Pharmacological Assay Methods. Isolated Guinea Pig Trachea. The male guinea pig trachea was prepared according to the procedure of Persson and Olsson.[10] Initial tension was adjusted to 2 g. Relaxations in the muscle were recorded by means of a Grass force displacement transducer (FT 03) and a Grass polygraph. The effect of the racemate, (−) isomer, and (+) isomer was compared to that of adrenaline. Dose–response curves were obtained and concentrations causing 50% relaxation were determined (EC 50).

Isolated Guinea Pig Auricle. Isolated left auricles were prepared from guinea pigs (0.5–0.7 kg) according to the method of Persson and Olsson.[10] Initial tension was adjusted to 1 g. Contractions in the muscle were recorded by means of a force displacement transducer and a Grass polygraph. Dose–response curves were obtained and the concentration causing 20% increase in the force of contraction was graphically determined. The effect of the compounds was compared to that of (−)-adrenaline.

Acknowledgment. The author wishes to thank Dr. H. Persson for the pharmacological tests, Miss L. Knutsson for preparing (+)- and (−)-dibenzoyltartaric acids, and Miss. E. Larsson for measuring the optical rotations.

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Syntheses and Pharmacological Actions of 2-[(2-Chloroethyl)methylamino]ethyl Acetate and Some of Its Derivatives on the Isolated Guinea Pig Ileum†

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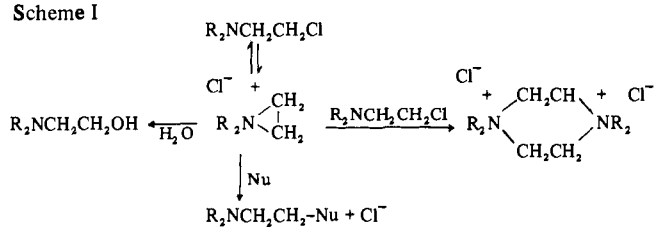
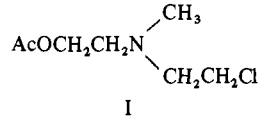
Aziridinium ions, formed from the internal SN2 reaction of tertiary β-haloethylamine precursors, have been shown to be consumed by four reactions:[1-3] reconversion to starting material by reaction with chloride ion; dimerization by reaction with starting material to a piperazinium derivative; hydrolysis to the corresponding alcohol and reaction with other nucleophiles present (Scheme I).

We recently reported[4] that aqueous solutions of 2-[(2-chloroethyl)methylamino]ethyl acetate consume sodium thiosulfate[5] and liberate chloride ion. These observations

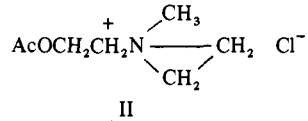
Scheme I



imply that this compound is capable of isomerizing to 1-(2-hydroxyethyl)-1-methylaziridinium chloride acetate II, an aziridinium analog of acetylcholine. As solutions of I are potent spasmogens of the guinea pig ileum,[4,6] we have suggested that the species II is of importance to the stimulant action of I.

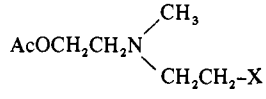


I

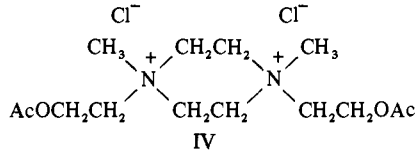


II

In order to confirm this proposal we have examined the potencies of the degradation products of II and some related compounds. By analogy with Scheme I the aziridinium ion may be hydrolyzed to 2,2'-(methylimino)diethanol acetate IIIa or dimerized to 1,4-bis(2-hydroxyethyl)-1,4-dimethylpiperazinium dichloride diacetate IV. The potencies of these compounds were investigated. As a means of indirectly assessing the activity of the uncyclized 2-chloroethylamine I, the methiodide salt Va was prepared and bioassayed. Also examined were 2,2'-(methylimino)diethanol diacetate IIIb and its methiodide salt Vb, the methiodide salt of IIIa, Vc, and 1-aziridineethanol acetate VI, the nor derivative of II.



