INVESTIGATION OF HOMO- AND HETERO-AROMATIC ANALOGUES
OF CYCLOHEXYL SUBSTITUTED ANTERGAN FOR
PHARMACOLOGICAL ACTIVITIES

by

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ABSTRACT

In the present study seven tertiary ethylenediamine derivatives were synthesized. They had the basic structure of N,N-dimethyl-N'-R₁-N'-R₂-ethylenediamine where R₁ = cyclohexylmethyl and R₂ = 2-pyridyl, 2-pyrimidyl, 2-pyrazyl, 1-naphthyl, 5-quinolyl; and R₁ = cyclohexanecarbonyl and R₂ = 2-pyrimidyl, 2-pyrazyl. Two oxetane derivatives, 2,2-diphenyl-3-chloro-oxetane and 2,2-diphenyl-3-(pyrrolidin-2-one-1-yl)oxetane, have also been synthesized as intermediates for further investigation.

The general reaction sequence followed was to start with the appropriate primary aromatic amine and build up to a secondary amine via a cyclohexanecarboxamide. The desired secondary amine was reacted with β-dimethylaminoethyl chloride. The tertiary diamine readily formed in good yields. In cases where the primary aromatic amines could not be built up to a secondary amine via the above route, the tertiary diamine derivative was obtained by two condensation reactions, i.e. the primary aromatic amine was first condensed with β-dimethylaminoethyl chloride to form the desired secondary amine which was then condensed with cyclohexylmethyl bromide to produce the tertiary diamine derivative. The overall yields were lower than those of the first route.

The oxetane derivatives were obtained by photocycloaddition of benzophenone to either vinyl chloride or N-vinyl-2-pyrrolidinone.
The antihistaminic activity and the possible anticholinergic activity of the tertiary diamine derivatives were studied and compared with those of diphenhydramine, atropine, and the cyclohexyl analogue of Antergan. These compounds exhibited negligible anticholinergic activity. However, their relatively potent antihistaminic activity was found to give more evidence in direct support of Nauta's antihistaminic receptor theory.

Signature of Examiners

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My sincere gratitude is extended to my major professor, Dr. T. H. Brown, for professional guidance, understanding and encouragement during the course of this study.

I must express my thanks to Dr. K. Y. Chang of the Department of Electrical Engineering, U. B. C. for the technical assistance in plotting the cumulative log dose-response curves using the IBM 360/67 Computer.

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DEDICATION

To my wife Sarko.
PART I
INTRODUCTION

In a search to understand the receptors for ethylene-diamine-type antihistamines, our laboratory has shown that one of the aromatic rings in Antergan (I) could be replaced by an alicyclic system (e.g. cyclohexyl, IIa) with no loss in antihistaminic activity (1, 2). This was in contrast to the conventional structure activity relationship which appeared to require the presence of both aromatic rings in order to have high activity. Replacement of both aromatic rings by cycloaliphatic rings greatly decreased the activity. Thus, it was apparent that the activity was due mostly to the remaining aromatic moiety. It was the purpose of this study to investigate how variations in size and structure of the aromatic moiety (R₂ group in II) in this alicyclic substituted system (II) would affect the activity at the receptor sites.

Attempts have also been made to synthesize oxetane derivatives (III or IV) which were structural analogues of diphenhydramine (V), however, synthetic difficulties prevailed and the results were unsuccessful.

All the compounds synthesized in the (II)-series have been found to be useful in further defining the structure-
activity relationships of antihistamines. The results obtained from pharmacological testing of these compounds give more evidence in direct support of the histamine/antihistamine receptor model (3) and the mode of binding of diphenhydramine (V) to the receptor as proposed by Nauta (3, 4) recently. Also, since the activity of antihistaminic agents overlaps with that for antiacetylcholine agents, these new compounds have also been investigated for their cholinolytic activity.
A. BIOGENESIS AND PHYSIOLOGY OF
HISTAMINE AND ANAPHYLAXIS

Until about 1960 it was believed that alterations in the histamine content of tissues, the release therefrom, and its excretion, would reflect the participation of histamine in normal reactions. Schayer (5) showed that in normal and pathological reactions great changes in the rate of endogenous histamine formation did occur without any corresponding changes in tissue histamine content. From then on, the concepts of what are referred to as "non-mast-cell histamine" (6, 7) and "histamine forming capacity (HFC)" (6) have readily become accepted. The latter term (HFC) refers to the rate at which histamine is formed in minced tissues, cell suspensions, or the whole body; it denotes histidine decarboxylase activity in numerical terms (6). Non-mast-cell histamine indicates the histamine formed in cells other than mast cells; in non-mast cells the HFC can be strikingly high and the resulting histamine is often "nascent" in nature (7). The nascent histamine is seemingly involved in certain kinds of rapid tissue growth. Its action appears to be linked to the very process of its formation. The action of nascent histamine is presumably not achieved by injected histamine (exogenous histamine), and its action is not prevented by antihistamines.

The biogenesis and physiology of histamine has recently been extensively reviewed (8, 13). The principal site of histamine synthesis is the mast-cell system (9). In the rat the evidence (8) appears overwhelming in support of the
view that the histamine contained in tissues is also formed therein. This was concluded from an experiment showing that germ-free rats, neither in any particular tissue examined, nor in the whole animal, contained less histamine than non-germ-free ones. Indeed, the uniformity in tissue histamine content between the two groups should be taken as conclusive evidence that even in non-germ-free rats fed on a histamine free diet, the histamine contained in the various tissues is endogenous in origin. Also mast cells were counted in the mesentery, and no difference in their number was found between germ-free and ordinary rats. In fact, every mammalian tissue that contained histamine, including white blood cells, appeared capable of manufacturing the amine from histidine.

It thus seems likely that the mast cells form histamine and store it in granules, from where it can be released into the surrounding tissues when a suitable stimulus is received by the cell (11). Such pre-formed histamine appears to be the main source of histamine released in the body, but Schayer (12) produced evidence that histamine can also be readily formed in response to physiological demand. This newly-formed histamine is apparently not produced in the mast cells and has variously been called non-mast cell histamine, induced histamine and nascent histamine (13).

Mast cells are connective-tissue cells widely distributed throughout the body, particularly in the lungs and liver (14), although there are places where they are not found such as the wall of the stomach, human-epidermis and
5.

the CNS. Histamine is also present in substantial amounts in such nonmast-cell sites. Besides histamine virtually all endogenous heparin was located in the mast-cell system. Rodents' mast-cell also contained 5-hydroxytryptamine (5-HT) (9).

At least two different enzymes are able to form histamine from histidine (15). One is a nonspecific L-aminoacid decarboxylase (dopa decarboxylase), and the other is a specific histidine decarboxylase. The former acts on many aromatic amino acids, such as 5-hydroxytryptophan and 3,4-dihydroxyphenylalanine. The latter (specific decarboxylase) acts only on histidine, and it is this enzyme that is found in the stomach, mast cells, and fetal liver of the rats; this enzyme is the principal enzyme involved in vivo requiring pyridoxal-5-phosphate as a cofactor. The enzyme can be inhibited by α-methylhistidine as well as by 4-bromo-3-hydroxybenzylxyloxyamine. Furthermore, this enzyme is inducible and the histamine-forming capacity at the nonmast-cell sites is subject to regulation by various physiological and other factors (13). Present evidence (35) indicates that nonspecific aromatic L-aminoacid decarboxylase does not participate in histamine synthesis in vivo.

In mast cells (rat) histamine is stored in basophil granules consisting mainly (over 95%) of a heparin-protein complex. This heparin-protein complex is water insoluble and is held together by the strong attractive forces between two macromolecules with opposite charges, the acid heparin and the basic protein (15). The sulphuric acid groups of the heparin
are essential for the formation of the heparin-protein complex, the desulphated heparin lacking complex forming capacity (17). The histamine binding sites in the granules are carboxyl groups belonging to the protein part of the heparin-protein complex (16). Recent observations (18) also indicate that carboxyl groups, and not sulphuric acid groups, are the most likely ionic binding sites for histamine in the mast cell granules. Uvnas (19) suggested a possible gross structure of the granular heparin-protein-histamine complex (VI): in the intact complex at pH 7 the acid groups of heparin should be linked to the amino (imino) groups of the basic protein, thus leaving only the COO⁻-group of the protein available for histamine binding.

\[
\text{Heparin} \quad \text{Protein} \quad \text{Histamine} \\
\text{(VI)}
\]

In their binding of histamine the granules show the properties of a weak cation exchange material, allowing an exchange with other cations (e.g. an equivalent exchange between histamine and sodium). Thus on degranulation the mast cells discharge histamine-containing granules and the weak cation exchange mechanism allows an immediate extracellular release of histamine from discharged granules (16) by exchanging histamine
with the cations present in the extracellular medium.

Histamine is also formed in the nervous system. Within the central nervous system, except for restricted areas (i.e. pineal body, portions of the hypophysis and choroid plexus), mast cells are absent and the histamine there must be held in neural cells, or supporting cells, or both (20, 21). It is of interest that the pattern of distribution of histamine in the brain is much like that of the other amines—norepinephrine, dopamine, and 5-HT; the concentration is highest in the hypothalamus (where indeed, there is twice as much histamine as 5-HT), intermediate in the thalamus and midbrain, and lowest in the cerebral cortex and white matter (20, 21). In the peripheral nerves histamine content is grossly correlatable with mast cell count (22, 23).

Recent in vivo investigations (24, 25, 26) have shown that histamine in the brain is formed by the action of a specific histidine decarboxylase. Campos and Jurupe (27, 28) suggest the involvement of a cholinergic mechanism in histamine synthesis. They found that electrical stimulation of rat paws depleted brain acetylcholine and concomitantly increased brain histamine levels. Intraperitoneal injections of cholinesterase inhibitors (physostigmine salicylate 0.2 mg./kg. or an equimolar dose of parathion) also increased brain acetylcholine and histamine. The brain acetylcholine released during central nervous stimulation apparently triggered a mechanism to accelerate brain histamine synthesis in vivo (27, 28).
8.

In the peripheral nerve it is likely that at least some of the decarboxylase is in the associated mast cells and therefore is a specific histidine decarboxylase (20). Ryan and Brody (29) studied the role of histamine as a sympathetic neurotransmitter released during active reflex vasodilation in the canine autonomic nervous system and found supportive evidence that histamine is liberated from sympathetic histaminergic fibers during reflex vasodilatation. The neurogenic stores of histamine appear to be non-mast cell in nature. There is still no certainty, however, that this histamine was intimately related to axons, and the existence of such histaminergic nerves is quite uncertain (21).

To what compound or compounds histamine is bound in particulate material of brain is not known (20). Histamine forms complexes with acidic lipids and other acidic substances, including sulfomucopolysaccharides to which histamine in mast cells is believed to be bound (30). Like histamine, sulfomucopolysaccharides are present in gray matter and in the microsomal fraction of brain (30). In the mast cells found in nerve fibers and in structures associated with brain, it might be assumed that histamine is bound as in other mast cells (19).

Most knowledge about catabolism of histamine was learned from the various $^{14}$C-labelled catabolites excreted in the urine after the injection of a small amount of $^{14}$C-histamine. Investigations of the urinary pattern of radioactive histamine catabolites have given results which are difficult to interpret, and perhaps, do not reflect plainly physiological events.
(8). Studies with injected histamine (exogenous histamine) cannot reveal precisely what is happening to endogenously formed histamine (15). It is assumed, although it is difficult to prove, that small amounts of exogenous histamine are catabolized in the same way as endogenous histamine. Otherwise their functional status is perhaps not the same in that endogenous histamine seems to exert functions which can not be reproduced by exogenous histamine.

In man and most laboratory animals there are two major pathways of histamine catabolism (21). The more important pathway involves ring N-methylation and is catalyzed by the enzyme histamine-N-methyl-transferase which methylates histamine without affecting other imidazoles or aromatic amines (31). Most of the product, methylhistamine, is converted by MAO to methylimidazole acetic acid. Reilly and Schayer (32) showed methylhistamine inhibits histamine methylation in vivo. In the other pathway histamine undergoes oxidative deamination catalyzed by the enzyme known as histaminase, with the production of imidazole acetic acid and its riboside. It is not agreed that histaminase is the same as diamine oxidase (15, 20). The various metabolites are then excreted in the urine. The relative roles of these enzymes in the metabolism of endogenous histamine have not yet been established, nor is it clear why histaminase activity of plasma rises sharply during pregnancy (13). As mentioned above, the catabolism of endogenous histamine, remains a topic for further study (8).

Ring methylation is a major pathway of histamine-
catabolism in brain (33). The brain contains N-methyltransferase and monoamine oxidase but does not contain diamine oxidase (20). After perfusion of cerebral ventricles in cats with histamine, both methylhistamine and methylimidazole acetic acid are detected. The regional distribution of histamine methyltransferase in monkey brain, measured in homogenates, was shown as follows: The hypothalamus, which is especially rich in histamine, showed high activity. The hypophysis, which has less histamine than the hypothalamus, had the greatest activity of all the areas measured (20).

There are numerous reasons for supposing that histamine has important functions in the body's economy. Histamine not only is an extremely active substance, capable of mimicking a variety of physiological and pathological phenomena, but also occurs naturally in tissues throughout the body, most of which contain the enzymes that both form it and inactivate it (21).

Although some of the most familiar hypotheses on the function of histamine have been concerned with pathological physiology and, in particular, with anaphylaxis, allergy, injury, and shock, indications of a normal physiological function are accumulating rapidly with the development of highly refined methods of study. For example, there is now impressive evidence that endogenous histamine is the final common mediator of gastric secretory responses, and there are some grounds for supposing it to be involved in tissue growth and repair, in regulation of the microcirculation, and in the functioning of the CNS (3, 13).
The mast-cell histamine has an important role in the pathological physiology of tissue responses to anaphylaxis, injury, and certain drugs (34). The histamine stored in mast cells is not actively metabolized, and turnover rate is slow. When tissues rich in these cells are depleted of their histamine stores, it might take weeks before they are returned to normal. On the other hand histamine in non-mast cell sites undergoes a brisk turnover and the histamine synthesized is released at once rather than stored. This histamine contributes importantly to the daily excretion of histamine and its metabolites in the urine. The histamine-forming capacity of such non-mast cell sites is subject to regulation by various physiological factors (13, 34). It is this non-mast cell histamine that was important in various physiological processes which are discussed here.

Endogenous histamine is believed to be the final common stimulator of the parietal cell of the gastric mucosa and therefore is the chemical mediator for gastric secretion. Feeding is the only hitherto recognized physiological circumstance in which preformed tissue histamine has been shown to be mobilized (8). In sections of the stomach the number of acid-secreting cells parallel the amount of histamine, which is held in enterochromaffin-like cells in the gastric mucosa (15). Furthermore, the output of histamine into the gastric juice parallels the volume of hydrochloric acid secretion, whether this secretion is induced by the injection of gastrin, by the ingestion of food, or by the injection of cholinergic
drugs (13). This output is paralleled by an increased synthesis of histamine in the stomach, which suggest a kind of feedback control (13). Moreover, the demonstration of a lowered mucosal histamine content associated with excitation of acid secretion is taken as strong evidence that endogenous histamine is the chemical mediator for gastric secretion (8).

Schayer (36) recently pointed out that histamine appears to be the most important amine in microcirculatory regulation. An earlier view that bradykinin was the mediator of functional vasodilatation has recently been rendered doubtful. The correlation between changes in histidine decarboxylase activity and circulatory changes was taken by Schayer (37) as a basis for the hypothesis that induced histamine serves as a governor of the functional state of the terminal vessels, induced histamine being formed at a rate required to maintain homeostasis. For example, induced histamine serves as an intrinsic mediator of vascular dilatation whose utilization rose during exercise (38). The intrinsic microcirculatory dilator effect of histamine indirectly influences every aspect of body economy (38). A moderate histamine excess increases nutritive blood flow and permits cells to reach their full potentialities. Conversely, a histamine insufficiency leads to impaired cell nutrition and to development of abnormalities in cell chemistry and function.

The first real indication that histamine might be involved in human reproduction was the discovery that the histaminase activity of the placenta increases greatly during
pregnancy (8). The enzyme is produced under the influence of progesterone and can be measured in the plasma of pregnant women (11). Determination of maternal histaminase has been used as a clinical guide in cases of threatened abortion, as it has been shown that plasma histaminase activity increasing according to the normal pattern indicates a good prognosis, whereas a fall below the normal range presages spontaneous abortion (39). The meaning of the histaminase increase in human pregnancy is open to question. Smith (40) has demonstrated that placental histaminase is capable of oxidizing a number of amines, several aliphatic diamines are oxidized even faster than histamine. This implies a more fundamental significance of histaminase in cell metabolism.

Much evidence indicates that a conspicuously high histamine-forming capacity is present in many tissues undergoing rapid growth or repair, such as embryonic tissue, regenerating liver, bone marrow, wound (15, 41) and granulation tissue, and malignant growths in various species, principally rats (8, 13). This implies the nascent histamine has a role in anabolic processes. Other supporting evidence (42, 43, 44) that neonatal rat brain contains a considerably higher concentration of histamine than adult rat brain and has both histamine synthesizing and catabolizing enzymes as early as 3 days after birth, suggest a function for histamine in modulating the growth processes of the neonatal brain.

Finally, the possible role of endogenous histamine in the nervous system has been investigated. The effect of
histamine on neural tissues has recently been reviewed (20). Specific methods for measuring brain histamine show that some psychotropic drugs cause changes in histamine levels. Reserpine in the dose range 0.5 to 10 mg./kg. does not affect histamine level in the hypophysis but lowers it in other areas, in hypothalamus to about 40%, in thalamus to 64% of control (13, 20). Chloropromazine, when given three times at a dose of 50 mg./kg., increases histamine levels in hypothalamus by about 50% and in the medial thalamus by 32%, by inhibiting histamine methyltransferase (13, 20). The phenothiazines fail to raise histamine levels in the hypophysis, perhaps because most of the histamine is in the mast cells which do not methylate histamine at a measurable rate (45). Also, it is pertinent that several antihistamines had central actions. Furthermore, histamine has a variety of central effects, including stimulation of sympathetic centers and convulsions when introduced directly into the cerebral ventricles or into the brain tissue (21). All this evidence hints that histamine has important functions within the brain.

In the peripheral nervous system, it has been suggested that the vasodilatation that follows elevation of blood pressure might be due to a liberation of histamine from the sympathetic nerves (20). Ryan and Brody (29) recently provided supportive evidence for the postulate that histamine was liberated from sympathetic histaminergic fibers during reflex vasodilatation. Studies with the exogenous histamine, however, were in accord with the idea that histamine was the vasodilator.
Thus, Beck et al (46) observed that norepinephrine-induced reflex dilatation resulted from the increased neural release of histamine into the venous effluent of dog gracilis muscle. Green (20) suggested that some of the histamine that appeared to function in peripheral nerves might derive from mast cells. These observations lead to the conclusion that histamine is a neuromediator for the baroreceptor depressor reflex. The histamine liberated by cell injury has a role to play in the initiation of sensory impulse evoking pain and itch. Also, the analgesic effect of locally administered antihistamine drugs and of compound 48/80 (potent histamine liberator) suggest that histamine is the mediator for cutaneous pain.

In conclusion, the principal actions of histamine, in the ranking as we see them at present, namely the part played in gastric secretion and in metabolic processes of tissue growth and protein synthesis, are not obviated by the common antihistamines. Recently Black et al (129) have found that burimamide competitively antagonizes histamine-stimulated gastric secretion. Endeavours for decades have not succeeded in demonstrating histamine release in any purely physiological event. Now, at last, by sheer chance, a case of physiological histamine release has been shown to occur, namely a lowering of the gastric mucosal histamine content on feeding a rat deprived of food. Although the physiological role of histamine is still poorly understood, there is enough evidence to justify further investigation into the role of histamine in participating in every aspect of body economy (38).
16.

In the following discussion the now-classical involvement of histamine release from mast cells in various pathological processes, particularly in anaphylaxis, will be considered. Histamine release in anaphylaxis has recently been extensively reviewed (47, 48, 49, 50, 51 and 52). In brief, the anaphylaxis picture which emerges is that of a system activated by contact with the antigen to which the animal had been specifically sensitized by previous exposure. An antigen might be a polysaccharide or protein or a simple compound that is covalently bound to a protein (53). As a defense against the specific antigen, the mammalian organism manufactures a specific antibody from the globulin fraction of the blood. Renewed contact of antigen and antibody at a later date initiates a chain of events resulting in release of histamine, serotonin, a lipid slow-reacting substance (SRS), bradykinin (polypeptides) and other compounds from mast cell, basophilic leucocytes, and platelets (53). There is no general requirement for hemolytic complement in this reaction (50).

The reaction involves at least two steps, i.e., fixation of bivalent immunoglobulin and activation of processes leading to release of vasoactive mediators from the cell (Table 1) (52). The actions of various mediators, and thus the prominence of these manifestations of shock, varies from one animal species to another (50). For example, in dogs, the more pronounced effects of anaphylactic shock are hypotension, portal venous constriction, and hemorrhage of the G-I tract. In guinea-pigs, bronchoconstriction is most prominent, and
anaphylaxis in man might be manifested by a combination of urticaria, hypotension, and bronchoconstriction (50, 52).

