SYNTHETIC AND STRUCTURAL STUDIES ON QUATERNARY AMMONIUM COMPOUNDS

A THESIS

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by

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SUMMARY

Part I of the thesis describes the preparation of some linear tris-onium ethers and Part II spectral studies on acetylcholine and related compounds. The influence of chemical structure upon biological activity is the unifying concept.

In Part I a general review of the development and use of neuromuscular blocking agents serves as an introduction to the receptor theory of neuromuscular blocking activity and to a survey of natural and synthetic neuromuscular blocking agents containing ether linkages. To extend previous studies on the influence of aliphatic ether linkages on the muscle relaxant activity of bis- and tris-onium salts some linear tris-onium compounds possessing an asymmetric ether linkage have been synthesised.

A new method has been devised for the synthesis of 3-alkoxypropylamines and 3-alkoxybutylamines. The base catalysed addition of dialkylaminoethanols and dialkylamino-propanols to N-alkylacrylamides and N-alkylcrotonamides afforded respectively 3-dialkylaminoalkoxy-propion-N-alkyl-amides and -butyr-N-alkylamides. The relationship between these additions and the Michael addition is discussed. Reduction of the propion-N-alkylamides and butyr-N-alkylamide with lithium aluminium hydride gave the 3-alkoxypropylamines and 3-alkoxybutylamines but cleavage of the ether linkage also occurred. The reduction of 3-(2-diethylaminoethoxy)-propion-N-ethylamide yielded 3-(2-diethylaminoethoxy)propyl-ethylamine, N,N'-diethyl-1,3-diaminopropane, 2-diethylamino-ethanol, a polymeric amide and an unidentified amine. A mechanism for the cleavage is proposed. Some bis-(3-alkoxy-propyl)-alkylamines and a bis-(3-alkoxybutyl)ethylamine, the triamines required for quaternisation, have similarly

been synthesised.

The attempted preparation of 3-(2-diethylaminoethoxy)-propionic acid by the addition of 2-diethylaminoethanol to ethyl acrylate gave chiefly 2-diethylaminoethyl acrylate, 2-diethylaminoethyl 3-ethoxypropionate, and 2-diethylamino-ethyl 3-(2-diethylaminoethoxy)propionate. Acid hydrolysis of 3-(2-diethylaminoethoxy)propionitrile caused appreciable cleavage of the ether linkage. Tentative mechanisms have been advanced for both reactions.

The ultraviolet spectra of acrylamide, crotonamide and some of their N-substituted derivatives have been recorded. N-Alkyl and N,N-dialkyl substitution in the acrylamide series is accompanied by the appearance of new bands. The effect of N-substitution is less obvious in the crotonamide series.

The triamines were readily quaternised but many of the salts were hygroscopic. These tris-onium ethers have not yet been tested pharmacologically but may provide information on the suggested anti-bonding effect of ether oxygen atoms at the myoneural synaptic receptor.

In Part II the biological actions of acetylcholine are reviewed and the possible role of conformational isomerism in its biological activity is discussed. Kinetic and infrared studies in the literature may be reinterpreted to mean that the quasi-ring form of acetylcholine exists in ethanolic and aqueous solution. The hypothesis is advanced that the quasi-ring conformation is involved at the "muscarin receptor and that the extended conformation is complementary to the "nicotinic" receptor.

To establish whether two conformations of acetylcholine co-existed in solution infrared studies of the N-methyl C-H stretching and deformation frequencies and of the carbonyl stretching frequencies of acetylcholine and related compounds were undertaken.

No suitable solvent could be found for the examination of the C-H stretching and deformation frequencies. Infrare spectra were accordingly measured in the solid state for the trimethylammonium salts and these were compared with the spectra of some of the corresponding tertiary bases measured in the liquid state. The spectra were complex in the 3100-2700 cm^{-1} region. No effect attributable to the influence of either a quaternary nitrogen atom or to intramolecular NC-H----O hydrogen bonding on the C-H stretching frequencies was apparent. The examination of the C-H deformation frequencies provided some evidence for NC-H----O bonding in acetylcholine, acetyl-β-methylcholine and succinylcholine but the results are not unambiguous.