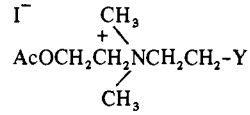
IIIa, X = OH
IIIb, X = OAc



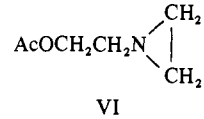
IV

Results and Discussion

Table I summarizes the activity of the compounds tested on the guinea pig ileum in the form of equipotent molar ratios (EPMR), against acetylcholine iodide as the standard drug.



Va, Y = Cl
Vb, Y = OAc
Vc, Y = OH



VI

†Supported by Grant MA-3359 from the Medical Research Council of Canada. C. H. J. was the recipient of a Medical Research Council Fellowship during this study.

Table I. Muscarinic Activities on the Guinea Pig Ileum Expressed as EPMR's Relative to Acetylcholine

No.	Compound	EPMR ± SEM[a]	n[b]
	Acetylcholine iodide	1.0	
I	2-[(2-Chloroethyl)methylamino]-ethyl acetate	6.1 ± 0.12[c]	3
IIIa	2,2'-(Methylimino)diethanol acetate	$(8.9 \pm 1.3) \times 10^2$	3
IIIb	2,2'-(Methylimino)diethanol diacetate	$(3.0 \pm 0.38) \times 10^3$	3
IV	1,4-Bis(2-hydroxyethyl)-1,4-dimethylpiperazinium dichloride diacetate	$>5.0 \times 10^3$	3
Va	(2-Chloroethyl)dimethyl(2-hydroxyethyl)ammonium iodide acetate	$(1.1 \pm 0.14) \times 10^3$	3
Vb	Bis(2-hydroxyethyl)dimethylammonium iodide diacetate	$(1.3 \pm 0.11) \times 10^2$	3
Vc	Bis(2-hydroxyethyl)dimethylammonium iodide acetate	64 ± 8.3	3
VI	1-Aziridineethanol acetate	$>5.0 \times 10^3$	3

[a] EPMR ± SEM = equipotent molar ratio ± standard error of mean. [b] n = number of bioassays performed. [c] Ref 4.

Table I reveals that of the synthetized compounds, 2-[(2-chloroethyl)methylamino]ethyl acetate I is by far the most potent agonist on the guinea pig ileum. The EPMR's of IIIa and IV, expected transformation products of II, are high indicating that these compounds are not agonist species which effectively contribute to the potency of I.

The difference in potency between I and its methiodide salt Va is very significant, for the methiodide is almost 200 times less active than the base. This behavior contrasts to that demonstrated by IIIa and IIIb which are 14 and 23 times less potent than their respective methiodide salts Vc, Vb. As quaternary methyl salts of cholinergic compounds are commonly more active than their parent nor derivatives,[7] it would seem that the uncyclized isomer I is not primarily responsible for its potency.

Compound VI, the nor analog of the aziridinium ion II, proved to be an ineffective stimulant. This finding, which compares qualitatively with the demonstrated difference in potencies of acetylcholine and its nor derivative,[8] further indicates the importance of cationic species for stimulant activity.

These results present confirmatory evidence for II being the agonist species responsible for the potent cholinomimetic activity of I.

Experimental Section‡

Biological Methods. Bioassays were performed on guinea pig ilea[9] suspended in an 8-ml aerated, water-jacketed, organ bath maintained at 37° and containing Tyrode solution 0.1 m*M* in hexamethonium dichloride. Drug solutions used in the bioassays were prepared in this modified Tyrode solution. Acetylcholine iodide was used as the standard reference substance against which the synthetized compounds were assayed. Four-point assays were performed using two dose levels of the standard compound S_1 and S_2 and two dose levels of the synthesized compound U_1 and U_2 such that $[S_1]/[S_2] = [U_1]/[U_2]$. The final concentrations used in the assay were such that the responses to the low and high concentrations of the standard were similar in magnitude to the low and high concentrations of the unknowns. All drug-induced contractions lay on the vertical portions of the log dose-response curves. The order of addition of the drug solutions was based on a 4 × 4 "Latin Square," the muscle being exposed to a drug solution for 30 sec in each 3-min cycle. The recorded contractures were measured and the EPMR determined by the method of Gaddum.[10,11] Bioassays of a particular compound were performed three times, using at least two different preparations.

