Physical and Pharmacological Properties of an Irreversible Local Anesthetic[1]

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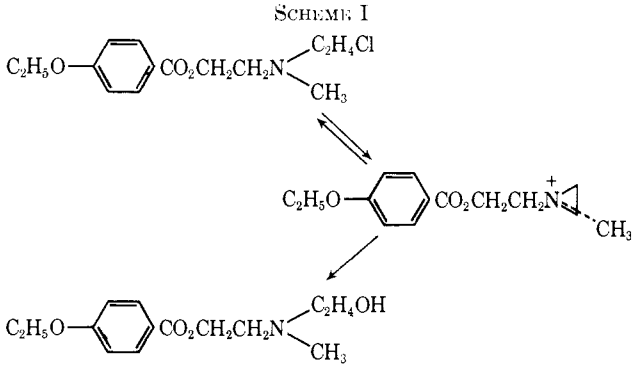
A systematic investigation of a new class of ultralong-acting local anesthetic was undertaken to determine the active pharmacophore. The thermodynamic constants for the solvolysis of the aziridinium ion (E_a, ΔH^*, ΔS^*) compared favorably with those reported in the literature for other aziridinium type compounds. 2-[(2-Chloroethyl)methylamino]ethyl 4-ethoxybenzoate·HCl upon neutralization in a physiological buffered solution blocked irreversibly the indirectly elicited twitch of the rat phrenic nerve–diaphragm preparation without altering either nerve or muscle excitability, suggesting a selective action at the neuromuscular junction at 0.2% and at an exposure time of 30 min. At 0.5%, this compound upon neutralization blocked axonal conduction of the sciatic nerve of the frog irreversibly whereas comparable blockade by procaine (1%) or lidocaine (2%) of this preparation was fully reversible upon washing. It is proposed that the aziridinium ion forms an irreversible bond with a tissue "acceptor" and the stability of this ion determines the effectiveness of drug action.

In a previous article,[4] we reported the development of a new class of ultralong-acting local anesthetics. These compounds were prepared on the principle of using an established pharmacologically active molecule (*eg.*, lidocaine) as the carrier for a potential alkylating moiety (2-haloethylamine).

We suggested that, like the α-adrenergic blocking agents of the 2-haloethylamine type, the formation of the aziridinium ion led to the alkylation of a site implicated in nerve conduction, thus forming an irreversible bond. The aziridinium ion is usually formed from the hydrohalide salt of the 2-haloethylamine when it is dissolved in a physiological buffered solution. Hydrolysis to the alcohol may also occur. Thus at any time there may be three pharmacologically active species present, the precursor, the aziridinium ion, and the hydrolysis product. It is important to establish whether the irreversible effects are due to the aziridinium ion alone or to some combination of the above species.

SCHEME I



Since it is likely that this aziridinium ion might be the active pharmacophore, it is of importance to investigate the formation and stability of this species. Such a study might offer substantial information about the design of more potent irreversible local anesthetics and is considered of fundamental importance in obtaining drugs which have useful therapeutic activity. Compounds which form the aziridinium ion too slowly or those which hydrolyze too rapidly might have limited usefulness. Likewise, aziridinium ions which react too slowly with the tissue nucleophiles might have too low an activity. The local anesthetic chosen to test our concepts was 2-[(2-chloroethyl)methylamino]ethyl 4-ethoxybenzoate·HCl.[4]

A variety of analytical techniques have been used for the analysis of 2-haloethylamines and for the formation of the aziridinium ion. Friedman and Boger[5] used colorimetry to determine the concentration of the aziridinium ion in H_2O. Bartlett, *et al.*,[6] used acid titration and the Volhard Cl^- method. Golumbic, *et al.*,[7] developed a thiosulfate titration method for measuring the aziridinium ion. Wagner and Berg[8] reported the use of a polarographic catalytic wave technique for measuring the concentration of the aziridinium ion. Beroza and Borkovec,[9] Pettit, *et al.*,[10] Levins and Papanastassiou,[11] and Sowa and Price[12] used nmr spectroscopy to study the formation and hydrolysis of the aziridinium ion.