Solution spectra of acetylcholine and acetyl-β-methyl-choline in dry dioxan showed split carbonyl absorptions. Low intensity absorption, indicative of hydrolysis, precluded definite assignment of these absorptions to confor ational effects. A series of acetoxyalkyl sulphones formally related to acetylcholine have been synthesised. From measurements of their carbonyl absorptions in dry dioxa the high frequency carbonyl absorption of acetylcholine is best interpreted by inductive effects.

The N.M.R. spectra of acetylcholine and acetyl-β-methy choline in D_2O solution gave no indication of intramolecular NC-H----O hydrogen bonding.

ACKNOWLEDGMENTS

I wish to express my sincere thanks to Professor J. B. Stenlake for suggesting the problem and for his inspiring guidance and supervision throughout, and to Dr. M. Martin-Smith for his guidance and willing contributions to the work on acetylcholine. I am also indebted to my colleagues on the Pharmaceutical Chemistry staff of the Department of Pharmacy of this University for many helpful discussions.

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Last, but not least, to my wife goes a husband's thanks for extending the syllabus of housework and childcare to include thesis work.

CONTENTS

PART I

THE SYNTHESIS OF LINEAR TRIS-ONIUM ETHERS WITH POTENTIAL NEUROMUSCULAR BLOCKING ACTIVITY.

INTRODUCTION.

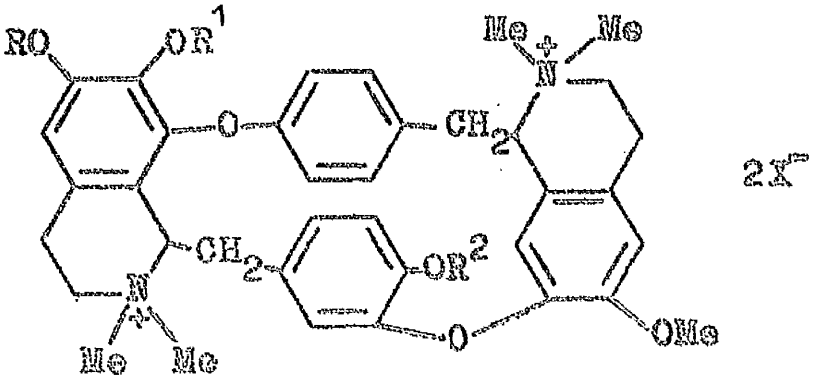
Analgesia, induction of unconsciousness and muscle relaxation are regarded as prime requisites of good surgical anaesthesia.[1,2] Prior to 1942 muscle relaxation was obtained only by deep anaesthesia and other techniques which were hazardous to the patient and exacting on the anaesthetist. Initially ether and chloroform were in general use and with both these agents relaxation was satisfactory, but undesirable side effects combined with an inability to meet the demands of an ever-widening surgical field hastened the introduction of better general anaesthetics. The advantages of these newer agents were purchased at the cost of adequate muscular relaxation. The success of curare preparations in controlling various spastic conditions[3,4] and the convulsions in shock therapy for psychiatric patients[5] suggested that this same drug might be a valuable adjunct to surgical anaesthesia[2]. In 1942 Griffith and Johnson[6] reported the successful use of a purified curare extract in producing muscular relaxation in conjunction with cyclopropane-induced anaesthesia and the following year Cullen[7] published more extensive experience with the drug.

