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Boulos, John, Ph.D.

City University of New York, 1991

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SYNTHESIS OF NOVEL MUSCARINIC AGONISTS

BY

JOHN BOULOS

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1991

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This manuscript has been read and accepted by the Graduate Faculty in Chemistry in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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ABSTRACT

Synthesis of Novel Muscarinic Agonists

By

John Boulos

Advisor: **Professor Jerome Schulman**

We have synthesized a series of imidazole and oxazole-containing heterocyclic compounds, based upon a theoretical model of the structural requirements for muscarinic receptor activity. The compounds are intended as mimics of acetylcholine and other cholinergic agonists (cholinomimetics) at muscarinic cholinergic receptors in the brain and the peripheral nervous system. Muscarinic receptors are of importance in Alzheimer's disease and problems of intestinal mobility. Compounds which act selectively at these receptors could be desirable therapeutic agents as well as research probes of cholinergic pharmacology. Biological data have been obtained for some of these compounds.

Dedicated to my beloved wife, Sarita, and to my two sons Alexander and John Michael; to my parents, Georgette and Fernand Boulos.

Acknowledgments

I would like to express my sincere appreciation to my mentor, Dr. Jerome Schulman, who helped and guided me throughout this project. I extend my gratitude to Dr. William Berkowitz who has greatly contributed to my success.

I thank all my colleagues, especially Mr. Kasthuri Rengan, for their sound advice and for providing an amicable and peaceful atmosphere in the laboratory.

I extend my thanks to Dr. David Locke for the GC/MS analyses and to Dr. George Axelrad for his financial support throughout the years.

Finally, I am extremely grateful to my wife Sarita Boulos for her patience, understanding and moral support. I am also grateful to my parents, Georgette and Fernand Boulos, for all the sacrifices they have made in order for me to complete this work.

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Chapter 1

General Introduction

I. Some Relevant Aspects of the Nervous System:[1] The nervous system is composed of the central nervous system (brain and spinal cord) and the peripheral nervous system (somatic and autonomic). Brain regions of particular interest here are the hippocampus, the cerebral cortex and the basal ganglia (e.g. nucleus of Meynert) which appear to be involved in memory and which contain muscarinic M_1 as well as other subtypes of muscarinic receptors. It is estimated that 80% of the muscarinic receptors in the brain are M_1. The autonomic peripheral nervous system is divided into sympathetic and parasympathetic subsystems based upon anatomical and physiological differences. The parasympathetic system utilizes presynaptic neurons which emanate from the cranial and sacral nerves and which synapse with postsynaptic neurons on nicotinic cholinergic receptors. These postsynaptic neurons in turn synapse with the muscarinic receptors of the various effector cells, using acetylcholine (**1**) as the neurotransmitter. An example is the tenth cranial or vagus nerve which innervates the lungs, trachea, esophagus, heart, stomach, pancreas, kidneys and intestine.

1

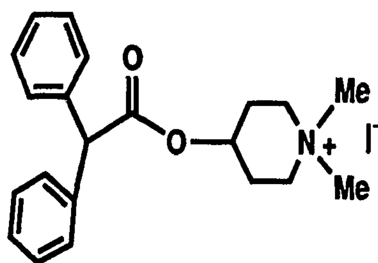
Acetylcholine and other muscarinic agonists bind to muscarinic receptors and effect intracellular changes by three mechanisms: opening ion channels selective to potassium[2] or calcium;[3] stimulating guanylate cyclase and inhibiting adenylate cyclase[4] through a pathway involving proteins that bind GTP (G proteins); and hydrolyzing inositolbiphosphate.[5] The latter two "second messenger" mechanisms also lead to the opening of ion channels that lead to depolarization of the cell membrane to facilitate an action potential. Hyperpolarized membranes inhibit action potentials.

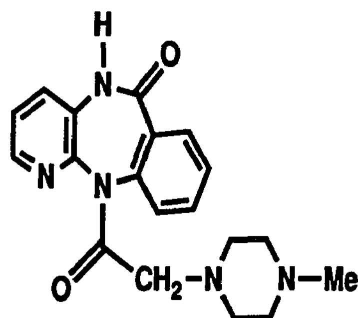
II. Muscarinic Receptor Subtypes: In the mid-1960's, suggestions were made that subtypes of muscarinic receptors exist. Burgen and Spero[6] examined the ratio of potencies of a variety of agonists at muscarinic receptors to stimulate contraction of guinea-pig ileum and potassium efflux from the same tissue. They reported that the properties of the receptors mediating these responses might be different, but they were unable to find selective antagonists for these receptors.

The earliest reports of selective antagonists at muscarinic receptors came from Fisher et al.[7] However, their compounds were not very potent antagonists. Barlow et al.[8] described a compound, 4-DAMP (**2**), that showed selectivity in blocking muscarinic receptor-mediated responses in the ileum, versus to those in the heart. Gardier et al.[9] presented physiological evidence for two distinct types of muscarinic receptors in the superior cervical ganglion of the cat.

In other experiments, Goyal and Rattan[10] measured the potencies of McN-A343 (**12**) and bethanechol at muscarinic receptors on interneurons in the lower esophageal sphincter of the opossum and compared them to the potencies for stimulation of muscarinic receptors in the smooth muscle of the esophageal sphincter itself. They suggested that the observed selectivity was consistent with the hypothesis that subtypes of muscarinic receptors exist proposing that ganglionic receptors be termed M_1, and smooth muscle receptors be termed M_2.

An advance in the characterization of muscarinic receptor subtypes came about with the discovery of pirenzepine (**3**), an M_1 selective antagonist.[11] M_1 and M_2 receptors have high and low affinity for pirenzepine, respectively.





2 **3**

Mutschler and Lambrecht[12] suggested that the M_2 receptors may need to be divided further into subgroups. Moreover, Giachetti et al.[13] have shown that the compound AF-DX 116 distinguishes between M_1 and M_2 subtypes and also recognizes cardiac M_2 receptors with an affinity 30-fold higher than for M_2 receptors located on smooth muscles or secretory glands. These data are consistent with the idea that more than two types of muscarinic receptors exist and that the receptors M_2 should be subclassified.

It is now generally agreed that there exist three pharmacologically defined muscarinic receptors:[14] M_1, with a high affinity for pirenzepine; M_2, with a high affinity for AF-DX 116 and gallamine; and M_3, having a high affinity for 4-DAMP and hexahydrosiladifenol (HHSD). (The 4-DAMP-sensitive receptor was previously designated as M_2.)

i) Molecular forms: Within the past few years five distinct muscarinic receptors have been cloned. Referred to as m_1 through m_5, they are single chain proteins with molecular weights ranging from 51,452 to 66,127 Daltons.[15] (The current practice is to denote pharmacologically defined receptors with a capital M, cloned molecular forms with a lower case m.) The m_1, m_3 and m_5 receptors share a sequence homology of over 40%; m_2 and m_4 receptors share an overall amino acid homology of 44%, but are only 32-33% identical to either receptor of the first group. The pharmacologically defined receptors M_1, M_2, M_3 correspond to their molecular forms m_1,

m_2, m_3, respectively.

The m_1 receptors have been shown to play a role in senile dementias of the Alzheimer's type. Thus treatment with selective m_1 agonists could prove efficacious as a replacement therapy to alleviate symptoms of the disease.

From their known sequences, it is believed that muscarinic receptors belong to the family of seven-helix receptors (Fig. 1, pg 18).[16] Such receptors span the cell plasma membrane seven times, creating four extracellular domains, o1-o4, seven helical hydrophobic transmembrane domains I-VII, and four intracellular domains i1-i4. Muscarinic receptors bind their cholinergic ligands at sites within the cell membrane; this results in intracellular signals by an unknown transduction mechanism to one or more species of cytoplasmic G proteins which interact with the receptor at its innerloop 3.

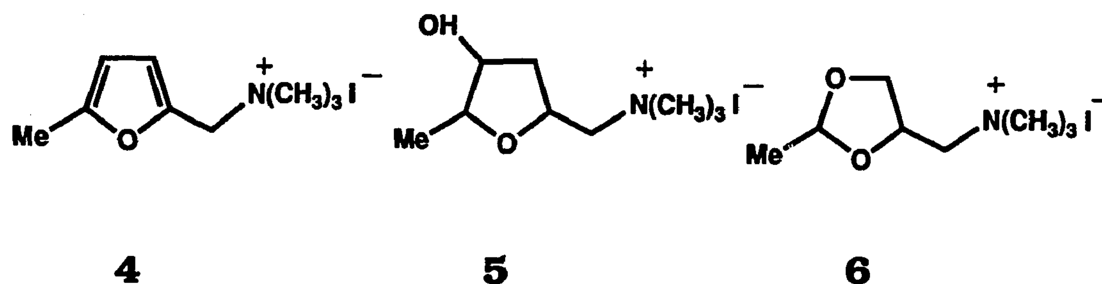
ii) Binding sites: The molecular basis for the pharmacologic selectivities of muscarinic receptors is unknown since the binding sites on the receptors have yet to be determined. Aspartate group (D) in transmembrane domain III is a candidate binding site for the positively charged head group of muscarinic agents. The serine (S), in transmembrane domain III, could also be involved in hydrogen bonding with regions of electrostatic potentials on cholinergic agents. These residues are conserved in all five cloned receptors.

III. Aspects of the Structural Chemistry of Muscarinic Agonists.

i) Classical muscarinic cholinergic agonists: Although five muscarinic receptors have been sequenced, their detailed three-dimensional features remain unknown. It therefore is still necessary to examine structure-activity data on acetylcholine and other agonists in order to infer information about the receptor binding sites.

Ing[17] has noted that for maximal muscarinic potency there should be a quaternary nitrogen atom having three methyl groups and a fourth group with a chain of five atoms, e.g , (C-C-O-C-C). This "five-atom rule" has received confirmation from the work Stephenson,[18] van Rossum and Ariens[19] who showed maximum activity for n=4 in $CH_3(CH_2)_nN(CH_3)_3^+$.

A series of compounds examined by Ing, Kordik and Tudor-Williams[20] had methylene groups replaced either by an oxygen atom or a carbonyl group at various loci in the chain. These analogues were found to be more potent than the alkyl compounds: maximal activity was observed with a divalent oxygen in position 3 of the chain. Moreover, activity was increased when the oxygen was incorporated into a furan ring **4**. It is worth noting that the 2-methyl group of the furan ring was found to be necesary for high activity, in accordance with the five-atom rule. It perhaps increases the electron density on the ether oxygen or leads to an increase in van der Waals binding interactions. Other potent muscarinic agonists are muscarine **5** and the 1,3-dioxolane **6**, shown here as their iodide salts.

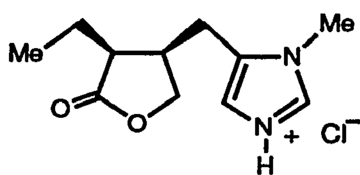


Based on those studies, it was concluded that a trimethylammonium group or other such group and a divalent oxygen are present in most active muscarinic agonists.

ii. Nonclassical muscarinic agonists lacking the NCCOCC chain:

One example is the partial agonist pilocarpine (**7**), an alkaloid obtained from the South American plant *genus pilocarpus* which is a peripheral stimulant of the parasympathetic system, having diaphoretic, miotic and CNS effects.[21] It possesses many characteristic actions of acetylcholine and its action is antagonized by atropine (**8**). Pilocarpine is currently used clinically to treat glaucoma.[21,22] In a recent study by Schulman et al. on the active conformation of pilocarpine,[23] the structure of pilocarpine was fitted to that of muscarine active conformer obtained in a previous theoretical study.[24] A computer search showed no low-energy conformers of pilocarpine which juxtapose the lactone or carbonyl oxygen with the ether oxygen of muscarine, while simultaneously superimposing nitrogens and terminal methyls of the two molecules. Although the muscarine ether-oxygen is important

for high efficacy, it is not a stringent requirement since weaker muscarinic agonists lacking this oxygen are known. Low energy conformers of pilocarpine are found when the carbonyl oxygen of the lactone ring is juxtaposed with the hydroxyl oxygen of muscarine. The NCC-CC spacing is maintained in part by the *cis* fusion of the two chiral groups in pilocarpine. The *cis* configuration (R,S) of the two chiral groups is essential for activity since its *trans* epimer (R,R), isopilocarpine is less active as a miotic.[25] The carbons of the ethyl group presumably play the role of atoms four and five of the "five-atom rule", as shown in the hypothetical agonist (**9**).



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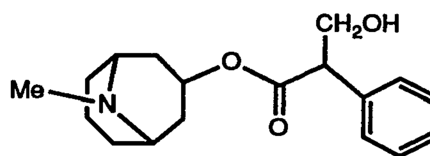

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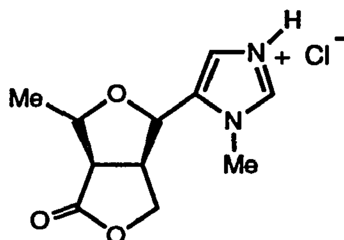

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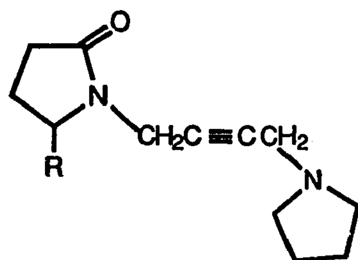
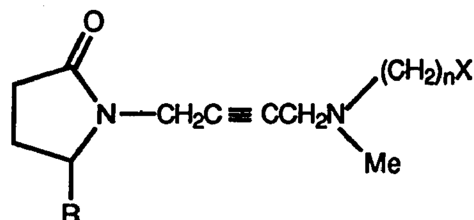
Oxotremorine (**10**) also has a structure quite different from that of acetylcholine and is equipotent with it. Cho et al.[26] suggested that its activity might be explained on the basis of an "induced fit" hypothesis; an alternative explanation was proposed by Bebbington, Brimblecombe and Shakeshaft[27] who noted that oxotremorine can exist in two possible planar forms, *cisoid* or *transoid*. They argued that if the groups involved in the interaction between drug and receptor were the pyrrolidine nitrogen, the acetylenic bond and the carbonyl group, these would be well placed in the *transoid* form to interact with the receptor. It was further suggested that the acetylenic bond, a region of high electron density, is capable of interaction with the same receptor site as the ether-oxygens of acetylcholine and muscarine.

Recent work by Ringdal[28] and Lambrecht[29] on oxotremorine analogues should be discussed briefly. In Ringdal's work, a series of tertiary 3- and 4-haloalkylamines (**11**) related to oxotremorine were synthesized and tested for activity. It was shown that central potency was critically dependent on the rate of *in vivo* cyclization of the parent haloalkylamines to the corresponding aziridinium ions (n=2), azetidinium ions (n=3), and pyrrolidinium ions (n=4). For example, the rapidly cyclizing 2-bromoethylamine was shown to be considerably more potent than its (2-chloroethyl)amino analogue in producing tremor and analgesia. However, these agonists effects generally were quite short lasting and were replaced by long-lasting anti-muscarinic effects due to alkylation of muscarinic receptors by the reactive

aziridinium ion.

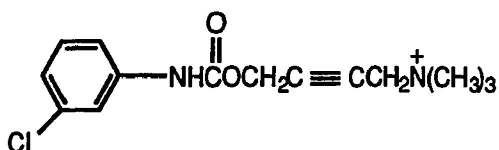


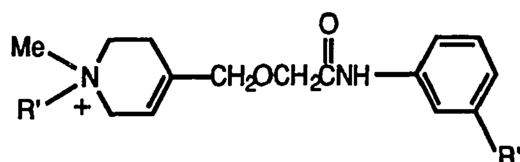


10

11

Lambrecht reported a series of tertiary and quaternary analogues of McN-A-343 (**12**), a somewhat selective M_1 agonist, which were synthesized and tested. It was shown that some activity was retained when the flexibility of the cationic head group was reduced by its incorporation into a ring system (**13**). However, no activity was observed when the (*E*) double bond was reduced. This suggests that the acetylenic group or at least some double bond character is involved in the interaction of the drug with the receptor.