Results and Discussion

1. Nmr.—Using nmr spectroscopy,[13] we studied the properties of the HCl salt of 2-[(2-chloroethyl)methylamino]ethyl 4-ethoxybenzoate. An AA′BB′ pattern was observed for the aromatic protons in which $2H^+$ (H_b)

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(13) Nmr spectra were run in D_2O using sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as internal standard. The CH_3–Si shifts of DSS and tetramethylsilane (TMS) are identical, and the shift values are reported in parts per million. The spectra were run on the Varian Associates 220 spectrometer. The chart paper and temperature controller were calibrated prior to each study.

appeared as a split singlet centered at 8.03 ppm with a coupling constant of 0.05 ppm, while the remaining $2H^+$ (H_a) appeared as a split singlet centered at 7.04 ppm with a coupling constant of 0.05 ppm. The ethoxymethyl protons appeared as a triplet at 1.36 ppm; the *N*-Me protons as a singlet at 2.96 ppm. This deshielding is primarily due to the disappearance of the lone-pair electrons on the N. The remaining CH_2 appeared as a multiplet from 4.30 to 3.68 ppm.

When the HCl salt of 2-[(2-chloroethyl)methylamino]ethyl 4-ethoxybenzoate was neutralized ($NaHCO_3$), the rate of formation of the aziridinium ion was too rapid to measure with the nmr spectrometer, but it could be determined that 98% had been converted into the aziridinium ion. The nmr spectrum[14] showed the formation of the aziridinium ion with the appearance of a split singlet at 2.50 ppm. Since little work has been done in considering the ground state electronic configuration of the aziridinium ion,[15] and since this ion is isoelectronic with cyclopropane, we might be able to suggest similar ground states. Even though cyclopropane's "banana bonding"[16,17] electrons contrast sharply with the π electrons of benzene which lie above and below the plane of the ring, it is possible for the aziridinium ion to lie beneath and coplanar to the benzene nucleus.[18] Considering this geometry, one might anticipate that either a split broad singlet or 2 singlets would be observed for the aziridinium ring's protons. The aromatic protons were shielded and appeared upfield at 7.73 ppm for H_b and 6.64 ppm for H_a. The aromatic π electrons were dislocated due to the anisotropic diamagnetic effects of the aziridinium ring, which caused the shielding of the H_a and H_b protons. It is worth noting that the change in shielding of the H_a protons is 0.40 ppm, whereas that of the H_b protons is 0.30 ppm. Thus, we suggest that the axis bisecting the aziridinium ring does not lie equidistant between the H_a and H_b protons. The ethoxymethyl protons shifted upfield to 1.12 ppm. It appears that the ethoxymethyl protons are influenced by the banana bonding electrons of the aziridinium ring, thus causing the increased shielding. The *N*-Me protons became shielded and moved to 2.06 ppm. This marked shielding suggests that the Me protons are located near the cavity of the benzene nucleus in which diamagnetic effects play an integral part in shielding the protons. The remaining protons appeared as distinguishable peaks at 4.08, 3.62, and 3.30 ppm. Molecular models substantiate these findings. An important observation is the increased stability of the aziridinium ring brought about by its interaction with the π electrons of the benzene ring. This enhanced stability suggests a valuable parameter by which drug action may be studied.

Solvolysis of the aziridinium ion was measured in neutral solution in order to obtain information about the mechanism of this reaction and also to compare the thermodynamic constants (E_a, ΔH^*, ΔS^*) with those reported in the literature[9] for other compounds of this type. As indicated above, we were unable to ascertain

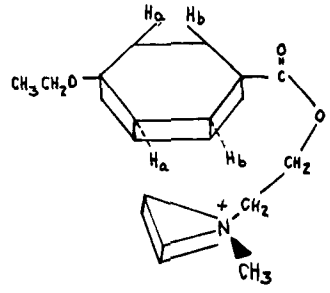

Figure 1.

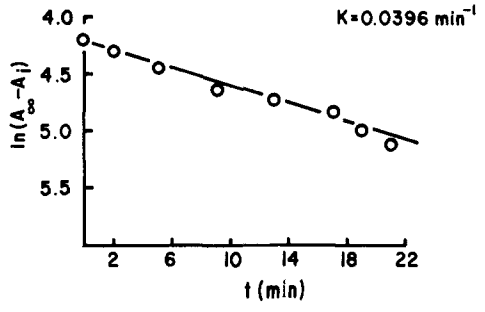

Figure 2.—First-order rate plot for the solvolysis of the aziridinium ion in D_2O.