**TABLE 1**

**PHARMACOLOGIC ACTIONS OF MEDIATORS OF ANAPHYLACTIC SHOCK**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Hypotension</th>
<th>Increased Capillary Permeability</th>
<th>Bronchoconstriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Serotonin</td>
<td>+ or -</td>
<td>+</td>
<td>+ or -</td>
</tr>
<tr>
<td>Slow-reacting substance (SRS)</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Note: + = present; - = absent

Some pharmacologically active agents that are stored in tissue mast cells have been implicated in anaphylactic shock. Mast cells contain proteolytic enzymes and have the capacity to form or liberate "slow-reacting substance" (SRS) (Table 2) (52). The amines are bound in inactive form to a complex of heparin and protein in mast cell granules (see previous discussion and Lit. (16)). SRS, a lipid spasmogen, has not been completely characterized, but it is distinct from prostaglandins (50, 52).

Release of histamine and other substances from mast cells can be induced by antigen-antibody reactions, by injury, or by various chemical agents (Table 3). The antigen-antibody interaction require fixation of "anaphylactic" (Immunoglobulin E, IgE) antibodies (50), but do not require complement (52).
<table>
<thead>
<tr>
<th>Granules</th>
<th>Particulate</th>
<th>Cytoplasm</th>
<th>Cell Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>Acid phosphatase</td>
<td>Lactic dehydrogenase</td>
<td>ATP</td>
</tr>
<tr>
<td>Serotonin (rat, mouse)</td>
<td>Alkaline phosphatase</td>
<td>Dopa decarboxylase</td>
<td>ATPase</td>
</tr>
<tr>
<td>Dopamine (hamster, ungulates)</td>
<td>Succinic dehydrogenase</td>
<td>Histidine decarboxylase</td>
<td></td>
</tr>
<tr>
<td>Heparin</td>
<td>Amine oxidase</td>
<td>5-Hydroxytryptophan decarboxylase</td>
<td></td>
</tr>
<tr>
<td>Chymotrypsin (rat)</td>
<td>Fumarase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trypsin (man, dog)</td>
<td>ATP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucineaminopeptidase (man, rat)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esteroprotease (mouse)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sulfurylase</td>
<td>SRS (?)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sulfokinase</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 3

**AGENTS THAT RELEASE SUBSTANCES FROM MAST CELLS**

<table>
<thead>
<tr>
<th>Selective</th>
<th>Non-selective</th>
<th>Undetermined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen-antibody</td>
<td>Tissue injury</td>
<td>Anaphylatoxin</td>
</tr>
<tr>
<td>Cationic proteins</td>
<td>Proteolytic enzymes</td>
<td>Bradykinin</td>
</tr>
<tr>
<td>Amines</td>
<td>Venoms</td>
<td>Endotoxin (?)</td>
</tr>
<tr>
<td>d-Tubocurarine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stilbamidine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymyxin B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound 48/80</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The reaction is selective in that constituents of the granules can be released without apparent damage to the cell membrane (60, 61). The release reaction can be blocked by low temperatures, by anoxia, and by a variety of other inhibitors of cell metabolism (Table 4) (54, 55). This has been taken as evidence

### TABLE 4

**AGENTS THAT INHIBIT HISTAMINE RELEASE AND MAST CELL DEGRANULATION INDUCED BY ANTIGEN AND 48/80**

<table>
<thead>
<tr>
<th>SH group inhibitors</th>
<th>NH$_2$ group inhibitors</th>
<th>Inhibitors of oxidative metabolism</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodoacetate</td>
<td>Ninhydrin</td>
<td>Anoxia</td>
<td>Cold (0-20°C)</td>
</tr>
<tr>
<td>p-Chloromercuribenzoate</td>
<td>Dinitrofluorobenzene</td>
<td>Cyanide</td>
<td>Heat (45°C)</td>
</tr>
<tr>
<td>N-ethylmaleimide</td>
<td>Phenylisocyanate</td>
<td>Dinitrophenol</td>
<td></td>
</tr>
<tr>
<td>o-Iodosobenzoate</td>
<td>Cyanate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
for an enzymatic process, but specific enzymatic pathways remain unidentified (52).

The most popular mechanism for release of histamine and other amines from mast cells by antigen has been proposed by Uvnas (56, 57 and 58) and has received supporting comments from other workers (21, 48, 51 and 52). Release of amines from mast cells involves at least two distinct steps, i.e., exocytosis of the granules and displacement of amines from heparin-protein matrix (16, 56). In the reaction of mast cells with antigen, peri-granular membranes become fused with each other and with the cell membrane so that the granule matrix is excluded from the interior of the cell (50, 59). Once the granules are exposed to the external medium through channels formed by fused membranes or by extrusion to the surface of the cells, the amines are rapidly displaced by cations from the extracellular fluid (Fig. 1) (16, 19, 52, 56).

The fundamental biochemical changes that induce this reaction are still obscure (52). The experimental findings so far have indicated that the biochemical mechanism is heat-sensitive, requiring calcium and narrow pH range for activity, is inhibited by SH reagents and other enzyme inhibitors, and lacks a requirement of complement (48). Probably deformation of the mast cell membrane by reaction of antigen with fixed antibody induce physical changes in the cell that stimulate rearrangement of intracellular membranes through one or more enzymatic steps, e.g. cleavage of ATP by membrane ATPase and liberation of calcium or other "intracellular messengers" (52).
Antigen interacts with specific immunoglobulins associated with mast cell membranes. Exocytosis (Step 1) exposes the mast cell granules to the external medium and results in formation of SRS. Amines are displaced from the granule matrix (Step 2) by cations in extracellular fluid.

FIG 1: General Mechanism of Histamine Release from Mast Cells.
B. HISTAMINE RECEPTORS

Little is known of the nature of the "receptors" for histamine in the different tissues, but two points are clear from studies of the structure-activity relationship and the effect of specific blocking agents (21). First, histamine receptors, whether on smooth muscles, capillary endothelium, nerves, ganglion cells, chromaffin cells, or gastric glands, are distinct from each of the various "receptors" activated by other autacoids such as the catecholamines, 5-HT, ACh, and the polypeptides bradykinin and angiotensin. Second, it is obvious that there is more than one pharmacologically identifiable histamine receptor. Ash and Schild (62) differentiate the histamine receptors into at least two classes. The specific antagonism of some actions of histamine by low concentrations of antihistamine drugs characterize one type of histamine receptor for which they suggest the symbol \( H_1 \). Such receptors occur in guinea-pig ileum and bronchi. Several other actions of histamine, for example, stimulation of gastric acid secretion, inhibition of rat uterus and stimulation of isolated atria, can not be antagonized by common antihistamines (129). These actions are likely to be mediated by non-\( H_1 \) receptors.

Histamine \( H_1 \) receptor was first envisaged by Rocha e Silva (63, 64). He proposed a model for histamine receptor in the ileum of the guinea-pig. The model was constructed on the basis of the following ideas then accepted: (a) the active form of histamine under physiological conditions was that in which hydrogen bonding was possible between the amine nitrogen
(N⁺) of the side chain and the pyridine nitrogen (N) of the imidazole ring; (b) the secondary anchorage group of histamine to fit its receptor site, could be the imine (=NH) radical of the imidazole ring and a carbonyl group in a polypeptide chain; (c) the pK of the receptor site was found to be around pH = 7.10-7.00 (this pH range was later confirmed by Ariens (81) and Gero (82)), thus suggesting a histidine moiety at the receptor site. The function of histamine would be to protonate the pyridine nitrogen of the receptor histidine, shifting the double bond and releasing a high energy radical hypothetically bound to the "pyrrole" nitrogen of the histidine moiety, as shown in Fig. 2.

FIGURE 2
Schematic representation of the receptor for histamine in the ileum of the guinea pig.

However, this model did not include the pyridine nitrogen of histamine which was important for H₁ histamine activity (3, 62). Kier (65) also pointed out in his molecular orbital calculations that the preferred conformations of
histamine could not form an intramolecular hydrogen bond between the side chain $N^+\text{-H}$ and the pyridine nitrogen ($N$) because of an unfavorable geometry and less than minimal energy consideration. Kier (65) further proposed a dual activity of histamine to be a consequence of the existence of the two preferred conformations in equilibrium. One of these conformations placed the quaternary nitrogen of the side chain and the pyridine nitrogen of histamine 4.55 Å apart, which was quite comparable to the 4.8 Å estimated for the internitrogen distance in the antihistaminic triprolidine. Therefore, this was the one specific for the histamine $H_1$ receptor. The second preferred conformation with an internitrogen distance of 3.60 Å was responsible for action other than those on the $H_1$ receptor such as stimulation of gastric acid secretion. For distinction, Kier designated the latter receptor as $H_2$. It needs a specific antagonist to define the structural characteristics of $H_2$ receptor. The recent finding of burimamide (129), which can competitively antagonize the actions of histamine on rat uterus and stomach, may help to unravel the histamine $H_2$ receptor.

In 1969 Rocha e Silva (66) improved his original $H_1$ receptor (63, 64) based on the findings by Kier (65). The model then suggested that histamine was attracted to the specific receptor site ($H_1$) by: (a) strong electrostatic interaction between the pyridine ($N^-$) nitrogen of the histidine moiety and the strongly charged protonated nitrogen ($N^+$) of the histamine cation, and (b) the reciprocally inverted dipoles in the peptide link of the receptor and the carbon ($C^+$)-
pyridine nitrogen (N\textsuperscript{-}) of the imidazole ring of the agonist (Fig. 3). These anchorage sites were almost the same as those proposed by Nauta et al (3).

FIGURE 3
Schematic representation of the forces involved in the interaction of histamine with its hypothetical receptor site. (E) electrostatic; (D-D) dipole-dipole interactions. Protonation of the pyridine (-N=) nitrogen of the histidyl moiety of the receptor would shift double bond to -C=N- position.

The French worker Allain (67) also proposed that histamine promoted the activation of a receptor by a transfer of a proton, very likely by accepting a proton from the receptor or, in a few cases, by giving a proton to the receptor. The analysis of the physico-chemical properties of histamine showed that it could exist in the body in tautomeric, ionic, and conformational forms (67). This fact might explain why histaminic receptors could not be absolutely similar. Allain's view gave some support for Kier's dual activity of
histamine at the receptors. Paiva et al (63) found, in aqueous solutions of histamine, that no intramolecular hydrogen bond was possible between the side chain amino group and an imidazole nitrogen atom.

However, Kier's work (65) has recently been severely criticized by Green et al (69). They (69) commented that Kier's work (65) was based on the extended Hückel method which has been described as giving "hopelessly inaccurate results" and "worthless as a procedure for predicting the structures or chemical behavior of molecules". Also, in Kier's work the dihedral angle was rotated every 60°, a procedure that could miss important conformers. In their studies the INDO (Intermediate Neglect of Differential Overlap) molecular orbital method was used. The total energy of the molecule was minimized as a function of two dihedral angles, $\theta_{4-\beta}$ and $\theta_{\alpha-\beta}$ for histamine (VII).

\[
\begin{array}{c}
\text{N} \\
\text{H} \\
\text{CH}_2-\text{CH}_2-\text{NH}_2 \\
\end{array}
\]

(VII)

The angles were rotated at 30°-intervals and when necessary every 15°. The result for histamine free base appeared that approximately 98% of the histamine would be in the configuration $\theta_{4-\beta} = 150^\circ$ and $\theta_{\alpha-\beta} = 330^\circ$. This conformation
would appear to be satisfactory for metal binding (Fig. 4)
and, moreover, rotation of one of the side-chain amino

FIGURE 4
Schematic representation of the binding
of a metal to one molecule of histamine.

hydrogens revealed that a suitable bond distance was attainable between this hydrogen and the N-3 of the imidazole ring to permit hydrogen bonding. An analysis of the histamine cation showed that one conformer had a substantially lower potential energy, namely, where \( \theta_{4-\beta} = 180^\circ \) (in the plane of the imidazole ring) and \( \theta_{\alpha-\beta} = 30^\circ \) or \( 330^\circ \) (the energy of the molecule was the same whether the quaternary nitrogen was \( 30^\circ \) above or below the plane of the imidazole ring). The quaternary nitrogen was close to N-3 of the imidazole ring to permit hydrogen bonding. This result was confirmed by Pullman et al (70) who reported that they found at physiological pH, in the cationic form, the folded structure of histamine seemed to be more stable; it included an intramolecular hydrogen bond.

Green et al (69) also found that the high frontier electron density of C-5 might permit interaction with an electron acceptor, e.g., the hydroxyl groups of heparin, an
OH-π interaction. Moreover, N-3 of the imidazole ring, being a locus of negative charge, might hydrogen-bond to N-1 of another imidazole ring (intermolecular hydrogen bond) (Fig. 5).

**FIGURE 5**

Intermolecular hydrogen bonding between two molecules of histamine.

![Intermolecular hydrogen bonding between two molecules of histamine.](image)

From the above evidence (Fig. 4) Green suggested that histamine was stored in the mast cell as a metal complex because of the fact that high concentration of zinc and iron had been found in the mast cells (compared with the previous discussion on histamine storage in mast cells, structure VI and Lit. (19)).

In conclusion, much work has to be done in the future to clarify the structure requirements at the histamine receptor sites.

As to the mechanism for the stimulant effect of histamine on smooth muscle, it has now been firmly established that an action on the cell membrane to facilitate calcium entry did explain the stimulant effect of histamine and other autacoids on smooth muscle contraction (21). Histamine
promoted calcium influx by actions on the membrane that resulted in increased permeability to ions (including calcium). The influx of calcium ions provided the immediate intracellular stimulus for activation of the actinmyosin system in smooth muscle (71).

Likewise, one could at present only speculate on the mode of action of histamine and other autacoids to produce smooth muscle relaxation, including vasodilatation. One permissible conjecture was that these agents somehow lowered calcium ion concentration, perhaps by diminishing membrane permeability and spontaneous influx, and thus relaxation (21).

C. ANTIHISTAMINES AND ANTIHISTAMINE RECEPTOR

Antihistamines are drugs with the ability to antagonize in varying degree most, but not all, of the pharmacologic actions of histamine (72). They fall into that large group of pharmacological antagonists that appear to act by occupying the receptor sites on the effector cells to the exclusion of the agonist (73). Apparently they bind with the histamine receptor without initiating a response. Most antihistamines act as competitive antagonists to histamine (73).

Antihistaminics seem to act as antiallergenic agents by more than one mechanism. The belief that they are antidotes to factors other than histamine in the allergic syndrome is supported by the overlapping of antihistaminic
and antiacetylcholine properties in many of these agents. Serotonin and bradykinin, known to be released during anaphylaxis in small animals, are also antagonized by some antihistaminics (53). However, the value of antihistamines in systemic allergies is variable (8, 21). The discovery that in anaphylaxis increased histamine formation takes place in all tissues investigated, and that the elevated HFC persists for a long time after histamine release has ceased, appear helpful in explaining the failure of histamine antagonists to afford protection in the later stage of anaphylaxis (8). Histamine antagonists do not interfere with actions exerted by histamine formed and acting within the cells. Moreover, in anaphylaxis agents other than histamine (e.g. SRS released in human lung) are believed to account for the symptoms which are not alleviated by histamine antagonists (8, 21, 53). Consequently, drugs such as theophylline, epinephrine and isoproterenol are necessary, acting directly to convert the bronchoconstriction to bronchodilatation (73).

Antihistamines act on the CNS to produce either a stimulant or depressive effect. Depression is the most common and is frequently used for its sedative effect as an adjunct to hypersensitivity therapy and or insomnia. Use of antihistamines in parkinsonism has led to lessened rigidity and improved spontaneous movement and speech. Theoretical discussions were presented recently (74) regarding the structural requirement for anticonvulsant activity in antihistaminic drugs. The effectiveness of different antihistamines in this disease roughly
paralleled their effectiveness in motion sickness. The anti-motion sickness action of certain antihistamine drugs appears to be more related to central anticholinergic activity than to peripheral antihistamine activity (73).

Maximum antihistaminic activity is found in the following structure (VIII), where $R_1$ and $R_2$ are aromatic or heteroaromatic rings, one of which may be separated from $X$ by a methylene group; however, work in this laboratory (1, 2) has shown that one of these aromatic rings may be replaced by an alicyclic system (e.g. cyclohexyl) and the activity is retained. $X$ is CO, N, or CH; $R_3$ is generally an ethylene group or a two-carbon fragment of a nitrogen heterocyclic system, and branching decreases activity. The aromatic rings $R_1$ and $R_2$ may or may not be ortho connected by $Y$, where $Y = \text{CH}_2$, a heteroatom, or $\text{CH}_2$-heteroatom. $R_4$ and $R_5$ are methyl groups, but a small planar cyclic group may be employed advantageously. The tertiary nitrogen is necessary since primary and secondary compounds are inactive and quaternary compounds tend to be anticholinergic.

The antihistamine receptor was first proposed by Nauta et al (3) in 1966 after their extensive studies on
alkyl substitutions at phenyl groups in diphenhydramine for antihistaminic and anticholinergic activities (75). They showed that one of the aryl group participated in an overlap interaction with the ether oxygen and also that it was this aryl group that was assumed to be more or less coplanar with the C-0 in order to have high antihistaminic activity. Supporting evidence was the potent antihistaminic tripolidine (IX) in which the pyridyl ring was coplanar with the olefinic bond (65); coplanarity was also known in the 2-(dimethylamino)-

![Chemical Structure IX](image)

ethylene ether of the rigid 10,11-dihydrodibenzo-(a, d)-cyclohepten-5-ol (X) which was 2-4 times more active than diphenhydramine (76). To account for their findings Nauta

![Chemical Structure X](image)
proposed an antihistamine receptor showing three anchorage sites to accommodate histamine or the diphenhydramine molecule:

(i) Histidine-$N_3$ in the receptor site was hydrogen-bonded with the protonated $NH_2$ and $N(CH_3)_2$ groups of the respective side chains;

(ii) Serine-OH was hydrogen-bonded with the $N_3$ atom of the histamine and the oxygen atom of the ether, respectively; and

(iii) Phenylalanine-phenyl group was in $\pi$-complexation with the imidazole ring of the histamine and one of the two aryl rings of the ether, respectively.

Recently, Kutter and Hansch (77) concluded through use of thermodynamically derived steric parameters ($E_s$) and hydrophobic constants ($\pi$) that substituents in the ortho and meta positions of the more highly substituted ring of diphenhydramine had parallel deactivating effects and substituents in the ortho and meta positions of the less substituted ring had little effect. Mono-para substitution had an activating effect up to an optimum size and then a deactivating effect; a second para substituent appeared to have a deactivating effect. These conclusions were in agreement with those of Nauta's (3).

Later, Nauta's research group (4) studied the thioether analogs of substituted diphenhydramine and found the in vitro antihistaminic activities of the thioether analogs were less than those of the corresponding $O$ analogs, whereas
the anticholinergic activities were greater. The decreased activity of the thio compounds may have two causes:

(i) Since the free electrons of sulfur showed little or no tendency to form associative structures, it was doubtful whether an overlapping interaction between these free electrons and the \( \pi \)-electrons of one of the aromatic rings, such as postulated in diphenhydramine, was a real possibility, here. If interaction did occur there were sound reasons for suspecting steric interference (bulk of the sulfur atom compared with oxygen) so as to lower the activity in the thio analogs.

(ii) Because hydrophilic bonds were less readily formed by sulfur than by oxygen, the thio compounds would be more weakly bound to the receptor than the ethers.

The differences in activity found in this series clearly supported the concept that in the diphenhydramines antihistaminic and anticholinergic activity was more or less complementary: factors that increased the antihistaminic activity lowered the anticholinergic effect, and vice versa. This complementarity between these two activities was also extendible to the chemical shift value of the central hydrogen atom (78) and to the configuration of the active optical isomers (79) of substituted diphenhydramines. The former (78) was shown by the fact that high antihistaminic activity was coupled with low shift value, whereas high anticholinergic activity was accompanied by a high shift value; and the latter
(79) by the fact that, in ortho-substituted compounds, the highest activities always resided in identical configurations, one predominantly antihistaminic and its antipode chiefly anticholinergic, whereas in the para derivatives, the same antipode was the more active one in both respects. The fact that stereoselective activity was observed only when the asymmetric carbon atom was α to the aromatic ring system indicated that regions adjacent to the histamine receptor ($H_1$) might also be asymmetric.

Further, antihistamines may be antagonists through a reversible allosteric type of molecular perturbation mechanism and may not be blocking by binding to the same site which binds histamine. Using bovine serum albumin (BSA) as a model in vitro system the binding of antihistamines and cortisol have been studied by employing "coupled" ion-exchange membrane electrodes (80). The data indicated that at appropriate concentrations antihistamines (e.g. diphenhydramine HCl) induced changes in BSA which prevented binding of histamine. It remained open to question how far BSA could be compared to the physiological receptor.
PART II
DISCUSSION OF THE CHEMISTRY

The present study is concerned with the synthesis of seven ethylenediamine derivatives (I) and two oxetane compounds (II and III). The ethylenediamine derivatives (I) have been investigated as potential antihistaminics and possible anticholinergic agents. The general reaction sequence for

\[
\begin{align*}
&\text{Cyclohexyl} - \text{R}_1 - \text{N} - \text{CH}_2 - \text{CH}_2 - \text{N} - \text{CH}_3 \\
&\text{R}_2
\end{align*}
\]

(I)

\[ R_1 = \text{CH}_2, R_2 = \text{(a) 2-pyridyl, (b) 2-pyrimidyl,} \]
\[ \text{(c) 2-pyrazyl, (d) 1-naphthyl,} \]
\[ \text{(e) 5-quinolyl.} \]

\[ R_1 = \text{C}, R_2 = \text{(f) 2-pyrimidyl, (g) 2-pyrazyl.} \]

the preparation of the ethylenediamine derivatives (I) started with the appropriate primary homo- and heterocyclic-aromatic amines. The primary amines were reacted with cyclohexanecarbonyl chloride to form the amides. Lithium aluminum
Hydride was used to produce the desired secondary amines by reduction of the amides. In cases where the primary amines did not form the amides or where the amides could not be reduced to the corresponding secondary amines, the primary aromatic amines were condensed with $\beta$-dimethylaminoethyl chloride to form the desired secondary amines. In the former case, the appropriate secondary amine was condensed with $\beta$-dimethylaminoethyl chloride to obtain the final ethylenediamine derivatives; while in the latter case, cyclohexylmethyl bromide was used to condense with the appropriate secondary amine to form the final products. Generally speaking, the reaction sequence involving reduction of the amides appeared to give the better yields.

