III) were also inactive at 1×10^{-3} *M*. These results are consistent with the previous report of Nelson and Wilson (8) who reported these analogs to be inactive in the rabbit ileum preparation.

Surprising results were obtained with the 5-acetoxy quaternary salts. The methiodide salt of the 5-*cis*-isomer (II) was inactive as a cholinergic agonist but competitively antagonized the effects of acetylcholine. When using the method of Gaddum (15), a pA_2 value of 4.4 was determined for II-methiodide. On the other hand, the methiodide salt of the 5-*trans*-isomer (IV) was found to possess cholinergic agonist activity at a 1×10^{-4} *M* concentration equivalent to the response produced by a 2.1×10^{-6} *M* solution of acetylcholine. Thus, IV-methiodide has an equipotent molar concentration of 47.6 compared to acetylcholine, with an intrinsic activity of 1.0. The action of IV-methiodide was completely blocked in the presence of 1.6 μg/ml of atropine. However, a 1-mg/ml solution of hexamethonium blocked approximately 80% of the contractile effects of IV-methiodide, indicating that IV-methiodide, is acting primarily at parasympathetic ganglia.

It is obvious that the relative configuration of the acetoxy groups in II-methiodide and IV-methiodide has a significant effect on biological activity. Seldom does one observe the large changes in biological activity among conformational isomers represented by II-methiodide and IV-methiodide. In addition to the separation of agonist and antagonist properties in these acetylcholine analogs, it is also somewhat surprising that cholinergic activity is absent in the ethanolamine series (I-methiodide and III-methiodide) but is present to opposite extremes in the propanolamine series. Acetyl-γ-homocholine is much less active than acetylcholine at both parasympathetic ganglia and postganglionic parasympathetic sites of action (16). Examination of Dreiding models of II-methiodide and IV-methiodide indicates that the average interatomic distance from nitrogen to ester oxygen is 3.5 Å in II-methiodide and 4.2 Å in IV-methiodide. The interatomic distance from nitrogen to carbonyl oxygen in IV-methiodide varies from 4.0 to 6.2 Å since the carbonyl group is free to adopt a number of conformations with respect to the ring system.

According to Beers and Reich (7), nicotinic activity is dependent upon a positive charge and a hydrogen acceptor group separated by 5.9 Å while a distance of 4.4 Å between these groups is postulated for muscarinic activity. In IV-methiodide, the ester oxygen to nitrogen distance is similar to that proposed for muscarinic activity and the carbonyl oxygen to nitrogen distance is in agreement with that proposed for nicotinic activity. Only the nitrogen and carbonyl oxygen are capable of assuming these interatomic distances in II-methiodide.

More detailed pharmacological investigations are in progress, particularly with respect to the pharmacological properties exhibited by IV-methiodide. Since the pharmacological results presented here for the methiodide salts of II and IV were conducted on the racemic mixtures, resolution of enantiomers and pharmacological testing of the optical isomers are also being explored.

EXPERIMENTAL[1]

6-*cis*-Hydroxy-2-methyl-2-azabicyclo[2.2.2]octane (V)—To a solution of 2-carbethoxy-2-azabicyclo[2.2.2]octa-5-ene (IX, 28.7 g, 0.15 mole) (17) in 60 ml of tetrahydrofuran was added sodium borohydride (4.2 g, 0.11 mole), followed by the dropwise addition of dimethyl sulfate (13.8 g, 0.11 mole) in 40 ml of tetrahydrofuran. The solution was maintained at 40° for 12 hr and then cooled to 5°. Water (20 ml) was added followed by 32 ml of 3 *N* NaOH and 32 ml of 30% hydrogen peroxide. The aqueous layer was saturated with sodium chloride and the organic layer was separated, washed with saturated sodium chloride (3 × 60 ml), dried over magnesium sulfate, and evaporated to give 25.5 g of a yellow oil.

A solution of 12.0 g of this oil in 75 ml of benzene was added slowly to 80 ml of sodium bis(2-methoxyethoxy)aluminum hydride (0.57 mole), and the resulting mixture was refluxed for 3 hr. Excess hydride was destroyed with ethanol and water, the resulting mixture was filtered, and the filtrate was dried over magnesium sulfate and evaporated. The residue was distilled to give 3.0 g of a clear liquid, bp 106–110° (20 mm); IR (0.002 *M* CCl_4): 3480 cm^{-1} (OH, associated); NMR (CCl_4): δ 1.06–2.7 [broad signals, 12, bicyclic envelope and N—CH_3 (s, 2.4)], 2.9–3.4 (broad signal, 1, H at C_1), and 3.5–4.1 (s over a broad signal, 2, OH and H at C_6). The picrate was prepared in the normal manner, mp 259–261°. The pot residue from distillation was shown by GLC to be a mixture of V and VIII.