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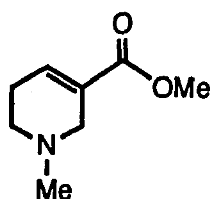
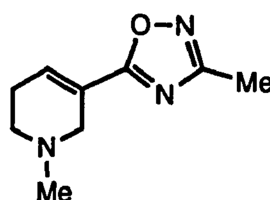
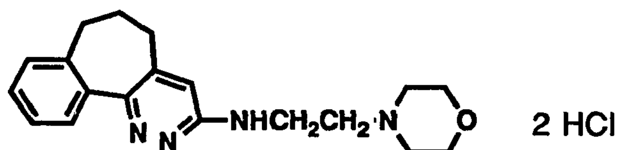
As part of a study aimed at improving the clinical profile of the muscarinic agonist arecoline (**14**), a Merck group[30] replaced the methyl ester functionality by methyloxadiazole (**15**). This replacement is claimed to have led to the discovery of the most potent muscarinic agonist, although no selectivity data for the compound has been reported.





14 **15**

Finally, Michaud et al.[31] synthesized SR 95639A (**16**), which is claimed to be a selective M_1 agonist at central muscarinic receptors.



16

IV. A Model of the muscarinic binding site: In 1983, Schulman et al.[24] made detailed conformational analyses of known muscarinic agonists to obtain both the muscarinic pharmacophore and the biogically active conformation of each agonist. It was based on the following assumptions:

(1) All potent agonists have a cationic head group usually, a trimethylammonium group or a protonated nitrogen head group, which can interact directly with an anionic receptor site, such as the carboxylate ion of an aspartate or glutamate. Assuming a specific orientation of the agonist head group to the receptor oxygen, one possibility is that the receptor oxygen lies on the three fold axis of the trimethylammonium group (or is nearly colinear with the NH bond in the case of protonated head group so as to provide a linear N-H---O hydrogen bond). In Figure **2**, P represents the anionic site of the receptor.

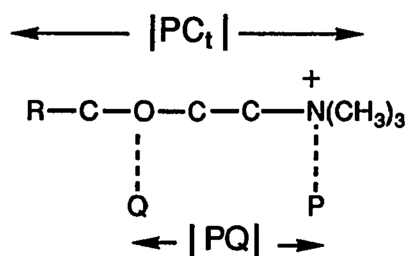


Fig. 2

(2) A region of negative electrostatic potential proximal to the ester or ether oxygen constitutes a second binding site. It interacts with a positive receptor residue or a hydrogen bond at point Q.

(3) Since agonist binding is of high affinity and stereoselectivity, it is reasonable to assume that the distance lPQl is a nearly invariant property of the receptor in its active conformation. A reasonable assumption is that lPQl varies by no more than 0.3 A over a set of agonists.

(4) In order to account for the relative orientation of the drug to the receptor, an "interaction dihedral angle" PNOQ should also remain nearly invariant over a set of similar agonists. This angle results from superimposing O->Q on a stationary N->P, and viewing along the non-bonded O->N direction. The new variables PNOQ and lPQl can be used as alternatives to the dihedral angles t_1 (NCCO) and t_2 (CCOC) which more customarily define the backbone of the drug.

(5) The model assumed that a conformation is accessible if its energy is within 3-4 kcal/mol of the minimum-energy conformer. Therefore, any agonist with a set of PNOQ and lPQl values that lie within this energy range should have considerable potency, assuming that there is no steric inhibition elsewhere. The range of accessible PNOQ values is 100° to 117°, with positive signs according to the Prelog convention.

(6) Finally, the relationship between chain-length and potency was taken into account through the distance PC_t,

where C_t is the terminal methyl group. A typical example of chain-length requirements is found in the furmethide series, where 5-methylfurmethide (PC_t = 8,5 A) is 35 times more potent than furmethide (PC_t=7.1A) itself and 105 times more potent than 5-ethylfurmethide (PC_t=9.7A). These results suggest that for high potency, the distance between the anionic receptor site P and the terminal atom, R, usually a methyl carbon, should be similar to that of 5-methylfurmethide. High potencies are also observed when R=Cl, Br , I in 5-R furmethide. When the terminal atom R is farther from P than 9.7 A, partial agonism or antagonism is usually observed. Conversely, when the terminal atom is much closer to P than 8.5 A, a full agonist of low potency is found.

V) Pharmacological attributes for muscarinic agonist activity:

(1) We desire compounds that exhibit full or partial agonist activity. A full agonist provides 100% response; a partial agonist does not reach the 100% response even at large dosages.

(2) Selective M_1 agonists could help alleviate symptoms of Alzheimer's desease while having little effect on muscarinic receptors in cardiac and smooth muscles which are largely of the M_2 type. The desired agonists should be highly selective toward the M_1 receptors in order to prevent side effects.

(3) The agonists must be able to penetrate the blood-brain barrier in order to reach the receptors. Tertiary amines are reasonable candidates since they can be protonated and deprotonated at physiological pH and can cross the blood-barrier in their neutral forms; charged organic species usually do not enter the brain.

(4) Since quaternary ammonium salts are unable to penetrate the blood-brain barrier they still could be used, for example pharmacologically as gastroprokinetic agents to increase gastrointestinal motility.

(5) An effective drug should not be a substrate for acetylcholine-esterase and should have a lifetime of at least several hours.

VI) Candidate Compounds (Figure 3, Pg 17): Our target compounds had the following general properties:

(1) They were tertiary amines having a basic head group, the terminal methyl or equivalent and oxygen functionalities which are arrayed so as to satisfy the theoretical model. Tertiary amines for Alzheimer's applications and quaternary ammonium salts for gastroprokinetic drugs.

(2) Selectivity for the M_1 receptors will be obtained by use of an imidazole ring or equivalent. The imidazole ring also provides two equivalent electrophilic sites for interaction with the receptor.

(3) The compounds contained no hydrolyzable or other reactive groups.

(4) The number of asymmetric centers was minimized as to entail as few optical and geometrical isomers as possible.

(5) All compounds contained the NCCOCC backbone, so as to satisfy the 5-atom rule. The nitrogen atoms of the imidazolium rings can interact with a carboxylate of an aspartate group of the receptor while the oxygen atom can form hydrogen bonding with a serine group; it is believed that both amino acid residues are located in transmembrane domain III of the receptor and are conserved in all 5 subtypes.

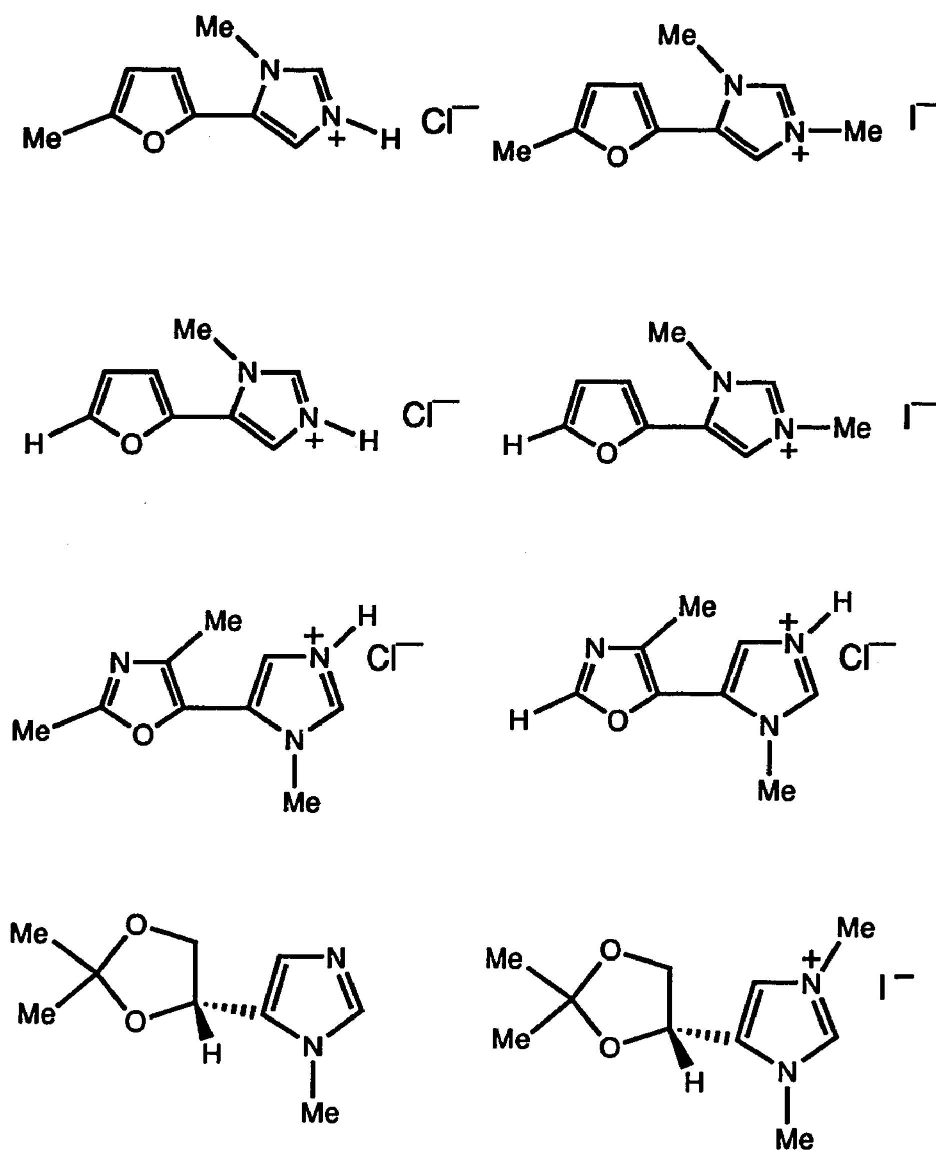


Figure 3

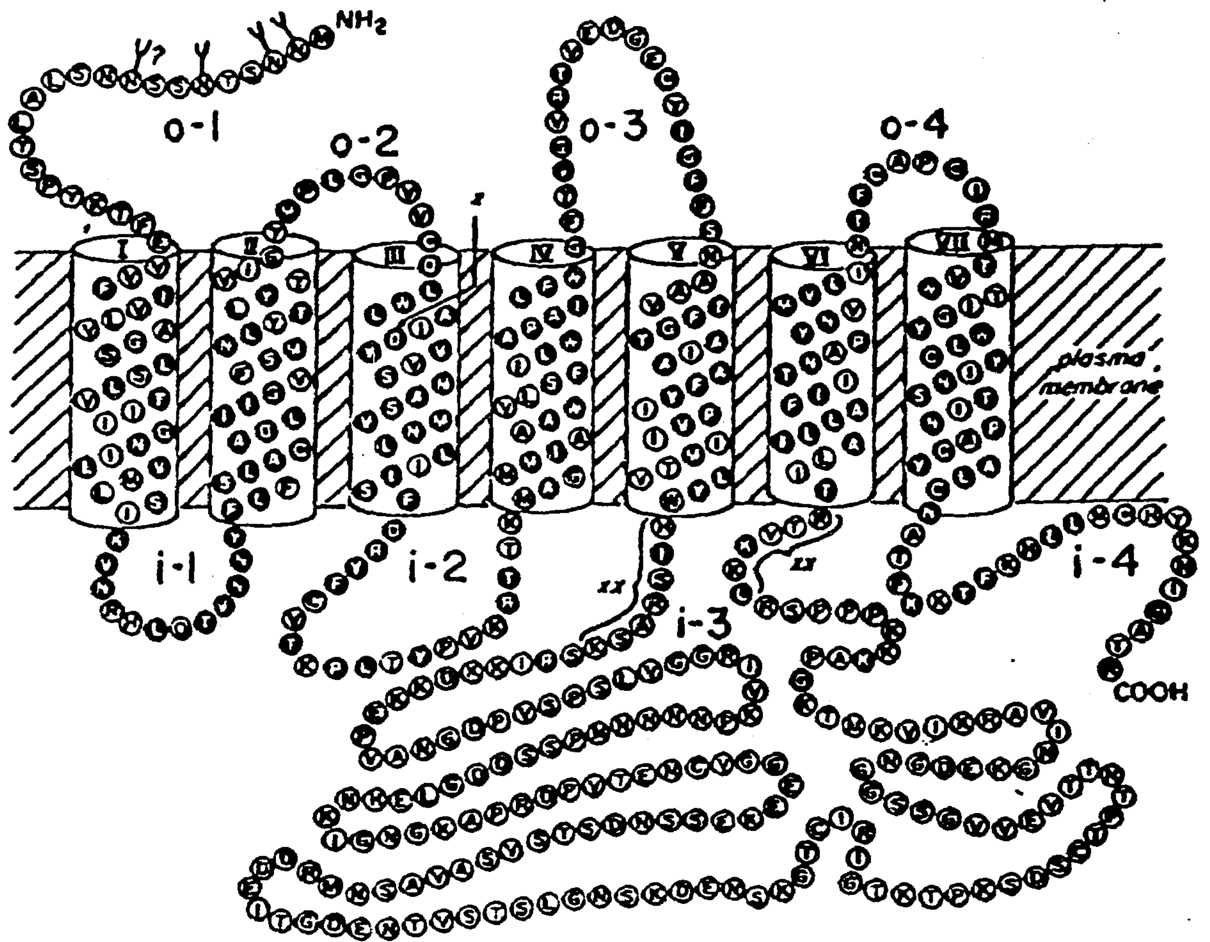


Fig. 1

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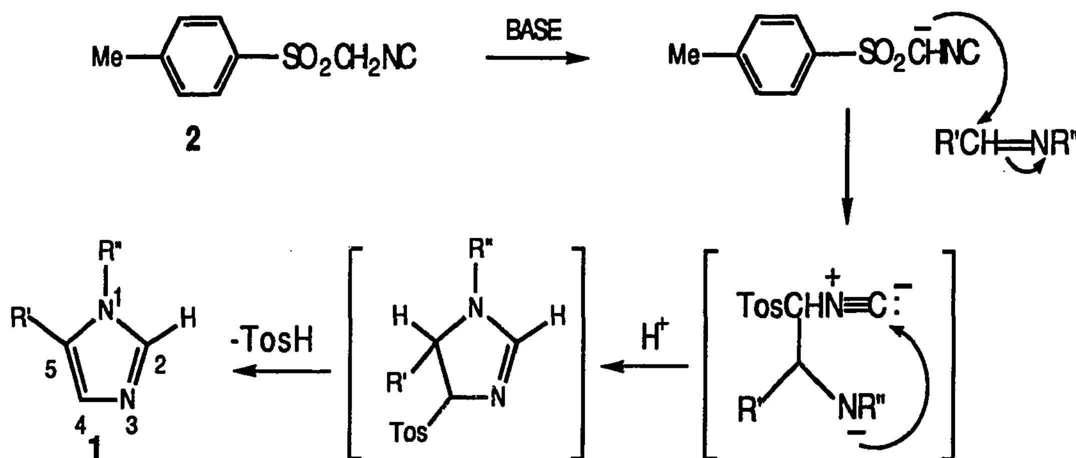
Chapter 2

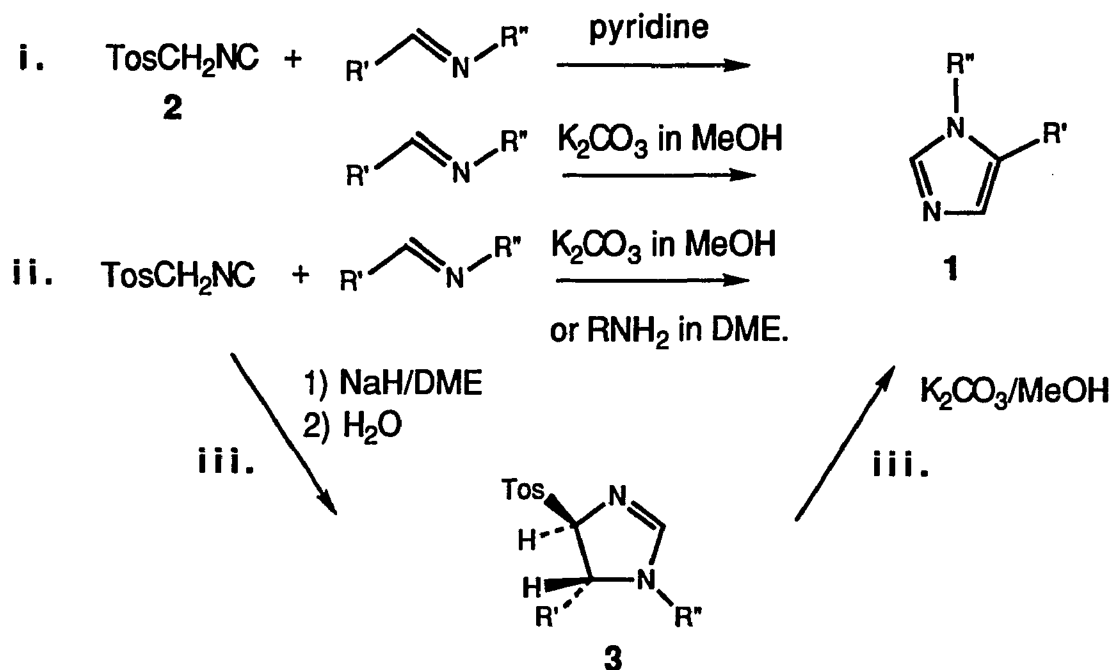
Furanylimidazoles: Syntheses, Mechanisms, pH measurements.