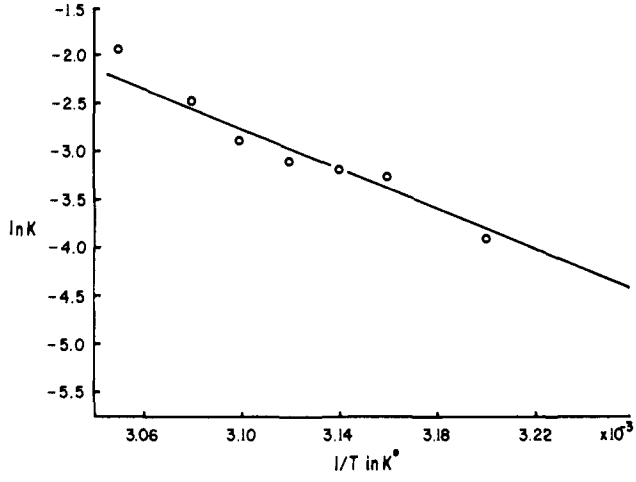

Figure 3.—A plot of ln K against $1/T$ for the solvolysis of the aziridinium ion.

the rate of ring closure, but the solvolysis of the aziridinium ion did obey measurable first-order kinetics in neutral solutions at various temperatures. Figure 2 represents a typical result. Levins and Papanastassiou[11] as well as Leonard, *et al.*,[19] have indicated that the rate-determining step of this reaction is ring opening; thus it is unlikely that the H_2O–D_2O kinetic isotope effect would be of any great significance.

Figure 3 represents an Arrhenius plot in which the activation energy (E_a) is 21 kcal/mole. The entropy of activation (ΔS^*, −13.94 cal/deg mole) and the enthalpy of activation (ΔH^*, 20.4 kcal/mole) agree with literature values for other mustards.[14]

2. Pharmacological Results.—The HCl salt of 2-[(2-chloroethyl)methylamino]ethyl 4-ethoxybenzoate was neutralized in physiological soln, and its ability to act as a local anesthetic on a variety of tissues was examined. At 0.2% and at an exposure time of 30

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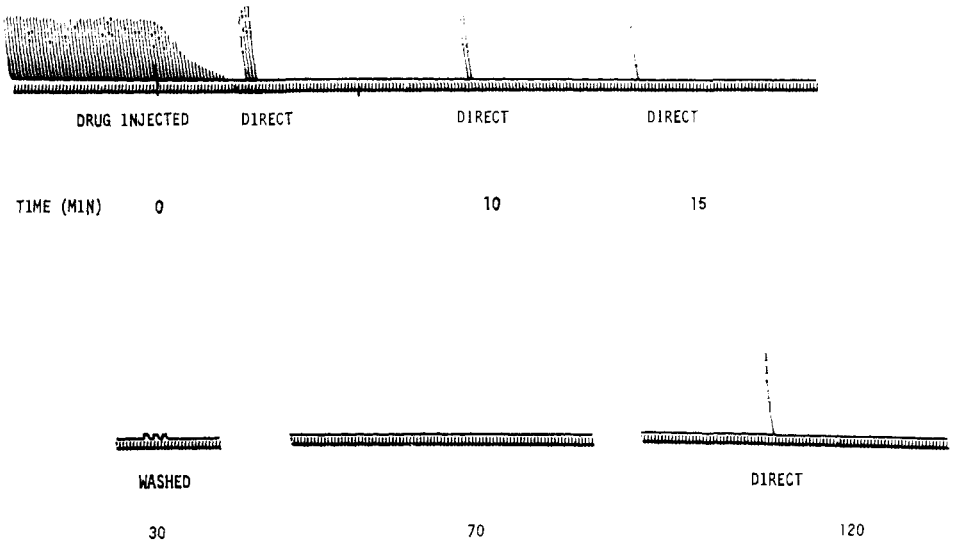

Figure 4.—Rat phrenic nerve-diaphragm preparation, indirect and direct stimulation are at 1.6 V and 60 V, respectively. Initial tension is at 5 g.

