zepam could not be thoroughly cleaned by washing with hot tap water and rinsing with water and/or rinsing with 0.1 *N* HCl followed by a water rinse since all animals tested were protected from pentylenetetrazol seizures when these procedures were employed. Although rinsing the mortar and pestle with absolute ethanol or washing with detergent reduced the amount of clonazepam remaining (two animals protected out of eight tested), neither procedure alone was adequate. Indeed, a clean mortar and pestle were obtained only when all of these procedures were employed in sequence (none protected out of eight tested).

The benzodiazepines in general (3) and clonazepam in particular are the most potent antipentylenetetrazol agents tested in this laboratory. The ED_{50} (and its 95% confidence interval) by the subcutaneous pentylenetetrazol test is 0.009 (0.0046–0.0165) mg/kg. Thus, a dose of only 180 ng is sufficient to protect 50% of the mice (20 g). Since the ED_{99} for clonazepam is approximately 0.013 mg/kg, at least 3.25 μg apparently was carried over on the mortar and pestle and subsequently incorporated in the polyethylene glycol suspension of the inactive substance. Chemical analysis[5] of 2.5 ml of 30% polyethylene glycol triturated in a contaminated mortar and pestle revealed that 66.25 μg (26.5 μg/ml) was, in fact, carried over in the solvent. This quantity is approximately 20 times the ED_{99} for clonazepam.

[5] Performed by Mr. Tom Jennison, Center for Human Toxicology, University of Utah.

The results presented reveal only a small fraction of the total problem. Many laboratories are working with highly potent, water-insoluble substances. Various methods such as a mortar and pestle, a sonicator, a homogenizer, a tissue grinder, and a micromixer are used to achieve a suitable solution or suspension. Subsequent use of this equipment to solubilize or suspend other chemicals could result in significant contamination unless appropriate precautions are taken. These precautions include the use of disposable laboratory utensils such as syringes, needles, test tubes, and spatulas and internal controls to determine when reused utensils are clean. One cannot help but wonder to what extent this problem has been unrecognized, ignored, or explained away on the basis of "biological variability."

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Quaternary Acetate and Propionate Esters of 3-Hydroxyquinolizidine

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Received February 16, 1977, from the **Department of Medicinal Chemistry and the* ‡*Department of Pharmacology, School of Pharmacy, Southwestern Oklahoma State University, Weatherford, OK 73096.* Accepted for publication April 18, 1978.

Abstract □ The preparation of the quaternary acetate and propionate esters of 3-hydroxyquinolizidine is described. Tentative structures are assigned on the basis of NMR data. Results of preliminary pharmacological screening of the methiodide and hydrochloride salts are given.

Keyphrases □ 3-Hydroxyquinolizidine esters, various—synthesized, structures assigned, cholinergic activity evaluated *in vitro* □ Cholinergic activity—various 3-hydroxyquinolizidine esters evaluated *in vitro* □ Structure–activity relationships—various 3-hydroxyquinolizidine esters evaluated for cholinergic activity *in vitro*

The quinolizidine ring system has been used to prepare semirigid analogs of several classes of biologically active compounds (1–4). The reported reversal of configuration of the *trans*-fused rings of quinolizidine to the *cis*-configuration upon quaternization of the acetate and propionate esters of axial and equatorial 1-hydroxyquinolizidine (1) led to the present investigation of the analogous esters at the 3-position.

This report describes the preparation of the acetate and propionate esters of axial and equatorial 3-hydroxyquinolizidine and their behavior upon quaternization with methyl iodide. These isomers were screened for cholinergic activity since they are structurally related to acetylcholine.

DISCUSSION

The synthesis of the desired compounds began with the preparation of 3-oxoquinolizidine (I) by the method of Counsell and Soine (5). Then, I was reduced according to the method of Aaron *et al.* (6). The structures of II and III (Scheme I) were assigned (6, 7) primarily on the basis of IR spectral data. The intermediate esters, IV and V (Scheme I), were obtained by reaction of the appropriate alcohol with either acetic or propionic anhydride and pyridine as a catalyst. The esters so prepared were assigned the same configuration as the respective parent alcohols since the conditions used for ester formation would not be expected to result in epimerization. The assignments appear valid since all esters exhibited Bohlmann bands (Table I) in their IR spectra, which is usually taken as empirical evidence for *trans*-ring fusion (6–8).