As 1 m*M* solutions of compounds IV and VI caused negligible tissue responses comparative potencies were not established.

Syntheses. 2,2'-(Methylimino)diethanol Acetate (IIIa). 2,2'-(Methylimino)diethanol (20 g, 0.16 mole, Aldrich) was dissolved in EtOAc (40 ml) and with cooling Ac_2O (8.58 g, 0.08 mole) was added dropwise. After stirring at room temperature for 30 min, the solution was extracted from Na_2CO_3 solution with $CHCl_3$. The $CHCl_3$ extracts were concentrated *in vacuo* and the residue distilled under reduced pressure to give 13 g (51%) of IIIa: bp 73° (0.55 mm) [lit.[3] bp 81–83° (1 mm)].

Bis(2-hydroxyethyl)dimethylammonium Iodide Acetate (Vc). To a cooled ethereal solution of IIIa (2 g), MeI (2 ml) was slowly added. The solid that separated was recrystallized from Me_2CO–Et_2O to give 2.3 g (61%) of Vc: mp 60–61°; ir (Nujol), 3450 (-OH), 1740 (ester >C=O) cm^{-1}. *Anal.* ($C_8H_{18}INO_3$) C, H, N.

2-[(2-Chloroethyl)methylamino]ethyl Acetate (I). To a cooled solution of IIIa (5 g, 0.031 mole) in $CHCl_3$ (10 ml) $SOCl_2$ (3 ml, 0.041 mole) was added dropwise with stirring. After stirring at room temperature for 1.5 hr, the solution was neutralized with cold 10% NaOH and extracted with $CHCl_3$. (Glassware used in the work-up from this point was flamed in order to minimize dimerization of the product.) The $CHCl_3$ extracts were filtered through Na_2SO_4 and concentrated *in vacuo*, and finally the residue was distilled at reduced pressure to give 3 g (55%) of I: bp 65–66° (0.5 mm) [lit.[3] bp 78–80° (1.5 mm)]; ir (neat), 1740 (ester >C=O) cm^{-1}.

(2-Chlorethyl)dimethyl(2-hydroxyethyl)ammonium Iodide Acetate (Va). This compound was prepared, as described for Vc, from I; mp 149–151° dec (lit.[3] mp 150° dec). *Anal.* ($C_8H_{17}ClINO_2$) C, H, N.

2,2'-(Methylimino)diethanol Diacetate (IIIb). 2,2'-(Methylimino)diethanol (60 g, 0.50 mole) was treated with Ac_2O (120 g, excess) at 0°. The solution was left at refrigerator temperature for 48 hr and then neutralized with concentrated Na_2CO_3. Extraction with $CHCl_3$ and distillation of the residue obtained from concentration *in vacuo* of the extracts gave 100 g (98%) of IIIb: bp 96° (1.5 mm) [lit.[12] bp 110° (4 mm)]; ir (neat), 1740 (ester >C=O) cm^{-1}.

Bis(2-hydroxyethyl)dimethylammonium Iodide Diacetate (Vb). This compound was prepared, as described for Vc, from IIIb: mp 115–116° (lit.[12] mp 117–118°).

1,4-Bis(2-hydroxyethyl)-1,4-dimethylpiperazinium Dichloride Diacetate (IV). Aged preparations of I inevitably deposited a colorless solid which was recrystallized from MeOH to give IV: mp 204–205.5° dec (lit.[9] mp 228° for cis isomer). *Anal.* ($C_{14}H_{28}Cl_2N_2O_4$) C, H, N.

1-Aziridineethanol Acetate (VI). 1-Aziridineethanol (90 g, Aldrich) was acetylated by a procedure described previously[13] to give 101 g (76%) of VI after distillation at reduced pressure: bp 58–59° (4.5 mm) [lit.[13] bp 99–100° (63 mm)].

‡Infrared spectra were recorded with a Hilger and Watts infragraph H1200 spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian T-60 spectrometer. All melting points were taken on a Kofler micro heating stage and are uncorrected. All boiling points are uncorrected. The microanalyses were performed by A. B. Gygli, Toronto, Canada, and were within ±0.3% of the theoretical values for C, H, and N.

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