THE INVESTIGATION OF DISPIRO AND FUSED RING TRICYCLIC ANALOGUES OF ETHYLENEDIAMINE TYPE ANTIHISTAMINICS

by

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to Anne

"...and with you there to help me

then it probably will."

- Ian Anderson

ABSTRACT

The synthesis and pharmacological testing of two series of compounds for use in the investigation of the steric and electronic nature of the antihistaminic receptor is described.

The dimethylaminoacetyl derivatives of 7-amino-14-azadispiro[5.1.5.2]-pentadecan-15-one and 8-amino-16-azadispiro[6.1.6.2]heptadecan-17-one and the methiodide salts of these compounds were synthesized. The dimethylaminoacetyl derivatives of the reduced ring systems, 7-amino-14-azadispiro[5.1.5.2]pentadecane and 8-amino-16-azadispiro[6.1.6.2] heptadecane, and the dimethiodide salts were also synthesized. Suitable compounds in this series were tested for local anesthetic activity by the guinea pig intradermal wheal method and for antihistaminic and antimuscarinic activity in isolated guinea pig ileum. Several of the compounds in this series were also screened for general activity and acute toxicity. The compounds in this series were inactive or showed only a very low order of activity.

The 2-dimethylaminoethyl derivatives of carbazole, 1,2,3,4-tetrahydrocarbazole, dodecahydrocarbazole, 1,2,3,4-tetrahydrocyclopent[b]-indole, dodecahydrocyclopent[b]indole, 5,6,7,8,9,10-hexahydrocyclohept-[b]indole, tetradecahydrocyclohept[b]indole, diphenylamine, dicyclohexylamine, fluorene, and 1,2,3,4,4a,9a-hexahydrofluorene were synthesized. The bis(2-dimethylaminoethyl) derivative of fluorene was also obtained.

All of these compounds were tested for antihistaminic and antimuscarinic activity in isolated guinea pig ileum. The compounds showed only non-competitive antimuscarinic activity which was probably due to a non-specific toxic effect. All of the compounds showed competitive

antihistaminic activity. The fully hydrogenated compounds and the di-substituted fluorene derivative showed non-competitive antihistaminic activity, again probably due to a non-specific toxic effect, as well as weak competitive antagonism.

The seven aromatic or indolic compounds showed a high degree of competitive activity. These compounds, followed by the pA_2 values, were: 5-(2-dimethylaminoethyl)-5,6,7,8,9,10-hexahydrocyclohept[b]indole (7.94), 9-(2-dimethylaminoethyl)-1,2,3,4,4a,9a-hexahydrofluorene (7.45), 9-(2-dimethylaminoethyl)-1,2,3,4-tetrahydrocarbazole (6.90), N-(2-dimethylaminoethyl)diphenylamine (6.67), 9-(2-dimethylaminoethyl)carbazole (6.38), 9-(2-dimethylaminoethyl)fluorene (6.12), and 4-(2-dimethylaminoethyl)-1,2,3,4-tetrahydrocyclopent[b]indole (6.12). The pA_2 value for diphenhydramine was found to be 7.75.

The results are discussed in terms of the steric nature and possible binding modes of the antihistaminic receptor.

Signatures of Examiners .....

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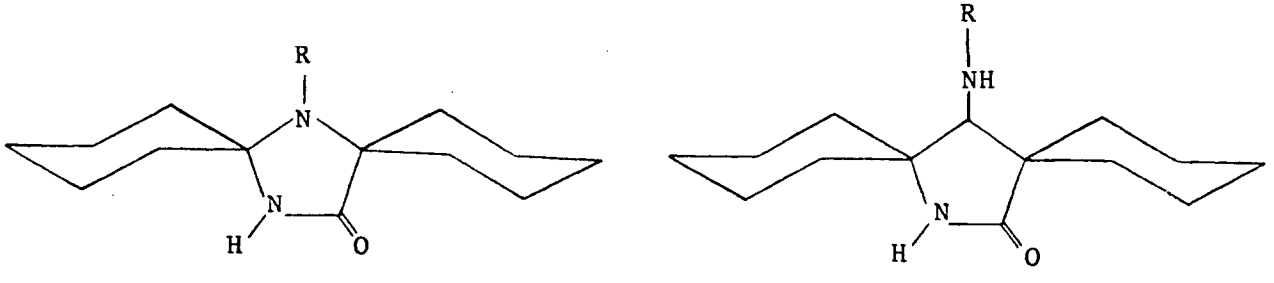
INTRODUCTION

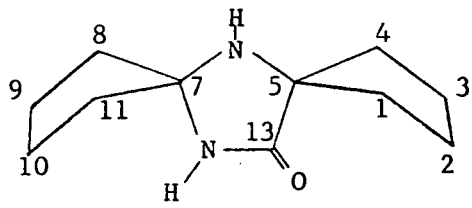
It is generally accepted (1,2) that, in order to obtain good antihistaminic activity in ethylenediamine compounds (see Table I, p 14), N^1 must be substituted with two aromatic functions or with one aromatic and one aromatic methyl function. However, work in this laboratory (3,4) has shown that at least one of these functions may be replaced with an alicyclic function and a reasonably high degree of activity is retained. With this in mind, work was started on a series of diazadispiro derivatives which, it was hoped, would allow further elucidation of the structural requirements for activity at the antihistaminic receptor.

These compounds are unique in that the two alicyclic rings attached to the central lactam ring are held perpendicular to the side chain which is substituted on the apex of the lactam ring. Figure 1 shows the steric relationship of the alicyclic rings and the side chain. Due to this relationship, the dispiro ring system should be a highly efficient blocking group if it is incorporated into a molecule which will bind at a receptor.

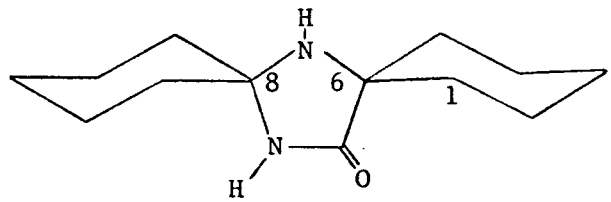


(a) (b)

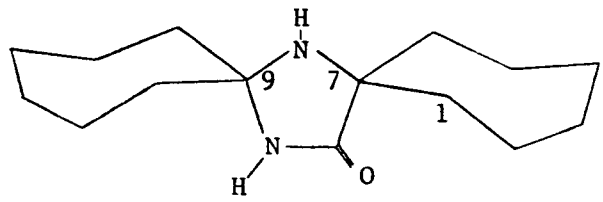
Figure 1: The steric relationship between the side chain and the alicyclic rings in diazadispiro (a) and azadispiro (b) compounds.



6,12-diazadispiro[4.1.4.2]tridecan-13-one 1



7,14-diazadispiro[5.1.5.2]pentadecan-15-one 2

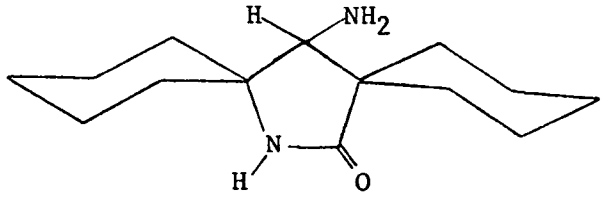


8,16-diazadispiro[6.1.6.2]heptadecan-17-one 3

Figure 2: Diazadispiro compounds

Substitution of a 2-dimethylaminoethyl group, which is typical of antihistaminics, on the secondary amino group of the lactam ring was therefore attempted (5,6). Probably because of the large steric effect of the alicyclic rings, this could not be accomplished in 2 (Figure 2). However, the 6-chloracetyl derivative of 1 was successfully prepared (7). Compound 3 could not be prepared (6).

Because of these difficulties, attention was focussed on azadispiro compounds (Figure 3) where the amino function has been removed from the lactam ring. A number of derivatives of these compounds were prepared



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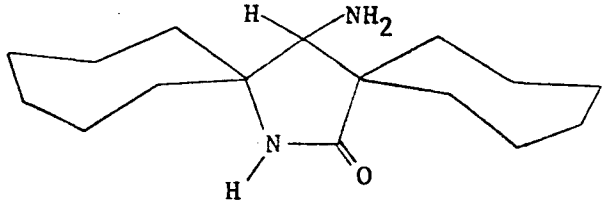


8-amino-14-azadispiro[6.1.6.2]heptadecan-17-one 5

Figure 3: Azadispiro compounds

and tested for pharmacological activity.

It was realized at the outset that the azadispiro compounds represent a major divergence from the typical ethylenediamine antihistaminics. However, it was also felt that the results would be of sufficient interest to warrant continuation of the project. These compounds also bear a resemblance to the ethanolamine antihistaminics (see Table I) where the oxygen atom has been replaced by nitrogen.

Although the 2-dimethylaminoethyl side chain is typical of antihistaminic agents there is considerable overlap of activities with local anesthetics and antimuscarinics. Since only minor changes in the side chain are required to make these latter activities predominant, compounds were also prepared in the azadispiro series which it was hoped would show these activities.

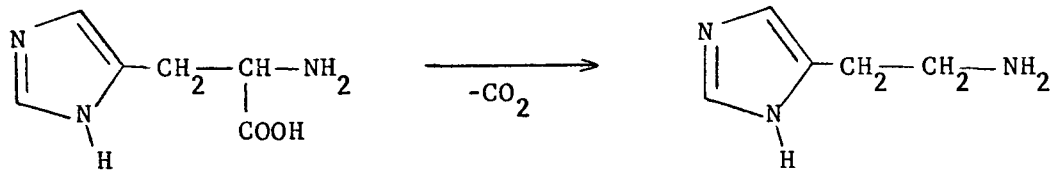
Preliminary pharmacological testing of the resultant compounds indicated a very low order of activity.

A series of derivatives and analogues of carbazole was then synthesized. It was felt that this approach offered a greater chance of success in obtaining compounds which could be used in the study of the electronic and steric requirements of the antihistaminic receptor.

HISTAMINE

Histamine 7 (2-(4-imidazolyl)ethylamine) was first synthesized in 1907 as a chemical curiosity. Soon after, its potent smooth muscle stimulant and intense depressor effects were discovered. However, it was not until 1927 that it was unequivocally demonstrated to be a constituent of normal mammalian tissue (2).

Histamine is synthesized in the body by decarboxylation of the amino acid histidine 6.



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This reaction may be mediated by a specific histidine decarboxylase or by non-specific aromatic amino acid decarboxylase which is responsible for the decarboxylation of dihydroxyphenylalanine and 5-hydroxytryptophane (5-HTP) to norephinephrine (NE) and serotonin (5-HT) (8). The rate at which decarboxylation occurs is dependent on the species and tissue studied. It appears that histamine is continuously synthesized in the body. Although there is some cellular uptake of exogenous histamine, notably after absorption from the gut of histamine formed by bacterial activity, this seems to be simply a means of rapidly reducing blood levels of circulating histamine and is followed by rapid catabolism of the exogenous amine (20).

Once formed, histamine may be catabolized or stored in either of

two sites: in mast cells, or in non-mast cell sites.

Mast cells are large, usually ovoid cells, 8-20 μ in length, which contain a large number of dark staining granules. They are widely distributed in the connective tissues and large concentrations of these cells are found around the small blood vessels. These cells are also found in large numbers in the mesentery, lung pleura, thymus, scrotum, and uterus of mammals, but are absent from the central nervous system. The number of mast cells is increased by chronic inflammation or by other conditions which result in increased local nutrition (9).

Histamine in the mast cells is primarily stored in granules which are separated from the cytoplasm by a perigranular membrane (10). The granules consist of a protein-heparin complex to which the histamine is electrostatically bound, as represented in Figure 4. The turnover rate

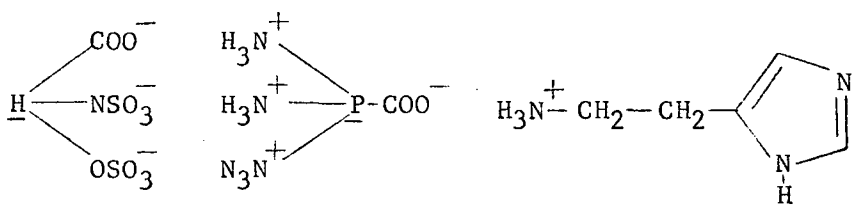


Figure 4: Hypothetical heparin-protein-histamine binding.
H = heparin; P = protein (from ref. 10)

of histamine in mast cell stores is very slow.

The protein portion of the granule consists of a number of enzymes which have protease activity (10). Zinc has also been implicated in the binding (15).

In response to a number of stimuli, the mast cell granules are

released from the cell into the surrounding tissue. This reaction may be elicited by an antigen-antibody reaction on the surface of the cell, or by a number of chemical substances which may be applied locally or systemically. One of the most potent of these histamine releasing chemicals is compound 48/80, a synthetic amine polymer.

The anaphylactic response is due to the massive release of histamine and other physiologically active substances from the mast cell (12). Release of lesser quantities of these substances in a localized area probably accounts for other allergic reactions (10).

The release of histamine from the mast cell involves: a) attachment of the releasing agent (antigen or 48/80) to the cell membrane, b) selective and extremely rapid degranulation of the cell by an energy requiring "exocytosis", liberating only the histamine containing granules without disruption of the cell membrane, and c) liberation of histamine from the histamine-heparin-protein complex by a process of ion exchange with cations of the extracellular fluid (18). Some evidence has been presented that adenosine 3',5'-monophosphate is involved in preventing the release of histamine from the mast cell in response to dextran or 48/80 (18,19).

The release of mast cell granules can be accomplished with adenosine 5'-triphosphate (ATP) and is dependent on the presence of calcium ion (11).

On release from the cell, the granule loses its membrane and histamine is released from the complex by exchange with (probably) sodium ions. The encapsulated granules are pharmacologically inert. Anticoagulant and chymase activity is also observed and is dependent on the rate

at which the granule dissolves (10).

In contrast to the mast cell stores of histamine, there are other stores which have a very fast turnover rate and which are not released by 48/80. For example, human epidermis, which is almost devoid of mast cells, contains large quantities of histamine (2). The half-life of hypothalamic histamine in the rat was found by radiotracer studies (8) to be about five minutes.

All mammalian tissue contains stores of histamine not depleted by 48/80 (13). It is these stores of histamine which have been implicated in autoregulation of body functions (13,14,20). Considering the high rate of synthesis at these sites, the so-called nascent histamine may account for the major portion of histamine in the body. Injection of small amounts of tritiated histamine indicated that the mast cells do not take up exogenous histamine but it is incorporated into other stores which have a half-life of 1-3 hours (13). The same study also indicated that some exocrine gland secretions are controlled by the release of nascent histamine. Local production and release of histamine has also been suggested as a control mechanism for the microcirculatory system. From this postulate Schayer (14) has developed unified theories which account for the glucocorticoid activity of the steroid hormones and for the activity of the thyroid hormone.

The main pharmacological effects of released histamine are on the vascular system, smooth muscles, and exocrine glands (2). The most important vascular effect in man is a powerful dilatation of capillaries and venules, which is accompanied by a weak constrictor effect on the larger veins. The arterioles are also dilated. This dilatation is

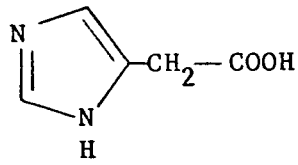
caused by a direct effect on the vessels, is resistant to atropine, and is only partially prevented by the antihistaminics although it can be reversed by sympathomimetic amines. Particularly with larger doses, the dilatation is accompanied by an increase in permeability of the vessel walls, resulting in edema due to exudation of plasma fluid and proteins into the extracellular spaces. Two commonly described phenomena attributable to this dilatory effect are the triple response and histamine headache. Histamine has no direct effect on the heart in the intact animal although reflex actions increase the cardiac output. With large doses, shock occurs.

Histamine causes constriction of smooth muscle tissue to a degree which is dependent on the species and particular tissue studied. Although in normal man bronchoconstriction due to endogenous histamine is not severe, it is in those suffering with asthma and other pulmonary diseases. This effect is readily countered by aminophylline, epinephrine, or isoproterenol while antihistaminics are less effective and slower in action. The contractile effect of histamine is dependent on the presence of calcium ion (2).

The exocrine glands, particularly those of the stomach, are stimulated to increased secretion by histamine. Actions on salivary, pancreatic, intestinal, bronchial, and lachrimal glands are much less prominent and appear to have a cholinergic component.

Metabolism of histamine (16,17) is mediated by histaminase, which may be the same as diamine oxidase, to give imidazole 4-acetic acid 8 via the acetaldehyde 9. The acid is excreted free or as the riboside. Other metabolites are 2-(1-methyl-4-imidazolyl)ethylamine 10, formed by

the action of histamine N-methyl transferase, and (1-methyl-4-imidazolyl)-acetic acid 11. Very little unmetabolized histamine is excreted.



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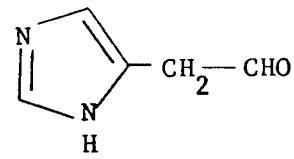

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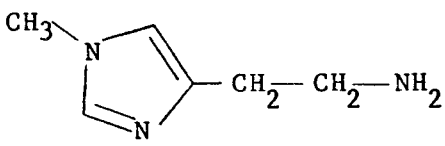

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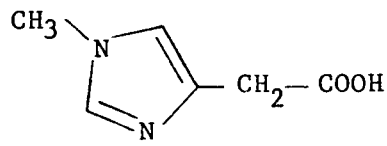


11

ANTIHISTAMINICS

As previously stated, the release of histamine from the mast cell response to an antigen-antibody reaction is associated with the allergic response and, in its most severe form, the anaphylactic syndrome. Although usually not of a severe nature, the allergic response is a nuisance and for this reason, drugs to counteract the effect of endogenously released histamine were sought. These effects may be reversed by administration of physiological antagonists such as ephedrine or epinephrine which have an effect diametrically opposed to that of histamine. This is the treatment of choice in anaphylactic reactions.

However, antihistaminics are usually defined (2,21) as drugs which antagonize some of the effects of histamine by a mechanism other than the production of a physiological antagonism. Antihistaminics do not act by a physical or chemical inactivation of histamine, and they do not prevent the synthesis of histamine nor its release from the mast cell sites (22). In fact some antihistaminics cause the release of histamine.

Antihistaminics will diminish, in varying degree, the bronchiolar and intestinal spasm, increased capillary permeability, salivation, cutaneous wheal, and release of epinephrine from the adrenals which are caused by histamine. These compounds have no effect on gastric secretion induced by histamine. A new class of compounds which competitively inhibit actions of histamine on gastric secretion has recently been reported (101).

Antihistaminics inhibit the effects of histamine by competing with the histamine molecule for the specific receptor at which histamine exerts its effect. This implies that a given dose of histamine will be countered by a sufficiently high dose of antihistaminic, and vice versa. For a further discussion of drug antagonism, see Pharmacological Testing,

Part Two, p 157.

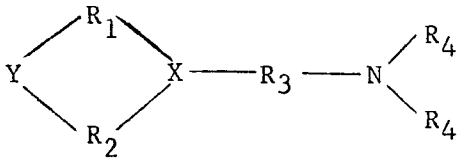
Other effects of antihistaminics include central nervous system effects (either depression or excitation), local anesthesia when applied topically, and antimuscarinic activity (2).

Antihistaminics are used in the treatment of a large number of mild to severe allergic conditions. They are of value in hay fever, particularly at the start of the season when exposure to the allergen is limited, and in skin reactions such as drug eruptions, pruritis, contact dermatitis, atopic dermatitis, and urticaria. The drugs may be used systemically or applied locally, although there is a risk of allergic reaction to topically applied antihistaminics (2,21). These drugs are useful in serum sickness but play a minor adjuvant role in anaphylactic reactions. Some antihistaminics are useful in motion sickness and other conditions causing nausea and vomiting, such as pregnancy and radiation therapy. This effect is probably the result of a central action of the compounds.

A number of side effects are common to the antihistaminics. There is a great variation in the severity and occurrence of these with the individual patient and drug. The most frequent side effect is sedation, although central stimulation is sometimes seen. Other central effects are dizziness, incoordination, blurred vision, diplopia, tinnitis, lassitude, fatigue, nervousness, tremors, and euphoria. Gastric effects ranging from nausea and vomiting through diarrhea or constipation are also seen. Drying of the mucous membranes of the mouth and respiratory system is due to the antimuscarinic effects of the antihistaminics.

STRUCTURE ACTIVITY RELATIONSHIPS (1,2,21)

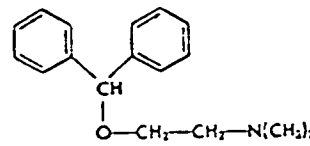
Innumerable compounds of exceedingly diverse structures have been tested for antihistaminic activity. From these studies has come a fairly well defined idea of the molecular structure required to attain activity. A general formula for antihistaminics may be represented as



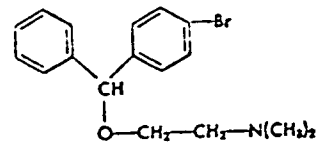
where X may be carbon, nitrogen, or an oxy ether to carbon (>CH-O). The use of a thio ether reduces activity (23). The nature of X is commonly used to classify antihistaminics, where R_3 is ethylene, as propylamine, ethylenediamine, or ethanolamine derivatives. Two subclasses of the ethylenediamines are the piperazine and phenothiazine derivatives. Representative examples of each class are given in Table I.

It is generally accepted that R_1 and R_2 must be aromatic or heteroaromatic rings, one of which may be separated from X by a methylene group. R_1 and R_2 may be linked in the ortho positions by Y, a methylene, a heteroatom, or a methylene-heteroatom function. Except in the cases where Y is present, R_3 must be ethylene to attain good antihistaminic activity. Lengthening or branching the chain markedly decreases activity although R_3 may be incorporated into a nitrogen containing ring as in the piperazine derivatives. For good antihistaminic activity, R_1 and R_2 should not be coplanar. That is, the two rings should not be directly ortho connected.

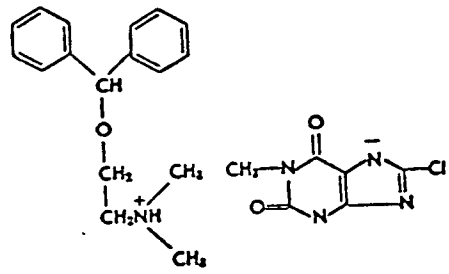
Diphenhydramine (Benadryl)



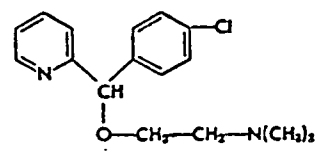
Bromodiphenhydramine HCl (Ambodryl)



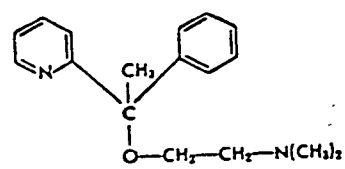
Dimenhydrinate (Gravol)



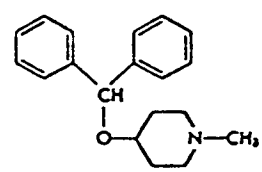
Carbinoxamine maleate (Clistin)



Doxylamine succinate (Decapryn)

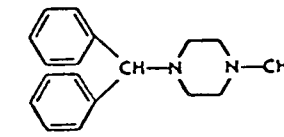


Diphenylpyraline HCl

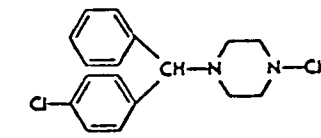


Ethanolamines

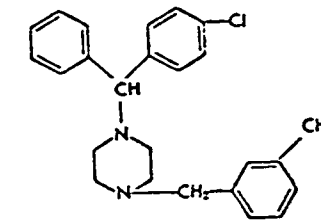
Cyclizine HCl U.S.P. (Marzine)



Chlorcyclizine HCl U.S.P. (Perazil)

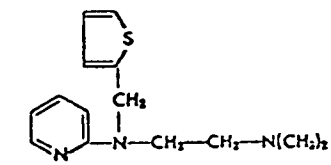


Meclizine HCl U.S.P. (Bonamine)

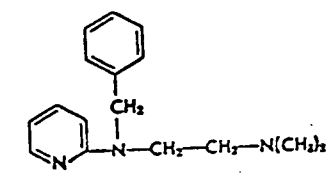


Piperazines

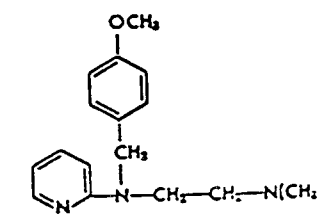
Methapyrilene HCl (Histadyl)



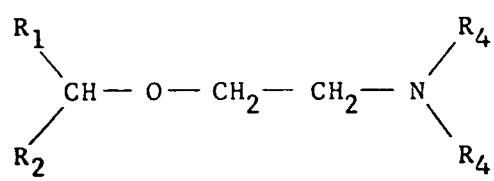
Pyrilamine maleate (Neo-Antergan)



Tripelennamine HCl (Pyribenzamine)



Ethylenediamines



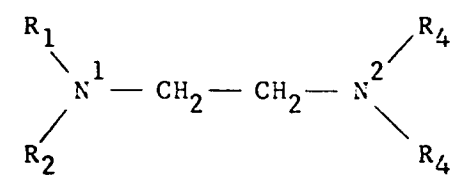
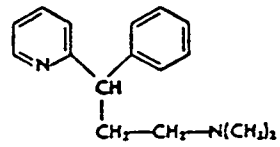
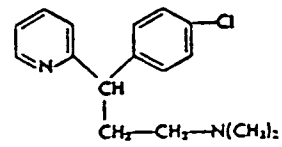


Table I: Representative Antihistaminics

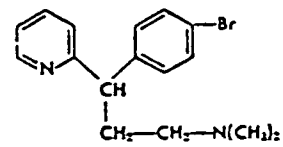
Pheniramine maleate N.F. (Trimeton)



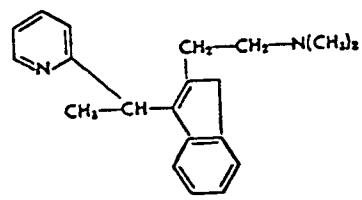
Chlorpheniramine maleate U.S.P. (Chlor-Tripolon)



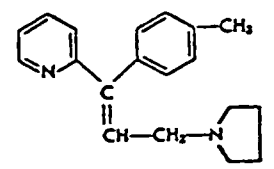
Brompheniramine maleate N.F. (Dimetane)



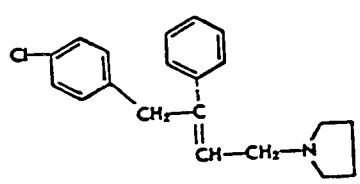
Dimethindene maleate N.N.D. (Forhistal)



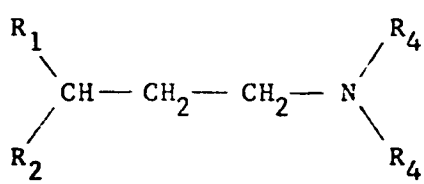
Triprolidine HCl N.N.D. (Actidil)



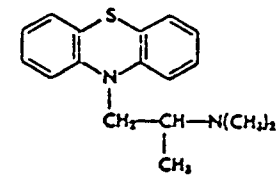
Pyrrobutamine phosphate N.N.D. (Pyronil)



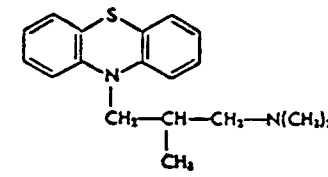
Propylamines



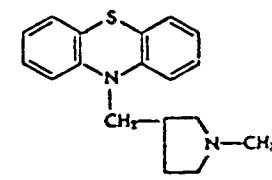
Promethazine HCl U.S.P. (Phenergan)



Trimeprazine tartrate N.N.D. (Panectyl)



Methdilazine HCl N.N.D. (Dilosyn)



Phenothiozines

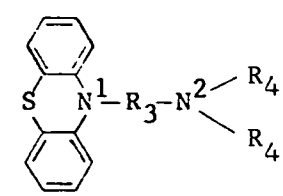


Table I (cont'd): Representative Antihistaminics

Where Y is present, R_3 may be branched α to the nitrogen atom, as in promethazine, or a propylene group may be used. In both cases, activity is increased over the analagous compound containing an ethylene group.

In general, R_4 are methyl groups, although $-N(R_4)_2$ may be incorporated into a small ring system. Use of ethyl groups for R_4 decreases the antihistaminic activity and increases the antimuscarinic activity.

A distance of 5-6 A from the amino nitrogen to the centre of one of the aromatic rings gives the strongest competitive activity.

Substitution of one of the aromatic rings with a methyl or a chloro group generally increases activity. Ortho substitution on both rings decreases activity and has little effect if only one of the rings is substituted. Nauta (24a) has explained this relationship in alkyl substituted diphenhydramine derivatives by proposing that one of the aromatic rings interacts with the oxygen lone pair electrons to decrease the electron density of the oxygen atom. Para alkyl substitution enhances the overlap interaction while ortho substitution decreases the interaction.

In optically active compounds the dextro isomer is generally more active than the levo isomer although the reverse is true in some cases. Few studies have been done on compounds whose absolute configurations are known. Stereoselectivity is observed only when the asymmetric carbon atom is α to the aromatic ring system. Since histamine itself is optically inactive, this information may indicate that an asymmetric area is adjacent to the histamine receptor, or that an asymmetric conformer of histamine is the active species.

Failure to relate physical properties such as solubility, relative surface activity, and ionization constants to activity of antihistaminics,

as well as isomer stereoselectivity, indicates that steric factors are very important in determining the activity of these compounds.

LOCAL ANESTHETICS (25,26)

Local anesthetics are drugs which block nerve conduction when applied to nerve fibres. All nerve fibres, sensory or motor, myelinated or unmyelinated, are affected by these agents. The action of local anesthetics is ideally completely reversible so that there is no permanent damage to the nervous tissue. Local anesthetics may be applied topically to whole or abraded skin, injected intra- or subcutaneously into the area to be anesthetized (infiltration anesthesia), or injected adjacent to nerve trunks and their branches or into the epidural or subarachnoid spaces (nerve block and spinal anesthesia). The fine nerve fibres and endings are blocked in the region of the injection by infiltration anesthesia while nerve impulses to and from areas remote from the injection are halted by nerve block and spinal anesthesia.

The effects of these drugs are sensory impairment and muscle relaxation. Their systemic effects are of no therapeutic value.

The mechanism of action is unknown although it appears that the site of action is the cell membrane with no effect on the axoplasm. Shanes (27a,b) has suggested that these compounds increase the surface pressure of the nerve membrane lipids, thus closing off the pores through which ions move in the propagation of the action potential. The drugs act by preventing the large transient increase, caused by a slight depolarization of the membrane, in permeability of the membrane to sodium ions. The resting permeability to potassium ions is also reduced. The cationic form of the molecule is responsible for the activity at the nerve membrane.

The duration of action of local anesthetics is largely determined

by the rate at which the drug is absorbed into the blood stream. In order to prolong anesthesia, vasoconstrictor substances are frequently added to preparations for injection. This has the added advantage of reducing the incidence and severity of systemic side effects since the rate of metabolism of absorbed compound is not affected. Most of the synthetic local anesthetics are esters of aromatic carboxylic acids and are metabolized by plasma or liver esterases.

The toxic effects of local anesthetics are caused by systemic extension of their therapeutic properties. Thus, there is decreased electrical excitability and conduction in the myocardium. The central nervous system is both depressed and excited, although it is thought that the excitation is due to a preferential depression of inhibitory fibres. Contractions of smooth muscle are also diminished. In rare cases, hypersensitivity occurs and may appear as a dermatitis, an asthmatic attack, or an anaphylactic reaction. Addition of a vasoconstrictor may result in tissue necrosis, edema, or delay in wound healing, particularly when sympathomimetic amines are used. Local side effects are neurolysis, and subsequent slough of the tissue, and necrosis of surrounding tissues.

Some examples of synthetic local anesthetics are given in Table II.

Structure Activity Relationships

Most of the structures which have been tested for local anesthetic activity may be related to a general representation as

lipophilic portion-intermediate chain-hydrophilic portion

or, more specifically,

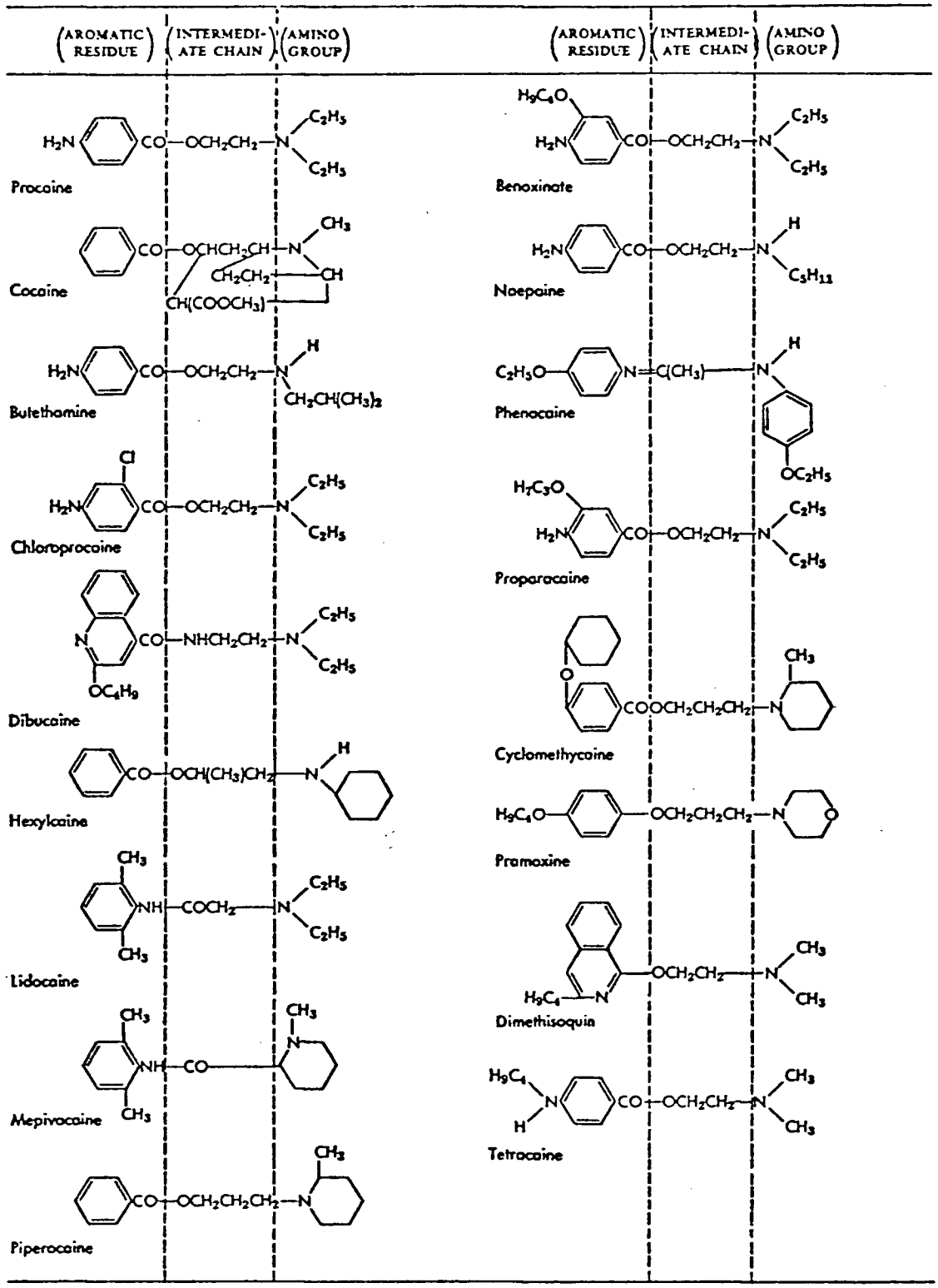


Table II: Representative Local Anesthetics

aromatic residue-intermediate chain-amino function

A balance between lipophilic and hydrophilic nature is necessary, as in all drugs, to attain distribution through the extracellular (aqueous) phase and the lipoidal membranes.

The amino group is usually tertiary but may be secondary. Increasing the size of the N-alkyl substituents increases activity and toxicity of the compound. The N-alkyl substituents may be the same or different. The amino function may also be incorporated into a ring system such as piperidino or morpholino.

The intermediate chain is usually an ethyl group which is linked to the aromatic residue via an ester. Amides, imides, ethers, and ketones may also be used. Lengthening of the chain increases activity and toxicity with the propyl group being optimal. The nature of the link to the aromatic function largely dictates the duration of activity since it is this link which is hydrolyzed in the body. Thus 2,6-disubstitution on the aromatic ring is often seen.

Generally, the aromatic residue is a benzoic acid derivative, although a large number of other functions can be used. Substitution of a para-amino group on benzoic acid increases activity. Subsequent alkylation increases activity further although toxicity also increases with the size of the alkyl group. A para-alkoxy also increases activity and toxicity in proportion to the size of the group.

ANTIMUSCARINICS (28,29)

The antimuscarinics are drugs which block the actions of acetylcholine (ACh) at structures which are innervated by postganglionic parasympathetic (cholinergic) fibres. They also block the effect on smooth muscles which respond to ACh but lack cholinergic innervation. Antimuscarinics generally have no effect on other actions of ACh but at high doses transmission at autonomic ganglia and neuromuscular junctions may be blocked. The antimuscarinics exert their effects by a competitive blockade of ACh at the muscarinic receptor.

These drugs are used in conditions characterized by glandular hypersecretion and spasm or hypermotility of smooth muscle. They are widely used in ophthalmology to produce mydriasis and cycloplegia. They are used as preanesthetic medication to depress salivary and respiratory tract secretions, and they are used in the treatment of gastric and duodenal ulcers. They may also provide symptomatic relief of acute rhinitis in hay fever and colds. All of the antimuscarinics have central effects which vary with the particular drug used. Only the belladonna alkaloids, atropine and scopolamine (the prototype compounds), and their semisynthetic derivatives are clinically useful in this respect. These compounds are shown in Table III.

These alkaloids are used in the treatment of parkinsonism, and scopolamine is one of the more useful drugs for prevention of motion sickness. The sedative and tranquilizing effects of scopolamine have been used in other states, including labor, toxic psychoses, and delirium tremens. The antimuscarinics are also used in the treatment of poisoning with organophosphate insecticides and the rapid type of mushroom poison-

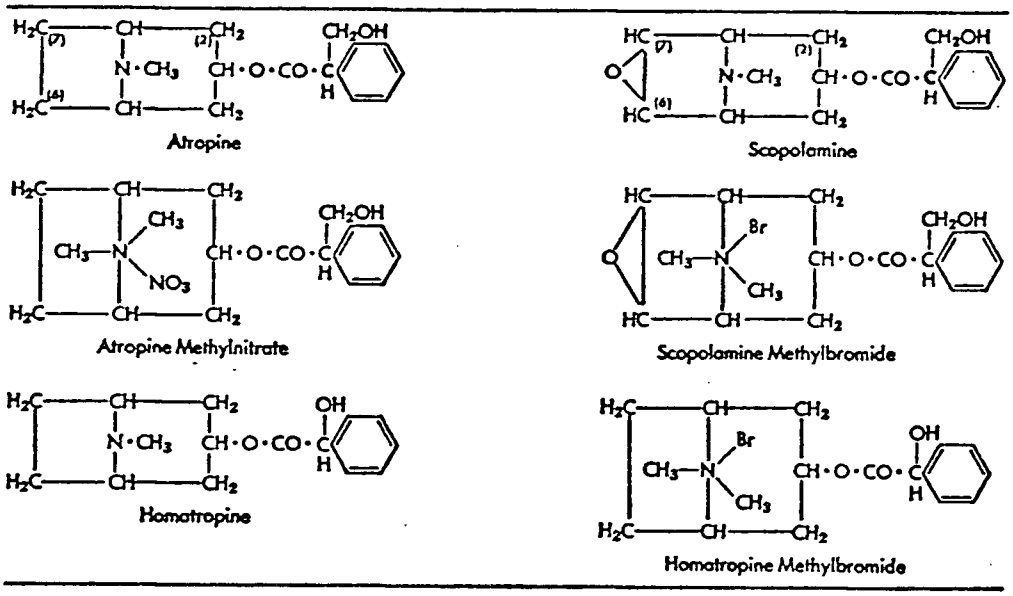
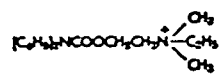
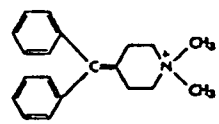
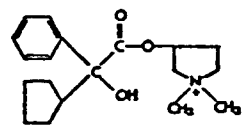
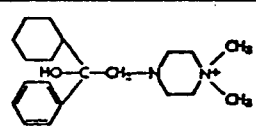
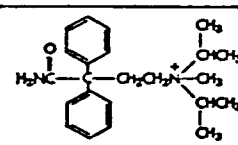
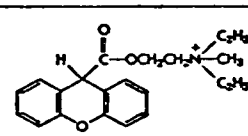
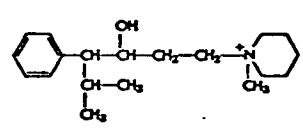
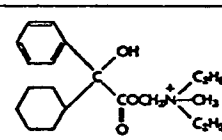
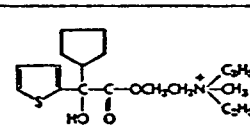
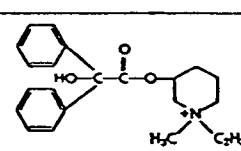
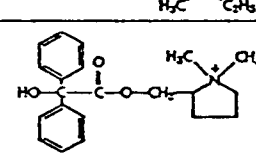


Table III: Antimuscarinic Belladonna Alkaloids and Semisynthetic Derivatives.

ing due to Amanita species. Examples of synthetic antimuscarinics are given in Table IV.

The susceptibility of the parasympathetic neuroeffector junction to the antimuscarinics varies. The order of sensitivity to the various drugs varies little although some compounds have a degree of specificity. Antimuscarinic agents, when administered systemically, tend to inhibit salivation and sweating most readily. This is followed, at increasing doses, by inhibition of the responses of the iris and ciliary body, vagal control of cardiac rate, gastrointestinal and urinary bladder tone and motility, and finally gastric secretion.

The side effects of these drugs are related to blockade of responses at organs other than the target of therapy. Side effects are seldom

NONPROPRIETARY NAME	TRADE NAMES	CHEMICAL STRUCTURES
Quaternary Ammonium Compounds		
Dibutoline	Dibuline	
Diphemanil	Prantal	
Glycopyrrolate	Robinul	
Hexocyclium	Tral	
Isopropamide	Darbid	
Methantheline	Banthine	
Mepiperphenidol	Darstine	
Oxyphenonium	Antrenyl	
Penthienate	Monodral	
Pipenzolate	Piptal	
Poldine	Nacton	

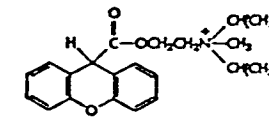
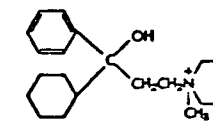
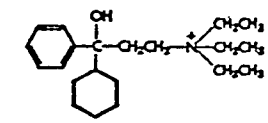
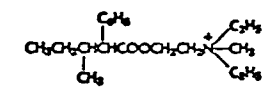
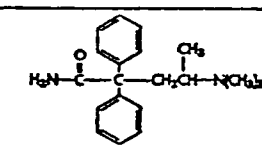
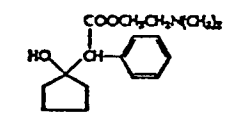
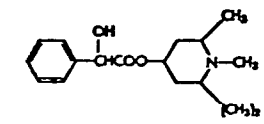
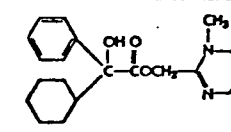
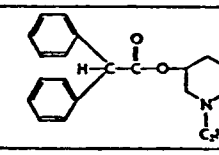
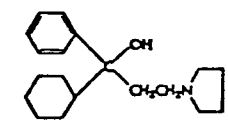
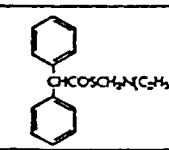
NONPROPRIETARY NAME	TRADE NAMES	CHEMICAL STRUCTURES
Propantheline	Pro-Banthine	
Tricyclamol	Elorine Tricoloid	
Tridihexethyl	Pathilon	
Valethamate	Murel	
Other Synthetic Antimuscarinic Compounds		
Aminopentamide	Centrine	
Cyclopentolate	Cyclogyl	
Eucatropine	Euphthalmine	
Oxyphencyclimine	Daricon Vio-thene	
Piperidolate	Dactil	
Procyclidine	Kemadrin	
Thiphenamil	Trocinate	

Table IV: Representative Antimuscarinics

serious but limit therapy because of subjective unpleasantness. Dryness of the mouth, blurred vision, photophobia, and tachycardia are the most common side effects. Inhibition of sweating may cause heat intolerance in some individuals in hot climates. Acute glaucoma may be precipitated by local or systemic use and it is of particular importance that care be taken in the use of these drugs in patients over 40 years of age. Constipation is not uncommon and complete blockage may occur. Urinary retention also occurs and may be aggravated by prostatic hypertrophy. Impotence is a less common side effect of compounds having some ganglionic blocking activity. Reduction of the volume of bronchial secretions may result in the formation of viscid plugs in the bronchial tree which may be particularly dangerous in individuals with chronic respiratory dysfunction. Hypersensitivity reactions, manifested usually as skin rashes which may proceed to exfoliation, are rare occurrences.

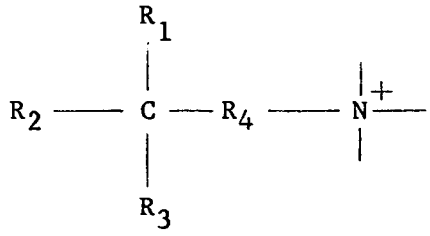
The belladonna alkaloids have a greater tendency to produce blurred vision than side effects related to the reduced tone and motility of smooth muscle while the converse is true of synthetic agents which are capable of producing considerable ganglionic blockade. Tolerance to the side effects is not uncommonly developed, although a parallel reduction in therapeutic effect is usually seen.

Structure Activity Relationships

Because of the difficulty in separating the effects of the antimuscarinics, vast numbers of compounds have been synthesized. The chemical structures vary a great deal and this has given rise to a highly complex set of structure activity relationships which can be

only superficially reviewed here. Even though a great variety of compounds has been synthesized, few of these have anything to offer in the way of increased specificity, with respect to the site of action, over the prototype alkaloids.

A general formula for the antimuscarinics may be represented as



Usually, R_1 is an aromatic function fulfilling certain spatial requirements, R_2 is a normal alkyl or alicyclic function, and R_3 is a group capable of hydrogen bonding, a small alkyl, or hydrogen. The group, R_4, linking the cyclic substituent to the cationic head is generally an alkyl ester of a specific length.

The one feature which is common to nearly all effective antimuscarinics is the cationic head. It is this group which is thought to be most important in the initiation of complex formation between the muscarinic receptor and the antagonist. This function is also common to the muscarinic agonists.

Usually the cationic head is a quaternary ammonium group, although tertiary amines having a pK_A such that a large portion of the dose used is protonated at physiological pH may also be used. Sulfonium and phosphonium groups are used infrequently. High basicity of the cationic function increases the stability of the drug-receptor complex and thus

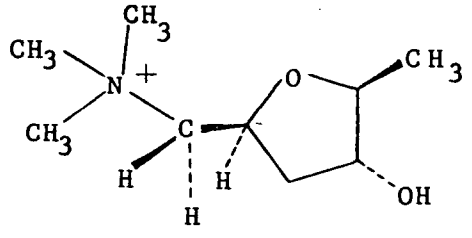
increases activity. However, steric factors are also important. The best activity is seen with di-iso-propyl- or diethylamino and trimethylammonium functions. Larger and smaller groups show a decrease in activity. The cationic head may be incorporated into a suitable ring system. The cationic charge is not absolutely necessary to activity, since the trimethylammonium group may be replaced by t-butyl to give active agonists and antagonists.

The cyclic substituents, R_1 and R_2, are most commonly phenyl rings. They may be incorporated into a polycyclic system such as fluorene, or heterocyclic systems may be used. Frequently, one of the rings is alicyclic C-5 or C-6 or the corresponding unsaturated carbocyclic system. A normal alkyl substituent may also be used but this decreases activity. The greatest activity is obtained in compounds with two cyclic substituents; a third decreases activity. Large cyclic groups such as naphthyl and biphenyl also decrease activity. One cyclic group will confer activity provided an alkyl or hydroxyl group is also present. Compounds with differing cyclic groups are usually more active and less toxic. Normal or branched alkyl substituents are often located at the same carbon atom as one or two cyclic groups.

These groups, R_1, R_2, and R_3, are felt to contribute to receptor binding by hydrophobic or van der Waals binding and thus increase the affinity for the receptor. If the groups are sufficiently large, they may overlap with adjacent receptors and thus give a greater degree of activity provided steric interaction does not prevent binding to the receptor.

The cationic head and the cyclic moiety are connected by a chain,

R_4, that must meet certain requirements. For compounds containing an ester function, five atoms must be contained in the chain for maximum activity. These are usually esters of substituted phenylacetic acids with trialkylammoniumethanol. Where the chain consists of an alkyl group, maximum activity is attained with the n-propyl function. It is thought that the ester compounds interact with the receptor in a skewed spatial conformation which has a structure similar to that of muscarine 12, while the alkylamines interact in an extended conformation. The distance from the cationic head to the carbon atom on which the cyclic function is substituted is the same in both cases. This conformational representation is shown in Figure 5.



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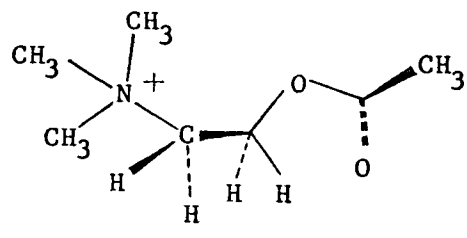
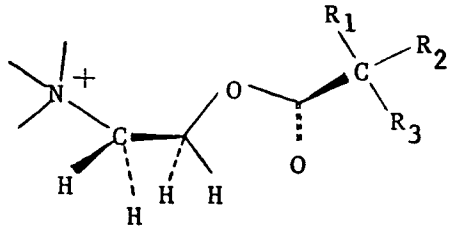
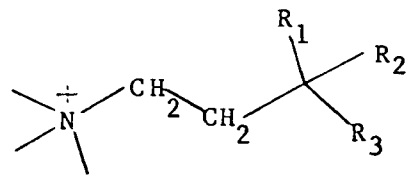


Acetylcholine



a



b

Figure 5: Active conformations of muscarine 12, acetylcholine and anti-muscarinics (ester (a) and alkylamine (b) types). (from ref. 29)

Branching of the chain decreases activity. The ester function is not necessary for activity although it may increase binding by interacting with the receptor. It may also influence the conformation of the compound and thus influence the degree of interaction of the essential groups with the receptor.

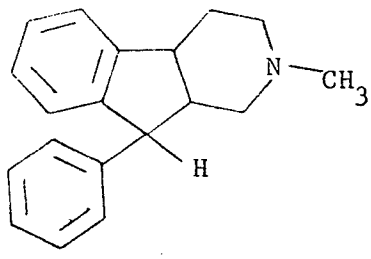
The presence of a hydroxyl group on the chain β to the carbonyl group of the ester increases activity. Compounds with an α-hydroxy function are also active. In the aminoalcohols (ie R_4 is an alkyl group), optimal activity is attained when the hydroxy is on the third carbon atom from the nitrogen. Generally, hydroxy functions are located at the same, or the carbon adjacent to, carbon to which the cyclic structures are attached. The hydroxy group may be replaced by cyano or primary carboxamide, but activity is decreased. Alkoxy and acetamido groups also lower activity.

Where a centre of asymmetry is present, the levo isomer generally has more activity than the dextro isomer. Although absolute configurations are not generally known and no specific inferences can be drawn from the data, it does point out that the muscarinic receptor is stereoselective.

DRUG-RECEPTOR INTERACTIONS

The mechanism by which homeostasis is maintained is dependent on a delicate balance between the effects of the active humoral agents normally present in the body. In disease states this balance is disrupted and drugs are administered which may mimic or antagonize the effects of one or more of the natural agents in an attempt to reattain the natural balance. It is generally assumed, for drugs which either directly mimic or competitively inhibit the actions of normal constituents of the body, that the drugs act at a "receptor" at which the humoral agents are normally active. The mechanism by which drugs exert their effect is for the most part unknown, although a great number of theories have been proposed. No direct evidence on the nature of the postulated receptors is available, but by indirect evidence obtained chiefly by the study of the relationship of chemical structure to activity it is possible to describe the steric and electronic requirements of the receptors and to theorize on the mechanism of drug action.