The desired quaternary salts were obtained by treating the purified esters with excess methyl iodide in refluxing acetone. The NMR spectra of each quaternized ester exhibited only one *N*-methyl signal, which appeared as a three-proton singlet (Table I). The appearance of only one *N*-methyl signal, along with the small differences in the chemical shifts of the signals, suggested that the quaternized esters were configurationally pure and probably all of the same configuration. These conclusions seem reasonable in view of the work of Williamson *et al.* (9), who showed a 12-Hz difference between the *N*-methyl groups of *trans*- (171 Hz) and *cis*- (183 Hz) fused *N*-methylquinolizidinium iodide. Also, since the *N*-methyl signals of the quaternary salts prepared have chemical shifts very close to the *N*-methyl signals of the analogous *cis*-fused quaternary salts substituted at the 1-position (see introduction and Ref. 1), the *cis*-configuration was tentatively assigned to the quaternary salts prepared.

Examination of Drieding models of the *cis*-fused products revealed that difficultly interconvertible conformers were probably in equilibrium as depicted in Scheme II. It was reasonable to assign a conformational preference to the products, and this assignment was made on the basis that less steric interaction would occur if the ester function was in the equatorial position in the *cis*-fused system. Therefore, XII and XVI (Table I) were tentatively assigned Structure VI, where R is methyl and ethyl, respectively. Likewise, XIII and XVII (Table I) were assigned Structure IX. Support for this conclusion was obtained by the observation

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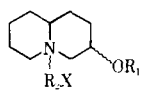
Table I—Quinolizidine Hydrochlorides and Methiodides



Compound	R_1	R_2	X	Melting Point	Analysis, %	Calc.	Found	Bohlmann Bands[a], cm^{-1}	NMR *N*-Methyl Signal[b], Hz
X	$OCOCH_3$ (ax)[c]	H	Cl	240–245°	C	56.53	56.70	2760, 2800	—
					H	8.63	8.64		
					N	5.99	6.15		
XI	$OCOCH_3$ (eq)[c]	H	Cl	195–197°	C	56.53	56.37	2760, 2805	—
					H	8.63	8.79		
					N	5.99	6.19		
XII	$OCOCH_3$ (eq)[d]	CH_3	I	175–178°	C	42.49	42.50	—	200
					H	6.54	6.61		
					N	4.13	4.08		
XIII	$OCOCH_3$ (eq)[d]	CH_3	I	200–205°	C	42.49	42.60	—	196
					H	6.54	6.48		
					N	4.13	4.21		
XIV	$OCOCH_2CH_3$ (ax)[c]	H	Cl	—[e]	C	58.18	—	2770, 2800	—
					H	8.95	—		
					N	5.65	—[e]		
XV	$OCOCH_2CH_3$ (eq)[c]	H	Cl	194–195°	C	58.18	58.28	2780, 2805	—
					H	8.95	8.98		
					N	5.65	5.76		
XVI	$OCOCH_2CH_3$ (eq)[d]	CH_3	I	148–150°	C	44.20	44.37	—	201
					H	6.85	6.74		
					N	3.97	3.92		
XVII	$OCOCH_2CH_3$ (eq)[d]	CH_3	I	190–192°	C	44.20	44.37	—	198
					H	6.85	6.76		
					N	3.97	3.91		

[a] Bohlmann bands in the IR were determined on the free bases, all of which were oils. [b] All signals were singlets integrating to three protons. [c] Axial or equatorial designation refers to the free base. [d] Equatorial designation refers to the *cis*-fused products as depicted in Scheme II. [e] The hydrochloride decomposed upon recrystallization.

that the NMR signals for the methine protons on the oxygen-bearing carbons of the quinolizidine ring all showed bandwidths at half-height (W_h) indicative of axial orientation (Table II). Only *cis*-fused products were obtained, probably as the result of small but steady-state concentrations of *cis*-fused esters that react preferentially due to much greater accessibility of the nitrogen electron pair[1].