Introduction: Substituted imidazoles can be prepared by several synthetic methods.[1] Few, however, offer a straightforward route to 1,5-disubstituted imidazoles **1** since, for one thing, the 2-position is susceptible to alkylation. One successful synthetic route to **1** is the dipolar cycloaddition of tosylmethylisocyanide (TosMIC) **2** to an aldimine in basic solution.[2] The $^{-}$CH-N=C moiety of TosMIC adds to the polarized imine double bond[3] as shown in Scheme 1:



Scheme 1

The TosMIC route to imidazole **1** has been implemented by van Leusen et al.[2] in three different ways (Scheme 2): (i) TosMIC and the aldimine were dissolved in pyridine; (ii) TosMIC and the aldimine were allowed to react in the presence of potassium carbonate in methanol or RNH_2 in dimethoxyethane; (iii) TosMIC and the aldimine were first treated with NaH in dimethoxyethane, then with potassium carbonate in methanol. In each case the reaction was run at room temperature and monitored by TLC.

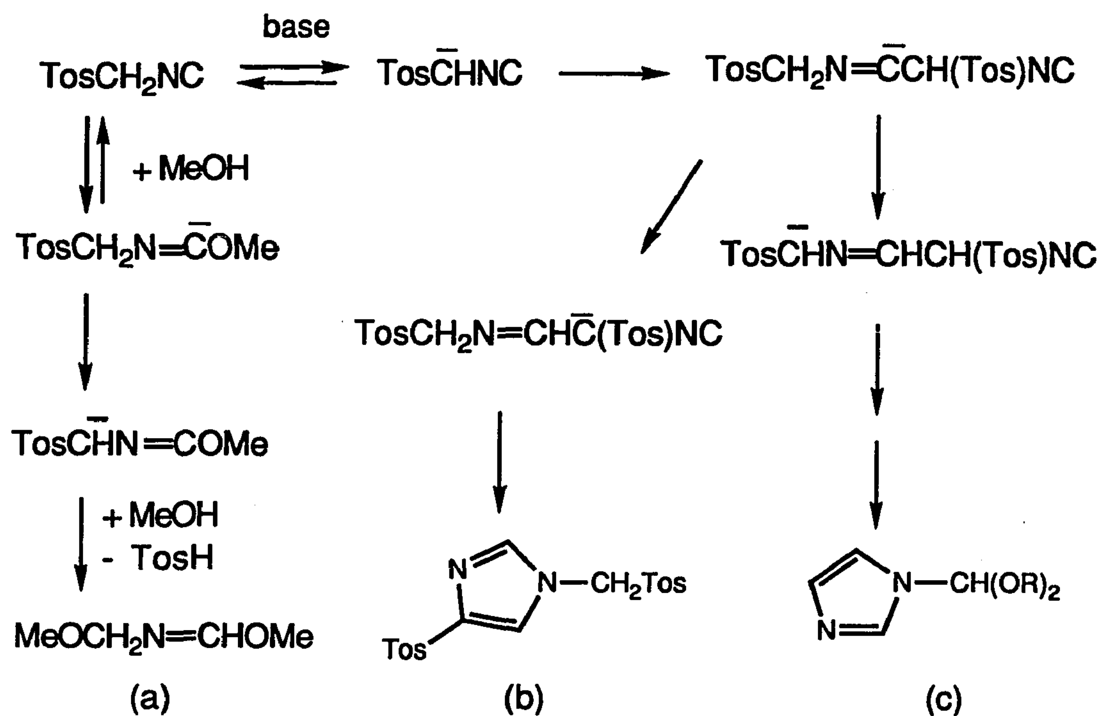


Scheme 2

Regardless of which method is used, the formation of the disubstituted imidazole occurs via deprotonation of the

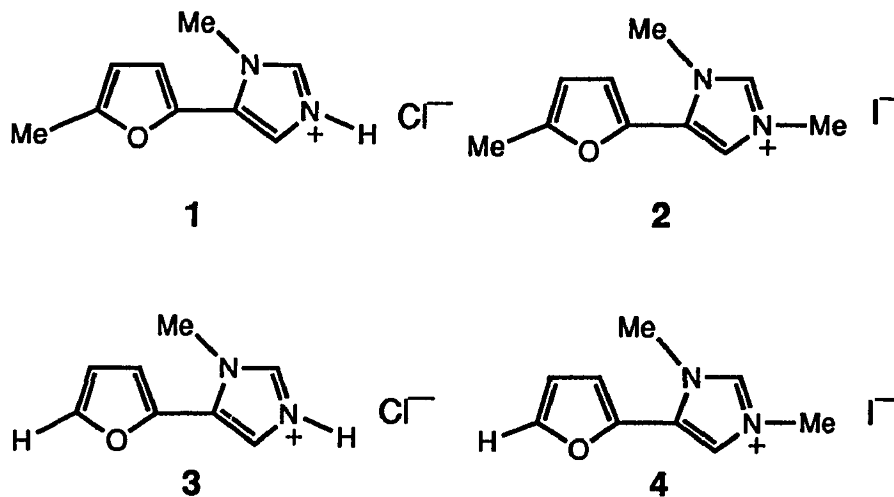
corresponding 2-imidazoline **3**. The acidity of the hydrogen on C5 depends on the nature of R'; for example, electron withdrawing groups R' allow facile elimination of the adjacent tosyl group. Finally, the yield of **1** also depends upon the stability of the aldimine since it is susceptible to hydrolysis. The yields of 1,5-disubstituted imidazoles **1** are greatly influenced by the relative rates of cycloaddition and decomposition of TosMIC itself into the side products shown in Scheme 3. Reactions carried out with potassium carbonate in methanol give all three side products (**a**, **b**, **c**).[3] With the pyridine route, **b** is the major by-product since the formation of **a** and **c** require alcohols.

Because cycloaddition is slower than formation of the side products, higher yields of **1** are obtained under condition where TosMIC is long-lived. The half-life of TosMIC (7 mM in MeOH-DME, 2:1) is 40 minutes when stirred with 2 equivalents of K_2CO_3 at room temperature in the absence of the aldimine; its half-life in dimethoxyethane with 1 equivalent of NaH is 15 minutes. On the other hand, TosMIC is stable at room temperature for at least 65 hours in methanol or dimethoxyethane with 0.5-20 equivalent of $t\text{-BuNH}_2$ and we have found it to be stable in pyridine for several days at room temperature. Of course, at higher temperatures the half-life of TosMIC decreases considerably in any basic medium. These considerations led us to run our reactions at room temperature with the reaction followed by TLC.

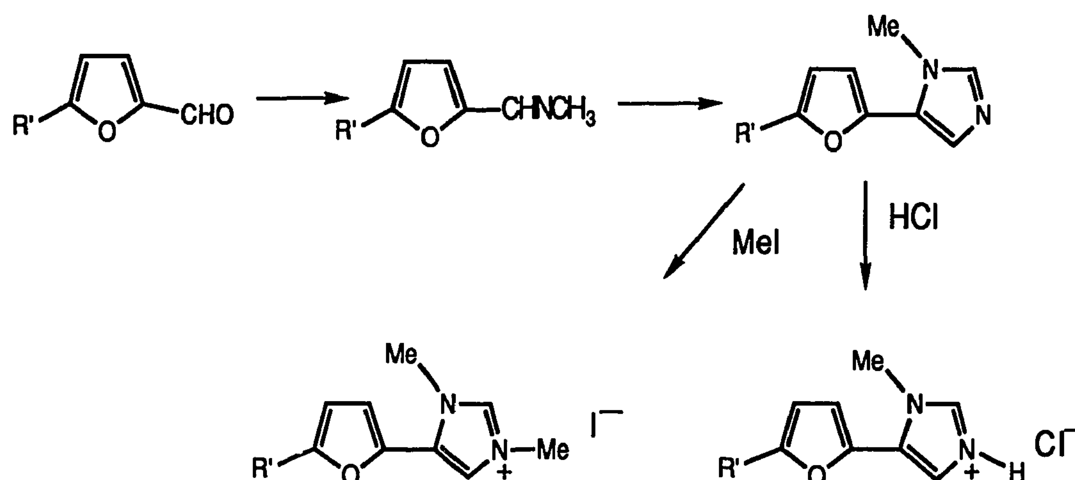


Scheme 3

Discussion and results: The first series of compounds synthesized were the 5-furanylimidazole salts **1-4** according to Scheme 4.



The aldimine precursors of the four cationic compounds were obtained by treating their corresponding aldehydes with 40% aqueous methylamine. The aldimines were isolated as liquids at room temperature and their structures were confirmed by IR and NMR spectroscopy. The aldimines were allowed to react with TosMIC in pyridine which served as both the base and solvent (route (i)). Both imidazole bases were obtained in low yields (26-38%), significant amounts of product being lost during work-up and purification. The structures of the bases were confirmed by NMR spectroscopy. The bases were converted to their hydrochloride salts by bubbling HCl gas into their methylene chloride solutions; the iodide salts were formed by treating the bases with methyl iodide in acetonitrile. Structures of the salts were confirmed by elemental analyses, NMR and mass spectroscopy.



Scheme 4

pH Measurements: Aqueous 0.50 N solutions of **1** and **3** were titrated with a 0.10 N solution of sodium hydroxyde. The pH measurements of the hydrochloride solutions were made after the addition of 0.50 ml of the base using a pH meter calibrated with buffers of pH 4 and 7 at 25°C. Both compounds came out of solution as oils at a pH value of ca. 6. Due to the low solubility of the bases in water, the pKa values could not be measured, even in 30% ethanol. Nonetheless, one expects these compounds (**1** and **3**) to be less basic than imidazole (pKa =7.4) and pilocarpine (pKa =7.2) due to the withdrawing effects of the furan ring.

Experimental: Reagents were purchased from Aldrich Chemical Co. unless otherwise noted and all starting materials were distilled before use. Silica gel (230-400 mesh) and neutral Alumina (Brockman activity I, 80-200 mesh) were used for flash chromatography. All NMR spectra were recorded at 60 MHz on a EM360 Varian or at 200 MHz on a IBM-Bruker WP200SY spectrometer. Solvents were used without further distillation except for methanol which was distilled over magnesium metal. Melting points are uncorrected. A Perkin-Elmer 1600 Series FT-IR was used to record IR spectra. Elemental analyses were carried out by Galbraith Laboratories and mass spectra were recorded by Dr. David Locke at Queens College on a Hewlett-Packard 5988 A GC/ quadruple MS with H-P 1000 data system.

I. Synthesis of 1 and 2

a. Aldimine (General Procedure, Ref. 4): 59.6 g of 40% aqueous methylamine were placed in a 125 mL erlenmeyer flask containing a magnetic bar. This solution was submerged in an ice bath and to it were added 55.3 g (0.50 mole) of 5-methylfurfural over 30 minutes, keeping the temperature between 15 and 20°C. The ice bath was removed after an additional 5 minutes of cooling and the reaction mixture stoppered and stirred at room temperature for 1/2 hour. Over a few minutes, 45 g of KOH pellets were added with cooling and stirring, keeping the temperature below 15°C. Two layers developed, a lower aqueous layer and an upper amber organic layer. The aqueous layer was

extracted with methylene chloride; all organic extracts were combined and dried over KOH pellets. The solution was filtered, evaporated and distilled under reduced pressure; the fraction boiling at 82-83°C/16-17 mm Hg afforded 46.4 g of the imine (76%). ^1H NMR ($CDCl_3$, 60 MHz) δ1.9 (s, 3H, CH_3C=), 3.0 (s, 3H, CH_3N=), 5.7 (d, 1H, =CH), 6.3 (d, 1H, =CH), 7.6 (s, 1H, HC=N).

b. Furanylimidazole base (General procedure, Ref. 2): A solution containing 11.18 g (60 mmol) of TosMIC, 8.20 g of the aldimine (70 mmole) and 70 mL of pyridine was stirred at room temperature for 12 days. The mixture was concentrated, dissolved in methylene chloride, and extracted with 10% HCl solution; the combined aqueous extracts was made basic with KOH pellets to pH 8. The solution was extracted 3 times with methylene chloride and the combined extracts were dried over $MgSO_4$. After filtration, the solution was concentrated to furnish a crude residue of the base contaminated with traces of pyridine. The base crystallized after the pyridine was removed; m.p. 30-33°C, yield 2.54 g (26%), Rf 0.5 in ethylacetate. The crude base was chromatographed on silica gel (230-400 mesh) with ethyl acetate. 12 fractions were collected (50 ml each). Fractions 7-12 were combined and concentrated to yield 1.60 g (16%) of clear yellow crystals, m.p. 33-35°C. ^1H NMR ($CDCl_3$, 60 MHz) δ2.2 (s, 3H, =CCH_3), 3.7 (s, 3H, NCH_3), 6.0 (d, 1H, =CH), 6.2 (d, 1H, =CH), 7.1(s, 1H, =CH), 7.3 (s, 1H, =CH).

c. Furanylimidazole hydrochloride salt 1. HCl gas was bubbled slowly into a solution of 0.75 g of the chromatographed base (5 mmole) in 20 mL of methylene chloride for 5 minutes. The solution was concentrated and the residue recrystallized from *n*-butanol to afford 0.50 g of **1** (60%), which decomposed at 235-237°C. ^{1}H NMR (D_2O, 60 MHz) δ 2.2 (s, 3H, CH_3C=), 3.8 (s, 3H, NCH_3), 6.1 (d, 1H, =CH), 6.6 (d, 1H, =CH), 7.5 (s, 1H, =CH), 8.6 (s, 1H, N=CH), **Fig. 1.** The molecular ion obtained from mass spectroscopy had a mass of 162, as expected for the free base with loss of HCl.

Elemental Analysis:

Calculated: C 54.41; H 5.58; N 14.10; Cl 17.85.

Found: C 53.98; H 5.57; N 14.17; Cl 17.83.

d. Furanylimidazole methyl iodide salt 2: To a solution containing 0.80 g of the chromatographed base (5 mmole) and 12 mL of acetonitrile, 2.20 g of methyl iodide (7 mmole) were added. The solution was stirred at room temperature for about 1/2 hour, concentrated and the residue recrystallized from 50 mL of *t*-butyl alcohol to afford 0.75 g of **2** (50%). The crystals were dried in a pistol dryer, m.p.174-177°C. ^{1}H NMR ($CDCl_3$, 60 MHz) δ2.4 (s, 3H, =CCH_3), 4.1 (s, 6H, =NCH_3), 6.1 (d, 1H, =CH), 6.7 (d, 1H, =CH), 7.7 (s, 1H, =CH), 10.1 (s, 1H, N=CH), **Fig. 2.**

Elemental analysis:

Calculated: C 39.49, H 4.31, N 9.21, I 41.70

Found: C 39.33, H 4.34, N 9.10, I 41.67

II. Synthesis of 3 and 4

a. Aldimine: The procedure was the same as described in **Ia** using the following reagents: 17.4 g of furfural (0.18 mole) and 20 ml of 40% aqueous methylamine solution. The reaction afforded 14.1 g of the aldimine (72 %), b.p 70-76°C/15-20 mm Hg. ^1H NMR ($CDCl_3$, 60 MHz) δ2.8 (s, 3H, NCH_3), 5.8 (m, 1H, =CH), 6.2 (d, 1H, =CH), 7.0 (s, 1H, N=CH), 7.4 (d, 1H, OCH).

b. Furanylimidazole base: A solution containing 6.72 g of the preformed aldimine (60 mmole), 10 grams of TosMIC (50 mmole) and 75 mL of pyridine was stirred at room temperature for 12 days. The work up was the same as described in **Ib**. The reaction afforded 3.5 g of crude base (38%). The base (3.1 g) was chromatographed on silica gel (230-400 mesh) with ethyl acetate. A total of 20 fractions (50 ml each) were collected. Fractions 4 to 12 were combined and concentrated to yield 1.65 g of pure base. ^1H NMR ($CDCl_3$, 60 MHz) δ3.7 (s, 3H, NCH_3), 6.4 (s, 2H), 7.2 (s, 1H), 7.4 (s, 2H).

c. Furanylimidazole hydrochloride salt 3: HCl gas was bubbled slowly into a solution containing 1.05 g of chromatographed base (7 mmole) in 20 mL of methylene chloride for 5 minutes. The reaction mixture was concentrated and the residue recrystallized 3 times from *n*-butanol to yield 0.47 gram of **3** (35%), m.p. 228-229°C. ^1H NMR (D_2O, 60 MHz) δ3.9 (s, 3H, NCH_3), 6.6 (m, 1H, =CH), 6.8 (d, 1H, =CH), 7.6 (s, 2H), 8.6 (s, 1H, N=CH), **Fig. 3**.