Except for 3-aminopyridazine, the starting primary aromatic amines were all commercially available (e.g. 2-aminopyridine, 2-aminopyrimidine, 2-aminopyrazine, 1-naphthylamine, and 5-aminoquinoline). 3-Aminopyridazine was unstable as a free base but the mono-hydrochloride or mono-picrate was stable (83). Therefore, 3-aminopyridazine was prepared from 3,6-dichloropyridazine according to the methods of Steck and co-workers (84). Reaction of 3,6-dichloropyridazine (IV) with ammoniacal ethanol at 125-130°C gave 3-amino-6-chloropyridazine (V) (this reaction was performed in a pressure reaction apparatus (i.e. a bomb) instead of a shaking autoclave which was specified in Steck's paper (84)). Hydrogenolysis of (V) with palladium-charcoal catalyst produced 3-aminopyridazine as the mono-hydrochloride salt (VI). The salt (VI) was converted to
the free base and purified just before using for the next reaction because of its unstable nature. A lower yield was obtained when using 3,6-dibromopyridazine instead of IV (83).

\[ \text{Cl} \quad \text{Cl} \quad \text{NH}_3 \quad \text{NH}_2 \quad \text{NH}_2 \cdot \text{HCl} \\
\text{Cl} \quad \text{Cl} \quad \text{H}_2, \text{Pd} \quad \text{Cl} \quad \text{Cl} \quad \text{N} \quad \text{N} \\
(\text{IV}) \quad (\text{V}) \quad (\text{VI}) \]

After obtaining the appropriate primary aromatic amine, the next step was to react the amine with cyclohexanecarbonyl chloride. Cyclohexanecarboxylic acid was commercially available and was used to prepare the acid chloride. Three reagents are commonly used for this purpose: thionyl chloride, \( \text{SOCl}_2 \); phosphorus trichloride, \( \text{PCl}_3 \); and phosphorus pentachloride, \( \text{PCl}_5 \).

\[
\begin{align*}
\text{R-CO-OH} + \text{SOCl}_2 & \rightarrow \text{R-CO-Cl} + \text{SO}_2 + \text{HCl} \\
3\text{R-CO-OH} + \text{PCl}_3 & \rightarrow 3\text{R-CO-Cl} + \text{H}_3\text{PO}_3 \\
\text{R-CO-OH} + \text{PCl}_5 & \rightarrow \text{R-CO-Cl} + \text{HCl} + \text{POCl}_3
\end{align*}
\]

Thionyl chloride was chosen as the reagent for the preparation of cyclohexanecarbonyl chloride not only because the products formed besides the acid chloride were gases and thus easily separated from the acid chloride, but because the acid chloride formed was found to have higher boiling points than this reagent; any excess of the low-boiling thionyl chloride (b.p. 79°C, 1 atm.) was easily removed by distillation.
Two precautions were taken for running this reaction. The first was to protect the reaction and the acid chloride from moist air; the second was to avoid high temperatures which may cause pyrolysis of the acid chloride by using a water bath during the distillation.

2-Aminopyridine, 2-aminopyrazine, 1-naphthylamine, and 5-aminoquinoline readily formed amides with cyclohexanecarbonyl chloride in the presence of pyridine. Pyridine was added to neutralize the hydrogen chloride formed during the reaction. The cyclohexanecarboxamides thus formed were found to be water-insoluble, and on completion of the reaction, water was added to wash away the pyridine hydrochloride. In the case of 5-aminoquinoline it was found that the product amide could also abstract the hydrogen chloride through its N₁-quinoline. This meant that the N-5-quinolyl-cyclohexanecarboxamide-HCl salt and pyridine-HCl were both present in the water washings. The aqueous solution was, therefore, made basic with excess 10% NaOH solution, the crude amide (base) was precipitated out and could be separated from the pyridine. All the amides readily formed in good yields (Table 5).

\[
\text{C}_6\text{H}_{11}\text{C}=\!\!\text{O} + \text{R-NH}_2 \xrightarrow{\text{pyridine}} \text{C}_6\text{H}_{11}\text{C}=\!\!\text{O} + \text{Pyridine-HCl}
\]
40.

**TABLE 5**

**CYCLOHEXANECARBOXAMIDES**

<table>
<thead>
<tr>
<th>R</th>
<th>MELTING POINT (°C.)</th>
<th>% YIELD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Pyridyl</td>
<td>91.0-92.5</td>
<td>93.9 (New compd.)</td>
</tr>
<tr>
<td>2-Pyrazyl</td>
<td>165.9-166.8</td>
<td>91.5 (New compd.)</td>
</tr>
<tr>
<td>1-Naphthyl</td>
<td>189.6-190.8</td>
<td>90.5</td>
</tr>
<tr>
<td></td>
<td>(Lit. (85), 188°C.)</td>
<td></td>
</tr>
<tr>
<td>5-Quinolyl</td>
<td>202.9-204.0</td>
<td>86.3 (New compd.)</td>
</tr>
</tbody>
</table>

N-1-Naphthyl-cyclohexanecarboxamide had been prepared by Gilman and Furry (85) by reacting cyclohexylmagnesium bromide and 1-naphthyl isocyanate:

![Chemical Reaction](image)

The reaction between 2-aminopyrimidine and cyclohexanecarbonyl chloride was unsuccessful. However, some solid precipitates were obtained after washing away the pyridine-HCl. The crude solid precipitates were found to contain most of the unreacted starting 2-aminopyrimidine and some other products. To get rid of the starting material, the solid precipitates were suspended in 1000 ml. of water and the mixture was brought to pH 8-9 with dilute ammonium hydroxide solution (86). The insoluble product was again collected by filtration,
washed with cold water and was purified by recrystallization from n-hexane to constant melting point (120.5-122.3°C.). The IR of the purified product showed two carbonyl peaks: one at 1680 cm\(^{-1}\) corresponding to the secondary amide carbonyl band, and the other at 1740 cm\(^{-1}\) corresponding to the ketone carbonyl peak. The elemental analysis result suggested that it may have the following structural formula:

```
  N
 C\(\text{H}_2\text{N}\)C\(\text{H}_2\)N
  C
```

Calcd. for C\(_{18}\)H\(_{25}\)N\(_2\)O\(_2\): C, 68.54; H, 7.99; N, 13.33.
Found: C, 68.66; H, 7.81; N, 13.39.

The reaction between 3-aminopyridazine and cyclohexanecarbonyl chloride was not attempted, because some of the nitrogen-containing aromatic amides when reduced with lithium aluminum hydride did not form the corresponding secondary amine (see later discussion) (87).

In order to obtain the secondary aromatic amine, the corresponding cyclohexanecarboxamide was reduced with lithium aluminum hydride. The reagent discovered by Finholt, Bond and Schlesinger (88) in 1947 (4LiH + AlCl\(_3\) \(\text{ether}\) \(\rightarrow\) LiAlH\(_4\) + 3LiCl) has proved to be a remarkable reducing agent for the carbonyl group in amides and similar carbonyl compounds (89). Amides were not readily reducible to pure amines by other chemical methods. Hydrogenation with a catalyst at high temperatures and pressures could be accomplished, but
usually resulted in a mixture of products.

The powdered LiAlH₄ can be safely handled, even in very humid air, probably because of the formation of a protective coating of aluminum hydroxide (88). It is generally used in solution or suspension in dry ether (25-30 g, solid hydride dissolves in 100 g ether at 25°C). In the normal procedure the substance to be reduced is added to an ethereal solution or slurry of the hydride. If the substance to be reduced is an ether-soluble liquid or solid, the solution is added dropwise to produce gentle reflux. For moderately soluble materials, a Soxhlet extractor or a continuous-return type of extractor is used.

In the reduction of the amides, an excess (2- to 3-times the stoichiometrical quantities) of LiAlH₄ was used. Water was then added to destroy the excess hydride with the

\[
2 \overset{\text{NHR}}{\text{C}} = 0 + \text{LiAlH}_4 \rightarrow 2 \overset{\text{CH}_2 - \text{NHR}}{\text{C}} = 0 + \text{LiAlO}_2
\]

evolution of hydrogen, and the precipitation of lithium- and aluminum-hydroxide.

\[
\text{LiAlH}_4 + 4\text{H}_2\text{O} \rightarrow \text{LiOH} + \text{Al(OH)}_3 + 4\text{H}_2 \uparrow
\]

As the amine was ether soluble, the mixture was treated with strong hydroxide solution to dissolve the precipitated alumina. This allowed a clear-cut separation of phases on centrifugation.

N-2-Pyridyl-cyclohexanecarboxamide, N-1-naphthyl-
cyclohexanecarboxamide and N-5-quinolyl-cyclohexanecarboxamide were found to be readily reduced to the corresponding secondary amine (Table 6). However, when N-2-pyrazyl-cyclohexanecarboxamide was reduced with excess lithium aluminum hydride,

**TABLE 6**

SECONDARY AROMATIC AMINES OF CYCLOHEXYLMETHYL DERIVATIVE.

<table>
<thead>
<tr>
<th>R</th>
<th>MELTING POINT °C.</th>
<th>BOILING POINT °C. (mm. Hg.)</th>
<th>% YIELD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Pyridyl</td>
<td>91.2-92.2</td>
<td>---</td>
<td>98.2 (New compd.)</td>
</tr>
<tr>
<td>1-Naphthyl</td>
<td>---</td>
<td>149-152 (0.25)</td>
<td>86.4 (New compd.)</td>
</tr>
<tr>
<td>5-Quinolyl</td>
<td>---</td>
<td>186-190 (0.7-0.75)</td>
<td>83.3 (New compd.)</td>
</tr>
</tbody>
</table>

Instead of the corresponding secondary amine a mixture of cyclohexylcarbinol and 2-aminopyrazine was obtained.

![Chemical diagram]

Similar reactions were found in the following three examples (87):

1) ![Chemical diagram]
Upon obtaining the appropriate secondary aromatic amines (Table 6), the next portion of the molecule to be attached was the \( \beta \)-dimethylaminoethyl side chain to completely form the final tertiary ethylenediamine products. Our previous work (2) had shown that the cycloalkyl analogues of Antergan could be synthesized in good yield through the following reaction pathways.
The secondary aromatic amines we obtained in Table 6 were similar in structure to (VII) except that the aromatic group was different. Except for the 1-naphthyl derivative, all the others contained nitrogen heteroatom(s) in the aromatic rings. An attempt was made to get 2-cyclohexylmethylaminopyridine to react with chloroacetyl chloride under the same conditions as (VII) in the above scheme. However, a tarry residue soon resulted after the addition of chloroacetyl chloride with no desired product formation:

\[
\begin{align*}
2 & \quad \text{CH}_2\text{N} & \quad \text{CH}_2\text{Cl-COCl} & \quad \text{No product} \\
& \quad \text{Ice-salt bath} \\
\end{align*}
\]

This could be due to the extra nitrogen present in the aromatic ring (pyridine-$N_1$ atom) which may serve as a hydrogen chloride acceptor or may react with chloroacetyl chloride.

Leung (1) had succeeded in obtaining the teriary
diamine (VIII) by condensing the secondary amine (VII) with 
\( \beta \)-dimethylaminoethylbromide HBr in the presence of sodamide 
but the yield was very poor (21.8%).

\[
\text{CH}_2\text{NH} + \text{Br-CH}_2\text{CH}_2\text{-N} < \text{CH}_3\cdot\text{HBr} \rightarrow 2 \text{NaNH}_2
\]

\[
\text{(VII)} \quad \text{(VIII) 21.8% yield}
\]

The poor yield could be due to the unstable character of the 
free base of \( \beta \)-dimethylaminoethylbromide in the presence of 
the strong base sodamide.

Hutter and co-workers (90) in their original syn-
thesis of Neo-Antergan (X) used a small excess of lithium amide 
to react with p-methoxybenzylaminopyridine (IX) first, and a 
few hours later the free base of \( \beta \)-dimethylaminoethyl chloride 
equal to the stoichiometrical quantity of lithium amide was 
added to the reaction mixture. The condensation effectiveness 
was greatly increased and their yield for Neo-Antergan was ex-
cellent (81%). Other later workers (91, 92) also employed 
the free base of \( \beta \)-dimethylaminoethyl chloride to synthesize 
the secondary aromatic amines bearing the pyridine (91) and 
pyrimidine rings (92), and their results were all better than 
that of Leung's (1).
The free base of \( \beta \)-dimethylaminoethyl chloride was unstable in the air and dimerized easily to become a precipitate which made the clear base solution cloudy. However, if the free base was freshly distilled from its hydrochloride salt and flake sodium hydroxide (93) and stored in an ice-bath or in the refrigerator, it was quite stable for some time.

For all the reasons cited above, the secondary aromatic amines (Table 6) were condensed with the freshly distilled \( \beta \)-dimethylaminoethyl chloride using sodamide as condensing agent. Hutterer's (90) and Adams' methods (92) were followed and a stream of dry nitrogen gas was bubbled through the reaction mixture so as to prevent the sodamide from reacting with atmospheric carbon dioxide and more important to drive away the ammonia gas formed in favor of the desired reaction.

The sodium salts of the secondary amines (XI) were insoluble in dry benzene or toluene and could be isolated from the solution if adequate precautions were taken to prevent contact
from the moist air (e.g. worked up in a dry box under dry nitrogen atmosphere). For the present purpose, the sodium salts did not have to be isolated, the freshly distilled $\beta$-dimethylaminoethyl chloride in a little dry benzene or dry toluene was added to the reaction mixtures (at room temperature) and then refluxed for 24 hours. The tertiary amines (XII) were distilled at reduced pressure and obtained as a light yellow or yellow oil in relatively good yield (Table 7).

![Chemical structure of XII](image)

**TABLE 7**

<table>
<thead>
<tr>
<th>R</th>
<th>BOILING POINT °C. (mm.Hg.)</th>
<th>% YIELD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Pyridyl</td>
<td>145-146.5 (1.0-1.1)</td>
<td>83.1</td>
</tr>
<tr>
<td></td>
<td>(Lit. (94), 160-165 (13 mm.))</td>
<td></td>
</tr>
<tr>
<td>1-Naphthyl</td>
<td>188-190 (1.5)</td>
<td>71.2</td>
</tr>
<tr>
<td>5-Quinolyl</td>
<td>164-168 (0.15-0.2)</td>
<td>60.9</td>
</tr>
</tbody>
</table>

Kyrides and co-workers (94) had obtained the tertiary diamine, $N,N$-dimethyl-$N'$-cyclohexylmethyl-$N'$-2-pyridyl-ethylenediamine (i.e. XII, $R=2$-pyridyl), as a trihydrochloride salt. However, their elemental analysis result showed only
the chloride percentage, they did not mention how to obtain the trihydrochloride salt and the solvent used for recrystallization, and their starting materials were different. They used \( N,N\text{-dimethyl-N'-2-pyridylethylenediamine (XIII) to condense with cyclohexylmethyl bromide, a method which was found to give poor yields (usually 10-40\%) in a similar reaction (see later discussion). Also, it was found that the hydrochloride salt of the tertiary diamine was too hygroscopic to handle and a suitable solvent or solvent pairs for recrystallization could not be found. Therefore, the diperchloric acid salt derivative was prepared.

\[
\text{[CH}_2\text{Br + }\text{HN-CH}_2\text{-CH}_2\text{-N-C\text{-CH}_3 \xrightarrow{\text{NaNH}_2 \text{ or LiNH}_2}}}\text{CH}_3\text{ (XIII) }
\]

For those other compounds that could not form the secondary aromatic amines of cyclohexylmethyl derivative (Table 6), usually two successive condensation reactions using Adams' method (92) were required to obtain the desired tertiary diamine products from the primary aromatic amines. The overall results obtained showed much lower yields than the above synthetic method (Table 5, 6 and 7).

The appropriate primary aromatic amines were reacted with freshly prepared \( \beta\text{-dimethylaminoethyl chloride using} \)
sodamide as condensing agent. The secondary amines were obtained as a yellow oil (Table 8).

\[
\text{R-NH}_2 + \text{Cl-CH}_2\text{-CH}_2-\text{N-CH}_3 \xrightarrow{\text{NaNH}_2} \text{R-N-CH}_2\text{-CH}_2-\text{N-CH}_3
\]

\(\text{(XIV)}\)

<table>
<thead>
<tr>
<th>R</th>
<th>BOILING POINT °C. (mm.Hg.)</th>
<th>% YIELD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Pyrimidyl</td>
<td>94 (0.9)</td>
<td>37.1</td>
</tr>
<tr>
<td></td>
<td>(Lit. (90), 85-90 (0.02));</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Lit. (90), 24.0; (92), 90-95 (3.0))</td>
<td></td>
</tr>
<tr>
<td>2-Pyrazyl</td>
<td>105-106 (0.95)</td>
<td>32.4 (New compd.)</td>
</tr>
<tr>
<td>3-Pyrizidazyl</td>
<td>107-108 (1.1)</td>
<td>25.1 (unstable)</td>
</tr>
</tbody>
</table>

The N,N-dimethyl-N'-3-pyridazylethylenediamine (XIV, R = 3-pyridazyl) was not stable in the air, but the NMR of freshly distilled product (Fig. 6) looked similar to that of N,N-dimethyl-N'-2-pyrazylethylenediamine (Fig. 7). The -NH-proton of either amine was exchangeable with deuterium when D₂O was added. However, when efforts were made to purify the compound by column chromatography (silica gel), the amine was decomposed. The unstable character of 3-aminopyridazine had been described (83)
FIG. 6: NMR spectrum of freshly distilled N,N-dimethyl-N'-3-pyridazylethylenediamine dissolved in CDCl$_3$ (10%). The integration was done after D$_2$O added.
FIG. 7: NMR spectrum of N,N-dimethyl-N'-2-pyrazylethlenediamine dissolved in CC14 (10%).
and its secondary amine derivative (XIV, R = 3-pyridazyl) appeared to be even more unstable.

In order to obtain the final tertiary diamine products, another condensation reaction was required. The appropriate secondary amine (Table 8) was condensed with cyclohexylmethyl bromide in the presence of sodamide. The yellow oily liquid tertiary diamines (XV) were obtained by vacuum-distillation (Table 9).

\[
\text{H} \quad \text{R-N-CH}_2\text{-CH}_2\text{-N} \quad \text{CH}_3 \quad + \quad \text{Br-CH}_2\text{-CH}_2\text{-N} \quad \text{CH}_3 \quad \text{NaNH}_2 \quad \rightarrow
\]

(XIV)

\[
\text{CH}_2\text{-N-CH}_2\text{-CH}_2\text{-N} \quad \text{CH}_3 \quad \text{R}
\]

(XV)

**TABLE 9**

TERTIARY ETHYLENEDIAMINE DERIVATIVES (XV)

<table>
<thead>
<tr>
<th>R</th>
<th>BOILING POINT °C. (mm.Hg.)</th>
<th>% YIELD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Pyrimidyl</td>
<td>129-130 (0.5)</td>
<td>10 (New compd.)</td>
</tr>
<tr>
<td>2-Pyrazyl</td>
<td>126-136 (0.25-0.35)</td>
<td>38.6 (New compd.)</td>
</tr>
</tbody>
</table>

Owing to the low yields of the tertiary diamines (XV, Table 9) efforts were made to increase the yields by other synthetic routes. Since the aromatic secondary amines of cyclohexylmethyl derivative (Table 6) seemed to condense better with \(\beta\)-dimethylaminoethyl chloride (Table 7), 2-amino- pyrimidine condensed first with cyclohexylmethyl bromide and
then with $\beta$-dimethylaminoethyl chloride. However, the result from the reaction between 2-aminopyrimidine and cyclohexylmethyl bromide also gave a low yield of product (20%).

The secondary aromatic amines (Table 8, XIV, $R=$ 2-pyrimidyl, and 2-pyrazyl) were reacted with cyclohexanecarbonyl chloride and then the amides were reduced to produce the corresponding tertiary diamine products as listed in Table 9. It was found that the amides (XVI) readily formed in moderate yields (Table 10).

![Chemical Structures](image_url)

**TABLE 10**

**TERTIARY CYCLOHEXANECARBOXAMIDES (XVI)**

<table>
<thead>
<tr>
<th>$R$</th>
<th>BOILING POINT °C. (mm. Hg.)</th>
<th>% YIELD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Pyrimidyl</td>
<td>137-141 (0.35)</td>
<td>50.1 (New compd.)</td>
</tr>
<tr>
<td>2-Pyrazyl</td>
<td>130 (0.20)</td>
<td>47.6 (New compd.)</td>
</tr>
</tbody>
</table>
However, when the amides (XVI) were reduced with excess lithium aluminum hydride, the same thing happened as when N-2-pyrazyl-cyclohexanecarboxamide was reacted (see previous discussion) and a mixture of cyclohexylcarbinol and the respective secondary amine (XIV) was obtained instead of the corresponding tertiary diamines (Table 9).

\[
\text{R-N-CH}_2-\text{CH}_2-N-\text{CH}_3 \xrightarrow{\text{Excess LiAlH}_4} \text{CH}_2\text{OH} + \text{R-N-CH}_2-\text{CH}_2-N-\text{CH}_3
\]

(XIV)

Finally, the two oxetane derivatives (II and III) synthesized for the present study were two intermediates and not the final products for antihistaminic study purpose. The desired products (XVII and XVIII) were oxetane analogues of the potent antihistamine, diphenhydramine (XIX).
Aromatic and aliphatic ketones and aldehydes may form oxetanes on irradiation in the presence of olefins (95). Although the yields of oxetanes are generally only about 5 - 10% and the reaction is very slow, the simplicity of the method makes it useful. In addition, photocycloaddition is frequently the method of choice for the preparation of oxetanes since they can be difficult to prepare by more classical methods.