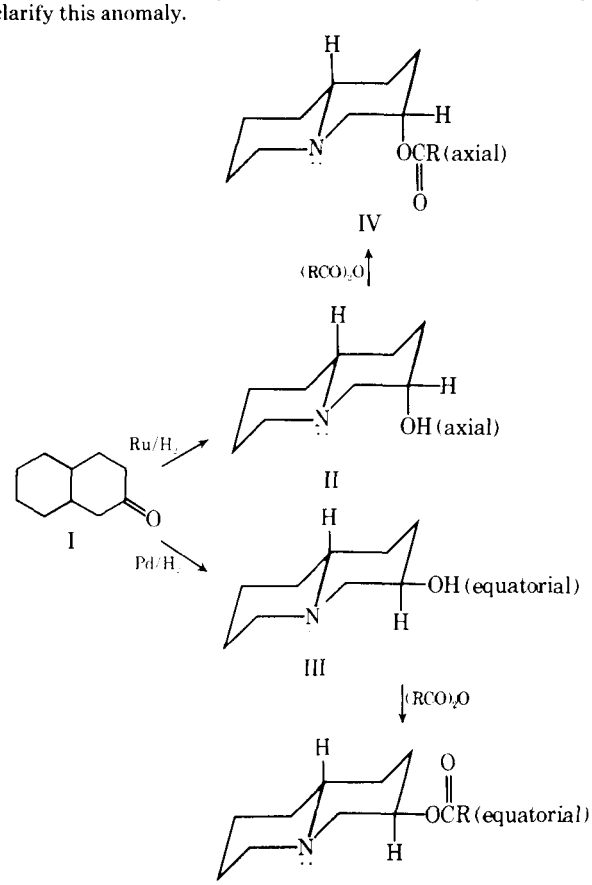
Of the seven compounds tested, two exerted powerful, consistent, and reproducible effects on the rat jejunum. Compound XVII (Table I) inhibited contractile activity of the muscle. This effect was essentially instantaneous following injection and was reproducible at bath concentrations as low as 1 μg/ml. The IC_{50} of XVII was calculated to be 8 μg/ml (2.3×10^{-5} *M*), determined from seven experiments (*SD* ±4 μg/ml). Both spontaneous contractions and acetylcholine-induced contractions were inhibited.

Conversely, X (Table I) exerted a spasmogenic effect at concentrations as low as 25 μg/ml. This effect was not immediate but took 5–15 min to become maximal and was readily reversed by atropine sulfate. An average concentration of 62 μg/ml (2.7×10^{-4} *M*) determined from six experiments was required to produce contractions equal to approximately 0.03 μg/ml (1.3×10^{-7} *M*) of acetylcholine. Thus, X was approximately 0.0005 as potent as acetylcholine in this preparation.

The remaining compounds elicited weak, inconsistent, and nonreproducible effects at all concentrations tested up to the maximum of 100 μg/ml and were thus deemed inactive.

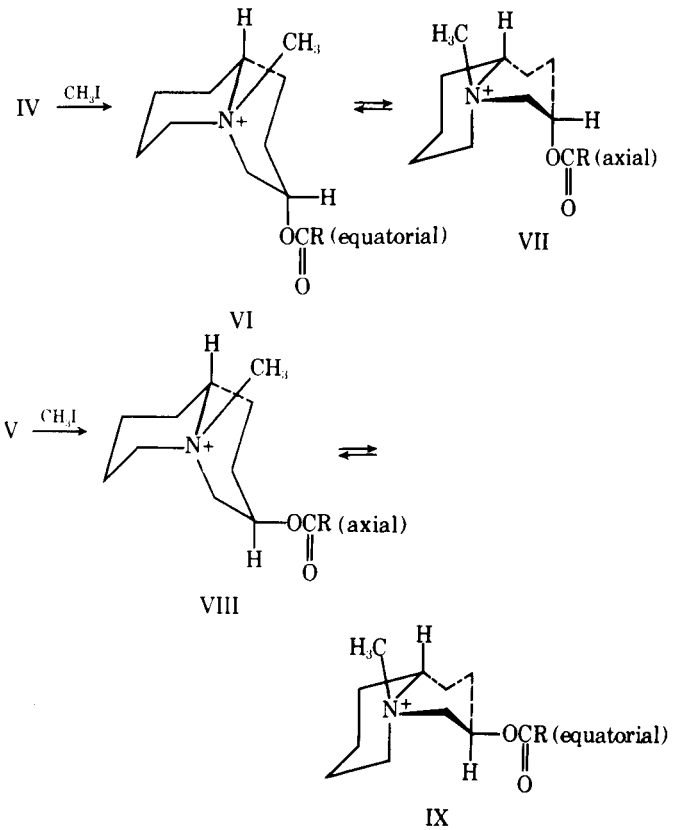
The lack of agonist activity displayed by the prepared quaternary salts was unexpected since all active decalin acetylcholine analogs have the antiplanar nitrogen–ester oxygen orientation (10). Drieding models of the quaternary salts prepared show that, regardless of the product formed (Structure VI or IX of Scheme II), there will be an antiplanar nitrogen–ester oxygen relationship. Even more enigmatic is the agonist activity of X (Table I), a hydrochloride salt. If it is assumed that the quinolizidine ring in the compound remains in the *trans*-fused configuration (since it is not quaternized), then Drieding models demonstrate that the nitrogen–ester oxygen relationship is synclinal. The synclinal arrangement is also thought to be present in acetylcholine (10) and thus may account for the agonist activity of the hydrochloride salt. However, acetylcholine-like agonists are normally quaternized. This finding is rather unusual, especially since the other amine salts prepared did not show activity of any sort. The nature of the agonist activity displayed by this hydrochloride salt will be explored in the future.