Elemental analysis:

Calulated: C 52.04, H 4.91, N 15.17, Cl 19.20

Found: C 52.30, H 5.00, N 15.15, Cl 18.53

d. Furanylimidazole methyl iodide salt 4: To a solution containing 0.60 g of the pure base (4 mmole) in 20 mL of acetonitrile was added 0.90 g (6 mmole) of methyl iodide. The reaction mixture was stirred at room temperature for 30 minutes, concentrated and the residue recrystallized from a mixture of *t*-butanol/*n*-butanol (5:1) to yield 0.5 g of **4**, m.p. 152-153°C. ^1H NMR (D_2O, 60 MHz) δ3.9 (2s, 6H, NCH_3), 6.6 (m, 1H), 6.9 (d, 1H), 7.7 (m, 2H), **Fig. 4**. The molecular ion obtained from mass spectroscopy had a mass of 148, as expected for the free base with loss of MeI.

Elemental Analysis:

Calculated: C 37.26; H 3.82; N 9.66; I 43.74.

Found: C 37.25; H 3.94; N 9.58; I 44.73.

Spectrum Ampl 100/4

Filter 1 sec

RF Power .05 mG

Sweep Time 5 min.

Sweep Width 10 ppm or Hz

End of Sweep 0 ppm or Hz

Solvent D_2O

Fig. 1

Spectrum Ampl 100/8

Filter .1 sec

RF Power .05 mG

Sweep Time 5 min.

Sweep Width 10 ppm or Hz

End of Sweep 0 ppm or Hz

Solvent CDCl3

Fig. 2

34

Spectrum Ampl. 100/5

Filter 1 sec

RF Power .05 mG

Sweep Time 5 min

Sweep Width 10 ppm or Hz

End of Sweep 0 ppm or Hz

Solvent D_2O

Fig 3

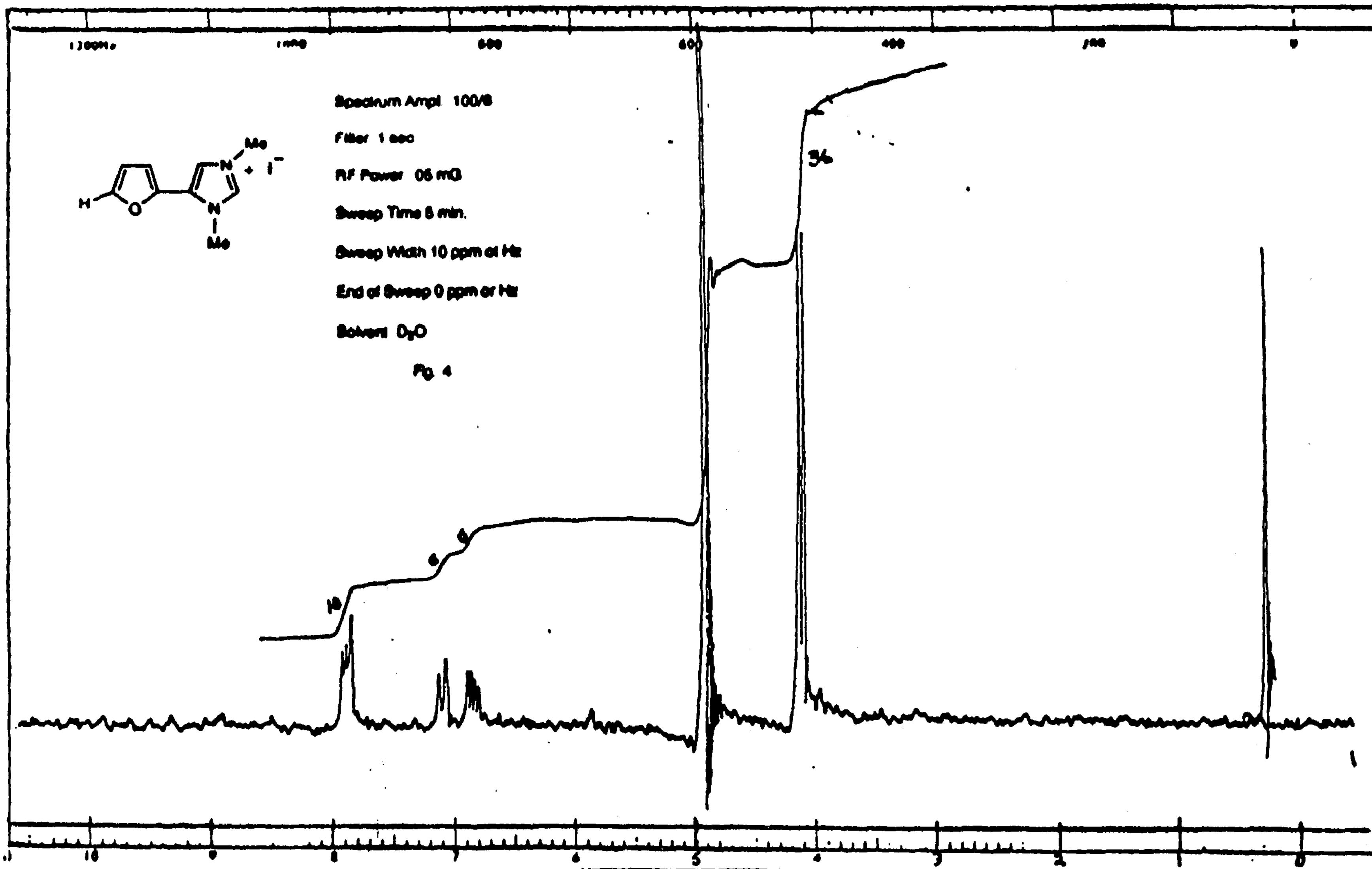


Fig. 4

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Chapter 3

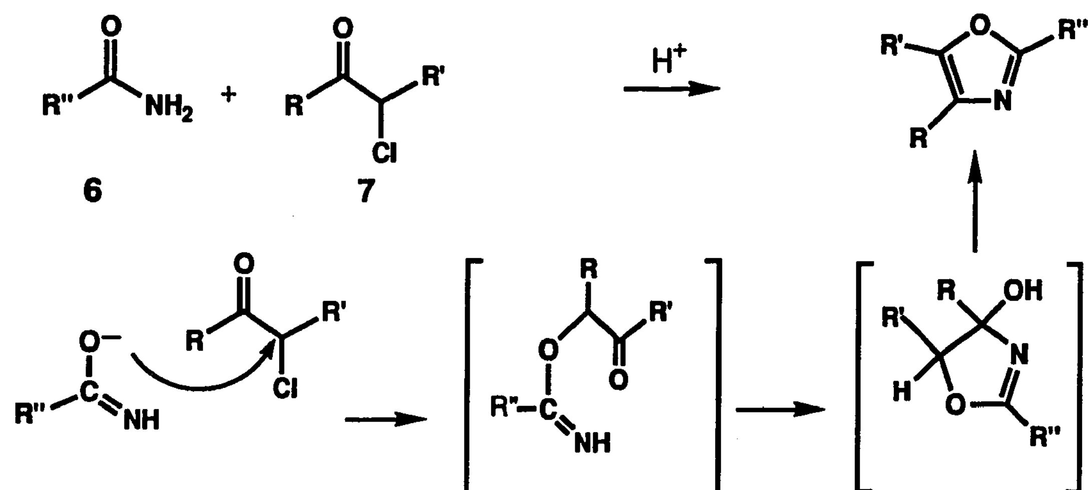
Oxazolylimidazoles: Syntheses, Mechanisms, pH measurements.

Introduction: There are several synthetic routes to 4,5-disubstituted oxazoles **1**. One convenient method[1] is treatment of α-metalated alkyl isocyanides **3** (prepared from isocyanides **2**) with acylating agents such as acyl chlorides, carboxylic esters, and N,N-disubstituted amides (Scheme 1). Intermediates in this synthesis are the 2-oxoalkyl isocyanides **4** which cyclize during work up by way of their enolic forms **5**.

$$RCH_2NC \ (\mathbf{2}) \xrightarrow{BuLi} R\bar{C}HNC \ (\mathbf{3}) \xrightarrow{R'COX} \mathbf{4} \rightarrow \mathbf{5} \rightarrow \mathbf{1}$$

Scheme 1

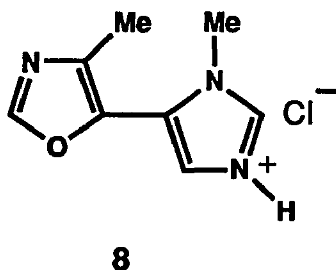
A second route[2] is the treatment of alkylamides **6** with α-chlorocarbonyl compounds **7** in acidic medium (Scheme 2).

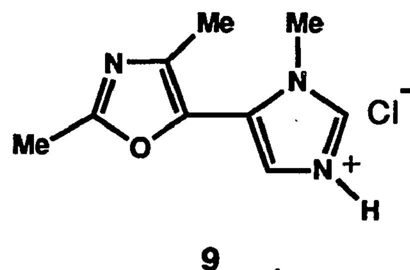


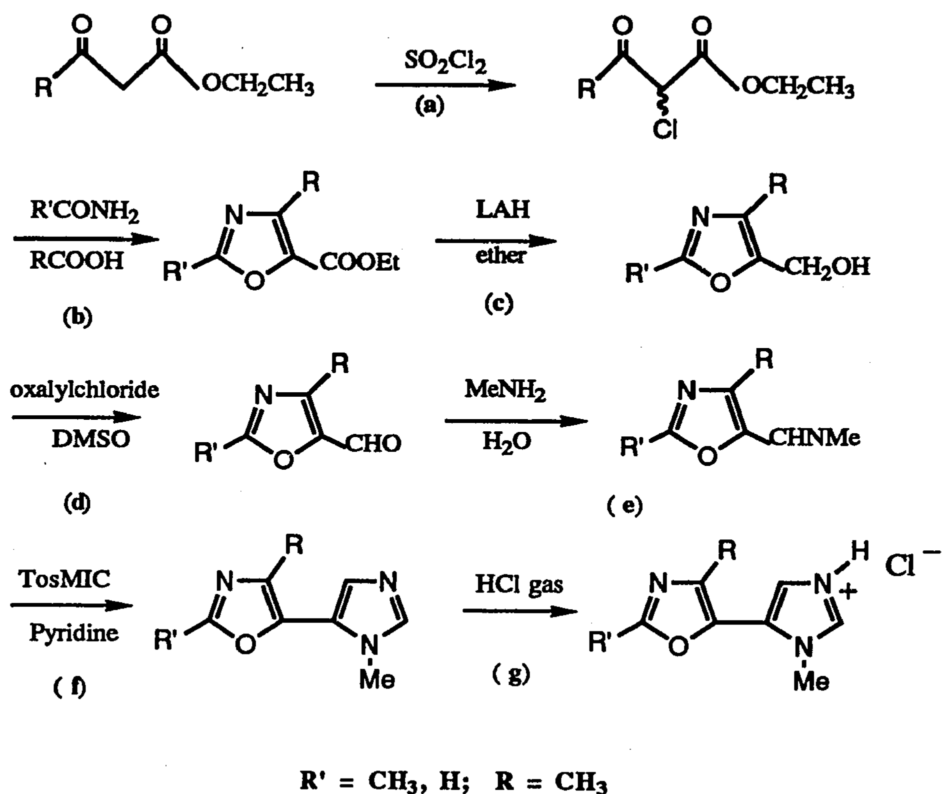
Scheme 2

Discussion and results: The strategy employed in our laboratory for synthesizing compounds **8** and **9** (Scheme 3) was to form the oxazole moiety according to Scheme 2 and then build on the imidazole ring via the TosMIC route. The oxazolylimidazolium salts described in this chapter were synthesized first by treating the appropriate amides with ethylchloroacetoacetate to form the corresponding esters in 25 to 26% yield (Scheme 3). The esters were then reduced with LAH in anhydrous ether at -10°C to the corresponding alcohols in 60 to 80% yield. At lower temperatures, the esters precipitated out of solution leading to much lower yields of alcohols. The alcohols were then oxidized to the corresponding aldehydes by the Swern method[3] (DMSO/oxalylchloride) in 50 to 65% yield. Attempts at oxidizing the alcohols by other methods such as the Moffat oxidation, pyridinium dichromate, MnO_2, or nitric acid resulted either in a

very low yield of aldehyde or recovery of starting materials. The aldehydes were then treated with 40% aqueous methylamine to obtain the aldimines in 38 to 49% yield. The reaction mixture was extracted 5 minutes after the addition of the C2 unsubstituted oxazole aldehyde to the aqueous methylamine solution. With longer reaction times, the yield dropped considerably. It is believed that C2 of the oxazole ring is the site for nucleophilic attack; prolonged exposure to methylamine leads to structure **10** according to Scheme 4. While structure **10** was not confirmed in our laboratory, the observation that methyl substitution at C2, which would prevent such attack, furnished a yield independent of reaction time, suggests that compund **10** is indeed the side product. The aldimines were then treated with TosMIC/pyridine and the oxazolylimidazole bases were obtained in 3 to 20% yield. All structures were confirmed by NMR spectroscopy and some by IR and elemental analysis. HCl gas was bubbled through methylene chloride solutions of the bases to furnish the corresponding hydrochloride salts. Structures of the salts were confirmed by NMR, mass spectroscopy and by elemental analysis.

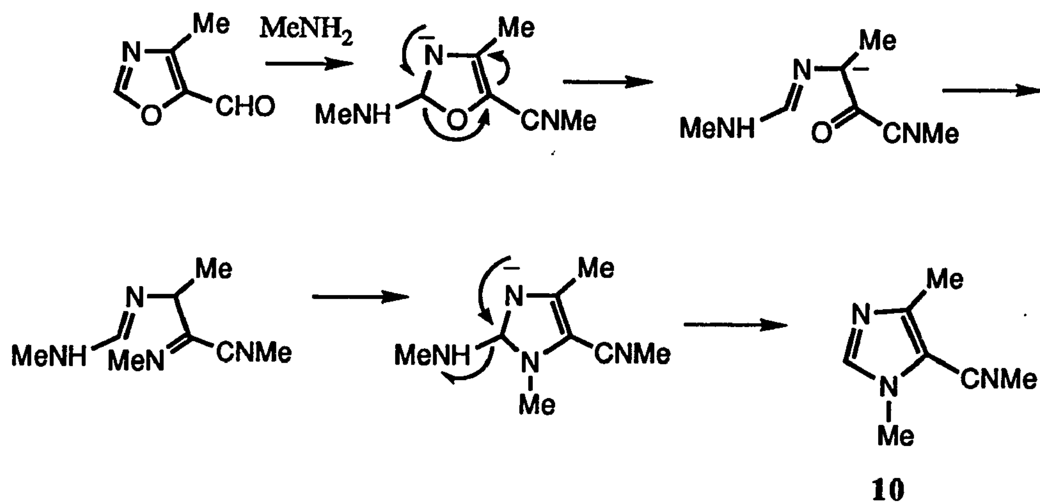






R' = CH_3, H; R = CH_3

Scheme 3



Scheme 4

pH measurements: In order to measure the pH of **9**, 300 mg of the compound were dissolved in 4.7 g of distilled water and the resulting solution titrated with a 0.10 N aqueous sodium hydroxide solution. The pH measurements of the hydrochloride salt were made after the addition of 0.50 ml of the base with a pH meter calibrated with buffers of pH 4 and 7 at 25°C. The pH values were plotted against volume of NaOH (Figure 1) and a pKa value of ca. 5.2 was obtained from the inflection point.