The introduction of drugs which produce muscle relaxation has eliminated the need for deep anaesthesia during most surgical procedures and as a consequence the clinical interest in anaesthetics has altered in the last twenty years.[8] Anaesthesia need only be of a depth sufficient to produce analgesia since the muscular flaccidity which facilitates the surgeons' task has become the function of a second drug. Despite criticisms[9] levelled at the use of these agents as substitutes rather than adjuvants to anaesthesia,[10] unless some unforeseen development occurs in the field of anaesthetics the place of the relaxant drugs in surgery seems assured.[11]

Historical Development

Although 1942 marked the surgical debut of curare, the drug had been known for over three hundred years. Its progress from the poisoned arrows of the Indians of Amazonia to modern clinical and surgical practice constitutes the subject matter of a number of publications.[12] At present the term curare is confined to those arrow poisons which act by muscle paralysis. Boehm[13] considered the poison containers to be a diagnostic aid to their active contents and thus differentiated calabash-curare, pot-curare and tube-curare, but this classification is no longer valid. It has been established[14] that curares from Eastern Amazonia contain material derived from Strychnos species (Loganiaceae) whereas those from the more westerly areas contain extracts from several varieties of Chondodendron (Menispermaceae). Calabash curare was mainly derived from the former while tube-curare largely depended on Menispermaceous sources for its composition.

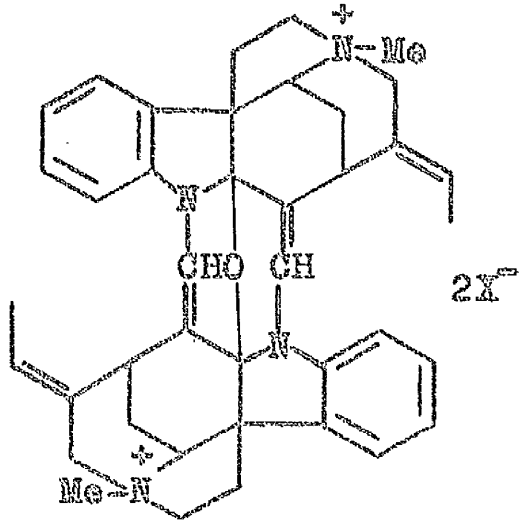
Tube-curare. Boehm[15] recognised that the active principle of tube-curare was a water-soluble quaternary alkaloid and was also aware of the presence of a crystalline tertiary base. Späth, Leithe, and Ladek[16] identified the base and King[17] isolated and elucidated the structure of (+)-tubocurarine (I; R=Me, R^1=R^2=H). Wintersteiner and Dutcher[18] isolated (+)-tubocurarine from authenticated specimens of



(I)

Chondodendron tomentosum thereby confirming the major botanical source of tube-curare. King[19] found the laevorotatory enantiomorph in samples of the vine but this remains the sole instance of its isolation. An isomeric alkaloid, (+)-chondocurarine (I; $R=R^2=H, R^1=Me$), was isolated by Dutcher.[20] The chemistry of these alkaloids has been reviewed by Dutcher[21] and Wintersteiner.[22]

Calabash-Curare. The majority of the alkaloids of calabash-curare are almost certainly derived from the bark of different species of Strychnos. The prefix C- is used to differentiate the curare alkaloids obtained from the arrow poisons from those isolated directly from plant sources.[23] The problems of isolation and the complex chemistry involved are shown by the presence of some forty one alkaloids in a sample of calabash curare[24] and at least thirty in the single plant material S.toxifera.[25] It is now recognised that the alkaloids are either mono- or bis-quaternary,[26] those with the highest physiological activity being in the latter class. C-curarine I (II) was the first calabash alkaloid to be isolated[27] and its structure has recently been described.[28] Toxiferine I (III) is the most potent curarising alkaloid of known structure[29,30] being fifteen to thirty times as potent as (+)-tubocurarine. Numerous reviews have been published on the complex botany[14,31]