The possible mechanism for the photochemical synthesis of oxetanes has been discussed but not systematically studied. Buchi has suggested a step-wise process, initiated by photoactivation of the carbonyl compound to the diradical \( n,\pi^* \) triplet state (96). Subsequent addition to the olefin would give 1,4-diradical intermediates, followed by ring closure. As it seems reasonable that the most stable diradical would be that with the unpaired electrons on the most substituted carbon atoms (in other words, the addition reaction took place with the oxygen atom becoming attached to the less-substituted of the doubled bonded carbon atoms), this mechanism
appears to be in agreement with the structures of the oxetanes obtained (Note: there are, however, exceptions).

The initial excitation is of the carbonyl compound, rather than the olefin (97), since the reaction can be brought about by irradiation in a region where only the carbonyl compound absorbs. The simple olefins are essentially transparent at wavelengths longer than 250 μm. In order to excite the carbonyl compound exclusively without affecting the olefin molecule, a pyrex filter was used to filter out all light below 290 μm (=2900 Å).

In addition to the usually desired characteristics, solvents for the photocycloaddition reaction should be
(a) transparent to the wavelengths used (usually > 300 μm),
(b) inert (no easily abstractable hydrogen atoms), and
(c) have no quenching properties. Table 11 indicates the variety of solvents that have been used successfully for the photocycloaddition reaction.
**TABLE 11**

SOME SOLVENTS WHICH HAVE BEEN USED SUCCESSFULLY FOR THE PHOTOCYCLOADDITION REACTION

<table>
<thead>
<tr>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
</tr>
<tr>
<td>Pyridine</td>
</tr>
<tr>
<td>Acetic acid</td>
</tr>
<tr>
<td>Acetonitrile</td>
</tr>
<tr>
<td>Excess olefin (2-methyl-2butene, cyclohexene, etc.)</td>
</tr>
<tr>
<td>Excess carbonyl compound (acetone, perfluorocarbonyl compds.)</td>
</tr>
<tr>
<td>Saturated hydrocarbons (hexane, pentane, etc.)</td>
</tr>
<tr>
<td>Ethers (dioxane)</td>
</tr>
</tbody>
</table>

In the present study, two oxetane derivatives (II and III) have been synthesized (130). 2,2-Diphenyl-3-chlorooxetane (II) was obtained by photolyzing the benzophenone (through a pyrex filter) in the presence of vinyl chloride using benzene as solvent. The process of the reaction was followed by TLC analysis of aliquots and by IR which indicated the decreased benzophenone carbonyl absorption band at 1670 cm$^{-1}$. The formation of the oxetane ring was indicated by the presence of a strong absorption band at 980 cm$^{-1}$. Guepet and co-workers (98) have shown that the bands corresponding to the symmetrical and anti-symmetrical vibrations of the oxetane ring, affecting the C-O-C bond, have a constant frequency within the limits 960 - 980 cm$^{-1}$, regardless of the substituents on the ring. The isomer obtained was assigned the structure (II) on the basis of NMR data. High chemical shift values for the C$_4$-methylene hydrogens ($\delta$4.80-5.02 ppm) is in accord with being adjacent to the ether oxygen (99).
The same method was used to irradiate benzophenone in the presence of N-vinyl-2-pyrrolidinone and 2,2-diphenyl-3-(pyrrolidin-2-one-1-yl)oxetane (III) was formed in 1.3% yield. Although oxetane (III) was synthesized independently in our laboratory, Ogata and co-workers (99) published the synthesis.

Ogata (99)
In order to obtain the desired oxetane end products (XVII and XVIII), a number of photosynthetic reactions were attempted. However, due to the easy ring-opening of the oxetanes by common chemical reactions and to the major difficulty which was usually encountered during efforts to isolate and purify the products, the results were unsuccessful. The unsuccessful reactions are listed in the following equations for the reference of future workers.

1) \[
\text{aryl Cl} + \text{NH}_3 \xrightarrow{\text{Neat}} \text{Ring opened} \]
   \[
   + \text{NaNH}_2 \xrightarrow{\text{In xylene}} \text{Ring opened} \]
   \[
   \xrightarrow{\text{Mg, dry ice}} \text{No reaction} \]

2) \[
\text{(C}_6\text{H}_5\text{)}_2\text{C}=\text{O} + \text{CH}_2=\text{CH}-\text{CH}_2-\text{N}^+\text{CH}_3 \xrightarrow{\text{hv in AcOH}} \text{Seemed to have oxetane formation but could not isolate them.} \]

3) \[
\text{(C}_6\text{H}_5\text{)}_2\text{C}=\text{O} + \text{CH}_2=\text{CH}-\text{CH}_2-\text{N}^-\text{H} \xrightarrow{\text{hv in AcOH}} \text{Seemed to have oxetane formation but could not isolate them.} \]

4) \[
\text{(C}_6\text{H}_5\text{)}_2\text{C}=\text{O} + \text{CH}_2=\text{CH}-\text{CH}_2-\text{Cl} \xrightarrow{\text{hv in Benzene}} \text{Seemed to have oxetane formation but could not isolate them.} \]

5) \[
\text{(C}_6\text{H}_5\text{)}_2\text{C}=\text{O} + \text{H}_2\text{C}=\text{C}^+\text{Br} \xrightarrow{\text{hv in Benzene}} \text{No oxetane formation} \]
In conclusion, the compounds in Table 5 – 10 as well as two oxetane compounds (II and III) were synthesized in the present study. If the compounds were solid, pure and recrystallized substances were analyzed for percentage composition of carbon, hydrogen, nitrogen and halogen. No derivatives were made. For those liquid compounds, hydrochloride-, picrate- or perchlorate-derivatives were made for elemental microanalysis. It was found that most of the perchlorate derivatives had a large melting point range (more than 2°C.) but nevertheless, they were pure compounds according to the elemental analysis results. To supplement the analytical results of a derivative, an infrared spectrum and NMR spectrum were also taken on the synthesized compound.
PART III
ANALYTICAL METHODS

Melting points were determined using a Thomas-Hoover Capillary Melting Point Apparatus (Arthur H. Thomas Co., PA., U.S.A.). All melting points and boiling points are reported uncorrected.

A Beckman IR-10 Infrared Spectrophotometer (Beckman Instruments, Inc., California, U.S.A.) was used to record the infrared spectra.

The NMR spectroscopy was performed by Miss Phyllis Watson of the Department of Chemistry, U.B.C., using a Varian A-60, T-60 or XL-100 Spectrometer. The concentration of solutions was about 10% and tetramethylsilane served as the internal standard. Solvents are specified.

Elemental microanalyses were performed by Alfred Bernhardt, Mikroanalytisches Laboratorium, 5251 Elbach über Engelskirchen, Fritz-Pregl-Straße 14-16, West-Germany.

Cumulative log dose-response curves were plotted by using a Calcomp Plotter with the aid of an IBM 360/67 Computer (see Appendix A for an example for plotting 3 figures, and the plotting routine, Superplot, which was used for plotting). The pD₂, pA₂ and pD₂ values were calculated by using the WANG's advanced programming calculator (600 series) (see Appendix B for Program Title: Mean, Variance, Standard Deviation (Ungrouped Data)).
PART IV

EXPERIMENTAL\(^{(a)}\)

A. Synthesis of \(N,N\)-Dimethyl-\(N^\prime\)-Cyclohexylmethyl-\(N^\prime\)-2-Pyridylethlenediamine:

1. Cyclohexanecarbonyl Chloride:

Cyclohexanecarboxylic acid (128 g., 1.0 mole) was placed in a 1-liter, three-necked flask fitted with a reflex condenser and drying tube, a 250 ml.-dropping funnel and a mechanical stirrer. Thionyl chloride (238 g., 2.0 moles) was added dropwise with stirring. The flask was placed on a heating mantle and heated at reflux for 1.5-2 hours. After that time, the mixture was distilled, and the crude product collected from 160-185°C. This fraction was redistilled under reduced pressure and the fraction boiling at 82-85°C. (14-15 mm.) was collected to yield 118 g. (0.8 mole, 80.5%) of product (Lit. (100), b.p. 67-67.5°C. (14 mm.), 81%; (101), b.p. 76°C. (17 mm.)).

2. \(N\)-2-Pyridyl-Cyclohexanecarboxamide:

A mixture of 2-aminopyridine (75.3 g., 0.8 mole), pyridine (63.2 g., 0.8 mole), and dry benzene (120 ml.) was placed in a 1-liter three-necked flask fitted with a mechanical stirrer, a reflux condenser (drying tube) and a dropping funnel (250 ml.). The mixture was cooled in an ice-bath and cyclohexanecarbonyl chloride (118 g., 0.8 mole) was added

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\(^{(a)}\) A table of all the chemicals and reagents used for the EXPERIMENTAL PART together with their grades and company names was listed in Appendix C.
dropwise with stirring. After the addition was complete, the reaction was refluxed with stirring for 1 hour, cooled and washed with water (150 ml.). The solid which precipitated was suction filtered and the layers were separated. The aqueous phase was extracted with two 100 ml. portions of ether. The ether extracts were combined with the benzene layer, and dried over anhydrous sodium sulfate. The solvents were removed by flash evaporation to recover more of the amide in a total yield of 153.3 g. (0.75 mole, 93.9%). The amide was recrystallized from benzene-petroleum ether (b.p. 30-60°C.) mixtures; m.p. 91.0-92.5°C.

Infrared spectrum of the solid amide (KBr) showed a strong C=O stretching band at 1675 cm\(^{-1}\) and the characteristic cyclohexyl C-H stretching vibration at 2750 cm\(^{-1}\) and 2820 cm\(^{-1}\); also the absence of N-H stretching for 2-amino-pyridine at 3300 cm\(^{-1}\) and 3440 cm\(^{-1}\) indicated the amide structure. NMR signals (60 MHz, CDCl\(_3\)): \(\delta 0.90\) - 2.50 ppm (multiplet, 11 H, C\(_6\)H\(_{11}\)), 6.82 - 8.40 ppm (multiplet, 4 H, C\(_5\)H\(_4\)N) and 8.70 ppm (singlet, 1 H, NH).

**Anal.** Calcd. for C\(_{12}\)H\(_{16}\)N\(_2\)O: C, 70.55; H, 7.90; N, 13.72. Found: C, 70.74; H, 7.75; N, 13.91.

3. **2-Cyclohexylmethylaminopyridine:**

To a 2-liter three-necked flask fitted with a dropping funnel (500 ml.), a reflux condenser (drying tube) and a mechanical stirrer was placed 1 liter of dry ether. Lithium aluminum hydride (38 g., 1 mole) was added to the ether, and the mixture was gently refluxed with stirring for
4 hours. The mixture was cooled to room temperature and a solution of N-2-pyridyl-cyclohexanecarboxamide (102 g., 0.5 mole) in dry ether (700 ml.) was added from the dropping funnel at such a rate as to maintain gentle reflux. After the addition was complete, the mixture was stirred and refluxed for 3 days. At the end of this time, the reaction was cooled in an ice-bath and 100 ml. of water was added slowly to decompose the excess hydride; then sufficient 40% NaOH solution was added to allow complete separation. The ethereal layer was separated by decantation and excess benzene solvent (800 ml.) was used to extract more of the amine from the white solid residues. The organic layers were combined and dried over anhydrous sodium sulfate and reduced in volume to yield 93.3 g. (0.49 mole, 98.2%) of product. The crude product was re-crystallized from n-hexane; m.p. 91.2-92.2°C.

Infrared spectrum for the solid amine (KBr) showed the absence of the C=O band at 1675 cm.\(^{-1}\) indicating complete reduction of the amide. NMR signals (60 MHz, CDCl\(_3\)): 0.60-2.00 ppm (multiplet, 11 H, C\(_6\)H\(_{11}\)), 2.90-3.20 ppm (triplet, 2 H, CH\(_2\)), 4.50 ppm (singlet, 1 H, NH), and 6.10-6.60 ppm, 7.10-7.50 ppm and 7.95-8.10 ppm (multiplet, 4 H, C\(_5\)H\(_4\)N).

Anal. Calcd. for C\(_{12}\)H\(_8\)N\(_2\): C, 75.74; H, 9.53; N, 14.73. Found: C, 75.58; H, 9.45; N, 14.62

4. N,N-Dimethyl-N'-Cyclohexylmethyl-N'-2-Pyridylethylene-diamine:

A mixture of 2-cyclohexylmethylaminopyridine (38 g., 0.2 mole), sodium amide (11.7 g., 0.3 mole) and dry benzene
(300 ml.) was placed in a 0.5-liter three-necked flask fitted with a mechanical stirrer, reflux condenser (drying tube), a dropping funnel (250 ml.) and a dry nitrogen gas inlet tube. The mixture was bubbled with dry nitrogen gas and refluxed for two hours. The mixture was then allowed to cool and the nitrogen was discontinued. A solution of β-dimethylaminoethyl chloride (32.3 g., 0.3 mole) freshly distilled from its hydrochloride salt and flake sodium hydroxide (Lit. (93); (102), b.p. 109°C. at 750 mm.), in 70 ml. of dry benzene was added from the dropping funnel to the mixture which was then refluxed for another 24 hours. The mixture was cooled and filtered and the solvent was removed by flash evaporation. The residue was fractionated under reduced pressure. The tertiary amine was obtained as a light yellow oil, b.p. 145-146.5°C. at 1.0-1.1 mm., yield 43.4 g. (0.17 mole, 83.1%). (Lit. (90); (94), b.p. 160-165°C. at 13 mm.).

A tertiary amine (neat) was indicated from the IR spectrum by the absence of N-H stretching band at 3250 cm\(^{-1}\) and the presence of the characteristic C-H stretching for R-N(CH\(_3\))\(_2\) at 2840 cm\(^{-1}\), 2790 cm\(^{-1}\), 2750 cm\(^{-1}\). NMR signals (60 MHz, CDCl\(_3\)): 0.40-1.80 ppm (multiplet, 11 H, C\(_6\)H\(_{11}\)), 2.22 ppm (singlet, 6 H, N(CH\(_3\))\(_2\)), 2.40-2.55 ppm (doublet, 2 H, CH\(_2\)), 3.20-3.78 ppm (two triplet, 4 H, (CH\(_2\))\(_2\)), and 6.30-6.50, 7.20-7.50 and 8.05-8.20 ppm (multiplet, 4 H, C\(_5\)H\(_4\)N).

**Di-perchlorate Derivative:**

A solution of the tertiary amine (2.61 g., 0.01 mole) in 15 ml. of 95% ethanol was treated with a solution of
70% perchloric acid (1.44 g.) in 5 ml. of 95% ethanol (103). The clear reaction mixture was saturated with excess dry ether to force the perchloric acid salt to precipitate out. After standing overnight in the refrigerator, the mixture was filtered and the salt recrystallized from dry ethanol and dry ether (using a water bath at 65°C); m.p. 87-110°C.

Anal Calcd. for C16H29N3Cl2O8: C, 41.56; H, 6.32; N, 9.09; Cl 15.34. Found: C, 41.76; H, 6.50; N, 8.89; Cl 15.10.

B. Synthesis of N,N-Dimethyl-N'-Cyclohexylmethyl-N'-2-Pyrimidylethylenediamine:

1. 2-Cyclohexylmethylaminopyrimidine

A mixture of 2-aminopyrimidine (9.5 g., 0.1 mole), sodium amide (5.85 g., 0.15 mole) and dry toluene (150 ml.) was placed in a 250-ml. three-necked flask fitted with a mechanical stirrer, reflux condenser (drying tube), a dropping funnel (125 ml.) and a dry nitrogen gas inlet tube. The mixture was bubbled with dry nitrogen gas and refluxed at 125°C. in an oil-bath for 10 hours. The mixture was cooled and the nitrogen flow discontinued. A solution of cyclohexylmethyl bromide (17.7 g., 0.1 mole) in 20 ml. of dry toluene was added from the dropping funnel. The mixture was refluxed and stirred for 16 hours, cooled and washed with 100 ml. of water. The toluene layer was separated and the water layer saturated with potassium carbonate and extracted three times (3 x 75 ml.) with ether. The combined extracts and the toluene layer were distilled through a Claisen flask to get rid of the excess 2-aminopyrimididine and the residue fractionated under reduced
pressure to yield 3.82 g. (0.02 mole, 20.0%) of product, b.p. 112-116°C. at 0.75 mm. (Lit. (91)).

A secondary amine (neat) was shown from the IR spectrum by the presence of one sharp peak at 3260 cm.\(^{-1}\); also the presence of the characteristic cyclohexyl C-H stretching bands at 2910 cm.\(^{-1}\) and 2850 cm.\(^{-1}\) indicated the amine structure. NMR signals (60 MHz, CDCl\(_3\)): 1.10-1.90 ppm (multiplet, 11 H, C\(_6\)H\(_{11}\)), 3.15-3.35 ppm (triplet, 2 H, CH\(_2\)), 5.55 ppm (singlet, 1 H, NH), and 6.35-6.55 ppm and 8.20-8.30 ppm (triplet and doublet respectively, 3 H, C\(_4\)H\(_3\)N\(_2\)).

**Mono-picrate Derivative:**

A sample of the secondary amine (0.5 g.) was added to 95% ethanol (10 ml.). This solution was then added to 10 ml. of a saturated solution of picric acid in 95% ethanol, and was heated to boiling. The solution was allowed to cool slowly, and the bright yellow crystals of the picrate were isolated by suction filtration. The solid was then recrystallized from 95% ethanol; m.p. 161-163°C.

**Anal.** Calcd. for C\(_{17}\)H\(_{20}\)N\(_6\)O\(_7\): C, 48.57; H, 4.80; N, 20.00. Found: 48.48; H, 4.87; N, 19.80.

2. **N,N-Dimethyl-N'-'2-Pyrimidylethylenediamine:**

A mixture of 2-aminopyrimidine (33.9 g., 0.36 mole), sodium amide (15.6 g., 0.4 mole) and dry toluene (280 ml.) was placed in a 0.5-liter three-necked flask fitted with a mechanical stirrer, reflux condenser (drying tube), a dropping funnel (250 ml.) and a dry nitrogen gas inlet tube. The mixture was bubbled with dry nitrogen gas and refluxed at 125°C.
in an oil-bath for 12 hours. The mixture was cooled and the nitro-
gen flow discontinued. A solution of β-dimethylaminoethyl-
chloride (43 g., 0.4 mole), freshly distilled from its hydro-
chloride salt and flake sodium hydroxide (Lit. (93)), in 50 ml. of dry toluene was added from the dropping funnel to the mixture which was then refluxed another 24 hours. At the end of this time, the mixture was cooled, filtered, and the filtrate dried over anhydrous potassium carbonate and kept in the refrigerator overnight. The cooled mixture was filtered and the solvent removed from the filtrate by flash evaporation. The concentrated liquid mixture was heated under reduced pressure (70°C. at 0.9 mm.) to get rid of more of the solid starting amine by sublimation. The liquid residue was fractionated under reduced pressure and the fraction boiling at 94°C. (0.9 mm.) was collected to yield 22.0 g. (0.13 mole, 37.1%) (Lit. (90), b.p. 85-90°C. at 0.02 mm., 24%; (92), b.p. 90-95°C. at 3 mm., 29.4%).

The infrared spectrum (neat) showed the characteristic C-H stretching for R-N(CH₃)₂ at 2830 cm⁻¹, 2780 cm⁻¹ and 2750 cm⁻¹. The N-H stretching vibration with one sharp peak at 3260 cm⁻¹ was characteristic of a secondary amine. NMR signals (60 MHz, CS₂): δ2.20 ppm (singlet, 6 H, N(CH₃)₂), 2.30-2.50 ppm (triplet, 2 H, CH₂N), 3.30-3.55 ppm (quartet, 2 H, CH₂NH), and 6.25-6.40 ppm and 8.10-8.15 ppm (triplet and doublet respectively, 4 H, NH-C₄H₂N₂).
3. **N,N-Dimethyl-N'-Cyclohexanecarbonyl-N'-2-Pyrimidyl-ethylenediamine**:

A mixture of N,N-dimethyl-N'-2-pyrimidylethylene-diamine (8.4 g., 0.05 mole), pyridine (7.9 g., 0.1 mole) and dry benzene (20 ml.) was placed in a 100-ml. three-necked flask fitted with a mechanical stirrer, a reflux condenser (drying tube) and a dropping funnel (50 ml.). Freshly prepared cyclohexanecarbonyl chloride (8.8 g., 0.06 mole) was added dropwise to the stirred mixture in the flask (cooled in an ice-bath). After the addition was complete, the reaction was refluxed with stirring for 2 hours, cooled and washed with two 30 ml. portions of 20% NaOH. The layers were separated and the aqueous layer was extracted with two 60 ml. portions of ether. The ethereal extract was combined with the benzene layer and dried over anhydrous sodium sulfate. The solvents were flashed off and the concentrated liquid fractionated under reduced pressure to yield 7.0 g. (0.025 mole, 50.1%) of product, b.p. 137-141°C. at 0.35 mm..

