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NOTES

Muscarinic Agents: The Isomeric 6-Acetoxy-2-methylisoquinuclidine Methiodides

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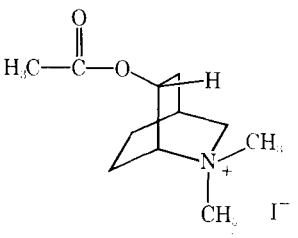
Abstract □ Preparation of 6-*endo* and 6-*exo*-acetoxy-2-methylisoquinuclidine methiodides are described. Muscarinic assay data are reported. Neither of the compounds showed activity when compared to acetylcholine and 3-acetoxyquinuclidine methiodide.

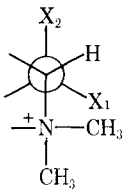
Keyphrases □ 6-Acetoxy-2-methylisoquinuclidine methiodides—synthesis □ Pharmacological screening—6-acetoxy-2-methylisoquinuclidine methiodides □ IR spectrophotometry—identity, structure □ NMR spectroscopy—structure

Hypotheses delineating the architectural features of cholinergic receptors have been based on observations of pharmacological activity of various substituted derivatives of the neurohormone, acetylcholine. Differences in the activity of these analogs of acetylcholine have long been explained on the basis of molecular steric and electronic effects in the drug-receptor interaction (1, 2). Spectral data concerned with conformational aspects of acetylcholine have also been studied and developed in recent attempts to describe receptor site architecture (3–7).

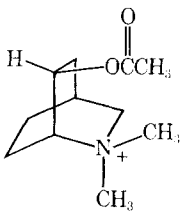
In further studies to determine the conformational requirements of the drug-receptor complex, in which the authors' assume a large degree of complementarity of the drug and receptor in this interaction, a number of conformationally rigid or semirigid analogs of acetylcholine have been prepared (8–13). Each, although incorporating the essential features for cholinergic activity, also inherently must be constructed of an additional number of carbons to maintain the desired conformational rigidity. Comparison of activities of agents structurally similar to each other seems valid.



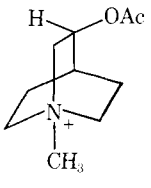
I



IA, $X_1 = H, X_2 = OAc$
IIA, $X_1 = OAc, X_2 = H$



II



VIII

PREPARATION OF ANALOGS

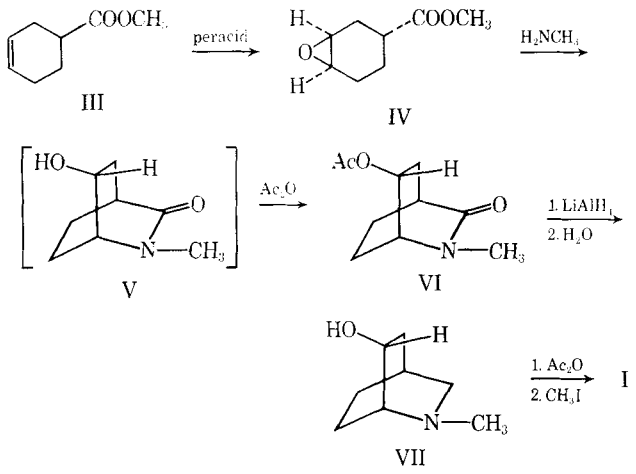
In a program of preparation of agents to further determine the geometric requirements of the muscarinic agents, it was decided to prepare cholinergic analogs in the isoquinuclidine system in which

the quaternary ammonium head is held in a fixed position by the carbocyclic skeleton. The compounds, 6-*endo*- and 6-*exo*-acetoxy-2-methylisoquinuclidine methiodides,[1] I and II, represent *anti* and *gauche* conformations of acetylcholine, IA and IIA, respectively, in a fused system.

Preparation of I was accomplished beginning with methyl 3-cyclohexencarboxylate (III) (Scheme I). Epoxidation of III with *m*-chloroperbenzoic acid produced epoxide IV. The authors could not detect the presence of more than a single epoxide, which has been assigned the *trans* stereochemistry by Henbest (14). It would not be unusual to expect both epoxides, which may be both dependent on solvent and oxidizing agent (15).

The epoxide (IV) was allowed to react with methylamine, with the expected *trans*-diaxial opening by this nucleophile. The reaction mixture was heated at reflux to effect ring closure to the isoquinuclidone, V, as well as dimeric and polymeric products. Similar opening of this epoxide has been reported by Huffman (15), who readily sublimed a quinuclidone from the mixture when benzylamine was used. In this case, only a sticky glass was obtained, and the sublimation failed. Crude V was acetylated to produce VI, 6-*endo*-acetoxy-2-methyl-3-isoquinuclidone.