Nauta and Harms (30) have noted that rigid and semi-rigid structures such as phenindamine 13 and chlorcyclizine 14 are highly active antihistaminics, while ortho substituted benzhydryl ethers 15 are nearly inactive as antihistaminics but antimuscarinic activity is increased. They have proposed that the active conformation of the antihistaminics is one in which the side chain is folded as in 16. Ortho substitution inhibits folding of the side chain. Studies of models show that nearly all of the practically useful antihistaminics can assume this conformation. They have also proposed that the extended conformer



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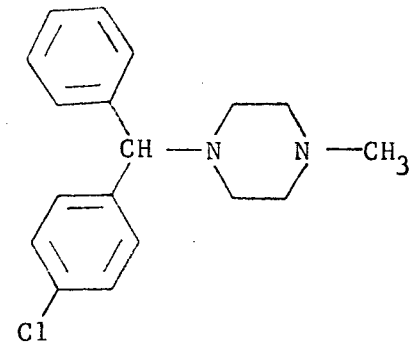

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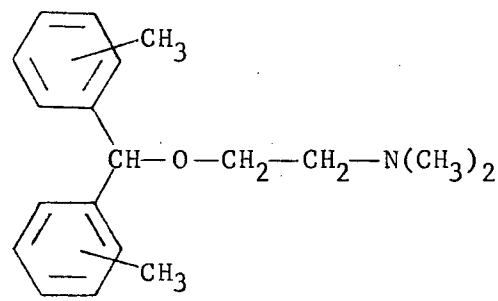


15

Substitution at 2

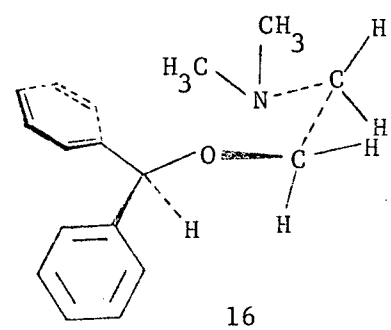
2,2'

2,6

2,6,2'

2,6,2'6'

17 is the inhibitory species at the muscarinic receptor. The distance from the amino nitrogen to the central carbon atom in 17 falls within



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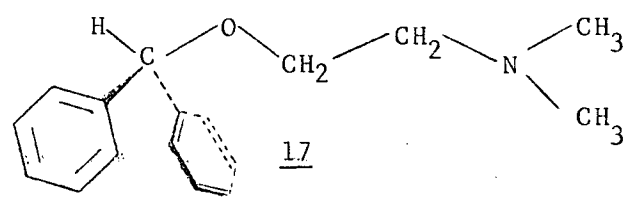


17

the range common to the antimuscarinics. The concept of a close fit at a receptor is strengthened when the relative activities of optical antipodes is considered.

Ham (31) has recently shown that appreciable quantities of the gauche conformers are present in aqueous solutions of the salts of some antihistaminics.

Through a series of papers, Nauta et al (24b) have further developed their receptor theory to the point where specific anchoring groups have been proposed. Their speculative receptor model is shown in Figure 6. The minimum requirements of a receptor surface for antihistaminics, and therefore for histamine, would appear to be an anionic site to accommodate the basic centre which is protonated at physiological pH, and a flat region, at a more or less fixed distance from the anionic site, which is capable of van der Waals binding with one of the aromatic rings. The two binding sites must be approximately co-planar. Other binding models which may be important at the receptor surface are London forces, hydrogen bonds, and hydrophobic bonds.

Throughout the work of Nauta et al the concept of complementarity of the histaminic and muscarinic receptors appears. They have found that in nearly every case an increase in antihistaminic activity is accompanied by a decrease in antimuscarinic activity (23). This effect extends to the stereoisomers of the compounds they have prepared such that, in a series of ortho substituted compounds, one set of antipodes is predominantly antihistaminic while the other set is predominantly antimuscarinic (32). A similar effect is seen in the para substituted compounds although the pattern is not as rigid as in the ortho substituted

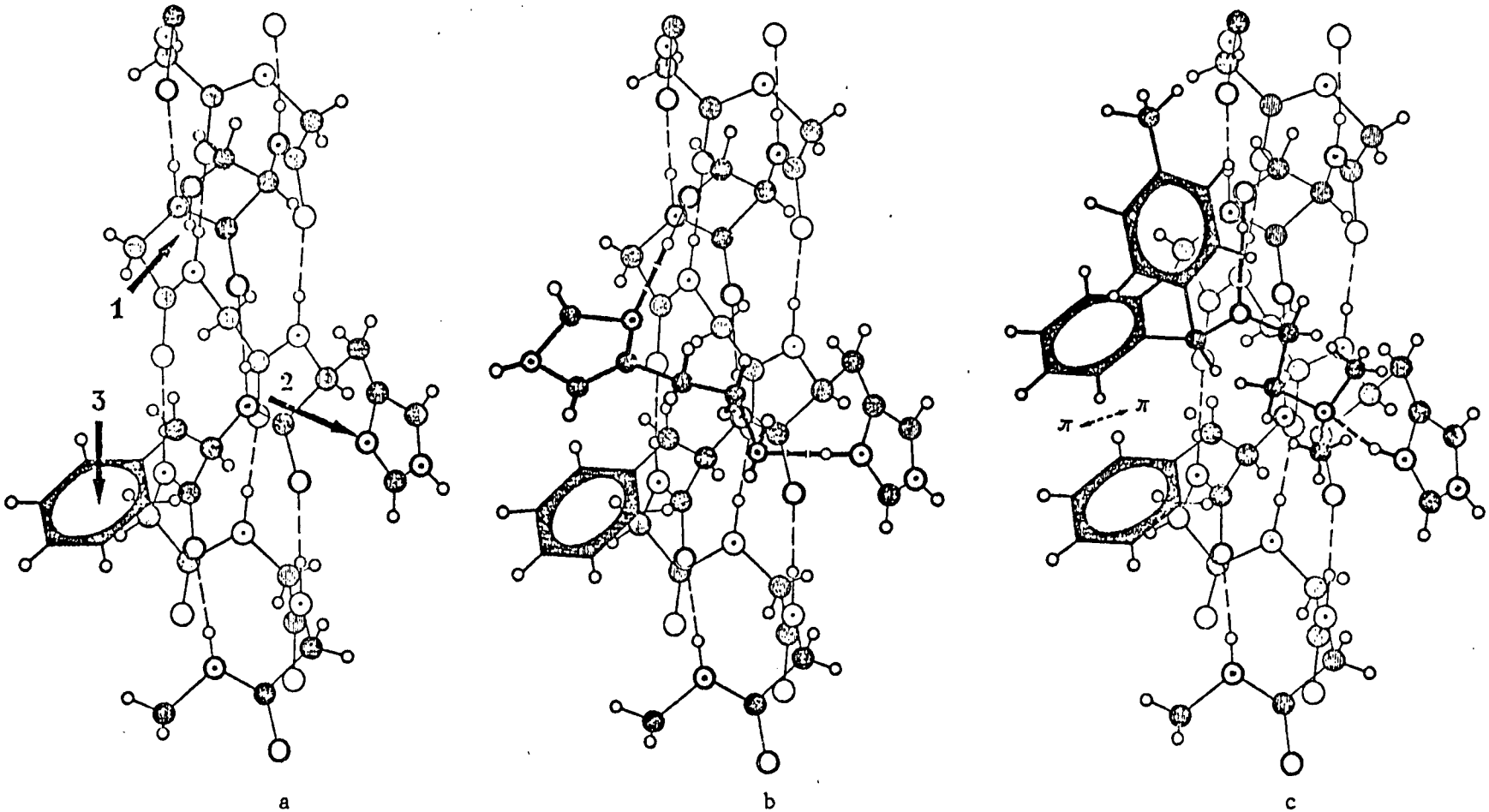


Figure 6: a) α-Helix receptor model showing three anchorage sites: 1) serine -OH; 2) histidine -N^3; 3) phenylalanine -phenyl group. Small circles = H; large shaded circles = C; large open circles = O; centred circles = N; broken lines = H-bonds.

b) Receptor model accommodating histamine.

c) Receptor model accommodating 4-methyldiphenhydramine.

(from ref. 24)

series.

It appears that Nauta has now abandoned the concept of folded and extended conformers in favour of a receptor theory which embodies the concept of complementarity between the antihistaminic and antimuscarinic receptors (32). The electron density on the oxygen atom is the most critical factor in determining the type of activity demonstrated with a particular compound and is influenced by the manner in which the aromatic rings are presented to the receptor surface. It was pointed out that topologically identical receptors are not implied in this theory. It is felt that both receptors have no bulk tolerance to accommodate protruding para substituents at the binding site for the aromatic ring while the receptors will accommodate ortho substituents. Substituents on the unbound aromatic nucleus alter the electron density on the oxygen atom and thus alter the activity of the compound. A balance between antihistaminic and antimuscarinic activity is thus obtained. In the case of para alkyl substituents a positive mesomeric effect increases the overlap interaction resulting in decreased electron density about the oxygen atom and increased antihistaminic potency. The ortho alkyl substituents sterically inhibit the interaction causing decreased antihistaminic effect with a concomitant increase in the antimuscarinic effect. The positive inductive effect of ortho alkyl groups tending to increase the oxygen electron density must also be considered.

This concept is at odds with the previously mentioned theory for which the receptor site shown in Figure 6 was proposed. As the electron density about the oxygen atom decreases, the tendency to form a hydrogen bond would also be expected to decrease, thus lowering the

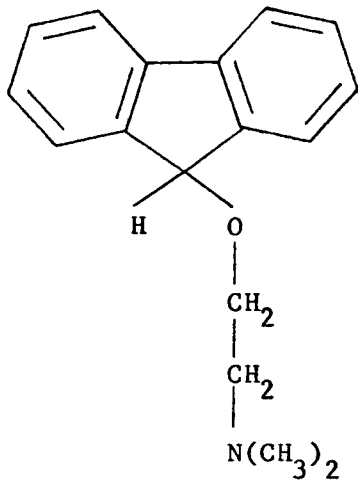
total binding energy of the drug-receptor complex which would result in a decrease in activity.

Nauta's work has been primarily concerned with the effect of alkyl substitution in the aromatic rings of diphenhydramine and it is difficult to determine the applicability of his theories to other series of antihistaminics.

It was previously stated that the aromatic rings of antihistaminics should not be coplanar. This conclusion has been reached from studies of dimethylaminoethyl 9-fluorenyl ether 18, which shows only 1% of the activity of diphenhydramine, and of a number of compounds where the two aromatic rings are ortho linked by one or more atoms, which demonstrate good antihistaminic activity. However, 9-(2-dimethylaminoethyl)fluorene 19, synthesized in this work, shows specific activity of a fairly high order. Two other compounds synthesized in this work, 9-(2-dimethylaminoethyl)carbazole 20 and N-(2-dimethylaminoethyl)diphenylamine 21 show comparable activities.

The contribution to receptor binding for a five, six, or seven-membered alicyclic ring is expected to be less than that of an aromatic ring. The factors which influence the degree to which a ring is bound are the size and planarity of the ring and the angle between the substituent chain and the plane of the ring.

In the azadispiro compounds synthesized in this work, the two outer alicyclic rings are held perpendicular to the central ring and can assume a position coplanar with the substituent chain amino function only at the expense of considerable non-bonded interaction. In this coplanar configuration the interatomic distance from the terminal nitrogen to



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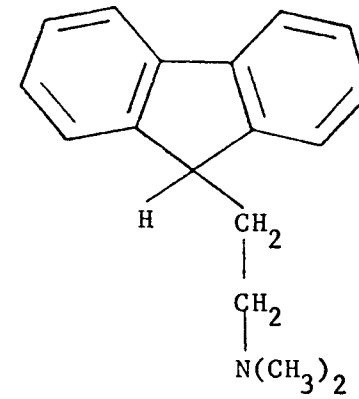

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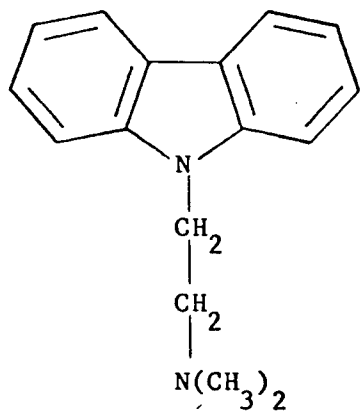

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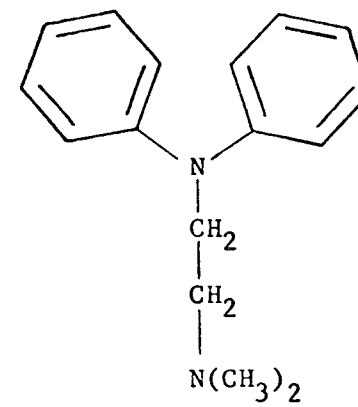

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the apex carbon of the lactam ring is about 4-4.5 A, which is in close agreement with the corresponding distance in chlorcyclizine and phenindamine. The distance from the terminal nitrogen to the centre of one of the coplanar rings is about 5 A while the distance to the centre of the other ring is about 7 A. Corresponding distances to the centre of either ring in chlorcyclizine are 5.5 A and in phenindamine are 5.5 A. A similar distance is noted in the folded conformer of the carbazole analogues which were synthesized.

It would appear from a study of models of active antihistaminics and the compounds proposed in this work that the two series of compounds would be useful tools with which to investigate the steric and electronic requirements of the antihistaminic receptor.

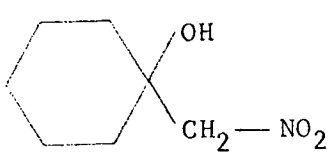
DISCUSSION OF THE CHEMISTRY

PART ONE

DERIVATIVES AND ANALOGUES OF 7-AMINO-14-AZADISPIRO[5.1.5.2]PENTADECAN-15-ONE

The compounds synthesized in this work are shown in Table V. Those tested for pharmacological activity were 23, 24, 25, 26, 28, and 30. The general reaction sequence used to synthesize the compounds is shown in Scheme I, using the 14-azadispiro[5.1.5.2]pentadecan-15-one series as an example. Synthesis of the corresponding 16-azadispiro[6.1.6.2]-heptadecan-17-one derivatives was accomplished by replacement of cyclohexanone with cycloheptanone in the first step of Scheme I.

The initial dispiro product, 14-hydroxy-14-azadispiro[5.1.5.2]-pentadec-9-en-7,15-dione 7-oxime 32, was first prepared, although not identified, in 1941 by Nightingale, et al (33) in 8.3% yield by heating nitromethane and cyclohexanone at 105° in the presence of piperidine or di-n-propylamine catalyst. Analogous solid products were also obtained from 4-methyl- and 3-methylcyclohexanone, but not from 2-methylcyclohexanone (34). Lambert and Lowe (39) obtained the same product using diethylamine as the catalyst and assigned the formula $C_{14}H_{20}N_2O_3$. They also isolated 1-nitromethylcyclohexanol 33, 1-nitromethylcyclohexene 34, and 1,1-bis(nitromethyl)cyclohexane 35 from the reaction.



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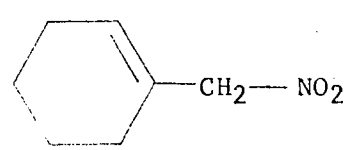

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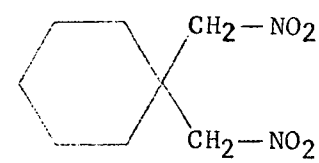

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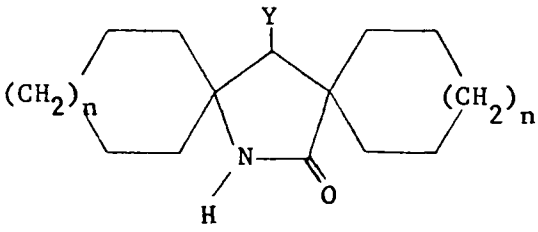


A

$R_1 = -NH_2$

$R_2 = -NH-CO-CH_2Cl$

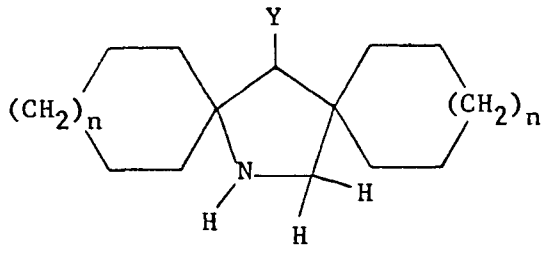
$R_3 = -NH-CO-CH_2-N(CH_3)_2$

$R_4 = -NH-CO-CH_2-N(CH_3)_2.MeI$

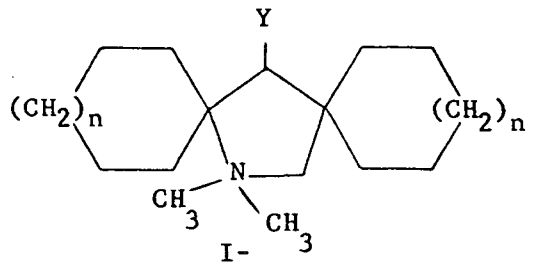
Compound

No.	nucleus	n	Y	Compound Name
4	A	1	R_1	7-amino-14-azadispiro[5.1.5.2]pentadecan-15-one
22	A	1	R_2	7-chloroacetamido-14-azadispiro[5.1.5.2]-pentadecan-15-one
23	A	1	R_3	7-dimethylaminoacetamido-14-azadispiro-[5.1.5.2]pentadecan-15-one
24	A	1	R_4	7-dimethylaminoacetamido-14-azadispiro-[5.1.5.2]pentadecan-15-one methiodide
5	A	2	R_1	8-amino-16-azadispiro[6.1.6.2]heptadecan-17-one
27	A	2	R_2	8-chloroacetamido-16-azadispiro[6.1.6.2]-heptadecan-17-one
28	A	2	R_3	8-dimethylaminoacetamido-16-azadispiro-[6.1.6.2]heptadecan-17-one
29	A	2	R_4	8-dimethylaminoacetamido-16-azadispiro-[6.1.6.2]heptadecan-17-one methiodide

Table V: Azadispiro compounds synthesized in this work.



B

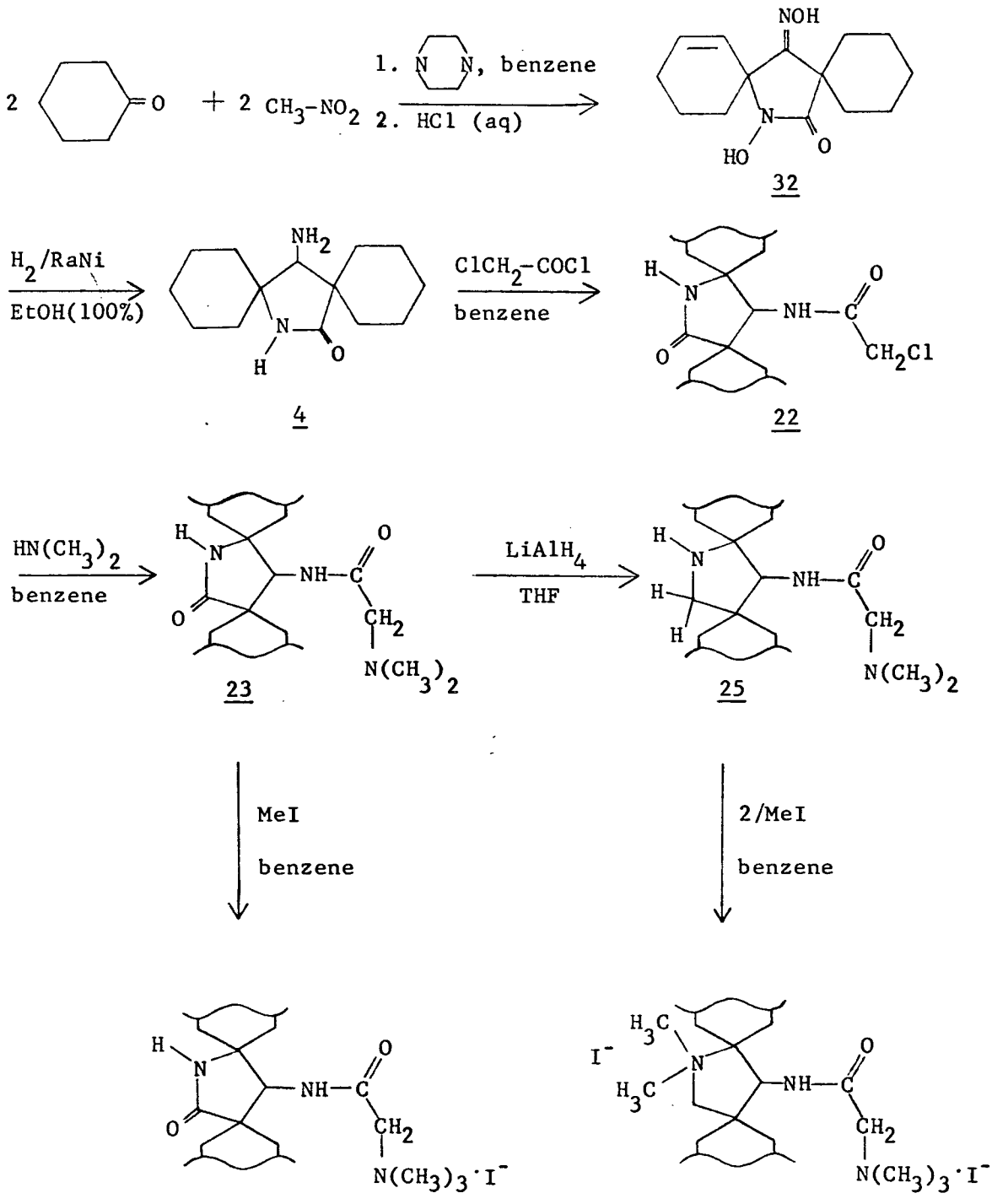


C

25	B	1	R_3	7-dimethylaminoacetamido-14-azadispiro-[5.1.5.2]pentadecane
26	C	1	R_4	7-dimethylaminoacetamido-14-azadispiro-[5.1.5.2]pentadecane dimethiodide
30	B	2	R_3	8-dimethylaminoacetamido-16-azadispiro-[6.1.6.2]heptadecane
31	C	2	R_4	8-dimethylaminoacetamido-16-azadispiro-[6.1.6.2]heptadecane dimethiodide

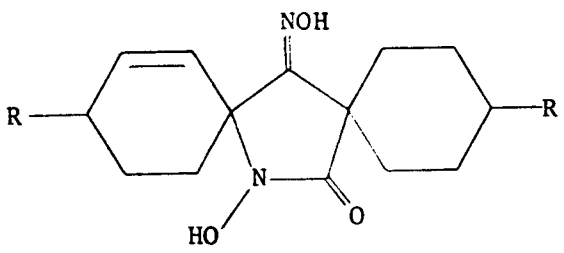
Table V (cont'd): Azadispiro compounds synthesized in this work.

Scheme I: Synthesis of derivatives of 7-amino-14-azadispiro[5.1.5.2]-pentadecan-15-one



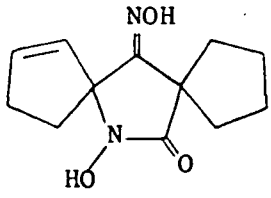
Nightingale, et al (34) obtained low yields of 32 from 33 and from 34 in the presence of secondary amine catalysts. Increased yields (14%) of 32 from cyclohexanone were obtained by addition of benzene to the reaction mixture to allow azeotropic removal of the water formed in the condensation reaction. Nitroethane, nitropropane, and phenylnitromethane did not give a solid product with cyclohexanone and no solid was obtained with the use of sodium ethoxide as the catalyst in a reaction with nitromethane. Cyclopentanone gave an analogous solid product with nitromethane (34).

A thorough investigation into the effect of the amount and type of catalyst used was carried out by Nightingale, et al (35,36,40). In general, piperazine was found to be the best catalyst for the synthesis of 32, its 3,11-dialkyl derivatives 36, and the analogous compounds (37,38, and 39 respectively) prepared from cyclopentanone, cycloheptanone, and cyclooctanone.



36

R = Me
Et
n-Pr
n-Bu
i-Pr
s-Bu
t-Bu



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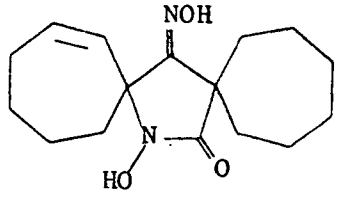

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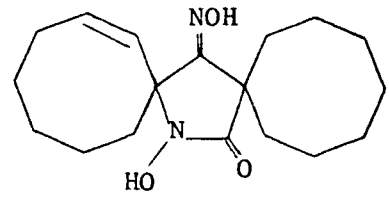


39

Other catalysts used include piperidine, morpholine, 2-methylpiperazine, pyrrolidine, hexamethylenamine, and diethylamine. Methylamine, triethylamine, tetramethylammonium hydroxide, and sodium ethoxide do not catalyze the reaction.

A study of the reactions of 32 led Nightingale, et al (34) to propose the partial structure $(C_{13}H_{18}NO)$-CONHOH, a hydroxamic acid. They also suggested the presence of non-conjugated $\geq C{=}C\leq$ and $\geq C{=}N$- functions.

The structure of 32 was determined in 1962 by Noland and Sundberg (37) by functional group determination and degradation experiments. These experiments are summarized in Charts 1,2, and 3. These Charts are copied directly from (37a); compounds I and II in the Charts correspond to 32 and 4, respectively, in this thesis.

In Nightingale's proposed formula, hydrolysis of the hydroxamic acid should lead to a carboxylic acid $(C_{13}H_{18}NO)$-COOH. Failure of acid catalyzed esterification of the hydrolysis product IV from I (32) and the low frequencies of ir absorption bands possibly attributable to a carboxyl function cast doubt on the presence of hydroxamic and carboxylic acid groups.

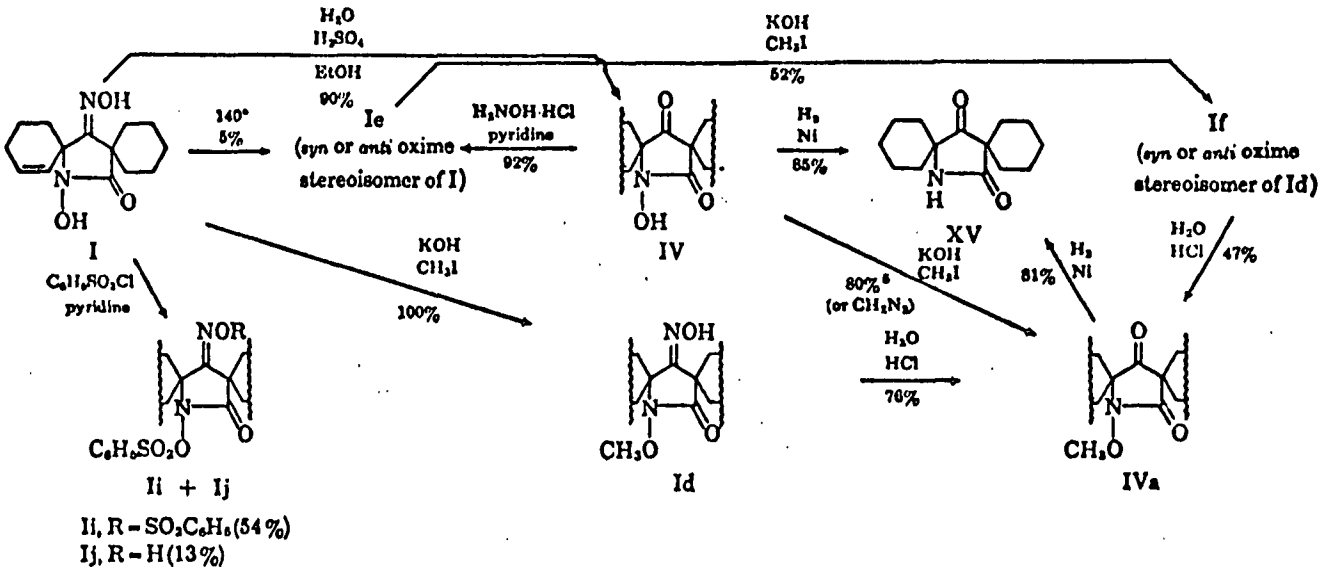


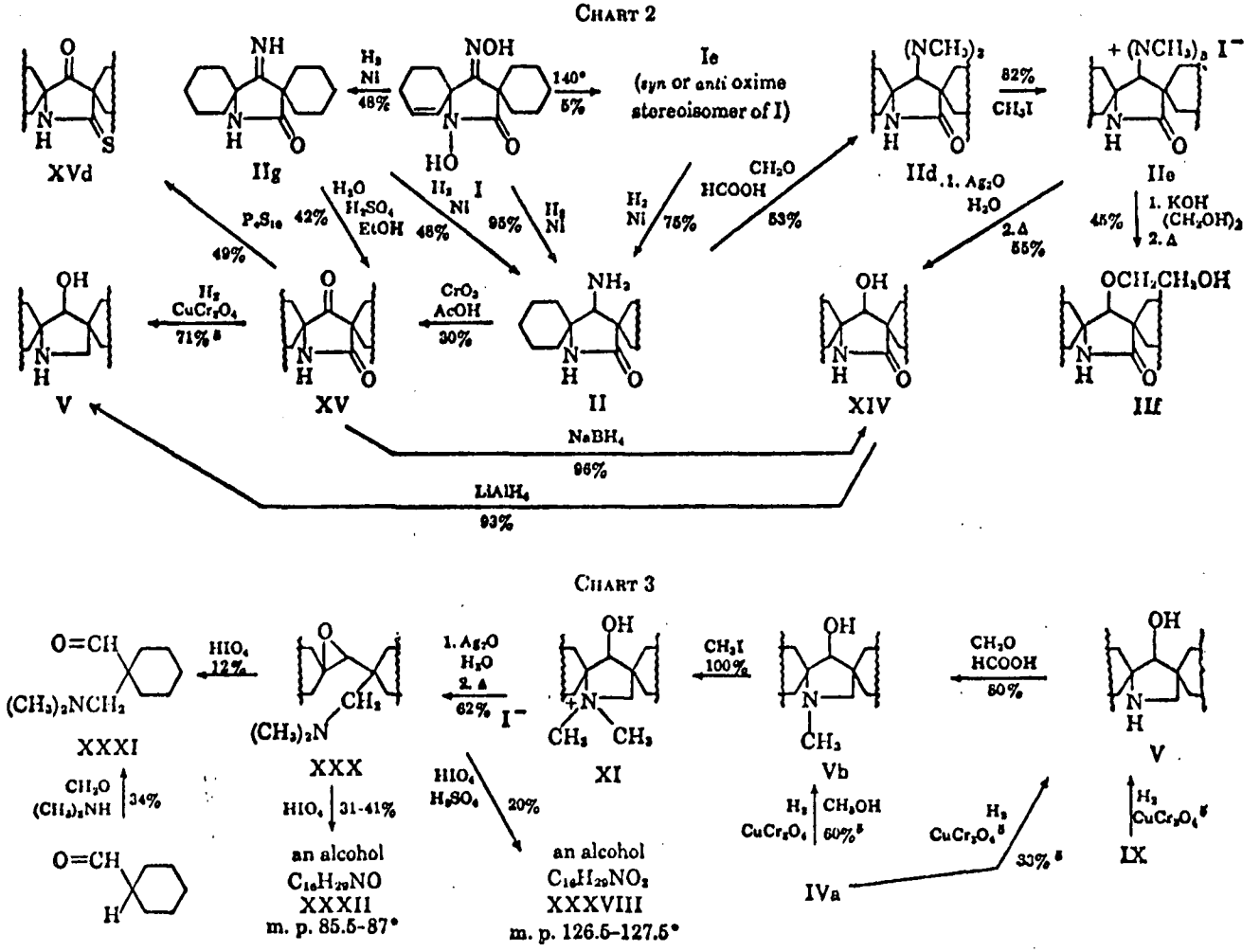
Chart 1: Determination of the functional groups present in the 2:2 condensation product of cyclohexanone and nitromethane (from (37a))

Low pressure hydrogenolysis of the acidic hydroxyl group of IV and the related methoxyl group of IVa were incompatible with the formulation of IV as a carboxylic acid and of IVa as its methyl ester. However, the data were consistent with the presence of a five-membered ring N-hydroxylactam which was resistant to hydrolysis. An oximino group would then account for the remaining N and O atoms in I and the hydrolysis in acid to IV. The ir spectra of I,IV,IVa, and XV all were consistent with the hypothesis that a ketoxime in I had undergone hydrolysis to a five membered ring ketone.

Attempts to convert IV back to I gave instead the syn or anti stereoisomer Ie. I could be partially isomerized to Ie by refluxing in xylene and by incomplete oxidation of I with aqueous $KMnO_4$. Catalytic hydrogenation of I and Ie gave II. Methylation of I and Ie gave isomeric derivatives Id and If respectively which both gave IVa on hydrolysis, proving that the difference between I and Ie was in the stereochemistry of the oxime.

In some hydrogenation experiments, reduction of I stopped at an iminolactam $C_{14}H_{22}N_2O$, IIg, which could be reduced over fresh catalyst to II. Hydrolysis of IIg gave XV, as did oxidation of II. XV could be converted to II by oximation followed by catalytic hydrogenation. This data confirmed that I was an unsaturated, five-membered ring ketoxime N-hydroxylactam.

Degradation experiments were then carried out on the primary aminolactam II (Chart 2) and on the hydroxyamine V (Chart 3). Attempted Hofmann degradation of II, via IId and IIe, led instead to a product XIV where hydroxyl had replaced the trimethylammonium group. A similar



Charts 2 and 3: Degradation experiments on the 2:2 condensation product of cyclohexanone and nitromethane (from (37a))

displacement product IIf was obtained from IIe when the reaction was run in ethylene glycol. Production of the displacement products rather than olefinic elimination products indicated that the carbon bearing the amino group in II was attached to carbons bearing no hydrogen atoms. Hofmann degradation of V, via Vb and XI, gave an epoxydimethylamine XXX via an intramolecular displacement reaction which is characteristic of β-hydroxyamine methiodides. Consequently, the hydroxyl group in V, which corresponds to the amino group of II, was assigned a position beta to the amino nitrogen. Assignment of structure XXX to the epoxyamine $C_{16}H_{30}NO$ permitted the assignment of structures to its precursors and the key compounds II and XV. The structural assignment was verified by the synthetic pathway shown in Chart 4. The synthetic sample of XV was identical to that obtained previously from I. As XV had been converted to II, and since II was derivable from I by low pressure catalytic hydrogenation under mild conditions, it was assumed that the atomic skeleton proved to be present in II was also present in I.

Action of strong alkali at 200° on IV gave cyclohexane carboxylic acid. This cleavage of the β-ketolactam and subsequent hydrolysis of the N-hydroxyamide proved that the olefinic double bond was not in the alicyclic ring attached α to the carbonyl carbon of the lactam but that it was in one of two positions in the ring attached α to the lactam nitrogen. Nmr spectra of IVa and the 3,11-dimethyl analogue showed clearly that the double bond was in the 9,10-position.

On completion of the structural assignment to 32, Noland and Sundberg proposed the mechanism given in Chart 5 for its formation from the starting materials. The key intermediate LXXI, they feel, could

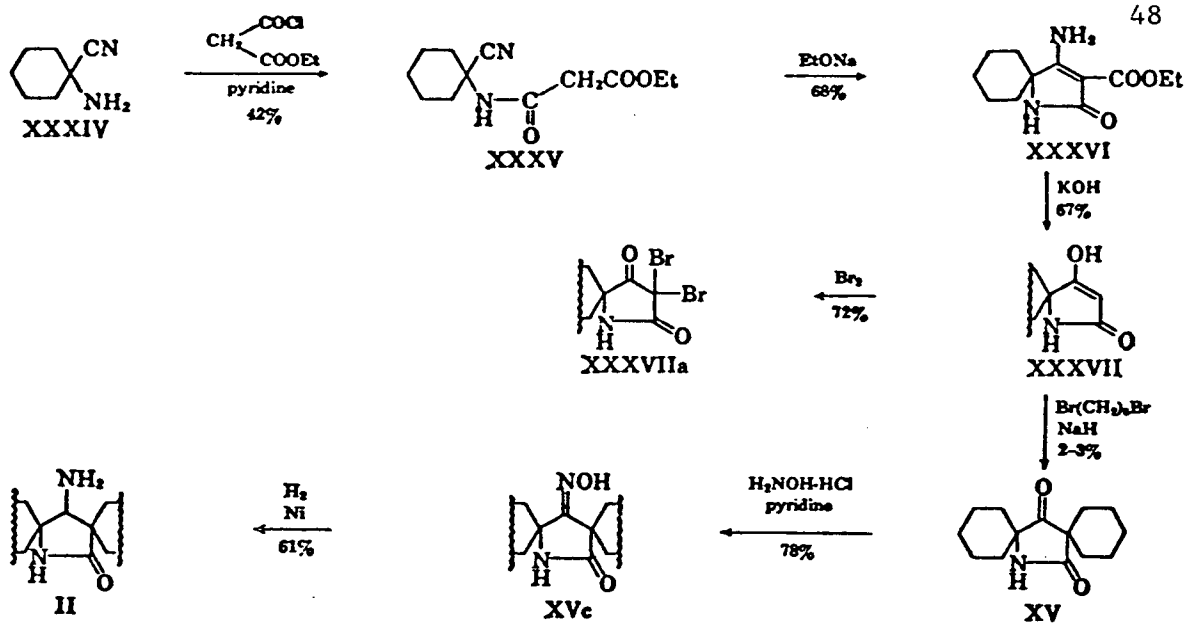


Chart 4: An alternate synthesis of the reduced 2:2 condensation product of cyclohexanone and nitromethane (from (37a))

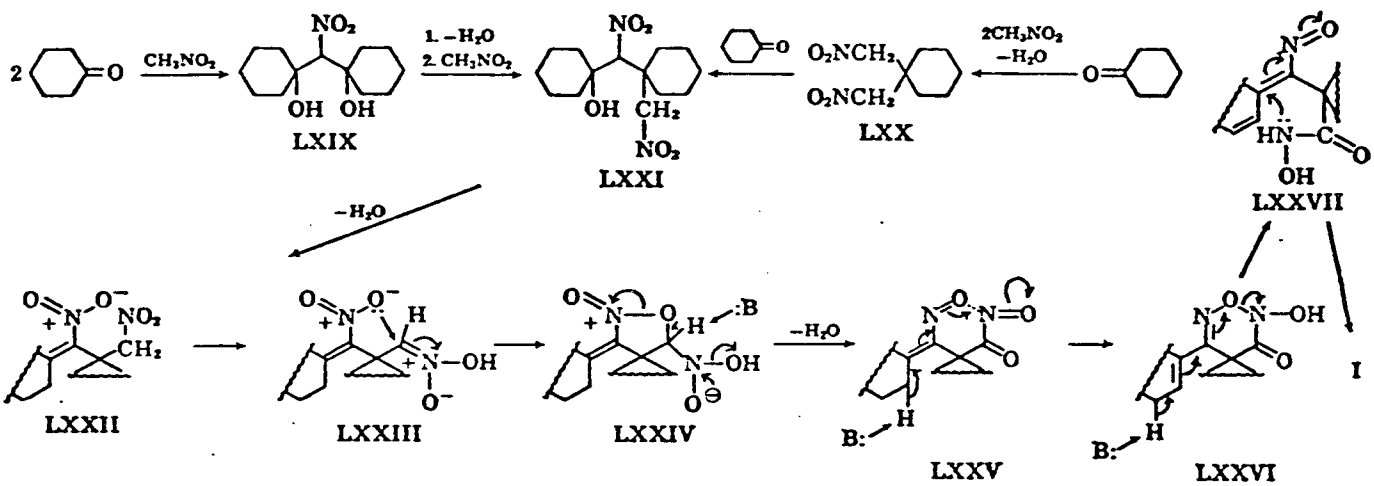


Chart 5: A proposed mechanism for the formation of the 2:2 condensation product of cyclohexanone and nitromethane (from (37a))

be formed either from LXX, which has been isolated from a reaction where I also formed, or from LXIX. From LXXI there would follow a series of steps, possibly in the order shown, involving intramolecular oxygen transfer, dehydration, and reduction-oxidation leading to the second key intermediate LXXVII which could isomerize to I.

House and Magin (38) independently arrived at the same atomic skeleton by a different and complementary degradative procedure.

In this work 32 was prepared in 47% yield, M.P. 272-274°. The maximum reported yield was 67%, M.P. 273-274° (35). A value of 47%, M.P. 263-265.5°, not recrystallized, has also been reported (40).

The cycloheptyl analogue 38 was probably first synthesized by Eckstein, et al (41) in 1957. A crystalline by-product in the reaction of cycloheptanone and nitromethane in the presence of piperidine catalyst was noted, but no physical constants, yield, or elemental analysis was reported. Nightingale, et al (34) attempted to prepare this compound but found that no solid precipitated when piperidine or di-n-propylamine were used as the catalyst. It was later found (35) that the compound had been formed but had remained in solution. Addition of acid to the reaction mixture precipitated 38 in 46% yield, M.P. 247-249°. In this work, the use of piperazine as the catalyst resulted in the precipitation of the piperazinium salt of 38 which was extracted with hot dilute HCl (1:1) to give 38 in 41% yield. The literature value using piperazine as the catalyst is 44.3% of the piperazinium salt.

The hydrogenation of 32 has been reported using Raney nickel at 2900 psi, heated to 160° (4240 psi), to give 92% of 4 and using copper chromium oxide at 70-90° and 3400 psi to give 85% of 4 (34). Noland

and Sundberg (37a) reported this reduction at two atmospheres (ca 30 psi) and room temperature for 67 hours over Raney nickel to give 95% of 4. However, they stated that in several instances the reduction stopped partially or completely before saturation of the carbon-nitrogen double bond, giving an iminolactam (IIg in Chart 2) which could be reduced to 4 over fresh catalyst. In this work, the reduction was carried out at room temperature over 24 hr at 400-500 psi to give 86% of 4, M.P. 192-193°. Lower pressures almost always gave the iminolactam. A commercial active nickel catalyst (W.R.Grace, Raney nickel No. 28 active catalyst in water) was used which may not have been as active as the preparation used by Noland and Sundberg. The commercial catalyst was purchased for the reduction of a nitrile compound in other work and was claimed to be less active than the usual Raney nickel catalysts. Janis (6) reports the hydrogenation of 32 over freshly prepared Raney nickel W-4 at room temperature and 30 psi to give 4 in 45.5% yield, M.P. 192-193°. In some instances the iminolactam was obtained.

The reduction of 38 was carried out over Raney nickel at 400 psi and room temperature for 24 hr to give 89% of 5, M.P. 188-190°.

The chloroacetamido- derivatives 22 and 27 were prepared by adding chloroacetyl chloride to a benzene solution of 4 or 5 respectively. The ratio of acid halide to amine was 1:2. The yields were 80%, M.P. 286-287° for 22 and 84%, M.P. 210-212° for 27. Janis (6) reports a low yield of 27, M.P. 210-212°, using a 1:1 ratio of acid halide and amine with an excess (25% over theory) of potassium carbonate as the HCl acceptor. For the reaction with 5, the excess of amine in the reaction mixture could be recovered as the precipitated hydrochloride salt.

Compound 27 could be recrystallized from benzene while 22 was much less soluble in refluxing benzene and was recrystallized from ethanol. Filtration of the reaction mixture from 22 gave a filter cake containing a mixture of 4 (as the hydrochloride) and 22. This was extracted with water in a mortar and pestle and the aqueous suspension was filtered. The solid 22 was recrystallized from ethanol while 4 was recovered by precipitation with NaOH. The recovery was only 50-75% of the excess of 4 used, due in part to mechanical losses.

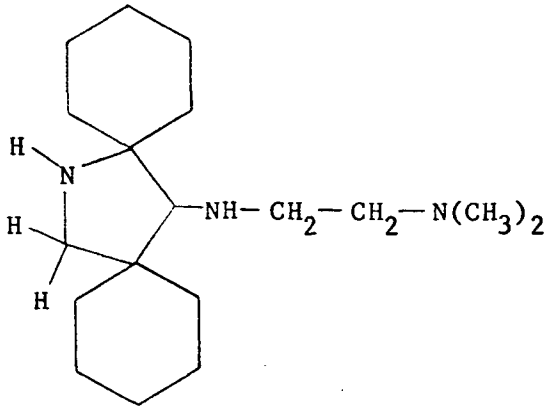
Synthesis of the dimethylaminoacetamido derivative 23 was accomplished by adding an excess of dimethylamine to a suspension of 22 in benzene. The yield was 44.5%, M.P. 203-204°, and 53.5% of 22 was recovered. The low yield of 23 is probably attributable to low solubility of 22 in the reaction solvent. A longer reaction time may have markedly improved the yield. This reaction was also attempted with ethanol (100%) as the solvent. The yield was 53% and no 22 was recovered.

Synthesis of the analogous 28 was accomplished by addition of an excess of dimethylamine to a benzene solution of 27. The yield was 82%, M.P. 173.5-174.5°.

The methyl iodide salts of 23 and 28 were prepared by addition of methyl iodide to a benzene solution of each of the compounds. Compound 24, the methyl iodide salt of 23, was recrystallized from glacial acetic acid, M.P. 160-161°. Recrystallization of 29, the methyl iodide salt of 28, from ethanol (100%)/anhydrous ether gave two crystalline forms which could be separated manually. Recrystallization of each of the dimorphs gave a mixture of the dimorphs.

The next compounds proposed for synthesis were 7-(2-dimethylamino-

ethylamino)-14-azadispiro[5.1.5.2]pentadecane 40 and 8-(2-dimethylamino-ethylamino)-16-azadispiro[6.1.6.2]heptadecane 41.



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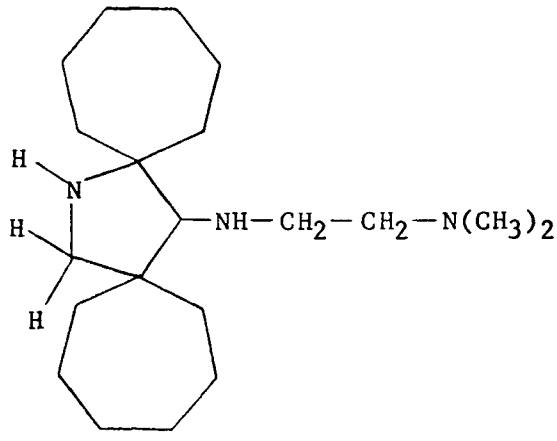
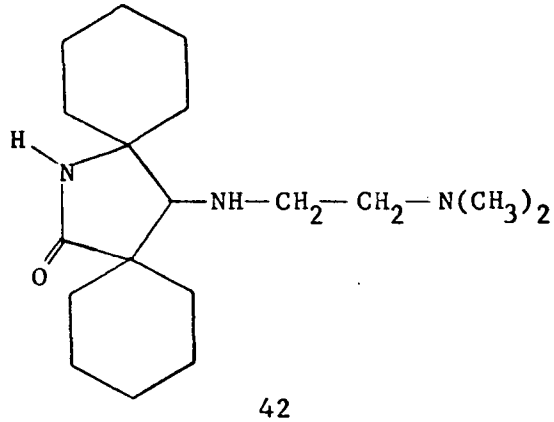


41

However, reduction of 23 with lithium aluminum hydride gave a partially reduced material whose ir spectrum showed one carbonyl group. Elemental analyses were consistent with the isomeric structures 42 and 25.



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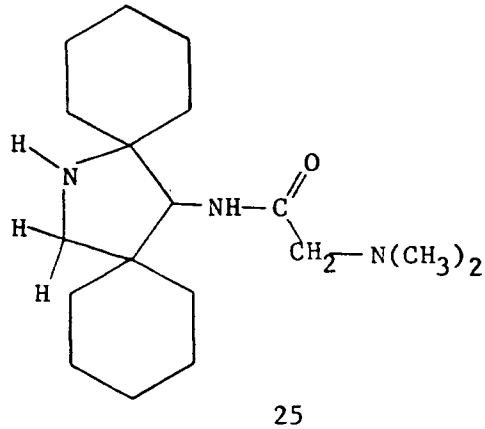
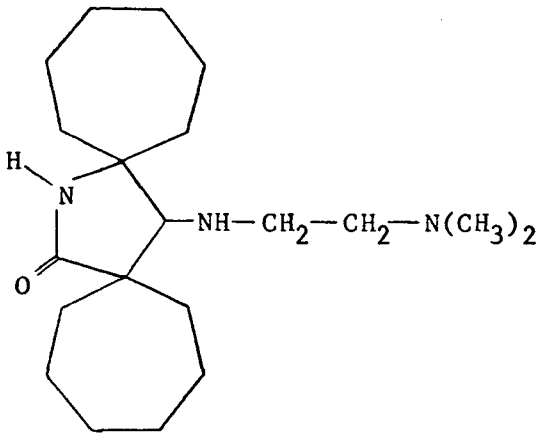


25

Reduction of 28 with lithium aluminum hydride gave a similar compound with elemental analyses consistent with the isomers 43 and 30.



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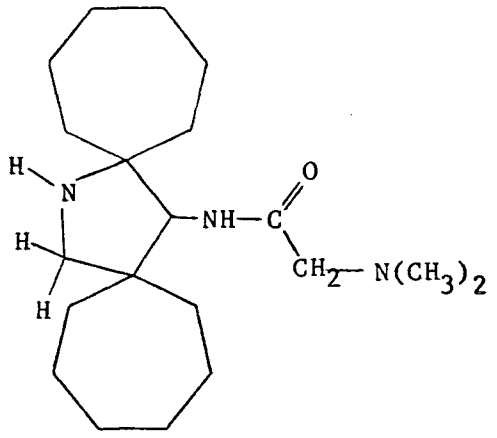
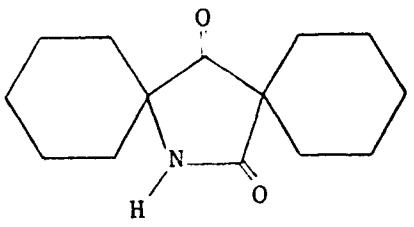


30

Reduction of 28 with diborane in tetrahydrofuran also gave the partially reduced compound. Attempted reduction of the partially reduced compound 43 or 30 over copper chromium oxide at 1320 psi and 220° was unsuccessful.

Although Nightingale, et al (34,35,36), Noland and Sundberg (37) and House and Magin (38) make reference to lithium aluminum hydride reduction of 44 and/or 45 to give the aminoalcohol 46, they do not mention attempted reduction of 4.



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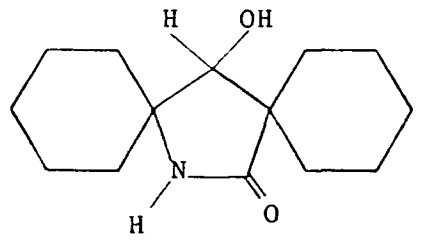

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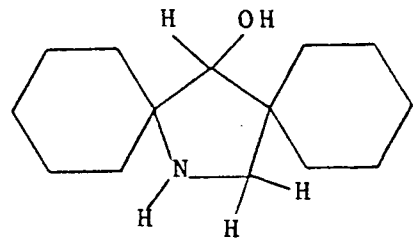
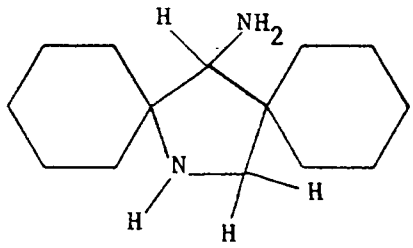


46

Reduction of 4 and 5 was attempted in this work to determine whether the lactam or the acyclic amide had been reduced in the sets of isomers 42-25 and 43-30. A solution of 4 was added to a suspension of lithium aluminum hydride in tetrahydrofuran and was refluxed for 48 hours. The expected 7-amino-14-azadispiro[5.1.5.2]pentadecane 47 was not obtained. Likewise, a solution of 5 added to a suspension of lithium aluminum hydride in tetrahydrofuran and refluxed for 40 hours did not give the expected 8-amino-16-azadispiro[6.1.6.2]heptadecane 48. Only starting material was isolated from these reactions.