Infrared spectrum of the product (neat) indicated an amide by the absence of the N-H stretching vibration band at 3260 cm.\(^{-1}\), and by the presence of a strong carbonyl absorption at 1670 cm.\(^{-1}\). NMR signals (60 MHz, CCl\(_4\)): \(0.90-1.90\) ppm (multiplet, 11 H, \(C_6H_{11}\)), \(2.20\) ppm (singlet, 6 H, N(CH\(_3\))\(_2\)), \(2.30-2.50\) ppm (triplet, 2 H, CH\(_2\)), \(3.92-4.15\) ppm (triplet, 2 H, CH\(_2\)), and \(6.85-7.00\) and \(8.50-8.60\) ppm (triplet and doublet respectively, 3 H, \(C_4H_3N_2\)).
The tertiary amine (2.8 g., 0.01 mole) in 15 ml. of 95% ethanol was treated with a solution of 70% perchloric acid (1.44 g.) in 5 ml. of 95% ethanol and worked up as described for the N,N-dimethyl-N'-cyclohexylmethyl-N'-2-pyridyl-ethylenediamine di-perchloric acid derivative. The white tertiary amine mono-perchlorate was recrystallized from dry ethanol; m.p. 153-155.5°C.

Anal. Calcd. for C₁₅H₂₅N₄ClO₅: C, 47.80; H, 6.69; N, 14.87; Cl, 9.41. Found: C, 47.77; H, 6.76; N, 14.67; Cl, 9.32.

4. N,N-Dimethyl-N'-Cyclohexylmethyl-N'-2-Pyrimidyl-ethylenediamine:

A mixture of N,N-dimethyl-N'-2-pyrimidylethylene-diamine (16.6 g., 0.1 mole), sodium amide (4.3 g., 0.11 mole) and dry toluene (80 ml.) was placed in a 150-ml. three-necked flask fitted with a mechanical stirrer, reflux condenser (drying tube), a dropping funnel (50 ml.) and a dry nitrogen gas inlet tube. The mixture was bubbled with dry nitrogen gas and refluxed at 125°C. in an oil-bath for 15 hours. The reaction mixture was cooled and the nitrogen discontinued. A solution of cyclohexylmethyl bromide (35.4 g., 0.2 mole) in 10 ml. of dry toluene was added from the dropping funnel to the mixture, which was then refluxed another 48 hours. At the end of this time, the mixture was cooled, filtered and the filtrate fractionated under reduced pressure after flash evaporation of the solvent. The tertiary amine was obtained
as a yellow oil, b.p. 129-130°C. at 0.5 mm., yield 2.6 g. (0.01 mole, 10%).

A tertiary amine (neat) was indicated from the IR spectrum by the absence of the sharp secondary amine N-H stretching vibration band at 3260 cm.$^{-1}$ and the presence of the characteristic cyclohexyl C-H stretching bands at 2920 cm.$^{-1}$ and 2850 cm.$^{-1}$. NMR signals (60 MHz, CCl$_4$ and trace of CDCl$_3$): $\delta$1.00-1.75 ppm (multiplet, 11 H, C$_6$H$_{11}$), 2.25 ppm (singlet, 6 H, N(CH$_3$)$_2$), 2.43-2.60 ppm and 3.35-3.75 ppm (multiplet, 6 H, (CH$_2$)$_2$ and CH$_2$), and 6.25-6.50 and 8.15-8.22 ppm (quartet and doublet respectively, 3 H, C$_4$H$_3$N$_2$).

Because a trace of the starting secondary amine (b.p. 94°C. at 0.9 mm.) was carried over during the distillation of the tertiary amine, it was necessary to purify the tertiary amine by column chromatography. A suspension of 80 g. silica gel (specification MIL-D-3716, Davison Commercial Grade, Fisher Sci. Co.) in petroleum ether (b.p. 30-60°C.) was packed into a pyrex-glass column (3 x 60 cm.) in vertical position. When the suspension settled down in the column, the height of the silica gel was about two-thirds of the total column length. A solution of 2 g. of the impure tertiary amine in petroleum ether was poured into the column and eluted with benzene, benzene and chloroform (1:1), and chloroform. The eluate was received into a 2.5 x 20 cm. test tube (about 70 ml. in volume) and checked by both TLC using sublimed iodine as the spotting agent and by IR spectrum. The pure tertiary amine came out in the chloroform fractions.
Di-perchlorate Derivative:

The white di-perchloric acid salt of the purified tertiary amine was prepared as described for the N,N-dimethyl-N'-cyclohexylmethyl-N'-2-pyridylethylenediamine di-perchloric acid derivative; m.p. 182.5-184°C.

Anal. Calcd. for C_{15}H_{28}N_{4}Cl_{2}O_{8}: C, 38.88; H, 6.09; N, 12.10; Cl, 15.31. Found: C, 38.99; H, 6.23; N, 11.91; Cl, 15.26.

C. Synthesis of N,N-Dimethyl-N'-Cyclohexylmethyl-N'-2-Pyrazylethylenediamine:

1. N-2-Pyrazyl-Cyclohexanecarboxamide:

A mixture of 2-aminopyrazine (47.6 g., 0.5 mole), pyridine (39.5 g., 0.5 mole), and dry benzene (250 ml.) was placed in a 0.5-liter three-necked flask fitted with a mechanical stirrer, a reflux condenser (drying tube) and a dropping funnel (250 ml.). To the stirred mixture in the flask (cooled in an ice-bath) was added freshly prepared cyclohexanecarbonyl chloride (73.3 g., 0.5 mole) dropwise. After the addition was complete, the reaction was refluxed with stirring for 1.5 hours, cooled and washed with water (200 ml.). The solid which precipitated was suction filtered and the layers were separated. The aqueous phase was extracted with two 200 ml. portions of ether. The ethereal extracts were combined with the benzene layer, and dried over anhydrous sodium sulfate. The solvents were flashed off to recover more of the amide in a total yield of 93.9 g. (0.46 mole, 91.5%). The amide was recrystallized from benzene; m.p. 165.9-166.8°C.
The IR spectrum of the solid amide (KBr) showed a strong C=O stretching band at 1700 cm\(^{-1}\) and the characteristic cyclohexyl C-H stretching vibration at 2870 cm\(^{-1}\) and 2940 cm\(^{-1}\); also the absence of N-H stretching for 2-aminopyrazine at 3160 cm\(^{-1}\) and 3340 cm\(^{-1}\) indicated the amide structure. NMR signals (60 MHz, CDCl\(_3\)): \(\delta 1.00-2.20\) ppm (multiplet, 11 H, C\(_6\)H\(_{11}\)), 8.10-8.40 ppm (multiplet, 4 H, C\(_4\)H\(_4\)N\(_2\)), and 9.65 ppm (singlet, 1 H, NH).

**Anal.** Calcd. for C\(_{11}\)H\(_{15}\)N\(_3\)O: C, 64.36; H, 7.37; N, 20.48. Found: C, 64.52; H, 7.33; N, 20.63.

2. **N,N-Dimethyl-N’-2-Pyrazylethylenediamine**

A mixture of 2-aminopyrazine (43.4 g., 0.46 mole), sodium amide (19.5 g., 0.5 mole) and dry toluene (320 ml.) was placed in a 0.5-liter three-necked flask fitted with a mechanical stirrer, reflux condenser (drying tube), a dropping funnel (250 ml.) and a dry nitrogen gas inlet tube. The mixture was bubbled with dry N\(_2\) gas and refluxed at 125°C in an oil-bath for 24 hours. The reaction mixture was then cooled and the nitrogen discontinued. A solution of \(\beta\)-dimethylaminoethyl chloride (53.8 g., 0.5 mole), freshly distilled from its hydrochloride salt and flake sodium hydroxide (Lit. (93)), in 30 ml. of dry toluene was added from the dropping funnel to the mixture, which was then refluxed another 24 hours. At the end of this time, the mixture was cooled and filtered and the filtrate concentrated by flash evaporation. The concentrated liquid was kept in the refrigerator overnight. The cooled mixture was filtered and the filtrate heated under
reduced pressure (90°C, at 0.95 mm.) to get rid of more of the solid starting amine by sublimation. The liquid residue was fractionated under reduced pressure to yield 24.6 g. (0.15 mole, 32.4%), b.p. 105-106°C. at 0.95 mm. (Lit. (92)).

Infrared spectrum (neat) showed the characteristic C-H stretching for R-N(CH$_3$)$_2$ at 2820 cm.$^{-1}$, 2780 cm.$^{-1}$ and 2730 cm.$^{-1}$. N-H stretching vibration with one broad peak at 3300 cm.$^{-1}$ (conjugated NH) indicated the presence of a secondary amine. NMR signals (60 MHz, CCl$_4$): 62.20 ppm (singlet, 6 H, N(CH$_3$)$_2$), 2.35-2.55 ppm (triplet, 2 H, CH$_2$), 3.20-3.50 ppm (quartet, 2 H, CH$_2$-NH), 5.30 ppm (singlet, 1 H, NH), and 7.65-7.90 ppm (multiplet, 3 H, C$_3$H$_6$N$_2$).

**Mono-picrate Derivative:**

The bright yellow picric acid salt was prepared as described for the 2-cyclohexylmethylaminopyrimidine mono-picric acid derivative. The N,N-dimethyl-N'-2-pyrazylethylenediamine mono-picrate was recrystallized from reagent acetone; m.p. 158-159.5°C.

**Anal.** Calcd. for C$_{14}$H$_{17}$O$_7$N$_7$: C, 42.53; H, 4.34; N, 24.81. Found: C, 42.37; H, 4.45; N, 25.00.

3. **N,N-Dimethyl-N'-Cyclohexanecarbonyl-N'-2-Pyrazylethylenediamine:**

A mixture of N,N-dimethyl-N'-2-pyrazylethylenediamine (4.1 g., 0.025 mole), pyridine (3.2 g., 0.04 mole) and dry benzene (20 ml.) was placed in a 100-ml. three-necked flask fitted with a mechanical stirrer, a reflux condenser (drying tube) and a dropping funnel (50 ml.). To the stirred
mixture in the flask (cooled in an ice-bath) was added freshly prepared cyclohexanecarbonyl chloride (5.9 g., 0.04 mole) dropwise. After the addition was complete, the reaction was refluxed with stirring for 2 hours, cooled and washed with 20 ml. of 20% NaOH. The layers were separated. The aqueous layer was extracted with two 30 ml. portions of ether. The ethereal extracts were combined with the benzene layer and dried over anhydrous sodium sulfate. The solvents were removed by flash evaporation to yield 3.24 g. (0.012 mole, 47.6%) of product, b.p. 130°C. at 0.20 mm.

The amide structure (neat) was indicated by the presence in the IR spectrum of a strong carbonyl absorption band at 1670 cm⁻¹. NMR signals (60 MHz, CCl₄): 1.00-1.80 ppm (multiplet, 11 H, C₆H₁₁), 2.15 ppm (singlet, 6 H, N(CH₃)₂), 2.30-2.52 ppm (triplet, 2 H, CH₂), 3.86-4.00 ppm (triplet, 2 H, CH₂), and 8.35 and 8.65 (two singlet, 3 H, C₄H₃N₂).

**Mono-picrate Derivative:**

The bright yellow picric acid salt was prepared as described for the 2-cyclohexylmethylaminopyrimidine mono-picric acid derivative, and was recrystallized from water; m.p. 158-159.5°C.


4. **N,N-Dimethyl-N'-Cyclohexylmethyl-N'-2-Pyrazyl-ethylenediamine:**
A mixture of N,N-dimethyl-N'-2-pyrazylethylene-diamine (11.5 g., 0.07 mole), sodium amide (3.9 g., 0.1 mole) and dry toluene (100 ml.) was placed in a 250-ml. three-necked flask fitted with a mechanical stirrer, reflux condenser (drying tube), a dropping funnel (125 ml.) and a dry nitrogen gas inlet tube. The mixture was bubbled with dry nitrogen gas and refluxed at 125°C. in an oil-bath for 22 hours. The reaction mixture was cooled and the nitrogen discontinued. A solution of cyclohexylmethyl bromide (17.7 g., 0.1 mole) in 10 ml. of dry toluene was added from the dropping funnel to the mixture, which was then refluxed another 24 hours. At the end of this time, the mixture was cooled, filtered and the filtrate fractionated under reduced pressure after removal of the solvent by flash evaporation. The tertiary amine was obtained as a yellow oil, b.p. 126-136°C. at 0.25-0.35 mm., yield 7.0 g. (0.026 mole, 38.6%).

A tertiary amine (neat) was indicated by the absence in the IR spectrum of the N-H stretching vibration at 3300 cm.⁻¹ and the presence of the characteristic cyclohexyl C-H stretching bands at 2920 cm.⁻¹ and 2850 cm.⁻¹. NMR signals (60 MHz, CCl₄): 0.90-1.70 ppm (multiplet, 11 H, C₆H₁₁), 2.15 ppm (singlet, 6 H, N(CH₃)₂), 2.30-2.50 ppm and 2.90-3.55 ppm (multiplet, (CH₂)₂ and CH₂), and 7.50-7.75 ppm multiplet, 3 H, C₄H₃N₂).

Because a trace of the starting secondary amine (b.p. 105-106°C. at 0.95 mm.) was brought over during the distillation of the tertiary amine, it was necessary to purify
the tertiary amine by column chromatography. The tertiary amine was purified by silica gel column as described for N,N-dimethyl-N'-cyclohexylmethyl-N'-2-pyrimidylethylenediamine. The pure N,N-dimethyl-N'-cyclohexylmethyl-N'-2-pyrazylethylenediamine came out in the benzene-chloroform (2:8) fractions.

**Di-perchlorate Derivative:**

The light-yellow di-perchloric acid salt of the purified tertiary amine was prepared as described for the N,N-dimethyl-N'-cyclohexylmethyl-N'-2-pyridylethylenediamine di-perchloric acid derivative, and was recrystallized from dry ethanol and dry ether (using a water bath at 60°C); m.p. 150-157°C.

**Anal.** Calcd. for C\textsubscript{15}H\textsubscript{28}N\textsubscript{4}Cl\textsubscript{2}O\textsubscript{8}: C, 33.88; H, 6.09; N, 12.10; Cl, 15.31. Found: C, 33.99; H, 6.19; N, 11.94; Cl, 15.07.

**D. Synthesis of N,N-Dimethyl-N'-Cyclohexylmethyl-N'-1-Naphthylethylenediamine:**

1. **N-1-Naphthyl-Cyclohexanecarboxamide**

A mixture of 1-naphthylamine (43.0 g., 0.3 mole), pyridine (23.8 g., 0.3 mole), and dry benzene (200 ml.) was placed in a 0.5-liter three-necked flask fitted with a mechanical stirrer, a reflux condenser (drying tube) and a dropping funnel (250 ml.). Freshly prepared cyclohexanecarbonyl chloride (49.5 g., 0.34 mole) was added dropwise and with stirring to the reaction flask cooled in an ice-bath. After the addition was complete, the reaction was refluxed
with stirring for 1 hour, cooled and washed with 200 ml. of water. The solid which precipitated was suction filtered and the layers were separated. The aqueous phase was extracted with two 200 ml. portions of ether. The ether extracts were combined with the benzene layer, and dried over anhydrous sodium sulfate. The solvents were removed by flash evaporation to recover more of the amide in a total yield of 68.6 g. (0.27 mole, 90.5%). The amide was recrystallized from 95% ethanol; m.p. 189.6-190.8°C. (Lit. (85), m.p. 188°C.).

Infrared spectrum of the solid amide (KBr) showed a strong C=O stretching band at 1660 cm.\(^{-1}\) and the characteristic cyclohexyl C-H stretching vibration at 2860 and 2940 cm.\(^{-1}\).

2. *N*-Cyclohexylmethyl-\(N\)-1-Naphthylamine:

In a 1-liter three-necked flask equipped with a mechanical stirrer, a Soxhlet extractor (drying tube) and a dropping funnel (250 ml.) was placed lithium aluminum hydride (11.4 g., 0.3 mole) in dry ether (700 ml.). The mixture was stirred with gentle reflux on a heating mantle for 4 hours. After this time, \(N\)-1-naphthyl-cyclohexanecarboxamide (30.4 g., 0.12 mole) was packed into the Soxhlet extractor whose bottom was lined with glass wool and a filter paper to prevent the blockage of the siphon arm. Three glass rods were inserted into the powder as channels for the extracting solvent. Refluxing was continued until all the amide had been carried into the flask. The reaction was then refluxed for 3 days. The reaction was then worked up with water (50 ml.) and sufficient 40% NaOH solution for complete separation. The
ethereal layer was separated by decantation and dried over anhydrous sodium sulfate. The solvent was removed by flash evaporation to yield 24.8 g. (0.1 mole, 86.4%) of product, b.p. 149-152°C. at 0.25 mm. The light yellow oil solidified after standing overnight.

The secondary amine (neat) was indicated in the IR spectrum by the sharp N-H stretching vibration at 3440 cm$^{-1}$ and the absence of a strong carbonyl absorption at 1660 cm$^{-1}$. NMR signals (60 MHz, CCl$_4$): 60.90-1.90 ppm (multiplet, 11 H, C$_6$H$_{11}$), 2.92-3.02 ppm (doublet, 2 H, CH$_2$), 4.15 ppm (singlet, 1 H, NH), and 6.35-6.50 ppm and 7.00-7.80 ppm (multiplet, 7 H, C$_{10}$H$_7$).

**Mono-hydrochloride Derivative:**

Dry hydrogen chloride was passed from a cylinder into a solution of the secondary amine (1 g.) in dry ether (50 ml.). When precipitation was complete, the solid was suction filtered under a stream of dry nitrogen gas to prevent it from contacting air moisture and was washed with a small amount of dry ether. The pale-brown HCl salt was recrystallized from dry ethanol and n-pentane (using a water bath at 65°C.); m.p. 165-178°C.

**Anal.** Calcd. for C$_{17}$H$_{22}$NCl: C, 74.02; H, 8.04; N, 5.08; Cl, 12.86. Found: C, 73.96; H, 7.90; N, 5.21; Cl, 12.77.
3. **N,N-Dimethyl-N'-Cyclohexylmethyl-N'-1-Naphthyl-ethylendiamine.**

A mixture of N-cyclohexylmethyl-N-1-naphthyl-amine (12.0 g., 0.05 mole), sodium amide (2.3 g., 0.06 mole) and dry toluene (80 ml.) was placed in a 150-ml. three-necked flask fitted with a mechanical stirrer, reflux condenser (drying tube), a dropping funnel (50 ml.) and a dry nitrogen gas inlet tube. The mixture was bubbled with dry nitrogen gas and refluxed at 125°C. in an oil-bath for 12 hours. The mixture was cooled and the nitrogen flow discontinued. A solution of β-dimethylaminoethyl chloride (6.5 g., 0.06 mole), freshly distilled from its hydrochloride salt and flake sodium hydroxide (Lit. (93)), in 30 ml. of dry toluene was added from the dropping funnel to the mixture, which was then refluxed another 24 hours. At the end of this time, the mixture was cooled, and filtered and the filtrate fractionated under reduced pressure after removal of the solvent by flash evaporation. The tertiary amine was obtained as a yellow oil, b.p. 188-190°C. at 1.5 mm., yield 11.1 g. (0.036 mole, 71.2%).

A tertiary amine (neat) was indicated in the IR spectrum by the absence of the sharp secondary amine N-H stretching vibration band at 3440 cm.⁻¹ and the presence of the characteristic C-H stretching for R-N(CH₃)₂ at 2820 cm.⁻¹, 2780 cm.⁻¹ and 2730 cm.⁻¹. NMR signals (60 MHz, CCl₄): δ 0.90-1.90 ppm (multiplet, 11 H, C₆H₁₁), 2.10 ppm (singlet, 6 H, N(CH₃)₂), 2.20-2.50 ppm and 2.95-3.30 ppm (multiplet, 6 H, (CH₂)₂ and CH₂), and 7.00-7.80 ppm and 8.20-8.35 ppm.
Because a trace of the starting secondary amine (b.p. 149-152°C. at 0.25 mm.) was brought over during distillation of the tertiary amine, it was necessary to purify the tertiary amine by column chromatography. The tertiary amine was purified on a silica gel column as described for the N,N-dimethyl-N'-cyclohexylmethyl-N'-2-pyrimidylethylenediamine. The pure N,N-dimethyl-N'-cyclohexylmethyl-N-1-naphthylethylenediamine came out in the chloroform fractions.

Di-perchlorate Derivative:

The grayish white di-perchloric acid salt of the purified tertiary amine was prepared as described for the N,N-dimethyl-N'-cyclohexylmethyl-N'-2-pyridylethylenediamine di-perchloric acid derivative, and was recrystallized from dry ethanol and dry ether; m.p. 106.5-108.5°C..

Anal. Calcd. for C_{21}H_{22}N_{2}Cl_{2}O_{8}: C, 49.32; H, 6.31; N, 5.48; Cl, 13.87. Found: C, 49.14; H, 6.46; N, 5.62; Cl, 13.73.