In an alternate method, a 0.45 M solution of the hydrochloride salt was titrated with a 0.10N sodium hydroxide solution. The NMR spectra of the salt were made after the addition of 0.50 ml of the base. The chemical shifts of the most acidic hydrogen C2 of the imidazole ring were plotted against the pH measurements (Figure 2), leading to a pKa value of ca. 5.0.

Experimental

I. Synthesis of 8

a. Ethyl chloroacetoacetate (Procedure was taken from reference 4): A 500 mL 3-necked flask, fitted with a dropping funnel and mechanical stirrer, was connected to a gas-absorption trap. 130 g (1 mole) of ethyl acetoacetate were added to the flask. 135 g of sulfuryl chloride (1 mole) were then added dropwise with external cooling (ice-bath) for 2 hours. The solution was allowed to stand overnight and remaining SO_2 and HCl were removed by evaporation. The solution was then distilled with a Vigreux column at a pressure of 25 mm Hg and the fraction boiling at 95-100°C collected to afford 95 g of ethylchloroacetoacetate (64%); literature values: b.p. 85-89°C/17 mm Hg, 93-97%. ^1H NMR ($CDCl_3$, 60 MHz) δ1.1-1.5 (t, 3H, OCH_2**CH_3**), 2.4 (s, 3H, **CH_3**CO), 4.1-4.5 (q, 2H, O**CH_2**CH_3), 4.8 (s, 1H, C**H**Cl).

b. Oxazolylester (Procedure was taken from reference 2): A mixture of 66.1 g (0.40 mol) of ethyl chloroacetoacetate, 36.3 g of formamide (0.80 mole) and 110 g of 88% aqueous formic acid was refluxed at 140-145°C for 6 hours. The residual dark solution was allowed to cool to room temperature, then submerged in an ice bath and made alkaline with 6N sodium hydroxide. The mixture was extracted with ether and the extracts combined and dried over sodium sulfate. After filtration, the ether was removed and the remaining black residue (30 g) distilled under vacuum at 75°C/4-5 mm Hg to give 18.8 g of a

colorless liquid. The distillate was shaken with 25 ml of ice cold half-concentrated sulfuric acid. Two layers were formed, the upper one unreacted ethylchloroacetoacetate and the lower sulfuric acid containing the oxazole. The sulfuric layer was diluted with cold water and made alkaline with 6N KOH. The solution was then extracted with ether and the combined ether extracts were dried over magnesium sulfate. The solvent was removed and the residue was distilled at 60-62°C/1-2 mm Hg to afford 15 g of the ester (25%). Literature values: b.p 98°C/13 mm Hg, 35%. ^{1}H NMR ($CDCl_3$, 60 MHz) δ1.3-1.6 (t, 3H, $OCH_2C\mathbf{H}_3$), 2.6 (s, 3H, $=CC\mathbf{H}_3$), 4.3-4.7 (q, 2H, $OC\mathbf{H}_2$), 8.0 (s, 1H, N=C**H**). IR (CCl_4): 1720 (C=O), 1610, 1490, 1450 cm^{-1}.

c. **Oxazolylalcohol** (Procedure was taken from reference 2): To about 50 ml of dry ether at -10°C under nitrogen were added simultaneously, with mechanical stirring, a solution of 11.5 g (74 mmole) of the ester in 15 mL of anhydrous ether and a solution of 2.66 g (70 mmole) of LAH in 56 mL of anhydrous ether. After 2.5 hours (including one hour for initial dropwise addition), 11 mL of ethyl acetate were added slowly and the solution was allowed to warm to room temperature. Excess LAH was destroyed with 95% ethanol. The reaction mixture was hydrolyzed with 19 g of tartaric acid in water and then made alkaline with 6N NaOH. The solution was saturated with K_2CO_3 and the two layers were separated. The aqueous layer was extracted with benzene and the benzene solution was dried over $MgSO_4$; the ether layer was also dried over $MgSO_4$. Both layers

were concentrated and the residues combined and distilled; the fraction boiling at 119-120°C/13-14 mm Hg afforded 4.3 g of the alcohol (60%). Literature values: b.p 120°C/13 mm Hg, 63%. ^{1}H NMR ($CDCl_3$, 60 MHz) δ2.2 (s,3H, =C**H**$_3$), 3.1 (s, 1H, O**H**), 4.6 (s, 2H, C**H**$_2$O), 7.8 (s, 1H, N=C**H**). IR (CCl_4): 3600-3200 (OH), 1600, 1490, 1440 cm^{-1}.

d. Oxazolylaldehyde (General procedure, Ref. 3): A solution containing 75 mL of methylene chloride and 3 mL (33 mmol) of oxalyl chloride was added to a 250 mL 3-necked flask equipped with a mechanical stirrer and two pressure-equalizing funnels. One contained a solution of 5.1 mL of dimethylsulfoxide (66 mmol) in 15 mL of methylene chloride; the other contained a solution of 3.4 g (30 mmole) of the alcohol in 30 mL of methylene chloride. The flask was submerged in a dry ice-acetone bath and the dimethyl sulfoxide solution was added over 5 minutes. Immediately thereafter, the alcohol was added over 5 minutes; stirring was continued for an additional 15 minutes. Triethylamine (21 mL, 50 mmol) was then added and the reaction mixture was allowed to warm to room temperature. 150 mL of water was then added and stirred with the mixture. The solution was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with methylene chloride and all organic extracts were combined and washed with: 1) saturated sodium chloride, 2) 5% HCl, 3) water, 4) dilute Na_2CO_3, and 5) water. The organic solution was dried over $MgSO_4$, filtered and concentrated to afford 2.2 g of the

aldehyde (65%). ^1H NMR ($CDCl_3$, 60 MHz) δ2.6 (s, 3H, =C**H**$_3$), 8.2 (s, 1H, N=C**H**), 9.9 (s,1H, C**H**O). IR (CCl_4): 1690 (C=O), 1595, 1490, 1475, 1440 cm^{-1}.

e. Oxazolylaldimine (General procedure, Ref. 5): A solution containing 10 g of the aldehyde (90 mmol) in distilled water was added slowly to excess 40% aqueous methylamine solution with stirring and cooling. After exactly 5 minutes additional stirring, the solution was quickly extracted with methylene chloride and the combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was distilled; the fraction boiling at 40-41°C/3 mm Hg afforded 4.24 g of the aldimine (38%). ^1H NMR ($CDCl_3$, 60 MHz) δ2.3 (s, 3H, =C-C**H**$_3$), 3.5 (s, 3H, =N-C**H**$_3$), 7.8 (s, 1H, N=C**H**), 8.2 (s, 1H, C**H**N). IR (CCl_4): 1655, 1640, 1490,1430 cm^{-1}.

f. Oxazolylimidazole base (General procedure, Ref. 6): A solution containing 4.1 g of the aldimine (33 mmol), 7.4 g of TosMIC (38 mmol) and 56 mL of pyridine was stirred at room temperature for 7 days. The reaction mixture was concentrated, diluted with 40 mL of methylene chloride and extracted twice with 40 mL of 10% aqueous HCl. The combined aqueous extracts were backwashed with methylene chloride and then made alkaline with 6 N KOH solution with stirring and cooling. The basic mixture was extracted with methylene chloride and the combined organic extracts dried over $MgSO_4$, filtered and concentrated to afford 900 mg of crude base. The base was

chromatographed from a mixture of 2:1:1 ethanol/ hexane/ methylene chloride on silica gel (grade 950, 60-200 mesh). A total of 23 fractions (20 mL each) were collected. Fractions 2-22 showed a single spot and together afforded 150 mg of base (2.8%). ^1H NMR ($CDCl_3$, 60MHz) δ2.3 (s, 3H, =CCH_3), 3.8 (s, 3H, NCH_3), 7.2 (s, 1H, imidazole), 7.5 (s, 1H, oxazole), 7.9 (s, 1H, imidazole).

g. Oxazolylimidazole hydrochloride salt 8: HCl gas was slowly bubbled into a solution containing 150 mg (0.9 mmole) of the base in 20 mL of methylene chloride. After 5 minutes, the solvent was evaporated and the residual solid was recrystallized twice from a 1:1 mixture of butanol and hexane. About 120 mg of the hydrochloride salt were recovered (yellowish, 226-229°C). Sublimation under vacuum afforded 80 mg of the white salt, m.p. 232-234°C. ^1H NMR (D_2O, 60 MHz) δ2.2 (s, 3H, oxazole), 3.8 (s, 3H, imidazole), 4.8 (s, D_2O), 7.7 (s, 1H, imidazole), 8.2 (s, 1H, oxazole), 8.8 (s, 1H, imidazole), **Fig.3**. The downfield signals disappeared after a while due to deuterium exchange. The molecular ion obtained from mass spectroscopy had a mass of 163, as expected for the free base with loss of HCl.

Elemental Analysis:

Found (%): C 48.10; H 4.93; N 20.71; Cl 17.78

Calculated (%): C 48.12; H 5.01; N 21.05; Cl 17.79

II. Synthesis of 9

a. Oxazolylester: A solution containing 33.05 g of ethyl chloroacetoacetate (0.20 mole), 23.7 g of acetamide (0.40 mole) and 73 g of glacial acetic acid (1.20 mole) was refluxed for 22 hours. Work up was the same as described in **Ib**. The ester was collected at 60°C/4-5 mm Hg, 8.9 g (26%). ^1H NMR ($CDCl_3$, 60 MHz) δ1.2-1.6 (t, 3H, $OCH_2\mathbf{CH_3}$), 2.4 (s,3H, $C{=}C\mathbf{CH_3}$), 2.5 (s, 3H, $N{=}C\mathbf{CH_3}$), 4.2-4.6 (q, 2H, $O\mathbf{CH_2}$). IR (CCl_4): 1720 (C=O), 1610, 1560, 1440 cm^{-1}.

Elemental analysis:

Calculated (%): C 56.80, H 6.55, N 8.28

Found (%): C 56.96, H 6.57, N 8.19

b. Oxazolylalcohol: 13 g of the ester (70 mmole) and 3.47 g of LAH (90 mmole) were used. The procedure was the same as describe in **Ic**. About 7.67 g of the alcohol were recovered (80%). ^1H NMR ($CDCl_3$, 60 MHz) δ2.1 (s, 3H, $C{=}C\mathbf{CH_3}$), 2.4 (s, 3H, $N{=}C\mathbf{CH_3}$), 4.1-4.3 (broad singlet, 1H, **OH**), 4.6 (s, 2H, $\mathbf{CH_2}OH$). IR (CCl_4): 3600-3200 (OH), 1640, 1570, 1440 cm^{-1}.

Elemental analysis:

Calculated (%): C 56.68, H 7.13, N 11.02

Found (%): C 56.75, H 6.98, N 11.15

c. Oxazolylaldehyde: The same Swern procedure as describe in **Id** was followed. From 7.67 g of the alcohol (70 mmole) used, 3.70 g of the aldehyde were recovered as yellowish crystals (49%), m.p 29-30°C. ^1H NMR ($CDCl_3$, 60MHz) δ2.5 (s, 3H,

C=C-CH_3), 2.6 (s, 3H, N=C-CH_3), 9.8 (s, 1H, CHO). IR (CCl_4): 1690 (C=O), 1670, 1590, 1550, 1440 cm^{-1}.

Elemental analysis:

Calculated (%): C 57.59, H 5.64, N 11.19

Found (%): C 57.83, H 5.58, N 11.09

d. Oxazolylaldimine: A solution containing 3.70 g of the aldehyde (30 mmol) in 5 mL of water was added dropwise to excess 40% aqueous methylamine solution with cooling and stirring. The reaction mixture was stirred at room temperature for an additional 30 minutes. The mixture was cooled again and KOH pellets were added until two layers formed. The aqueous layer was extracted with methylene chloride and all organic extracts were combined and dried over KOH pellets. The solution was concentrated, distilled under vacuum and the fraction boiling at 65-66°C/4-5 mm Hg afforded 1.84 g of the aldimine (49%). ^1H NMR ($CDCl_3$, 60MHz) δ2.3 (s, 3H, C=CCH_3), 2.5 (s, 3H, N=CCH_3), 3.6 (s, 3H, C=NCH_3), 8.2 (d, 1H, CH=N). IR (CCl_4): 1650, 1615, 1560, 1440 cm^{-1}.

e. Oxazolylimidazole base: A solution containing 2.5 g of the aldimine (20 mmole), 8.8 g of TosMIC and 45 mL of pyridine was stirred for 5 days. Work up was the same as described in **1f.** About 1.1 g of crude base was recovered. The crude base was chromatographed on 25 g of silica gel (grade 950, 60-200 mesh) with *n*-butanol. A total of 39 fractions (8 mL each) were collected. Fractions 4 to 23 were combined and concentrated to

afford 700 mg of pure base (20%). ^1H NMR ($CDCl_3$, 60 MHz) δ2.2 (s, 3H, C=CC**H**$_3$), 2.5 (s, 3H, N=CC**H**$_3$), 3.7 (s, 3H, NC**H**$_3$), 7.2 (broad singlet, 1H, C=C**H**), 7.5 (broad singlet, 1H, N=C**H**). The downfield broad singlets were due to deuterium exchanged.

f. Oxazolylimidazole hydrochloride salt 9: Excess 20% aqueous HCl was added to 700 mg of the chromatographed base and the mixture was stirred for 10 minutes. The reaction mixture was concentrated to dryness and the solid residue was recrystallized from a mixture of ethyl acetate and dimethylsulfoxide. First, 10 mL of ethyl acetate were added to the residue and warmed, then just enough dimethylsulfoxide was added to completely dissolve the solid. The solution was allowed to cool to room temperature and the crystals filtered off, washed with ethyl acetate, and dried under vacuum. 170 mg of pure salt were recovered (20%). ^1H NMR ($CDCl_3$, 60 MHz) δ2.2 (s, 3H, C=CC**H**$_3$), 2.5 (s, N=CH$_3$), 4.0 (s, 3H, NC**H**$_3$), 7.5 (s, 1H, C=C**H**), 10 (s, 1H, N=C**H**), **Fig.4**. The molecular ion obtained from mass spectroscopy had a mass of 177, as expected for the free base with loss of HCl.

Elemental Analysis:

Calculated (%): C 50.59, H 5.66, N 19.67, Cl 16.59.

Found (%): C 50.44, H 5.72, N 19.67, Cl 16.63.

Figure 1.

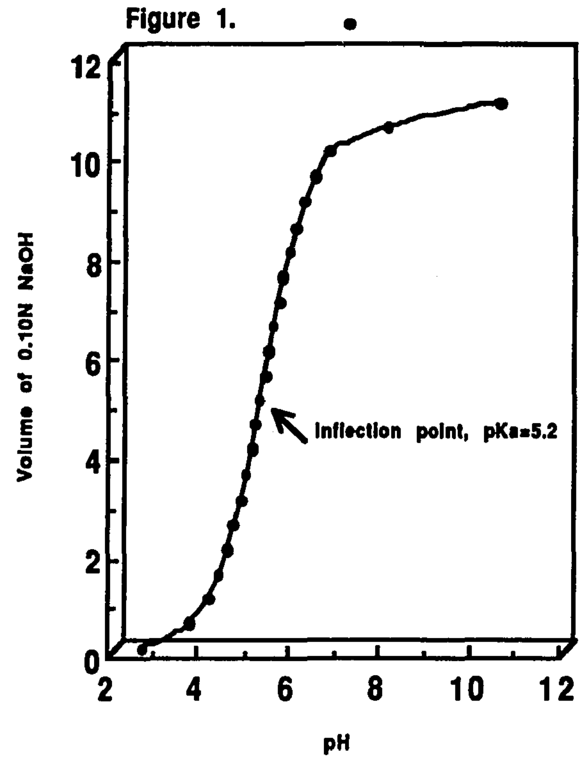
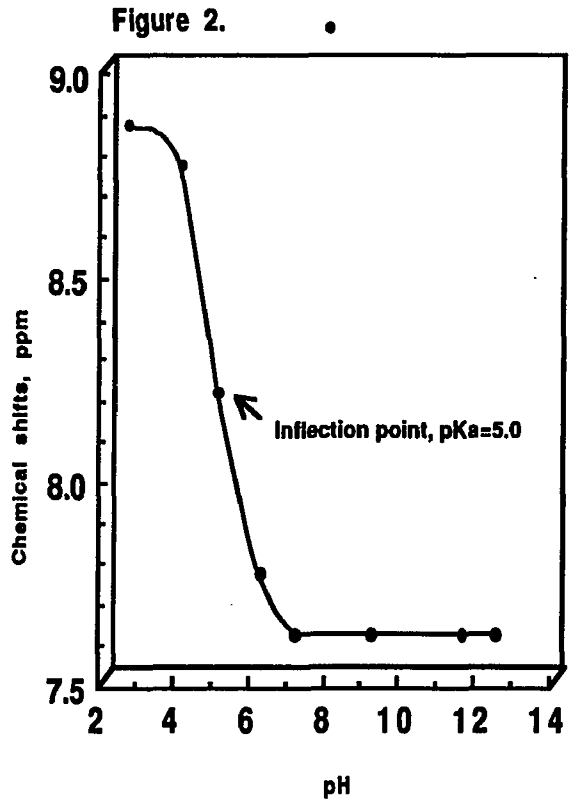


Figure 2.