Lithium aluminum hydride reduction of VI provided VII. Considerable losses were encountered in this reduction, probably due to the water solubility of the product. The crude amino alcohol was acetylated and then was allowed to react with methyl iodide to afford I.



Scheme I

During the early part of the work on I, a method appeared for the preparation of II from the Diels-Alder adduct of 1,3-cyclohexadiene and *N*-methylidene-urethan (16). This method obviated the intended conversion of VII to an intermediate ketone useful to reach II.

Of interest is the difference in NMR spectra of I and II. Whereas the spectrum of I shows magnetic equivalence of the *N*-methyl groups at 3.28 δ, two different signals are observed for the methyl groups in II at 3.26 and 3.35 δ, respectively. This difference reflects the long range effect of the acetoxy group (*vide infra*), although it is not possible to assign it a shielding or deshielding effect in this case, although the latter is most probable.

The best models available are 2β-substituted steroids (effects on the C-19 methyl group) and 17β-substituted compounds (effects on the C-18 methyl groups) (17, 18). Deshielding effects of the acetoxy group are noted in each case. However, the geometry of the isoquinuclidine ring is not completely analogous.

PHARMACOLOGICAL TESTING

The isoquinuclidines, I and II, as racemates, were tested for muscarinic activity in rabbit ileum. Neither of the compounds showed activity at concentrations up to 10^{-2} *M*. Acetylcholine showed half maximal activity at 1×10^{-7} *M* ($pD_2 = 7$, $\alpha = 1.0$).

For comparison, a structurally related compound, 3-acetoxyquinuclidine methiodide VIII was also tested in this system. This compound showed a dose-response curve parallel to that of acetylcholine, and a pD_2 of 4.2, $\alpha = 1.0$. Similar results have been reported (19).

Two rational explanations may be advanced for the lack of activity in the isoquinuclidines. The conformations of acetylcholine, which these compounds represent, may not be those in the drug-receptor complex at the muscarinic site. A more valid possibility is that the carbocyclic skeleton present in these compounds may prevent either of the compounds from forming a drug-receptor complex leading to pharmacological action. The observed lack of activity may be due to this hydrocarbon portion of the molecules, which may interact with hydrophobic regions outside the site, or prevent interaction of the quaternary ammonium head and acetoxy moieties.

Examination of Dreiding models of II shows a great deal of steric hindrance about the acetoxy group, to one of the *N*-methyl groups. The NMR spectrum supports some type of interaction between these groups, at least an electronic effect. Additionally, each of these compounds can be analyzed as an α,β-di-alkyl-substituted acetylcholine, with both an isopropyl and neopentyl group attached to nitrogen. One of these factors, or both, may be responsible for the lack of pharmacological activity.

EXPERIMENTAL

Melting points were determined on a calibrated Thomas-Hoover Unimelt and are corrected. IR spectra were recorded on Beckman IR-8 and IR-20 spectrophotometers. NMR spectra were determined with Varian Associates A-60 and T-60 spectrometers using tetramethylsilane as an internal standard in organic solvents and dimethylsilapentanoic acid sodium salt in aqueous solution. Elemental analyses were conducted by Drs. G. Weiler and F. B. Strauss, Oxford, England.

3-Carbomethoxy-7-oxabicyclo[4.1.0]heptane (IV)—To a cooled solution (0°) of 137 g. (0.98 mole) of methyl 3-cyclohexencarboxylate in 200 ml. of $CHCl_3$ was slowly added a $CHCl_3$ solution of 180 g. (1.02 moles) of *m*-chloroperbenzoic acid. After the addition, the mixture was stirred at room temperature for 2 hr. then worked with 5% aqueous sodium sulfite solution, 5% aqueous sodium hydroxide solution, water, and dried ($MgSO_4$). Evaporation of the $CHCl_3$ afforded a colorless oil, identical in IR spectral qualities with those reported by Henbest (14).

6-*endo*-Acetoxy-2-methyl-3-isoquinuclidine (VI)—To a cooled (0°) solution of 93.0 g. (0.60 mole) of crude epoxide, IV, in 200 ml. of CH_3OH was slowly added 77.5 g. (1.00 mole) of 40% aqueous methylamine. The mixture was allowed to stand at room temperature for 3 days, heated to reflux for 1 hr., then evaporated *in vacuo* to remove solvents and excess methylamine. The crude amino alcohol was refluxed for 3 hr. to effect ring closure to lactam VI. The residue, 87.0 g. of a viscous glass, resisted all attempts at sublimation, a technique that had been successful in similar compounds (15). The IR spectrum showed both ester (5.80 μ) and amide (6.10 μ) carbonyl bands.