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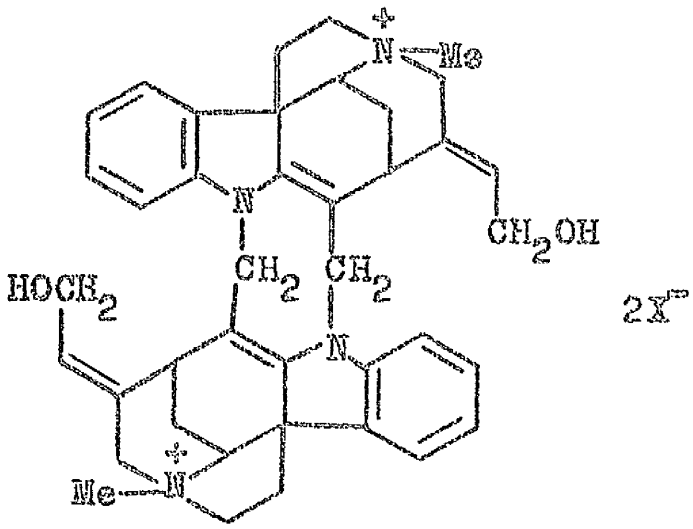


(III)

and chemistry[14,31,32] of calabash-curare and Strychnos species.

Pharmacological Considerations

The brilliant experiments of Claude Bernard and Pelouze[33,34,35] localised the site of action of curare to some point between nerve and muscle. The current view[36-40] of the sequence of events occurring on passage of an impulse from nerve to muscle has been reached by a variety of means including electron microscopy, histological and chemical studies and electrophysiological measurements. The arrival of the impulse at the nerve ending results in the liberation of acetylcholine[41,42] from presynaptic, intra-axonal storage sites which are probably the vesicles[42-46] occurring at the terminal.[47-49] The liberated acetylcholine traverses the cleft between nerve ending and motor end plate by a process of diffusion[50,51] and combines with specific receptors at the post-synaptic membrane.[52] The permeability of this membrane for sodium, potassium and other ions is profoundly altered by the acetylcholine-receptor interaction[53] and the concomitant depolarisation establishes a non-propagated end plate potential. This potential on reaching a threshold value overcomes the resistance of the surrounding muscle membrane, and the resulting propagated action potential[42,50,54] produces a contracture by a mechanism[55,56,57] which at the moment remains obscure. The polarised state of the end plate is restored by the rapid hydrolysis of the transmitter by acetylcholinesterase in the junctional region.[58] Koelle[59] has introduced a modified view in which acetylcholine liberated by the impulse acts at the nerve ending to release additional quanta of acetylcholine which then effect transmission.

Nachmansohn[60-64] envisages axonal conduction and neuromuscular transmission as taking place by similar mechanisms. In this case, currents generated in the nerve endings cause the liberation of acetylcholine at the end plate. The absence of permeability barriers at the junctional region accounts for the blocking action of drugs on neuromuscular transmission only, leaving axonal conduction unimpaired. A considerable body of evidence exists to substantiate these views.[65-72]

The precise mechanism of neuromuscular transmission is not relevant as far as the present work is concerned. What is important is that acetylcholine reacts with some specific receptor at the end plate to initiate a sequence of events which results in muscle contraction. On this basis the classification of neuromuscular blocking agents can be approached rationally.

The Classification of Neuromuscular Blocking Agents

Loss of tone in skeletal muscle can be achieved by blocking nerve impulses either centrally or at the neuromuscular junction. The generic term muscle relaxant is applied to drugs which produce muscular flaccidity by either mechanism.[73] Neuromuscular blocking agents are those drugs which act peripherally at the myoneural junction. Interference with the mechanism of transmission must result in paralysis and a number of types of block are recognised.

1. Deficiency Block

A deficiency of acetylcholine can arise either from depression of its synthesis from choline (by acetylation with choline acetylase[74,75]) or by prevention of its release. The hemicholiniums (page 33) and triethylcholine[76] (IV) belong to the former category. Prevention of release can be caused by deficiency of calcium ions.[77]