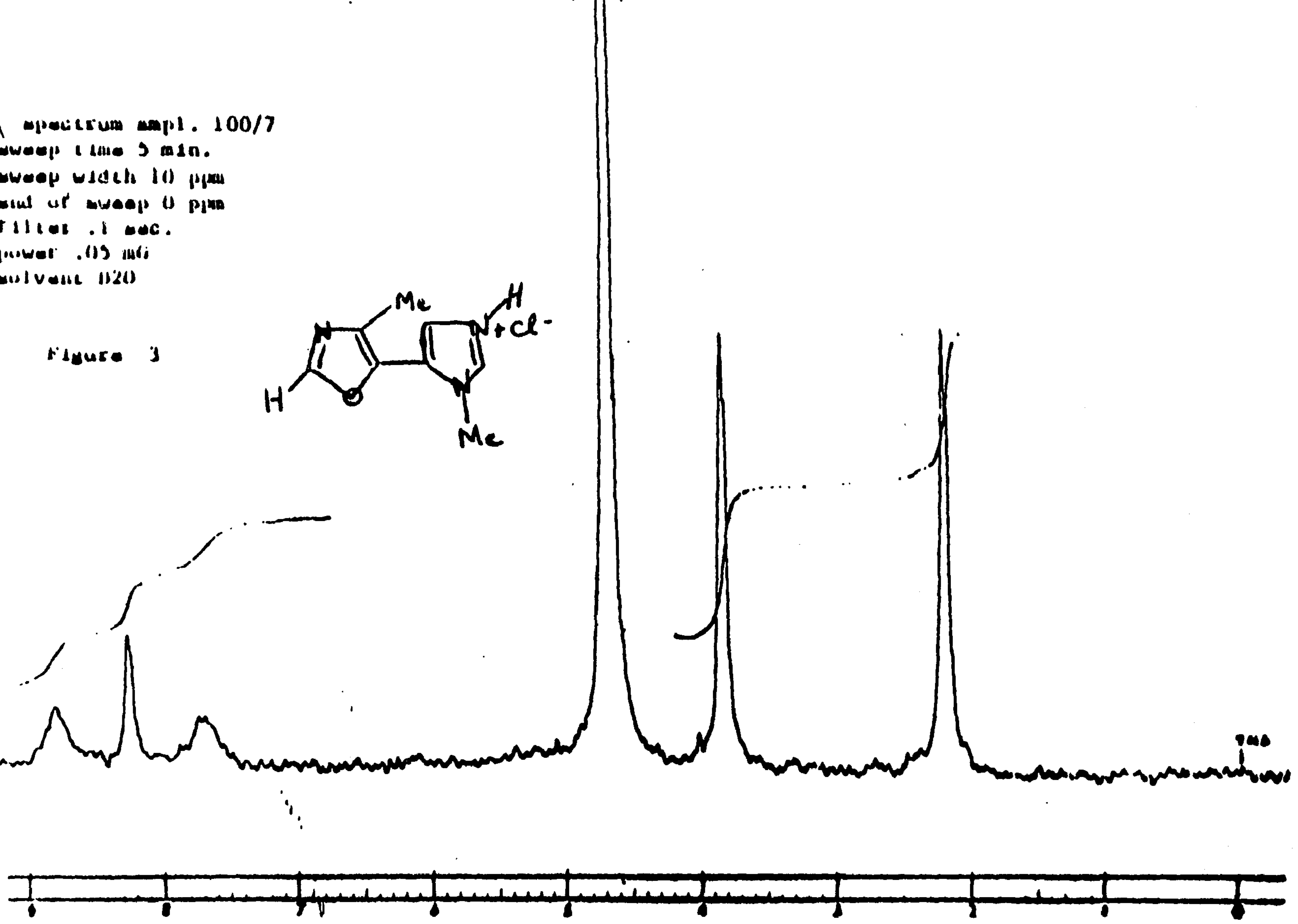


Figure 3

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sweep width 10 ppm
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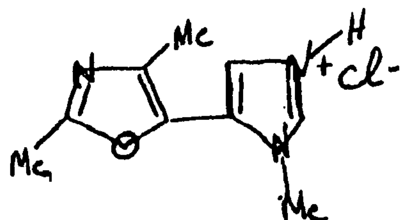
solvent D2O

Figure 4



D_2O

9 8 7 6 5 4 3 2 1 0

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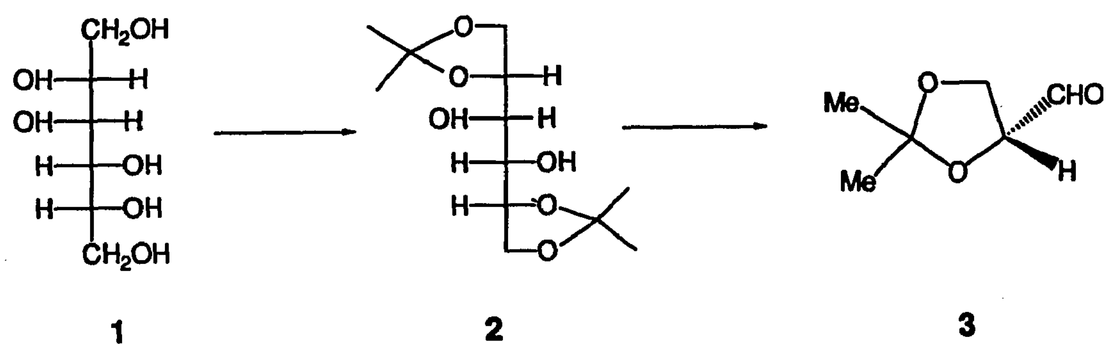
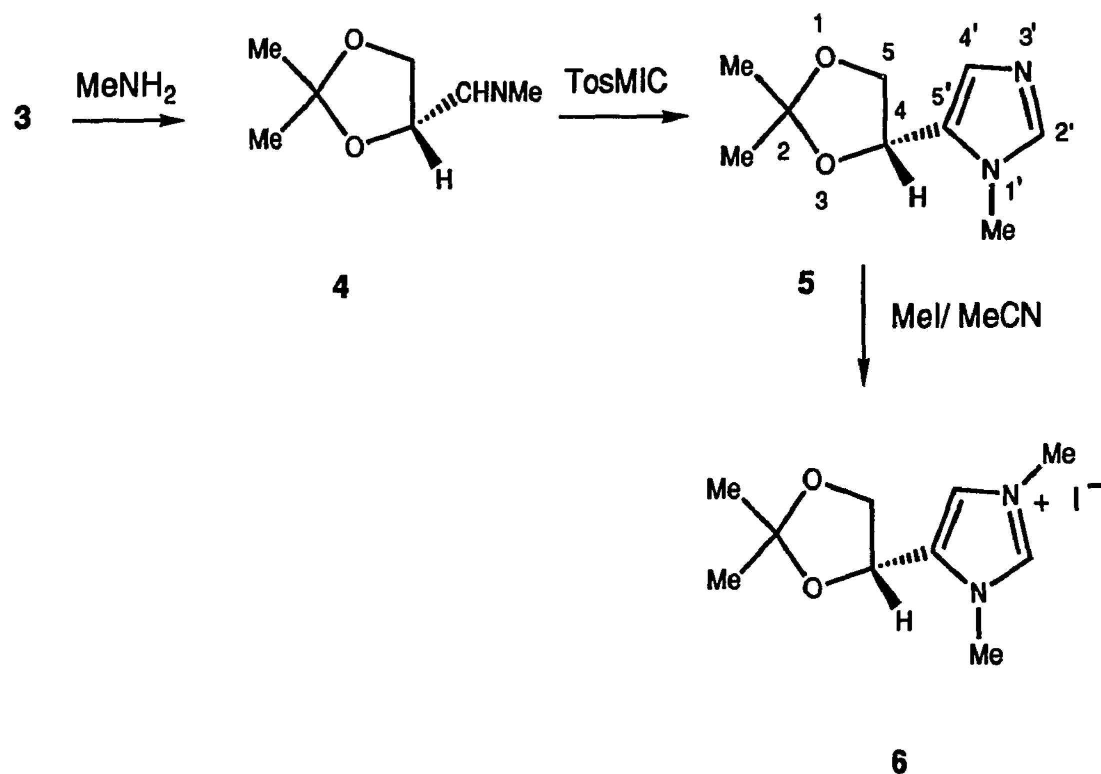
Chapter 4

Imidazolyl-1,3-dioxolane: Synthesis

Introduction: Following the procedure of Baer and Fisher,[1] D-mannitol **1** was treated with zinc chloride to form diacetone D-mannitol **2**, which was then cleaved with lead tetraacetate to give 4(R)-formyldioxolane **3** (Scheme 1). Treatment of aldehyde **3** with 40% aqueous methylamine afforded the corresponding aldimine **4**. Compound **5** was obtained in less than 1% (Scheme 2) by treating **4** with a solution of TosMIC and liquid methylamine for 16 hours at room temperature. Attempts to form **5** by all three TosMIC routes described in chapter 2 failed. Also, attempts to convert the free base to its hydrochloride salt with HCl gas failed, perhaps due to decomposition of the hydrochloride salt. The base was successfully converted to its N-methyl iodide salt **6**, but could not be obtained pure enough for elemental analysis due to the fact that we could not find an appropriate solvent for recrystallization. Compound **6** was observed to be soluble in slightly polar organic solvents such as chloroform and also in water and alcohols. The structures of **2**, **3**, **4**, **6** were confirmed by NMR spectroscopy; **3**, **4** by IR spectroscopy. Compound **5** was also confirmed by NMR, IR, mass spectroscopy and elemental analysis.



Scheme 1



Scheme 2

EXPERIMENTAL

a. 1,2,5,6-diacetone D-mannitol 2 (Procedure was that of Ref. 1): A solution of 120 g of anhydrous $ZnCl_2$ (0.88 mole) dissolved in 600 mL of acetone was filtered to remove undissolved $ZnCl_2$. D-mannitol (20 g, 0.11 mole) were added to the filtrate; the mixture was stirred at room temperature for 3 hours and allowed to stand overnight. A solution of 140 g of K_2CO_3 (0.90 mole) in 140 mL of water was added, followed by 600 mL of ether and the mixture was stirred for about 30 minutes. The acetone-ether layer was removed and the remaining $ZnCO_3$ by-product was washed twice with 200 mL of a 1:1 mixture of acetone-ether solution. All organic fractions were combined and concentrated to dryness. The residue was then refluxed five times in ligroin. After each reflux, the solution was rapidly filtered through a steam-heated funnel. All ligroin fractions were combined and allowed to cool at room temperature. The crystals which formed were filtered off and washed with cold ligroin to afford 9.18 g of **2** (32%), m.p. 117-119°C. Literature values: m.p. 119-122°C, 54.8% yield. ^1H NMR ($CDCl_3$, 60 MHz) δ1.3 (s, 3H, CCH_3), 1.4 (s, 3H, CCH_3), 3.6-4.4 (m, 8H).

b. 1,3-dioxolane 4-carboxaldehyde 3 (Procedure was that of Ref. 1): A solution containing 12.4 g of **2** (50 mmole) in 440 mL of thiophene-free benzene was treated with 21.3 g of lead tetraacetate (50 mmole) and stirred for 30 minutes. The residue in the mixture was crushed to a fine powder and filtered off. The

remaining solution was concentrated and distilled 35-40°C/8-11 mm Hg to afford 8.1 g of **3** (68%). Literature values: b.p. 35-42°C/8-11 mm Hg (79.3%). ^1H NMR ($CDCl_3$, 60 MHz) δ1.4 (s, 3H, CCH_3), 1.5 (s, 3H, CCH_3), 4.0-4.5 (m, 3H, dioxolane), 9.8 (d, 1H, CHO). IR: 1710 (C=O) cm^{-1}.

c. 1,3-dioxolane 4-aldimine 4 (General procedure, Ref. 2): 8.0 g of the aldehyde (0.07 mole) were added dropwise with stirring to excess ice-cold 40% aqueous methylamine solution. The reaction mixture was then stirred at room temperature for 30 minutes. The solution was cooled again and KOH pellets were added with stirring until two layers formed. The aqueous layer was extracted 3 times with chloroform. All organic extracts were combined and dried over KOH pellets. The solution was filtered, concentrated and distilled 45-50°C/ 2-3 mm Hg to yield 6.5 grams of **4** (65%), $[\alpha]^{25}_D$ + 45.2° (ethanol). ^1H NMR ($CDCl_3$, 200 MHz) δ1.4 (s, 3H, CCH_3), 1.5 (s, 3H, CCH_3), 3.3 (s, 3H, $C{=}NCH_3$), 3.9-4.0 (q, 1H, dioxolane), 4.1-4.2 (q, 1H, dioxolane), 4.5-4.6 (q, 1H, dioxolane), 7.7 (s, 1H, CH=N), **Figure 1**. IR (cm^{-1}): 1675 (C=N), 1453, 1380, 1372, 1252, 1213, 1150, 1064.

d. Imidazolyl-1,3-dioxolane 5 (General procedure, Ref. 3): A solution containing 7.0 g of the aldimine (50 mmole), 19.5 g of TosMIC (0.1 mole), 6.8 g of liquid methylamine in 10 mL absolute ethanol, and 50 mL of methylenechloride was stirred at room temperature for 16 hours. The solution was concentrated,

the residue diluted with methylene chloride and extracted several times with a 1% HCl solution. The combined aqueous extracts were made alkaline with 6 M NaOH solution and extracted with methylene chloride. All organic extracts were combined, dried over $MgSO_4$, filtered and concentrated to afford about 1 g of the crude base. The base was chromatographed on 20 g of neutral alumina (Brockman Activity I, 80-200 mesh) with a mixture of 40:1 chloroform and absolute ethanol. One 200 mL fraction was collected and concentrated to yield 0.50 g of by-product, Rf value of 0.54 on alumina plates (40:1 chloroform:ethanol). The column was then eluted with absolute ethanol to recover the compound at the origin; about 300 mg were collected and rechromatographed on 10 g of silica gel with a mixture of 3:1 ethyl acetate and *n*-butanol. 10 fractions were collected (5 ml each). Fractions 3 to 6 were combined and concentrated to yield 70 mg (0.8%) of pure base $[\alpha]^{25}_D$ -20.8° (ethanol). A molecular ion of mass 182 was obtained from MS, as expected for the free base. ^1H NMR ($CDCl_3$, 200 MHz) δ1.3 (s, 6H, $C(C\mathbf{H}_3)_2$), 3.6 (s, 3H, $NC\mathbf{H}_3$), 3.9 (t, 1H, $OC\mathbf{H}_2$), 4.2 (q, 1H, $OC\mathbf{H}_2$), 5.1 (t, 1H, OC**H**), 6.9 (s, 1H, C=C-**H**), 7.4 (s, 1H, N=C**H**), **Figure 2**.

Elemental analysis:

Found (%): C 59.00, H 7.75, N 15.21.

Calculated (%): C 59.32, H 7.74, N 15.37.

e. N-methyl-Imidazolyl 1,3-dioxolane iodide salt 6: To a solution containing 150 mg of the chromatographed base in 20 mL of acetonitrile was added excess of methyl iodide with stirring. The reaction mixture was refluxed for 20 minutes, concentrated and the residual solid was dissolved in chloroform. Then, just enough anhydrous ether was added to the mixture to precipitate out the salt. This process was repeated several times until the solid was clean enough for NMR analysis; about 40 mg of the salt were recovered. ^1H NMR (D_2O, 60 MHz) δ1.4 (s, 6H, $C(CH_3)_3$), 3.1 (s, 3H, NCH_3), 3.8 (s, 3H, $=NCH_3$), 7.5 (s, 1H, imidazole), 8.7 (s, 1H, imidazole), **Figure 3**.