The crude mixture (87.0 g., 0.40 mole) was dissolved in 86.4 g. (1.10 moles) of pyridine and 110.7 g. (1.10 moles) of acetic anhydride was added. The solution was refluxed for 2 hr.; excess pyridine, acetic anhydride, and acetic acid were removed utilizing a water aspirator. Aqueous 2% HCl was added and after 1 hr. the mixture was extracted with EtOAc. The EtOAc layer was dried ($MgSO_4$) and solvent evaporated. The residue oil, was distilled, b.p. 110° (1.0 mm.) affording 31.5 g. (17% of theory) of VI; IR (neat), 3.02, 3.41, 5.82, 6.05, 6.96, 7.19, 7.33, 8.12, 8.55, 8.79, 9.53, 9.80, 11.0, 12.15 μ; NMR δ ($CDCl_3$), 5.10 (sextet, H_6, $J_{6,1} \sim J_{6,5} \sim 4$ c.p.s., $J_{6,5'} = 10$ c.p.s.), 3.75 (multiplet, H_1 proton, $W_h = 8$ c.p.s.), 3.05 (singlet, $N—CH_3$ proton), 2.18 (singlet, $CH_3—\overset{O}{\overset{\|}{C}}—$), 1.6–2.7 (multiplet, seven protons, methylene-methine envelope).

6-*endo*-Acetoxy-2-methylisoquinuclidine Methiodide (I)—A solution of isoquinuclidone VI, 29.9 g. (0.165 mole) in 100 ml. of anhydrous ether was added dropwise to a slurry of 12.0 g. (0.316 mole) of lithium aluminum hydride in 150 ml. of ether. After the addition, the mixture was refluxed for 4 hr. Excess lithium aluminum hydride was destroyed with a 40% aqueous solution of Rochelle salt. The mixture was filtered (Celite), dried, and the solvent removed. The crude product was partitioned between ethyl acetate

[1] An *exo* substituent is defined as one which is *cis* to the nitrogen containing bridge to the isoquinuclidine ring, and an *endo* substituent is *trans* to the bridge.

and 5% aqueous hydrochloric acid. The acidic solution was made alkaline with 20% aqueous NaOH and extracted with EtOAc, which was dried, and solvent removed affording crude VII, 6.5 g. (22%) as a dark orange oil; IR (neat), 3.05 (broad), 3.47, 6.12, 6.58, 6.98, 8.65, 8.83, 9.38, 9.88, 10.55, 11.05, and 12.21 μ.

A mixture of crude VII, 1.5 g. (11 mmoles), and 1.0 g. (10 mmoles) of acetic anhydride was refluxed for 1 hr. and then allowed to stand overnight at room temperature. Sufficient 10% aqueous NaOH was added to make the mixture alkaline, and the ester extracted with ether. The crude ester was partitioned between ether and aqueous acid again, the acidic solution neutralized, and the basic ester isolated, 500 mg (30%); IR (neat), 2.91, 3.42, 5.78, 6.13, 6.96, 7.10, 7.32, 8.09, 8.60, 8.79, 9.29, and 9.65 μ.

To a solution of 500 mg. (3.0 mmoles) of the crude acetate ester of amino alcohol VII in 10 ml. of methanol was added 10 ml. of methyl iodide and the mixture allowed to stand overnight. The solvent and excess methyl iodide were removed, the solution was decolorized (Norite) in methanol, and crystallized twice from methanol–ethyl acetate affording 300 mg. (53%) of colorless crystals, m.p. 165–165.5°; IR (KBr) 3.29, 3.36, 5.73, 6.79, 6.88, 7.69, 8.02, 8.64, 9.30, 9.55, 9.63, 10.49, 10.77, and 11.03 μ; NMR (D_2O) δ, 5.48 (sextet, H_6 proton, $J_{6,1} \sim J_{6,5} = 4$ c.p.s., $J_{6,5'} = 10$ c.p.s.); 3.60 (broadened quartet, H_1, $J_{1,7} \sim 3$ c.p.s.), 4.45 (broadened singlet, CH_3 protons), 3.28 [singlet, $\overset{+}{N}(CH_3)_2$], 2.12 (singlet, $CH_3-\overset{O}{\overset{\|}{C}}-$), 1.26–3.0 (multiplet, seven protons, methylene-methine envelope).

Anal.—Calcd. for $C_{11}H_{20}INO$: C, 40.60; H, 6.15; N, 4.31. Found: C, 40.67; H, 6.02; N, 4.21.

6-*exo*-Acetoxy-2-methylisoquinuclidine Methiodide (II)—This compound was prepared by the method of DeGraw and Kennedy (16).

3-Acetoxyquinuclidine Methiodide (VIII)—This compound was prepared by the method of Grob (20, 21) from 3-hydroxyquinuclidine.

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