E. Synthesis of N,N-Dimethyl-N'-Cyclohexylmethyl-N'-5-Quinolylethylenediamine:

1. N-5-Quinolyl-Cyclohexanecarboxamide:

A mixture of 5-aminoquinoline (30 g., 0.21 mole), pyridine (17.4 g., 0.22 mole), and dry benzene (100 ml.) was placed in a 250-ml. three-necked flask fitted with a mechanical stirrer, a reflux condenser (drying tube) and a dropping funnel (125 ml.). To the stirred mixture in the flask (cooled in an ice-bath) was added dropwise freshly prepared cyclo-
hexanecarbonyl chloride (32.3 g., 0.22 mole). The reaction was refluxed for two hrs., cooled and made basic with 100 ml. of 10% NaOH solution. The crude amide was precipitated as a yellowish white solid, which was collected by suction filtration and washed with water until the water washing was neutral to litmus paper, yield 45.7 g. (0.18 mole, 86.3%). The amide was recrystallized from 95% ethanol, m.p. 202.9-204.0°C.

Infrared spectrum of the solid amide (KBr) showed a strong C=O stretching band at 1650 cm.⁻¹ and the characteristic cyclohexyl C-H stretching vibration bands at 2750 cm.⁻¹ and 2820 cm.⁻¹; also the absence of N-H stretching for 5-aminoquinoline at 3180 cm.⁻¹ and 3330 cm.⁻¹ proved the amide structure. NMR signals (60 MHz, CDCl₃): 0.00-2.60 ppm (multiplet, 11 H, C₆H₁₁), 7.20-8.25 ppm (multiplet, 6 H, C₉H₆N), and 8.90 ppm (doublet, 1 H, NH).

Anal. Calcd. for C₁₆H₁₈ON₂: C, 75.56; H, 7.13; N, 11.02. Found: C, 75.50; H, 7.02; N, 11.20.

2. 5-Cyclohexylmethylaminoquinoline:

In a 1-liter three-necked flask equipped with a mechanical stirrer, a Soxhlet extractor (drying tube) and a dropping funnel (250 ml.) was placed lithium aluminum hydride (9.5 g., 0.25 mole) in dry ether (600 ml.). The mixture was stirred with gentle reflux for 4 hours. After this time N-5-quinolyl-cyclohexanecarboxamide (25.5 g., 0.1 mole) was packed into the Soxhlet extractor as described for the reduction of N-1-naphthyl-cyclohexanecarboxamide under section PART IV D-2. Refluxing with stirring was continued until all
the amide had been dissolved. The reaction was refluxed for 3 days and then worked up with water (50 ml.) and sufficient 40% NaOH solution to give complete separation. The separated organic layer was dried with anhydrous sodium sulfate and reduced in volume to yield 20.0 g. (0.08 mole, 83.3%) of 5-cyclohexylmethylaminoquinoline, b.p. 186-190°C. at 0.70-0.75 mm. The light yellow oil solidified after standing overnight.

The infrared spectrum (neat) indicated complete reduction by the absence of the carbonyl absorption band at 1650 cm.\(^{-1}\) and by the shift of the N-H stretching vibration to 3320 cm.\(^{-1}\). NMR signals (60 MHz, CDCl\(_3\)): \(\delta\) 1.10-1.90 ppm (multiplet, 11 H, C\(\text{H}_{11}\)), 3.05-3.15 ppm (doublet, 2 H, CH\(_2\)), 4.80 ppm (singlet, 1 H, NH), and 6.50-6.65 ppm, 7.15-7.70 ppm, 8.15-8.32 ppm and 8.80-8.85 ppm (multiplet, 6 H, C\(\text{H}_6\)N).

**Mono-hydrochloride Derivative:**

The blood red crystals of the secondary amine mono-hydrochloride salt were prepared as described for the N-cyclohexylmethyl-N-1-naphthylamine mono-HCl derivative; m.p. 240-242°C. (decomposed).

**Anal.** Calcd. for C\(_{16}\)H\(_{21}\)N\(_2\)Cl: C, 69.42; H, 7.65; N, 10.12; Cl, 12.81. Found: C, 69.56; H, 7.72; N, 10.01; Cl, 12.65.

**3. N,N-Dimethyl-N'-Cyclohexylmethyl-N'-5-Quinolylethylenediamine:**

A mixture of 5-cyclohexylmethylaminoquinoline (12.0 g., 0.05 mole), sodium amide (2.4 g., 0.06 mole) and dry toluene (100ml.) was placed in a 150-ml. three-necked flask
fitted with a mechanical stirrer, reflux condenser (drying tube), a dropping funnel (50 ml.) and a dry nitrogen gas inlet tube. The mixture was bubbled with dry nitrogen gas and refluxed at 125°C. in an oil-bath for 20 hours. The reaction was cooled and the nitrogen flow discontinued. A solution of dimethylaminoethyl chloride (6.5 g., 0.06 mole), freshly distilled from its hydrochloride salt and flake sodium hydroxide (Lit. (93)), in 20 ml. of dry toluene was added from the dropping funnel to the mixture, which was then refluxed another 24 hours. At the end of this time, the reaction was cooled and filtered and the filtrate fractionated under reduced pressure after flash evaporation of the solvent. The tertiary amine was obtained as a yellow oil, b.p. 164-168°C. at 0.15-0.20 mm., yield 9.5 g. (0.03 mole, 60.9%).

A tertiary amine (neat) was indicated by the absence of the secondary amine NH stretching vibration band at 3320 cm.⁻¹ in the infrared spectrum and the presence of the characteristic C-H stretching vibration for R-N(CH₃)₂ at 2820 cm.⁻¹, 2780 cm.⁻¹ and 2730 cm.⁻¹. NMR signals (60 MHz, CDCl₃): 0.90-1.80 ppm (multiplet, 11 H, C₆H₁₁), 2.10 ppm (singlet, 6 H, N(CH₃)₂), 2.24-2.50 ppm and 2.90-3.30 ppm (multiplet, 6 H, (CH₂)₂ and CH₂), and 7.10-7.85 ppm and 8.55-8.85 ppm (multiplet, 6 H, C₉H₆N).

Because a trace of the starting secondary amine (b.p. 186-190°C. at 0.70-0.75 mm.) was carried over during distillation of the tertiary amine, it was necessary to purify the tertiary amine by column chromatography. The tertiary
amine was purified on a silica gel column as described for the N,N-dimethyl-N'-cyclohexylmethyl-N'-2-pyrimidylethylene-
diamine. The pure N,N-dimethyl-N'-cyclohexylmethyl-N'-5-
quinoxylethylene diamine came out in the chloroform-methanol (9:1) fractions.

**Di-hydrochloride Derivative:**

The orange-red crystals of the purified tertiary amine di-HCl salt were prepared as described for the N-cyclo-
hexylmethyl-N-1-naphthylamine mono-HCl derivative; m.p. 147-
150°C. (decomposed).

**Anal.** Calcd. for C_{20}H_{31}N_{3}Cl_{2}: C, 62.49; H, 8.13;
N, 10.93; Cl, 18.45. Found: C, 62.55; H, 8.33; N, 10.75;
Cl, 18.30.

**Synthesis of N,N-Dimethyl-N'-Cyclohexylmethyl-N'-3-**
**Pyridazylethylene diamine:**

1. **3-Amino-6-Chloropyridazine:**

A mixture of 3,6-dichloropyridazine (30.0 g., 0.2 mole), absolute ethanol (400 ml.) and liquid ammonia (35.3 g.
2.08 mole, trapped from an ammonia cylinder into a flask cooled in an acetone-dry ice bath) was heated and stirred for 10 hrs. in a pressure reaction apparatus (Parr Instrument Co., Inc., Moline, Ill., U.S.A., Pat. No. 2625296). The starting pressure built up inside the apparatus was around 160 psi and at the end of 10 hours the pressure went down to about 40 psi. The reaction was cooled and the solvent and excess ammonia removed by flash evaporation. The brownish residue (34.4 g.) was then extracted in a Soxhlet extractor with ethyl acetate
solvent (350 ml.) overnight. Crystallization from the extraction solvent gave 13.0 g. (0.1 mole, 50.0% yield) of 3-amino-6-chloropyridazine, m.p. 206-208°C. (decomposed). (Lit. (84), m.p. 210-212°C. decomposed, 70% yield).

A primary amine (KBr) was indicated in the IR spectrum by the presence of N-H stretching vibration at 3160 cm.\(^{-1}\), 3300 cm.\(^{-1}\) and 3340 cm.\(^{-1}\); also the absence of C-Cl absorption band at 790 cm.\(^{-1}\) indicated the substitution of 3-chloro group by an amino group.

2. 3-Aminopyridazine

A suspension of 3-amino-6-chloropyridazine (20.8 g., 0.16 mole), sodium hydroxide (6.4 g., 0.16 mole) and 2.4 g. of 7% palladium-charcoal catalyst in 600 ml. of absolute ethanol was subjected to hydrogenation at 3 atmospheres pressure (i.e. 45 psi) in a pressure reaction apparatus (Parr Instrument Co., U.S.A.) stirring was continued for 4 days. After this time, the mixture was warmed, filtered and then excess hydrogen chloride was added before concentration of the filtrates. The solvent was flashed off and the yellowish white residue recrystallized from ethanol-pentane produced 13.7 g. (0.144 mole, 90.0% yield) of 3-aminopyridazine as white microcrystals, m.p. 174.0-176.0°C. (Lit. (84), m.p. 175.5-176.5°C., 91.5% yield). The hydrochloride was converted to the base by sodium bicarbonate and the crude product crystallized from ethyl acetate. Transparent blades were obtained, m.p. 169-171°C. (Lit. (84), m.p. 170-171°C.; (104) 168-170°C.; (105) 172°C.).
3. N,N-Dimethyl-N'-3-Pyridazylethylenediamine

A mixture of 3-aminopyridazine (12.3 g., 0.13 mole), sodium amide (5.46 g., 0.14 mole) and dry toluene (110 ml.) was placed in a 150 ml. three-necked flask fitted with a mechanical stirrer, reflux condenser (drying tube), a dropping funnel (50 ml.) and a dry nitrogen gas inlet tube. The mixture was bubbled with dry nitrogen gas and refluxed at 125°C. in an oil-bath for 20 hours. The reaction was cooled and the nitrogen discontinued. A solution of β-dimethylaminoethyl chloride (15.06 g., 0.14 mole), which was freshly distilled from its hydrochloride salt and flake sodium hydroxide (Lit. (93), in 20 ml. of dry toluene was added from the dropping funnel to the reaction mixture, which was then refluxed another 24 hours. At the end of this time, the reaction was cooled and filtered and the filtrate, after removal of solvent by flash evaporation was fractionated under reduced pressure to yield 5.4 g. (0.03 mole, 25.1%) of N,N-dimethyl-N'-3-pyridazylethylenediamine, b.p. 107-108°C. at 1.1 mm.

A secondary amine (neat) was indicated in the IR spectrum by the presence of a broad N-H stretching vibration band (conjugated) at 3280 cm.⁻¹ and by the presence of the characteristic C-H stretching vibration for R-N(CH₃)₂ at 2820 cm.⁻¹, 2780 cm.⁻¹ and 2730 cm.⁻¹. NMR signals (60 MHz, CDCl₃): 2.30 ppm (singlet, 6 H, N(CH₃)₂), 2.60-2.80 ppm (triplet, 2 H, CH₂), 4.05-4.30 ppm (triplet, 2 H, CH₂), 5.55 ppm (singlet, 1 H, NH), and 6.55-6.60 ppm and 7.25-7.35 ppm (multiplet, 3 H, C₄H₃N₂).
The secondary amine was not stable and decomposed after standing overnight. Column chromatography (silica gel) was tried to purify the secondary amine but the efforts were in vain.

G. Synthesis of 2,2-Diphenyl-4-Dimethylaminomethyloxetane

1. 2,2-Diphenyl-3-Chlorooxetane:

Benzophenone (2.00 g., 0.011 mole) was dissolved in 150 ml. of reagent benzene in a 200-ml. photochemical reaction vessel (Hanovia, U.S.A.). A quartz immersion well was fitted into the reaction vessel, and vinyl chloride was bubbled from a vinyl chloride cylinder into the solution through a small diameter PVC tube, until the volume reached the maximum level (total volume 200 ml. which contained about 40 ml. of vinyl chloride). The gas was turned off and the solution was cooled with running water and irradiated with a 450-w. mercury lamp (Hanovia, U.S.A.) through a pyrex filter tube (which filtered out all light below 2900 Å wave length) for 2 1/2 hours. The benzophenone carbonyl absorption band at 1670 cm.⁻¹ in IR was almost gone at the end of this time. The solution was then suction filtered and the filtrate concentrated by flash evaporation. The yellowish white residue was extracted several times with excess hot petroleum ether (b.p. 30-60°C.). The petroleum ether extracts were concentrated and crystallized from hexane to give 400 mg. (0.0016 mole, 14.8% yield) of the adduct, m.p. 85.5-87.0°C.

Infrared spectrum of the solid oxetane (KBr) showed a strong absorption band at 980 cm.⁻¹ which was characteristic
of an oxetane ring. NMR signals (60 MHz, CDCl₃): 64.50-4.72 ppm and 4.80-5.02 ppm (two triplet, 2 H, CH₂), 5.30-5.50 ppm (triplet, 1 H, CHCl), and 7.20-7.55 ppm (multiplet, 10 H, C₆H₅-C-C₆H₅). The low chemical shift values of CH₂ proved the structure.

Anal. Calcd. for C₁₅H₁₃OCl: C, 73.62; H, 5.35; Cl, 14.49. Found: C, 73.70; H, 5.51; Cl, 14.43.

2. 2,2-Diphenyl-3-(Pyrrolidin-2-one-1-yl)oxetane:

Benzophenone (2.00 g., 0.011 mole) and 5 ml. of N-vinyl-2-pyrrolidinone (about 5 g., 0.047 mole) were dissolved in 180 ml. of reagent benzene in a 200-ml. photochemical reaction vessel (Hanovia, U.S.A.). A quartz immersion well was fitted into the reaction vessel, and the solution was bubbled with dry nitrogen gas for 10 minutes. The gas flow was turned off and the solution was cooled with running water and irradiated with a 450-w. mercury lamp (Hanovia, U.S.A.) through a pyrex filter tube (which filtered out all light below 2900 Å wave length) for 3 hours. The benzophenone was almost gone by the end of this time (IR showed the benzophenone carbonyl absorption band at 1670 cm.⁻¹ had almost disappeared). The solvent was flashed off giving 6.5 g. of an oil residue. The oil was extracted by shaking several times with hexane and decanting the hexane soluble portions. The hexane insoluble residue was dissolved in a small amount of ethanol. Water was then added to the ethanolic solution giving a precipitate which was collected by suction filtration. The precipitate was separated on 4 TLC plates (20 x 20 cm.) using silica gel G (Art. 7731, E. Merck, Germany) and solvent system CHCl₃ (98%).
MeOH (2%), and a large band was collected (Rf = 0.7, 170 mg.) and crystallized from ether-hexane mixture giving 42 mg. (0.00014 mole, 1.3% yield) of 2,2-diphenyl-3-(pyrrolidin-2-one-1-yl)oxetane, m.p. 140-142°C. (Lit. (99), m.p. 141.5-143°C.).

Infrared spectrum of the solid oxetane (KBr) showed the strong oxetane ring absorption band at 980 cm.⁻¹, also the strong amide carbonyl absorption band at 1670 cm.⁻¹, indicated the adduct formation. NMR signals (60 MHz, CDCl₃): 6 1.30-3.40 ppm (multiplet, 6 H, C₆H₅NO), 4.75-5.02 ppm (two triplet, 2 H, CH₂), 5.65-5.90 ppm (triplet, 1 H, CHN), and 7.20-7.80 ppm (multiplet, 10 H, C₆H₅-C-C₆H₅).
PART V

PHARMACOLOGICAL TESTING AND ANTIHISTAMINIC RECEPTOR STUDIES

A. INTRODUCTION

Formal analysis of the dose-response relationships is a fundamental tool in receptor studies; one should remember, however, that the information so obtained shows only how closely relationships proposed on the basis of a certain theoretical concept are able to mimic the real situation (106). Dose-response curves of various drugs and of combinations of drugs in isolated organs have provided a deeper insight into the mode of action of various classes of drugs (107, 108). For example, most antihistamines act competitively and antagonize the action of histamine on guinea-pig ileum by means of a reversible union with a common receptor site. This could be studied by making dose-response curves and by characterizing their maximum height, the slope of the curves and their positions on the dose-axis (108).

Previous work in this laboratory (1, 2) had shown that, in the ethylenediamine-type antihistaminic receptor, one of the aromatic rings in Antergan (I) could be replaced by an alicyclic system (e.g. cyclohexyl, IIa) and the antihistaminic activity was fully retained. Therefore, it was of interest to study how variations in size and structure of the aromatic moiety ($R_2$ in II) in this alicyclic substituted system (II) would affect the activity at the receptor site.
B. EXPERIMENTAL

Drug-receptor interactions were studied and evaluated according to the kinetic methods of van Rossum (107, 108) and the technique for the making of cumulative dose-response curves was followed (108). Histamine acid phosphate (British Drug Houses, Poole, England) and acetylcholine bromide (Eastman Kodak, U.S.A.) were used as the reference agonists for studying the antihistaminic and anticholinergic activities, respectively, of a series of eight ethylenediamine-type antihistaminic compounds, i.e. (IIa) and (IIf) as mono- and di-hydrochloride salts respectively, (IIg) and (IIh) as mono-perchloric
acid salts, and (IIB), (IIC), (IID) and (IIe) as di-perchloric acid salts. Diphenhydramine hydrochloride (Sigma Chem. Co., Missouri, U.S.A.) and atropine sulfate (British Drug Houses, London, England) were used as a standard antihistaminic and anticholinergic agent, respectively. All compounds tested (i.e. agonists and antagonists) were freshly prepared in normal saline solutions and calculated as molar concentration of the respective salt in terms of the salt used. All donor guinea-pigs were fasted for 24-48 hours prior to sacrifice. Strips of guinea-pig's terminal ileum 2-4 cm. long were aerated with a mixture of 95% oxygen and 5% carbon dioxide in a 25-c.c. bath containing Tyrode solution (8.0 g. NaCl, 0.2 g. KCl, 0.1 g. CaCl₂, 0.1 g. MgCl₂, 0.05 g. NaH₂PO₄, 1.0 g. glucose, and 1.0 g. NaHCO₃, water to 1000 ml.). The bath fluid was kept at a constant temperature (37 ± 0.5°C.) by pumping water from a thermostatically-controlled reservoir through a jacket surrounding the bath. The longitudinal contractions of the intestine were recorded on a sooted drum with the aid of a light isotonic lever (i.e. a kymograph). Cumulative dose-response curves were obtained by stepwise addition of agonist (histamine or acetylcholine) to the organ bath, such that the next dose of agonist was added just as the gut strip had reached a steady maximum contraction due to the previous dose. Doses were incremented at half log units for histamine and at log units for acetylcholine, i.e. histamine was added so that the total bath concentration was $10^{-8}$ M/l, $3 \times 10^{-8}$ M/l, $10^{-7}$ M/l, $3 \times 10^{-7}$ M/l, ...... $3 \times 10^{-4}$ M/l without washing the preparation until
maximum contraction was reached; while the total bath concentration for acetylcholine was $10^{-8}$ M/l, $10^{-7}$ M/l, ...... $10^{-3}$ M/l. The height of contraction for every cumulative dose was measured in mm. and calculated in percentages of the maximum height of the reference compound. The percentages were plotted on a linear scale as mm. (100% was 100 mm.) on the ordinate while the doses were plotted on a logarithmic scale by using 30 mm. for every log 10 interval on the abscissa (see Appendix A for the computer program, Superplot, which was used for plotting the log dose-response curves).

Cumulative dose-response curves for the agonist were made until at least two consecutive, reproducible dose-response curves had been obtained. A 15-20 min. wash period was established between each curve utilizing three 100 ml. washings of fresh perfusate. Antagonistic effects were then determined by allowing the antagonist to incubate with the ileum for 15 min. (115) prior to documenting the cumulative log dose-response curve for the agonist in its presence. The intestine was then washed out with four 100 ml. of fresh Tyrode solution during a 25-35 min. interval for complete recovery. In this manner the cumulative dose-response curve of the reference agonist was made, alternatively in the presence and in the absence of a constant concentration of the test compound (antagonist), and the procedure was repeated three times. A fresh piece of gut was used for each concentration of the test compounds. Each compound was tested at three dose concentrations (generally $\frac{1}{2}$ log unit apart) and
each concentration was tested in four separate organ preparations. The dose-response curves after adding the test compounds were compared to the control curves, and from the displacement and/or depression of the test curve from the control, values for the $pA_2$ and $pD_2$ were calculated (for detailed procedures see Results and Discussion). Values for the $pD_2$ of the agonists were determined from the control curves.

C. RESULTS AND DISCUSSION

Within the concentrations tested, the antihistaminic activity of the test compounds (IIa-f) were shown to exhibit two types of antagonistic actions, i.e. compounds (IIa-d) exhibited pure competitive antagonism (Fig. 8-19, page 118-123) while compounds (IIe-f) exhibited mixed competitive-noncompetitive activity (Fig. 20-25, page 124-126). At high concentrations most clinically used antihistamines become non-competitive antagonists of histamine. For example, diphenhydramine hydrochloride is usually thought to be a highly potent competitive antagonist of histamine, but when the concentration is equal to and higher than $1 \times 10^{-6}$ M/l, it becomes a noncompetitive antagonist of histamine on isolated guinea-pig ileum (109).

With respect to anticholinergic activity on the isolated guinea-pig ileum, all compounds tested (IIa-f) displayed noncompetitive antagonism of acetylcholine (Fig. 26-45,
Compounds (IIg) and (IIh), however, were found to be inactive in both antihistaminic and anticholinergic tests even when the concentration used was as high as $1 \times 10^{-3}$ M/l.