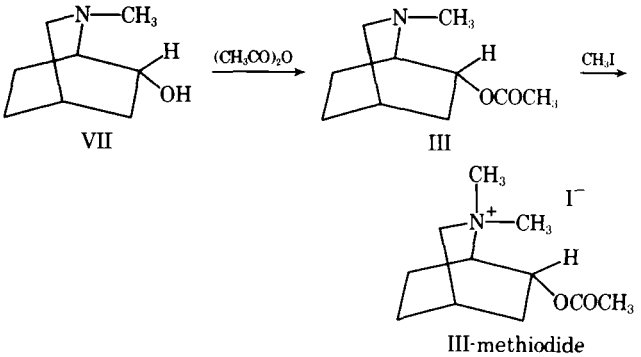
6-*cis*-Acetoxy-2-methyl-2-azabicyclo[2.2.2]octane (I)—A solution of V (1.0 g, 0.007 mole) and 5 ml of acetic anhydride in 20 ml of benzene was stirred at room temperature for 12 hr. Water (50 ml) was added and stirring continued for 1 hr. The solution was made basic with potassium carbonate and extracted with ether. The ether extracts were combined, dried over magnesium sulfate, and evaporated. The resulting oil was chromatographed on 5.0 g of silica gel, with benzene as eluent, to yield the desired ester (0.8 g, 60%) as a clear liquid; IR (CCl_4): 1740 cm^{-1} (C=O); NMR (CCl_4): δ 2.5 (s, 3, N—CH_3) and 2.17 (s, 3, $COCH_3$).

6-*cis*-Acetoxy-2-methyl-2-azabicyclo[2.2.2]octane Methiodide (I-Methiodide)—A solution of I (1.0 g, 0.005 mole) and 5 ml of methyl iodide in 20 ml of dry benzene was stirred at room temperature for 2 hr. The mixture was filtered and the solid was dried and recrystallized from ethanol to give white needles (1.0 g, 62%), mp 165–167°; IR (KBr): 1750 cm^{-1} (C=O); NMR (D_2O): δ 2.17 (s, 3, $COCH_3$), 3.23 (s, 3, N—CH_3), and 3.33 (s, 3, N—CH_3).

Anal.—Calc. for $C_{11}H_{20}INO_2$: C, 40.63; H, 6.20; N, 4.30. Found: C, 40.50; H, 6.25; N, 4.32.

6-*trans*-Acetoxy-2-methyl-2-azabicyclo[2.2.2]octane (III)—A solution of 2.0 g (0.014 mole) of VII, prepared as described previously (10), was treated with 10 ml of acetic anhydride in a manner similar to that described for the synthesis of I. Chromatography of the crude acetate on silica gel gave 1.4 g (55%) of III as a clear liquid; IR (CCl_4): 1740 cm^{-1} (C=O); NMR (CCl_4): δ 2.51 (s, 3, $COCH_3$) and 2.50 (s, 3, N—CH_3).

6-*trans*-Acetoxy-2-methyl-2-azabicyclo[2.2.2]octane Methiodide (III-Methiodide)—A solution of 1.0 g (0.005 mole) of III and 10 ml of methyl iodide in 20 ml of dry benzene was stirred at



VII III III-methiodide

Scheme III

Table II—NMR Data

Isomer	Δδ[a], ppm
I-Methiodide	0.10
II-Methiodide	0.13
III-Methiodide	0.00
IV-Methiodide	0.08

[a] Chemical shift difference between methyl signals.

[1] Melting points were determined on a Thomas-Hoover Uni-Melt apparatus and are corrected. IR spectra were determined with a Perkin-Elmer model 257 or a Beckman IR-33 spectrophotometer. Solution spectra were taken in sodium chloride cells of widths 1.058 mm (reference) and 1.09 mm (sample). Dilution studies were performed using matched sodium chloride cells of widths 10 mm for 0.01 *M* solutions and 25 mm for 0.002 *M* solutions. All NMR spectra were obtained on a Jeolco model C-60-HL spectrometer, and all values are reported in parts per million from tetramethylsilane or sodium 4,4-dimethyl-4-silapentanesulfonate as internal standards. Elemental analyses were performed by Chemalytics, Inc., Tempe, Ariz. GLC was performed on a Varian Aerograph model 600D chromatograph equipped with a flame-ionization detector and a 1.5-m × 0.3-cm (5-ft × 0.125-in.) column containing 3% SE-30 on Varaport 30 (100–120 mesh). Sodium bis(2-methoxyethoxy)aluminum hydride was purchased from Aldrich Chemical Co.