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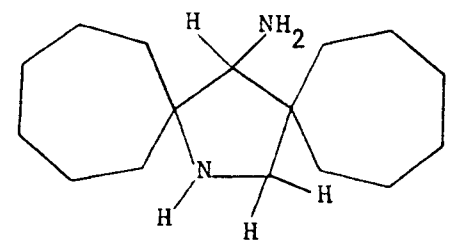
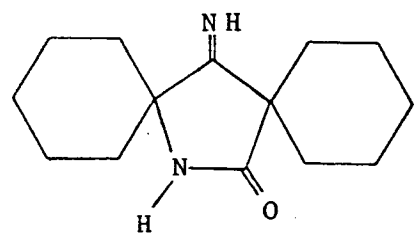


48

Hydrolysis of the remaining amide bond in the isomer pair 43-30 was then attempted in 30% sulfuric acid. Only starting material was recovered from this reaction. Noland and Sundberg (37a) report the hydrolysis of 7-imino-14-azadispiro[5.1.5.2]pentadecan-15-one 49 in sulfuric acid:ethanol:water (1:2:3) for 20 hr to give the 7-keto compound 44 in 48% yield. They do not mention formation of a product where the lactam bond had hydrolyzed.



49

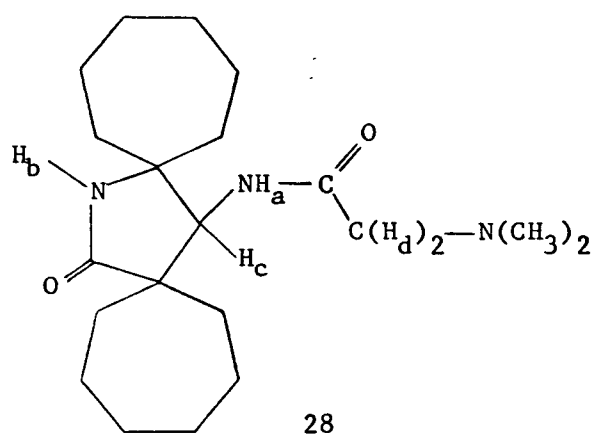
On the basis of this evidence, and a degree of bias on the part of the experimenter, the partially reduced compounds were assigned structures 42 and 43. The methyl iodide and hydrochloride salts of these two compounds were formed and pharmacological testing was carried out.

However, during the writing of this thesis, a review of the spectral data indicated that the two compounds were 7-dimethylaminoacetamido-14-azadispiro[5.1.5.2]pentadecane 25 and 8-dimethylaminoacetamido-16-azadispiro[6.1.6.2]heptadecane 30.

The presence of an ir absorption band at 1510-1520 cm^{-1}, which may be an Amide II band, indicated that the products of the reduction reactions were 25 and 30 since lactams containing less than nine atoms

in the ring do not show an Amide II band (42). The position for the Amide I band of a five-membered lactam is ca 1700 cm^{-1} while that for an acyclic secondary amide is ca 1680 cm^{-1} in the free state and ca 1655 cm^{-1} in the associated state (42). The spectrum of 23 has absorption bands at 1660 and 1700 cm^{-1} while that of 28 has absorption bands at 1680 and 1695 cm^{-1}. In the reduced products, the one absorption band remaining in this area is at 1680 cm^{-1} for the product from 23 and at 1670 cm^{-1} for the product from 28. This data also indicated that the lactam reduction products 25 and 30 had been obtained.

The nmr spectrum of 28 has two downfield signals (δ = 7.78 and 6.92) corresponding to the two amide protons H_a and H_b respectively. The signal at δ = 7.78 is a broad 1:1 doublet, J = 10Hz. There is a 1:1 doublet, J = 11Hz, at δ = 4.34 corresponding to H_c, and sharp singlets at δ = 3.06 (H_d) and at δ = 2.38 (N-methyl protons).

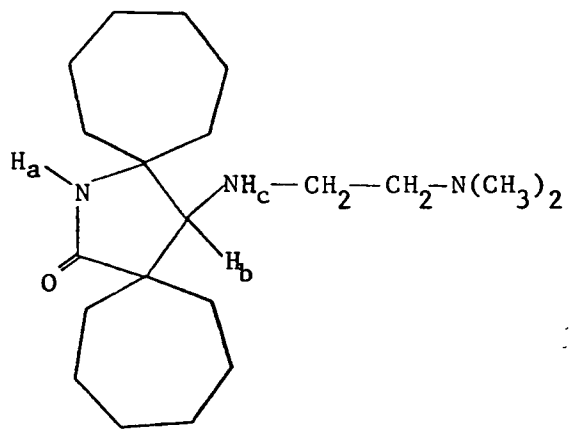


28

An envelope band centred at δ = 1.59 accounts for the protons in the alicyclic rings. After addition of D_2O the spectrum was essentially the same except that the 1:1 doublet at δ = 4.34 became a 1:0.75:0.85 triplet, J = 5.5Hz, centred at δ = 4.34. It appears that partial

exchange of the amide protons (H_a and H_b) has occurred, giving rise to a mixture of two species. In that species where H_a has exchanged, H_c appears as a singlet which appears at the same chemical shift as does the doublet due to the species where H_a has not exchanged. The signal to noise ratio in the spectra did not allow quantitation of the degree of exchange but the integral over the region in which H_a and H_b absorb had decreased by approximately 50%.

The nmr spectrum of the reduction product has a poorly defined signal at δ = 7.3 to 7.7, and a 1:1 doublet at δ = 3.85, J = 11Hz, initially assigned to H_a and H_b respectively in 43.



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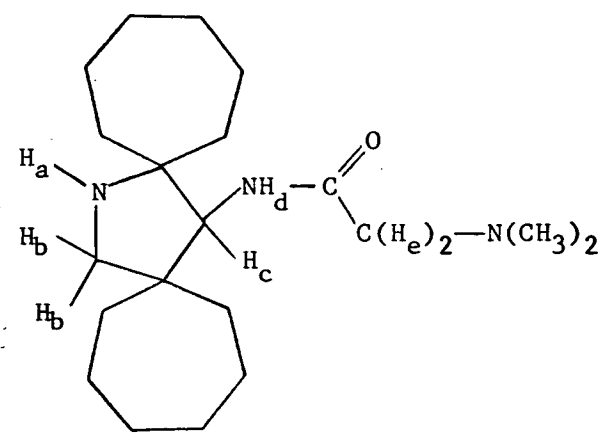


30

Sharp singlets at δ = 3.00 and 2.74 were assigned to the two pairs of ethylene protons, and sharp singlets at δ = 2.36 and 2.10 were assigned to the N-methyl protons and H_c respectively. After addition of D_2O, the singlet at δ = 2.10 disappeared and the doublet at δ = 3.85 collapsed to a singlet at δ = 3.76. The entire spectrum had shifted upfield by 5-6Hz so that the chemical shift of this singlet was actually 3.85 which corresponds to the value of the doublet in the spectrum be-

fore exchange. No explanation was offered for the lack of a triplet pattern for the two signals assigned to the ethylene protons.

The re-examination of the spectra which revealed the upfield shift also indicated that the amide proton had exchanged. The signal to noise ratio in the region of absorption of the amide proton did not allow quantitation of this exchange although the absorption signal was much reduced. The spectra were further confused by the presence of an absorption signal at δ = 7.3 ($\pm$ 0.05) due to chloroform impurity present in the deuterchloroform used as solvent. Although amide protons do not undergo rapid chemical exchange (77a) no other hypothesis explained all of the data. The spectra were then readily assigned to 30. The poorly defined downfield signal was seen to be a doublet, J = 8-12Hz, and was assigned to H_d, the doublet at δ = 3.85 to H_c, the two sharp singlets at δ = 3.0 and 2.74 to H_e and H_b respectively, and the singlet at δ = 2.10 to the amine proton H_a.

The exchange of the amide protons in 23 and 25 was found to be time dependent. Immediately after addition of D_2O, the protons were about 50% exchanged. The acyclic amide proton in 23 was more readily exchanged than the lactam proton. After 16 hr at room temperature exchange of the three amide protons in 23 and 25 was nearly complete.

In the reduction of a carbonyl group by lithium aluminum hydride, a hydride ion is transferred to the carbonyl carbon (43). It is therefore possible to account for the lack of reduction of 4 and 5 with lithium aluminum hydride, while 45 does reduce, on the basis of substituent effects. The amino group (σ_I = 0.10) is much less electron withdrawing than the hydroxyl group (σ_I = 0.25) (44) and therefore will

not stabilize the intermediate formed in the reduction of the carbonyl group in 4 and 5 as well as the hydroxyl group in 45. Acetylation of either functional group increases the electron withdrawing effect of the substituent. The value for the acetamido group is $\sigma_I = 0.28$ which is in the range of the value for the hydroxyl group (44). It would therefore be expected that the effect of the acetamido and, by extension, the dimethylaminoacetamido, function would be similar to that of the hydroxyl group, ie the reaction would proceed to give the secondary amine from the lactam.

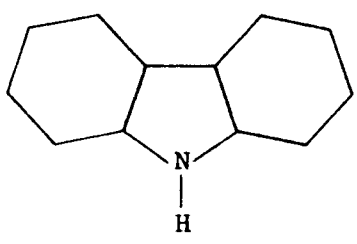
No explanation for lack of reduction or hydrolysis of the acyclic amide comes to mind.

PART TWO

DERIVATIVES AND ANALOGUES OF CARBAZOLE

The compounds which were synthesized in this series are shown in Table VI. All of the compounds in Table VI were tested for antihistaminic and antimuscarinic activity. A typical synthesis is shown in Scheme II.

The initial object of this work was the synthesis of appropriately substituted derivatives of dodecahydrocarbazole 49 and the compounds wherein the two alicyclic rings fused to the azolidine ring had been varied to contain five and seven carbon atoms, as in 50 and 51. Compounds containing isolated and conjugated double bonds were also to be synthesized.



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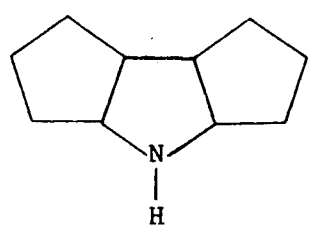

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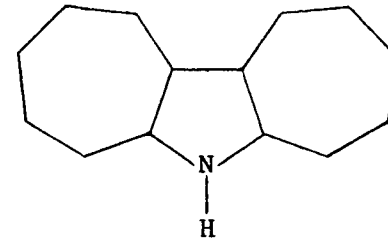
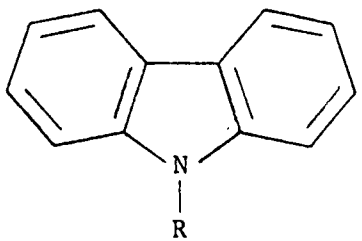


51

A search of the literature revealed only one method of preparation of 49 which may have been applicable to the synthesis of 50 and 51. Jager, et al (45) have reported a product, which they have identified as 49, from the reaction of cyclohexylidene cyclohexanone with formamide in ethylene glycol. This synthesis is an example of the Leuckart-



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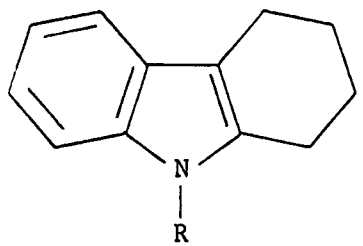

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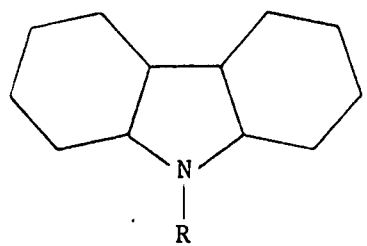

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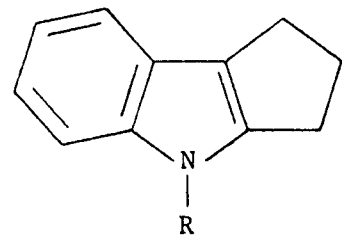

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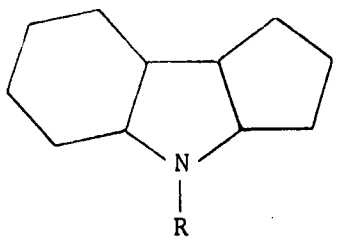

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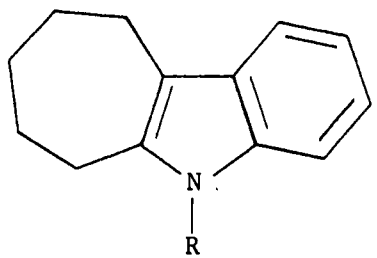

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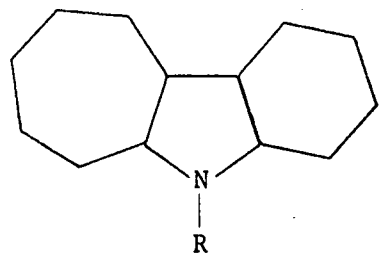

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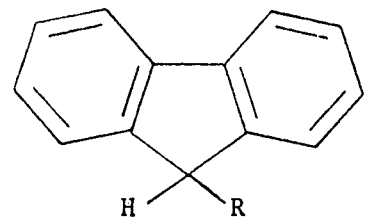

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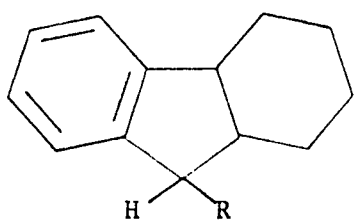

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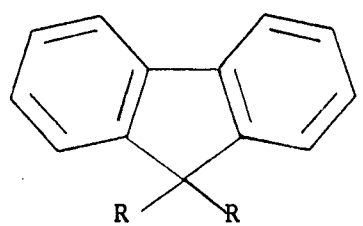

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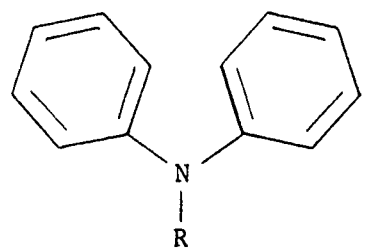

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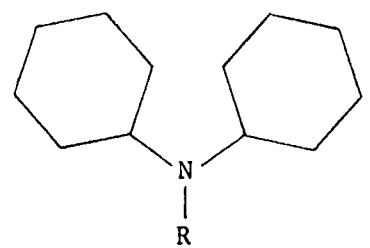

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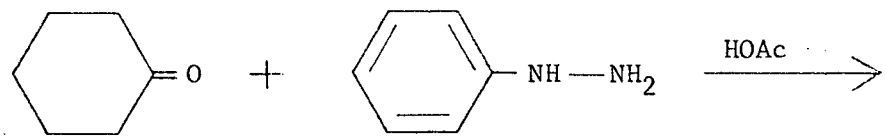
Table VI: Derivatives and Analogues of Carbazole synthesized in this work.

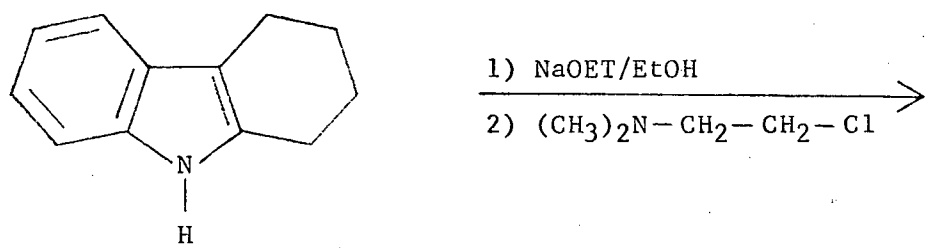
R = $-CH_2-CH_2-N(CH_3)_2$

Compound Number	Compound Name
52	9-(2-dimethylaminoethyl)carbazole
53	9-(2-dimethylaminoethyl)-1,2,3,4-tetrahydrocarbazole
54	9-(2-dimethylaminoethyl)dodecahydrocarbazole
55	4-(2-dimethylaminoethyl)-1,2,3,4-tetrahydrocyclopent[b]indole
56	4-(2-dimethylaminoethyl)dodecahydrocyclopent[b]indole
57	5-(2-dimethylaminoethyl)-5,6,7,8,9,10-hexahydrocyclohept[b]indole
58	5-(2-dimethylaminoethyl)tetradecahydrocyclohept[b]indole
59	9-(2-dimethylaminoethyl)fluorene
60	9-(2-dimethylaminoethyl)-1,2,3,4,4a,9a-hexahydrofluorene
61	9,9-bis(2-dimethylaminoethyl)fluorene
62	N-(2-dimethylaminoethyl)diphenylamine
63	N-(2-dimethylaminoethyl)dicyclohexylamine

Table VI: (cont'd) Derivatives and Analogues of Carbazole synthesized in this work.

Scheme II: Synthesis of 9-(2-dimethylaminoethyl)-1,2,3,4-tetrahydrocarbazole.





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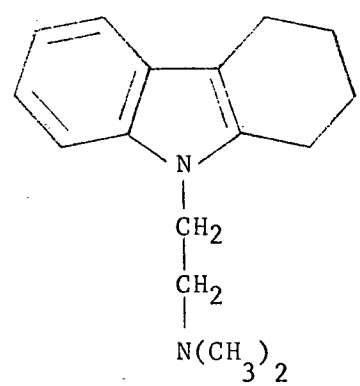


53

Wallach reaction, which involves the reductive alkylation of ammonia or primary or secondary amines with carbonyl compounds in the presence of formic acid which acts as the reducing agent. In a review, Moore (46) states that the method is unsuitable for application to α,β-unsaturated ketones, because of the formation of resinous by-products. The liquid product, b_{12} 130-132°, obtained by Jager, et al (45) in 32% yield, formed a hydrochloride, M.P. 265-268°; a picrate, M.P. 186°; an ethyl iodide, M.P. 187°; and a benzoyl derivative, M.P. 179°. Elemental analyses were carried out on the hydrochloride (Cl), the ethyl iodide (I), and the benzoyl derivative (C,H,N).

The values obtained by various workers for the melting and boiling points of dodecahydrocarbazole and some of its derivatives are shown in Table VII.

Adkins and Coonradt (47) had reported the high pressure catalytic hydrogenation of carbazole, obtaining 83-87% yield of 49 as a liquid, b_{10} 124-125°, or as a solid, M.P. 73-74.5°, with the same boiling range. The picrate of the solid was prepared, M.P. 167-168°. Elemental analyses (C,H) were carried out on the hydrochlorides, M.P. 208-209°.

Perkin and Plant (48) had previously reported the synthesis of 49, M.P. 65°, in "very good" yield by the electrolytic reduction of 1,2,3,-4,5,6,7,8-octahydrocarbazole. Elemental analyses (C,H) were carried out on the solid free base. The picrate was also prepared, M.P. 187°.

Masamune, et al (49) hydrogenated cis-hexahydrocarbazole and octahydrocarbazole over platinum in acetic acid to give 49, b_{15} 134-136°; hydrochloride, M.P. 209-211°, and 49; hydrochloride, M.P. 208-210° respectively. The picrate was also prepared, M.P. 175-177°.

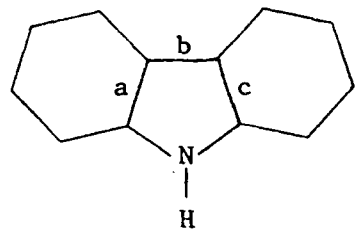
	dodecahydro-carbazole	hydrochloride	picrate	benzamide
Jager, et al (45)	b_{12} 130-132°	265-268°	186°	179°
Adkins and Coonradt (47)	b_{10} 124-125° M.P. 73-74.5°	208-209°	167-168°	
Perkin and Plant (48)	M.P. 65°		187°	
Masamune, et al (49)	b_{15} 134-136°	209-211° 208-210°	175-177°	
This work a	$b_{0.2}$ 62° M.P. 38.5-39°	255-257°d		175-176°
b	M.P. 75.5-76.5°	211.0-212.0°	168-168.5°	130.5-131.7°

a - constants for the unidentified product obtained by the method of Jager, et al (45).

b - constants for the product obtained from high pressure hydrogenation of carbazole.

Table VII: Melting and boiling points of dodecahydrocarbazole and its derivatives obtained by various workers

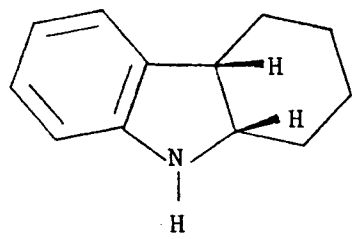
After a consideration of the above data it seemed doubtful that Jager, et al had obtained the reported compound. However, a number of geometrical isomers of 49 exist with respect to the configuration about the bonds labelled a, b, and c.



There are two internally compensated isomers: cis-syn-cis and trans-syn-trans and four pairs of diastereoisomers: trans-syn-cis; cis-anti-cis; trans-anti-cis; and trans-anti-trans. Generally, catalytic reductions occur to give the cis orientation about the reduced bond, although trans hydrogenation does occur (50). The expected products from the catalytic hydrogenation of carbazole are therefore the cis-syn-cis isomer and a lesser quantity of the cis-anti-cis isomer. The electrolytic reduction of Perkin and Plant and the synthetic route of Jager, et al would be expected, from first considerations, to lead predominantly to the trans-anti-trans isomer.

However, a study of models indicates that the cis-syn-cis or the cis-anti-cis isomers are less strained than the expected trans-anti-trans isomer. An investigation of the isomers of 1,2,3,4,4a,9a-hexahydrocarbazole 65 (51) revealed that reduction of 1,2,3,4-tetrahydrocarbazole 64 with tin and hydrochloric acid gave less than 2% of the trans isomer. The structural assignment was not rigidly proven but was made on the basis of a considerable amount more strain in the trans than the cis

isomer, as demonstrated with the aid of models.



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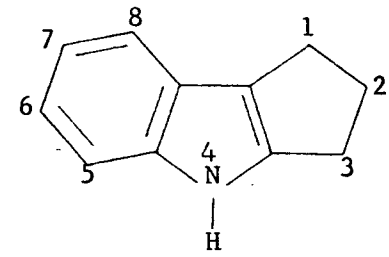

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Other examples of strained trans fused ring systems were mentioned by the authors (51). A similar relationship may be noted in models of 1,2,3,4,4a,5,6,7,8,9a-decahydrocarbazole, the expected intermediate in the electrolytic reduction of octahydrocarbazole, as well as in dodecahydrocarbazole. Booth, et al (52) also suggest the cis-fused rings are present in the dihydrogenated products following reduction of tetrahydrocarbazole and 1,2,3,4-tetrahydrocyclopent[b]indole 66 over Raney nickel.

During the course of this work, several attempts were made to prepare 49 by the method of Jager, et al (45). Cyclohexylidene cyclohexanone was prepared by the method of Gault, et al (53) to give a liquid, $b_{1.5}$ 105-108° (literature b_{18-20} 150-155°) in variable yield depending on the reaction conditions. The yield reported in the literature was 83% based on the amount of cyclohexanone consumed in the reaction.

The yields of cyclohexylidene cyclohexanone obtained under various conditions are summarized in Table VIII. The low yields obtained may have been due to the use of a somewhat too concentrated sulfuric acid

Reaction	Weight (g) cyclohexanone	Reaction Temperature	Reaction Time (hr)	Yield (%)
A	100	R.T.	24	27.5
B	500	R.T.	24	7.4
C	100	R.T.	5	38
D	100	0° [a]	24	35.6
E	165.5[b]	R.T.	3.5	45
F	100[c]	R.T.	5	36
G	200[d]	R.T.	5.5	30

Table VIII: Yields of cyclohexylidene cyclohexanone obtained under various reaction conditions.

Reaction A was separated by extraction with ether and the ether solution was washed with water.

Reaction B was steam distilled after dilution with two volumes water.

Reactions C-G were quenched by the addition of two volumes water and were extracted with ether.

a after 24 hours very little upper layer had separated; the reaction was allowed to warm to room temperature over seven hours and was worked up as above.

b cyclohexanone recovered from previous reactions.

c two portions of 50 g each were centrifuged at 1200 rpm, mixed, and worked up as above.

d two portions of 100 g each were centrifuged at 1400 rpm, mixed, and worked up as above.

solution, since Gault, et al stated that concentrations in excess of 60% caused a decrease in yield, while more dilute acid solutions (20, 30,40 and 50%) possessed only "feeble" catalytic activity.

Jager (54) prepared cyclohexylidene cyclohexanone in an average 70% yield by refluxing cyclohexanone with p-toluenesulfonic acid in benzene and removing water produced in the reaction by azeotropic distillation. He stated that the compound readily isomerized to 2-(cyclohexen-1-yl)cyclohexanone which does not react in the same manner as the desired compound.

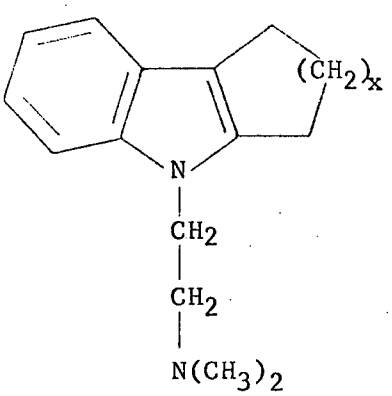
Attempts to condense cyclohexylidene cyclohexanone with formamide in ethylene glycol gave only unidentified products. A solid, M.P. 38.5-39°, was obtained from the distillate, $b_{0.2}$ 62°, of the product from the reaction. The Hinsberg procedure indicated that the solid was a secondary amine. A solution of the solid in acetone spontaneously isomerized to a mixture of two materials. The isomerization could be followed by vapour phase chromatography. On evaporation of the acetone the solid, contaminated with a small amount of the other component of the mixture, could be recovered. The nmr spectrum of the solid material had a signal at δ = 5.40 which may have been due to a vinylic proton, and a broad, poorly defined absorption signal centred at δ = 1.16. The spectrum showed no exchangeable proton although the amine proton signal may have been lost in the δ = 1.16 signal. The hydrochloride salt, M.P. 255-257°d, and the benzoyl derivative, M.P. 175-176°, were prepared.

No useful data could be derived from the nmr integration curve or the ir spectrum. Elemental analyses carried out on the solid and the

hydrochloride indicated a mixture of products even through vapour phase chromatography of the solid initially showed only one peak. The mass spectrum also indicated a mixture of products, one of which may have had a molecular weight of 179 or 180.

The liquid distillate from the reaction was a less pure fraction of the same material. Although the ir spectra of the solid and liquid materials were identical, they varied markedly from the spectrum of a reference sample of 49 prepared by high pressure hydrogenation of carbazole.

Following failure to obtain 49 by a chemical synthesis, the approach to the problem via derivatives of 49, 50, and 51 was abandoned in favour of an approach via the indole derivatives 67, where x = 1, 2, or 3. The fully hydrogenated derivatives of these compounds, as well as the



67

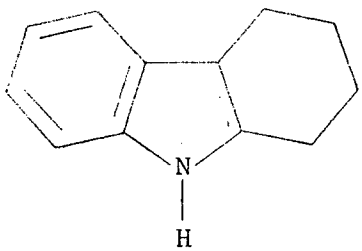
aromatic and saturated carbazole and fluorene analogues, were also to be synthesized. Four of the object compounds--52, 53, 57, and 62--as well as the indole bases 64, 66, and 68 have been previously reported, primarily in the patent literature. Testing of the reported compounds has been for psychotropic activity and for antihistaminic activity.

The synthesis of 9-(2-dimethylaminoethyl)carbazole 52 was accomplished by the reaction of 2-dimethylaminoethyl chloride hydrochloride with the carbazole anion, prepared by addition of a solution of carbazole in dimethylformamide to an equimolar quantity of sodium methoxide in anhydrous methanol. The yield after purification by reduced pressure sublimation was 35%, M.P. 40.5-41°. This compound has been reported in the patent literature (55) in 36% yield from the reaction of carbazole with 2-dimethylaminoethyl chloride in dimethyl sulfoxide using aqueous sodium hydroxide (50%) as the base. The melting point of the compound was reported to be 240°; this was obviously the hydrochloride salt which was found to melt at 243-244.5° in this work. Another reported (56) synthesis of 52 involved the reaction of carbazole with sodium amide in dry benzene followed by the addition of 2-dimethylaminoethyl chloride to give 60% of 52, b_{4-6} 190-210°, hydrochloride M.P. 240-242°d. At that time the product was tested for antihistaminic activity and was found to be ineffective in guinea pigs when administered intraperitoneally in doses of 25 mgm/kgm.

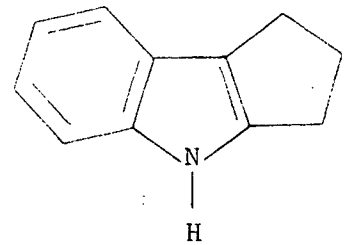
This compound was also found to be inactive in preventing dextran-induced edema or deposition of India ink carbon particles at sites of edema caused by histamine or dextran in rats (57,58).

Tetrahydrocarbazole 64, 5,6,7,8,9,10-hexahydrocyclohept[b]indole 68, and 1,2,3,4-tetrahydrocyclopent[b]indole 66 were prepared by the Fischer indole synthesis.

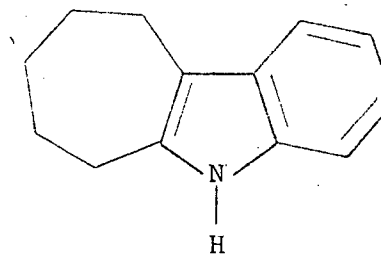
The Fischer indole synthesis involves the elimination of ammonia from the arylhydrazone of a ketone or aldehyde with formation of an indole nucleus. The reaction is carried out in the presence of a



64



66



68

catalyst, which may be an acid or a metal or metal salt. It is not necessary to isolate the arylhydrazone. The mechanism of the reaction, shown in Chart 6, may be considered to consist of three essentially separate stages: a) hydrazone-enehydrazine equilibrium (69 $\rightleftharpoons$ 70); b) formation of the new carbon-carbon bond (70 $\longrightarrow$ 71); and c) elimination of ammonia by either of two possible routes to give the indole compound 72. There is good experimental evidence, which has been reviewed (59, 60), to support steps a and b of the above mechanism and the elimination of N^2 of the arylhydrazone, although the mechanism of elimination is still in doubt.

Further detailed kinetic studies of the Fischer indole synthesis, under various conditions of catalysis, are needed to further investigate the mechanism and to determine the rate controlling step which may vary with the experimental conditions (60).

Tars are often formed in the Fischer indole synthesis and it has been suggested (59) that in some cases these materials arise from the para-rearrangement of the substitution on the aryl nucleus since the products so formed would be unstable under the reaction conditions.

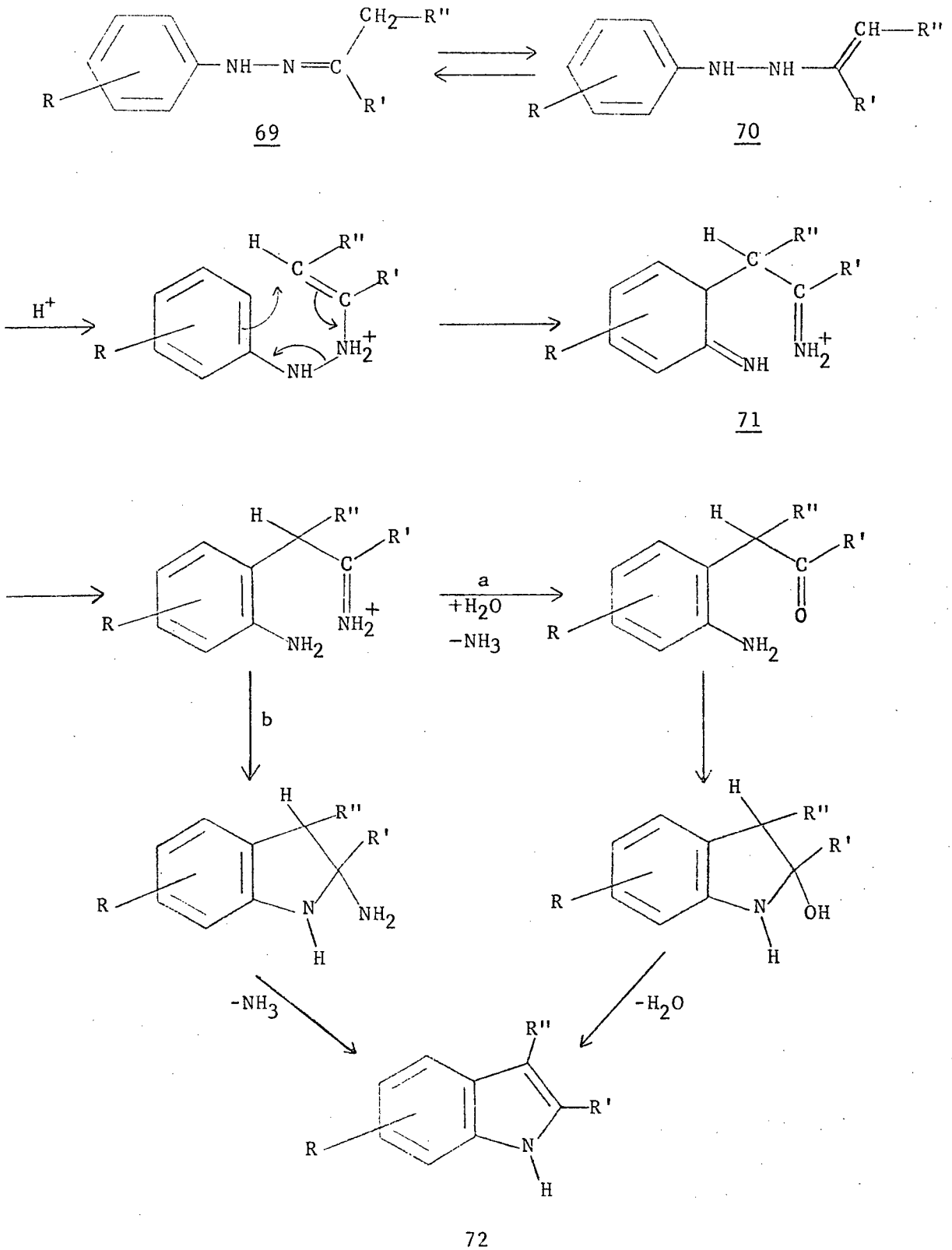
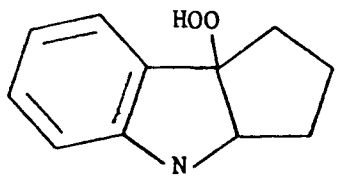


Chart 6: Mechanism of the Fischer indole synthesis (from ref. 59)

In the synthesis of 64 and 68, refluxing the appropriate alicyclic ketone with an equimolar quantity of phenylhydrazine in glacial acetic acid gave 66%, M.P. 113-117°, and 74%, M.P. 142.5-143.5°, respectively, of the desired compounds. The literature values for 64 are 82%, M.P. 116-117° (61) and for 68 are 74%, M.P. 142-144° (62).

Synthesis of 66 was accomplished by stirring cyclopentanone phenylhydrazone, prepared from equimolar quantities of cyclopentanone and phenylhydrazine, in dilute aqueous sulfuric acid while heating below the boiling point of the mixture. It was not necessary to purify the phenylhydrazone before carrying out the cyclization. The yield was 41.5%, M.P. 106.5-107.5°. The literature values were 45%, M.P. 108-109° (63). Both cyclopentanone phenylhydrazone and 66 were unstable in air and light, the rate of decomposition being increased by heat. The use of strongly acidic media in the cyclization step of the reaction resulted in extensive degradation of the compound to tars. Perkin and Plant (63) state that the use of hydrochloric or acetic acids, or sulfuric acid solutions more concentrated than 20 ml in 360 ml water leads to considerable hydrolysis of the hydrazone and there is a tendency to the formation of much tar.

Witkop, et al (64) found that 66 was unstable in polar solvents, decomposing to the lactam 74 presumably via the 8b-hydroperoxide 73.



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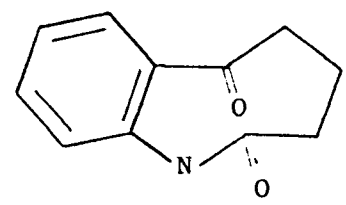


74

The six- and seven-membered ring homologues 64 and 68 were stable in solution but homologous products were obtained when the solutions were catalytically oxygenated (64). The 4a-hydroperoxide of 64 has been isolated.

The test compounds 53, 55, and 57 were then prepared by reaction of the anions of 64, 66, and 68, respectively, with 2-dimethylaminoethyl bromide hydrobromide or with 2-dimethylaminoethyl chloride. Addition of 64 in dimethylformamide to a solution of sodium ethoxide in ethanol (100%) and subsequent addition of 2-dimethylaminoethyl bromide hydrobromide, dissolved in dimethylformamide, gave 36.5% of 53, $b_{1.2}$ 155-160°, hydrochloride M.P. 244.5-246.0°d. The ratio of 64 to the bromide salt was 2:1 and 70% of the excess of 64 was recovered. The liquid product partially crystallized on standing and was purified by reduced pressure sublimation, M.P. 47.5-48.5°.

Compound 53 has been previously synthesized, the hydrochloride reported as M.P. 243-244° (66,67) and 248-250° (68). Yields were not quoted. No biological data were reported although the two patents (66, 67) claim this compound, among others, to be useful as an intermediate in the manufacture of dyes and pharmaceuticals, particularly antihistaminics and antispasmodics.

Addition of 68, dissolved in benzene, to a suspension of sodium amide in benzene and subsequent addition of 2-dimethylaminoethyl chloride gave 59% of 57, $b_{0.5}$ 154-158°, hydrochloride M.P. 226.5-228.5°. The ratio of 68 to halide was 2:1 and 40% of the excess was recovered.

Compound 57 has been previously synthesized (69) from the reaction of 68 with 2-dimethylaminoethyl chloride in dimethylformamide using sodium

hydride. No yield was quoted, $b_{0.05}$ 131-136°. The patent claims a series of compounds for use in the treatment of depression although no specific biological data was given.

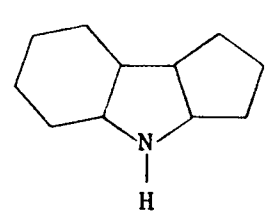
Although 64 and 68 fairly readily formed the anion necessary for the above reactions to occur, the synthesis of 55 was much more difficult due to the inherent instability of 66. Compound 66 was reasonably stable in the crystalline state, although decomposition was accelerated in air and probably by light. Recrystallization of the compound could be accomplished from petroleum ether, a non-polar solvent, but heating a solution of 66 in a polar solvent led to extensive degradation. The sodium derivative of 66 was prepared by very vigourous stirring of sodium metal with a xylene/ether (4:1) solution of 66. Addition of dimethylaminoethyl bromide hydrobromide in dimethylformamide gave 17.5% of 55, $b_{0.5}$ 136.5-139.5°, picrate M.P. 198.0-198.5°. The hydrochloride was unstable in ethanol solution and could not be successfully recrystallized. The yield might have been improved if 2-dimethylaminoethyl chloride had been used as the alkylating agent, either neat or in a non-polar solvent. The ratio of 66 to halide salt was 2:1. None of the excess starting material could be recovered.

High pressure hydrogenation of carbazole over 5% rhodium on alumina catalyst gave 36.5% of 49, M.P. 75.5-76.5°, hydrochloride M.P. 211.0-212.0°. The picrate and N-benzoyl derivatives were also prepared, M.P. 168-168.5° (literature (47) M.P. 167-168°) and 130.5-131.7°, respectively. Adkins and Coonradt (47) obtained 83-87% of 49, M.P. 73-74.5°, hydrochloride M.P. 208-209°, by reduction of carbazole at 220-260° over Raney nickel or copper chromium oxide catalysts at pressures of 3750 to 4500

psi. The equipment available in this laboratory dictated an upper limit of 1800 psi and therefore the highly active rhodium catalyst was used. This catalyst is inhibited by the presence of basic materials (70) and it was therefore necessary to add acetic acid in sufficient quantity to neutralize the amine products. The nature of the carrier on which the catalyst is supported has some effect on the reactions, charcoal being the preferred support for hydrogenations while alumina is favored for hydrogenolyses (70). The low yields obtained in some of the reactions carried out using this catalyst may in part be due to hydrogenolysis.

Although an upper limit of 1800 psi was obtainable, most of the hydrogenations in this work were carried out at pressures between 1000 and 1500 psi. At these pressures difficulty was experienced in maintaining an effective seal in the apparatus due to leakage about the stirrer shaft. The apparatus used (Paar 4511) was designed for use at pressures less than 1000 psi. Quantities of hydrogen taken up during the various reactions could not be calculated with any degree of accuracy. The course of the reaction could, however, be followed by withdrawal of aliquots of the reaction mixture.

Hydrogenation of 66 under conditions similar to those used for the hydrogenation of 64 gave 84% of dodecahydrocyclopent[b]indole 75, $b_{0.8}$ 64-66°, hydrochloride M.P. 241.5-243.0°.



75

Reaction of 49 with sodium amide in benzene followed by addition of dimethylaminoethyl bromide in benzene gave 50% of 54, $b_{0.7}$ 115-116°, hydrochloride M.P. 246-247°. The ratio of 49 to bromide was 10:6 and 52.5% of the excess starting material was recovered.

The addition of 75 to metallic sodium in dioxane gave no reaction. However, addition of 2-dimethylaminoethyl chloride to 75 in benzene gave 57% of 56, $b_{0.5}$ 97-100°, hydrochloride M.P. 209-210.7°. The ratio of 75 to halide was 2:1. The recovery of excess starting material was not calculated.

It is doubtful that the inclusion of sodium amide in the reaction of 49 with dimethylaminoethyl bromide served any useful purpose. It was necessary to use a strongly basic substance to ionize the indolic and aromatic compounds because the nitrogen atom is very weakly basic due to the inductive effect of the aromatic rings. In the fully hydrogenated bases, the nitrogen atom is much more basic and is capable of reacting with the alkyl halides provided that steric effects do not inhibit the reaction. Since 75 was not sufficiently acidic to react with sodium metal it is doubtful that 49 reacted with sodium amide.

High pressure hydrogenation of 57 over the rhodium catalyst gave 77% of 58, $b_{1.2}$ 126-127.5, picrate M.P. 204.0-205.5°d. The hydrochloride precipitated as an oil which could not be recrystallized.

The analogous N-(2-dimethylaminoethyl)diphenylamine 62 and N-(2-dimethylaminoethyl)dicyclohexylamine 63, in which the two rings attached to the nitrogen atom are not ortho-linked, were also synthesized. Reaction of diphenylamine with sodium amide in benzene and subsequent addition of 2-dimethylaminoethyl bromide hydrobromide in dimethylformamide

gave 22% of 62, $b_{0.8}$ 127-130°, hydrochloride M.P. 255.0-256.5°. The ratio of diphenylamine to halide salt was 2:1 and 73.5% of the diphenylamine was recovered. This compound had previously been reported (71) in 36% yield, hydrochloride M.P. 244-247°d, from reaction in the solid state between diphenylamine and 2-dimethylaminoethyl chloride hydrochloride. Other reported values for the hydrochloride are M.P. 246-247° (66) and 252-254° (69). Biological data are not reported (69,71) although the patent (66) claims this compound, among others, for antihistaminic and antispasmodic activity. Another report (56) gives a yield of 80% from the reaction of diphenylamine with sodium amide followed by addition of 2-dimethylaminoethyl chloride. The product was found to be about one-tenth as active as diphenhydramine when injected subcutaneously in guinea pigs.

An attempt to form the anion of dicyclohexylamine by refluxing with metallic potassium in dioxane was unsuccessful, due to the very low acidity of the amine. Attempted condensation of dicyclohexylamine with 2-dimethylaminoethyl chloride, in the absence of a basic reagent, was also unsuccessful. This was probably because of steric inhibition of the alkylation reaction due to the large bulk of the cyclohexyl rings. Although the rate of the reaction with dicyclohexylamine would be slow, the alkylating agent would be quickly removed from solution by dimerization and no appreciable quantity of the desired product could be formed.

This steric inhibition of reaction would not be a large factor in the reaction with the hydrogenated cycloalk[b]indoles 64 and 66 since the rings are rigidly held to one side of the nitrogen atom.

Reaction of dicyclohexylamine with chloroacetyl chloride, followed

by dimethylamination and subsequent reduction of the amide gave 60.5% overall yield of 63, $b_{0.1}$ 94-98°, hydrochloride M.P. 216.8-217.5°. This synthetic approach would probably have given improved yields of 54, 56, and 58 from 49, 75, and tetradecahydrocyclohept[b]indole, respectively. However, it was felt that the saving in time attained by using the 2-dimethylaminoethyl halide route overshadowed considerations of yield. Reaction of chloroacetyl chloride with the aromatic and indolic compounds would very likely have been unsuccessful.

The reaction of fluorene with solid potassium t-butoxide in anhydrous benzene followed by addition of 2-dimethylaminoethyl bromide hydrobromide in dimethylformamide gave only 12% of 59, $b_{0.5}$ 140-145°, picrate M.P. 179.5-180.3°.

Scherf and Brown (72) have reported the preparation of 9-fluorenyl potassium by reaction of fluorene with metallic potassium in dioxane. The product was sufficiently stable to be isolated as a reddish-brown solid, rapidly decomposed by moisture and oxygen in the air. It appears that the metallated derivative is formed via a radical anion which is stable below -50°C in tetrahydrofuran solution (73).

An attempt to increase the yield of 59 utilizing this procedure with equivalent amounts of fluorene, potassium, and 2-dimethylaminoethyl chloride in purified dioxane led to a mixture of the mono- and disubstituted fluorenes 59 and 61. Separation of the two components could not be accomplished with the equipment available in this laboratory.

Scherf and Brown (72) have reported that six absorption peaks in the ir spectrum of fluorene are attributable to the methylene group and they have used ir spectra to identify mono- and disubstituted derivatives

of fluorene. None of these bands was of any use in this work. The spectra of 59 and 61 were identical as thin films and as solutions in carbon tetrachloride. Structural assignment was made on the basis of the nmr spectra of the previously prepared 59 and pure 61 prepared later, as well as the elemental analyses of hydrochlorides and picrates.

Reaction of the sodium derivative of fluorene, prepared by refluxing equivalent quantities of fluorene and sodium amide in purified decalin and suggested (72) as a highly satisfactory method of preparing 9-monosubstituted fluorenes, with an equivalent amount of 2-dimethylaminoethyl chloride also gave a mixture of 59 and 61. Repetition of this reaction with fluorene, sodium amide, and 2-dimethylaminoethyl chloride in the ratio 25:20:7.5 gave only the disubstituted derivative in 72% yield. The desired compound was finally synthesized in 30% yield from the reaction in dioxane solution of fluorene, potassium, and 2-dimethylaminoethyl chloride in the ratio 3:2:1.

The presence of large quantities of 61 in the reaction products is difficult to rationalize. Reduction of fluorene to hydrofluorenes in the presence of alkali metals in ethereal solvents has been noted. This reduction may occur by abstraction by the radical anion from the 9-position of fluorene or from the solvent, or by liberation of hydrogen radicals from the radical anion during the metallation process. Eisch and Kaska (74) reported that a portion of the starting material was reduced during the reaction of fluorene with lithium in tetrahydrofuran. The stoichiometry of the reaction requires that one equivalent of fluorene react with 0.80 equivalent of lithium to give rise to 0.80 equivalent of 9-fluorenyllithium and tetrahydrofluorene. The experimental values

(74) showed that one equivalent of fluorene reacted with 0.83 equivalent of lithium to give 0.83 equivalent of metallated product plus a neutral oil which was determined to be a mixture of tetra- and hexahydrofluorene. The use of more than the calculated amount of metal in the reaction could therefore result in the formation of bi-metallated fluorene in the reaction mixture, leading to the formation of 61. The presence of reduction products may have accounted for the difficulty encountered in purifying fluorene recovered from these reactions although no reduction products were isolated.

The ratio of products (59:61) obtained in the reaction using equimolar quantities of fluorene, potassium, and 2-dimethylaminoethyl chloride in dioxane indicated that approximately 25% (10 g) of the fluorene would have been reduced if this mechanism were operative. It is highly unlikely that this amount of reduction products would have been overlooked. Furthermore, Scherf and Brown (72) reported that the reaction of fluorene with potassium in dioxane was accompanied by the evolution of hydrogen gas and not by reduction.

It is also unlikely that the fluorenyl potassium resulting from the reaction of equimolar quantities of fluorene and potassium was actually a mixture of fluorene with the mono- and dipotassium derivatives (72). The monopotassium compound has been isolated and is reasonably stable in the absence of air and moisture while the product of the reaction of two equivalents of potassium with one equivalent of fluorene was too highly reactive to be isolated (72).

Greenhow, et al (75), Scherf and Brown (72), and other authors (loc. cit.) have all reported the synthesis of monosubstituted fluorenes

from the reaction of equimolar quantities of fluorene with basic metallating reagents and an alkylating agent. Weissgerber obtained 9,9-dibenzylfluorene from the reaction of equimolar quantities of fluorenyl potassium and benzyl chloride in toluene (76,75). The fluorenyl potassium was prepared by fusion of fluorene with potassium hydroxide at 280°, a procedure found unsatisfactory by other workers (72).

The temperature and the nature of the solvent are described as critical (75) in the metallation of fluorene with sodium amide, refluxing decalin at 180° being the suggested system. The 9-fluorenyl sodium which precipitated in good yield during the described work (75) was a brownish yellow powder and an acceptable value for sodium was found on elemental analysis. Other authors (72) have not been able to duplicate the good yield and high quality obtained in (75), the product (72) being described as a black amorphous solid adhering to the lower portion of the reaction flask. In work carried out in this laboratory the precipitate was a black gummy mass which solidified on cooling into a substance resembling bitumen.

It is possible that the sodium amide, being of unknown vintage, had decomposed to the point where unexpected reactions occurred. However, the material was not visibly different from a recently purchased and freshly opened sample of the same reagent. It is more likely that the gummy mass precipitated in the reaction entrapped a portion of the sodium amide which was insoluble in the solvent, thus preventing the reaction from going to completion with respect to fluorene. Removal of the solvent and subsequent washing of the precipitate would then remove fluorene from the reaction. Addition of the alkylating agent and

stirring would then break up the matrix of the precipitate, freeing any entrapped sodium amide to react with 59 formed in the reaction. Subsequent reaction of metallated 59 would give rise to 61. Although Greenhow, et al (75) did not report the presence of disubstituted fluorenes, Scherf and Brown (72) had to separate 9,9-dimethylfluorene from the 9-methylfluorene prepared by this method.

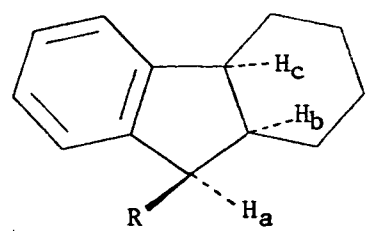
The results of the reactions involving 9-fluorenyl potassium (A) may perhaps be best explained by postulating an equilibrium reaction between A and 59 to give fluorene and 9-(2-dimethylaminoethyl)fluoren-9-yl potassium (C). Although such an equilibrium would be in favour of A and 59, the removal of C by irreversible reaction with the alkylating agent would result in the accumulation of an appreciable quantity of 61. C would be expected to react with the alkylating agent at a rate comparable to that of A. The reaction of the alkylating agent with 9-fluorenyl potassium was slow enough to allow such an equilibrium to occur since only a 30% yield of 59 was obtained over three hours when the alkylating agent was added over a period of approximately one minute. Dropwise addition of the alkylating agent to the reaction mixture would be expected to increase the proportion of 61 obtained and this was in fact observed.

This mechanism would also account for the presence of dideuterated fluorene in monodeuterated fluorene prepared by this method (72). The reaction would of course not be irreversible in the case of deuterated fluorene and the mixture obtained may represent an equilibrium mixture. Scherf and Brown (72) suggested that the presence of dideuterated fluorene was due to isotope exchange with excess D_2O mediated by KOD. It is

doubtful however that this base is a strong enough reagent to cause this to occur under the conditions used.

Attempted hydrogenation of a mixture of 59 and 61 gave the starting materials as well as 51% of 9-(2-dimethylaminoethyl)-1,2,3,4,4a,9a-hexahydrofluorene 60, $b_{0.05}$ 99°, hydrochloride M.P. 190.2-191.5°. Several other compounds were present in small quantities, as determined from vapour phase chromatography of the partially purified reaction mixture. The safe limits of the reaction apparatus were exceeded during this unsuccessful attempt to obtain the fully hydrogenated derivatives of 59 and 61 so no further attempts were made. The minor products from the reaction were probably partially hydrogenated derivatives of 59 although some 61 may have been partially reduced. No attempt was made to isolate or identify these minor constituents.

By analogy with the reduction products of carbazole, the expected configuration of 60 is as shown.



Since H_a and H_b are cis oriented on a cyclopentane ring the dihedral angle approaches zero degrees and the coupling constant for these protons is expected to be about 8 cps (77). A similar relationship holds between H_b and H_c. Unfortunately, the nmr spectrum of 60 was not well enough resolved to support this assignment. Other splitting

patterns greatly confuse the spectrum.

A study of models reveals no steric reasons for failure of reduction of the remaining aromatic ring.