The activity of any particular drug will depend on its affinity for the receptor upon which it acts and on its intrinsic activity at that receptor (110, 111).

Intrinsic activity is defined as the ratio between the maximum effect of a drug and the maximum possible effect in that system with any other reference compound. Both histamine and acetylcholine have intrinsic activity equal to unity.

The affinity of an agonist is determined as the negative logarithm of the dose which produces a response equal to 50% of the maximal response and has been called the $\text{pD}_2$ value (106, 108).

$$\text{pD}_2 = - \log A_{50} \ldots \ldots \ldots \ldots \ldots (1)$$

where $A_{50}$ is the agonist concentration which produces a 50% effect ($E_{50}$). The affinity $\text{pD}_2$ of histamine can be estimated from the log dose-response curve (e.g. Fig. 8 and 9) by drawing a vertical from $E_{50}$ to the abscissa to obtain the $A_{50}$ and then applying the above equation (1). Because the sensitivity of the gut strips varied a certain amount even in different segments of the same gut (112), the $\text{pD}_2$ values obtained from Fig. 8 and 9 also varied. However, this could be overcome by repeating the experiment and by random selection of
experimental animals (112). Therefore, 222 control plots have been performed in the present study to obtain the average $pD_2$ value for histamine, i.e. $pD_2 = 6.41$ (Table 12).

### TABLE 12

RESULTS FOR ANTIHISTAMINIC AND ANTICHOLINERGIC TESTS

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$pA_2$ vs Histamine$^d$</th>
<th>$pD_2$ vs Histamine$^e$</th>
<th>$pD_2$ vs Ach.$^f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIa-monoHC1</td>
<td>8.46 (0.04)$^c$</td>
<td></td>
<td>5.55 (0.05)$^c$</td>
</tr>
<tr>
<td>IIb-diHClO$_4$</td>
<td>7.77 (0.03)</td>
<td></td>
<td>5.01 (0.05)</td>
</tr>
<tr>
<td>IIc-diHClO$_4$</td>
<td>7.24 (0.05)</td>
<td></td>
<td>4.56 (0.02)</td>
</tr>
<tr>
<td>IID-diHClO$_4$</td>
<td>6.37 (0.02)</td>
<td></td>
<td>4.45 (0.04)</td>
</tr>
<tr>
<td>IIe-diHClO$_4$</td>
<td>7.19 (0.03)</td>
<td>6.04 (0.06)$^c$</td>
<td>6.18 (0.05)</td>
</tr>
<tr>
<td>IIf-diHCl</td>
<td>7.23 (0.03)</td>
<td>5.96 (0.04)</td>
<td>5.11 (0.02)</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>8.27$^g$ (0.03)</td>
<td></td>
<td>6.58$^h$ (0.02)</td>
</tr>
<tr>
<td>Atropine</td>
<td></td>
<td></td>
<td>8.92$^{hi}$ (0.04)</td>
</tr>
</tbody>
</table>

- **a**: average of 222 control plots.
- **b**: average of 265 control plots.
- **c**: numbers in parentheses are standard error.
- **d**: mean of 30 to 48 values.
- **e**: mean of 24 values.
- **f**: mean of 30 to 48 values (Ach. = Acetylcholine).
- **g**: Literature value 7.7 (108), 8.0 (112).
- **h**: $pA_2$ vs Acetylcholine.
- **i**: Literature value 8.9 (rat intestine) (108), 8.6 (guinea-pig ileum) (112).
The pD₂ value for acetylcholine bromide (e.g. Fig. 26) was obtained in the same way as for histamine acid phosphate, the value being pD₂ = 6.70 (Lit. (113), Acetylcholine pD₂ = 7.0) (Table 12).

It should be noted that the parameters so obtained were empirical and not corrected for a possible non-linearity between stimulus and effect and that they therefore might deviate from the exact values. To find the exact value would be an enormously laborious procedure (108).

Pure competitive antagonists are inactive as agonists. They have affinity towards the specific receptors but no intrinsic activity and thus have no ability to generate a stimulus. Independently of the relationship between stimulus and effect, the dose-response curves of the agonist in the presence of a constant concentration of the competitive drug have a shape essentially identical with the curve when the antagonist is absent (106, 108, 110). A similar effect as for instance, 50%, is reached only at higher doses. The curves are parallelly shifted to the right on the log dose axis (e.g. Fig. 8-10). The extent of shifting of the dose-response curve of a stimulant drug can be used for determining the affinity of the antagonist (111). The pA₂ value was introduced by Schild (112, 114) to measure the affinity of the competitive drug and was defined as the negative logarithm of the molar concentration of antagonist which caused a shift of a value 2. A certain dose of the antagonists causes a shift x (e.g. x₁, x₂, and x₃ in Fig. 8, 9, and 10, respectively) and its
negative logarithm is denoted as $pA_x$ where the relationship $pA_x$ and $pA_2$ is as follows:

$$pA_2 = pA_x + \log (x - 1) \ldots \ldots \ldots (11)$$

The molar concentration of the antagonist (e.g. $B \times 10^{-p} \text{ M/l}$) tested in the experiment was used to calculate the $pA_x$ value, thus $pA_x = p - \log B$. The distance in mm. between the 50% effect point in the control curve and that in the test curve was denoted as $x$ (e.g. $x_1$, $x_2$, and $x_3$ in Fig. 8, 9, and 10 respectively) and by the aid of Table 13 (see Appendix D, from reference 108) $x$ was converted into $\log (x - 1)$. The $pA_2$ value was then read according to the above equation (11).

However, it was pointed out by Schild (112) that when the activity of the antagonist had to be defined in terms of some other drug, the results were not equally reproducible since the apparent activity varied in successive experiments, even though conditions were kept as constant as possible. This was what was found for the result of diphenhydramine. Its $pA_2$ value was equal to 8.27 while the literature value was 7.7 (108) and 8.0 (112) (Table 12). To obtain a representative value of $pA_2$ for a given tissue, and an antagonistic drug, the mean of a random sample of determinations on different individual animals must be determined. Therefore, $pA_2$ is a statistical constant (112). Variability may be reduced by comparing the activity of one
antagonist with that of another (112). For this reason the experimental conditions were kept as constant as possible and the pA$_2$ values of a series of antagonists were compared with that of diphenhydramine hydrochloride. The potency in decreasing order of the compounds tested (pA$_2$ value in parenthesis) was as follows:

\[
\text{IIa} \ (8.46) \ > \ \text{Diphenhydramine} \ (8.27) > \\
\text{IIb} \ (7.77) > \ \text{IIc} \ (7.24) > \ \text{IID} \ (6.37).
\]

The pA$_2$ values for both diphenhydramine and atropine (Table 12) as antagonists for acetylcholine in guinea-pig ileum were determined in the same way as that of diphenhydramine vs histamine (i.e. used equation ii). Raina and Das (125) showed that diphenhydramine was 56-times more active in antagonizing histamine than acetylcholine. This was similar to the present results which indicated that diphenhydramine was 49-times as effective in antagonizing histamine than acetylcholine.

A pure noncompetitive antagonist, like a competitive antagonist, does not produce a response in the tissue. It has no affinity for the specific receptor of the agonist but interacts with different receptors and so influences stimulus formation or stimulus effectuation. Therefore, it has intrinsic activity with a negative sign. The affinity of the noncompetitive antagonist for its receptors can be calculated from the depression of the maximal response to
the agonist in the presence of the antagonist. The pD$_2$ value is defined as the negative log of the molar concentration of antagonist which causes a depression of the maximal response to the 50% level. From the depression ($x$) caused by a certain dose of the non-competitive antagonist the pD$_2'$ value can be calculated as follows:

$$pD'_2 = pD_x' + \log (x - 1) \ldots \ldots \ldots \ldots \ (iii)$$

From the molar concentration of the antagonist $B \times 10^{-p}$ the pD$_x'$ value is calculated as $pD_x' = p - \log B$. The maximum effect of the test curve is expressed in percentage of the maximum effect of the control. With the aid of Table 14 (see Appendix E, from Reference (108)) the percentage is converted into $\log (x - 1)$. The pD$_2'$ value is easily found according to equation (iii).

The results for the non-competitive action of compounds IIa-f against acetylcholine were shown in Table 12 and Fig. 26-45. These compounds merely caused a depression of the dose-response curves without shifting which indicated that they were pure non-competitive antagonists.

It is possible to get dualism of antagonism, i.e. a drug may act as both a competitive and a non-competitive antagonist. In this case both components show their own characteristic effect: the competitive interaction shifts the dose-response curve to the right in relation to $pA_2$, the non-competitive antagonist component depresses the maximum in
relation to $pD^2_2$ (106). Thus, both $pA^2_2$ and $pD^2_2$ values may be calculated, provided that the competitive antagonism is of greater order than the non-competitive (108).

Compounds (IIe) and (IIf) were found to exhibit dualism of antagonism (Fig. 20-25 and Table 12). At concentration less than $1 \times 10^{-7}$ M/l, both (IIe) and (IIf) displayed competitive antagonism towards the specific histamine receptor; while at concentration equal to or higher than $1 \times 10^{-7}$ M/l, they antagonized histamine non-competitively. In fact, this type of interaction could often be seen, as a large number of competitive antagonists produced non-competitive depressive activity when used in very high concentrations (106, 108, 109, 110, 112).

Recently several workers have studied some of the variables which might influence the experimental results in the guinea-pig ileum preparation. Bruno and co-workers (116) reported that after prolonged contact with the ileum the activity of diphenhydramine (as hydrochloride and pamoate) decreased. Schild (112) in his original paper also found that the $pA^2_2$ values were different between the 2 min. and 14 min. contact times of the antagonist with the ileum. In the present study the contact time was set at 15 min. as suggested by Reuse (115). However, as mentioned by Reuse (115) the procedures involved the assumption that after 15 min. the response to the agonist would remain unchanged. The fact that this is not certain may account for some of the variations in the results; nevertheless if all
antagonists tested were incubated with the ileum for the same period of time, the pA\textsubscript{2} values so obtained still were valuable for comparing the relative activity of each antagonist towards the histamine receptor sites.

Rocha e Silva (117) have found that the recovery of the histamine-induced contractile response of isolated guinea-pig following inhibition of this response with diphenhydramine (2-3 \textmu g/ml.) was faster at 4\textdegree C. than at 37\textdegree C.. In the present study the ileum was washed with Tyrode solution warmed through a 37\textdegree C.-jacket and found that after 25-35 min. of resting period most ilea could repeat the original response before adding any antagonists. Cirstea (118) reported that the contractions induced by histamine and acetylcholine in the guinea-pig intestinal musculature were larger and equal respectively at 27\textdegree C., as compared to 37\textdegree C.. He concluded that the unexpected effect of low temperature on the contraction induced by histamine was due to a functional blocking of some inhibitory ganglion cells from the enteric nervous plexuses.

Cortese (119) mentioned that the survival time of isolated guinea-pig intestine showed characteristic differences according to the parameters or function used for the study. Thus frequent washings with fresh Tyrode solution which supplied constantly the ions and nutrition needed for the ileum could make the intestine live longer. In the present work it was found that although the ilea were immersed in Tyrode solution and kept in the refrigerator, they could not function properly 10 hours after sacrificing
the animal. For this reason all the ilea were used within 8 hours after sacrificing the animal.

Tamarit (120) reported that the dose-effect of acetylcholine and histamine on the isolated guinea-pig ileum were represented by a sigmoid-type cure. He used different equations from those of van Rossum's (108) to measure the relative activity for histamine and acetylcholine acting on the ileum; the results were that acetylcholine was found to be about 1.8-fold more active than histamine in the ileum. This agreed with the present result where it was found that acetylcholine was 1.9-fold more active than histamine in the ileum.

D. ANTIHISTAMINIC RECEPTOR STUDIES

The most comprehensive study on the antihistaminic receptor was that carried out by Nauta and co-workers (3, 4, 75, 78, 79). They proposed an antihistaminic receptor in the smooth muscle cells of ileum to accommodate both histamine and diphenhydramine molecules. The high antihistaminic activity of diphenhydramine was attributed to the fact that firstly, one of the aryl groups participated in an overlap interaction with the ether oxygen; secondly, it was also this aryl group that was assumed to be more or less coplanar with the C-O; thirdly, one of the aryl groups was in \( \pi \)-complexation with the phenyl group of phenylalanine in the polypeptide chain; and finally, the protonated-N\((\text{CH}_3)\)\(_2\) group interacted with the anionic site on the receptor through a potential hydrogen-bond between itself and the N\(_2\)-
atom of histidine residue. The fact that compound (IIa) is highly potent (1.5-times more active than diphenhydramine) made us believe that the phenyl group of (IIa) participates in an overlap interaction with the amino nitrogen, is co-planar with C-N, and was in \( \pi \)-complexation at the anchorage site in the receptor. The overlap interaction is far more important due to the fact that 4-methyldiphenhydramine (Neobenodine, III) enhanced the overlap interaction through the positive mesomeric effect induced by the 4-CH\(_3\) group and therefore (III) was 10-fold more potent than diphenhydramine itself (3); also, it is relevant that the thio ether analogues of substituted diphenhydramine exhibited less antihistaminic activity due to the fact that the free electrons of sulfur are not as available as those of oxygen for overlapping interaction with the \( \pi \)-electrons of one of the aromatic rings and the steric interference of the bulkier sulfur atom also interfered with this interaction (4). As a result, any factors which interfered with this overlap interaction would decrease the antihistaminic activity. This was further supported by compounds (IIc) and (IID)
which were 10.7-fold, and 79.4-fold, respectively, less potent than diphenhydramine. The reason for less activity was due to the fact that both compounds (IIc) and (IId) contain two electron-withdrawing centers (i.e. two electro-negative nitrogens). In compound (IIc), the carbon flanking the two nitrogens (i.e. C<sub>2</sub>-atom) in the pyrimidine ring is highly positively charged (IV) (121). This positively charged C<sub>2</sub>-atom attracts the lone pair electrons from the amino nitrogen in the ethylenediamine chain towards itself so that the overlapping interaction between the π-electrons of the pyrimidine ring and the lone pair electrons of the amino nitrogen is greatly reduced. Consequently, the observed antihistaminic activity was decreased. Compound (IId) is less active than (IIc) while the C<sub>2</sub>-atom in (V) is less positively charged than that of (IV). This is probably because the activity of (V) is different from that of (IV) at the receptor sites.

Compound (IIb) contains only one electron-withdrawing center in the pyridine ring such that the C<sub>2</sub>-atom of the pyridine is the least positively charged (VI) as compared with C<sub>2</sub>-atoms
of pyrimidine (IV) and pyrazine (V) rings. Thus (IIb) was approximately as active as diphenhydramine (although the pA$_2$ value calculation showed that (IIb) was 3.1-fold less active than diphenhydramine, the fact that Bruno and co-workers (116) had shown that the activity of diphenhydramine pamoate was less potent than the corresponding HCl salt supported the belief that because (IIb) was tested as the di-HClO$_4$ salt rather than the corresponding HCl salt, the larger salt derivative had less antihistaminic activity). Moreover, compound (IIb) resembled the structure of the potent antihistamine Tripelennamine (VII) except that the benzyl group of the latter was replaced by a cyclohexylmethyl group in the former structure.

![Chemical Structure](attachment:image.png)

Although previous work (2) had shown that it is not necessary to have both aromatic rings in order to have high antihistaminic activity, yet the ring size of the alicyclic substituted system (VIII) was important in the interaction with the complementary groups on the receptor sites. Cyclohexyl derivative (VIIIb = IIa) was the most active one, and cyclobutyl (VIIIc), cyclopentyl (VIIIc), and cycloheptyl (VIIIa)
derivatives were 10.7-fold, 23.4-fold, and 27.5-fold, respectively, less active than the cyclohexyl derivative. Compounds (VIIIc and f) were inactive. This could not be explained by the lipophilic character of the alicyclic substitutions since the highest ring size cycloheptyl derivative (VIIIa) was the least active in the series. It was probably that the fit of the cyclohexyl ring in the complementary receptor sites contributed most of the influence on the activity. This was further supported by the present study where compound (IIb) containing a cyclohexyl ring was more or less as active as diphenhydramine (see above discussion). Moreover, if the R group in (VIII) was replaced with a cyclohexanecarbonyl group as in compounds (IIg) and (IIh), the antihistaminic activity was completely lost. The loss of activity in compounds IIg and IIh may have two causes: (a) the electron-withdrawing effect of both the slightly positive carbonyl carbon atom \( \delta^+ \) and the positively charged \( C_2 \)-atom
in the respective pyrimidine and pyrazine rings makes the lone pair electrons of the amino nitrogen even less available for attaining the overlap interaction with the π-electrons of the hetero-aromatic rings; (b) the bulkier carbonyl group may show steric interference and thus reduce the "fit" of the adjacent cyclohexyl ring into the complementary receptor sites.

In ethylenediamine-type antihistamines only one ring was found 4.8-6.0 Å from the amino nitrogen, whereas the other ring was 6.0-7.2 Å away, depending on its conformation when the molecule was in the trans form (53, 122). Similarly in the trans conformation of aminoalkyl ether-type antihistamines and monoaminopropyl compounds a distance of 6.0-6.8 Å exists (53). It appeared that the strongest competitive antagonism occurred when at least one ring was capable of assuming a position 5-6 Å from the amino nitrogen. That only one ring may be necessary was evidenced by the high antihistaminic activity of 4-(2-dimethylaminoethylamino)quinoline (IX) (123), 3- or 4-(2-(dimethylamino)ethoxy)quinoline (X) (124, 125) and 4-(2-(1-pyrrolidinyl)ethoxy)quinoline (XI) (125) on the isolated guinea-pig ileum. Compound (XI) was the most active one among the
three quinoline compounds and the in vitro studies (guinea-pig ileum) indicated that (XI) was 4 times less potent than diphenhydramine but the antagonism to histamine was more specific (125). Compound (X) was 20 times less active than diphenhydramine (125). All this evidence is relevant to our present compounds (IIa) and (IIb) both of which contain only one aromatic ring and appeared to be capable of assuming the antihistaminic distance (5-6 Å). Thus (IIa) and (IIb) have been shown to be highly potent antihistamines.

However, if the remaining aromatic ring size is larger than either of the phenyl ring (IIa) or the pyridyl ring (IIb), the resulting compounds (e.g. IIE and IIF) exhibit mixed competitive-noncompetitive antagonism (Fig. 20-25). At concentrations less than 1 x 10^{-7} M/l, both (IIE) and (IIF) displayed competitive antagonism towards the H_{1} receptor on
the ileum, while at concentrations equal to or larger than $1 \times 10^{-7} \text{M/l}$, they exhibited non-competitive antagonism. A similar example was that compound (X) (side chain at 3-position) antagonized histamine non-competitively (124).

Both (IIe) and (IIf) were found to be equipotent towards either the specific or the non-competitive receptor (see Table 12 for $pA_2$ and $pD_2'$ values). Their activities towards the specific histamine receptor were shown to be 11-12 times less active than diphenhydramine. At higher concentrations they interacted with the non-specific receptor sites and the activities were 170-200 times less active than diphenhydramine. Since the bulky naphthaline and quinoline groups and hindrance from surrounding chemical groups in the biophase could prevent specific receptor interaction, this may account for the non-competitive antihistaminic activity of both (IIe) and (IIf).

Since the activity of antihistaminic agents overlaps with that for anticholinergic agents, the compounds synthesized were also investigated for possible anticholinergic activity.

The structural requirement for anticholinergic activity in antihistaminic drugs has not been reported in the literature. All that is known is that histamine and antihistamines and acetylcholine and cholinolytic drugs bear one thing in common, i.e. cationic groups at physiological pH. It seems probably that antihistamines compete with acetylcholine for the anionic site on the receptor. But because the rest of the molecules
in antihistamines could not fit well into the complementary groups on the receptor sites, antihistamines show very weak and non-specific cholinolytic activity. Lands and Luduena (126) studied the cholinolytic action of a series of dialkylamino-alkanes (XII, \( R_1 = H \)) and dialkylamino-alkanols (XII, \( R_1 = -OH \)) on isolated segments of rabbit ileum. Shortening of the appendage

![Chemical Structure](image)

(XII)

\[ R_1 = H, \text{ or } OH \]

\[ R_2 = \text{cyclohexyl, cyclopentyl, cyclopentenyl, phenyl, or alkyl groups.} \]

side chain structure by one carbon atom resulted in a marked reduction in antispasmodic potency. Branching of the side chain also led to a distinct reduction in spasmolytic potency. These SAR relationships were same as those of antihistamines. A benzene ring may replace the cyclohexyl ring without changing the order of magnitude of cholinolytic action (note the structural similarities with the present compounds (II)). Also, cyclopentyl and cyclopentenyl substitution provided compounds that were of comparable potency to those of the various cyclohexyl and
phenyl analogs. An increase in the distance between the amino group and the terminal carbon by the addition of an ether oxygen between the second and terminal carbon (note the structural similarity with diphenhydramine - page 106, III without 4-CH$_3$ group) provided compounds that were essentially equivalent in spasmolytic potency to that of compounds of corresponding length of carbon side chain. Methyl quaternization of the amino side chain increased potency. However, the most striking difference in spasmolytic potency resided on the terminal hydroxyl group. Without this hydroxyl group (e.g. XII, $R_1 = H$) the potency dropped drastically to less than 1% or 2-3% that of atropine sulfate. With the hydroxyl group on the terminal carbon (e.g. XII, $R_1 = OH$), the potency was significantly increased to 20-90% that of atropine. This suggested that the hydroxyl groups played an important role, possibly by forming a second point of attachment to the receptor. In this respect, they resembled the corresponding anticholinergic esters. Since the present systems (compounds II) were without the hydroxyl group, they were therefore very weak anticholinergic agents (generally the potencies were 2,344-29,512 times less than that of atropine except compound (IIe) which was 550 times less potent than atropine). They all displayed non-competitive antagonism against acetylcholine on guinea-pig ileum. In compounds with the same aromatic ring size as that of phenyl group (e.g. compounds IIa-d), the anticholinergic potency decreased in the same direction as that of antihistaminic activity, i.e. IIa > IIb > IIc > IId
in decreasing potency. In compounds with larger aromatic ring size (e.g. compounds IIe and IIf), the spasmolytic potency did not follow the same pattern as that of antihistaminic action. Compound (IIe) was more potent than (IIf) (12 times difference) as a spasmolytic agent, while as antihistaminic agents, they had the same potency. Compound (IIe) was the most potent anticholinergic agent among the compounds tested but the reason was not known. Compounds (Ilg) and (IIh) were found to be inactive.