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DATE 4-7-90

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O1 3300.000
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TD 16384
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HZ/PT .365

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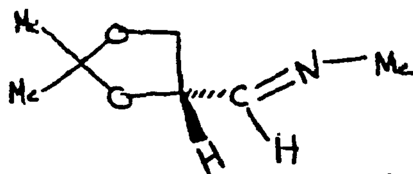

Figure 1

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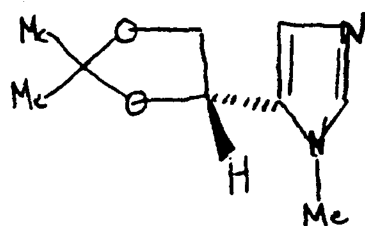
Figure 2



7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
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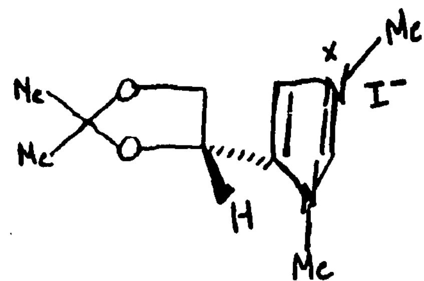

Figure 3

References

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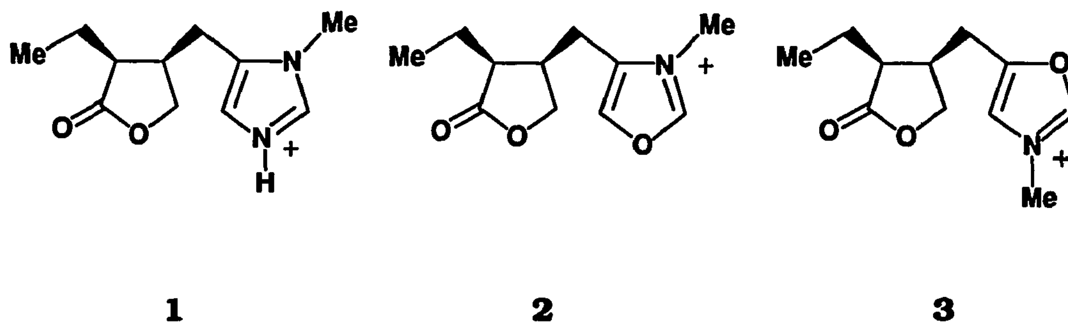
Chapter 5

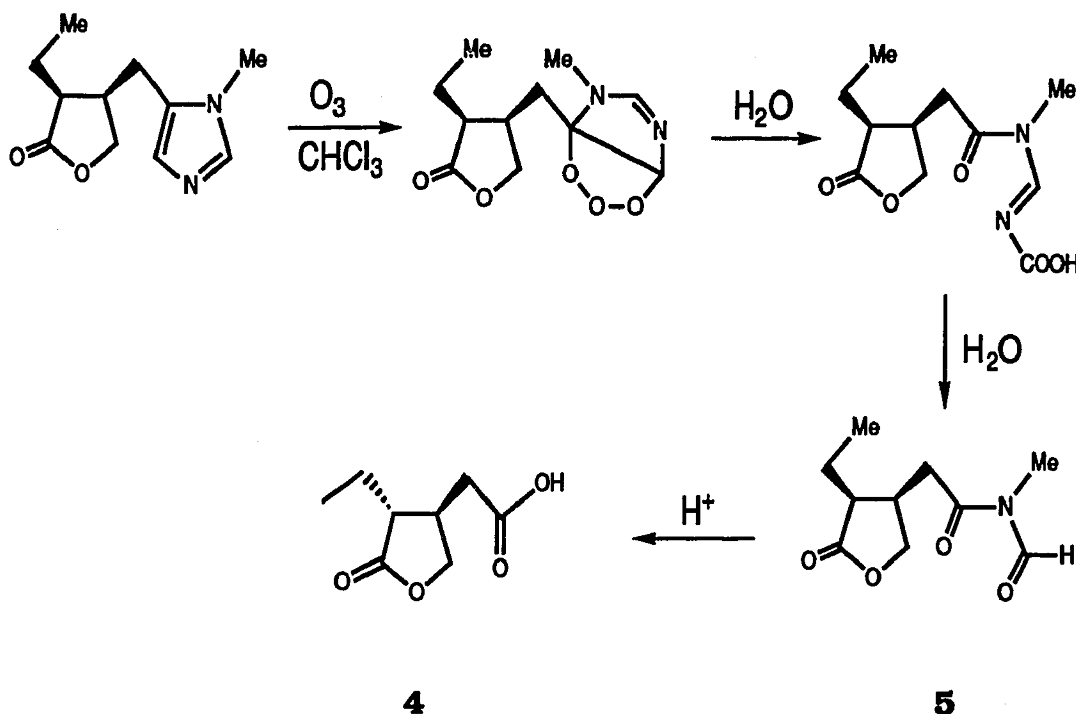
N-Methyl Oxazolium-substituted α, β-Butyrolactone

Introduction: The muscarinic partial agonist (+)-pilocarpine **1** continues to be of interest because of its pharmacological properties.[1,2] From the structural standpoint, pilocarpine lacks the traditional NCCOCC backbone of natural muscarinic agonists. Instead, it uses an imidazolium nitrogen and its lactone methyl and carbonyl oxygen to bind to the muscarinic receptor.[3] To determine which of the two nitrogens is utilized in the interaction, it is desirable to replace each nitrogen atom of the imidazolium ring in turn by oxygen to obtain oxazolium cations **2** and **3**, compounds whose pharmacological activity could be tested.



The route we have chosen for the synthesis of **2** is derived partly from the work of Hill et al.[4] who obtained homoisopilopic acid **4** by acid hydrolysis of N-methyl-N formylhomopilopamide **5** in good yield (Scheme 1).[5] While the

configuration of the α-carbon is lost during acid hydrolysis, it is known from the work of Rapoport,[6] that the desired *cis* lactone can be regenerated from the enolate anion under kinetically controlled re-protonation (Scheme 2). The absolute structure of homoisopilopic acid has been confirmed by the degradation studies of Hill.[4]

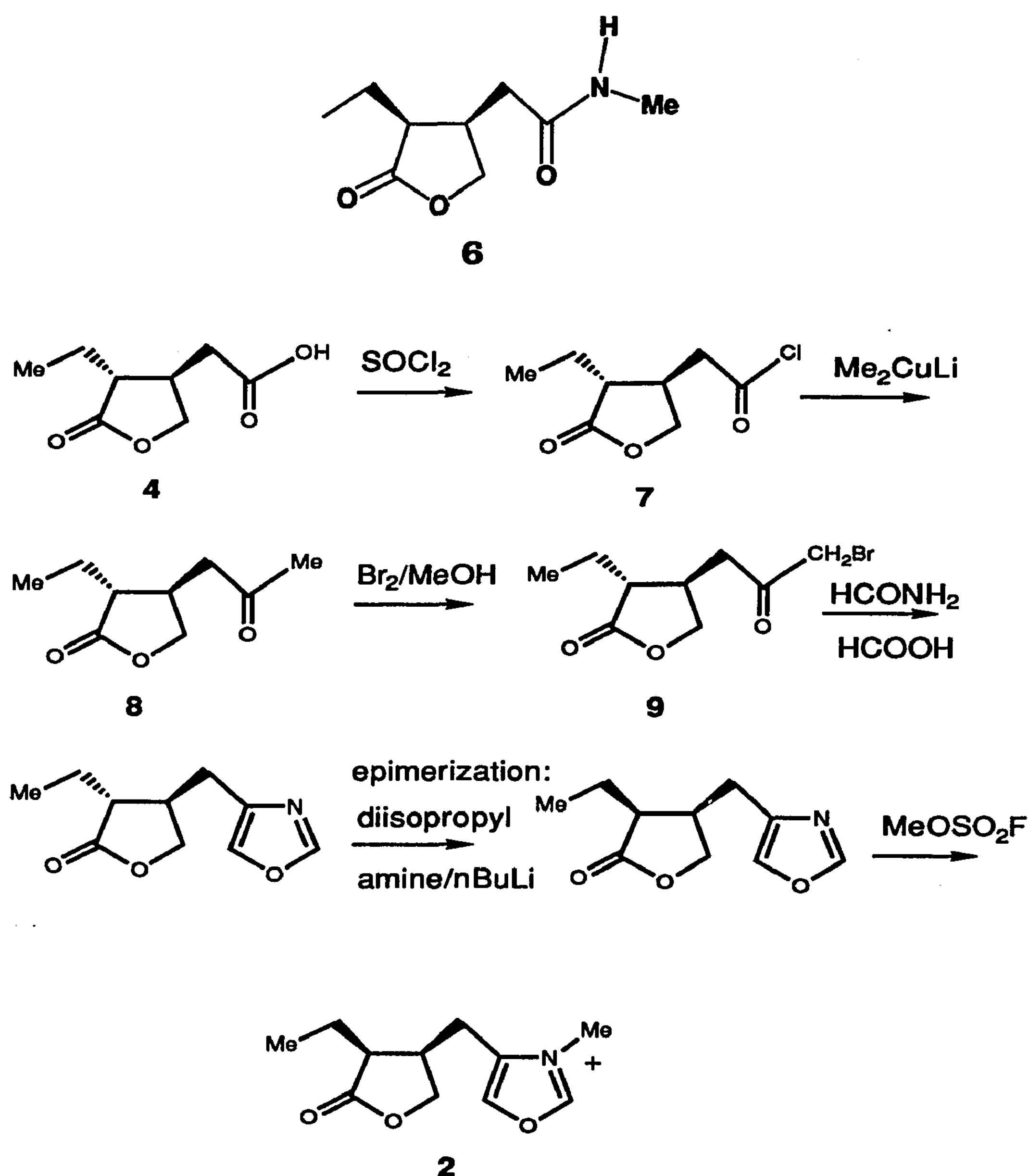


Scheme 1

(+)Pilocarpine was ozonized and converted to N-methyl-N-formylhomopilopamide **5** (not N-methylhomo pilopamide **6** as had been suggested by Langenbeck).[5] The structure of **5** was confirmed in our laboratory by NMR, MS and IR. The amide obtained was hydrolyzed with dilute HCl to homoisopilopic acid which was then converted to the acyl chloride **7**. The acyl carbon was coupled to a methyl group using

Me_2CuLi to generate the ketone **8**. Compound **8** was selectively brominated at the terminal carbon with bromine in methanol to furnish the α-bromoketone **9** (Scheme 2). All structures were confirmed by NMR, IR and **8** was further characterized by mass spectrometry and elemental analysis.

While we were unable to complete the synthesis of **2**, a plausible route for doing so is shown in Scheme 2.



Scheme 2

EXPERIMENTAL

1-(+)pilocarpine: 10g of (+)pilocarpine hydrochloride (41 mmol) were dissolved in distilled water and the solution was made basic with excess 50% NaOH solution with stirring and cooling. The mixture was extracted with chloroform and the organic extracts were combined and dried over $MgSO_4$. The solution was filtered and concentrated to yield about 6.2 g of the free base (73%).

2- N-methyl-N-formylhomopilopamide 5 (procedure was that of reference 5): A solution of 6 g (29 mmol) of (+)pilocarpine base in 50 mL of anhydrous chloroform was ozonized for about 3 hours. Water was added and the mixture was refluxed gently for 45 minutes. The reaction mixture was allowed to cool to room temperature and extracted with chloroform, dried over $MgSO_4$, filtered and concentrated to afford 5.5 g of the formylamide (90 %); Literature value: 79 %. ^1H NMR (200 MHz, $CDCl_3$) δ 1.05-1.15 (t, 3H, MeC); 1.25-1.55 (m); 1.65-1.95 (m); 2.55-2.85 (m); 3.15 (s, 3H, NMe); 4.0-4.1 (q, 1H, OCH); 4.35-4.45 (q, 1H, OCH); 9.25 (s, 1H, CHO), **Figure 2.** FT-IR: (C=O) 1771, 1724, 1680 cm^{-1}. The molecular ion obtained from mass spectroscopy had a mass of 213, as expected for the N-formylamide.

3- Homoisopilopic acid 4 (procedure was that of reference 4): A mixture of 4 g of the amide (19 mmol) and 48 mL of 2 M HCl was refluxed for 6 hours. The solution was extracted with

chloroform, dried over $MgSO_4$, filtered and concentrated to yield 1.4 g of the acid (43%). ^1H NMR ($CDCl_3$, 200MHz) δ 1.0-1.1 (t, 3H, Me); 1.2-1.5 (m); 1.7-1.9 (m); 2.5-2.6 (m); 2.9-3.1 (m); 4.0-4.2 (q, 1H, OCH); 4.4-4.5 (q, 1H, OCH); 11.3 (s, 1H, COOH). FT-IR: 3600-3048 (OH) cm^{-1}; 1778, 1707 (C=O) cm^{-1}.

4- <u>Trans</u> 4-ethyl-5-oxotetrahydrofuran-3-ethanoyl chloride (7) (procedure was that of reference 5): A mixture of 8 mL of distilled thionyl chloride and 1.4 g (8.1 mmol) of homoisopilopic acid was refluxed for 3 hours. The reaction mixture was concentrated, followed by the addition and evaporation of two 10-ml portions of toluene to yield 1.3 g of the acid chloride (85%).

5- <u>Trans</u> 3-ethyl-4-(2-oxopropyl)-tetrahydrofuran-2-one (8) (general procedure, reference 7): To a dry 3-necked flask, kept under nitrogen atmosphere, was added 2.3 g (12 mmol) of CuI. The solid was heated with a flame, the flask was cooled in an ice bath and 60 mL of anhydrous ether were added to the flask with stirring. After 10 minutes, 23 mL of 1.4 M MeLi in ether (24 mmol) were injected through a rubber septum. After an additional 5 minutes of cooling and stirring, the temperature was lowered to -78°C and a solution of 2 g (10.5 mmol) of the acyl chloride in anhydrous ether was injected through the septum. The solution was stirred for an additional 15 minutes; 5 mL of absolute methanol were added and the solution was

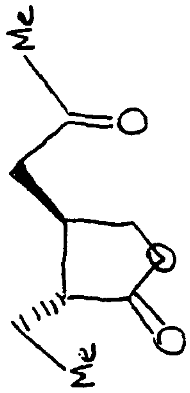
allowed to warm to room temperature. The reaction mixture was poured into 250 mL of saturated ammonium chloride and extracted with ether, dried over $MgSO_4$, filtered and concentrated to yield 910 mg (51%) of a mixture containing mostly the ketone. All 910 mg were chromatographed over silica gel using a 20:1 mixture of carbon tetrachloride and absolute ethanol. A total of 22 fractions were collected (3 ml each). Fractions 11-13 were combined and concentrated to furnish 800 mg of pure ketone (R_f 0.5), m.p. 45-47°C. Fractions 14-17 were also combined and concentrated to afford 80 mg of the slightly impure ketone. Fractions with R_f values higher than 0.5 and lower than 0.35 contained only impurities. ^1H NMR ($CDCl_3$, 200 MHz) δ 1.05 (t, 3H, Me); 1.3-1.5 (m); 1.67-1.85 (m); 2.2 (s, 3H, MeCO); 2.4-2.7 (m); 2.95-3.1 (m); 3.9-4.0 (q, 1H, OCH); 4.2-4.38 (q, 1H, OCH), **Figure 1.** The molecular ion obtained from GC/MS had a mass of 170 (retention time of 26.5 minutes) as expected for the ketone. GC data: Injection port-150°C; source temperature 270°C; column temperature 35°C to 100°C at 20°/min., then to 250°C at 10°/min.; electron multiplier 2130 V; column: 30 m DB-5, J. W. Scientific, Folson, CA. FT-IR: 1778, 1719 (C=O) cm^{-1}.