The absence of appreciable quantities of reduction products of 61 may be explained on the basis of steric interactions of the protruding dimethylaminoethyl groups on both sides of the plane of the rings preventing complex formation with the catalyst surface.

ANALYTICAL METHODS

Melting points were determined, unless otherwise indicated, in open capillaries in a Thomas-Hoover Unimelt apparatus. Some melting points were determined on a Mettler SP2 microscope hot stage and are indicated as such. All melting points are reported uncorrected.

Infrared (ir) spectra were determined on a Beckmann IR-10 spectrophotometer. Liquid samples were scanned as thin films between NaCl plates and solids were incorporated in KBr discs.

Nuclear magnetic resonance (nmr) spectra were recorded by Miss P. Watson, Department of Chemistry, U.B.C., on a Varian Associates A-60 or T-60 instrument. Solutions were about 10% and solvents are specified. Tetramethylsilane (TMS) was used as an internal standard. Peaks in the nmr spectra are reported according to the following format: chemical shift from TMS (peak multiplicity, number of protons, coupling constant where applicable). The following abbreviations are used for the peak multiplicity: s, singlet; d, doublet; t, triplet; m, multiplet; e, envelope - a wide indistinct band, covering up to 1 ppm.

Vapour phase chromatography (vpc) was carried out on a Micro-Tek Model 220 instrument equipped with a flame ionization detector. Peak areas were measured by a Model 222 Disc Integrator.

Microanalyses were performed by Alfred Bernhardt Mikroanalytisches Laboratorium, West Germany.

Benzene sulfonamide derivatives were often formed to determine the presence of primary and secondary amines in distillation fractions. The presence of halogen was determined by burning a sample of the compound under test on a copper wire in a bunsen burner.

Yields are based on the amount of purified compound isolated.

EXPERIMENTAL

PART ONE

SYNTHESIS OF DERIVATIVES AND ANALOGUES OF 7-AMINO-14-AZADISPIRO[5.1.5.2]PENTADECAN-15-ONE

1. 14-Hydroxy-14-azadispiro[5.1.5.2]pentadec-9-en-7,15-dione 7-oxime 32

This compound was prepared essentially by the method used by Nightingale, et al (35). A mixture of cyclohexanone (196 g,2 mole), nitromethane (122 g,2 mole), and anhydrous piperazine (172 g,2 mole) in 400 ml anhydrous benzene was refluxed for five days in a one litre boiling flask fitted with a Dean-Stark tube, reflux condenser, and drying tube. During the reflux period, 75 ml water was collected.

The reaction mixture was filtered and the solid material (piperazinium salt of the desired compound) was washed with hot benzene. The solid was then extracted with 500 ml hot, dilute HCl (1:1) for 40 min. The resultant solid was filtered off, washed with water, dried in vac, and recrystallized from ethanol to give colourless crystals, M.P. 272-274°. Yield 124 g (47%) (lit (35) M.P. 273-274°, 67%)

ir (KBr); 3110,2930,2680,1700,1650,1440,1415,1350,1160,1045,1000,980, 935,920,815 cm^{-1}

2. 7-Amino-14-azadispiro[5.1.5.2]pentadecan-15-one 4

14-Hydroxy-14-azadispiro[5.1.5.2]pentadec-9-en-7,15-dione 7-oxime (44 g,0.17 mole) was dissolved in 400 ml ethanol (100%) in a Paar 4511 pressure reaction apparatus. Raney nickel No. 28 (W.R.Grace) (4 tsp) was washed with ethanol (100%) to remove water and added to the solution. The reaction mixture was stirred at room temperature under 500

psi hydrogen for 48 hr and filtered, and the catalyst was washed with hot ethanol. The solvent was removed on a rotary evaporator to give a white solid residue. This was recrystallized from dilute ethanol to give colourless crystals, M.P. 192-193°. Yield 34 g (86%). (lit. (37a) M.P. 193-195°, 95%)

ir (KBr); 3180,2940,2860,1690,1450,1405,1260,810 cm^{-1}.

nmr ($CDCl_3$); δ 7.20 (s,1) lactam proton; 2.76 (s,1) C-7 proton; 1.60 (e,22)

After addition of D_2O the spectrum was as above except δ 7.20 absent.

3. 7-Chloroacetamido-14-azadispiro[5.1.5.2]pentadecan-15-one 22

A solution of chloroacetyl chloride (11.3 g,0.10 mole) in 50 ml anhydrous benzene was added dropwise over 1.5 hr to a gently refluxing solution of 7-amino-14-azadispiro[5.1.5.2]pentadecan-15-one (47.3 g, 0.20 mole) in 500 ml anhydrous benzene in a one litre three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. The reaction mixture was refluxed for a further 2.5 hr and stirred at room temperature for 12 hr. The reaction mixture was then filtered and the solid was washed with benzene. The solvent was removed on a rotary evaporator to give a white solid which was recrystallized from ethanol to give colourless crystals, M.P. 286-287°.

The precipitated solid which had been filtered from the reaction mixture was extracted with water in convenient portions in a mortar and pestle. The aqueous suspension was filtered and the solid material was recrystallized from ethanol to give colourless crystals, M.P. 286-287°. Total yield 25 g (80%, based on chloroacetyl chloride).

ir (KBr); 3375,3210,2960,2780,1695,1670,1550,1270,790 cm^{-1}.

Analysis for $C_{16}H_{25}ClN_2O_2$ M.W. 312.85

calculated C,61.43; H,8.06; Cl,11.33; N,8.95

found C,61.62; H,8.20; Cl,11.31; N,8.79

The filtered aqueous solution was made basic with NaOH (40%) and the resultant precipitate was filtered off, dried in vac and recrystallized from ethanol to give colourless crystals of 7-amino-14-azadispiro[5.1.5.2]pentadecan-15-one. Recovery ranged from 50-75% of the excess used.

4. 7-Dimethylaminoacetamido-14-azadispiro[5.1.5.2]pentadecan-15-one 23

A. With benzene as solvent

Dimethylamine (100 ml,1.66 mole) was collected from a compressed gas cylinder in a graduated cylinder in an acetone/dry ice bath and was dissolved in 100 ml anhydrous ether. This solution was then added dropwise to a suspension of 7-chloroacetamido-14-azadispiro[5.1.5.2]pentadecan-15-one (82.7 g,0.27 mole) in 1500 ml anhydrous benzene in a two litre three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. The reaction mixture was refluxed for seven hr and stirred at room temperature for 15 hr. Since crystalline material was still present in the reaction, a further 50 ml portion of dimethylamine was added and the reaction mixture was refluxed for 18 hr.

The reaction mixture was filtered and solvents were removed on a rotary evaporator to give a white solid. The residue and the solid material from the reaction were suspended in 300 ml HCl (5%) and filtered to recover solid unreacted 7-chloroacetamido-14-azadispiro[5.1.5.2]-pentadecan-15-one (recovery 43.3 g,53.5%). The acidic filtrate was made

basic with NaOH (50%) and precipitated solid was filtered off. The solid material was dried in vac and recrystallized from benzene/hexane to give colourless crystals, M.P. 203-204°. Yield 38 g (44.5%)

ir (KBr); 3290,3200,3100,2940,2870,2840,2780,1695,1660,1560,1460,1410, 1165,1055,870 cm^{-1}.

nmr ($CDCl_3$); δ 7.64(d,1,J = 11Hz) amide proton; 7.45(s,1) lactam proton; 4.32(d,1,J = 11Hz) C-7 proton; 3.05(s,2) methylene protons; 2.35(s,6) N-methyl protons; 1.57(e,20)

After addition of D_2O the spectrum was as above except δ 7.64 absent, δ 4.32 became 4.32(s,1).

Analysis for $C_{18}H_{31}N_3O_2$ M.W. 321.47

calculated	C,67.30;	H,9.72;	N,13.07;	O,9.95
found	C,67.48;	H,9.61;	N,12.92	

B. With ethanol (100%) as solvent

Dimethylamine (90 ml,1.5 mole) was collected in a graduated cylinder in an acetone/dry ice bath and dissolved in 100 ml ethanol (100%). This solution was then added dropwise to a suspension of 7-chloroacetamido-14-azadispiro[5.1.5.2]pentadecan-15-one (40 g,0.128 mole) in 1000 ml ethanol (100%) in a two litre three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. The reaction mixture was then refluxed for 15 hr, cooled to room temperature, and the solvent was removed on a rotary evaporator to give a white solid. The residue was dissolved in 500 ml HCl (5%) and the solution was filtered. The acidic solution was made basic with NaOH (50%) and the solid precipitate was filtered off. Recrystallization

from benzene/hexane gave colourless crystals, M.P. 202-204°. Yield 21.8 g (53%).

5. 7-(2-Dimethylaminoethylamino)-14-azadispiro[5.1.5.2]pentadecane (attempted)

Lithium aluminum hydride (9.5 g,0.25 mole) was suspended in 1000 ml anhydrous tetrahydrofuran in a two litre three-neck flask fitted with a mechanical stirrer, Soxhlet extractor, condenser, and drying tube. 7-Dimethylaminoacetamido-14-azadispiro[5.1.5.2]pentadecan-15-one (38 g,0.12 mole) was continuously extracted into the hydride suspension over 16 hr. The reaction mixture was then cooled and the excess hydride was destroyed by dropwise addition of 10 ml NaOH (10%) followed by an excess of water. The reaction mixture was filtered, the solid material was washed with ether, and the solvents were removed from the filtrate on a rotary evaporator to give a slightly grey-brown oil which crystallized after drying in vac. This material was recrystallized from hexane to give colourless crystals, M.P. 110-111°, and was subsequently identified as 7-dimethylaminoacetamido-14-azadispiro[5.1.5.2]-pentadecane 25. Yield 21.5 g (60%)

ir (KBr); 3375,3140,2930,2860,2790,1685,1515,1460,1410,1345,1280,1180, 1155,1105,1050,870,540 cm^{-1}.

nmr ($CDCl_3$); δ 7.30(d,1,J = 11Hz) amide proton; 3.76(d,1,J = 11Hz) C-7 proton; 3.01(s,2) methylene protons; 2.90(s,1) and 2.88(s,1) C-15 protons; 2.35(s,6) N-methyl protons; 2.05(s,1) amine proton; 1.5(e,20)

After addition of D_2O the spectrum was as above except δ 7.30 and 2.05 absent, 3.76 became 3.76(s,1)

Analysis for $C_{18}H_{33}N_3O$ M.W. 307.48

calculated C,70.38; H,10.74; N,13.67; O,5.21

found C,70.13; H,10.65; N,13.35

A second reaction with 7-dimethylaminoacetamido-14-azadispiro-[5.1.5.2]pentadecan-15-one (2.05 g,0.0064 mole) and lithium aluminum hydride (0.57 g,0.015 mole) in tetrahydrofuran was refluxed for six days and gave only the partially reduced compound.

6. 7-Dimethylaminoacetamido-14-azadispiro[5.1.5.2]pentadecan-15-one methiodide 24

To a solution of 7-dimethylaminoacetamido-14-azadispiro[5.1.5.2]-pentadecan-15-one (10 g,0.03 mole) in 100 ml anhydrous benzene in a 250 ml three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube, was added dropwise a solution of methyl iodide (8.5 g,0.06 mole) in 25 ml anhydrous benzene. The reaction mixture was refluxed for two hr after addition was completed. The mixture was cooled and filtered to give a yellow, amorphous powder, which on recrystallization from glacial acetic acid gave dark yellow crystals, M.P. 160-161°d. Yield not calculated.

ir (KBr); 3230,3180,2935,2860,1665,1560,1450,1280,930 cm^{-1}.

Analysis for $C_{19}H_{34}IN_3O_2$ M.W. 434.61

calculated C,49.28; H,7.34; I,27.40; N,9.07; O,6.91

found C,49.00; H,7.22; I,27.41; N,8.97

7. 7-Dimethylaminoacetamido-14-azadispiro[5.1.5.2]pentadecane dimethiodide 26

To a solution of 7-dimethylaminoacetamido-14-azadispiro[5.1.5.2]-

pentadecane (10 g,0.03 mole) in 100 ml anhydrous benzene in a 250 ml three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube, was added dropwise a solution of methyl iodide (8.5 g,0.06 mole) in 25 ml anhydrous benzene. The reaction mixture was refluxed for two hr and filtered to give an orange amorphous powder. A suitable solvent for recrystallization could not be found.
ir (KBr); 3440,3260,2940,2860,1680,1545,1455,1025 cm^{-1}.

8. 16-Hydroxy-16-azadispiro[6.1.6.2]heptadec-10-en-8,17-dione 8-oxime 38

A mixture of cycloheptanone (112 g,1 mole), nitromethane (61 g,1 mole) and anhydrous piperazine (43 g,0.5 mole) in 150 ml anhydrous benzene was refluxed for four days in a one litre boiling flask fitted with a Dean-Stark trap, condenser, and drying tube. During the reflux period 40.5 ml water was collected.

The reaction mixture was cooled and the solid piperazinium salt was filtered off, washed with ether, then with hot benzene, and dried *in vac*. The salt was then extracted with hot dilute HCl (1:1) for 40 minutes and the crude buff-coloured product was filtered off. Recrystallization from ethanol gave colourless crystals, M.P. 246-249°d.
Yield 54.5 g (41.2%) (lit (35) M.P. 250-251°d, 51.5%)
ir (KBr); 3400,3120,2940,2870,1690,1655,1470,1425,1190,1175,945,920, 690 cm^{-1}.

9. 8-Amino-16-azadispiro[6.1.6.2]heptadecan-17-one 5

16-Hydroxy-16-azadispiro[6.1.6.2]heptadec-10-en-8,17-dione 8-oxime (40 g,0.14 mole) was dissolved in 400 ml ethanol (100%) in a Paar 4511 pressure reaction apparatus. Raney nickel No. 28 (W.R. Grace) (4 tsp)

was washed with ethanol (100%) to remove water and added to the solution. The reaction mixture was stirred at room temperature under 400 psi hydrogen for 24 hr and filtered, and the catalyst was washed with hot ethanol. The solvent was removed on a rotary evaporator to give a white solid which was recrystallized from dilute ethanol to give colourless crystals, M.P. 188-190°d. Yield 32.2 g (89%)

ir (KBr); 3200,3080,2940,2865,1690,1465,1390,1350,925,860 cm^{-1}.

nmr ($CDCl_3$); δ 7.45(s,1) lactam proton; 2.14(s,1) C-8 proton; 1.63 (e,26)

After addition of D_2O, δ 7.45 absent, 1.63 became 1.63(e,24).

10. 8-Chloroacetamido-16-azadispiro[6.1.6.2]heptadecan-17-one 27

A solution of chloroacetyl chloride (2.5 g,0.02 mole) in 50 ml anhydrous benzene was added dropwise to a gently refluxing solution of 8-amino-16-azadispiro[6.1.6.2]heptadecan-17-one (10.6 g,0.04 mole) in 100 ml anhydrous benzene in a 250 ml three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. The reaction mixture was refluxed for five hr after addition was completed, and the precipitated solid was filtered off and washed with hot acetone. The solvents were removed from the filtrate on a rotary evaporator to give a white solid residue which was recrystallized from benzene to give colourless crystals, M.P. 210-212°. Yield 6.2 g (84%)

ir (KBr); 3330,2940,2870,1695,1670,1550,1470 cm^{-1}

nmr ($CDCl_3$); δ 6.90(s,1) lactam proton, overlapping 6.96(d,1,J = 9.5Hz) amide proton; 4.33(d,1,J = 10Hz) C-8 proton; 4.15(s,2) methylene protons; 2.6(e,24)

There was no change on addition of D_2O.

The excess of 5 was recovered from a filtered aqueous solution of the precipitate from the reaction by addition of NaOH (40%) and collection of the resultant precipitate by filtration, followed by drying in vac. Recovery was 90% of the excess used.

11. 8-Dimethylaminoacetamido-16-azadispiro[6.1.6.2]heptadecan-17-one 28

Dimethylamine (66 ml,1 mole) was collected in a graduated cylinder in an acetone/dry ice bath and was dissolved in 100 ml reagent ether. This solution was then added dropwise to a solution of 8-chloroacetamido-16-azadispiro[6.1.6.2]heptadecan-17-one (51 g,0.15 mole) in 1000 ml reagent benzene in a two litre three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. The reaction mixture was refluxed for three hr and stirred at room temperature for 34 hr. The reaction was then filtered and solvent was removed on a rotary evaporator to give a slightly grey-green solid. The residue was dissolved in 500 ml benzene and 800 ml HCl (5%) was added. The white precipitate was filtered off and the aqueous phase was separated from the benzene solution. The white precipitate was suspended in the aqueous phase and sufficient NaOH (40%) was added to give a persistent basic reaction. The basic suspension was filtered, the solid was washed with water, dried in vac, and recrystallized from benzene/hexane to give colourless crystals, M.P. 173.5-174.5°. Yield 43 g (82%)

ir (KBr); 3330,3190,3080,2935,2860,2795,1695,1680,1530,1470,1390,1350, 1055 cm^{-1}.

nmr ($CDCl_3$); δ 7.79(d,1,J = 10Hz) amide proton; 6.93(s,1) lactam proton; 4.35(d,1,J = 11Hz) C-8 proton; 3.05(s,2) methylene protons; 2.38(s,6) N-methyl protons; 1.59(e,24)

After addition of D_2O, partial exchange of δ 7.79 occurred so that δ 4.35 became 4.35(d,J = 11Hz) overlapped with 4.35(s), total 1 proton.

Analysis for $C_{20}H_{35}N_3O_2$ M.W. 349.52

calculated C,68.75; H,10.09; N,12.02; O,9.13

found C,69.12; H,10.36; N,11.70

12. 8-(2-Dimethylaminoethylamino)-16-azadispiro[6.1.6.2]heptadecane (attempted)

A. With lithium aluminum hydride

A solution of 8-dimethylaminoacetamido-16-azadispiro[6.1.6.2]-heptadecan-17-one (27 g,0.08 mole) in 600 ml anhydrous tetrahydrofuran was added dropwise to a suspension of lithium aluminum hydride (5.7 g, 0.15 mole) in 600 ml anhydrous tetrahydrofuran in a two litre three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. After addition was completed, the reaction mixture was refluxed for 42 hr and cooled, and excess hydride was destroyed by dropwise addition of 10 ml NaOH (5%) and 10 ml water. The mixture was filtered, the solid was washed with ether, and the combined filtrates were dried over $MgSO_4$. The solution was filtered and solvents were removed on a rotary evaporator to give a white solid which was recrystallized from hexane to give colourless crystals, M.P. 104-105°, subsequently identified as 8-dimethylaminoacetamido-16-azadispiro[6.1.6.2]-heptadecane 30. Yield 19.5 g (75%).

ir (KBr); 3350,3250,2920,2850,2780,1675,1520,1465,1335,1275,1150,1055, 965,925,885,860,550 cm^{-1}

nmr ($CDCl_3$); δ 7.52(d,1,J = 10Hz) amide proton; 3.85(d,1,J = 11Hz)

C-8 proton; 3.00(s,2) methylene protons; 2.75 (s,2) C-17 protons; 2.36(s,6) N-methyl protons; 2.10(s,1) amine proton; 1.52(e,24)

After addition of D_2O, the spectrum was as above except δ 7.52 and 2.10 absent, 3.85 became 3.85(s,1).

Analysis for $C_{20}H_{37}N_3O$ M.W. 335.54

calculated C,71.57; H,11.14; N,12.52; O,3.77

found C,71.48; H,11.17; N,12.29

B. With diborane (78)

A solution of 8-dimethylaminoacetamido-16-azadispiro[6.1.6.2]-heptadecan-17-one (1.4 g,0.004 mole) in 35 ml anhydrous tetrahydrofuran was added dropwise, over one hour at 0°C, to a solution of diborane in anhydrous tetrahydrofuran (30 ml, approximately 0.3M in diborane) in a 100 ml four-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. After addition was completed, the reaction mixture was refluxed for two hr and cooled to room temperature, and 25 ml HCl (10%) was added dropwise to decompose excess reagent. Tetrahydrofuran was removed from the reaction solution on a rotary evaporator, and the aqueous residue was made basic with NaOH (40%) and then extracted with 3x75 ml ether which was dried over $MgSO_4$. The ether solution was filtered and solvent was removed on a rotary evaporator to give a grayish oil. Recrystallization from hexane was unsuccessful. The crude oil was distilled at reduced pressure to give a colourless oil, $b_{0.3}$ 175-180°, which solidified on standing. The product was found to be 8-dimethylaminoacetamido-16-azadispiro[6.1.6.2]heptadecane. Yield 0.7 g (52%).

C. By reduction over copper chromium oxide (78,79)

Copper chromium oxide catalyst (2.6 g) was added to a solution of 8-dimethylaminoacetamido-16-azadispiro[6.1.6.2]heptadecane (3.1 g, 0.0093 mole) in 75 ml purified dioxane in a Paar 4511 pressure reaction apparatus. The reaction mixture was stirred at 220°C and 1320 psi hydrogen for 22 hr. The reaction mixture was cooled and filtered, and the solid material was washed with ether. Solvents were removed from the filtrate on a rotary evaporator to give an oily residue which was dissolved in 100 ml ether.

The ether solution was extracted with 3x50 ml HCl (5%). The acidic solution was made basic with NaOH (40%) and was extracted with 3x75 ml ether. The final ether extract was dried over $MgSO_4$, filtered, and solvent was removed on a rotary evaporator to give a light brown oil which partially solidified on standing. Only starting material could be recovered from this residue.

13. 8-Dimethylaminoacetamido-16-azadispiro[6.1.6.2]heptadecan-17-one methiodide 29

Methyl iodide (4.0 g,0.028 mole) was added dropwise to a magnetically stirred solution of 8-dimethylaminoacetamido-16-azadispiro[6.1.6.2]-heptadecan-17-one (5 g,0.014 mole) in 100 ml anhydrous benzene in a 250 ml erlenmeyer flask. The yellow amorphous precipitate was filtered off and recrystallized from ethanol (100%)/anhydrous ether to give two distinct crystalline forms, which could be manually separated.

Recrystallization of each crystalline form, after manual separation, gave a mixture of both forms.

A) light yellow, fine, broken needles, M.P. 169.1-170.0° (Mettler

SP_2 microscope hot stage).

B) darker yellow, spherical clumps of needles (a hedgehog appearance), M.P. 239.3-239.4° (Mettler SP_2 microscope hot stage). Yield not calculated.

Each of the two crystalline forms gave a single spot with the same R_f value when chromatographed on silica gel using ethanol as the developing solvent.

Analysis for polymorphism by differential scanning calorimetry gave no thermal peak for either crystalline form, probably because the compounds melt with decomposition.

Ir spectra for both crystal types were identical.

ir (KBr); 3230,3080,2930,2860,1680,1660,1545,1465,1280,1240,980,935, 910,760 cm^{-1}

Analysis for $C_{21}H_{38}IN_3O_2$ M.W. 492.51

calculated	C,51.21;	H,7.79;	I,25.77;	N,8.53;	O,6.70
A) found	C,50.91;	H,8.22;	I,25.89;	N,8.33	
B) found	C,51.05;	H,7.92;	I,25.85;	N,8.35	

14. 8-Dimethylaminoacetamido-16-azadispiro[6.1.6.2]heptadecane dimethiodide 31

Methyl iodide (0.85 g,0.006 mole) was dissolved in 25 ml anhydrous benzene and was added dropwise to a refluxing solution of 8-dimethylaminoacetamido-16-azadispiro[6.1.6.2]heptadecane (1 g,0.003 mole) in 50 ml anhydrous benzene in a 250 ml three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel and drying tube. The reaction mixture was refluxed for one hr and was stirred at room temperature for 14 hr.

The dark yellow precipitate from the reaction was recrystallized

from glacial acetic acid to give yellow-orange crystals, M.P. 186.3° (Mettler SP_2). Yield not calculated.

ir (KBr); 3420,3260,2930,2860,1690,1540,1465,1235 cm^{-1}

Analysis for $C_{23}H_{45}I_2N_3O$ M.W. 605.43

calculated	C,43.61;	H,7.16;	I,40.07;	N,6.63;	O,2.53
found	C,41.23;	H,7.05;	I,40.10;	N,6.67	

15. 8-Dimethylaminoacetamido-16-azadispiro[6.1.6.2]heptadecane methyl sulfate (attempted)

Dimethyl sulfate (2.52 g,0.02 mole) was added to a solution of 8-dimethylaminoacetamido-16-azadispiro[6.1.6.2]heptadecane (6.7 g,0.02 mole) in 45 ml anhydrous benzene in a 250 ml erlenmeyer flask. The reaction mixture was heated on a steam bath for three hr and allowed to stand for 16 hr. During this interval a gummy translucent material separated. The benzene solution was decanted and the gum was washed with ether which was decanted into the benzene solution. The solvents were evaporated on a steam bath and the residual syrup and the original gum were dried in vac to give hygroscopic glasses. A suitable solvent for recrystallization could not be found.

MISCELLANEOUS REACTIONS

1. Diborane in tetrahydrofuran (78)

Sodium borohydride (17.1 g,0.45 mole) was dissolved in 340 ml anhydrous diglyme (previously distilled from lithium aluminum hydride), and the cloudy solution was filtered.

Boron trifluoride etherate (42.6 g,0.30 mole) was dissolved in 300 ml anhydrous diglyme in a one litre boiling flask fitted with a dropping funnel, a gas inlet tube, and a gas outlet tube leading through a mercury safety trap to a 500 ml erlenmeyer flask containing 250 ml anhydrous tetrahydrofuran. The outlet tube was positioned about five mm from the bottom of the erlenmeyer. The erlenmeyer was fitted with a drying tube and was cooled in ice water.

The system was flushed with a stream of dry nitrogen for one hour previous to and during the course of the reaction. The borohydride solution was added dropwise to the reaction flask while stirring magnetically. When addition was completed the reaction mixture was slowly heated to boiling to drive off dissolved diborane. It was necessary to add ice to the ice water cooling bath several times during the course of the reaction. Yield of diborane: 2 g increase in weight of erlenmeyer and contents (50%) 0.072 mole in 250 ml, 0.29M.

2. Copper Chromium Oxide catalyst (79,80)

Cupric chloride dihydrate (91.5 g,0.5 mole) and barium nitrate (15.5 g,0.06 mole) were dissolved in water and the solution was made up to a total volume of 450 ml. This solution was heated to 80°C and poured in a thin stream into a room temperature aqueous solution

containing sodium dichromate (VI) dihydrate (89 g,0.3 mole) and ammonium hydroxide (28%) (112.5 ml) in a total volume of 450 ml. The mixture was stirred by hand during addition and for ten minutes after addition was completed. The orange-brown precipitate was filtered off at a water aspirator until as dry as possible. The solid was then dried for two hours in a vacuum oven at 110° and 31 inches vacuum.

The product, in lump form, was divided into two equal portions in evaporating dishes and was decomposed for one hour at 350-400°C in a muffle furnace. The now black catalyst was powdered and leached with 500 ml acetic acid (10%) for 30 minutes. The suspension was filtered and the crude product was washed with 4x100 ml distilled water. The solid was filtered off at a water aspirator until as dry as possible and dried in vac (110°, 31 inches vacuum). The catalyst was stored in a screw cap jar. Yield 85.2 g.

3. 8-Amino-16-azadispiro[6.1.6.2]heptadecane (attempted)

A solution of 8-amino-16-azadispiro[6.1.6.2]heptadecan-17-one (5.28 g,0.02 mole) in 60 ml anhydrous tetrahydrofuran was added dropwise to a suspension of lithium aluminum hydride (1.52 g,0.04 mole) in 20 ml anhydrous tetrahydrofuran in a 100 ml four-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. The reaction mixture was refluxed for 40 hr, excess hydride was destroyed by addition of 1.5 ml NaOH (10%) and an excess of water, and the reaction mixture was filtered. The solid was washed with ether and the combined filtrates were dried over $MgSO_4$. The solution was filtered and solvents were removed on a rotary evaporator to give a white solid residue. Recrystallization from ethanol gave colourless crystals of the starting

material, M.P. 187-190°d. The infrared spectrum showed no changes from the starting material and the mixed melting point with authentic starting material was not depressed.

4. 7-Amino-14-azadispiro[5.1.5.2]pentadecane (attempted)

A solution of 7-amino-14-azadispiro[5.1.5.2]pentadecan-15-one (2.36 g,0.01 mole) in 100 ml anhydrous tetrahydrofuran was added dropwise to a suspension of lithium aluminum hydride (0.38 g,0.01 mole) in 50 ml anhydrous tetrahydrofuran in a 250 ml three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. The reaction mixture was refluxed for 48 hr and cooled to room temperature. Excess hydride was destroyed by the dropwise addition of 3 ml water and 3 ml NaOH (5%). The mixture was filtered and the solid was washed with ether. The combined filtrates were dried over $MgSO_4$, filtered, and the solvents were removed on a rotary evaporator to give a white solid. Recrystallization from ethanol gave colourless crystals of starting material, M.P. 191-193°.

5. Hydrolysis of 8-dimethylaminoacetamido-16-azadispiro[6.1.6.2]-heptadecane (attempted)

A solution of 8-dimethylaminoacetamido-16-azadispiro[6.1.6.2]-heptadecane (1.25 g,0.004 mole) in 20 ml H_2SO_4 (30%), in a 50 ml boiling flask fitted with a condenser, was refluxed for four hr, cooled, and made basic with NaOH (40%). The alkaline suspension was extracted with 3x100 ml ether and the solution was dried over $MgSO_4$. The ether solution was filtered and solvent was removed on a rotary evaporator to give a colourless oil which crystallized on standing. Recrystal-

lization of the residue from hexane gave colourless crystals of starting material, M.P. 109-110°.

PART TWO

SYNTHESIS OF DERIVATIVES AND ANALOGUES OF CARBAZOLE

1. 9-(2-Dimethylaminoethyl)carbazole 52

A solution of carbazole (33.4 g,0.2 mole) in 200 ml dimethylformamide was added over ten min to a solution of sodium methoxide prepared by reacting freshly cleaned sodium metal (4.6 g,0.2 g atom) with 50 ml anhydrous reagent methanol in a one litre three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. The reaction mixture was refluxed for ten min and a solution of 2-dimethylaminoethyl chloride hydrochloride (14.4 g,0.1 mole) in 500 ml dimethylformamide was added dropwise to the reaction mixture. The reaction mixture was refluxed for eight hr, cooled, and filtered. The solvent was removed on a rotary evaporator and the solid residue was suspended in 800 ml HCl (2%) and filtered to remove the excess of carbazole. The acidic solution was extracted with 3x150 ml ether, was made basic with NaOH (30%), and was then extracted with 3x150 ml ether. The latter ether extract was dried over $MgSO_4$ and filtered, and the solvent was removed on a rotary evaporator to give a brown solid.

After several unsuccessful attempts to recrystallize the residue, reduced pressure sublimation gave colourless crystals, M.P. 40.5-41°. Yield 8.3 g (35%).

ir (KBr); 3045,2970,2940,2815,2760,1585,1475,1445,1340,1320,1250,1220, 1175,1145,845,820 cm^{-1}

nmr (CCl_4); δ 7.93(disturbed doublet,2,J = 6.5Hz); 7.25 (m,6); 4.25 (t,2,J = 8Hz); 2.55(t,2,J = 8Hz); 2.22(s,6)

There was no change after addition of D_2O.

Analysis for $C_{16}H_{18}N_2$ M.W. 238.33

calculated C,80.63; H,7.61; N,11.75

found C,80.45; H,7.41; N,12.04

A portion of the crystals was dissolved in ether, the solution was dried over $MgSO_4$ and filtered, and the salt was precipitated by addition of HCl gas. Recrystallization from ethanol (100%)/anhydrous ether gave colourless crystals, M.P. 243-244.5°.

ir (KBr); 3050,3010,2940,2650,2580,2520,2485,1595,1485,1455,1355,1330, 1235,1160,1020,975,755,730 cm^{-1}

2. 9-(2-Dimethylaminoethyl)-1,2,3,4-tetrahydrocarbazole 53

A. 1,2,3,4-Tetrahydrocarbazole 64

A solution of cyclohexanone (78 g,0.79 mole) in 270 ml glacial acetic acid in a one litre three-neck flask, fitted with a mechanical stirrer, dropping funnel, and condenser, was heated to reflux temperature, heat was removed, and freshly distilled phenylhydrazine (81 g, 0.75 mole) was added at a rate sufficient to maintain refluxing. The reaction mixture was refluxed for one hr further and was then poured into a beaker and stirred vigourously while cooling to 8°C in ice.

The slurry was filtered and the red-brown solid was washed with 75 ml water, suspended in 200 ml water, and filtered. The filtrates were discarded. The solid was washed with 100 ml ethanol, suspended in 200 ml ethanol, and filtered. The combined ethanolic filtrates were diluted to 1500 ml with water, allowed to stand for one hr, and filtered.

The combined solid materials were air dried (12 hr) and recrystallized from methanol, after decolourizing with charcoal, to give

slightly yellow crystals, M.P. 113-117°. Yield 89 g (66%) (lit (61) M.P. 116-117°, 82%)

ir (KBr); 3400,3065,2940,2865,1470,1450,1440,1370,1330,1305,1240,1150, 745,640,570,485 cm^{-1}

nmr ($CDCl_3$); δ 7.44(m,2); 7.16(m,3); 2.66(m,4); 1.86(m,4)

There was no change after addition of D_2O.

B. 9-(2-dimethylaminoethyl)-1,2,3,4-tetrahydrocarbazole

Freshly cleaned sodium metal (4.6 g,0.2 g atom), cut into small pieces, was reacted with 50 ml ethanol (100%) in a one litre three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. When most of the sodium had reacted, a solution of 1,2,3,4-tetrahydrocarbazole (34.2 g,0.2 mole) in 130 ml dimethylformamide was added over a period of ten min. The reaction was refluxed for 30 min and a solution of 2-dimethylaminoethyl bromide hydrobromide (23.2 g,0.1 mole) in 300 ml dimethylformamide was added dropwise to the refluxing reaction mixture. The reaction mixture was refluxed for 12 hr after addition was completed.

The reaction mixture was cooled to room temperature and filtered, and solvents were removed on a rotary evaporator. The semisolid residue was suspended in four litres HCl (5%) and was extracted with 4x500 ml ether which was then dried over $MgSO_4$. The excess of starting material was recovered from this solution (70%).

Insoluble material suspended in the acidic phase was filtered off and partitioned between 200 ml ether and 50 ml NaOH (30%) on a magnetic stirrer. The ether solution was extracted with 2x150 ml water and was dried over $MgSO_4$.

The acidic phase was made basic with NaOH (30%) and was extracted with 3x400 ml ether which was then dried over $MgSO_4$.

The two ether solutions were filtered (separately) and the solvents were removed on a rotary evaporator to give brown oils. Infrared spectra indicated that the two residues were the same. The combined residues were distilled at reduced pressure to give a light yellow oil, $b_{1.2}$ 155-160°. Yield 6.25 g (36.5%).

ir (liquid film); 3055,2930,2845,2780,1460,1370,1315,1175,1150,1040, 740 cm^{-1}

nmr (CCl_4,50%); δ 7.25(m,1); 6.96(m,3); 3.66(t,2,J = 7Hz); 2.48(e,4); 2.20(t,2,J = 7Hz); 2.00(s,6); 1.70(e,4).

There was no change after addition of D_2O.

A portion of the distillate was dissolved in ether, the solution was dried over $MgSO_4$ and filtered, and the salt was precipitated by addition of HCl gas. Recrystallization from ethanol (100%)/anhydrous ether gave colourless crystals, M.P. 244.5-246.0°d.

ir (KBr); 3420,2930,2850,2660,2585,2480,1455,1370,1230,1150,1010,965, 735 cm^{-1}

Analysis for $C_{16}H_{23}ClN_2$ M.W. 278.83

calculated	C,68.92;	H,8.32;	Cl,12.72;	N,10.05
found	C,68.77;	H,8.19;	Cl,12.67;	N,10.14

The remaining distillate partially crystallized on transferring the distillate to a vial for storage. Reduced pressure microsublimation of this material gave a liquid and a solid, M.P. 47.5-48.5°. The ir spectra of both solid and liquid were identical with that of the distillate. Hydrochloride salts of the solid and liquid were prepared.

Their melting points were the same as that of the salt of the distillate. Mixed melting points of all combinations of the three salts showed no depression from the melting point of the salt of the distillate.

3. 9-(2-Dimethylaminoethyl)dodecahydrocarbazole 54

A. Dodecahydrocarbazole 49

i) from 2-cyclohexylidene cyclohexanone (attempted)

a) 2-Cyclohexylidene cyclohexanone (53)

A solution of cyclohexanone (100 g,1.01 mole) in 100 g H_2SO_4 (60%) in a 500 ml erlenmeyer flask was tightly stoppered and allowed to stand at room temperature for 24 hr. The two layers were separated and 148 ml water was added to the aqueous (lower) layer, resulting in the separation of more organic layer. The combined organic material was washed with 5x35 ml saturated Na_2SO_4 solution. Since this procedure gave a poor separation, the combined aqueous phases were extracted with 3x150 ml ether, and ether was added to the organic phase to facilitate separation of the aqueous phase. The combined ether solutions were dried over $MgSO_4$ and filtered, and solvent was removed on a rotary evaporator to give a reddish oil. Unreacted cyclohexanone was recovered from the residue by distillation at atmospheric pressure. The residue was distilled at reduced pressure to give a yellow oil, $b_{1.5}$ 105-108°. Yield 24.5 g (27.5%) (lit (53) b_{760} 155-158°, 76-83% based on cyclohexanone consumed in the reaction).

This reaction was repeated several times using different reaction times and temperatures, and was also run in a centrifuge for five hr. The best yield obtained was 58.5%, based on the amount of cyclohexanone consumed in the reaction (Table VIII, p 68).

b) Dodecahydrocarbazole (45)

2-Cyclohexylidene cyclohexanone (24.5 g,0.137 mole) was mixed with formamide (36 g,0.8 mole) and 25 ml ethylene glycol in a 100 ml four-neck reaction flask fitted with a mechanical stirrer, condenser, and drying tube. The reaction mixture was gently refluxed at 120° for eight hr, then at 180° for two hr. The ethylene glycol and excess formamide were then distilled out of the reaction mixture at reduced pressure to give a thick brown oil. The residue was washed by decantation with an equal volume (35 ml) of water. An equal volume of HCl (38%) was added and the mixture was refluxed for three hr. Volatile material was then removed on a rotary evaporator, two volumes (50 ml) of water were added to the dark brown residue, and the mixture was extracted with 4x25 ml ether. The aqueous phase was then made basic with NaOH (30%) and extracted with 3x100 ml ether, which was dried over K_2CO_3. The ether solution was filtered and solvent was removed on a rotary evaporator to give a brown oil. Distillation of the residue at reduced pressure gave an unidentified product, $b_{2.5}$ 96-100°. (lit (45) b_{12} 130-132°, 32%, hydrochloride, M.P. 265-268°, analysis for Cl only; A different synthesis (47) gives b_{10} 124-125°, hydrochloride M.P. 208-209°, analysis for $C_{12}H_{22}ClN$ (C,H).

ii) from carbazole (47,70)

Glacial acetic acid (17.0 g,0.283 mole), and 5% rhodium on alumina catalyst (10 g) were added to a solution of carbazole (47 g, 0.28 mole) in 450 ml tetrahydrofuran in a Paar 4511 pressure reaction apparatus. The reaction mixture was stirred at room temperature under 1060 psi hydrogen. After 14 hr the pressure was 900 psi. The pressure

was increased to 1040 psi and the reaction was heated to 125°. After eight hr the pressure was 520 psi at room temperature. The pressure was increased to 1040 psi and the reaction was stirred at room temperature for 14 hr, after which time the pressure was 1000 psi. The reaction mixture was filtered and the solid material was washed with ether. The solvents were removed on a rotary evaporator to give a light yellow oil.

The solid material was suspended in 500 ml water, the residue from the evaporation was added to the aqueous suspension, and the suspension was made acidic with HCl (5%). The acidic suspension was filtered through dry keiselguhr and the filtrate was extracted with 3x300 ml ether.

The acidic solution was made basic with NaOH (30%) and was extracted with 300 ml ether. The aqueous phase was filtered and the gel which had formed at the interface was filtered through dry keiselguhr with the ethereal phase. The gel was washed with ether and this was added to the filtrate. The aqueous phase was then extracted with 2x300 ml ether. The combined ether extracts were dried over $MgSO_4$ and filtered, and the solvent was removed on a rotary evaporator to give a slightly yellow solid. The residue was sublimed in vac and recrystallized from hexane to give colourless crystals, M.P. 75.5-76.5°.

Yield 18.1 g (36.5%). (lit (47) 73-74.5°, 83-87%)

ir (KBr); 3250,2930,2860,1450,1095,955,905,845 cm^{-1}

nmr (CCl_4); δ 3.1(e); 2.32(s); 1.50(e) The integration curve could not be interpreted.

After addition of D_2O, the spectrum was as above except 2.32 absent.

Analysis for $C_{12}H_{21}N$ M.W. 179.31

calculated C,80.38; H,11.81; N,7.81

found C,80.35; H,11.64; N,7.79

A portion of the solid was dissolved in ether, the solution was dried over $MgSO_4$ and filtered, and the salt was precipitated by addition of HCl gas. Recrystallization from ethanol (100%)/anhydrous ether gave colourless crystals, M.P. 211.0-212.0°. (lit (47) 208-209°) ir (KBr); 2950,2880,2750,2720,2545,1580,1445,1065,1040,1010,980,935 cm^{-1}

B) 9-(2-Dimethylaminoethyl)dodecahydrocarbazole

A solution of dodecahydrocarbazole (18.1 g,0.1 mole) in 250 ml reagent benzene was added to sodium amide (3.9 g,0.1 mole) in a one litre three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube, and the reaction mixture was refluxed for two hr.

2-Dimethylaminoethyl bromide hydrobromide (23.3 g,0.2 mole) and flake sodium hydroxide (16.0 g,0.4 mole) were placed in a 250 ml three-neck flask fitted with a mechanical stirrer, Claisen still head, and a condenser set for distillation into a tared, ice-cooled receiver. This apparatus was connected to a water aspirator and the pressure was reduced to about 35 mm Hg. The solid reactants were slowly mixed together, turning the stirrer shaft by hand. When the reaction was proceeding smoothly, the motor of the stirrer was started. Reaction of the product occurred during the distillation, as evidenced by precipitation of a fine white solid in the receiver. Benzene (100 g), previously cooled in ice, was added to the distillate and the suspension was filtered into a tared, ice-cooled flask. Yield 9.3 g (0.061 mole,

39%) of 2-dimethylaminoethyl bromide. The solution was used immediately.

A solution of 2-dimethylaminoethyl bromide (9.3 g,0.061 mole) in 100 g benzene was added dropwise to the refluxing dodecahydrocarbazole reaction mixture and the mixture was refluxed for 24 hr. The reaction mixture was cooled and filtered, and the solvent was removed on a rotary evaporator to give an orange solid. Recrystallization of the residue from hexane gave colourless crystals of dodecahydrocarbazole, yield 3.7 g (52.5% of excess used).

The amorphous gray solid which was filtered out of the reaction mixture was decomposed by cautiously adding the solid to 700 ml water. The strongly basic solution was extracted with 4x200 ml ether and the combined extracts were dried over $MgSO_4$. The ether solution was filtered and the solvent was removed on a rotary evaporator to give a yellow oil which was distilled at reduced pressure to give a colourless liquid, $b_{0.7}$ 115-116°. Yield 10 g (50%).

ir (liquid film); 2940,2870,2830,2780,1455,1360,1270,1170,1160,1130, 1110,1050,1035,865,840 cm^{-1}

nmr ($CDCl_3$); δ 2.4(m,4); 2.13(s,6); 1.40(e,20)

There was no change after addition of D_2O.

A portion of the distillate was dissolved in ether, the solution was dried over $MgSO_4$ and filtered, and the salt was precipitated by addition of HCl gas. Recrystallization from ethanol (100%)/anhydrous ether gave colourless crystals, M.P. 246-247°.

ir (KBr); 2950,2870,2660,2490,1635,1450,1425,1375,1275,1000 cm^{-1}

Analysis for $C_{16}H_{32}Cl_2N_2$ M.W. 323.36

calculated	C,59.43;	H,9.98;	Cl,21.93;	N,8.66
found	C,59.19;	H,9.72;	Cl,21.80;	N,8.84

4. 4-(2-Dimethylaminoethyl)-1,2,3,4-tetrahydrocyclopent[b]indole 55

A. 1,2,3,4-Tetrahydrocyclopent[b]indole 66

A mixture of cyclopentanone (42 g,0.5 mole) and freshly distilled phenylhydrazine (54 g,0.5 mole) was shaken in a 250 ml erlenmeyer flask and allowed to stand for 20 minutes, during which a highly exothermic reaction occurred. The mixture was then heated on a steam bath for ten min and the red syrup was poured into a solution of H_2SO_4 (58 ml) in water (1039 ml). This mixture was heated for 45 min while stirring vigourously, and was then cooled in ice. The red solid was filtered off and dried in vac over sulfuric acid. Recrystallization of the dry product from petroleum spirit (60-80°) gave slightly pink crystals, M.P. 106.5-107.5°. Yield 32.5 g (41.5%) (lit (63) 108-109°, 45%).

ir (KBr); 3400,3060,2980,2950,2770,1580,1470,1450,1370,1320,1300,750, 640 cm^{-1}

nmr (CCl_4); δ 7.20(m,2); 6.97(m,3); 2.63(m,6)

There was no change after addition of D_2O.

B. 4-(2-Dimethylaminoethyl)-1,2,3,4-tetrahydrocyclopent[b]indole

i) using sodium amide as the base (attempted)

A solution of 1,2,3,4-tetrahydrocyclopent[b]indole (20.2 g, 0.129 mole) in 250 ml dimethylformamide was refluxed with sodium amide (6.6 g,0.17 mole) for four hr under a dry nitrogen atmosphere in a one litre three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, drying tube, and gas inlet tube. The dark brown suspension was cooled to room temperature and 2-dimethylaminoethyl chloride (18.5 g,0.17 mole) was added dropwise. The reaction mixture was refluxed

for three hr and stirred at room temperature for 24 hr. The reaction mixture was cautiously diluted to two litres with water and filtered, and the resultant basic solution was extracted with 3x300 ml ether. The ether solution was extracted with 3x200 ml HCl (3%) and was dried over $MgSO_4$. No 1,2,3,4-tetrahydrocyclopent[b]indole could be recovered from this ether solution.

The acidic solution was made basic with NaOH (30%) and was extracted with 3x200 ml ether which was dried over $MgSO_4$. The ether solution was filtered and the solvent was removed on a rotary evaporator to give a small amount of nearly black resinous material, from which none of the desired product could be isolated.

ii) using sodium ethoxide as the base (attempted)

Freshly cleaned sodium (2.9 g,0.125 g atom), cut in small pieces, was reacted with 50 ml ethanol (100%) in a 500 ml three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. The apparatus was wrapped in aluminum foil to protect the indole from light. A solution of 1,2,3,4-tetrahydrocyclopent[b]-indole (19.7 g,0.125 mole) in 90 ml ethanol (100%) was added dropwise to the slowly refluxing solution of ethoxide. A solution of 2-dimethylaminoethyl bromide hydrobromide (14.5 g,0.063 mole) in 200 ml warm ethanol (100%) was then added dropwise, over 2.5 hr, to the gently refluxing reaction mixture. The reaction mixture was stirred at room temperature for 12 hr and filtered, and the solvent was removed on a rotary evaporator. The residue was suspended in 600 ml water and the basic suspension was extracted with 3x150 ml ether. The ethereal solution was extracted with 3x150 ml HCl (3%) and dried over $MgSO_4$. None

of the starting material could be recovered from this solution.

The acidic solution was made basic with NaOH (30%) and was extracted with 3x150 ml ether which was dried over $MgSO_4$. The ether solution was filtered and solvent was removed on a rotary evaporator to give a small amount of black oil from which none of the desired product could be isolated.

iii) without using a base (attempted)

A solution of 2-dimethylaminoethyl bromide hydrobromide (11.65 g,0.05 mole) in 200 ml ethanol (100%) was heated to a slow reflux in a 500 ml three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. The apparatus was wrapped in aluminum foil to protect the indole from light. A solution of 1,2,3,4-tetrahydrocyclopent[b]indole (7.85 g,0.05 mole) in 80 ml ethanol (100%) was added over a period of three hr to the reaction flask. The reaction mixture was refluxed for 12 hr, cooled to room temperature, and the solvent was removed on a rotary evaporator. The solid residue was suspended in 250 ml ether and was extracted with 3x90 ml HCl (5%). The ether solution was dried over $MgSO_4$. None of the starting material could be recovered from this solution.

The acidic solution was made basic with NaOH (30%) and was extracted with 3x100 ml ether which was dried over $MgSO_4$. The ether solution was filtered and the solvent was removed on a rotary evaporator to give a brown liquid residue, which was not identified. None of the desired product could be isolated from the residue.

iv) using sodium metal as the base

Freshly cleaned sodium metal (3 g,0.13 g atom) was very finely

dispersed in 150 ml xylene, previously dried over sodium wire, in a one litre three-neck flask fitted with a mechanical stirrer (Hershberg type), dropping funnel, condenser, and drying tube. The apparatus was wrapped in aluminum foil to protect the indole from light. A solution of 1,2,3,4-tetrahydrocyclopent[b]indole (32.5 g,0.15 mole) in 100 ml xylene/anhydrous ether (1:1) was added to the reaction flask over 2.5 hr, while stirring very vigourously. A solution of 2-dimethylaminoethyl bromide hydrobromide (16.3 g,0.07 mole) in 200 ml dimethylformamide was then added dropwise to the vigourously stirred reaction mixture and the reaction was stirred for 14 hr at room temperature.

The reaction mixture was filtered, the precipitate was washed with benzene, and the solvents were removed from the combined filtrates on a rotary evaporator. The residue was dissolved in 500 ml ether and was extracted with 3x150 ml water which had been made slightly basic with NaOH (5%). The ether solution was then extracted with 3x150 ml HCl (3%) and was dried over $MgSO_4$. None of the starting material could be recovered from this solution.

The acidic solution was made basic with NaOH (30%) and was extracted with 3x150 ml ether which was dried over $MgSO_4$. The ether solution was filtered and solvent was removed on a rotary evaporator to give a brown oil. Distillation of the residue at reduced pressure gave a slightly yellow oil, $b_{0.55}$ 136.5-139.5°. Yield 5.97 g (17.5%).

ir (liquid film); 3050,2950,2860,2830,2780,1460,1380,1355,1295,1175, 1150,1035,745 cm^{-1}

nmr (CCl_4); δ 7.23(m,1); 6.99(m,3); 3.85(t,2,J = 7.5Hz); 2.65 overlapped with 2.42 (e and t respectively, total 8, J of t = 7Hz);

2.12(s,6)

A portion of the distillate was dissolved in ether, the solution was dried over $MgSO_4$ and filtered, and the salt was precipitated by addition of HCl gas. The mauve precipitate was recrystallized from ethanol (100%)/anhydrous ether to give light purple crystals, M.P. 220-226.5°d. This salt decomposed on recrystallization. The picrate was prepared and recrystallized from ethanol to give yellow crystals, M.P. 198.0-198.5°.

ir (KBr); 2940,2860,2640,1630,1560,1460,1430,1365,1315,1270,1165,1085, 745 cm^{-1}

Analysis for $C_{21}H_{23}N_5O_7$ M.W. 457.44

calculated C,55.14; H,5.07; N,15.31; O,24.48

found C,55.14; H,5.13; N,15.26

5. 4-(2-Dimethylaminoethyl)dodecahydrocyclopent[b]indole 56

A. Dodecahydrocyclopent[b]indole 75

Glacial acetic acid (15.0 g,0.25 mole) and 5% rhodium on alumina catalyst (10 g) were added to a solution of 1,2,3,4-tetrahydrocyclopent[b]indole in 550 ml anhydrous tetrahydrofuran in a Paar 4511 pressure reaction apparatus. The reaction mixture was stirred at room temperature under 1300 psi hydrogen for 24 hr. The apparatus was then heated to 130° for 16 hr. After a further 20 hr at room temperature, the reaction mixture was filtered and the solid was washed with ether.

The solvents were removed from the combined filtrates on a rotary evaporator to give a gray solid residue. The residue was suspended in 250 ml ether and was extracted with 3x125 ml HCl (3%). The ether solution was then dried over $MgSO_4$. No starting material could

be recovered from this solution.