Lands and Lunduena (126) suggested the following cholinolytic receptor to accommodate the series of compounds (XII) on the isolated segments of rabbit ileum. Post-ganglionic parasympathetic fibers of the autonomic nervous system supplied a single type of receptor which had the characteristics of a crevice or trough-like structure in which the active cholinolytic drug was held by electrostatic attraction to an anionic area by the cationic head and by a second point of attachment to the receptor through a hydroxyl (polar) group, this latter being at a distance of 4-7 Å from the cationic head. The overall length of the reactive receptor surface was about 7-8 Å, measured from the anionic site. Quaternization of the amine with a methyl- or ethyl-halide increased cholinolytic potency, possibly by providing a more favorable fit to the receptor site and / or by increasing the electrostatic charge on the nitrogen. Thus, it was apparent that our present compounds (II) with only one cationic head but without both hydroxyl group and quaternization appeared
to have little anticholinergic activities on the guinea-pig ileum.

Finally, where is the site of action of histamine and/or acetylcholine on the guinea-pig ileum? Both acetylcholine and histamine have a direct mode of action on smooth muscle. Brownlee (127, 128) in two successive papers described the evidence for the site of action of histamine and acetylcholine on the guinea-pig ileum. Both histamine and acetylcholine could stimulate the intramural plexuses of the ileum as shown by their action on the circular muscle strip. The longitudinal muscle preparation was more sensitive to the direct muscle stimulating action of histamine and acetylcholine than to their nerve plexus stimulating actions; this made it unlikely that any plexus stimulating action would contribute to the response (128).
NOTE FOR FIGURE 8 - 45 (Cumulative Dose-Response Curves = DRC):

Since the figures on the following pages are computer write-outs, there is insufficient space for complete captions for each figure. The following description is therefore necessary:

Three or four figures are used to illustrate the activities of each test compound. In each figure the cumulative DRC for the agonist alone (i.e. either Histamine or Acetylcholine = Ach.) is represented by the solid line (Δ—Δ). The cumulative DRC for the agonist in the presence of the test compound is represented by dotted line (x•••x). The concentration of the test compounds is indicated by the numbers which are typed just beside each curve, for example, 0, 1, 3, 10,...... etc. :

0 means no test compound present;
1 means 1 times the concentration of the test compound listed on each figure (e.g. 10⁻⁹ M/l);
3 means 3 times the concentration of the test compound listed on each figure, etc.
Fig. 8-10: Effect of IIa on DRC of histamine, note the progressive and parallel shift of DRC (competitive antagonist). See page 117 for detail.
Fig. 12:

IIb  
$10^{-8}$ M/L

Fig. 13:

IIb  
$10^{-8}$ M/L

Fig. 11-13: Effect of IIb on DRC of histamine, note the progressive and parallel shift of DRC (Competitive antagonist). See page 117 for detail.
Fig. 14:
IIC
$10^{-8}$ M/l
p. 121.

Fig. 15
IIC
$10^{-8}$ M/l
Fig. 14-16: Effect of IIc on DRC of histamine, note the progressive and parallel shift of DRC (competitive antagonist). See page 117 for detail.
Fig. 17-19: Effect of IID on DRC of histamine, note the progressive and parallel shift of DRC (competitive antagonist). See page 117 for detail.

Fig. 18:
IID
$10^{-7}$ M/L
0 3

Fig. 19:
IID
$10^{-7}$ M/L
0 10

Histamine (log molar concentration)

% O/D contraction
Fig. 20-22: Effect of IIe on DRC of histamine, showing a dualism in antagonism, note the combination of parallel shift (competition) and a reduction of the max. response (non-competitive antagonism). See page 117 for detail.
Fig. 23-25: Effect of IIf on DRC of histamine, showing a dualism in antagonism, note the combination of parallel shift (competition) and a reduction of the max. response (non-competitive antagonism). See page 117 for detail.
Fig. 26-29: Effect of IIa on DRC of Ach., note the progressive depression of DRC (non-competitive antagonist). See page 117 for detail.
Fig. 30-32: Effect of I1b on DRC of Ach., note the progressive depression of DRC (non-competitive antagonist). See page 117 for detail.

Fig. 33: I1c
10^{-6} M/l

Fig. 32: I1b
10^{-7} M/l
Fig. 33-35: Effect of IIc on DRC of Ach, note the progressive depression of DRC (non-competitive antagonist). See page 117 for detail.
**Fig. 36:**

IId

$10^{-6}$ M/l

**Fig. 37:**

IId

$10^{-6}$ M/l

---

ACETYLCHOLINE (LOG MOLAR CONCENTRATION)

D/D CONTRACTURE

---

ACETYLCHOLINE (LOG MOLAR CONCENTRATION)
Fig. 36-38: Effect of IID on DRC of Ach., note the progressive depression of DRC (non-competitive antagonist). See page 117 for detail.
Effect of He on DRC of Ach., note the progressive depression of DRC (non-competitive antagonist). See page 117 for detail.
Fig. 43-45: Effect of IIf on DRC of Ach. Note the progressive depression of DRC (non-competitive antagonist). See page 117 for detail.
PART VI
SUMMARY

Previous work (2) had shown that the cyclohexyl analogue of Antergan, N,N-dimethyl-N'-cyclohexylmethyl-N'-phenylethlenediamine (I), was a very potent antihistaminic agent. In order to gain more insight into the antihistaminic activity in relation to the remaining aromatic ring, five aromatic systems varying in size and structure were used in the present study to substitute for the remaining phenyl ring. Seven tertiary ethylenediamine compounds were synthesized. They are as follows: (A) N,N-dimethyl-N'-cyclohexylmethyl-N'-2-pyridylethlenediamine, (B) N,N-dimethyl-N'-cyclohexylmethyl-N'-2-pyrimidylethlenediamine, (C) N,N-dimethyl-N'-cyclohexylmethyl-N'-2-pyrazylethlenediamine, (D) N,N-dimethyl-N'-cyclohexylmethyl-N'-1-naphthylethlenediamine, (E) N,N-dimethyl-N'-cyclohexylmethyl-N'-5-quinolylethlenediamine, (F) N,N-dimethyl-N'-cyclohexanecarbonyl-N'-2-pyrimidylethlenediamine, and (G) N,N-dimethyl-N'-cyclohexanecarbonyl-N'-2-pyrazylethlenediamine. Two oxetane derivatives, 2,2-diphenyl-3-chlorooxetane and 2,2-diphenyl-3-(pyrrolidin-2-one-1-yl)-oxetane, were also synthesized as intermediates in the present study, but the final products necessary for the antihistamine study could not be obtained.

The general reaction sequence for the preparation of the ethylenediamine derivatives started with the appropriate primary homo- and heterocyclic-aromatic amine. The primary amine was reacted with cyclohexanecarbonyl chloride to form
the amide. Lithium aluminum hydride was used to produce the desired secondary amine. In cases where the primary aromatic amines did not form the amides or if they did form the amides which could not be reduced to the required amines with LiAlH$_4$, the primary aromatic amines were condensed with $\beta$-dimethylaminoethyl chloride to form the desired secondary amines. In the former case, the appropriate secondary amine was condensed with $\beta$-dimethylaminoethyl chloride to obtain the final tertiary diamine compounds; while in the latter case, cyclohexylmethyl bromide was used to condense with the appropriate secondary amine to form the final products. Generally speaking, the products obtained by the first method appeared to give better yields than those of the second.

These ethylenediamine derivatives were isolated as the free base. The hydrochloride, picrate, and perchlorate salts of these amines were prepared for elemental microanalysis.

The two oxetane derivatives were obtained by photocycloaddition of benzophenone (a carbonyl compound) to either vinyl chloride (an olefin) or N-vinyl-2-pyrrolidinone (an olefin). Benzene was used as the inert solvent and a pyrex filter was used to filter out all light below 290 $\mu$m.

All the intermediates synthesized were characterized through their physical constants, boiling point, melting point, infrared and NMR spectra, and were verified by elemental microanalyses of the compounds (for solid intermediates) or their hydrochloride, picrate, or perchlorate salt derivatives (for liquid intermediates).
The antihistaminic activity and the possible anticholinergic activity of all the seven tertiary ethylenediamine compounds (i.e. (A)-(G) above) were studied by the cumulative dose-response curves' method of van Rossum (107, 108). The dose-response curves of each compound so obtained were used to analyze their mode of action and their relative antihistaminic and anticholinergic activities towards the respective receptor sites. Their relative activities were compared with those of diphenhydramine, atropine, and the cyclohexyl analogue of Antergan (I). It was found within the concentrations studied that compounds containing the aromatic ring size comparable to that of phenyl ring (i.e. compounds (A), (B), (C), and (I)) displayed competitive antagonism towards the antihistaminic receptor, and compounds containing larger ring size than phenyl group (i.e. compounds (D) and (E)) exhibited mixed competitive-noncompetitive antagonism activities. The possible electronic and steric effects of these nitrogen-containing aromatic systems on their antihistaminic activity at the receptor sites were explained. The overlapping interaction between the lone pair electrons of nitrogen and \( \pi \)-electrons of the aromatic system seemed to be the determinant factor governing the antihistaminic effect of compounds (A), (B), (C) and (I). The presence of two electron-withdrawing centers (i.e. nitrogen atoms) in the aromatic ring decreased this overlap interaction and thus decreased their antihistaminic activity. This gave more evidence in direct support of Nauta's antihistaminic receptor theory (3, 4). Therefore, (I) was
shown to be the most active antihistaminic agent (1.5-fold more active than diphenhydramine), (A) was more or less as active as diphenhydramine, and (B) and (C) were less active. The bulky aromatic ring of compounds (D) and (E) seemed to impose steric hindrance more important than the overlapping interaction in the biophase and thus prevents the receptor interaction. This in turn may account for their non-competitive activity at concentrations $\geq 10^{-7} \text{ M/l}$ and their relatively less antihistaminic activity at the receptor sites.

As to their anticholinergic activity on the isolated guinea-pig ileum, all compounds tested (i.e. (A), (B), (C), (D), (E), and (I)) showed non-competitive antagonism towards the cholinergic receptor sites. However, their activity was generally several thousand-fold less active than atropine. Compounds (F) and (G) were inactive in both antihistaminic and anticholinergic tests.
PART VII
APPENDICES

A. SUPERPLOT - A GENERAL PURPOSE PLOTTING ROUTINE AND AN EXAMPLE FOR PLOTTING THREE FIGURES.

B. PROGRAM TITLE: MEAN, VARIANCE, STANDARD DEVIATION (UNGROUPED DATA).

C. CHEMICALS AND REAGENTS USED FOR THE EXPERIMENTAL PART (IV).

D. TABLE 13 - COMPETITIVE ANTAGONISTS

E. TABLE 14 - NON-COMPETITIVE ANTAGONISTS.
A. SUPERPLOT - A GENERAL PURPOSE PLOTTING ROUTINE
(By E.J. Tarnai)

SUPERPLOT is an object program which prepares a "plotfile" to draw lines through sets of points. To invoke it

$RUN TARN: SUPERPLOT 4=dataf. 5=pagef. 6=printf. 9=plotf.

Logical unit 4 contains data relating to the sets of points. It may be convenient to define it to be *SOURCE*.

Logical unit 5 contains information relevant to the page size, and the axes to which the lines are to be drawn. If pagef. is not specified logical unit 5 defaults to *SOURCE*.

Logical unit 5 is the file on which error messages, etc., are written. Unit 5 is usually left to default to *SINK*.

Logical unit 9 is the file on which the "plotfile" is written. The use of plotfiles is described in several UBC write-ups (e.g., PLOT, REPLOT, etc.).

A detailed description of the input data-lines follows.

************

Reading begins on logical unit 5 and continues through six data lines. These lines are as follows:

1) PAGE (12), ORIENTATION (A4)

PAGE is an integer number (0 ≤ PAGE < 100). For each new value of this argument a new page is started. If the value is the same as it was at the last read operation a new set of axes is plotted on the same page.

ORIENTATION is ignored if new page is not started. If a new page is being started this parameter may take on one of three values:
ORIENTATION = HORI specifies an 11" x 8.5" page.

ORIENTATION = specifies an 8.5" x 11" page.

ORIENTATION = NONE will suppress the drawing of page boundaries.

2) METHOD (A4). NARCS (I2), R (F10.0), IEND (I2)

METHOD may take one of the three values PARA, CIRC, EXPO. This argument specifies the type of curve-fitting to be done. For details see UBC writeup SKETCH. If the field of this argument is left blank the default value PARA is assumed.

NARCS specifies the number of arcs to be fitted between consecutive data points. (0 < NARCS < 51). If NARCS is outside the above range the number of arcs is set to be 25. Note: If NARCS = 1 the line segments joining the points are straight.

R is the radius of curvature for method EXPO. (1.0 ≤ R ≤ 2.0). For R outside this range R is set to be 1.5. For methods other than EXPO this parameter is not used, and need not be specified.

IEND specifies whether or not the first and last line segments are to be drawn.

IEND = 0 end segments are left unaltered.

IEND = 1 end segments are straightened.

IEND ≠ 1 or 0 end segments are dropped.

3) XOR (1) (G5.0), XOR (2) (G5.0), XLEN (G5.0), XMIN (G5.0), DX (G5.0), XTITLE (13A4).

XOR (1) & XOR (2) are the coordinates (in inches) of the starting point of the X-axis with respect to the lower left corner of the page. The default values are (1.0, 0,5). (for XOR (I) the default values are the coordinates XOR (I)).

1) Throughout this writeup default value refers to that number which is set equal to the variable which have input value specified to be zero or left blank.
XLEN specifies the length of the X-axis in inches. The default value is 7.0 (or 10.0 for YLEN).

XMIN specifies the minimum value to be assigned to the X-axis. The default value is zero for linear axis or one for logarithmic axis.

DX specifies the increment in value by which the major scale divisions are to be labeled. The default value for DX is 1.0. Note that for a logarithmic axis the increment is used as a multiplying factor so that the value of 10.0 is assumed if DX = 1.0 is specified.

XTITLE is the Hollerith literal (not more than 52 characters) to be used as the title of the X-axis.

DIVX specifies the length in inches between the major scale division of the X-axis. The default value is 1.0.

NTICS specifies the number of scale subdivisions and whether or not the labeling is done using a scale factor. For NTICS > 0 no scale factor is used. NTICS = 0 defaults to the value 5. If NTICS < 0 the absolute value is used to determine the number of scale subdivisions and the labeling is done using 10^x scale factor, where x = NDIGX.

NDIGX specifies the number of decimal digits to be used in labeling the major scale divisions of the X-axis. The default value for this argument is 2. If NDIGX = -1 decimal digits and decimal point are suppressed, and if NDIGX = -2 the labeling of the X-axis scale divisions is suppressed.

NLABLEX specifies the maximum number of characters, including negative sign and decimal point, if applicable, in the labeling of the major scale division of the X-axis. The
default value for NLABLEX is 6. If NLABLEX < 0 the labeling of the major scale divisions of the X-axis will be suppressed.

FHIX specifies the angle, in degrees, of the labels on the major scale divisions of the X-axis. The default value is 0.0. (Note: the default value for FHIX is 90.0. If FHIX = 0.0 is required, an arbitrary negative number must be specified.)

LINEARX specifies linear scale division for LINEARX = 0, or logarithmic scale increment for LINEARX = 1.

THETAX specifies the angle of the X-axis in degrees. The default value is 0.0. (Note: the default value for THETAY is 90.0. If THETAY = 0.0 is required, a negative number must be specified.)

CSIZEX specifies the height size, in inches, of the characters used in labeling the X-axis. The default value is 0.1".

ISAX is normally 0. If ISAX = 1 the plotting of the X-axis will be suppressed.

5) & 6) contain information regarding the Y-axis. The data on these lines are arranged identically as the data on lines 3 and 4.

************

Reading continues on logical unit 4. Each line contains information regarding one point. Reading continues until an $ENDFILE is reached. This terminates the set of points through which a line is being drawn. Reading continues on this same unit and other sets of points are determined and lines drawn until an $ENDFILE is reached (i.e., two consecutive $ENDFILES) at which point reading continues on logical unit 5. The datalines in unit 4 are in the following form:

X (I) (G10.0), Y (I) (G10.0), SYMB (I2), LINET (I2), LSQ (I3).

X (I) & Y (I) are the actual coordinates of the data points. These values will be scaled by the program to the previously specified axes.
SYMB specifies the type of symbol to be drawn at each data point.

SYMB < 0 specifies no symbol
SYMB = 0 defaults to the most recent value of SYMB
(initially set to be -1).

SYMB = 1 specifies \( \triangle \)
SYMB = 2 specifies \( \times \)
SYMB = 3 specifies \( \circ \)
SYMB = 4 specifies \( \Diamond \)
SYMB = 5 specifies \( \ast \)
SYMB = 6 specifies \( \times \)

LINET specifies the type of line to be drawn through the points. The default value is 1. The various lines are as follows:

LINET = 1 specifies

LINET = 2 specifies

LINET = 3 specifies

LINET = 4 specifies

LINET = 5 specifies

LINET = 6 specifies

LSQ specifies whether or not least square fitting with an orthogonal polynomial of degree M. If LSQ = 0 no least square fitting will be done. If LSQ is a positive number between 1 and 30 M will be set by the program to that value which will give the best fit such that M ≤ LSQ if LSQ < 0 M will be set to ABS (LSQ) if LSQ > -30. If ABS (LSQ) > 30 M will be set to 0.

Note: The parameters SYMB, LINET, LSQ are set by the dataline containing X (1) and Y (1). I.e. These parameters need to be specified only once per line to be drawn.
If reading returns to logical unit 5 and the first data line encountered is an $\text{ENDFILE}$ it will cause execution to terminate.

If the user requires calling a subroutine during execution in order to do more plotting or to calculate the coordinates of the points on unit 4, etc., he may add the subroutine RTWAIT (PAGE). E.g. $\text{RUN TARN:SUERPLOT+LOAD#}$ where -LOAD# contains the object deck containing subroutine RTWAIT. The MAIN routine calls RTWAIT between the time reading is completed on unit 5 and reading begins on unit 4 if new page is being started. If the user does not supply this routine, a UBC-library routine is called which in batch mode does nothing and in terminal mode does very little (waits less than 0.3 sec. in real time).

The writeup UBC-SKETCH describes a subroutine OUT which "draws" lines through the points generated by SKETCH. Such a routine is part of SUERPLOT and may be used independently by a program which calls SKETCH. The line type is passed to OUT by the labeled common COMMON/LINETP/LINET for the exact location of OUT within SUERPLOT $\text{RUN *OBJSCAN PAR=TARN:SUERPLOT.}$

Note: See an example for plotting three figures on the next page.
This is an example for plotting three figures (i.e. Fig. 8, 9, and 10).

This FORTRAN program is to generate control cards for axes, 

IT = Total numbers of figures ( = 3 in this example).

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$RUN PLOT:Q PAR=-PLOT
$SIGNOFF

(End)
B. PROGRAM TITLE: MEAN, VARIANCE, STANDARD DEVIATION
(UNGROUPED DATA)

No.: 1014-2-ST1

Programmer: E. Thibeault

Formulas Used:

\[ \bar{x} = \frac{\sum x_1}{n} \]

\[ \sigma^2 = \frac{\sum x_1^2 - n (\bar{x})^2}{n - 1} \]

\[ \sigma = \sqrt{\sigma^2} \]

where \( x_1 \) = any of the data values being used (\( x_1, x_2, x_3, \ldots, x_n \))

\( n \) = number of values

\( \bar{x} \) = arithmetic mean of these values

\( \sigma^2 \) = variance (square of standard deviation)

\( \sigma \) = standard deviation from the mean (68.27% of all values should fall within \( \pm 1 \) deviation from the mean)

Then, Standard Error = \( \frac{\sigma}{\sqrt{n}} \)
C. CHEMICALS AND REAGENTS USED FOR
THE EXPERIMENTAL PART (IV)

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### COMPETITIVE ANTAGONISTS

Relationship between $x$ in mm and $\log (x-1)$ to be used for the calculation of $pA_2$ values according to the equation: $pA_2 = pA_x + \log (x-1)$

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NON-COMPETITIVE ANTAGONISTS

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PART VIII

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122. N.S. Ham, J. Pharm. Sci. 60 (11), 1764-5 (1971).
123. S.M. Deshpande and K. Nain, Indian J. Chem. 6 (11), 628-30 (1968).

130. I must express my thanks to Dr. G. Bauslaugh of the College of New Caledonia for designing the photochemical reactions.