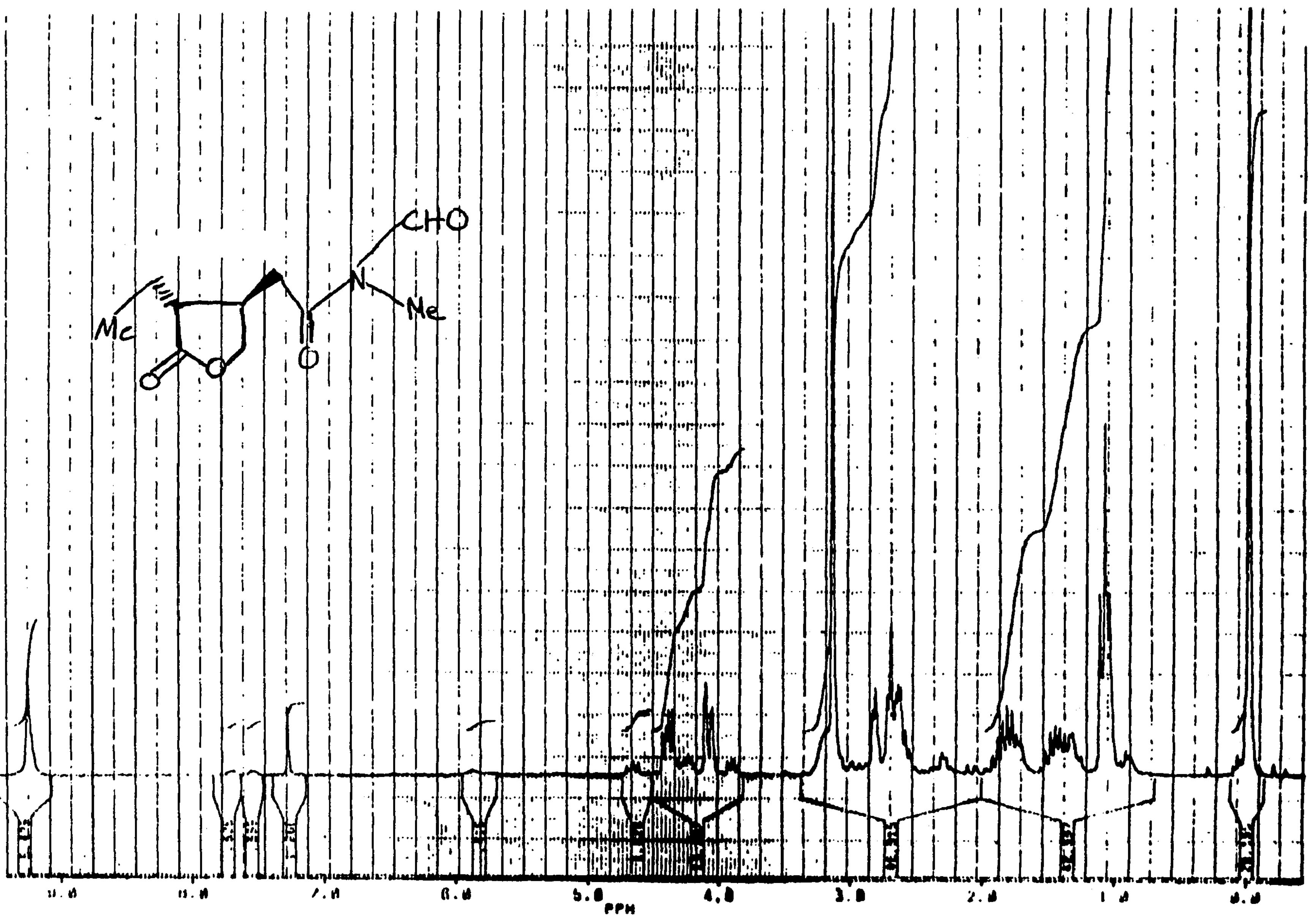
Elemental analysis:

Calculated (%): C 63.51; H 8.29

Found (%): C 64.14; H 8.51

6. Trans-3-ethyl-4-(3-bromo-2-oxopropyl) tetrahydrofuran-2-one (9) (general procedure, reference 8): To a solution of 0.9 g (5.3 mmol) of the ketone in dried methanol was added 0.85 g (5.3 mmol) of bromine with stirring. After the bromine was completely consumed (solution turned clear), 10 mL of water were added followed by the addition of 1 mL of concentrated sulfuric acid. The reaction mixture was stirred for 2 hours and then extracted with ether. The combined ether extracts were extracted with water and dried over Na_2SO_4. The solution was filtered and concentrated to yield 0.7 g of the bromoketone (54%). Based on NMR spectroscopy, bromination at the α' position was 100% selective as shown by the complete disappearance of the methyl peak (ketone, δ 2.2) and formation of a new methylene peak at δ 4.8.


Me
Me


CHO
N
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O
Me
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PPM
3.0
2.0
4.0
5.0

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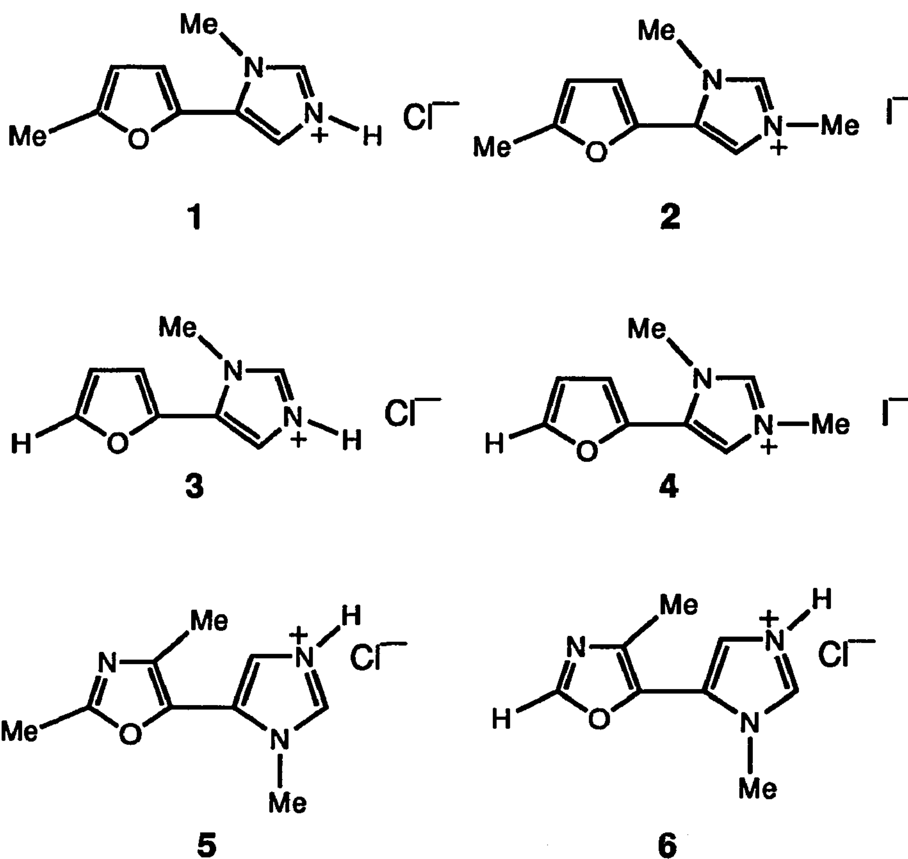
Chapter 6

Pharmacology

Introduction: Compounds **1-6** were evaluated for muscarinic activity by Goyal and Rattan of Beth Israel Hospital, Boston, by the Medical Research Division of Lederle Laboratories, Pearl River, New York, by the Mitsubishi Kasei corporation, and by the Lilly Research Laboratories. Two kinds of tests were made. The first measured the affinity of the compounds for the muscarinic receptors by allowing them to compete against a known muscarinic agonist (*cis*-methyldioxolane) and two antagonists (quinuclidinylbenzilate (QNB) and pirenzepine). The second class assayed the compounds for their biological activities.



I. Class 1 (binding assays):

a. [^{3}H-Quinuclidinyl Benzilate (QNB) Binding Assay:[1] This assay is the Lederle primary screen for central nervous system cholinergic agents. It determines the IC_{50} of the drug, that concentration of it which is required to occupy half of the receptor sites available to the tritiated antagonist quinuclidinebenzilate (^{3}H-QNB). Compounds **1-4** were also screened at 50 mM for their abilities to displace ^{3}H-QNB from rat cortical muscarinic acetylcholine cholinergic receptors. The results are displayed as % inhibition of the QNB response.

b. [^{3}H]-Cis-methyldioxolane (CD) Binding:[2,3] This assay is used in conjunction with the ^{3}H-QNB binding assay to infer agonist properties of CNS cholinergic agents. The log of the ratio of IC_{50} values for labeled QNB and *cis*-dioxolane (an agonist) are thought to be an indicator of agonist activity. Values of the log ratio of 3 to 4 are found for potent agonists, which are said to have high intrinsic activity.

c. [^{3}H]-Pirenzepine (PZ) Binding:[2] In this assay, a compound competes for the receptor with pirenzepine, a selective antagonist of M_1 muscarinic receptors. A compound that successfully competes with pirenzepine can be considered a possible M_1 agonist or antagonist.

d. [^{3}H]-Oxotremorine binding: Oxotemorine is an muscarinic agonist used by the Lilly Research group to induce tremors in

mice. Our compounds were tested for their ability to inhibit oxotremorine induced tremors. Compounds that inhibit these tremors are said to be M_2 antagonists.

II. Class 2 (assays for biological activity):

a. Test on the esophageal sphincter muscle of the oppossum: This assay was developed by Goyal and Rattan. Contraction of the muscle is indicative of M_2 activity, and relaxation of M_1 activity.

b. Stimulation of inositol Phosphate Release (SIPR):[4] This biochemical assay is performed to assess the intrinsic activity (agonist properties) of muscarinic compounds. Binding of muscarinic agonists to M_1 cholinergic receptors stimulate inositol phosphate release and diacylglycerol formation in cholinoceptive neurons.

c. Reversal of Scopolamine: Amnesia was induced in retired breeder mice with the muscarinic antagonist scopolamine. Reversal of this amnesia was studied by co-administering our cholinergic compound **1** with scopolamine.

III. Results and Discussion

Class 1: The Lederle results of the receptor binding assays with compounds **1-4** are summarized in Table 1. The compound with the highest affinity for the central muscarinic cholinergic receptors was **2** with 77% inhibition of the antagonist ^{3}H-QNB

and an IC_{50} of 13 mM. Lowest affinity was found for **3** with only 5% inhibition and an IC_{50} of 1221 mM. An attempt was made to investigate pharmacological muscarinic cholinergic receptor subtype selectivity utilizing ^{3}H-pirenzepine binding to rat cortical M_1 receptors. As seen from Table 1, our compounds **1-4** did not show high affinity for the cortical receptors. Again, compound **2** had the highest affinity for these receptors with an IC_{50} of 1410 nM and compound **3**, the lowest with an IC_{50} of 281600 nM.

Compounds **1-5** were tested by the Mitsubishi Kasei Corporation for affinity and selectivity on cholinergic muscarinic receptors in the rat cortex. The results are summarized in Table 2. Again, within the furan series, compound **2** was found to have the highest affinity in displacing ^{3}H-pirenzepine and compound **3** the lowest affinity. However, **3** was found to be the most selective with 25% M_1 receptor selectivity and **1** the least with 11% M_1 receptor selectivity. Compound **5** showed no affinity and selectivity for the receptors.

Compounds **1-6** were tested by Lilly Research Laboratories for affinity on cholinergic muscarinic receptors of the rabbit vas deferens (Table 3). Again, compound **2** was found to have the highest affinity in displacing ^{3}H-perenzepine (IC_{50} 1400 nM) and produced greatest inhibition of oxotremorine induced tremors (54%). Although compound **5** showed little affinity for the receptors, it inhibited oxotremorine tremors by 28%.

Class 2: Compound **1** was tested by Goyal and Rattan on the lower esophageal sphincter of anesthetized opossum, a region containing both M_1 and M_2 muscarinic receptors. When injected into the arterial supply of the sphincter, relaxation of the sphinter was observed. Compound **1** was also tested on guinea-pig trachea and showed virtually no potency in contracting the trachea muscle, a region consisting mostly of M_2 receptors. These results would indicate that **1** is selective for M_1 receptors.

Compounds **1-4** were assayed for intrinsic activity in the rat cortex (Table 4). Of the four submitted compounds, **3** and **4** were slightly active with 14% and 12% stimulation of inositolphosphate release, respectively. Since compound **1** had the largest log IC_{50} ratio (2.11), one would expect it to have greater intrinsic activity. This was not the case here with no stimulation of inositolphosphate release. Nevertheless, compound **1** was considered to be the most interesting of the four and further tests were conducted with it to determine its degree of muscarinic activity. The activity of **1** in the reversal of scopolamine is summarized in Table 5. Amnesia was induced in retired brider mice with scopolamine (1.5 mg/Kg) and attempts to reverse this amnesia were carried out by co-administering compound **1**. As can be seen from Table 5, **1** was most effective in reversing the amnesia at both 0.005 and 0.01 mg/Kg. However, it did not return the latency to control level.

IV. Conclusion

Several conclusions may be drawn from these data. With the exception of **3**, **5** and **6**, the remaining compounds possesed affinity for the muscarinic receptors. However, none of these compounds could displaced ^{3}H-pirenzepine with high affinity. The results of Goyal and Rattan, the reversal of scopolamine-induced amnesia, the SIPR data and the log (QNB/CD) ratios are consistent with the possibility that compounds **1-4** maybe weak partial agonists. Furthermore, the contention that **1** is a weak partial agonist is further supported by the behavioral data. Compound **2** was found to have the highest affinity for the receptors and **3** the most M_1 selective. It is suspected that the low basicity of **1** and **3**, as indicated by the measured pka's, could play a major role in their low affinities for the receptor.

Table 1: Muscarinic acetylcholine receptor pharmacology

Compound	^{3}H-QNB % Inhibition @ 50 μM	^{3}H-QNB μM (IC_{50})	^{3}H-CD nM (IC_{50})	IC_{50} Ratio logQNB/CD	^{3}H-PZ nM (IC_{50})
Pilocarpine hydrochloride	82	11	10	3.04	1053
Methylfurmethide	71	12	2	3.78	1078
1	22	187	1460	2.11	21590
2	77	13	588	1.34	1410
3	5	1221	25380	1.68	281600
4	26	154	8421	1.26	11520
Pilocarpine N-methyl iodide	50	49	3007	1.21	3730
Carbachol	57	70	4	4.24	14
Oxotremorine	95	0.8	0.5	3.20	167
Arecoline	71	29	14	3.32	1630
Atropine	100	0.0012	0.6	0.30	------
Pirenzepine	-----	0.123	57	0.33	2.7

Table 2: The Mitsubishi Kasei results of acetylcholine receptor pharmacology

Compound	^{3}H-PZ IC_{50} (M)	^{3}H-QNB IC_{50} (M)	% M1 receptor selectivity
1	1.2×10^{-5}	1.3×10^{-4}	11
2	1.5×10^{-6}	2.4×10^{-5}	16
3	9.7×10^{-5}	2.4×10^{-3}	25
4	2.0×10^{-5}	3.1×10^{-4}	16
5	3.8×10^{-4}	$> 10^{-3}$	---
Carbachol	8.0×10^{-6}	5.6×10^{-5}	7
AF102B[2]	4×10^{-7}	1×10^{-5}	25

Notes:

1) M_1 receptor selectivity is represented as IC_{50} (QNB-binding)/ IC_{50} (PZ-binding).

2) The values for AF102B2, a M_1 selective receptor stimulant, were cited from Japan Patent.

Table 3: Lilly results on receptor pharmacology

Compound	Pirenzepine IC_{50} (nM)	oxotremorine IC_{50} (nM)	inhibition % @100μM
1	16600	9120	10
2	1400	1935	54
3	> 10000	> 10000	18
4	>10000	> 10000	22
5	> 10000	> 10000	28
6	> 10000	> 10000	0

Table 4: Muscarinic acetylcholine receptor biochemical pharmacology

Compound	IC_{50} Ratio logQNB/CD	SIPR Rat Cortex % Stimulation	SIPR Rat Parotid % Stimul.
Pilocarpine hydrchloride	3.04	24	199
Methylfurmethide	3.78	175	709
1	2.11	0	0
2	1.34	3	0
3	1.68	14	0
4	1.26	12	0
Pilocarpine N-methyliodide	1.21	0	0
Carbachol	4.24	424	9005
Oxotremorine	3.20	56	385
Arecoline	3.32	39	354

Table 5: Reversal of scopolamine-induced amnesia

Compound	Dose (mg/Kg)	Mean Retention Latency (sec.)
Scopolamine	1.5	52 (n= 176)
Saline	--	173 (n= 73)
Arecoline	0.1	83
	0.5	84
	1.0	86
	5.0	62
Oxotremorine	0.01	60
	0.05	138
	0.1	20
1	0.001	84
	0.005	109
	0.01	105
	0.05	58
	0.1	51
	0.5	45

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