The acidic solution was made basic with NaOH (30%) and was extracted with 3x100 ml ether, which was then dried over $MgSO_4$. The ether solution was filtered and solvent was removed on a rotary evaporator to give a mobile brown liquid. The residue was distilled at reduced pressure to give a colourless light oil, $b_{0.8}$ 64-66°. Yield 28.5 g (84%).

ir (liquid film); 3275,2920,2855,1450,1403,1335,1280,1235,1210,1115, 1100,945,865,850,715 cm^{-1}

nmr (neat); several envelope bands in the region 1.0 to 3.8. One sharp peak at 1.70 which disappeared after addition of D_2O. The integration curve was indecipherable.

A portion of the distillate was dissolved in ether, the solution was dried over $MgSO_4$ and filtered, and the salt was precipitated by the addition of HCl gas. Recrystallization from benzene gave colourless, feathery crystals, M.P. 241.5-243.0°.

ir (KBr); 2940,2870,2760,2715,2560,1580,1450,1420,1390,1070,945,585, 470 cm^{-1}

Analysis for $C_{11}H_{20}ClN$ M.W. 201.74

calculated	C,65.49;	H,9.99;	Cl,17.58;	N,6.94
found	C,65.66;	H,9.76;	Cl,17.50;	N,7.12

B. 4-(2-Dimethylaminoethyl)dodecahydrocyclopent[b]indole

i) using sodium metal as the base (attempted)

A solution of dodecahydrocyclopent[b]indole (24.8 g,0.15 mole) in 200 ml purified dioxane was added, under a nitrogen atmosphere, to freshly cleaned sodium metal (3.4 g, 0.15 g atom), cut into small

pieces, in a 500 ml three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, drying tube, and gas inlet tube. The reaction was refluxed for 70 hr, during which time only a small amount of the sodium had reacted.

An excess of ethanol (100%) was cautiously added to the cooled reaction mixture to destroy the unreacted sodium and the mixture was diluted to one litre with water. The resultant suspension was filtered and the filtrate was extracted with 3x250 ml ether. The ether solution was extracted with 3x150 ml HCl (3%) and discarded.

The acidic solution was made basic with NaOH (30%) and was extracted with 3x100 ml ether which was then dried over $MgSO_4$. The ether solution was filtered and the solvent was removed on a rotary evaporator to give a light brown oil. Distillation of the residue at reduced pressure gave 92% recovery of dodecahydrocyclopent[b]indole.

ii) without using a base

a) A solution of 2-dimethylaminoethyl bromide hydrobromide (2.3 g,0.01 mole) in 20 ml ethanol (100%) was heated to reflux in a 50 ml boiling flask fitted with a condenser, dropping funnel, and drying tube. The solution was stirred magnetically. A solution of dodecahydrocyclopent[b]indole (1.65 g,0.01 mole) in 10 ml ethanol (100%) was added dropwise to the reaction flask, giving an immediate white precipitate. The reaction mixture was refluxed for two hr after addition was completed and was then cooled to room temperature and filtered. The filtrate was diluted with 250 ml water and was made acidic with HCl (5%). The acidic solution was extracted with 3x50 ml ether which was discarded.

The acidic solution was made basic with NaOH (30%) and was extracted with 3x50 ml ether which was dried over $MgSO_4$. The ether solution was filtered and solvent was removed on a rotary evaporator to give a colourless oil. Distillation of the residue at reduced pressure gave 73% recovery of dodecahydrocyclopent[b]indole.

The amorphous white solid which precipitated during the reaction had a melting point in excess of 303° and contained halogen (Br). It was insoluble in ether, but was freely soluble in water. No precipitate was formed when the aqueous solution was made basic with NaOH (5%). The infra-red spectrum (KBr) showed very weak NH stretching and did not have the characteristic fingerprint region which is common to the saturated cycloalk[b]indoles. It was tentatively identified as the dimer (or polymer) of 2-dimethylaminoethyl bromide hydrobromide.

b) A solution of dodecahydrocyclopent[b]indole (1.6 g,0.01 mole) in 20 ml ethanol (100%) was heated to reflux in a 100 ml four-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. A solution of 2-dimethylaminoethyl bromide hydrobromide (2.33 g,0.01 mole) in 50 ml ethanol (100%) was added dropwise to the reaction mixture and the mixture was refluxed for one hr and stirred at room temperature for 42 hr. The reaction was worked up as in a) above to give 46% recovery of dodecahydrocyclopent[b]indole.

c) A suspension of 2-dimethylaminoethyl chloride (6.5 g,0.06 mole) in 100 ml anhydrous benzene was added dropwise to a refluxing solution of dodecahydrocyclopent[b]indole (20 g,0.12 mole) in 50 ml anhydrous benzene in a 250 ml three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. The reaction mixture was

refluxed for 34 hr, cooled, and diluted to 500 ml with ether. The suspension was then extracted with 3x250 ml water, adding sufficient HCl (5%) to each extraction to give an acidic reaction to litmus.

The acidic solution was made basic with NaOH (30%) and was extracted with 3x125 ml ether which was dried over $MgSO_4$. The ether solution was filtered and solvent was removed on a rotary evaporator to give a light yellow oil. Attempted distillation of the residue at atmospheric pressure resulted in the precipitation of a white solid. The residue was refluxed for three hr and the solid was filtered off and washed with ether. The infra-red spectrum and melting point of the solid indicated that it was dodecahydrocyclopent[b]indole hydrochloride.

The combined filtrates were distilled at atmospheric pressure to remove the ether wash solvent and then at reduced pressure to give a colourless oil, $b_{0.5}$ 97-100°. Yield 8.1 g (49.5%). Recovery of dodecahydrocyclopent[b]indole 12.5 g (62.5%)

ir (liquid film); 2940,2865,2830,2780,1455,1275,1220,1155,1110,1055, 950,860 cm^{-1}

The nmr spectrum (neat) showed several envelope bands in the region 0.8-3.0 with the characteristic dimethylamino band (sharp singlet) at 2.20. There was no change after addition of D_2O. The integration curve was indecipherable.

A portion of the distillate was dissolved in ether, the solution was dried over $MgSO_4$ and filtered, and the salt was precipitated by addition of HCl gas. The precipitate was recrystallized from ethanol (100%)/anhydrous ether to give colourless flattened needles, M.P.

209.0-210.7°.

ir (KBr); 3460,3390,2930,2860,2600,2470,1635,1450,1410,1260,1070,1045, 1005 cm^{-1}

Analysis for $C_{15}H_{30}Cl_2N_2$ M.W. 309.33

calculated	C,58.24;	H,9.78;	Cl,22.93;	N,9.06
found	C,58.60;	H,9.41;	Cl,22.87;	N,9.07

6. 5-(2-Dimethylaminoethyl)-5,6,7,8,9,10-hexahydrocyclohept[b]-indole 57

A. 5,6,7,8,9,10-Hexahydrocyclohept[b]indole 68

A solution of cycloheptanone (16.8 g,0.15 mole) in 55 ml glacial acetic acid was heated to reflux in a 250 ml three-neck flask fitted with a mechanical stirrer, condenser, and dropping funnel. Phenylhydrazine (16.0 g,0.15 mole) was added dropwise to the reaction flask and the reaction mixture was refluxed for one hr after addition was completed. The hot reaction mixture was then poured into a beaker and stirred vigourously while cooling to 5° in ice. The slurry was filtered and the solid material was washed with water. Decolourization and recrystallization from methanol gave colourless crystals, M.P. 142.5-143.5°. Yield 20.5 g (74%) (lit (61) 142-144°, 74%).

ir (KBr); 3400,3070,2920,2860,1470,1440,1430,1340,1320,1240,1190,1020, 960,750,590,510 cm^{-1}

nmr ($CDCl_3$); δ 7.20(m,5); 2.76(e,4); 1.87(e,6)

There was no change after addition of D_2O.

B. 5-(2-Dimethylaminoethyl)-5,6,7,8,9,10-hexahydrocyclohept[b]-indole

A solution of 5,6,7,8,9,10-hexahydrocyclohept[b]indole (19.4 g,0.105 mole) in 250 ml benzene was added, under a nitrogen atmosphere, to a suspension of sodium amide in 50 ml of benzene in a one litre three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, drying tube, and gas inlet tube. The reaction mixture was refluxed for eight hr, giving a brown suspension. 2-Dimethylaminoethyl chloride (11.3 g,0.105 mole) was added dropwise to the hot suspension and the reaction mixture was refluxed for 12 hr. The mixture was cooled to room temperature, 500 ml water was cautiously added, the mixture was filtered, and the phases were separated.

The organic phase was extracted with 2x250 ml water which was discarded, and then with 3x250 ml HCl (3%). The organic phase was dried over $MgSO_4$ and filtered, and solvent was removed on a rotary evaporator to give a light brown solid. The solid was decolourized and recrystallized from methanol to give 20% recovery of 5,6,7,8,9,10-hexahydrocyclohept[b]indole.

The acidic solution was made basic with NaOH (30%) and was extracted with 3x250 ml ether which was then dried over $MgSO_4$. The ether solution was filtered and solvent was removed on a rotary evaporator to give a brown oil. The residue was distilled at reduced pressure to give a light yellow oil, $b_{0.5}$ 154-158°. Yield 16.3 g (59%).

ir (liquid film); 2920,2855,2830,2780,1465,1375,1320,1275,1210,1175, 1160,1050,1025,740 cm^{-1}

nmr (CCl_4); δ 7.00(m,4); 4.08(t,2,J = 8Hz); 2.74(e,4); 2.40(t,2,J = 8Hz); 2.22(s,6); 1.83(e,6)

There was no change after addition of D_2O.

A portion of the distillate was dissolved in ether, the solution was dried over $MgSO_4$ and filtered, and the salt was precipitated by addition of HCl gas. Recrystallization from ethanol (100%)/anhydrous ether gave colourless crystals, M.P. 226.5-228.5°.

ir (KBr); 2935,2860,2660,2600,2480,1470,1380,1320,1220,1165,975,745 cm^{-1}

Analysis for $C_{17}H_{25}ClN_2$ M.W. 292.86

calculated	C,69.72;	H,8.61;	Cl,12.11;	N,9.57
found	C,69.52;	H,8.50;	Cl,12.30;	N,9.47

7. 5-(2-Dimethylaminoethyl)tetradecahydrocyclohept[b]indole 58

Glacial acetic acid (3.6 g,0.06 mole) was added to a solution of 5-(2-dimethylaminoethyl)-5,6,7,8,9,10-hexahydrocyclohept[b]indole (8 g, 0.031 mole) in 200 ml reagent methanol in a Paar 4511 pressure reaction apparatus. A suspension of 5% rhodium on alumina catalyst (3 g) in 50 ml reagent methanol was added and the reaction mixture was stirred under 900 psi hydrogen for 20 min. The apparatus was heated to 150° and stirred for 50 hr. The apparatus was then cooled to room temperature over 12 hr, at which time the pressure was 820 psi.

The suspension was filtered and the solid material was washed with ether and with water. The combined filtrates were diluted to 1500 ml with water and were made basic with NaOH (5%). The phases were separated and the aqueous phase was extracted with 3x200 ml ether, which was added to the organic phase from the filtrate.

The combined ethereal solutions were dried over $MgSO_4$ and filtered, and the solvent was removed on a rotary evaporator to give a yellow oil. The residue was distilled at reduced pressure to give a light yellow oil, $b_{1.2}$ 126-127.5°. Yield 5.7 g (77%).

ir (liquid film); 2920,2850,1440,1355,1255,1140,1060,1040 cm^{-1}

The nmr spectrum was very poorly defined, with all the absorption peaks overlapping in the region δ 0.6-3.5.

A portion of the distillate was dissolved in ether, the solution was dried over $MgSO_4$ and filtered, and the salt was precipitated by the addition of HCl gas. The salt precipitated as an oil which could not be recrystallized.

The picrate salt was formed and recrystallized from ethanol to give dark yellow crystals, M.P. 204.0-205.5°d.

ir (KBr); 2925,2860,1625,1610,1565,1430,1365,1320,1270,1165,1080,920 cm^{-1}

Analysis for $C_{29}H_{38}N_8O_{14}$ M.W. 722.66

calculated	C,48.20;	H,5.30;	N,15.51;	O,31.00
found	C,48.34;	H,5.38;	N,15.36	

8. 9-(2-Dimethylaminoethyl)fluorene 59

A. using potassium t-butoxide as the base

Freshly cleaned potassium metal (10 g,0.25 g atom), cut into small pieces, was reacted with 150 ml reagent t-butyl alcohol in a one litre boiling flask fitted with a condenser, dropping funnel, and drying tube. The reaction mixture was stirred magnetically. When all the potassium had reacted the excess solvent was distilled off at reduced pressure. A solution of fluorene (41.7 g,0.25 mole) in 300 ml anhydrous benzene was then added to the solid butoxide and the resultant solution was refluxed for ten min. A solution of 2-dimethylaminoethyl bromide hydrobromide (28.8 g,0.125 mole) in 300 ml dimethylformamide was then added dropwise to the refluxing reaction mixture. The mixture was refluxed for two hr after the addition was completed and stirred at room

temperature for 14 hr.

The reaction mixture was filtered and the solvents were removed on a rotary evaporator. The reddish brown solid residue was suspended in one litre ether and was extracted with 3x150 ml NaOH (5%), which was discarded. The ether phase was then extracted with 3x150 ml HCl (5%) and was dried over $MgSO_4$. The ether solution was filtered and solvent was removed on a rotary evaporator to give a yellow solid residue of unreacted fluorene. This was decolourized and the excess fluorene was recovered by recrystallization from ethanol and reduced pressure sublimation.

The acidic solution was made basic with NaOH (25%) and was extracted with 3x100 ml ether which was then dried over $MgSO_4$. The ether solution was filtered and solvent was removed on a rotary evaporator to give a brown oil. Distillation of the residue at reduced pressure gave a yellow oil, $b_{0.5}$ 140-145°. Yield 3.5 g (12.4%).

ir (liquid film); 3080,3050,3030,2950,2870,2830,2780,1460,1450,1265, 1145,1105,1045,850,745 cm^{-1}

nmr (CCl_4); δ 7.24(m,8); 3.93(t,1,J = 5Hz); 2.09(strong s overlapping m,10)

A portion of the distillate was dissolved in ether, the solution was dried over $MgSO_4$ and filtered, and the salt was precipitated by addition of HCl gas. The gummy brown solid could not be recrystallized. The picrate was prepared and recrystallized from ethanol to give feathery yellow needles, M.P. 179.5-180.3°.

ir (KBr); 3105,3060,2855,2780,1630,1620,1570,1495,1485,1440,1400,1370, 1335,1315,1270,1160,1145,1090,920,800,755,725 cm^{-1}

Analysis for $C_{23}H_{22}N_4O_7$ M.W. 466.45

calculated C,59.23; H,4.75; N,12.01; O,24.01

found C,59.18; H,4.66; N,12.20

B. using sodium hydride as the base (attempted)

A solution of fluorene (41.7 g,0.25 mole) in 300 ml anhydrous benzene was added dropwise to a refluxing suspension of sodium hydride (6.0 g,0.25 mole) in 75 ml anhydrous benzene in a one litre three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. The reaction mixture was then refluxed for five days. A solution of 2-dimethylaminoethyl bromide hydrobromide (28.8 g,0.125 mole) in 300 ml dimethylformamide was added dropwise to the refluxing reaction mixture. The mixture was refluxed for three hr after addition was completed, then cooled and filtered. The solvents were removed from the filtrate on a rotary evaporator to give a yellow oil which was dissolved in 1500 ml ether.

The ether solution was extracted with 3x500 ml water which was discarded. The ether solution was then extracted with 3x100 ml HCl (5%) and dried over $MgSO_4$. The ether solution was filtered and solvent removed on a rotary evaporator to give a dark yellow residue of unreacted fluorene which was decolourized and purified by sublimation.

The acidic solution was made basic with NaOH (30%) and was extracted with 2x100 ml ether which was dried over $MgSO_4$. The ether solution was filtered and solvent was removed on a rotary evaporator to give a negligible amount of yellow oil.

C. using potassium metal as the base (72,73)

Freshly cleaned potassium metal (10 g,0.25 g atom), cut in small pieces, was suspended in 100 ml purified dioxane in a one litre three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, drying tube, and gas inlet tube. A solution of fluorene (41.7 g,0.25 mole) in 350 ml purified dioxane was added and the reaction mixture was refluxed under a dry nitrogen atmosphere until all the potassium had reacted (about five hr), giving a dark red-brown suspension. The reaction mixture was then cooled to room temperature and a solution of 2-dimethylaminoethyl chloride (27.0 g,0.25 mole) in 100 ml purified dioxane was added dropwise to the reaction mixture which was then refluxed for 14 hr. The reaction mixture was cooled and filtered, the solid was washed with ether, and the solvents were removed from the combined filtrates on a rotary evaporator, giving a yellow-green oil.

The residue was suspended in 900 ml water and was extracted with 4x200 ml ether. The ether solution was extracted with 3x175 ml HCl (5%) and was dried over $MgSO_4$. The ether solution was filtered and solvent was removed on a rotary evaporator to give a dark yellow solid. Unreacted fluorene was recovered from the residue by reduced pressure sublimation after decolourization.

The acidic solution was made basic with NaOH (25%) and was extracted with 3x200 ml ether which was then dried over $MgSO_4$. The ether solution was filtered and solvent was removed on a rotary evaporator to give an amber oil. The residue was distilled at reduced pressure to give two fractions, both yellow oils, containing a mixture of the desired compound with 9,9-bis(2-dimethylaminoethyl)fluorene 61.

Fraction A, 14 g, $b_{0.5}$ 145-150°, 65% of 59; 35% of 61,

from the nmr spectrum.

Fraction B, 13.2 g, $b_{1.2}$ 170-175°, nearly pure 61, from the nmr spectrum.

nmr (CCl_4, Fraction B); δ 7.60(m,2); 7.34(m,6); 2.21 and 2.17 (overlapped t,4 protons total, large intensity buildup, J = 6 and 5Hz); 1.93(s,12); 1.43 (overlapped t,4,J = 6,7)

The infra-red spectra (10% in CCl_4, 0.5 mm cell) were identical for both fractions, except for slight variations in intensity of several of the minor peaks. The spectra of both fractions as thin films, and as CCl_4 solutions, were the same as the spectrum obtained for the compound as previously synthesized (8,A).

Separation of Fraction A was attempted unsuccessfully by preparative vapour phase chromatography. The stationary phase was UCON polar 50 HB660 (33% on ultraport, 60/70 mesh) in a 5 foot by 5/8 inch nickel-coated brass column, in a Beckmann GC-2 instrument. The carrier gas was helium at an inlet pressure of 40 psi; column temperatures were 160° and 190°. The distillate was injected as a 50% solution in ethanol. Only the solvent peak was detected.

Fraction B partially crystallized on standing at room temperature. Recrystallization was attempted from a number of solvents without success.

ir (KBr, Fraction B); 3070,3040,2995,2960,2940,2860,2820,2780,1460,1450, 1260,1180,1165,1150,1105,1045,865,785,750 cm^{-1}

A portion of Fraction B was dissolved in ethanol and the picrate was precipitated. Recrystallization from ethanol gave very

fine, yellow, matted needles, M.P. 230.0-231.5°.

ir (KBr); 3030,2740,1635,1615,1570,1550,1440,1370,1325,1280,1170,1085, 925,755,725 cm^{-1}

Analysis for $C_{33}H_{34}N_8O_{14}$ M.W. 766.68

calculated	C,51.70;	H,4.47;	N,14.62;	O,29.22
found	C,51.58;	H,4.40;	N,14.38	

D. using sodium amide as the base (72,75)

A solution of fluorene (41.7 g,0.25 mole) in 400 ml decalin, previously distilled from sodium, was gently refluxed, under a dry nitrogen atmosphere, with sodium amide (10.0 g,0.25 mole) in a one litre three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, drying tube, and gas inlet tube. After four hr a black, gummy mass had precipitated. This material interfered with the stirrer so stirring was discontinued while the reaction mixture was refluxed for one more hr. The mixture was then cooled and the solvent was removed through a sintered glass gas dispersion tube attached to an aspirator.

The black partially crystalline mass was washed twice, by decantation, with 75 ml hexane previously dried over sodium. The solid was suspended in 75 ml dried hexane and broken up by stirring vigourously. A solution of 2-dimethylaminoethyl chloride (29 g,0.25 mole) in 100 ml dried hexane was added dropwise and the mixture was refluxed for 15 hr. The reaction mixture was cooled and 700 ml water was cautiously added. The resultant suspension was mixed with 500 ml ether and then filtered. The phases were separated and the ether solution was washed with 3x150 ml water which was added to the aqueous

phase. The strongly basic aqueous solution was then extracted with 3x150 ml ether which was added to the ether solution.

The ether solution was extracted with 3x150 ml HCl (3%) and dried over $MgSO_4$. The ether solution was filtered and solvent was removed on a rotary evaporator to give a dark yellow residue. A small amount of fluorene was recovered from the residue by reduced pressure sublimation after decolourizing.

The acid solution was made basic with NaOH (30%) and was extracted with 3x150 ml ether which was dried over $MgSO_4$. The ether solution was filtered and the solvent was removed on a rotary evaporator to give a dark brown oil. The residue was distilled at reduced pressure to give two fractions, both yellow oils.

Fraction 1, 22.1 g $b_{0.25-0.4}$ 133-138°, 85% of 59 and 15% of 61 (from nmr)

Fraction 2, 5.0 g, $b_{0.4}$ 138-152°, 45% of 59 and 55% of 61 (from nmr)

Fractions 1 and 2 were combined with Fractions A and B from the reaction of fluorene using potassium metal (8,C) and the mixture was distilled at reduced pressure very slowly through a heated Vigreaux column. Six fractions were collected, all containing a mixture of the mono- and di-substituted fluorene compounds.

E. using sodium amide as the base

A solution of fluorene (41.7 g,0.25 mole) in 400 ml anhydrous decalin was gently refluxed, under a nitrogen atmosphere, with sodium amide (8 g,0.2 mole) in a one litre three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, drying tube, and gas

inlet tube. After five hr the reaction mixture was cooled and the nearly clear yellow decalin solution was decanted from the rust coloured mass which had precipitated as a gum and solidified on cooling. The precipitate was washed twice with 75 ml sodium dried hexane and then broken up by stirring very vigourously with 75 ml hexane. A solution of 2-dimethylaminoethyl chloride (8 g,0.075 mole) in 50 ml anhydrous hexane was added dropwise to the reaction mixture and the mixture was refluxed for 14 hr.

The reaction mixture was cooled and 500 ml water was cautiously added. The mixture was stirred with 250 ml ether and filtered, and the phases were separated. The aqueous phase was extracted with 2x150 ml ether which was added to the initial ether phase. The combined ether solution was extracted with 3x200 ml HCl (3%) and dried over $MgSO_4$. The ether solution was filtered and solvent was removed on a rotary evaporator to give a dark yellow solid. Unreacted fluorene was recovered from the residue by reduced pressure sublimation after decolourizing.

The acidic solution was made basic with NaOH (30%) and was extracted with 3x150 ml ether. The ether solution was dried over $MgSO_4$ and filtered, and the solvent was removed on a rotary evaporator to give a yellow oil which deposited cubic crystals on standing. Recrystallization from hexane gave a colourless amorphous powder, M.P. 93-100°. Recrystallization from several solvents did not improve the melting point. Distillation of the liquid residue at reduced pressure gave a light yellow oil $b_{0.6}$ 141-144°, which partially solidified on standing. Ir and nmr spectra were consistent with the di-substituted fluorene derivative. Yield 8.3 g (72% based on 2-dimethylaminoethyl

chloride).

The solid was eventually successfully recrystallized from dilute acetone to give a colourless amorphous powder, the main part of which melted at 83.4-85.0°, but which had a melting range of 79-89°.

Analysis for $C_{21}H_{28}N_2$ M.W 308.47

calculated C,81.78; H,9.15; N,9.08

found C,81.88; H,8.94; N,8.98

A portion of the solid material was dissolved in ether, the solution was dried over $MgSO_4$ and filtered, and the salt was precipitated by the addition of HCl gas. Recrystallization of the gummy precipitate from ethanol (100%)/anhydrous ether gave colourless crystals, M.P. 238.0-239.2°.

ir (KBr); 3015,2940,2670,2470,1470,1450,1420,1300,1165,1005,970,780, 745 cm^{-1}

Analysis for $C_{21}H_{30}Cl_2N_2$ M.W. 381.40

calculated C,66.13; H,7.93; Cl,18.59; N,7.35

found C,66.08; H,7.95; Cl,18.74; N,7.21

F. using potassium metal as the base

A solution of fluorene (50.0 g,0.3 mole) in 500 ml purified dioxane was reacted, under a nitrogen atmosphere, with freshly cleaned potassium (8.0 g,0.2 g atom) in a one litre three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, drying tube, and gas inlet tube. The reaction mixture was refluxed until no potassium was visible (approximately three hr) and gave a deep red colloidal suspension. A solution of 2-dimethylaminoethyl chloride (10.7 g,0.1 mole) in 100 ml purified dioxane was then run into the cooled reaction mixture

and the mixture was stirred at room temperature for three hr. Excess fluorenyl potassium was destroyed by cautious addition of 100 ml water, solvents were removed on a rotary evaporator, and the residue was partitioned between 1000 ml ether and 500 ml water. The phases were separated and the ether solution was extracted with 2x250 ml water. The ether phase was then extracted with 3x150 ml HCl (3%) and dried over $MgSO_4$. The ether solution was filtered and the solvent was removed on a rotary evaporator to give a yellow solid. Unreacted fluorene was recovered from this residue by sublimation at reduced pressure after decolourizing.

The acidic solution was made basic with NaOH (30%) and was extracted with 3x150 ml ether, which was dried over $MgSO_4$. The ether solution was filtered and solvent was removed on a rotary evaporator to give a yellow oil. Distillation of the residue at reduced pressure gave a light yellow oil, $b_{0.1}$ 128°. Ir and nmr spectra were consistent with the desired compound. Yield 7.0 g (30%, based on 2-dimethylaminoethyl chloride).

A portion of the distillate was dissolved in ether, the solution was dried over $MgSO_4$ and filtered, and the salt was precipitated by addition of HCl gas. Recrystallization from ethanol (100%)/anhydrous ether gave colourless crystals, M.P. 154.9-156.3°.

ir (KBr); 3060,3005,2930,2660,2570,2470,1470,1450,1420,1170,1160,1140, 1030,970,760,750 cm^{-1}

Analysis for $C_{17}H_{20}ClN$ M.W. 273.81

calculated	C,74.57;	H,7.36;	Cl,12.95;	N,5.12
found	C,74.45;	H,7.18;	Cl,12.96;	N,5.10

9. 9-(2-Dimethylaminoethyl)dodecahydrofluorene and 9,9-bis(2-dimethylaminoethyl)dodecahydrofluorene (attempted)

An inseparable mixture (51.7 g) containing, as determined from the nmr spectrum, 9-(2-dimethylaminoethyl)fluorene (28.9 g,0.12 mole) and 9,9-bis(2-dimethylaminoethyl)fluorene (22.9 g,0.08 mole) was dissolved in 300 ml tetrahydrofuran in a Paar 4511 pressure reaction apparatus. Glacial acetic acid (17.2 g,0.28 mole) and 5% rhodium on alumina catalyst (10 g) were added and the mixture was stirred under 1475 psi hydrogen and heated to 85°, when the pressure was 1650 psi. After 20 hr the pressure had dropped to 1600 psi. The apparatus was heated to 108° for 20 hr (1650 psi) and then to 120° for 24 hr (1700 psi). The apparatus was cooled to room temperature. The pressure at this time was 1190 psi.

The reaction mixture was filtered and the solids were washed with 100 ml tetrahydrofuran. Fresh catalyst (3 g) was added and the mixture was stirred under 1500 psi hydrogen. The apparatus was heated to 68° for 22 hr (1675 psi), to 105° for 24 hr (1625 psi), and to 110° for 24 hr (1535 psi). After cooling to room temperature the pressure was 1125 psi. A slow leak in the apparatus was repaired and the reaction mixture was stirred under 1500 psi hydrogen and heated to 85° for 44 hr, at which time the pressure was 1800 psi. The apparatus was then cooled to room temperature (the pressure at room temperature could not be determined since the sealing cones on the stirrer shaft of the apparatus disintegrated during the cooling interval).

The reaction mixture was filtered, the solid was washed with ether, and the solvents were removed from the combined filtrates on a rotary

evaporator. The residual light brown oil was suspended in 500 ml water and the suspension was made acid with dilute HCl (1:1). The acidic phase was extracted with 3x150 ml ether which was discarded. The acidic solution was made basic with NaOH (30%) and was extracted with 3x150 ml ether which was then dried over $MgSO_4$. The ether solution was filtered and the solvent was removed on a rotary evaporator to give a nearly colourless oil. The residue was distilled at reduced pressure to give the two starting materials (recovery of 9,9-bis(2-dimethylaminoethyl)fluorene 14 g, 60%) and a slightly yellow oil, $b_{0.05}$ 99°. This was determined to be 9-(2-dimethylaminoethyl)-1,2,3,4,4a,9a-hexahydrofluorene 60. Yield 15 g (51%).

ir (liquid film); 3065,3040,3020,2920,2850,2810,2760,1460,1445,1385, 1270,1180,1160,1140,1105,1045,1030,855,750 cm^{-1}

nmr (CCl_4); δ 7.04(s,4); 3.02(e,3); 2.38(e,2); 2.20(s,6); 1.42(e,10)

Several other compounds were detected by vapour phase chromatography as impurities in the distillate fractions. These were not identified or isolated.

A portion of the partially reduced compound was dissolved in ether, the solution was dried over $MgSO_4$ and filtered, and the salt was precipitated by addition of HCl gas. Recrystallization from ethanol (100%)/anhydrous ether gave colourless crystals, M.P. 190.2-191.5°.

ir (KBr); 2920,2860,2670,1475,1460,1450,970,770,750 cm^{-1}

Analysis for $C_{17}H_{26}ClN$ M.W. 279.86

calculated	C,72.96;	H,9.37;	Cl,12.67;	N,5.01
found	C,73.33;	H,9.07;	Cl,12.51;	N,5.16

10. N-(2-dimethylaminoethyl)diphenylamine 62

A solution of diphenylamine (68 g,0.4 mole) in 200 ml benzene was refluxed for 24 hr under a nitrogen atmosphere with sodium amide (15.6 g,0.4 mole) in a one litre three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, drying tube, and gas inlet tube. Heating was discontinued and a solution of 2-dimethylaminoethyl bromide hydrobromide (46.5 g,0.2 mole) in 100 ml dimethylformamide was added dropwise. The reaction mixture was refluxed for 16 hr and filtered, and the solvents were removed on a rotary evaporator to give a dark brown solid.

The residue was suspended in 750 ml water and the strongly basic suspension was extracted with 3x250 ml ether. The ether solution was extracted with 3x250 ml HCl (5%), giving a large amount of precipitate in the aqueous phase. This material was filtered off and washed with ether. The ether solutions were combined and dried over $MgSO_4$. The ether solution was filtered and solvent was removed on a rotary evaporator to give a brown solid. The residue was purified by sublimation at reduced pressure to give 50 g (73.5% recovery) diphenylamine.

The acidic solution and the precipitate were combined and stirred magnetically while the suspension was made basic with NaOH (30%). The basic suspension was then extracted with 3x250 ml ether which was dried over $MgSO_4$. The ether solution was filtered and solvent was removed on a rotary evaporator to give a dark brown oil. The residue was distilled at reduced pressure to give a slightly yellow oil, $b_{0.8}$ 127-130°. The distillate progressively darkened through reddish-brown to nearly black on exposure to light in a desiccator. Yield 10.5 g (22% based on 2-dimethylaminoethyl bromide hydrobromide).

ir (liquid film); 2985,2960,2870,2835,2780,1590,1500,1465,1370,1255, 1185,1160,1050,760,705 cm^{-1}

nmr (CCl_4); δ 7.00(m,10); 3.77(t,2,J = 7.5Hz); 2.46(t,2,J = 7.5Hz); 2.18(s,6)

There was no change after addition of D_2O.

A portion of the distillate was dissolved in ether, the solution was dried over $MgSO_4$ and filtered, and the salt was precipitated by addition of HCl gas and recrystallized from ethanol (100%)/anhydrous ether to give colourless crystals, M.P. 255.0-256.5°.

ir (KBr); 2960,2680,2660,2595,2525,2490,1590,1500,1370,1270,1250,755, 710 cm^{-1}

Analysis for $C_{16}H_{21}ClN_2$ M.W. 276.81

calculated	C,69.42;	H,7.65;	Cl,12.81;	N,10.12
found	C,69.21;	H,7.45;	Cl,12.89;	N,10.07

11. N-(2-dimethylaminoethyl)dicyclohexylamine 63

A. without using a base (attempted)

A suspension of 2-dimethylaminoethyl chloride (5 g,0.047 mole) in 50 ml anhydrous benzene was added dropwise to a solution of dicyclohexylamine (17.0 g,0.095 mole) in 50 ml anhydrous benzene in a 150 ml four-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. The reaction mixture was refluxed for 68 hr during which a large amount of white solid precipitated. The suspension was cooled and filtered and the solvent was removed by distillation. During the distillation more white solid precipitated. When all the solvent had been removed, the condenser was inverted and the residue was refluxed for 16 hours. The reaction mixture was filtered and

the filtrate was distilled at reduced pressure to give dicyclohexylamine.

The white solid was found to be dicyclohexylamine hydrochloride.

B. using potassium metal as the base (attempted)

Freshly cleaned potassium (4.0 g,0.1 g atom), cut into small pieces, was suspended in 50 ml purified dioxane under a nitrogen atmosphere in a 150 ml four-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, drying tube, and gas inlet tube. A solution of dicyclohexylamine (18.1 g,0.1 mole) in 50 ml purified dioxane was added and the reaction mixture was refluxed for 20 hr. After this time, a large amount of potassium was still present. An additional 18 g dicyclohexylamine was added and the mixture was refluxed for 72 hr, after which only a small amount of potassium had reacted. The reaction mixture was cooled to room temperature, excess potassium was destroyed by the cautious addition of ethanol (100%), and dicyclohexylamine was recovered (85%) by distillation at reduced pressure.

C. α-chloro-N,N-dicyclohexylacetamide

A solution of chloroacetyl chloride (11.3 g,0.1 mole) in 75 ml anhydrous benzene was heated to a slow reflux in a 250 ml three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. Heat was removed and a solution of dicyclohexylamine (36.2 g,0.2 mole) in 75 ml anhydrous benzene was added at a rate sufficient to maintain refluxing. The reaction mixture was stirred, without heating, for one hr more and filtered.

The solid material was dissolved in 1600 ml water, to which 5 ml HCl (5%) had been added. The solution was extracted with 3x100 ml ether which was dried over $MgSO_4$.

The benzene solution (filtrate) was extracted with 200 ml HCl (5%), giving an interfacial emulsion. The emulsion was drained off with the aqueous phase and the benzene was extracted with 2x200 ml HCl (5%) which was added to the emulsion phase. The benzene solution was dried over $MgSO_4$.

The combined emulsion and acidic solution was diluted to 1600 ml with water and was extracted with 2x200 ml ether which was then dried over $MgSO_4$.

The three organic solutions were filtered and the solvents were removed on a rotary evaporator to give grey green solids, all containing halogen. The combined residues were recrystallized from ethanol to give colourless crystals, M.P. 112.6-113.2°.

Yield 19.3 g (75%).

ir (KBr); 2940,2865,1635,1475,1460,1450,1380,1325,1140,715 cm^{-1}

nmr (CCl_4); δ 3.90(s,2); 1.66(e,22)

Analysis for $C_{14}H_{24}ClNO$ M.W. 275.81

calculated	C,65.22;	H,9.38;	Cl,13.75;	N,5.43;	O,6.21
found	C,65.46;	H,9.51;	Cl,13.65;	N,5.29	

D. α-Dimethylamino-N,N-dicyclohexylacetamide

Dimethylamine (33 ml,0.5 mole) was collected in a graduated cylinder in an acetone/dry ice bath and was dissolved in 50 ml anhydrous benzene. This solution was added dropwise to a solution of α-chloro-N,N-dicyclohexylacetamide (16.7 g,0.065 mole) in 150 ml anhydrous

benzene in a 250 ml three-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. The reaction mixture was stirred at room temperature for 68 hr, refluxed for five hr, cooled to room temperature and filtered. The solid was washed with benzene and the solvent was removed from the combined filtrates on a rotary evaporator to give a yellow oil which crystallized on standing. The residue was purified by sublimation at reduced pressure to give colourless crystals, M.P. 58.5-59.5°. Yield 16.6 g (96%).

ir (KBr); 2925,2860,2820,2765,1630,1450,1440,1315,1130,1005,860 cm^{-1}

nmr (CCl_4); δ 2.88(s,2); 2.60(e,2); 2.19(s,6); 1.5(e,20)

Analysis for $C_{16}H_{30}N_2O$ M.W. 266.43

calculated	C,72.13;	H,11.35;	N,10.51;	O,6.01
found	C,72.48;	H,10.83;	N,10.62	

E. (2-Dimethylaminoethyl)dicyclohexylamine

A suspension of lithium aluminum hydride (1.5 g,0.04 mole) in 50 ml anhydrous ether was heated to reflux in a 150 ml four-neck flask fitted with a mechanical stirrer, condenser, dropping funnel, and drying tube. Heat was removed and a solution of α-dimethylamino-N,N-dicyclohexylacetamide (13.3 g,0.05 mole) in 60 ml anhydrous ether was added at a rate sufficient to maintain refluxing. The reaction mixture was refluxed for 19 hr after addition was completed, cooled to room temperature, and excess hydride was destroyed by the addition of 4.25 ml H_2SO_4 in 25 ml water. The solution was decanted and the sludge was basified with NaOH (5%). The resultant gel was filtered in four portions and the solid was magnetically stirred with 300 ml ether for 15 minutes. The ether was decanted and the combined ether solutions were

washed with 2x150 ml water and then extracted with 3x100 ml HCl (3%).

The acidic solution was made basic with NaOH (30%) and was extracted with 3x150 ml ether. The ether solution was dried over $MgSO_4$ and filtered, and the solvent was removed on a rotary evaporator to give a colourless oil. The residue was distilled at reduced pressure to give a colourless oil, $b_{0.1}$ 94-98°. Yield 10.7 g (84%).

ir (liquid film); 2920,2840,2805,2760,1445,1370,1330,1265,1120,1070, 1045,1030,895,855 cm^{-1}

The nmr spectrum was poorly resolved with all the absorption signals overlapping in the region 1.0-2.7.

A portion of the distillate was dissolved in ether, the solution was dried over $MgSO_4$ and filtered, and the salt was precipitated by the addition of HCl gas. Recrystallization from ethanol (100%)/ anhydrous ether gave colourless crystals, M.P. 216.8-217.5°.

ir (KBr); 2940,2865,2560,2450,1480,1450,1025,980 cm^{-1}

Analysis for $C_{16}H_{34}Cl_2N_2$ M.W. 325.38

calculated	C,59.06;	H,10.53;	Cl,21.80;	N,8.61
found	C,59.06;	H,10.33;	Cl,21.99;	N,8.55

MISCELLANEOUS REACTIONS

1. 2-Dimethylaminoethyl chloride

A 250 ml three-neck flask was fitted with a mechanical stirrer, Claisen still head, and a condenser set for distillation into an ice cooled, tared receiver. 2-Dimethylaminoethyl chloride hydrochloride (48.0 g,0.33 mole) was placed in the reaction flask and flake sodium hydroxide (27.0 g,0.66 mole) was added. The pressure in the system was reduced to about 35 mm Hg and the stirrer shaft was slowly rotated by hand. When the distillation was proceeding smoothly, the stirrer motor was started. When most of the solids had reacted, the flask was heated on a steam bath until no more distillate was collected. Yield 31.2 g (88%) of colourless, mobile liquid.

The product was generally used immediately after distillation, but could be stored in a freezer for periods up to four weeks.

2. 2-Dimethylaminoethyl bromide hydrobromide (81)

Hydrobromic acid (48%) (700 ml) was cooled in ice to between 0° and 5° in a two litre three-neck flask fitted with a mechanical stirrer, dropping funnel, and a Vigreaux still head connected to a condenser set for distillation. 2-Dimethylaminoethanol (146.0 g,1.64 mole) was then added dropwise, maintaining the temperature at less than 10°. The reaction mixture was then heated and 185 ml of distillate was collected. Heating was then decreased so that the mixture refluxed slowly in the Vigreaux column for one hr. A further 70 ml of distillate was then collected and the reaction was again refluxed for one hr. This procedure was repeated, collecting fractions of 85,30,10, and 5 ml between

successive reflux periods. Each fraction includes the amount of distillate collected during the preceding reflux period as well as the volume collected during actual distillation. The reaction mixture was then refluxed for four hr. The reaction may be interrupted at any stage up to this point.

Finally 230 ml of distillate was collected. The total volume of distillate should be 615-630 ml. However, if a faint brown or violet colour appears in the reaction flask, or if white fumes are given off, the distillation should be stopped immediately.

The hot contents of the reaction flask were poured into a one litre erlenmeyer flask and allowed to cool to about 80°. During this interval, the reaction flask was rinsed with 250 ml acetone which was then slowly added to the semi-solid mass in the erlenmeyer. Acetone was then added to a total volume of about 400 ml acetone. The contents of the erlenmeyer were then well mixed and cooled in a refrigerator. The crystals were filtered off, washed with acetone until colourless, and air dried to remove acetone. The crystals were stored in a desiccator. A further crop of crystals was collected by evaporating the acetone solution to about 100 ml and seeding the solution.

The crystals were recrystallized from ethyl alcohol/ethyl acetate (5:8) to give colourless crystals, M.P. 187-188.5°. Yield 288.6 g (75.5%) (lit (81) 187-188°, 87.5%).

ir (KBr); 3030,2950,2905,2680,2570,2495,1475,1325,1250,1020,965 cm^{-1}

3. Purification of dioxane (82)

Dioxane (2300 ml) was refluxed with 32 ml concentrated HCl and 230 ml water in a three litre boiling flask for eight hr while a slow

stream of nitrogen was bubbled through the reaction. The solution was cooled to room temperature and KOH pellets were added until some remained undissolved. The aqueous layer was separated and the organic phase was dried over fresh KOH pellets (200 g) for 12 hours.

The reaction mixture was then refluxed with sodium metal (30 g) for ten hours and then distilled from the sodium to give a colourless liquid, b_{760} 101° (lit (82) 101.5°).

PHARMACOLOGICAL TESTING

PART ONE

DERIVATIVES AND ANALOGUES OF 14-AZADISPIRO[5.1.5.2]PENTADECAN-15-ONE

The synthesis of the azadispiro compounds was completed fairly early in this work and these compounds were subjected to preliminary tests for pharmacological activity. The results did not indicate that further investigation was warranted.

As mentioned in a previous section 7-dimethylaminoacetamido-14-azadispiro[5.1.5.2]pentadecane 25 and 8-dimethylaminoacetamido-16-azadispiro[6.1.6.2]heptadecane 30 were incorrectly identified as the isomeric products 7-(2-dimethylaminoethylamino)-14-azadispiro[5.1.5.2]-pentadecan-15-one 42 and 8-(2-dimethylaminoethylamino)-16-azadispiro-[6.1.6.2]heptadecan-17-one 43, respectively. This error was not realized at the time and the tests for pharmacological activity were carried out as if the latter compounds had been obtained.

A. Local Anesthetic Activity

The method used was essentially that of Bulbring and Wajda (83). Fully grown guinea pigs of either sex were used. The day previous to the experiment the hair on the animals' backs was clipped as short as possible using electric clippers. This resulted in a degree of inflammation which subsided overnight.

The compounds tested for local anesthetic activity were 7-dimethylaminoacetamido-14-azadispiro[5.1.5.2]pentadecan-15-one 23 and 8-dimethylaminoacetamido-16-azadispiro[6.1.6.2]heptadecan-17-one 28, as the hydrochloride salts. The compounds were dissolved in 0.9% saline solution

at concentrations of 0.25, 0.5, 1.0, and 2.0%. Solutions of 0.5 and 1.0% pontocaine hydrochloride (tetracaine hydrochloride U.S.P., Winthrop) were also prepared. A constant volume of 0.25 ml of saline or of test solution was injected intracutaneously into the back of a guinea pig, resulting in the formation of a wheal approximately eight mm in diameter. This was delineated with ink and a stimulus was applied every five minutes for 30 minutes, and thereafter at 30 minute intervals for one hour. Stimulus was applied with a bipolar electrode attached to a C.H.Stoelting electronic stimulator delivering 10 millisecond pulses of 58 volts at 60 pulses per second. The maximum stimulus time was three seconds.

After five minutes, there was no difference between the response to stimulation of normal tissue and stimulation of a control wheal induced by an injection of saline. There was no decrease in response to stimulation of any of the wheals induced by injection of the test compounds. There was no response to stimulation for one hour after 1.0% tetracaine and for 30 minutes after 0.5% tetracaine.

B. Antihistaminic Activity (84)

Compounds tested for antihistaminic activity were 7-dimethylaminoacetamido-14-azadispiro[5.1.5.2]pentadecane 25 and 8-dimethylaminoacetamido-16-azadispiro[6.1.6.2]heptadecane 30, as the hydrochlorides. Solutions of agonist and test compounds were prepared in 0.9% saline.

A section of guinea pig ileum (described on p 163) was suspended in a 50 ml isolated organ bath containing Tyrode's solution maintained at 37°C by circulating water through the outer jacket of the organ bath with a thermostatic pump. The fluid in the organ bath was oxygenated

with 95% O_2/5% CO_2. Contractions of the intestine were amplified by means of a light isotonic lever with a frontal writing tip tracing on a smoked kymograph paper.

The lowest dose of histamine hydrochloride which elicited a maximal response was then determined. The intestinal strip was washed with Tyrode's solution and allowed to equilibrate between each dose. After the control dose of histamine had been determined and several control responses recorded, the intestinal strip was equilibrated for 15 minutes with a dose of the test compound. The control dose of histamine was then added to the bath and the response was recorded. The preparation was washed with Tyrode's solution, allowed to equilibrate, and the control dose of histamine was added to determine that the preparation was still contracting maximally. If the preparation showed signs of deterioration a fresh section of intestine was suspended in the bath and the control dose of histamine was again determined. This procedure was repeated for a number of doses of each of the two test compounds.

At a concentration of 3.25×10^{-4}M for 25·HCl, the response to histamine was reduced about 50%. At this concentration however, the response of the preparation became highly erratic. This was probably due to a non-specific toxic effect of the compound.

The maximum concentration tested for 30·HCl was 3×10^{-4}M. There was no reduction in the response to histamine at this dose level, but there was a more rapid, erratic relaxation of the tissue, compared with the relaxation after a control dose of histamine alone.

Neither of the compounds exhibited any agonistic activity.

C. Anticholinergic Activity (84)

Compounds tested for anticholinergic activity were 7-dimethylaminoacetamido-14-azadispiro[5.1.5.2]pentadecan-15-one methiodide 24 and 7-dimethylaminoacetamido-14-azadispiro[5.1.5.2]pentadecane dimethiodide 26. The heptadecane analogues 29 and 31 were synthesized for this purpose but lacked sufficient solubility.

The preparation and the procedure used were as described in the test for antihistaminic activity except that acetylcholine bromide was used as the agonist in place of histamine HCl.

The maximum concentration tested for both compounds was $2x10^{-4}$M. There was no decrease of the maximal response at this dose level. Compound 24 showed slight transient agonistic activity at high dose levels.

D. General Activity and Acute Toxicity Screen (85)

The compounds which were tested using this procedure were 7-dimethylaminoacetamido-14-azadispiro[5.1.5.2]pentadecan-15-one 23 and 7-dimethylaminoacetamido-14-azadispiro[5.1.5.2]pentadecane 25, as the hydrochlorides, and 7-dimethylaminoacetamido-14-azadispiro[5.1.5.2]-pentadecane dimethiodide 26. Solutions were prepared in 0.9% saline. A constant volume of 0.5 ml was injected intraperitoneally into a group of three adult female mice (30-40 g). Doses of test compound were increased using a 1/2 log unit scale, expressed in mgm of test compound (as the salt) per kg body weight.

Each group of animals was then housed in a round glass observation jar containing a small amount of litter. A control group of animals was injected with saline and used for comparison. The animals were

then observed for changes in a large number of physical responses which could indicate pharmacological activity of the compounds under study.

For example, central effects may be indicated by tremors, aggressive behaviour, or other signs. At the same time, purely physical responses such as defecation, urination, and changes in heart and respiratory rate may be observed.

Compounds 25 and 26 caused no observable changes in the animals at doses which did not induce convulsions leading to death. The compounds had an LD_{50} of 230 and 57 mgm/kg respectively. One animal survived convulsions induced by compound 25. The animal showed no other effects and its actions were not noticeably abnormal for a period of two weeks, after which it was destroyed. Compound 23 caused no observable changes in the animals and had an LD_{50} in excess of 500 mgm/kg.

PART TWO

DERIVATIVES AND ANALOGUES OF CARBAZOLE

A. Evaluation of drug parameters (86,87,88,90)

i. Agonists

In order to attain a response of a biological system to a drug, a reaction must take place between the drug molecule and a molecule in the biological object (the drug-receptor interaction). This interaction is usually represented as a bimolecular reaction

$$R + A \rightleftharpoons RA \qquad (1)$$

where R is the concentration of receptors in the biological object,

A is the concentration of drug in the biophase, and

RA is the concentration of the drug-receptor complex.

The dissociation constant for the reaction is K_A. The fraction of receptors occupied, RA/R, is dependent on A and on the affinity of the drug for the receptor ($1/K_A$). As a result of receptor occupation a stimulus is built up. By definition, the stimulus is directly proportional to the number of receptors occupied.

$$S_A/S_m = \alpha\, RA/R \qquad (2)$$

where the stimulus (S_A) is represented as a fraction of the maximal stimulus (S_m) which may be obtained in a particular biological object and α is the intrinsic activity of the drug, i.e. α is a measure of the ability of the drug to cause a stimulus.

The stimulus finally gives rise to an observable effect. This

may be represented by

$$E_A/E_m = f(S_A/S_m) \qquad (3)$$

where the effect of drug A (E_A) is given as a fraction of the maximal effect (E_m) which may be obtained with the particular biological object. This function may be linear, non-linear, or all-or-none. It should, however, be invariant in the particular biological object.

The affinity and intrinsic activity of an agonist may be determined from the dose-response curve. The pD_2 value, which is a measure of the affinity, has been defined as

$$pD_2 = -\log A_{50} \qquad (4)$$

where A_{50} is the molar concentration of agonist which induces 50% of the maximal response possible with that agonist, i.e. the concentration for which $E_{Am}/E_A = 2$.

The intrinsic activity is determined by comparing the maximal effect of the agonist A (E_{Am}) with the maximal effect obtainable in the same biological object using a reference compound R (E_{Rm}).

$$\alpha = E_{Am}/E_{Rm} \qquad (5)$$

It is customary to use the endogenous agonists, such as acetylcholine, histamine, and adrenaline, as the reference compounds. The choice of reference compound is of course dictated by the type of activity which is expected from the test compound.

Calculated dose-response curves for agonists with varying values of intrinsic activity and affinity are shown in Figure 7.

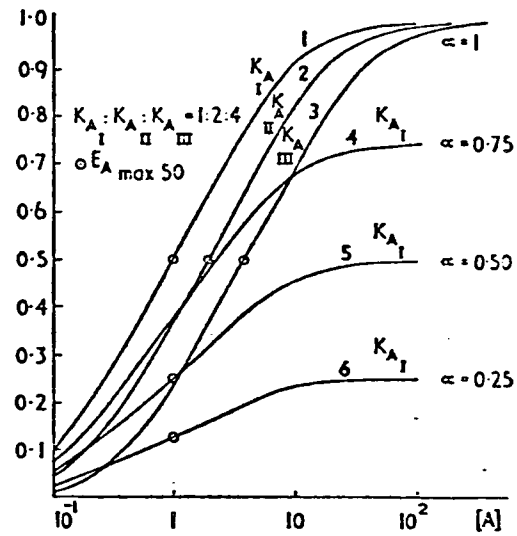


Figure 7: Dose-response curves for agonists having different affinities and intrinsic activities. Curves 1, 2, and 3 are for agonists with the same intrinsic activity (α) but differing affinities (K_A). Curves 4, 5, and 6 are for agonists with the same affinity but differing instrinsic activities. (from ref. 88, p 54)

ii. Antagonists

Antagonists are compounds which, when present in a biological preparation, decrease or abolish the effect of an agonist. Antagonists may act in a competitive or a non-competitive manner.

A competitive antagonist is a compound which, by virtue of its affinity for a receptor, interacts with the receptor and thus prevents the combination of the agonist with that receptor. The competitive antagonist has zero intrinsic activity and therefore does not itself give rise to a stimulus on interaction with the receptor.