room temperature for 12 hr. The solvent was evaporated and the residue was recrystallized from methanol–ethyl acetate to yield a colorless solid (1.1 g, 68%), mp 165–166° [lit. (8) mp 165–165.5°]; IR (KBr): 1745 cm^{-1} (C=O); NMR (D_2O): δ 2.20 (s, 3, $COCH_3$) and 3.50 (s, 6, N—CH_3).

Anal.—Calc. for $C_{11}H_{20}INO_2$: C, 40.63; H, 6.20; N, 4.30. Found: C, 40.66; H, 6.18; N, 4.21.

5-*cis*-Acetoxy-2-methyl-2-azabicyclo[2.2.2]octane (II)—A solution of 1.5 g (0.01 mole) of VI, prepared as previously described (10), was treated with 5 ml of acetic anhydride in a manner similar to that described for the preparation of I. Chromatography of the crude acetate on silica gel afforded 1.1 g (62%) of the desired ester as a clear liquid; IR (CCl_4): 1745 cm^{-1} (C=O); NMR (CCl_4): δ 2.15 (s, 3, $COCH_3$) and 2.52 (s, 3, N—CH_3).

5-*cis*-Acetoxy-2-methyl-2-azabicyclo[2.2.2]octane Methiodide (II-Methiodide)—A solution of II (1.0 g, 0.005 mole) and 5 ml of methyl iodide in 25 ml of dry benzene was stirred at room temperature for 3 hr. The resulting solid was collected and recrystallized from ethanol to give a white solid (0.5 g, 31%), mp 175–176°; IR (KBr): 1740 cm^{-1} (C=O); NMR (D_2O): δ 2.20 (s, 3, $COCH_3$), 3.30 (s, 3, N—CH_3), and 3.43 (s, 3, N—CH_3).

Anal.—Calc. for $C_{11}H_{20}INO_2$: C, 40.63; H, 6.20; N, 4.30. Found: C, 40.33; H, 6.15; N, 4.52.

5-*trans*-Acetoxy-2-methyl-2-azabicyclo[2.2.2]octane (IV)—A solution of 2.0 g (0.014 mole) of VIII, prepared as previously described (10), was treated with 10 ml of acetic anhydride in a manner similar to that described for the preparation of I. Chromatography of the crude acetate on silica gel gave the desired ester as a clear liquid; IR (CCl_4): 1745 cm^{-1} (C=O); NMR (CCl_4): δ 2.13 (s, 3, $COCH_3$) and 2.41 (s, 3, N—CH_3).

5-*trans*-Acetoxy-2-methyl-2-azabicyclo[2.2.2]octane Methiodide (IV-Methiodide)—A solution of 1.0 g (0.005 mole) of IV and 5 ml of methyl iodide in 25 ml of anhydrous methanol was stirred at room temperature for 6 hr. The solvent was evaporated and the residue was recrystallized from ethanol to give 0.9 g (56%) of IV-methiodide, mp 168–171°; IR (KBr): 1735 cm^{-1} (C=O); NMR (D_2O): δ 2.20 (s, 3, $COCH_3$), 3.24 (s, 3, N—CH_3), and 3.32 (s, 3, N—CH_3).

Anal.—Calc. for $C_{11}H_{20}INO_2$: C, 40.63; H, 6.20; N, 4.30. Found: C, 40.68; H, 6.08; N, 4.13.

Pharmacological Methods—The isolated guinea pig ileum assay (18) was employed to screen the cholinergic effects of I–IV and I-methiodide–IV-methiodide.The terminal ileum from guinea pigs (300–500 g) was cut into 2–3-cm strips and suspended in Tyrode's solution in a 30-ml organ bath. The solution was aerated with 95% oxygen and 5% carbon dioxide at 37°. Contractions of the ileum were recorded on a physiograph[2]. A dose–response curve for acetylcholine was determined with each piece of ileum before assaying the test compounds. Reported drug concentrations represent the final concentration in the organ bath. Compounds producing no contraction at 1×10^{-3} *M* were considered inactive. Evidence that II-methiodide competitively antagonized the effects of acetylcholine was determined by the parallel shift in the dose–response curve toward higher concentrations of acetylcholine in the presence of 1×10^{-4} *M* II-methiodide.

[2] E and M model 4.

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