It is also reasonable to anticipate antagonistic activity from the prepared quaternary salts because of the greater number of hydrophobic interactions of the quinolizidine ring relative to acetylcholine. However, only one member of the series is antagonistic. Intuitively, since structural differences between these compounds are so minimal, any biological activity should be shared by all members. Further study will be required to clarify this anomaly.



Scheme I

[1] This explanation was suggested by a reviewer.



Scheme II

EXPERIMENTAL[2]

3-Hydroxyquinolizidine—3-Oxoquinolizidine was prepared according to the method of Counsell and Soine (5) and reduced catalytically by the procedure of Aaron *et al.* (6). A 5% ruthenium-on-carbon reduction gave a 72:28 axial to equatorial alcohol ratio, and a 10% palladium-on-carbon reduction yielded a 3:97 axial to equatorial alcohol ratio (6, 7). The epimeric mixtures were separated by fractional distillation. Homogeneity of the separated epimers was easily established by TLC.

Esters (Table I)—Approximately 4 g (0.04 mole) of the respective 3-hydroxyquinolizidine was mixed with 10 ml (0.1 mole) of fresh acetic anhydride and 1 ml of pyridine. The mixture was stirred for 24 hr at room temperature. The dark brown-black solution was then poured over crushed ice, and the mixture was saturated with potassium carbonate while ice bath temperatures were maintained. The resulting mixture, pH 8, was extracted with four 50-ml portions of chloroform.

The combined chloroform extracts were dried over anhydrous sodium sulfate, and the solvent was removed under water aspirator vacuum. The resulting oil was then taken up in a mixture of petroleum ether (bp 60–90°)–ethyl acetate (9:1) and passed through a silica gel column. Hydrochloride salts were prepared by dissolving the ester in a minimum of ether and adding, dropwise, a saturated solution of hydrogen chloride gas in anhydrous ether until precipitation ceased. The precipitate was then crystallized from ethanol–ether.

Methiodides (Table I)—Approximately 0.02 mole of the ester and 5 ml (0.08 mole) of methyl iodide were dissolved in 25 ml of acetone. This mixture was refluxed overnight, and the solvent was removed and yielded yellow-white crystals. These crystals were readily recrystallized from ethanol or ethanol–ether.

Biological—The compounds were tested on strips of rat jejunum suspended in oxygenated Tyrode solution in a 100-ml bath maintained at 37°. The muscles were attached to a myograph transducer, and contractions were recorded on a physiograph. Responses to test compounds were observed in comparison to responses to standard solutions of acetylcholine chloride. Typical contractions in response to acetylcholine occurred at concentrations ranging from 0.01 to 0.1 μg/ml of bath solution.

In appropriate experiments, atropine sulfate (0.01-μg/ml final concentration) was utilized to block muscarinic receptors. Test compounds were injected into the bath in volumes of 1.0 ml or less to yield final bath concentrations of 100 μg/ml or less. All doses are expressed in micrograms per milliliter of bathing solution.

Dose–response curves were constructed for the pharmacologically active compounds. Average concentrations of antagonists required to inhibit acetylcholine-induced contractions 50% (IC_{50}) were calculated. Average concentrations of cholinomimetics required to equal acetylcholine-induced contractions also were calculated. Standard deviations were computed for such averages.

[2] Melting points were taken with a Thomas-Hoover Uni-Melt apparatus and are uncorrected. IR spectra were determined with a Beckman Accu Lab Spectrophotometer. NMR spectra were obtained with a Varian model A-60-A spectrometer relative to an internal standard of tetramethylsilane with deuterochloroform as solvent. Column chromatography was performed with 28–200-mesh silica gel. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn.

Table II—NMR Bandwidth Data of Methine Protons on Oxygen-Bearing Carbon

Compound	W_h, Hz	Chemical Shift, Hz
XII[a]	12	320
XIII[a]	15	320
XVI[a]	12	322
XVII[a]	18	320
Axial 3-hydroxyquinolizidine (II)[b]	6.5	225
Equatorial 3-hydroxyquinolizidine (III)[b]	19	217

[a] All measurements were obtained for 10% $CDCl_3$ solutions. [b] Data were taken from Ref. 6. Measurements were obtained for 10% carbon tetrachloride solutions.

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