For a pure competitive antagonist, the maximal response of the tissue to the agonist is not affected; however, higher doses of agonist are required to attain responses equivalent to those attained in the absence of the antagonist. The theoretical dose-response curves of Figure 8a show the effect of increasing concentrations of competitive antagonist.

Schild (87) has introduced a scale of drug antagonism, known as the pA scale, which he has proposed as a common method of reporting results, thus avoiding difficulties which arise in reporting results of antagonist activity in terms of another antagonist. The pA_x value is defined as the negative logarithm of the molar concentration of a competitive antagonist which will reduce the effect of a multiple dose of an agonist to the level of effect of a unit dose of the agonist in the absence of the antagonist. The values usually reported are pA_2 and pA_{10}, where the subscript refers to the multiple dose of agonist. For example, if pethidine (1.6×10^{-6}M) reduces the effect of 2 μg histamine to the level of effect of 1 μg histamine in the absence of

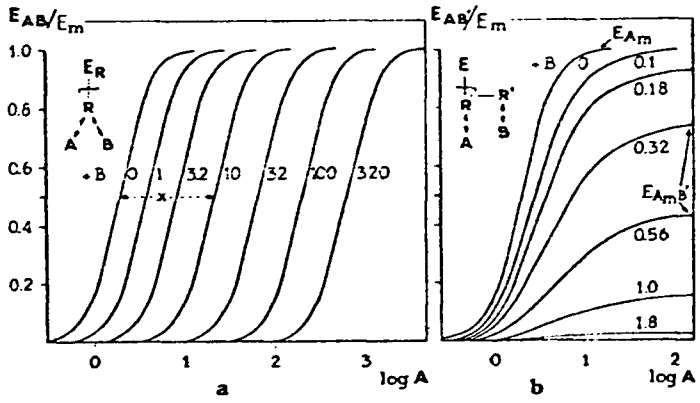


Figure 8a: Theoretical dose-response curves for an agonist (A) in the presence of constant, geometrically increasing doses of a pure competitive antagonist (B).

b: Theoretical dose-response curves for an agonist (A) in the presence of constant, but geometrically increasing doses of a pure non-competitive antagonist (B').

Abscissae are responses to A in the presence of B or B' relative to the maximal response to A alone. The ordinates are log A.

(from reference 86, p 311)

pethidine, then the pA_2 pethidine-histamine = 5.8.

The constant derived by this method is limited to the particular agonist-antagonist pair and to the biological object used. A mean of several determinations should be reported. The pA values are dependent on the length of time the antagonist is in contact with the biological object, but are independent of the method of experimentation and of the concentration of agonist and antagonist used, provided the unit dose of agonist causes a sub-maximal contraction.

The pA_2 value is equivalent to the negative logarithm of the dissociation constant for the receptor-antagonist complex (89).

Since the method used by Schild is somewhat tedious, van Rossum (86) has adapted the calculation of pA_2 values so that dose-response curves may be used.

A pure non-competitive antagonist has no affinity for the particular receptor with which the agonist interacts. Instead, the non-competitive antagonist interacts with another receptor in the biological object and in this way affects either stimulus formation or stimulus effectuation, resulting in a decrease in the effect of the agonist. An increase in the concentration of agonist cannot overcome the effect of a non-competitive antagonist, as is shown by the theoretical dose-response curves of Figure 8b.

A non-competitive antagonist has an intrinsic activity with a negative sign (since it must have some effect at its receptor to decrease the response to the agonist). A measure of the affinity (pD_2') of a non-competitive antagonist for the non-specific receptor may be calculated from the decrease in maximal height of the dose-response

curves.

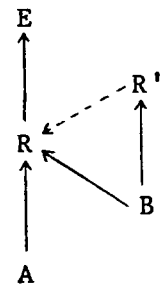
$$pD_2' = -\log B_{50} \qquad (6)$$

where B_{50} is the molar concentration of non-competitive antagonist which is required to cause a decrease of 50% in the maximal response to an agonist, i.e. the dose for which $E_m/E_{mB} = 2$.

The interactions of competitive and non-competitive antagonists with a receptor in the presence of an agonist may be represented diagrammatically as follows:

A = agonist; B = competitive antagonist; B' = non-competitive antagonist; E = effect; R = specific receptor for A; R' = non-specific receptor for B'.

In many cases, drugs are found to have multiple actions. For instance, a partial agonist ($\alpha < 1$) also exhibits competitive antagonism in the presence of a pure agonist ($\alpha = 1$). Dualism of antagonism, ie both competitive and non-competitive facets of antagonism, occurs when the antagonist has some affinity for the specific receptor of the agonist as well as affinity for some other receptor(s). This may be illustrated diagrammatically by:



In cases of multiple action, all of these drug parameters may be calculated from the dose-response curves by the application of techniques described by van Rossum (86). Where there is dualism of antagonism, the pA_2 value may only be calculated if the competitive antagonism is of greater order than the non-competitive antagonism.

B. Cumulative Dose-Response Curves (86)

Since it is very time consuming to determine a dose-response curve by measuring the effect of individual doses of a drug, a cumulative procedure was used. In this procedure, the total dose of drug in an organ bath is incremented in a stepwise fashion without washing out between doses. Dose-response curves prepared in this way are virtually identical with those prepared in the conventional manner. Table IX gives the volumes of agonist solutions which must be added to a 25 ml organ bath to give log 10 and 1/2 log 10 increments in the total concentrations.

In this work the dose response curves were always started at 10^{-8}M and successive increments were added until there was no further contraction of the intestinal strip. The time taken to attain maximal contraction in response to each increment rarely exceeded 20 seconds, and was

log 10				1/2 log 10			
to be added		total conc.	dose number	to be added		total conc.	dose number
ml	of conc.(M)	(M)		ml	of conc.(M)	(M)	
0.1	25×10^{-7}	10^{-8}	1	0.1	25×10^{-7}	10^{-8}	1
0.9	25×10^{-7}	10^{-7}	2	0.2	25×10^{-7}	3×10^{-8}	2
0.09	25×10^{-5}	10^{-6}	3	0.7	25×10^{-7}	10^{-7}	3
0.9	25×10^{-5}	10^{-5}	4	0.02	25×10^{-5}	3×10^{-7}	4
0.09	25×10^{-3}	10^{-4}	5	0.07	25×10^{-5}	10^{-6}	5
0.9	25×10^{-3}	10^{-3}	6	0.2	25×10^{-5}	3×10^{-6}	6
				0.7	25×10^{-5}	10^{-5}	7
				0.2	25×10^{-4}	3×10^{-5}	8
				0.7	25×10^{-4}	10^{-4}	9

Table IX: Sequence of doses to be added to a 25 ml organ bath for cumulative dose-response curves.

usually less than 10 seconds.

C. Experimental Procedure (84,86)

Adult guinea pigs of either sex were used. The animals were fasted for at least 12 but not more than 24 hours before use. A guinea pig was stunned by a blow to the base of the skull and exsanguinated. The abdomen was opened and the distal portion of the small intestine (ileum) was removed and placed in a beaker of cold Tyrode's solution. The upper end of the excised intestine was marked and sections 2-4 cm in length were cut from the lower portion. If necessary, the sections were allowed to relax in warm oxygenated Tyrode's solution and fecal material was gently washed out with a syringe filled with this solution. A section of the ileum was suspended in each of four 25 ml organ baths (labelled 1, 2, 3, and 4) containing Tyrode's solution and bubbled with 95% O_2/5% CO_2. The temperature was maintained at 37°C by means of a thermostatic pump circulating water through the outer jackets of the baths. Recordings were made on sooted paper on kymographs by means of a light isotonic lever fitted with a frontal writing tip.

The preparations were allowed to equilibrate for 10-20 minutes. Preparation 1 was then stimulated with a supramaximal dose of agonist and the agonist was washed out several times during a five minute interval. Preparation 2 was stimulated with the same dose of agonist five minutes after 1. This was repeated at five minute intervals for preparations 3 and 4, establishing a cycle of twenty minutes duration. The cycle was repeated at least three times while adjusting the recording system.

When the responses to the agonist were consistent within each preparation, cumulative control responses to the agonist were recorded, maintaining the 20 minute cycle. Generally three control responses for each preparation were recorded, although in a large number of cases four were recorded. If agreement between the control responses was particularly good, only two responses were recorded.

To maintain conditions as constant as possible, the preparations were not washed during the last 15 minutes of the interval between control responses.

While still maintaining the established 20 minute cycle, a dose of test compound was introduced into the muscle bath containing preparation 1 so that the preparation was in contact with the test compound for 15 minutes before the cumulative test response to the agonist was recorded. During this 15 minute interval the final control responses were recorded for preparations 2, 3, and 4, and the test compound was introduced into the muscle baths in the same manner as for 1. The cumulative responses to the agonist in the presence of test compound were then recorded. At least two of the four preparations were tested for return to control levels of response after the test response was recorded.

Using this procedure data for four cumulative dose-response curves could be collected in about 3.5-4 hr.

A fresh group of intestinal strips was used for each set of four experiments.

Solutions of agonists and antagonists were prepared in 0.9% NaCl. Histamine stock solutions (either histamine dihydrochloride, Nutritional

Biochemicals, or histamine acid phosphate, B.P., British Drug Houses) were prepared every second day and diluted to the required strength daily. Acetycholine solutions (acetylcholine bromide, Eastman) were prepared daily. Agonist solutions were stored in a refrigerator and 20 ml aliquots were withdrawn as needed. Solutions of antagonists (hydrochlorides) were prepared as needed and were kept in a refrigerator for a maximum of three days. (Two of the antagonists were not available as the hydrochlorides. These were dissolved in a minimum volume of HCl (1%) and the resultant solutions were diluted to the calculated volumes.) Standard antagonists used were diphenhydramine hydrochloride (Parke, Davis & Co.) which was tested against histamine and against acetylcholine, and atropine sulfate (B.P., British Drug Houses) which was tested against acetylcholine.

Each antagonist was tested at three doses, generally 1/2 log unit apart. Each dose was tested in four preparations, for a total of 12 determinations of activity for each compound against each of the two agonists.

Tests for antihistaminic activity were carried out using a 1/2 log 10 scale for incrementing the dose of agonist while tests for anticholinergic activity were carried out using a log 10 scale (see Table IX). Although a greater error was introduced in plotting the data for anticholinergic activity by using a log 10 scale, it was necessary to do so to prevent fading of the response at each increment of agonist.

A fairly large volume of agonist solution was added to the muscle baths during recording of the responses (Table IX). Since a high degree of error at the higher dose ranges would have been introduced by adding

this volume to 25 ml of Tyrode's solution in the muscle baths, the baths were filled to a line inscribed at the 23.5 ml level. In this way the error was distributed at the upper and lower dose limits and minimized at the more important middle dose range.

D. Manipulation of the Data

Examples are given from the data collected from tests for antihistaminic activity. Data collected from tests for anticholinergic activity were treated in the same manner.

Figure 9 is a scale drawing of the kymograph record obtained for one experiment. The height of contraction at each dose of agonist was measured and entered into a form designed for the purpose (Form 1). When the data for all experiments had been collected, a computer program was written to express the data as a percent of the maximal response. As well, the average control response and standard error for each experiment, and over the complete set of experiments was calculated at each dose of the two agonists.

The average control responses and the standard errors for the complete sets of experiments are given in Table X. From the data in Table X, the mean dose-response curve for control responses was drawn and the pD_2 value for the agonist was calculated by the method of van Rossum (86).

$$pD_2 = -\log A_{50}$$

From Figure 10, the dose of histamine which produces a 50% response is 5.5×10^{-7}, giving $pD_2 = 6.26$.

Pairs of dose response curves were then drawn, comparing the response in the absence of antagonist to that in the presence of antagonist for each experiment performed. See Figure 11. The distance from the 50% response level of the control curve to the 50% response level of the test curve was measured and entered in Form 2, which was adapted from the work of van Rossum. The percentage difference in the maximum response between the control curve and the test curve was entered in Form 3, also adapted from van Rossum.

Values for the pA_2 and pD_2' of the antagonists were then calculated, using tables of log(x-1) values given by van Rossum. Since log paper with a cycle of 51.5 mm was used, it was first necessary to express x in terms of a cycle of 30 mm (as used by van Rossum). The results are given in Table XI.

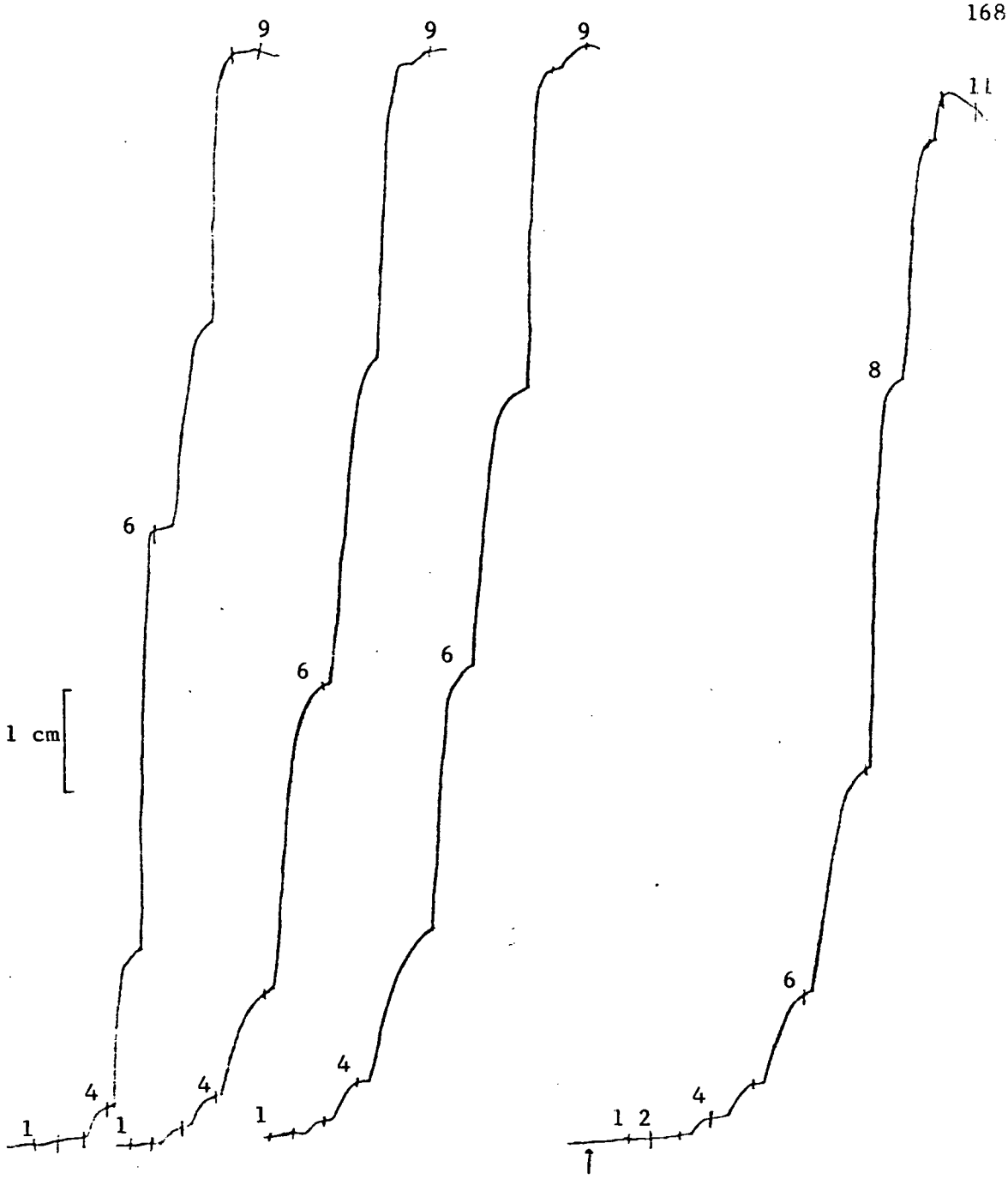


Figure 9: Responses of guinea pig ileum to cumulative doses of histamine. The numbers refer to dose of histamine, see Table IX. ↑-Addition of 3×10^{-5}M 5-(2-dimethylaminoethyl)-tetradecahydrocyclohept[b]indole hydrochloride to the muscle bath 15 minutes before the response was recorded.

Compound: 5-(2-dimethylaminoethyl)tetradecahydrocyclohept[b]indole

Dose: $3x10^{-5}M$ Agonist: Histamine Sequence: 1

Agonist Dose No.	Control Contraction (mm/%) 1	2	3	Average of control response %	SEM	Test Contraction mm	% of control (ave 107.3 mm)
1	0/0	---	---	0.0	---	---	---
2	0.5/0.5	1.5/1.4	1/0.9	0.9	0.27	0	0
3	3.5/3.2	5/4.6	4.5/4.2	4.0	0.43	1	0.9
4	19/17.6	16/14.9	20/18.8	17.1	1.15	3.5	3.3
5	61/56.5	46.5/43.3	46/43.3	47.6	4.42	12.5	11.6
6	81/75	77.5/72.1	73/68.6	71.9	1.87	36	33.5
7	107.5/99.4	106/98.5	104/97.7	98.6	0.54	72.5	67.5
8	108/100	107.5/100	106/99.6	99.8	0.16	97	90.4
9	108/100	107.5/100	106.5/100	100	0.0	102	95
10	---	---	--	---	---	102	95

Form 1: Experimental Data

Agonist Dose	Histamine Mean control response %	Histamine SEM	Acetylcholine Mean control response %	Acetylcholine SEM
10^{-8}	1.10	0.09	0.98	0.07
$3x10^{-8}$	3.52	0.20		
10^{-7}	12.02	0.46	13.76	0.55
$3x10^{-7}$	32.82	0.76		
10^{-6}	66.76	0.77	55.04	0.97
$3x10^{-6}$	86.92	0.47		
10^{-5}	98.22	0.12	86.07	0.45
$3x10^{-5}$	99.69	0.04		
10^{-4}	100	0.0	95.44	0.20
$3x10^{-4}$				
10^{-3}			100	0.0
Total no. of observations at each dose.	495		432	

Table X: Mean values of control responses for complete sets of experiments.

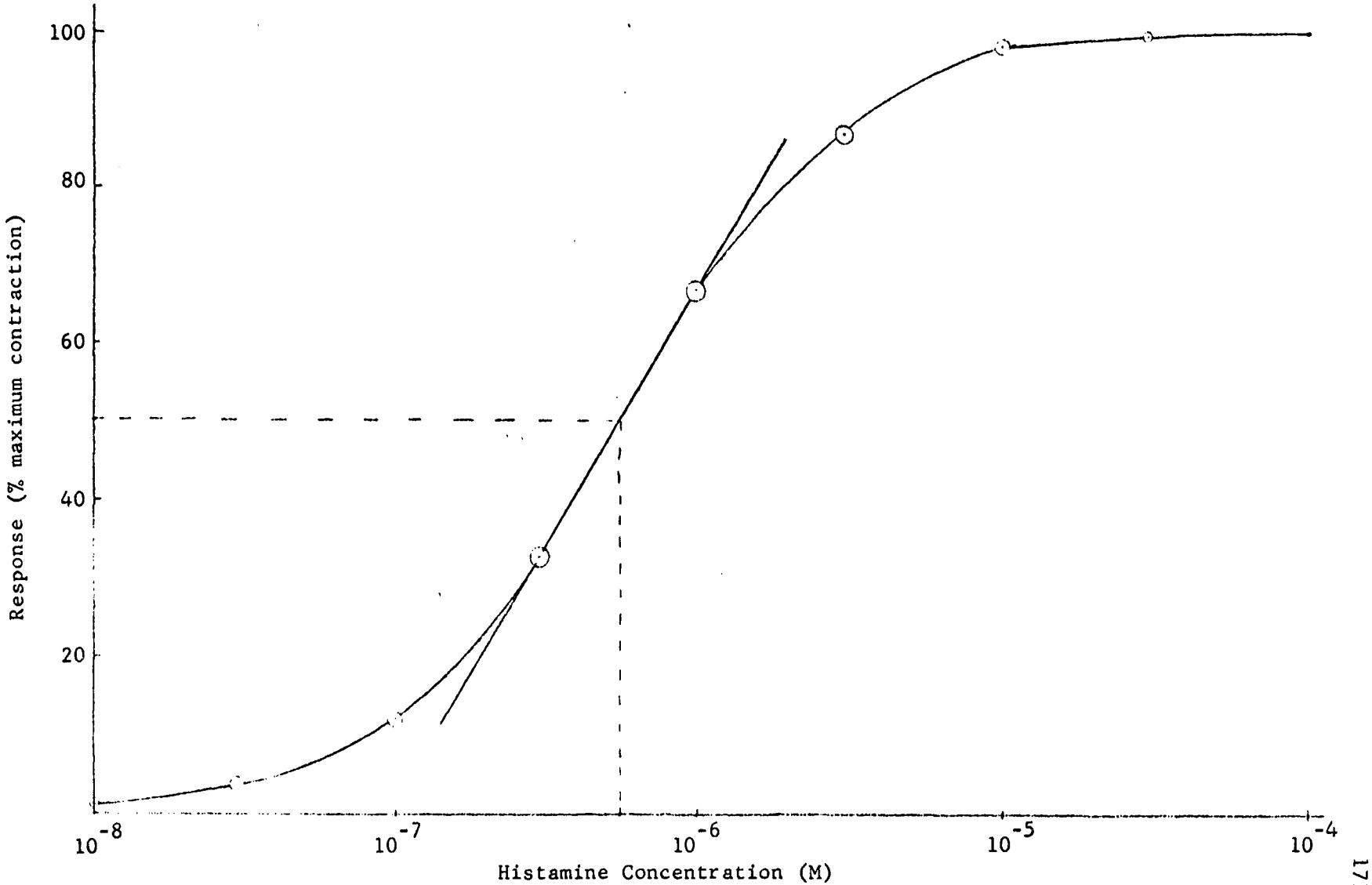


Figure 10: Average dose-response curve for the stimulation of guinea pig ileum with histamine. The size of the circle represents the standard error. Each point is the average of 495 observations.

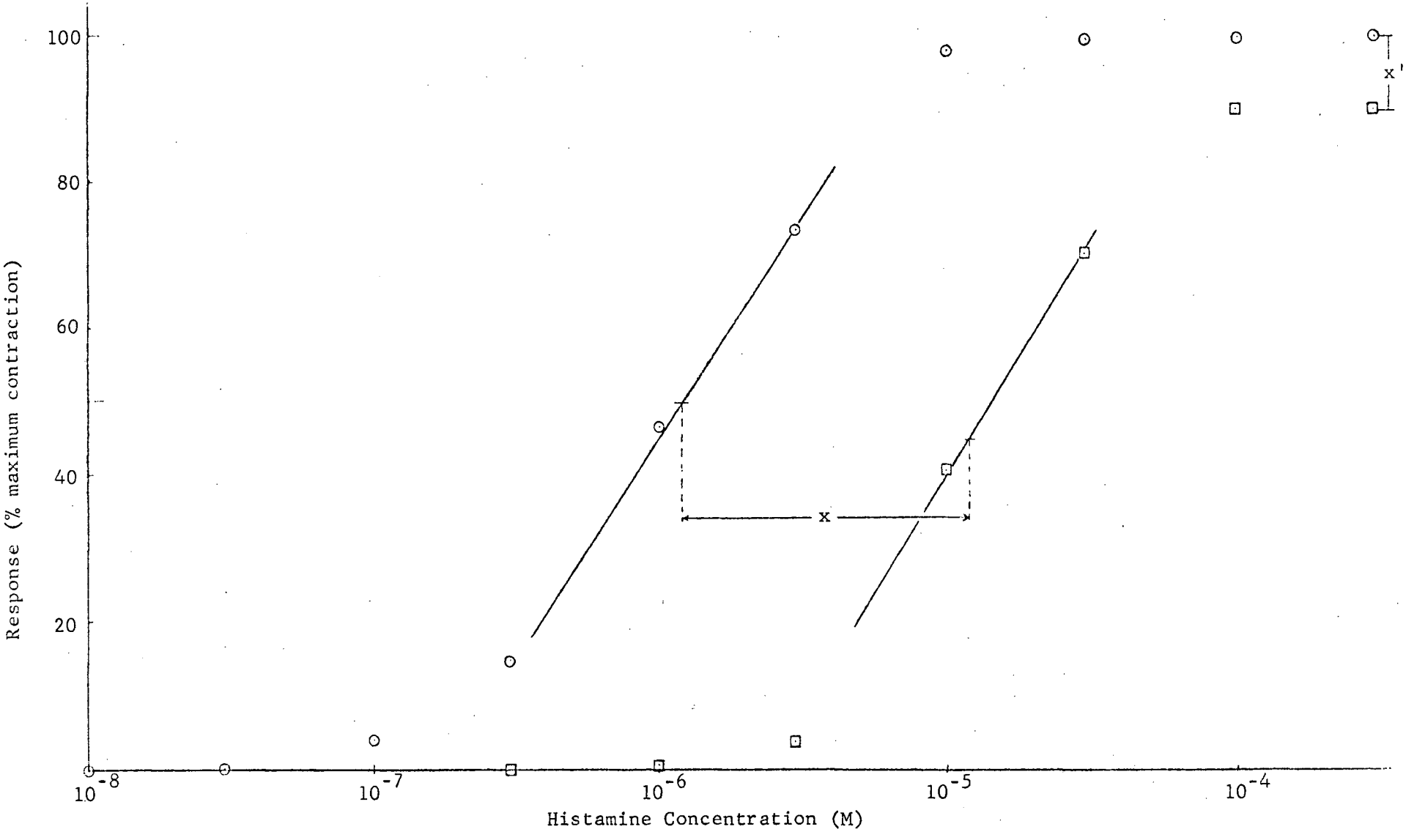


Figure 11: Dose-response curves in the presence (-o——o-) and in the absence (-o——o-) of 1-(2-dimethylaminoethyl)tetradecahydrocyclohept[b]indole (10^{-4}M), showing the measurements needed for the calculation of pA_2 and pD_2' values. Only the linear portion of the curve is shown. (x is in mm, x' is in %)

Competitive Antagonist (B):

1-(2-Dimethylaminoethyl)tetradeca-

hydrocyclohept[b]indole $pA_2 = 4.75 \pm 0.04$

Reference Agonist: Histamine $pD_2 = 6.26$

Experiment no.	E_{mB} %	antagonist concentration $x10^{-5}M$	pA_x	x mm.	log (x-1)	pA_2 relative
1_A	95	3	4.52	14.3	0.30	4.82
2_A	97	3	4.52	17.6	0.45	4.97
3_A	100	3	4.52	16.4	0.41	4.93
4_A	94	3	4.52	13.5	0.26	4.78
1_B	95	10	4.00	32.8	1.06	5.06
2_B	90	10	4.00	23.7	0.72	4.72
3_B	78	10	4.00	34.8	1.13	5.13
4_B	71	10	4.00	26.4	0.82	4.82
1_C	69	30	3.52	37.5	1.22	4.74
2_C	52	30	3.52	26.1	0.80	4.32
3_C	17	30	3.52	25.5	0.78	4.30
4_C	64	30	3.52	29.2	0.93	4.45
				Average value		4.75
				SEM		0.04

Form 2: Competitive Antagonists

Non-competitive Antagonist (B):

1-(2-Dimethylaminoethyl)tetradeca-

hydrocyclohept[b]indole $pD_2' = 3.46 \pm 0.14$

Reference agonist: Histamine $pD_2 = 6.26$

Experiment no.	E_{mB} %	Antagonist concentration $x10^{-5}M$	pD_2'	100-x' %	log (x'-1)	pD_2' relative
1_A	95	3	4.52	95	---	---
2_A	97	3	4.52	97	---	---
3_A	100	3	4.52	100	---	---
4_A	94	3	4.52	94	---	---
1_B	95	10	4.00	95	---	---
2_B	90	10	4.00	90	-0.96	3.04
3_B	78	10	4.00	78	-0.57	3.43
4_B	71	10	4.00	71	-0.39	3.61
1_C	69	30	3.52	69	-0.35	3.17
2_C	52	30	3.52	52	-0.04	3.48
3_C	17	30	3.52	17	0.69	4.21
4_C	64	30	3.52	64	-0.25	3.27
				Average value		3.46
				SEM		0.14

Form 3: Non-competitive Antagonists

Compound	pD_2	pA_2 vs Histamine	pD_2' vs Histamine	pD_2' vs ACh.
Histamine	6.26[a]			
Acetylcholine	6.08[b]			
57		7.94 (0.04)[c]	---	4.54 (0.10)
60		7.45 (0.03)	---	4.67 (0.11)
53		6.90 (0.07)	---	4.28 (0.16)
62		6.67 (0.06)	---	4.38 (0.09)
52		6.38 (0.05)	---	4.17 (0.12)
59		6.12 (0.08)	---	4.22 (0.04)
55		6.12 (0.07)	4.29 (0.17)	3.92 (0.06)
58		4.75 (0.04)	3.46 (0.14)	3.24 (0.10)
63		4.54 (0.10)	3.88 (0.12)	3.91 (0.11)
61		4.42 (0.04)	3.67 (0.16)	3.42 (0.07)
56		4.38 (0.07)	2.85 (0.16)	3.11 (0.07)
54		4.29 (0.13)	3.56 (0.26)	3.28 (0.06)
Diphenhydramine		7.75 (0.09)[d]	---	6.57 (0.14)[e,f]
Atropine		---	---	9.55 (0.05)[e,g]

Table XI: Drug parameters calculated from the experimental data.

(footnotes on page following)

Footnotes, Table XI.

a average of 495 control expts; literature value 6.6 (86)

b average of 432 control expts; literature value 7.0 (90)

The deviation from the literature value is probably due to the method of experimentation.

No cumulative dose-response curves have previously been reported for this agonist.

c numbers in parentheses are ± S.E.M.

d literature value 7.7 (86), 8.0 (87)

e pA_2 value vs acetylcholine

f literature value 6.6 (87)

g literature value 8.9 (rat intestine) (86)

8.8 (guinea pig intestine) (87)

DISCUSSION OF THE RESULTS

Biological manifestations, no matter how complex, are the result of chemical reactions and should therefore be subject to the laws of chemistry. The extreme complexity of even a simple biological event is the reason for only being able to describe chemical-biological relationships in almost all cases (91).

This basic premise underlies the concept of the drug-receptor interaction, although it is recognized that a reaction involving covalent bond formation does not necessarily take place (and in fact rarely occurs) between the drug and the receptor site. Although no direct evidence of the receptors is available, they are generally assumed to be distinct areas of biopolymers (eg proteins or enzymes) which may be localized at cellular or subcellular membranes (91). In many cases drugs are known to act in inhibiting enzymes and protein binding is a major consideration in the evaluation of drug bio-availability. The drug-receptor interaction has thus come to be considered as a parallel of the interaction which occurs between the active site of an enzyme and its substrate. Other factors such as decrease in activity with the introduction of bulky groups into a drug molecule and stereoselectivity where more than one isomer of a drug is available have further strengthened the analogy.

The molecular shape and charge distribution of a protein is determined by the tertiary and quaternary structure. The active site in an enzyme, and, by extension, a receptor site, consists of only a small number of amino acids which need not be adjacent in the primary structure of the protein--the folds and convolutions of the molecule bring

the necessary portions into close proximity (92,93,94a). In many cases a coenzyme or a prosthetic group is involved at the active site.

X-ray crystallography has revealed that enzymes have, on their roughly globular surfaces, pockets or clefts into which substrates may fit (94b). Extensive work on carboxypeptidase A (95) has shown that the active site consists of a pocket and a groove in the surface of the molecule into which the substrate fits. The amino acids present at this site have also been determined. During the binding of substrate to carboxypeptidase A, the phenolic -OH of a tyrosyl residue moves about 12 A nearer to the substrate. This and other conformational changes are concrete evidence to support the induced fit theory of enzyme-substrate and drug-receptor interaction. The active site of chymotrypsin has also been revealed as a hole in the surface of the globular enzyme (92). Using this, and other, data collected from X-ray crystallographic studies, several molecular mechanisms of enzyme action have been proposed (92,95).

There are certain obvious analogies between the problems of isolation of receptor material and that of isolation and identification of the components of the active sites of enzymes. However, the problem of isolation of a receptor is much more complex since the physiological activity of receptor systems, an essential criterion of their existence, is dependent on the integrity of the cellular system or, at least, of its membrane components while the activity of an enzyme preparation may be more or less readily monitored during the extraction and isolation procedures (96). Two basic techniques which have been used in attempts to isolate receptor substances are measurements of the ability of var-

ious fractions of receptor-containing tissues to reversibly bind ligands with affinities appropriate to the receptor system and the use of agents which will covalently bind to the receptor and subsequent fractionation of the tissues to obtain labelled material. Neither of these approaches has led to the isolation of a pure material which can be unambiguously stated to be the receptor, or a portion thereof, for a neurotransmitter (96) although some progress has been made in the isolation of, for example, the cholinergic (96) and estrogenic receptors (97).

The work done in this laboratory has been directed not at the isolation of an antihistaminic receptor, but rather at the elucidation of the steric and electronic characteristics of the receptor. The eventual aim of work such as this is, of course, the proposal of specific binding groups at the receptor site and, if possible, description of the molecular makeup of the receptor which would allow a better understanding of the molecular basis of drug action.

When tested for activity in several systems, the azadispiro compounds were found to have a very low order of activity. This lack of activity is probably due to the large bulk of the alicyclic rings preventing fitting of the molecule into a cleft on the receptor surface. Although a great many drugs in the pharmacological classes for which activity was tested contain bulky substituents, these are all capable of free rotation about one or several bonds thus decreasing the amount of surface area which is presented to the receptor. The azadispiro compounds are not capable of such conformational changes in the ring system and thus would not fit into a narrow cleft. It is also possible that the great degree of non-bonded interactions between the side chain

and the alicyclic rings prevented adoption of a conformation which could bind effectively to the receptor.

All of the compounds in the carbazole series exhibited non-competitive inhibition when tested for antimuscarinic activity. The fully hydrogenated compounds 54, 56, 58, and 63, as well as the disubstituted fluorene derivative 61 and the tetrahydrocyclopent[b]indole derivative 55, showed dualism of antagonism when tested for antihistaminic activity. The relative intensity of the non-competitive aspect of this inhibition caused by these six compounds parallels the relative intensity of the non-competitive inhibition of acetylcholine induced contraction by the same six compounds. This is represented diagrammatically in Figure 12. Except for 56, the lines are parallel within the limits of slope dictated by the S.E.M. This data may indicate that either a general toxic effect occurred or that the same non-competitive receptor site was involved in both inhibitions. The high doses at which these compounds were tested indicate that the former effect is more likely.

All of the compounds in the carbazole series exhibit competitive inhibitory activity against histamine. However, there is a distinct break in the series so that two groups of compounds are evident (Table XI, p 175). The first group contains all of the compounds which have an aromatic nucleus and show competitive activity of a fairly high order. The second group contains all the compounds, except 55, which show dualism of antagonism. These compounds all show a low order of competitive activity--they are 1.4-3.6 log units less active than the compounds in the first group. The competitive facet of activity of

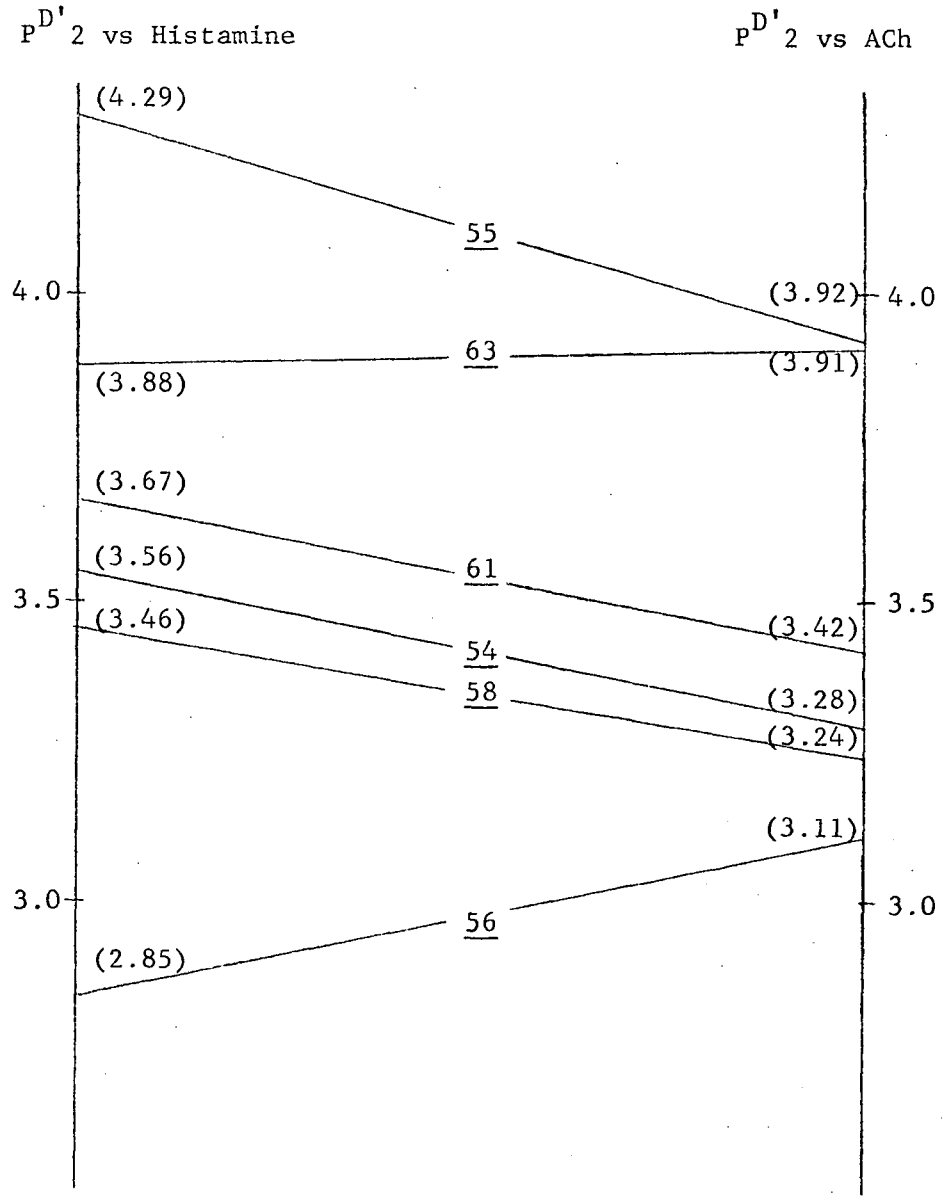


Figure 12: Comparison of the affinities of compounds in the carbazole series for non-specific receptors in the guinea pig ileum which cause inhibition of responses to histamine (H) and acetylcholine (ACh)

compounds in the second group is very likely due to the presence of the dimethylaminoethyl group which binds to the histaminic receptor. However, since no flat aromatic ring is available to reinforce the binding of 54, 56, 58, and 63, the drug-receptor complex is unstable and the compounds are readily replaced by histamine, the natural substrate. The steric effect of the second substituent in the disubstituted fluorene 61 is undoubtedly responsible for the instability of the receptor complex formed with this compound.

It would seem wise to point out at this time that although competitive inhibition presupposes binding at the same receptor site as the agonist, it is not necessary that all the groups to which the agonist binds be covered by the inhibitor. For example, if a three point binding mechanism for histamine is proposed to involve a π-complex with the imidazole electrons, an ionic bond between the protonated amino group and an anionic centre of the receptor, and a hydrogen bond involving either of the two possible sites in the imidazole ring, then binding of an inhibitor to any of the three receptor binding sites would be expected to result in competitive inhibition of the effects of histamine. It would be possible for the inhibitor to bind to any other convenient sites around the specific histamine receptor and, provided a portion of the receptor was covered, competitive activity would be observed.

The most important assumption inherent in the development of this theory is that histamine, being a natural substrate, will bind to the receptor more strongly than an inhibitor. As a result, the presence of a partially bound histamine molecule at a receptor which is partially

occupied by an inhibitor will decrease the binding energy between the inhibitor and the receptor as a consequence of strain introduced by the steric interaction. Belleau, in his macromolecular perturbation theory (98), has suggested the involvement of peripheral groups in the binding of an inhibitor to a receptor site. This interaction causes a non-specific conformational perturbation of the receptor protein, as opposed to the specific conformational perturbation induced by binding of an agonist to the receptor.

It seems likely that this occurs in the case of the fully hydrogenated derivatives prepared in this work since the puckered alicyclic ring systems would not be expected to bind to a flat aromatic region, but binding to a hydrophobic group on the periphery of the receptor may be expected to occur. Binding of the histamine molecule to the receptor would displace the amino function of the antagonist from the anionic site, weakening binding sufficiently so that ready dissociation of the antagonist-macromolecule complex would occur. It is assumed that the same anionic site is involved in binding the antagonists and histamine.

It would seem reasonable, because of the large increase in activity of those compounds containing an aromatic ring, to assume that the aromatic ring of the antagonists binds at the same site as does the imidazole ring of histamine. The assumption is made that at least two binding sites for histamine--a flat area for π-complex formation or van der Waals binding and an anionic site for Coulombic interaction--exist within a cleft in a protein. Then the relative activities of the highly active group of compounds may best be rationalized if N-(2-dimethylaminoethyl)diphenylamine 62 is considered to be the parent of the series.

All seven of these compounds are thought to interact with the same binding sites. In all cases the protonated dimethylamino group interacts with the anionic site while the aromatic ring binds at the flat area of the receptor. In 62, only one of the aromatic rings is involved in binding, the second aromatic ring being non-essential for binding to the receptor. This is in agreement with Nauta's proposal (32) and with the known activity of compounds containing only one aromatic ring, eg N-phenyl-N,N',N'-triethylethylenediamine (21). Although the second ring is non-essential it probably has a secondary role in receptor binding due to hydrophobic bond formation and may be important in determining the distribution characteristics of the compound.

Three of the compounds in this group--9-(2-dimethylaminoethyl)-carbazole 52, 4-(2-dimethylaminoethyl)-1,2,3,4-tetrahydrocyclopent[b]-indole 55, and 9-(2-dimethylaminoethyl)fluorene 59--are less active than 62. These compounds are all characterized by the presence of an essentially planar tricyclic ring system which suggests that a portion of the receptor adjacent to the flat area protrudes from the floor of the cleft and sterically interferes with the third ring in the system. A study of models shows that the dimensions of the cyclopentene ring in 55, in either a planar or puckered conformation, differ only slightly from those of the phenyl ring present in 52 and 59.

The three remaining compounds in the series--9-(2-dimethylaminoethyl)-1,2,3,4-tetrahydrocarbazole 53, 9-(2-dimethylaminoethyl)-1,2,3,4,4a,9a-hexahydrofluorene 60, and 5-(2-dimethylaminoethyl)-5,6,7,8,9,10-hexahydrocyclohept[b]indole 57--show increasing activity, in the order given, over 62. In 53 and 57 the partially saturated ring

may readily assume a boat conformation in which the ring is tipped upwards out of the plane of the aromatic ring and presumably away from the protruding portion of the receptor surface while in 60 the probable cis ring fusion more effectively removes the saturated ring from the area of steric repulsion. The assumption of a boat conformation in these molecules is easily accommodated by the induced fit theory of drug-receptor interactions.

The increased activity of these compounds over 62 may be rationalized on the basis of hydrophobic bonding to the roof of the cleft or by assuming that the second aromatic ring of 62 sterically interferes with receptor binding by interacting with the roof of the cleft. A gradation in the contribution of hydrophobic binding to total receptor binding must be invoked, in the order 57>60>53, to account for the differing activities of these compounds. The balance of the steric repulsive forces and hydrophobic binding forces will determine the activity of each compound.

The electron density about the nitrogen atom bonded to the aromatic ring system and about the corresponding carbon atom in the fluorene derivatives does not appear to be an important consideration in determining the activity of these compounds. Although no calculated values are available for the specific compounds synthesized in this work a sequence of relative electron densities may be determined by extrapolation from compounds with known values and by intuitive reasoning.

The calculated values of electron density about the nitrogen atom of pyrrole, indole, and carbazole are 1.52, 1.57, and 1.67 respectively (99). The presence of cycloaliphatic rings fused to the indole nucleus

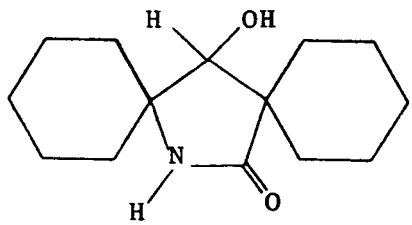
would not be expected to have a significant effect on the nitrogen electron density and the effect of the dimethylaminoethyl side chain may be neglected since it is present in all of the compounds in question (100). Consideration of the above values indicates that delocalization of the nitrogen electrons decreases as aromatic character increases over cyclopentadienyl character. Therefore, the diphenylamine nitrogen atom would be expected to have a higher electron density than those present in indole and carbazole. The fluorene derivatives would be expected to have a much lower electron density at the nine position than carbazole due to the lack of lone pair electrons. Of the two fluorene derivatives the inductive effect of two aromatic rings in 59 would be expected to decrease the electron density more than would occur in 60. The relative electron densities at the position considered would then be expected to fall in the order 62>52>53=55=57>60>59 which bears no resemblance to the activity series. Further work in this area would seem necessary in order to reconcile these results with the work of Nauta (32) on diphenhydramine derivatives.

Although the above simplistic discussion is of minimal use in understanding the molecular basis of antihistaminic activity, a much more detailed study than was undertaken in this work would be necessary to propose further details of the receptor site.

SUGGESTIONS FOR FUTURE WORK

Although it is doubtful that other derivatives of the azadispiro compounds will show appreciable activity, the 2-dimethylaminoethyl derivatives should be synthesized for the sake of completeness. The success achieved with the use of 2-dimethylaminoethyl chloride in the carbazole series suggests that this reagent would give the desired compounds when reacted with the 7- and 8-amino azadispiro compounds 4 and 5. Using this approach the appropriately substituted lactam and reduced lactam (imino) compounds should be readily available.

Since the hydroxy derivatives 45 and 76 are readily available the dimethylaminoethyl esters should be easily synthesized.



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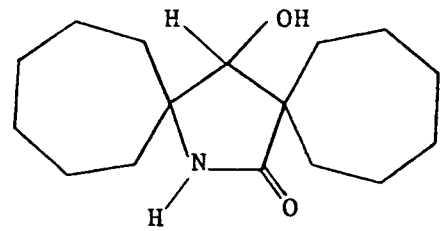


76

Ester derivatives are more commonly seen in the antimuscarinics and local anesthetics than are amides. Furthur to this, the esters of 45 and 76 would be the "reversed" esters. That is, they are esters of a complex alcohol with a simple acid rather than esters of a simple alcohol and a complex acid which are much more commonly seen in compounds having antimuscarinic and local anesthetic properties. The carboxylic acid derivatives of the azadispiro compounds should be

readily derivable from 45 and 76 by replacement of the hydroxyl group with halogen and subsequent carbonation of the Grignard reagent or lithiated compound derived therefrom.

In the carbazole series, a great many compounds come readily to mind as means of further refining the proposed receptor model. From the results obtained in this work it appears that further work in this area should include only those compounds containing at least one aromatic ring.

Those compounds which would perhaps be most interesting are the indene analogues of the cycloalk[b]indoles 53, 55, and 57 synthesized in this work. As well, the dihydrogenated derivatives of the indoles and the proposed indenes could be synthesized. Extension of the series to include 3-(2-dimethylaminoethyl)-1,2-dihydro-3H-cyclobut[b]indole, its indene analogue, and the respective dihydro derivatives would also be of interest as would the synthesis of the higher homologue 5-(2-dimethylaminoethyl)-6,7,8,9,10,11-hexahydro-5H-cyclooct[b]indole and its derivatives.

Further information on the steric nature of the receptor may be obtainable from a study of 1-(2-dimethylaminoethyl)-2,3-dialkylindoles.

The nature of the interaction with the receptor of the second aromatic ring of 62 may be deducible from a study of the activity of N-phenyl-N-cyclohexyl-N',N'-dimethylethylenediamine and derivatives containing isolated and conjugated double bonds in the cyclohexyl ring.

By far the most challenging possibility based on this work would be the separation of the R and S isomers of 60. In the activity series determined in this work, 60 was found to be less active than 57 while,

on the basis of a sterically interfering group adjacent to and in the same plane as the flat aromatic binding area of the receptor, 60 would be expected to be the most active of the compounds synthesized. Further to this, the configuration of 60 prepared in this work, and assumed to be the cis-syn isomer, should be determined, perhaps by high resolution or double resonance nmr studies. The synthesis of other isomers could also be considered.

Attempts to isolate a receptor substance are also suggested. Binding studies could then be carried out in order to determine whether ethylenediamine and ethanolamine type antihistaminics bind at the same receptor site.

BIBLIOGRAPHY

1. R.F.Doerge; Ch.23, Histamine and Antihistaminic Agents, in "Textbook of Organic Medicinal and Pharmaceutical Chemistry" 5th ed. (C.O. Wilson, O.Gisvold, and R.F.Doerge, eds) Lippincott, Philadelphia, 1966.

2. W.W.Douglas; Ch.29, Histamine and Antihistamines, in "The Pharmacological Basis of Therapeutics" 3rd ed. (L.S.Goodman and A. Gilman, eds) Macmillan, New York, 1965.

3. G.F.Q.Chan; Synthesis of Cycloalkyl Analogues of Diphenhydramine, Master's Thesis (T.H.Brown), U.B.C., 1964.

4. S.Y.S.Wang; Synthesis of Cycloalkyl Analogues of Antergan, Master's Thesis (T.H.Brown), U.B.C., 1970.

5. A.D.Blair; unpublished data, 1965-67.

6. R.A.J.Janis; Synthesis of Dispiro Compounds as Potential Medicinal Agents, Master's Thesis (T.H.Brown), U.B.C., 1969.

7. L.G.Stephanson; unpublished data, 1966.

8. K.M.Taylor and S.H.Snyder; Histamine in rat brain; Sensitive assay of endogenous levels, formation in vivo and lowering by inhibition of histidine decarboxylase, J.Pharmacol.Exptl.Ther., 179, 619 (1971).

9. G.B.West; Tissue mast cells and tissue amines, J.Pharm.Pharmacol., 11, 513 (1959).

10. B.Uvnas; Mast cells and Inflammation, p 221 in "Inflammation Biochemistry and Drug Interaction", Proceedings of an International Symposium, Como, Italy (1968) (A.Bertelli and J.C.Houck, eds) Excerpta Medica Foundation, Amsterdam, 1969.

11. KSugiyama; Calcium-dependent histamine release with degranulation from isolated rat mast cells by adenosine 5'-triphosphate. Jap. J.Pharmacol., 21, 209 (1971).

12. S.Norn; Anaphylactic histamine release and influence of antirheumatics. Acta Pharmacol. Toxicol., 30, Suppl 1, 13 (1971).

13. H.L.Johnson, M.A.Beaven, F.Erjavec, and B.B.Brodie; Selective labelling and release of non-mast cell histamine, Life Sci., 5, 115 (1966).

14. R.W.Schayer; The microcirculatory function of histamine and its role in glucocorticoid and thyroid hormone actions, p 33 in "Ovum Implantation", Proceedings of an Institute, 1967. (M.C.Schelesnyak

and G.J.Marcus, eds) Gordon and Breach, New York, 1969.

15. R.Keller; Mast cells and Inflammation, p 234 in "Inflammation Biochemistry and Drug Interaction", Proceedings of an International Symposium, Como, Italy (1968) (A.Bertelli and J.C.Houck, eds) Excerpta Medica Foundation, Amsterdam, 1969.

16. R.W.Schayer and J.A.D.Cooper; Metabolism of ^{14}C-histamine in man. J.Appl.Physiol., 9, 481 (1956).

17. R.W.Schayer; Catabolism of physiological quantities of histamine in vivo. Physiol.Rev., 39, 116 (1959).

18. L.J.Leffler, W.Lovenburg, and A.Sjoerdsma; Effects of dibutyryl-3',5'-adenosinemonophosphate, phosphodieterase inhibitors, and prostaglandin E_1 on compound 48/80-induced histamine release from rat peritoneal mast cells in vitro. Biochem.Pharmacol., 20, 2287 (1971).

19. J.H.Baxter, Z.Horakova, and M.A.Brown, Fedn.Proc., 29, 618 Abs., 2088 (1970).

20. G.Kahlson and E.Rosengren; "Biogenesis and Physiology of Histamine", Monographs of the Physiological Society, 21, 5 (1971) Edward Arnold, London.

21. D.T.Witiak; Ch.65, Antiallergenic Agents, in "Medicinal Chemistry" 3rd ed. (A.Burger, ed) Wiley Interscience, New York, 1970.