min, the compd blocked the indirectly elicited twitch of the rat phrenic nerve–diaphragm preparation;[4] prolonged washing did not restore transmission (Figure 4). It is worth noting that at this dil concn little if any effect on axonal conduction was observed on the phrenic nerve. This was ascertained by removing the nerve and examining its ability to conduct electrical impulses[20] at a time when the indirectly elicited twitch was blocked. Furthermore, at a time when transmission was blocked, contractions of the directly stimulated muscle were essentially normal (Figure 4). Thus, the junctional region, *i.e.*, nerve terminal and/or postsynaptic membrane receptors, constitutes the site(s) of action of the drug. At 0.5%, the compd blocked axonal conduction of the sciatic nerve of the frog irreversibly whereas comparable blockade of this preparation by procaine (1%) or lidocaine (2%) was fully reversed upon washing.

As was pointed out above, there are 3 different molecules that might possess the active pharmacophore: 2-[(2-chloroethyl)methylamino]ethyl 4-ethoxybenzoate, the precursor, the aziridinium ion, and 2-[(2-hydroxyethyl)methylamino]ethyl 4-ethoxybenzoate, the hydrolysis product. From nmr spectroscopy, it was noted that the precursor comprises only 2% of the equilibrium mixture. Although the hydrolysis product does possess local anesthetic activity, this is rapidly reversed upon washing. It is also worth noting that the half-life at 30° for the aziridinium ion is 83 min. Thus, it is suggested that the pharmacophore responsible for irreversible effects is only the aziridinium ion, and it is this ion that alkylates sites in the active membrane producing an irreversible block of excitation.

Experiments with this analog gave substantially the same pharmacological results as reported previously,[4] and thus the important parameters affecting the activity of these compounds are (1) the rate of reaction of the aziridinium ion with tissue nucleophiles and (2) the stereochemistry of this ion with respect to the geometry of the receptor site.

(20) The electrical impulses of the phrenic nerve were measured by placing the nerve in an isolated nerve chamber in which conducted impulses were monitored by a double beam oscilloscope.

TABLE I

RATE OF SOLVOLYSIS OF THE AZIRIDINIUM ION IN A NEUTRAL SOLUTION OF DEUTERIUM OXIDE[a]

Aziridinium ion, moles	$NaHCO_3$, moles	T, °C	K, min^{-1}
1.51×10^{-5}	1.51×10^{-5}	39.0	0.0190
		43.0	0.0377
		45.0	0.0396
		47.0	0.0444
		49.5	0.0551
		52.5	0.0832
		55.0	0.1460

[a] Integrating the proton bands at 1.12 and 1.36 ppm at each time gave the relative concentration of the aziridinium ion and its hydrolysis product.

Experimental Section[21]

2-[(2-Hydroxyethyl)methylamino]ethyl 4-Ethoxybenzoate.—*N*-Methyldiethanolamine (23.8 g, 0.200 mole) was dissolved in 200 ml of anhyd C_6H_6. Anhyd K_2CO_3 (27.6 g) was added, and the mixt was stirred mechan and heated to reflux. 4-Ethoxybenzoyl chloride (18.5 g, 0.100 mole) was added dropwise. Stirring and heating was continued for 3 hr, and then the mixt was allowed to stand overnight. The mixt was filtered, and the filtrate was washed with 100-ml portions of H_2O. The org layer was dried (K_2CO_3), and the solvent was removed *in vacuo*. The residual oil was distd to obtain 10.2 g of product, bp 157–161° (0.1 mm).

2-[(2-Chloroethyl)methylamino]ethyl 4-Ethoxybenzoate·HCl.—2-[(2-Hydroxyethyl)methylamino]ethyl 4-ethoxybenzoate (8.6 g) was dissolved in 25 ml of anhyd $CHCl_3$. A soln of 9 ml of $SOCl_2$ in 25 ml of anhyd $CHCl_3$ was added in small portions with stirring. The soln was refluxed for 3 hr and then concd *in vacuo* to give a thick oil. The residue was cooled in an ice bath and triturated with petr ether to induce crystn. The crude solid was recrystd from EtOAc. The purified product had mp 107.5–108.5°. *Anal.* ($C_{14}H_{21}Cl_2NO_3$): C, H, N.

(21) Melting points were taken in a Thomas-Hoover capillary melting point apparatus and are corrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.2% of the theoretical values.