22. J.Watt; A review of the actions of antihistamines. Can.Pharmaceut. J., 102, 275 (1969).

23. H.Timmerman, R.F.Rekker, A.F.Harms, and W.Th.Nauta; The effects of alkyl substitution in drugs. XXII, Arzneim.Forsch. (Drug Res.), 20, 1258 (1970).

24a. W.Th.Nauta, R.F.Rekker, and A.F.Harms; Diarylcarbinol ethers: structure activity relationships. A physico-chemical approach. p 305 in Proceedings of the Third International Pharmacological Meeting, 1966. Vol 7, "Physico-chemical Aspects of Drug Actions" (E.J.Ariens, ed) Permagon Press, Oxford, 1968.

24b. Reference 24a and references cited therein.

25. J.M.Ritchie, P.J.Cohen, and R.D.Dripps; Ch.20, Cocaine; Procaine and Other Synthetic Local Anesthetics, in "The Pharmacological Basis of Therapeutics" 3rd ed. (L.S.Goodman and A.Gilman, eds) Macmillan, New York, 1965.

26. B.H.Takman and G.Camougis; Ch.63, Local Anesthetics, in "Medicinal Chemistry" 3rd ed. (A.Burger, ed) Wiley Interscience, New York, 1970.

27a. A.M.Shanes; Electrochemical aspects of physiological and pharmacological action in excitable cells. Pharmacol.Rev., 10, 148-149 (1958).

27b. A.M.Shanes; Drugs and nerve conduction. Ann.Rev.Pharmacol., 3, 185 (1963).

28. J.R.Innes and M.Nickerson; Ch.25, Drugs Inhibiting the Action of Acetylcholine on Structures Innervated by Postganglionic Parasympathetic Nerves, in "The Pharmacological Basis of Therapeutics" 4th ed (L.S.Goodman and A.Gilman, eds) Macmillan, New York, 1970.

29. B.V.Rama Sastry; Ch.60, Anticholinergics: Antispasmodic and Antiulcer Drugs, in "Medicinal Chemistry", 3rd ed. (A.Burger, ed) Wiley Interscience, New York, 1970.

30. A.F.Harms, W.Th.Nauta; The effects of alkyl substitution in drugs, I. Substituted dimethylaminoethyl benzhydryl ethers. J.Med.Chem., 2, 57 (1960).

31. N.S.Ham; Solution conformations of antihistamines. J.Pharm.Sci., 60, 1764 (1971).

32. R.F.Rekker, H.Timmerman, A.F.Harms, and W.Th.Nauta; The antihistaminic and anticholinergic activities of optically active diphenhydramine derivatives: The concept of complementarity. Arzneim. Forsch (Drug Res.), 21, 688 (1971).

33. D.V.Nightingale, F.B.Erickson, and P.Shackelford; The reaction of nitroparaffins and alicyclic ketones. II. J.Org.Chem., 17, 1005 (1952).

34. D.V.Nightingale, D.A.Reich, and F.B.Erickson; Reaction of nitroparaffins with alicyclic ketones. III. The solid by-product from nitromethane and cyclohexanone. J.Org.Chem., 23, 236 (1958).

35. D.V.Nightingale, S.Miki, D.N.Heintz, and D.A.Reich; Reactions of nitroparaffins with alicyclic ketones. IV. J.Org.Chem., 28, 642 (1963).

36. D.V.Nightingale and D.N.Heintz; Reactions of 14-hydroxy-14-azadispiro[5.1.5.2]pentadec-9-en-7,15-dione and related compounds. I. The 3,11-dimethyl derivative. J.Org.Chem., 31, 361 (1966).

37a. W.E. Noland and R.J.Sundberg; Structure of the 2:2 condensation product of nitromethane and cyclohexanone. J.Org.Chem., 28, 3150 (1963).

37b. W.E.Noland and R.J.Sundberg; Structure of the 2:2 condensation product of nitromethane and cyclohexanone. Tet.Lett. (1962) 295.

38. H.O.House and R.W. Magin; The structure of the solid by-product

$C_{14}H_{20}N_2O_3$obtained from nitromethane and cyclohexanone. J.Org. Chem., 28, 647 (1963).

39. A.Lambert and A.Lowe; Aliphatic nitro-compounds. XVIII. Interaction of ketones and nitroparaffins. J.Chem.Soc., 1517 (1947).

40. S.Miki; The Reaction of Nitromethane with Alicyclic Ketones, Master's Thesis (D.V.Nightingale), U.of Missouri (1961).

41. Z.Eckstein, A.Sacha, and T.Urbanski; Properties and preparation of 1-cycloheptenylnitromethane. Bull.acad.polon.sci. Classe III, 5, 213 (1957) in Chem.Abst., 51, 16318i (1957).

42. K.Nakanishi; "Infrared Absorption Spectroscopy-Practical" p 45-47 Holden-Day, San Francisco (1962).

43. J.D.Roberts and M.C.Caserio; "Basic Principles of Organic Chemistry", p 456. Benjamin, New York (1965).

44. E.M.Kosower; "An Introduction to Physical Organic Chemistry", p 49, Wiley, New York (1968).

45. H.Jager, W.Farber, and N.Poonawalla; Cyclisierungen am 2-cyclohexyliden-cyclohexanone. Arch.Pharm. 295, 205 (1962).

46. M.L.Moore; The Leuckart Reaction, Ch.7 in "Organic Reactions" 5, 301 (1949) Wiley, New York.

47. H.Adkins and H.L.Coonradt; The selective hydrogenation of derivatives of pyrrole, indole, carbazole, and acridine. J.Amer.Chem. Soc., 63, 1563 (1941).

48. W.H.Perkin, Jr. and S.G.P.Plant; 1,2,3,4,5,6,7,8-Octahydrocarbazole and its derivatives. J.Chem.Soc., 125, 1503 (1924).

49. T.Masamune, M.Ohno, and M.Koshi; Condensed polynuclear perhydro compounds containing nitrogen. II. Dodecahydrocarbazoles. Nippon Kagaku Zasshi, 77, 1017 (1956) from Chem.Abst., 53, 5233e (1959).

50. S.Siegel; Stereochemistry and the mechanism of hydrogenation of unsaturated hydrocarbons. Advances in Catalysis, 16, Academic Press, New York, p 123 (1966).

51. J.Gurney, W.H.Perkin,Jr. and S.G.P.Plant; A new stereoisomeride (trans-) of hexahydrocarbazole. J.Chem.Soc. (1927) 2676.

52. H.Booth, F.E.King, and J.Parrick; Synthetic and stereochemical investigations of reduced cyclic bases. V. The exhaustive methylation of some partially reduced cyclic bases. J.Chem.Soc. (1958) 2302.

53. H.Gault, L.Daltroff, and J.Eck-Tridon; Preparation de la cyclo-

hexylidene-cyclohexanone a partir de la cyclohexanone. Mem.Pres. Soc.Chim., 12, 952 (1945).

54. H.Jager; Cyclisierungen am 2-cyclohexyliden-cyclohexanone. Chem. Ber., 242 (1962).

55. R.R.Schumaker; Substitution of heterocyclic amines. Fr.Pat. 1, 527,778 June 7, 1968 from Chem.Abst., 71, 81175b (1969).

56. J.H.Burckhalter, V.C.Stephens, and L.A.R.Hall; Antihistaminics: The N-β-dimethylaminoethyl derivatives of carbazole and diphenylamine. J.Amer.Pharm.Ass'n.Scientific Ed., 39, 271 (1950).

57. L.Kato and B.Gozsy; Effect of phenothiazine derivatives on dextran-induced edema. J.Pharmacol., 129, 231 (1960).

58. B.Gozsy and L.Kato; Investigations into the mechanism of action of neuroleptic drugs. Arch.Int.Pharmacodyn., 128, 75 (1960).

59. B.Robinson; The Fischer indole synthesis. Chem.Rev., 63, 373 (1963).

60. B.Robinson; Recent studies on the Fischer indole synthesis. Chem. Rev., 69, 227 (1969).

61. A.I.Vogel; "A Textbook of Practical Organic Chemistry", 3rd ed. Longmans, London (1957) p 852-3.

62. C.W.Muth, D.O.Steiniger, and Z.B.Papanastassiou; Azabenzazulenes. I. 1-Azadibenz[b,f]azulene. J.Amer.Chem.Soc., 77, 1006 (1955).

63. W.H.Perkin,Jr. and S.G.P.Plant; Dihydropentindole and its derivatives, Part I. J.Chem.Soc., 123, 3242 (1923).

64. B.Witkop, J.B.Patrick, and M.Rosenblum; Ring effects in autoxidation. A new type of Camps reaction. J.Amer.Chem.Soc., 73, 2641 (1951).

65a. B.Witkop and J.B.Patrick; The course and kinetics of the acid-base catalyzed rearrangements of 11-hydroxytetrahydrocarbazolenine. J.Amer.Chem.Soc., 73, 2188 (1951).

65b. B.Witkop and J.B.Patrick; On the mechanism of oxidation. I. A new type of peroxide rearrangement catalyzed by acid. J.Amer.Chem. Soc., 73, 2196 (1951).

66. J.W.Cusic; Aromatic aminoalkyl amines. U.S.Patent 2,687,414 Aug. 24, 1954 from Chem.Abst., 50, 1092d (1956).

67. J.W.Cusic and C.A.Dornfield; Tertiary aminoalkyl tetrahydrocarbazoles. U.S.Patent 2,541,211 Feb. 13, 1951 from Chem.Abst., 45, 7152i (1951).

68. G.Carrara, V.D'Amato, and R.Pagini; Histamine antagonists. Steric analogy and biological activity XII. Farm.Sci.e Tec. (Pavia), 3, 178 (1948) from Chem.Abst., 42, 6988c (1948).

69. L.M.Rice and M.E.Freed; Method of treating depression. U.S. Patent 3,329,571 July 4, 1967 from Chem.Abst., 68, 95679u (1968).

70. R.L.Augustine; "Catalytic Hydrogenation", Marcel Dekker, Inc., New York (1965), p 39-40.

71. H.H.Fox and W.Wenner; N,N'-substituted ethylenediamine derivatives. J.Org.Chem., 16, 225 (1951).

72. G.W.H.Scherf and R.K.Brown; Potassium derivatives of fluorene as intermediates in the preparation of C9-substituted fluorenes. I. Can.J.Chem., 38, 697 (1960).

73. R.L.Kugel, W.Hodgson and H.R.Allcock; The formation of radical anions in fluorene metallation. Chem. Ind. 1649 (1950).

74. J.Eisch and W.Kaska; Role of radical-anions in organo-lithium chemistry. Chem.Ind., 470 (1961).

75. E.J.Greenhow, E.N.White, and D.McNeil; The chemistry of fluorene. I. Condensations with 9-fluorenylsodium. J.Chem.Soc., 2848 (1951).

76. R.Weissgerber; Ueber eine Kaliumverbidung des Fluorens. Chem.Ber., 34, 1659 (1901).

77. J.R.Dyer, "Applications of Absorption Spectroscopy of Organic Compounds", Prentice-Hall, Engelwood Cliffs, N.J. (1965) a) p 95 b) p 117.

78. H.C.Brown and P.Heim; Diborane as a mild reducing agent for the conversion of primary, secondary, and tertiary amides into the corresponding amines. J.Amer.Chem.Soc., 86, 3566 (1964).

79. H.Adkins; "Reactions of Hydrogen with Organic Compounds over Copper Chromium Oxide and Nickel Catalysts". U. of Wisconsin Press, Madison, Wisconsin (1937) p 11-14.

80. W.H.Lazier and H.R.Arnold; Copper chromite catalyst, p 142-5 in "Organic Syntheses", Coll. Vol. 2, (A.H.Blatt, ed.) Wiley, New York (1943).

81. F.Y.T. Leung; "Synthesis of Cycloalkyl Analogues of Antergan" Master's Thesis (T.H.Brown) U.B.C. (1964) p 55.

82. A.I.Vogel; "A Textbook of Practical Organic Chemistry", 3rd ed., Longmans, London (1957) p 177.

83. E.Bulbring and I.Wajda; Biological comparison of local anesthetics.

J.Pharmacol., 85, 78 (1945).

84. "Pharmacological Experiments on Isolated Preparations", Staff of Dept. of Pharmacology, U. of Edinburgh, Livinstone, Edinburgh (1968) Ch.1,2,4, and appendices.

85. S.Irwin; Drug Screening and evaluation of new compounds in animals, Ch.4 in "Animal and Clinical Pharmacologic Techniques in Drug Evaluation", Vol. I. (J.H.Nodine and P.E.Siegler, eds.) Year Book Medical Pub., Chicago (1964).

86. J.M.van Rossum; Cumulative dose response curves. II. Arch.Int. Pharmacodyn.Ther., 143, 299 (1963).

87. H.O.Schild; pA, A new scale for the measurement of drug antagonism. Brit.J.Pharmacol., 2, 189 (1947).

88. D.J.Triggle, "Chemical Aspects of the Autonomic Nervous System" Vol. 4 of Theoretical and Experimental Biology, (J.R.Danielli, Consulting ed.) Ch.4, The analysis of drug receptor interactions, Academic Press, London (1965).

89. Ibid, p 59.

90. J.M.van Rossum; The relation between chemical structure and biological activity. J.Pharm.Pharmacol., 15, 285 (1963).

91. A.Burger; Introductory Comments, p 1-13 in "Fundamental Concepts in Drug-Receptor Interactions", Proceedings of the 3rd Buffalo-Milan Symposium on Molecular Pharmacology. Buffalo, New York. (J.F.Danielli, J.F.Moran, and D.J.Triggle eds.) Academic Press, N.Y. (1970).

92. A.Wiseman and B.J.Gould; "Enzymes, Their Nature and Role", Ch.6, Hutchinson Educational Ltd, London (1971).

93. D.M.Locke; "Enzymes - The Agents of Life" Ch.4, Crown Publishers, New York (1969).

94a. J.Westley; "Enzymic Catalysis" Ch.5, Harper & Row, New York (1969).

94b. Ibid, p 66.

95. W.N.Lipscomb; Structure and mechanism in the enzymatic activity of carboxypeptidase A and relations to chemical sequence. Accts. Chem.Res., 3, 81 (1970).

96. D.J.Triggle; "Neurotransmitter-Receptor Interactions", Ch.6, Academic Press, New York (1971).

97. J.Gorski, G.Shyamala, and D.Toft; The search for hormone receptors: Studies on estrogen binding in the uterus. p 215 in "Fundamental

Concepts in Drug-Receptor Interactions" (see reference 91) Academic Press, N.Y. (1970).

98. B.Belleau; A molecular theory of drug action based on induced conformational perturbations of receptors. J.Med.Chem., 7, 776 (1964).

99. C.A.Coulson and A.Streitwieser,Jr.; "A Dictionary of π-Electron Calculations", W.H.Freeman, San Francisco (1965) pp 238-241;318-321;344-348.

100. Dr.L.Weiler, Dept. of Chem., U.B.C. personal communication.

101. J.W. Black, W.A.M. Duncan, C.J. Durant, C.R. Ganellin, and E.M. Parsons; Definition and Antagonism of Histamine H_2-receptors. Nature, 236, 385 (1972).

APPENDICES

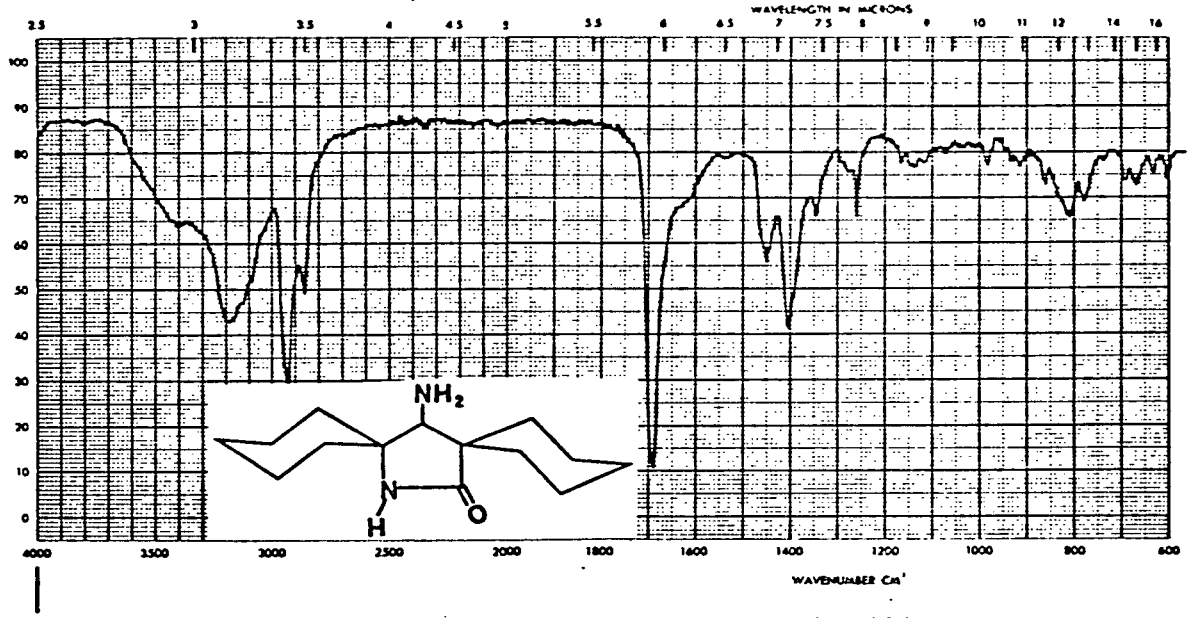
APPENDIX I

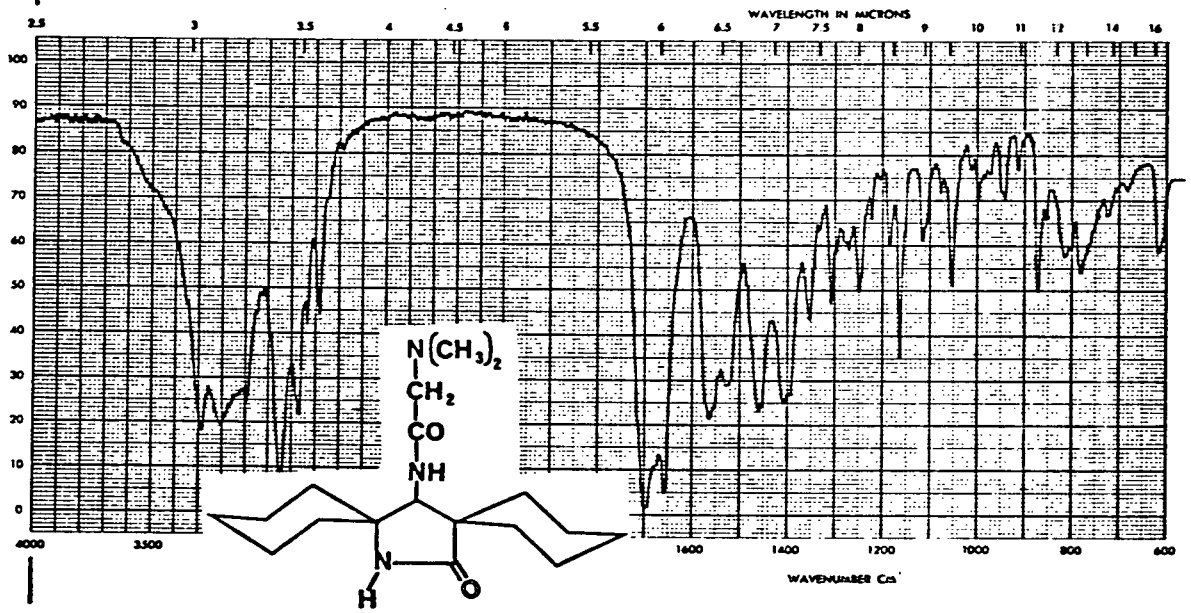
INFRARED SPECTRA

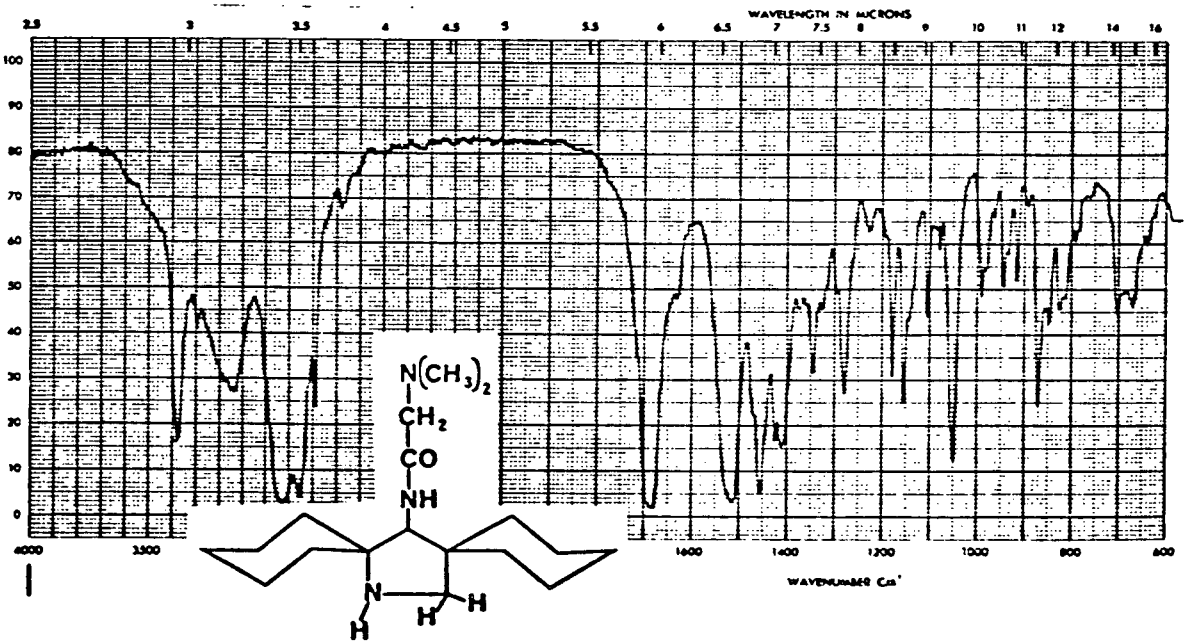
Spectrum 1 7-Amino-14-azadispiro[5.1.5.2]pentadecan-15-one (KBr disc)

Spectrum 2 7-Dimethylaminoacetamido-14-azadispiro[5.1.5.2]pentadecan-15-one (KBr disc)

Spectrum 3 7-Dimethylaminoacetamido-14-azadispiro[5.1.5.2]pentadecane (KBr disc)


WAVELENGTH IN MICRONS
WAVENUMBER Cm⁻¹
NH2
N
H
O

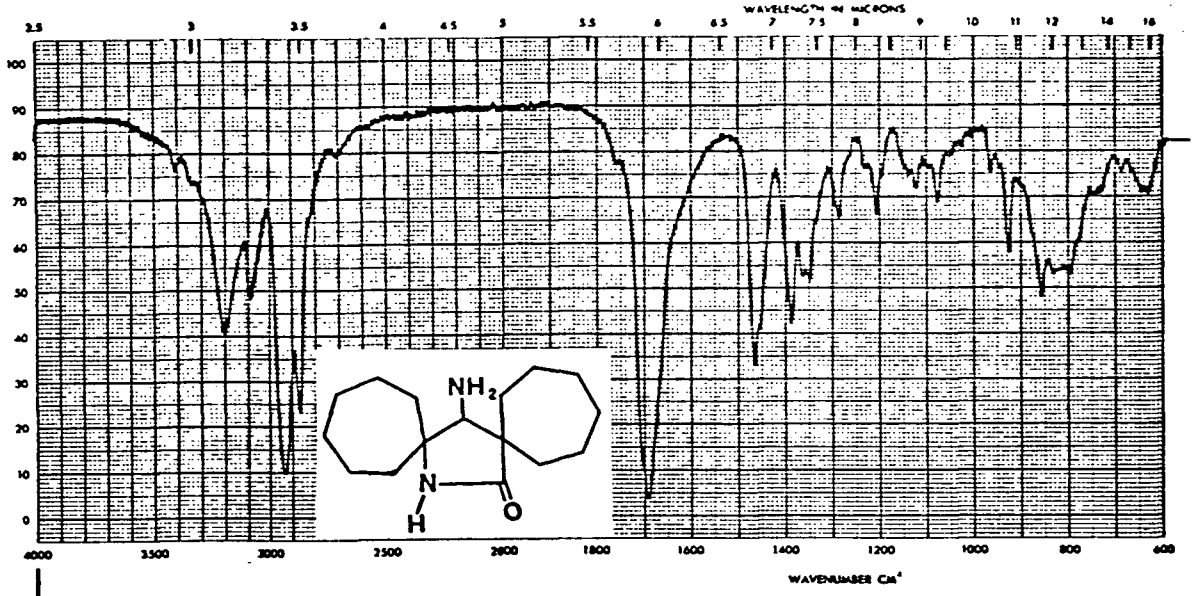

WAVELENGTH IN MICRONS
WAVENUMBER Cm⁻¹
N(CH3)2
CH2
CO
NH
N
H
O

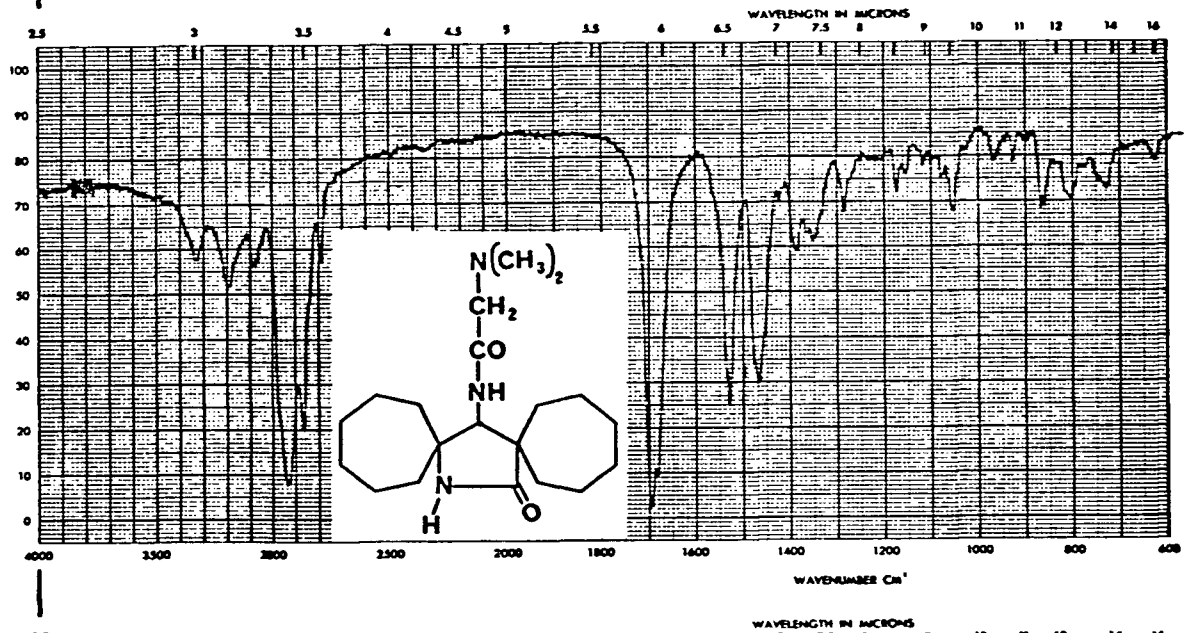

WAVELENGTH IN MICRONS
WAVENUMBER Cm⁻¹
N(CH3)2
CH2
CO
NH
N
H
H
H

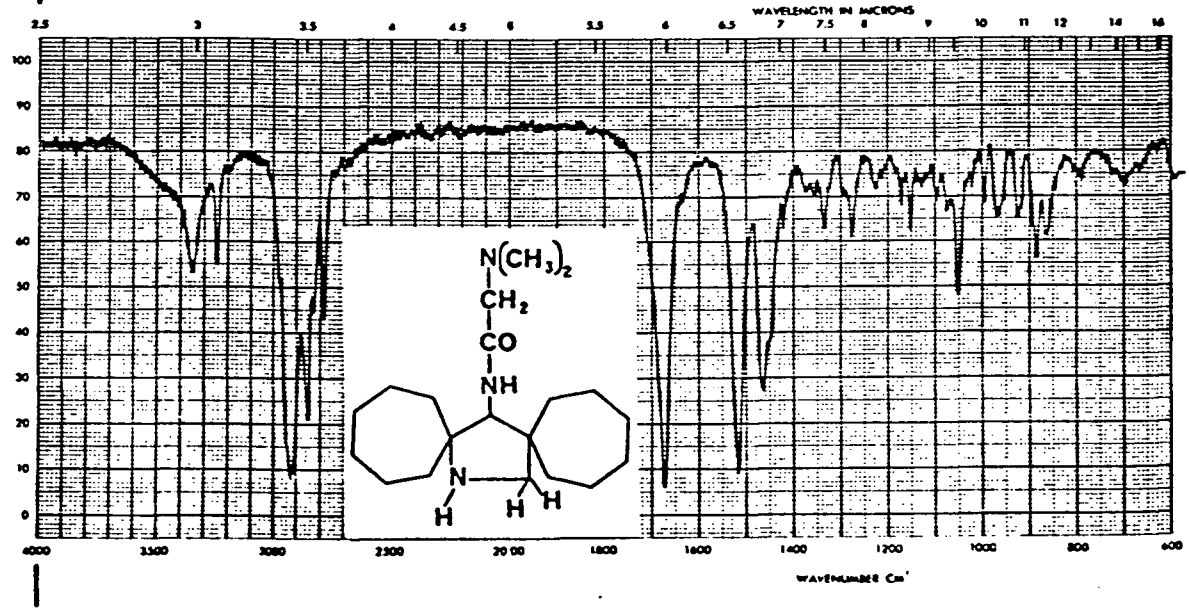
Spectrum 4 8-Amino-16-azadispiro[6.1.6.2]heptadecan-17-one (KBr disc)

Spectrum 5 8-Dimethylaminoacetamido-16-azadispiro[6.1.6.2]heptadecan-17-one (KBr disc)

Spectrum 6 8-Dimethylaminoacetamido-16-azadispiro[6.1.6.2]heptadecane (KBr disc)


WAVELENGTH IN MICRONS
WAVENUMBER Cm⁻¹
NH2
N
H
O


WAVELENGTH IN MICRONS
WAVENUMBER Cm⁻¹
N(CH3)2
CH2
CO
NH
N
H
O


WAVELENGTH IN MICRONS
WAVENUMBER Cm⁻¹
N(CH3)2
CH2
CO
NH
N
H
H
H

Spectrum 7 9-(2-Dimethylaminoethyl)carbazole

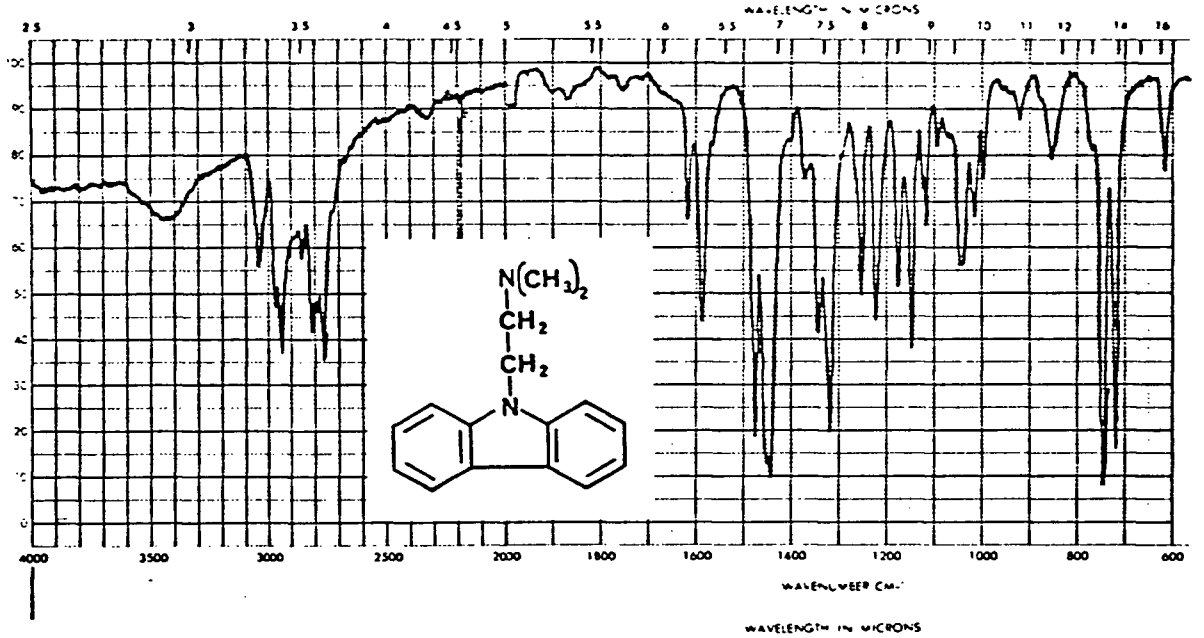
(KBr disc)

Spectrum 8 9-(2-Dimethylaminoethyl)-1,2,3,4-tetrahydrocarbazole

(liquid film)

Spectrum 9 9-(2-Dimethylaminoethyl)dodecahydrocarbazole

(liquid film)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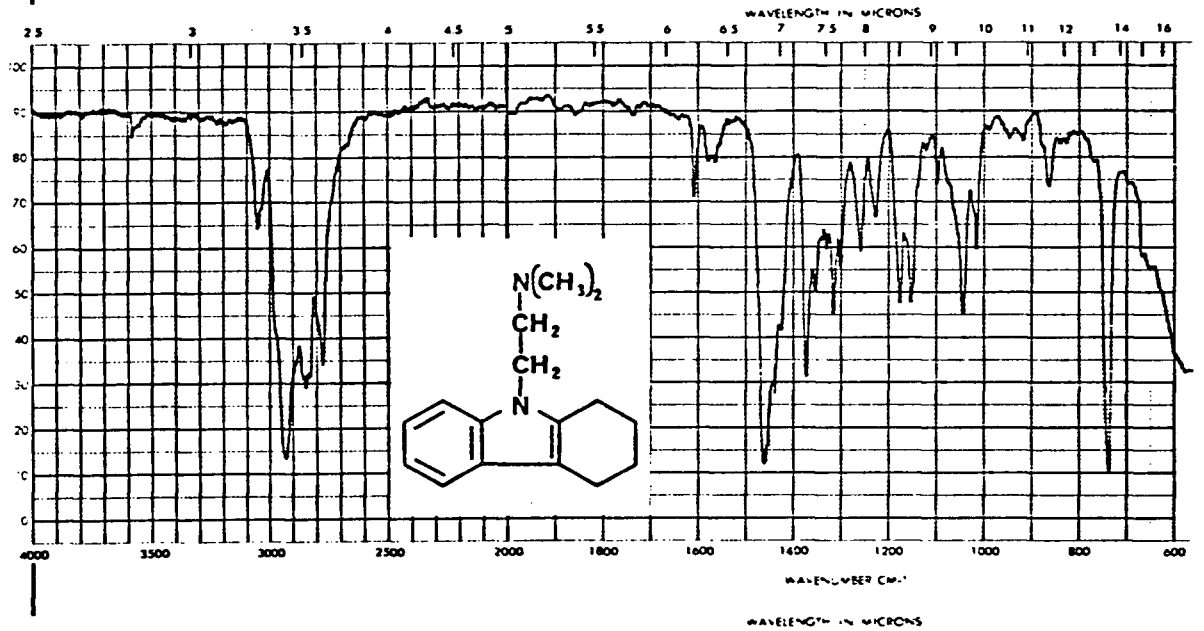
CH2
CH2
N
WAVENUMBER CM-1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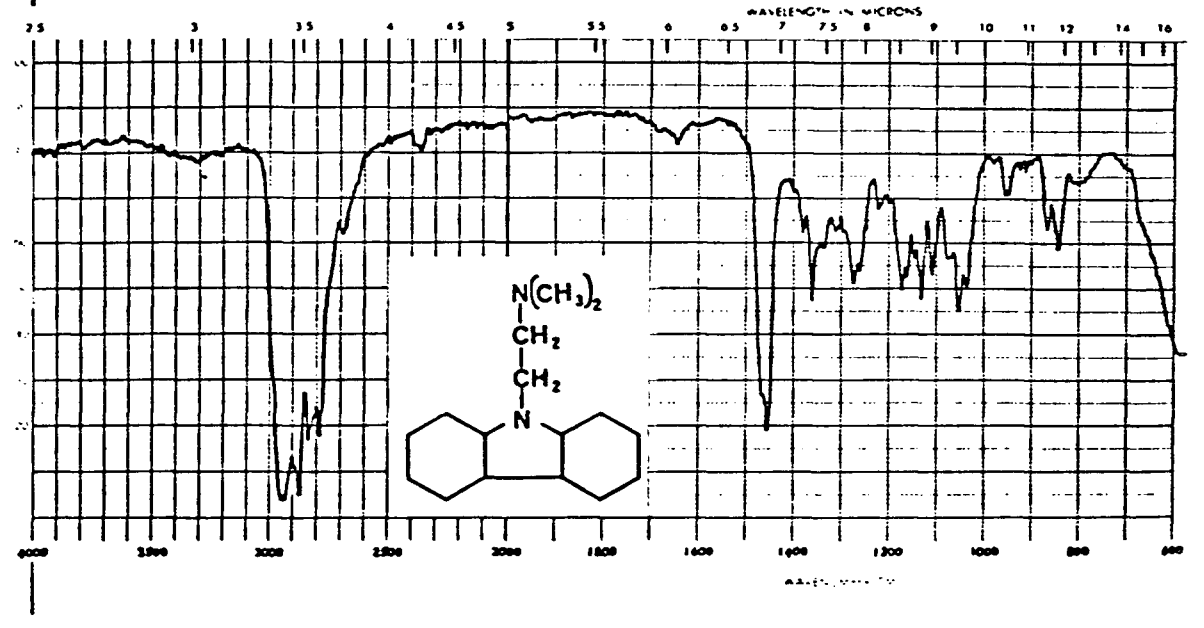
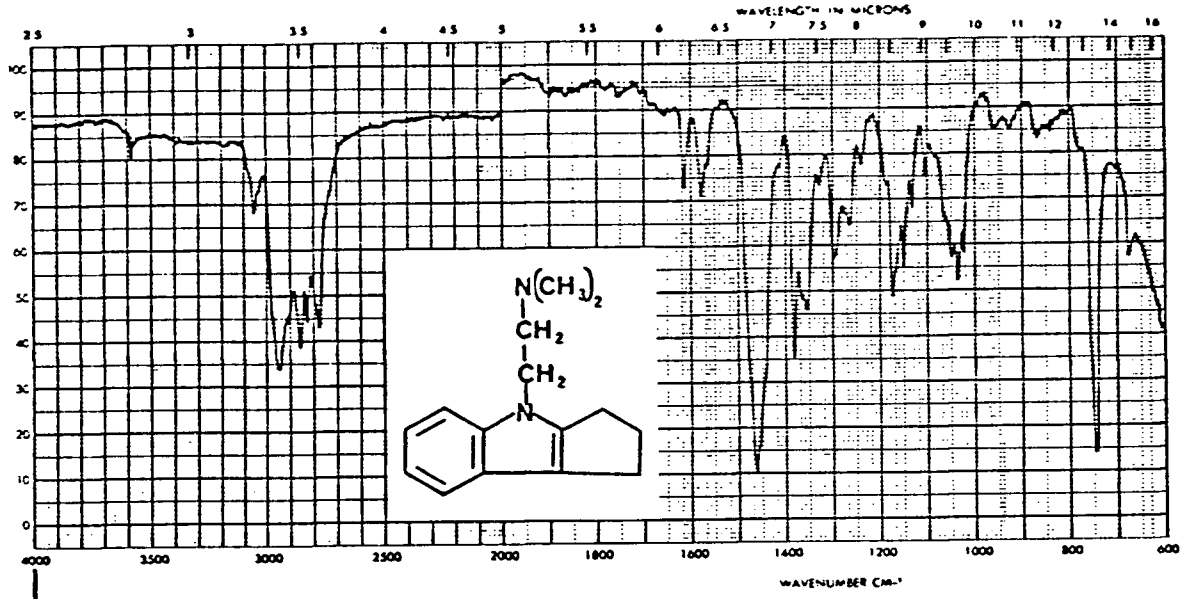
CH2
CH2
N
WAVENUMBER CM-1


WAVELENGTH IN MICRONS
N(CH3)2
CH2
CH2
N

Spectrum 10 4-(2-Dimethylaminoethyl)-1,2,3,4-tetrahydrocyclopent[b]indole (liquid film)

Spectrum 11 4-(2-Dimethylaminoethyl)dodecahydrocyclopent[b]indole (liquid film)

Spectrum 12 5-(2-Dimethylaminoethyl)-5,6,7,8,9,10-hexahydrocyclohept[b]-indole (liquid film)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WAVENUMBER CM⁻¹
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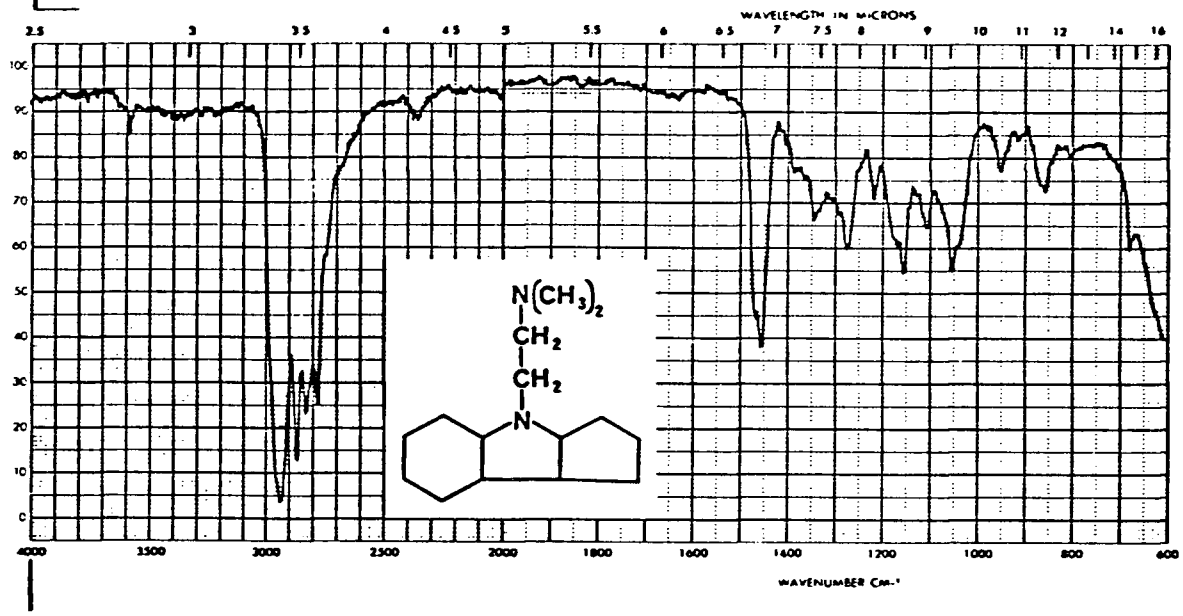
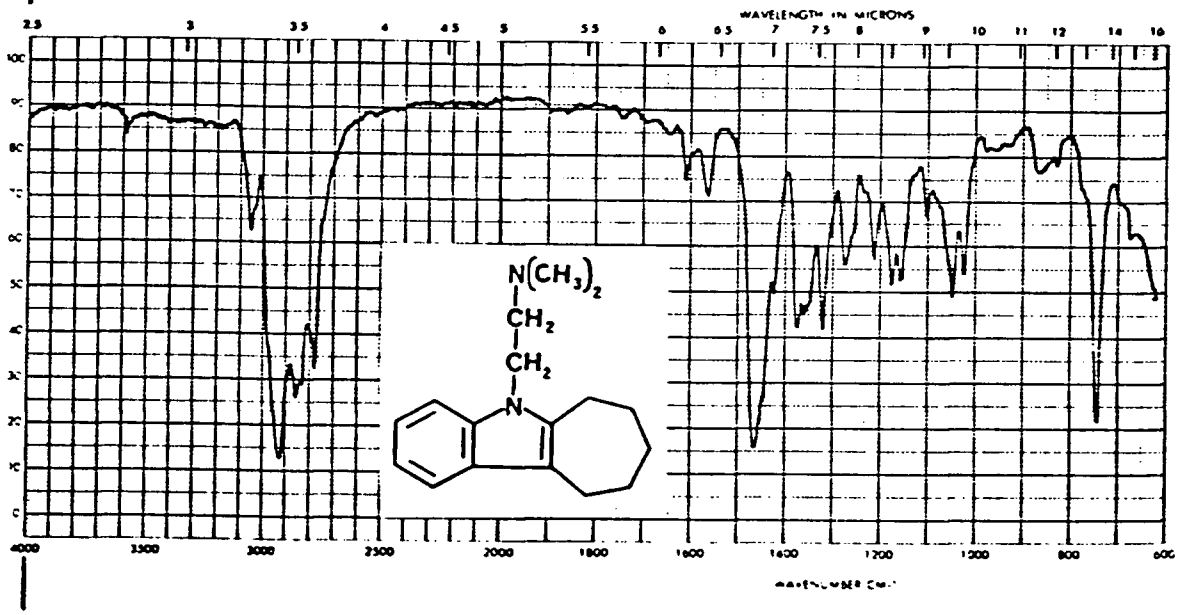
CH2
CH2
N

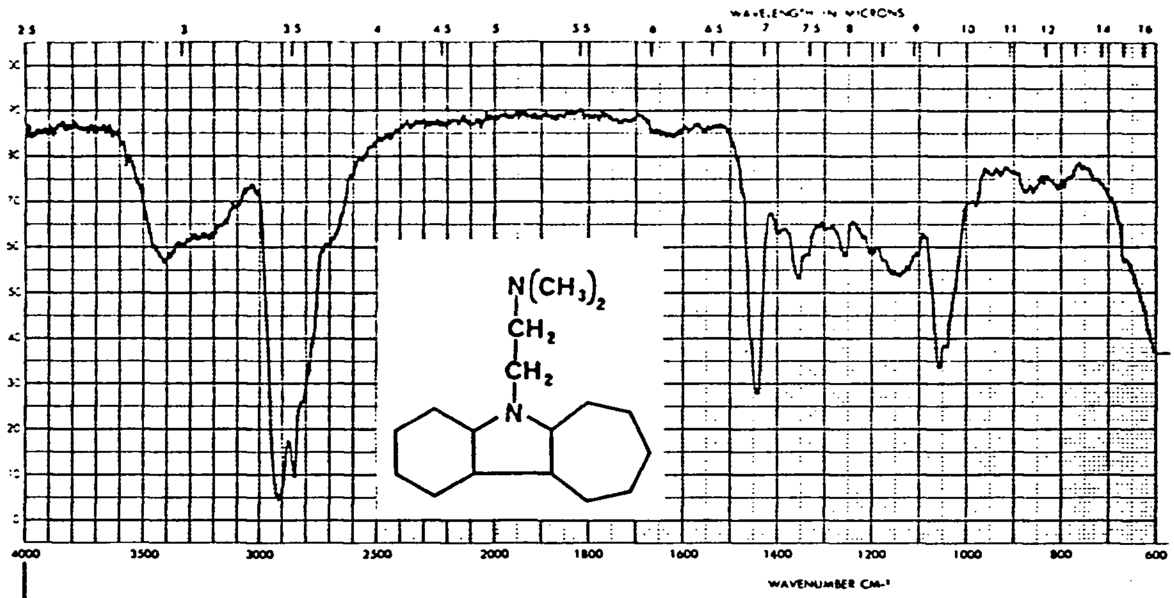

WAVELENGTH IN MICRONS
WAVENUMBER CM⁻¹
N(CH3)2
CH2
CH2
N


WAVELENGTH IN MICRONS
N(CH3)2
CH2
CH2
N

Spectrum 13 5-(2-Dimethylaminoethyl)tetradecahydrocyclohept[b]indole
(liquid film)

Spectrum 14 N-(2-Dimethylaminoethyl)diphenylamine
(liquid film)

Spectrum 15 N-(2-Dimethylaminoethyl)dicyclohexylamine
(liquid film)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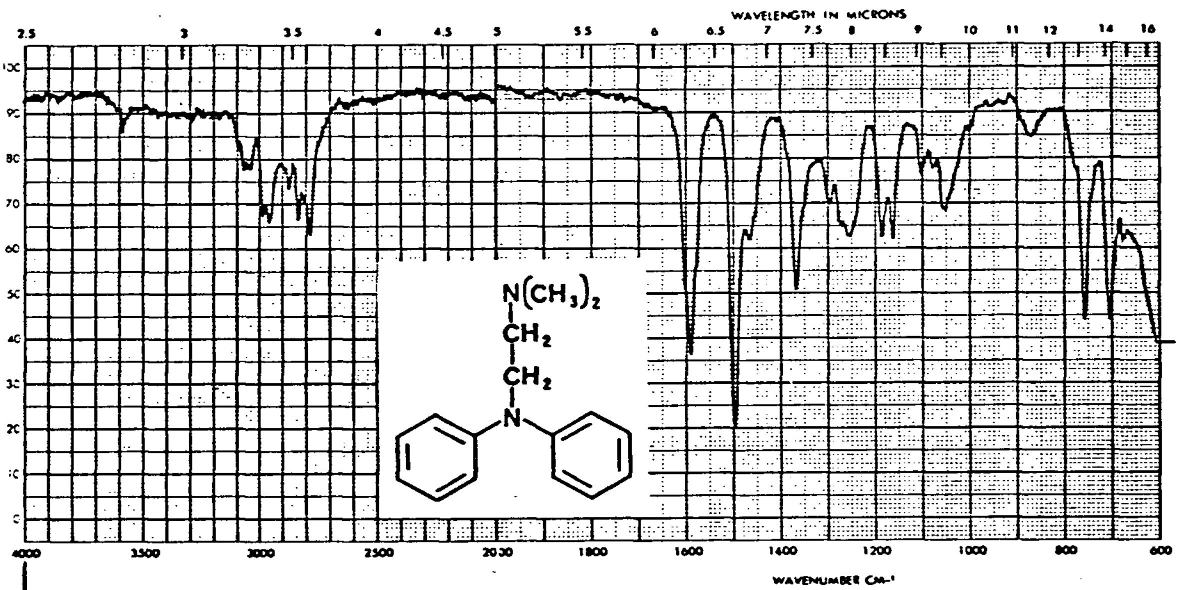
CH2
CH2
N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WAVENUMBER CM-1
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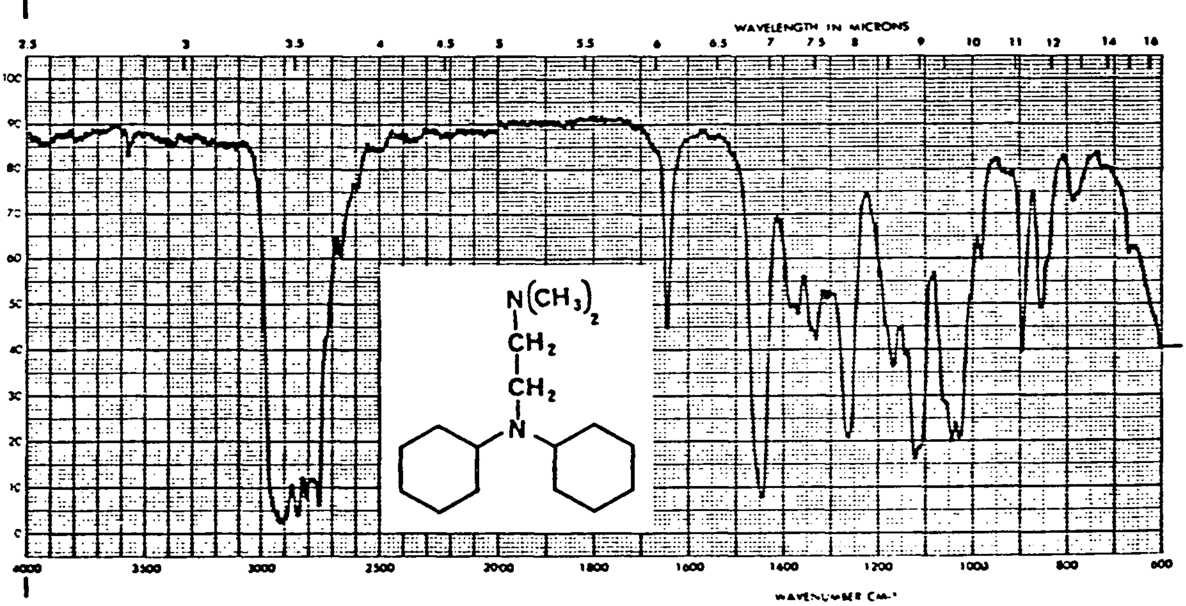
CH2
CH2
N


WAVELENGTH IN MICRONS
WAVENUMBER CM-1
N(CH3)2
CH2
CH2
N

Spectrum 16 9-(2-Dimethylaminoethyl)fluorene

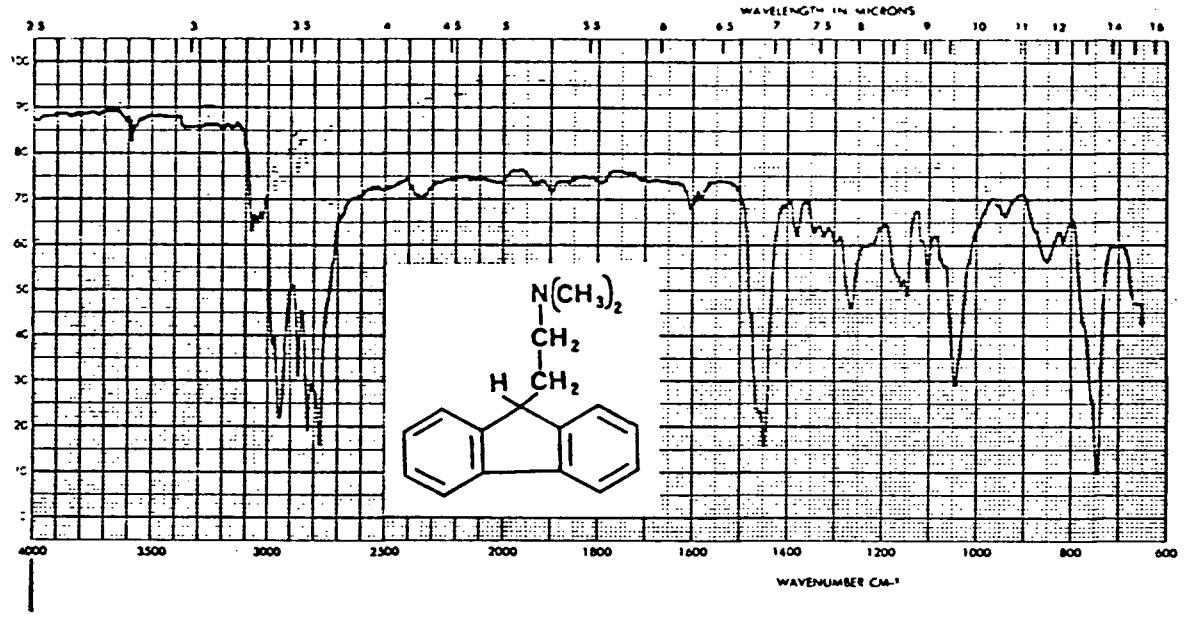
(liquid film)

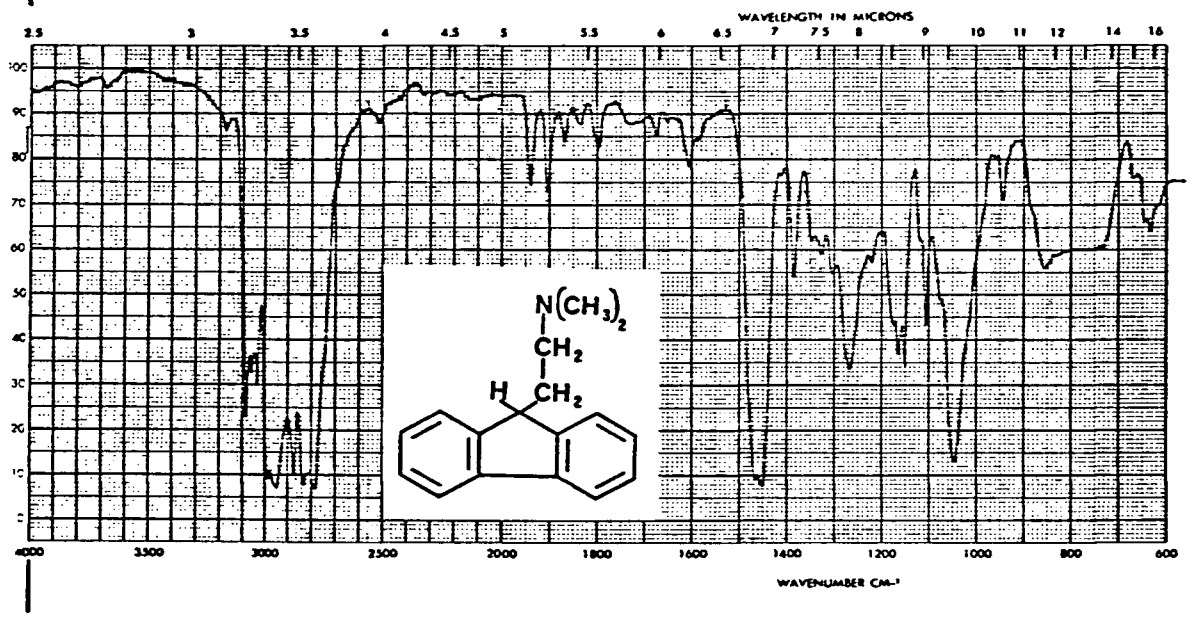
Spectrum 17 9-(2-Dimethylaminoethyl)fluorene

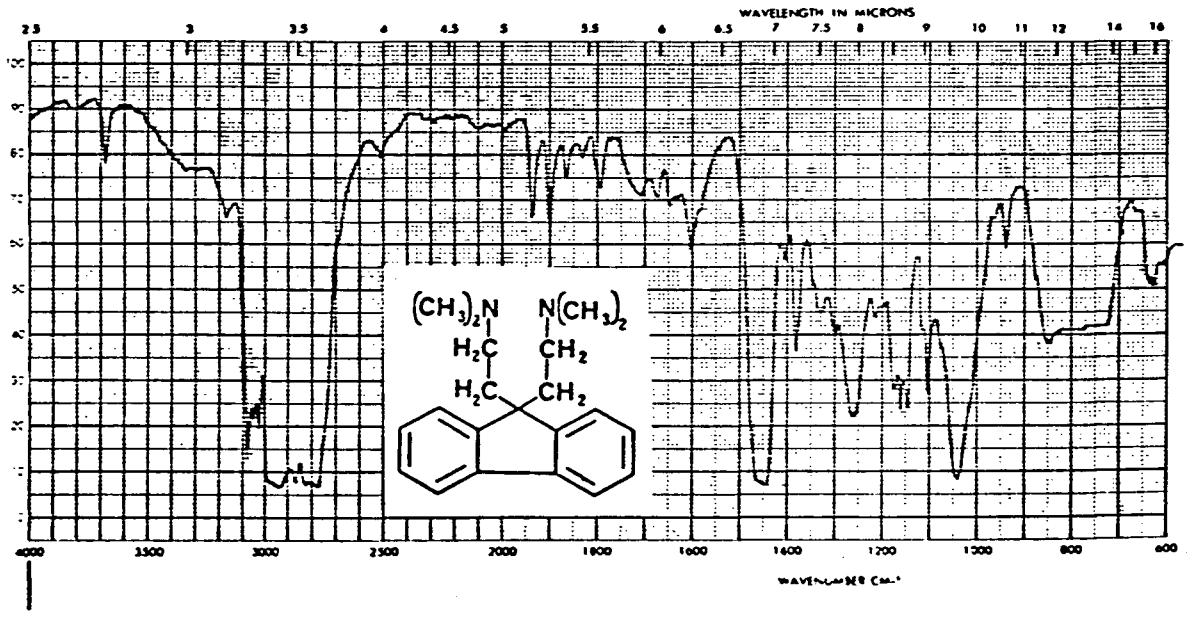
(10% in CCl_4, 0.5 mm cell)

Spectrum 18 9,9-Bis(2-dimethylaminoethyl)fluorene

(10% in CCl_4, 0.5 mm cell)


WAVELENGTH IN MICRONS
WAVENUMBER CM⁻¹
N(CH3)2
CH2
H
CH2


WAVELENGTH IN MICRONS
WAVENUMBER CM⁻¹
N(CH3)2
CH2
H
CH2

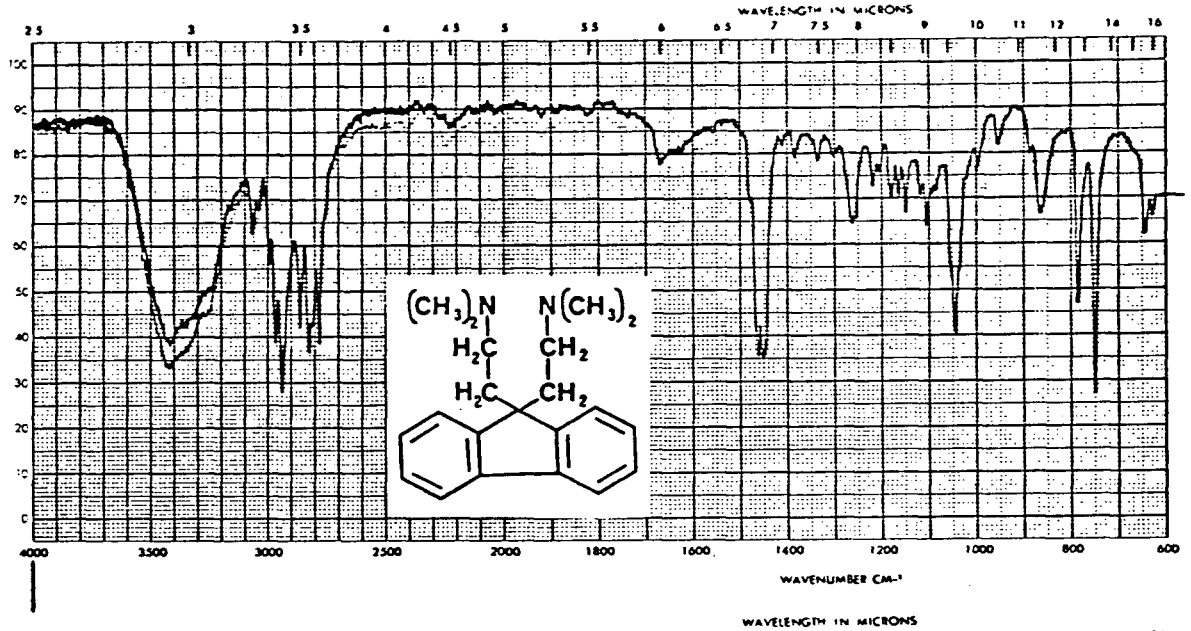

WAVELENGTH IN MICRONS
WAVENUMBER CM⁻¹
(CH3)2N
N(CH3)2
H2C
CH2
H2C
CH2

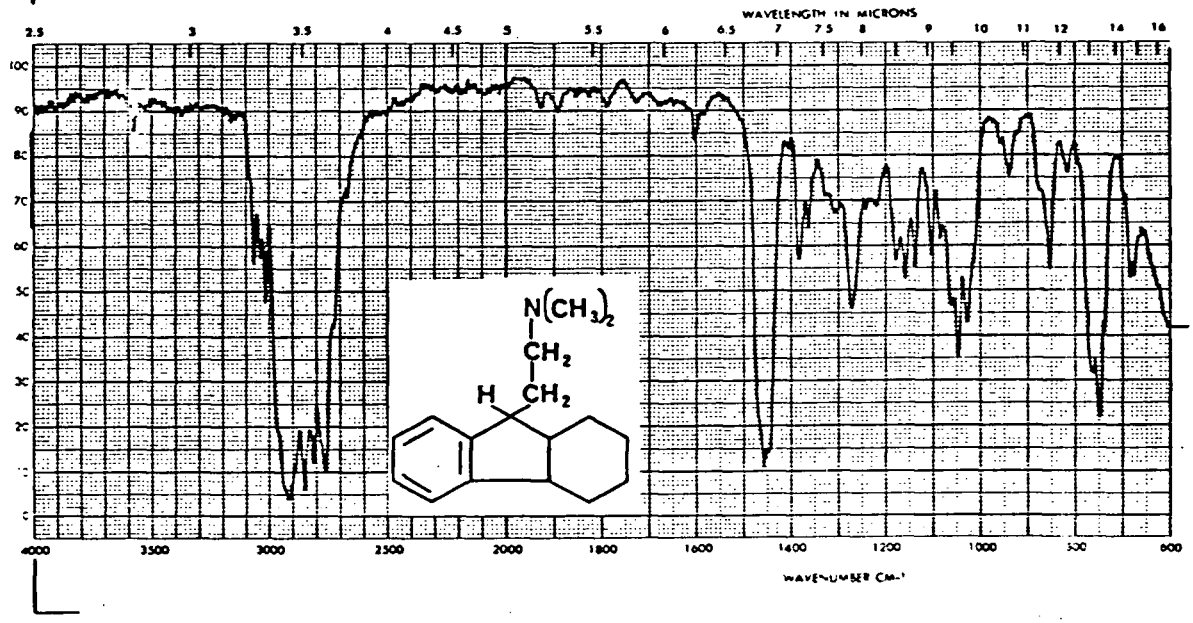
Spectrum 19 9,9-Bis(2-dimethylaminoethyl)fluorene

(KBr disc)

Spectrum 20 9-(2-Dimethylaminoethyl)-1,2,3,4,4a,9a-hexahydrofluorene

(liquid film)


WAVELENGTH IN MICRONS
WAVENUMBER CM⁻¹
(CH3)2N
N(CH3)2
H2C
CH2
H2C
CH2


WAVELENGTH IN MICRONS
WAVENUMBER CM⁻¹
N(CH3)2
CH2
H
CH2

APPENDIX II

MANUFACTURERS AND GRADES OF REAGENTS, SOLVENTS, AND GASES

A. REAGENTS

Chemical	Manufacturer	Grade or Purity
Acid, hydrobromic (48%)	Eastman	---
Acid, hydrochloric	Allied	A.C.S. reagent
Acid, sulfuric	Allied	A.C.S. reagent
Ammonium hydroxide	Allied	A.C.S. reagent
Barium nitrate	Mallinckrodt	analytical reagent
Boron trifluoride etherate	J.T. Baker	practical
Carbazole	Aldrich	99%
Chloroacetyl chloride	Aldrich	reagent
Cupric chloride dihydrate	Mallinckrodt	analytical reagent
Cycloheptanone	Aldrich	---
Cyclohexanone	Aldrich	puriss.
Cyclopentanone	Aldrich	puriss.
Dicyclohexylamine	Aldrich	b.p. 255°
Dimethyl sulfate	Eastman	practical
Dimethylaminoethanol	Eastman	reagent
Dimethylaminoethyl chloride hydrochloride	Aldrich	98%
Diphenylamine	Fisher	purified
Formamide	Aldrich	b_{10} 109°
Fluorene	Eastman	practical
Lithium aluminum hydride	Alfa Inorganics	---

Magnesium sulfate	British Drug Houses	anhydrous reagent
Methyl iodide	Eastman	C.P. reagent
Nitromethane	Aldrich	reagent
Phenylhydrazine	Fisher	certified reagent
Picric acid	Merck	N.F.
Piperazine (anhydrous)	Aldrich	practical
Potassium metal	British Drug Houses	reagent
Potassium hydroxide	Allied	A.C.S. reagent
Raney nickel No. 28	W.R.Grace	---
5% Rhodium on alumina	K & K Labs	---
Sodium metal	Allied	A.C.S. reagent
Sodium amide	Fisher	reagent
Sodium borohydride	Alfa Inorganics	98+%
Sodium dichromate dihydrate	Merck	Technical
Sodium hydride	Du Pont	---
Sodium hydroxide	British Drug Houses	analytical reagent
Sodium sulfate	Allied	A.C.S. reagent

B. SOLVENTS AND GASES

Acetone	Mallinckrodt	N.F.
Acid, acetic	Allied	A.C.S. reagent
Benzene	Mallinckrodt	reagent
Butanol, tertiary	Matheson	reagent
Decalin	Eastman	practical
Diglyme	Eastman	practical

Dimethylamine	Matheson	---
Dimethylformamide	Fisher	anhydrous reagent
Dioxane	J.T.Baker	reagent
Ethanol	Reliance Chemical	95%
Ethanol (100%)	Reliance Chemical	---
Ether	Fisher	N.F.
Ether, anhydrous	Fisher	anhydrous reagent
Ethylene glycol	Allied	reagent
Hexane	Matheson	practical
Hydrogen	Canadian Liquid Air	---
Hydrogen chloride	Matheson	---
Methanol	Fisher	anhydrous reagent
Nitrogen	Canadian Liquid Air	G
Petroleum spirit (60-80°)	British Drug Houses	analytical reagent
Tetrahydrofuran	British Drug Houses	reagent
Xylene	Mallinckrodt	reagent

Where anhydrous solvents are specified in experimental procedures, the solvent was first dried over sodium wire. Exceptions are anhydrous methanol, which was used as supplied, anhydrous ether used as supplied as a solvent in the recrystallization of hydrochloride salts, and anhydrous tetrahydrofuran which was distilled from lithium aluminum hydride.

In reactions which were run under a nitrogen atmosphere, a continuous slow stream of nitrogen was purified by bubbling the gas through concentrated sulfuric acid and passing it through a column loaded with potassium hydroxide pellets and thence into the reaction flask.

Other reagents were used as supplied except where noted in the text.

APPENDIX III

COMPUTER PROGRAMS

A. For interpretation of results from tests for antihistaminic activity.

```
 Card
number          Fortran statement

   1            DIMENSION P(4,9,37,4),S(9),N(9),SS(9),NN(9),AVG(9)
   2            DO 3 I=1,9
   3            DO 2 M=1,4
   4            DO 2 K=1,4
   5            DO 2 J=1,37
   6            P(M,I,J,K)=-1.0
   7        2   CONTINUE
   8            SS(I)=0.0
   9            NN(I)=0
  10        3   CONTINUE
  11            DO 150 K=1,4
  12            DO 4 L=1,9
  13            S(L)=0.0
  14            N(L)=0
  15            SSQ(L)=0
  16        4   CONTINUE
  17            DO 100 J=1,37
  18            READ (5,5) WMAX,XMAX,YMAX,ZMAX
  19        5   FORMAT (4F10.2)
  20            WRITE (6,6)J,K
  21        6   FORMAT (/'SUBSET',I3,',  GROUP',I2)
  22            WRITE (6,8)
  23        8   FORMAT (/10X,'WMAX',11X,'XMAX',11X,'YMAX',11X,'ZMAX'/)
  24            WRITE (6,9)WMAX,XMAX,YMAX,ZMAX
  25        9   FORMAT (4F15.3)
  26            WRITE (6,11)
  27       11   FORMAT (1X)
  28            WRITE (6,20)
  29       20   FORMAT (5X,'NO',6X,'W',9X,'X',9X,'Y',9X,'Z',9X,'A',
  30           1 9X,'B',9X,'C',9X,'D',8X,'SUM',6X,'MEAN',5X,'STD ERR'/)
  31            IF (ZMAX.NE.0.0) GO TO 70
  32            IF (YMAX.NE.0.0) GO TO 50
  33            DO 40 I=1,9
  34            READ (5,31) W,X
  35       31   FORMAT (2F10.2)
  36            A=(W/WMAX)*100.0
  37            P(1,I,J,K)=A
  38            B=(X/XMAX)*100.0
  39            P(2,I,J,K)=B
  40            SUM=A+B
  41            XMEAN=SUM/2
  42            SE=SQRT(((A-XMEAN)**2+ (B-XMEAN)**2)/2)
  43            N(I)=N(I)+ 2
  44            S(I)=S(I)+ SUM
  45            WRITE (6,35)I,W,X,A,B,SUM,XMEAN,SE
  46       35   FORMAT (I7,2(2F10.3,20X),3F10.3/)
```

```
47      40    CONTINUE
48            WRITE (6,11)
49            GO TO 100
50      50    DO 60 I=1,9
51            READ (5,51) W,X,Y
52      51    FORMAT (3F10.2)
53            A=(W/WMAX)*100.0
54            P(1,I,J,K)=A
55            B=(X/XMAX)*100.0
56            P(2,I,J,K)=B
57            C=(Y/YMAX)*100.0
58            P(3,I,J,K)=C
59            SUM=A+ B+ C
60            XMEAN=SUM/3
61            SE=SQRT(((A-XMEAN)**2+ (B-XMEAN)**2+ (C-XMEAN)**2)/6)
62            N(I)=N(I)+ 3
63            S(I)=S(I)+ SUM
64            WRITE (6,55)  I,W,X,Y,A,B,C,SUM,XMEAN,SE
65      55    FORMAT (I7,2(3F10.3,10X),3F10.3/)
66      60    CONTINUE
67            WRITE (6,11)
68            GO TO 100
69      70    DO 80 I=1,9
70            READ (5,71) W,X,Y,Z
71      71    FORMAT (4F10.2)
72            A=(W/WMAX)*100.0
73            P(1,I,J,K)=A
74            B=(X/XMAX)*100.0
75            P(2,I,J,K)=B
76            C=(Y/YMAX)*100.0
77            P(3,I,J,K)=C
78            D=(Z/ZMAX)*100.0
79            P(4,I,J,K)=D
80            SUM=A+B+C+D
81            XMEAN=SUM/4
82            SE=SQRT(((A-XMEAN)**2+ (B-XMEAN)**2 +(C-XMEAN)**2+
83           1 (D-XMEAN)**2)/12)
84            N(I)=N(I)+ 4
85            S(I)=S(I)+ SUM
86            WRITE (6,75)I,W,X,Y,Z,A,B,C,D,SUM,XMEAN,SE
87      75    FORMAT (I7,11F10.3/)
88      80    CONTINUE
89            WRITE (6,11)
90     100    CONTINUE
91            DO 110 I=1,9
92            AVG(I)=S(I)/N(I)
93            SS(I)=SS(I)+ S(I)
94     110    NN(I)=NN(I)+ N(I)
95            WRITE (6,115)
96     115    FORMAT (/20X,'VALUES FOR GROUP OF 37'//)
97            WRITE (6,118)
98     118    FORMAT (4X,'NO',4X,'NO OF OBS',11X,'SUM',11X,'MEAN',
```

```
 99           1 9X,'STD ERROR'/)
100            DO 130 I=1,9
101            DO 125 J=1,37
102            DO 120 M=1,4
103            1F (P(M,I,J,K).EQ.-1.0) GO TO 120
104            DIF=P(M,I,J,K)-AVG(I)
105            SSQ(I)=SSQ(I)+DIF**2
106     120    CONTINUE
107     125    CONTINUE
108            SE=SQRT(SSQ(I)/(N(I)*(N(I)-1)))
109            WRITE (6,128)I,N(I),S(I),AVG(I),SE
110     128    FORMAT (I6,I10,4X,2F15.3/)
111     130    CONTINUE
112            WRITE (6,11)
113            WRITE (6,11)
114     150    CONTINUE
115            WRITE (6,151)
116     151    FORMAT (//20X,'VALUES FOR COMPLETE SET'//)
117            WRITE (6,118)
118            DO 152 I=1,9
119            SSQ(I)=0.0
120     152    AVG(I)=SS(I)/NN(I)
121            DO 190 I=1,9
122            DO 180 K=1,4
123            DO 170 J=1,37
124            DO 160 M=1,4
125            IF (P(M,I,J,K).EQ.-1.0) GO TO 160
126            DIF=P(M,I,J,K)-AVG(I)
127            SSQ(I)=SSQ(I)+DIF**2
128     160    CONTINUE
129     170    CONTINUE
130     180    CONTINUE
131            SE=SQRT(SSQ(I)/(NN(I)*(NN(I)-1)))
132            WRITE (6,128)I,NN(I),SS(I),AVG(I),SE
133     190    CONTINUE
134            END
```

The above program was designed around the format in which data was collected from the experiments for antihistaminic activity. In order to use the program the experiment must be set up in the manner used in this work.

Thirty-seven separate experiments were run in each of four sets of apparatus. Each experiment involved the determination of two, three, or four cumulative responses to histamine alone and one determination

of the cumulative response to histamine in the presence of an antagonist. Up to nine cumulative doses of histamine were used for each response.

Essentially, this program computed the mean control response, with its standard error, at each dose for each individual experiment, for each group of 37 experiments in the same apparatus, and for the entire set (4x37) of experiments. Values for the test responses were not computed but were calculated with a slide rule.

The job data deck consisted of four groups of cards and each group consisted of 37 subsets which each represented one experiment. Each subset consisted of 10 data cards. The first of these 10 cards carried the values for the maximum response of the preparation, measured in mm from the kymograph record. The nine succeeding cards carried the measured responses at increasing doses of histamine. It was necessary to start each cumulative response at the same dose of histamine, preferably one which elicits zero response. In this work, 10^{-8}M histamine was found to be a satisfactory starting dose.

The data collected in the first experiment carried out using apparatus 1 is shown in Table XII.

It is necessary to have 10 cards in each subset. Therefore the responses at 10^{-4} histamine were assumed to be the same as those at $3x10^{-5}$M. The data cards thus carry the values listed below:

Card 1	106.0	108.0	114.0	117.0	Card 6	96.0	83.0	108.0	108.0
2	0.0	0.0	0.0	0.0	7	95.0	91.0	108.0	113.0
3	1.5	1.0	0.5	0.0	8	106.0	108.0	114.0	117.0
4	34.0	30.0	34.0	23.0	9	106.0	108.0	114.0	117.0
5	53.0	74.0	61.0	65.0	10	106.0	108.0	114.0	117.0

Histamine dose (M)	Contraction (mm) W	X	Y	Z	Contraction in presence of Diphenhydramine (10^{-7}M)
10^{-8}	0.0	0.0	0.0	0.0	0.0
$3x10^{-8}$	1.5	1.0	0.5	0.0	0.0
10^{-7}	34.0	30.0	34.0	23.0	0.0
$3x10^{-7}$	53.0	74.0	61.0	65.0	16.0
10^{-6}	96.0	83.0	108.0	108.0	46.0
$3x10^{-6}$	95.0	91.0	108.0	113.0	68.0
10^{-5}	106.0	108.0	114.0	117.0	103.0
$3x10^{-5}$	106.0	108.0	114.0	117.0	105.0
10^{-4}	-----	-----	-----	-----	107.0

Table XII: Data for the cumulative dose-response curves obtained in a typical experiment; specifically, the first experiment using apparatus 1.

In the program, the values across card one correspond to WMAX, XMAX, YMAX, and ZMAX, ie WMAX = 106.0. The values across the remaining nine cards correspond to values W, X, Y, and Z used as operands in the program.

This data was reduced to a percentage of maximal response and the mean control response and standard error at each dose was computed. In the computer print-out, A represents the percentage values calculated from W and B, C, and D similarly correspond to X, Y, and Z respectively. The format of the print-out is shown on page 218 for the data set given in Table XII. The values have been rounded off from the three decimal places given in the print-out. "NO" in the print-out refers to the dose of histamine where 1 is 10^{-8}M, progressing in 1/2 log unit steps (Table IX).

The next 36 subsets in group 1 were then printed out in the format given for subset 1, and this was followed by:

VALUES FOR GROUP OF 37

NO	NO OF OBS	SUM	MEAN	STD ERROR
1	123	244.589	1.989	0.249
2	123	650.111	5.285	0.484
3	123	1807.518	14.695	0.948
4	123	4217.223	34.286	1.461
5	123	8329.488	67.719	1.582
6	123	10811.082	87.895	0.798
7	123	12144.937	98.739	0.164
8	123	12266.863	99.731	0.059
9	123	12300.00	100.00	0.0

SUBSET 1, GROUP 1

WMAX	XMAX	YMAX	ZMAX
106.0	108.0	114.0	117.0

NO	W	X	Y	Z	A	B	C	D	SUM	MEAN	STD ERR
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	1.5	1.0	0.5	0.0	1.4	0.9	0.4	0.0	2.8	0.7	0.3
3	34.0	30.0	34.0	23.0	32.1	27.8	29.8	19.7	109.3	27.3	2.7
4	53.0	74.0	61.0	65.0	50.0	68.5	53.5	55.6	227.6	56.9	4.0
5	96.0	89.0	108.0	108.0	90.6	82.4	94.7	92.3	360.0	90.0	2.7
6	95.0	91.0	108.0	113.0	89.6	84.3	94.7	96.6	365.2	91.3	2.8
7	106.0	108.0	114.0	117.0	100.0	100.0	100.0	100.0	400.0	100.0	0.0
8	106.0	108.0	114.0	117.0	100.0	100.0	100.0	100.0	400.0	100.0	0.0
9	106.0	108.0	114.0	117.0	100.0	100.0	100.0	100.0	400.0	100.0	0.0

The values for groups 2, 3, and 4 were then printed out in the same format and were followed by:

VALUES FOR COMPLETE SET

NO	NO OF OBS	SUM	MEAN	STD ERROR
1	495	543.686	1.098	0.089
2	495	1743.600	3.522	0.197
3	495	5948.293	12.017	0.460
4	495	16244.668	32.818	0.755
5	495	33046.797	66.761	0.774
6	495	43027.152	86.924	0.468
7	495	48616.863	98.216	0.124
8	495	49345.602	99.688	0.041
9	495	49500.000	100.000	0.0

The values for the complete set were used to draw a control cumulative dose-response curve and from this curve the pD_2 value for histamine was calculated.

The values for the individual subsets were compared to the corresponding <u>test</u> cumulative responses to determine pA_2 and pD_2' values for each of the test compounds.

B. For interpretation of results from tests for antimuscarinic activity.

```
 Card
number        Fortran statement

   1           DIMENSION P(3,6,36,4),S(6),N(6),SS(6),NN(6),SSQ(6),AVG(6)
   2           DO 3 I=1,6
   3           DO 2 M=1,3
   4           DO 2 K=1,4
   5           DO 2 J=1,36
   6           P(M,I,J,K)=-1.0
   7      2    CONTINUE
   8           SS(I)=0.0
   9           NN(I)=0
  10      3    CONTINUE
  11           DO 150 K=1,4
  12           DO 4 L=1,6
  13           S(L)=0.0
  14           N(L)=0
  15           SSQ(L)=0
  16      4    CONTINUE
  17           DO 100 J=1,36
  18           READ (5,5) WMAX,XMAX,YMAX,ZMAX
  19      5    FORMAT (4F10.2)
  20           WRITE (6,6) J,K
  21      6    FORMAT (/'SUBSET',I3,',  GROUP',I2)
  22           WRITE (6,8)
  23      8    FORMAT (/10X,'WMAX',11X,'XMAX',11X,'YMAX',11X,'ZMAX'/)
  24           WRITE (6,9) WMAX,XMAX,YMAX,ZMAX
  25      9    FORMAT (4F15.3)
  26           WRITE (6,11)
  27     11    FORMAT (1X)
  28           WRITE (6,20)
  29     20    FORMAT (5X,'NO',6X,'W',9X,'X',9X,'Y',9X,'Z',9X,'A',
  30          1 9X,'B',9X,'C',9X,'D',8X,'SUM',6X,'MEAN',5X,'STD ERR'/)
  31           DO 80 I=1,6
  32           READ (5,71) W,X,Y,Z
  33     71    FORMAT (4F10.2)
  34           A=(W/WMAX)*100.0
  35           P(1,I,J,K)=A
  36           B=(X/XMAX)*100.0
  37           P(2,I,J,K)=B
  38           C=(Y/YMAX)*100.0
  39           P(3,I,J,K)=C
  40           D=(Z/ZMAX)*100.0
  41           SUM=A+B+C
  42           XMEAN=SUM/3.0
  43           SE=SQRT(((A-XMEAN)**2+(B-XMEAN)**2+(C-XMEAN)**2)/6)
  44           N(I)=N(I)+3
  45           S(I)=S(I)+SUM
```

```
46            WRITE (6,75) I,W,X,Y,Z,A,B,C,D,SUM,XMEAN,SE
47     75     FORMAT (I7,11F10.3/)
48     80     CONTINUE
49            WRITE (6,11)
50    100     CONTINUE
51            DO 110 I=1,6
52            AVG(I)=S(I)/N(I)
53            SS(I)=SS(I)+S(I)
54    110     NN(I)=NN(I)+N(I)
55            WRITE (6,115)
56    115     FORMAT (/20X,'VALUES FOR GROUP OF 36'//)
57            WRITE (6,118)
58    118     FORMAT (4X,'NO',4X,'NO OF OBS',11X,'SUM',11X,
59           1 'MEAN',9X,'STD ERROR'/)
60            DO 130 I=1,6
61            DO 125 J=1,36
62            DO 120 M=1,3
63            IF (P(M,I,J,K).EQ.-1.0) GO TO 120
64            DIF=P(M,I,J,K)-AVG(I)
65            SSQ(I)=SSQ(I)+DIF**2
66    120     CONTINUE
67    125     CONTINUE
68            SE=SQRT(SSQ(I)/(N(I)*(N(I)-1)))
69            WRITE (6,128)I,N(I),S(I),AVG(I),SE
70    128     FORMAT (I6,I10,4X,3F15.3/)
71    130     CONTINUE
72            WRITE (6,11)
73            WRITE (6,11)
74    150     CONTINUE
75            WRITE (6,151)
76    151     FORMAT (//20X,'VALUES FOR COMPLETE SET'//)
77            WRITE (6,118)
78            DO 152 I=1,6
79            SSQ(I)=0.0
80    152     AVG(I)=SS(I)/NN(I)
81            DO 190 I=1,6
82            DO 180 K=1,4
83            DO 170 J=1,36
84            DO 160 M=1,3
85            IF (P(M,I,J,K).EQ.-1.0) GO TO 160
86            DIF=P(M,I,J,K)-AVG(I)
87            SSQ(I)=SSQ(I)+DIF**2
88    160     CONTINUE
89    170     CONTINUE
90    180     CONTINUE
91            SE=SQRT(SSQ(I)/(NN(I)*(NN(I)-1)))
92            WRITE (6,128)I,NN(I),SS(I),AVG(I),SE
93    190     CONTINUE
94            END
```

In the tests for antimuscarinic activity, four groups of 36

experiments were carried out. Each experiment entailed the recording of three control cumulative responses and one test cumulative response. Only six doses of agonist were used for each cumulative response. Minor alterations of Program A were necessary so that the data from these experiments could be interpreted. The resultant Program B, above, printed out the same data as was obtained from Program A. In addition, Program B reduced the measured test responses to percentage values.

The data deck consisted of four groups, each containing 36 subsets. Each subset consisted of seven cards, each of which carried four values. An example of the data deck is given below for subset 1, group 1.

Card				
1	120.5	125.0	124.0	123.2
2	0.0	0.0	0.5	0.0
3	6.0	7.5	8.5	7.5
4	72.5	76.0	58.5	40.0
5	110.0	115.0	108.5	84.0
6	117.5	122.0	118.5	101.0
7	120.5	125.0	124.0	103.5

The values across card 1 are WMAX, XMAX, YMAX, the measured maxima of the three control responses, and ZMAX, the average of WMAX, XMAX, and YMAX. A portion of the print-out is shown on page 223. The values have been rounded off from the three decimal places given in the print-out. "NO" refers to the dose of agonist (1 = 10^{-8}M acetylcholine, log 10 increments). Columns W, X, and Y are measured values of the control responses, reduced to percentages in columns A, B, and C respectively.

SUBSET 1, GROUP 1

WMAX	XMAX	YMAX	ZMAX
120.5	125.0	124.0	123.2

NO	W	X	Y	Z	A	B	C	D	SUM	MEAN	STD ERR
1	0.0	0.0	0.5	0.0	0.0	0.0	0.4	0.0	0.4	0.1	0.1
2	6.0	7.5	8.5	7.5	5.0	6.0	6.9	6.1	17.8	5.9	0.5
3	72.5	76.0	58.5	40.0	60.2	60.8	47.2	32.5	168.1	56.0	4.4
4	110.0	115.0	108.5	84.5	91.3	92.0	87.5	68.6	270.8	90.3	1.4
5	117.5	122.0	118.5	101.0	97.5	97.6	95.6	82.0	290.7	96.9	0.7
6	120.5	125.0	124.0	103.5	100.0	100.0	100.0	84.0	300.0	100.0	0.0

Column Z contains measured values for test responses in the presence of antagonist while D is the percentage values calculated from Z. The agonist in this example is 9-(2-dimethylaminoethyl)-1,2,3,4-tetrahydrocarbazole (10^{-4}M).

Values for each group of 36 were printed out as in Program A. Values over the complete set are shown below.

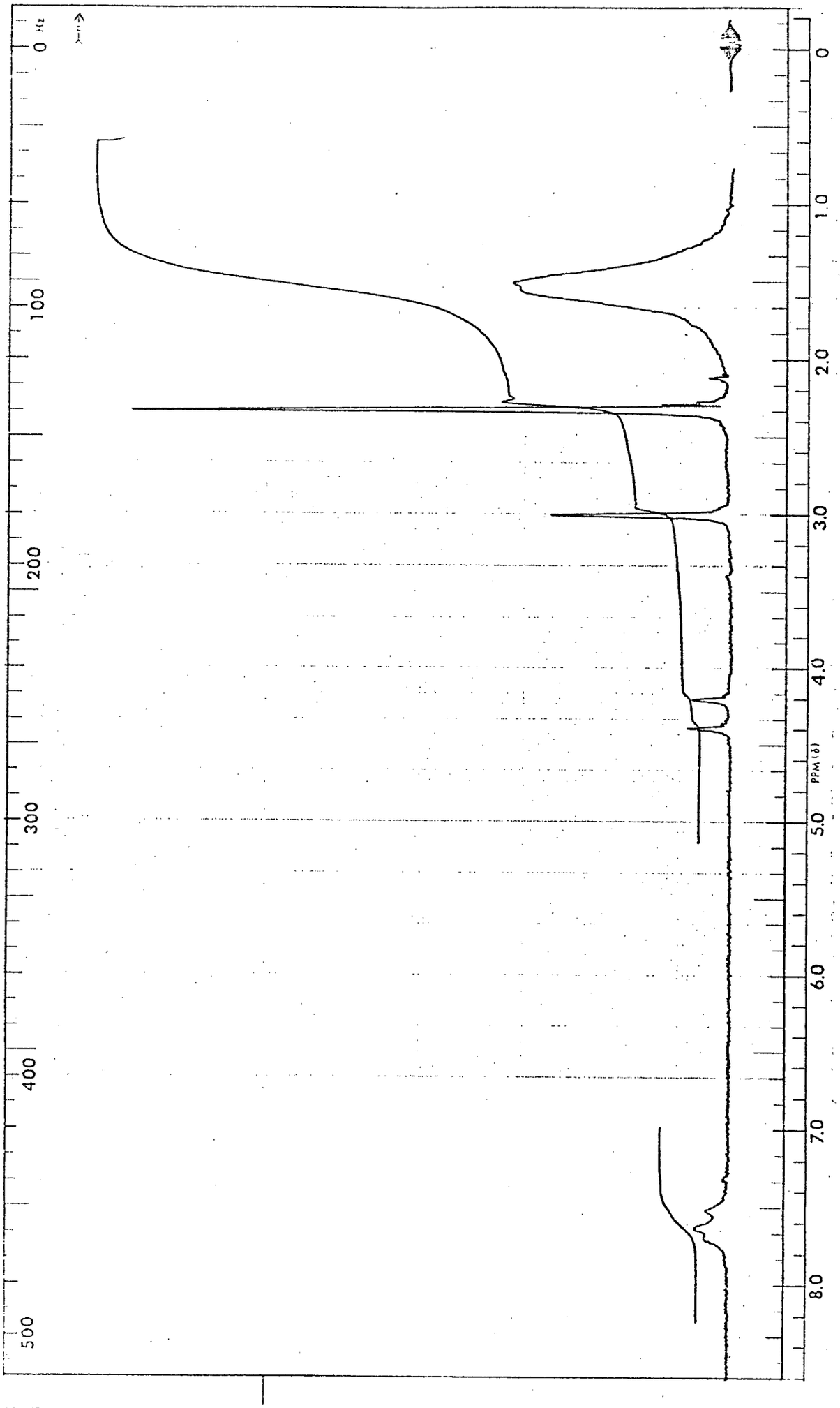
VALUES FOR COMPLETE SET

NO	NO OF OBS	SUM	MEAN	STD ERROR
1	432	423.936	0.981	0.065
2	432	5945.629	13.763	0.554
3	432	23775.645	55.036	0.971
4	432	37180.230	86.065	0.449
5	432	41229.961	95.440	0.196
6	432	43199.914	100.000	0.002

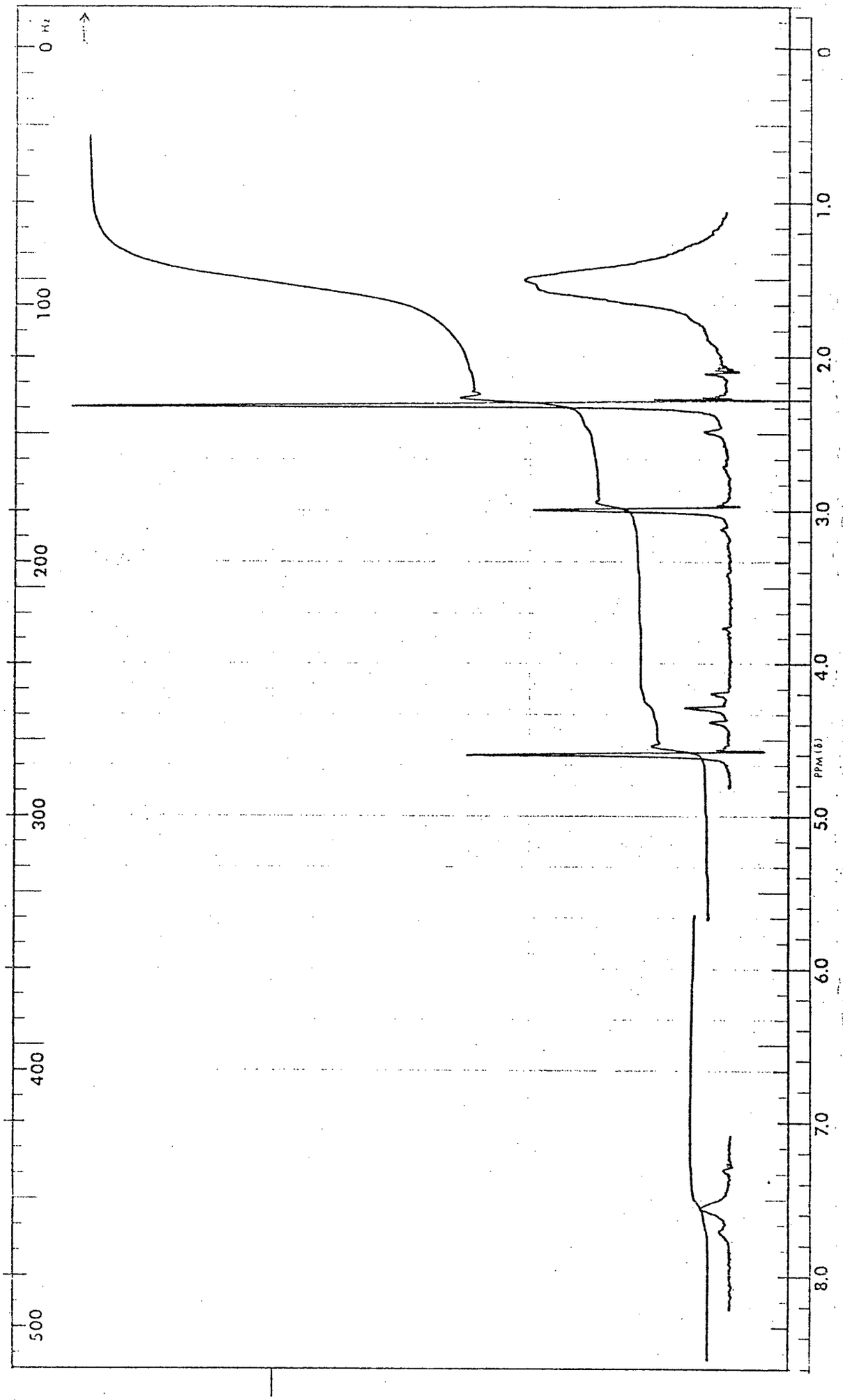
These values were used to draw an average cumulative dose-response curve for acetylcholine from which the pD_2 value was calculated. The values for the individual subsets were compared (test vs average of controls) to determine pD_2' values for each of the test compounds. Control data for atropine and diphenhydramine were calculated by hand.

APPENDIX IV

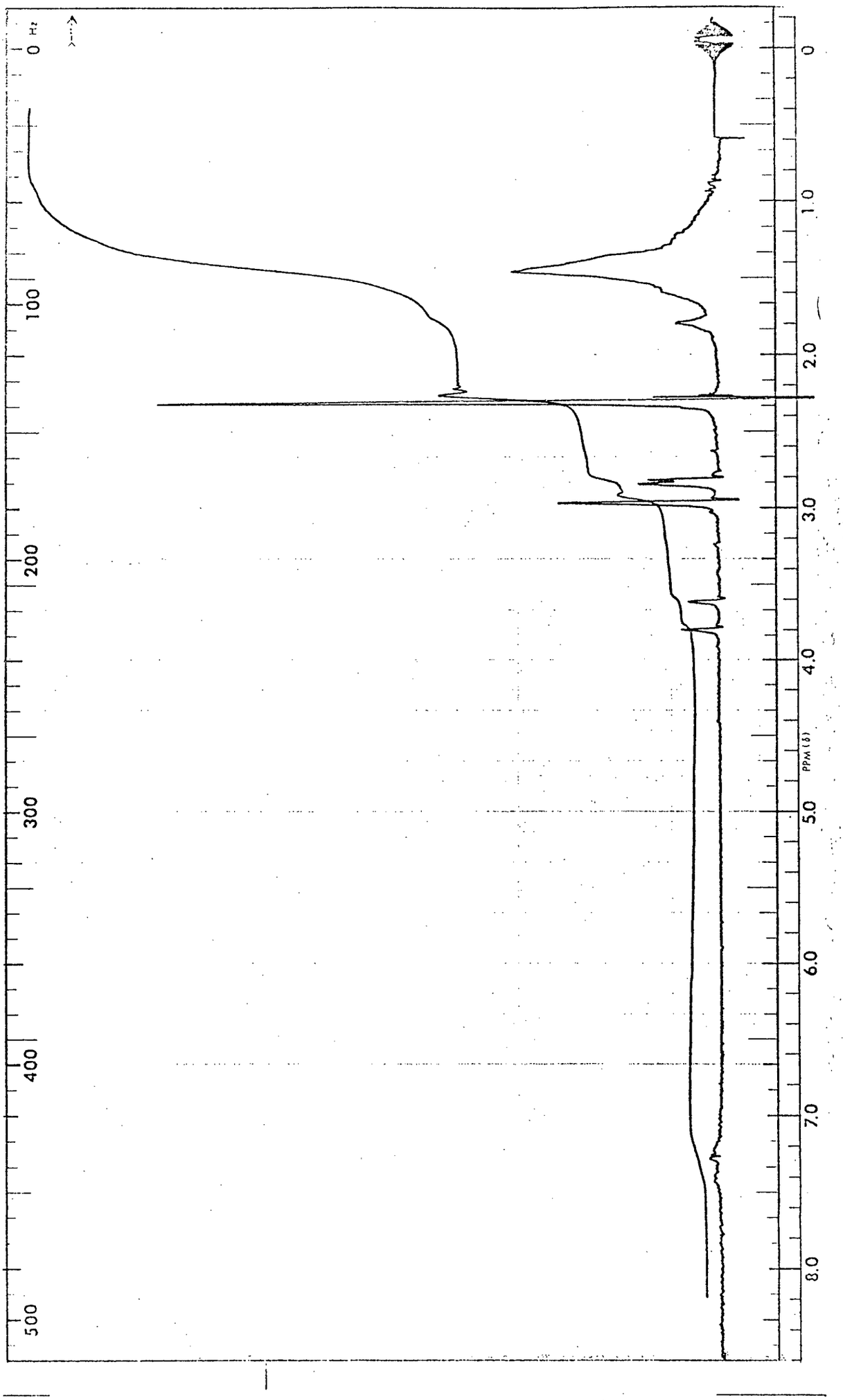
NUCLEAR MAGNETIC RESONANCE SPECTRA



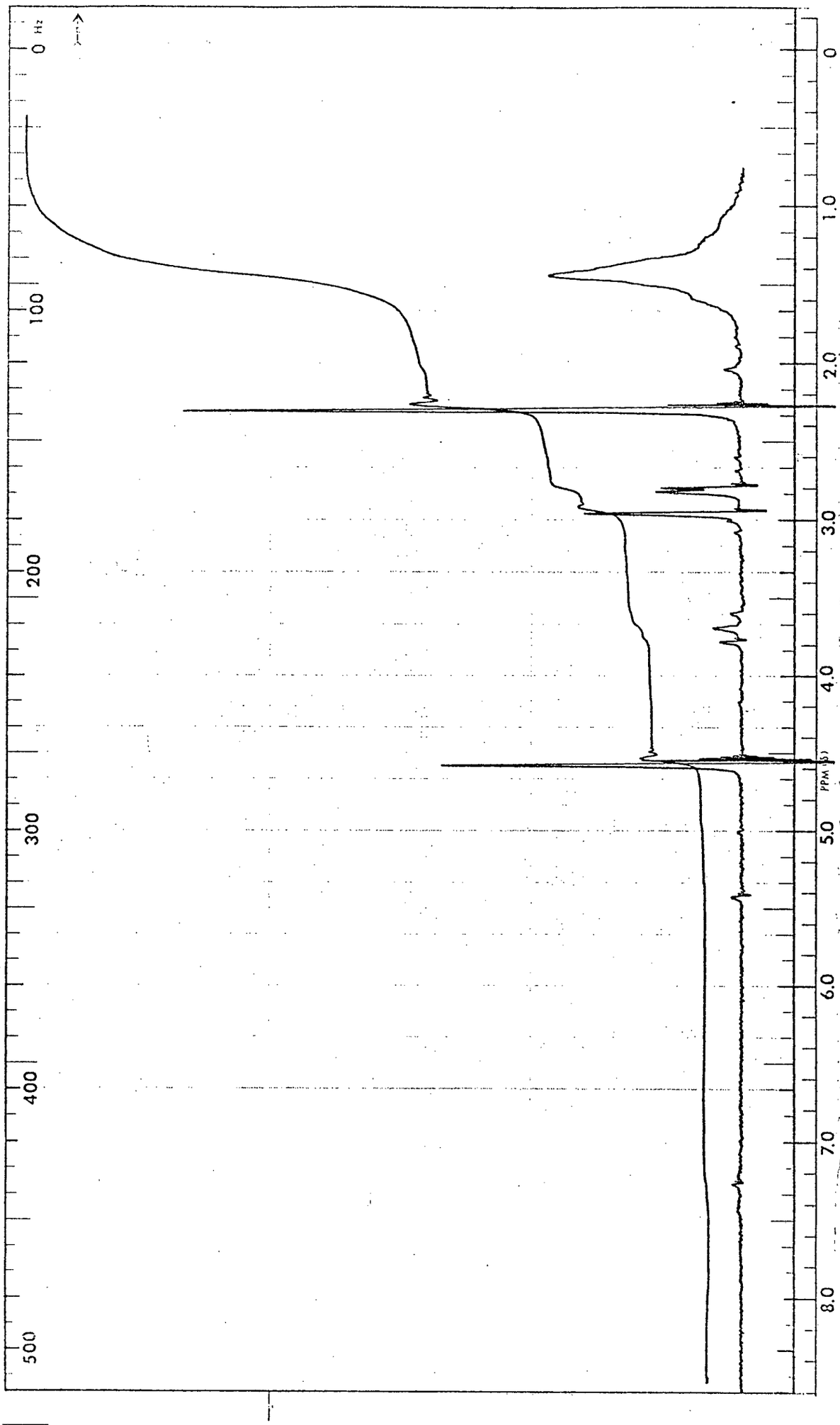
7-Dimethylazminoacetamido-14-azadispiro[5.1.5.2]pentadecan-15-one
(10%, $CDCl_3$)



7-Dimethylaminoacetamido-14-azadispiro[5.1.5.2]pentadecan-15-one
(10%, $CDCl_3$, D_2O added)



7-Dimethylaminoacetamido-14-azadispiro[5.1.5.2]pentadecane
(10%, $CDCl_3$)



7-Dimethylaminoacetamido-14-azadispiro[5.1.5.2]pentadecane
(10%, $CDCl_3$, D_2O added