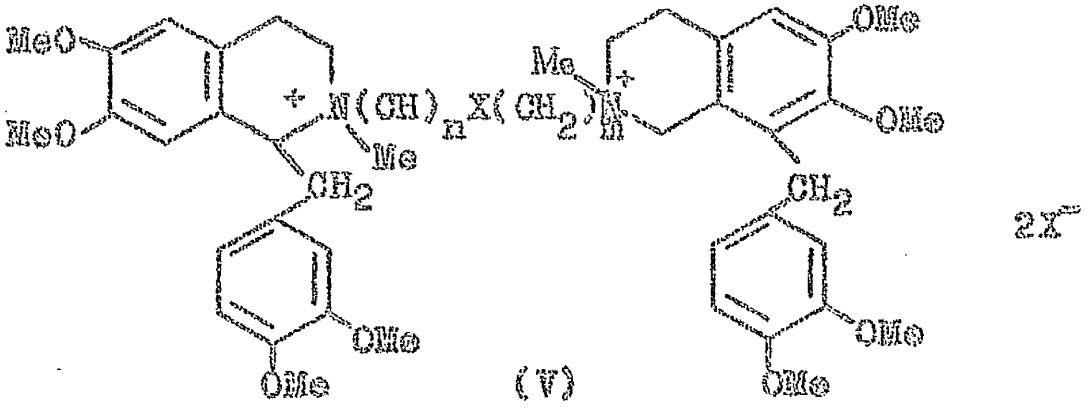
$$Et_3\overset{+}{N}CH_2CH_2OH \quad OH^-$$

(IV)

excess of magnesium[78] and phosphate ions,[79] by procaine[38,80] and by the toxin of Clostridium botulinum.[81,82]

2. Non-depolarising Block (Antidepolarising; Competitive)

In this type of block the drug competes with acetylcholine by combining with specific receptors in the postsynaptic muscle membrane. This combination does not cause depolarisation but merely reduces the end plate potential to a value below that necessary to initiate a propagated action potential[83] and hence a muscle response isprevented. Nerve and muscle retain their direct excitability and acetylcholine release is unimpaired.[41] Increase in the local concentration of acetylcholine by inhibition of acetylcholinesterase[84] or repetitive stimulation of the motor nerve[85] causes reversal of the block. The most important drugs in this group are (+)-tubocurarine (I; R=Me, $R^1=R^2=H$), (+)-tubocurarine dimethylether[18,86] (I; $R=R^1=R^2=Me$), C-toxiferine I(III), gallamine[87] (LXXVI) and laudexium[88] (V; n=4, m=5, $X=CH_2$).



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3. Depolarising Block

High concentrations of acetylcholine normally prevented by acetylcholinesterase, cause persistant depolarisation of the end plate and neuromuscular blockade results.[89] The

TABLE I

Comparison of Effects of Non-depolarisers and Depolarisers

	Non-depolarisers	Depolarisers
1. Species Sensitivity	Rat>hare>monkey>rabbit>man>dog>cat>hen	Hen>cat>man>rabbit>dog>monkey>hare>rat
2. Sensitivity of different muscles in one species		
Cat	Respiratory and soleus muscles (red muscles)) tibialis (white muscle)	Tibialis>respiratory and soleus
Man	Laryngeal, pharyngeal, ocular and hand muscles more sensitive than other muscles	Less difference between different sets of muscles
3. Initial excitation	None	Spontaneous fasciculations
4. Type of paralysis In avian muscle In frog muscle	Flaccid Antagonistic to contracture caused by depolarisers	Spastic Contracture
5. Effect of		
(+)-tubocurarine	Synergism	Antagonism
Ether	Potentiation	Antagonism
Tension during tetanus	Rapid waning	Well sustained
Anticholinesterases	Antagonism	Little effect
Potassium	Antagonism	No effect
Reduction of Temperature	Reduces block	Deepens and prolongs block
Myasthenia gravis	Hypersensitivity	No hypersensitivity, increased tolerance and initial strengthening

depolarisers such as decamethonium[90,91] (VI; n=10, R=Me), succinylcholine[92] (VII) and carbolonium bromide[93] (VIII) resemble acetylcholine in their action causing electrical inexcitability by persistent depolarisation. The main points of difference between non-depolarisers and depolarisers are summarised in Table 1.[94,95] Although depolarisers also compete with acetylcholine for the same receptors the misleading term "non-competitive" has been used to describe this block.

$$R_3\overset{+}{N}(CH_2)_n\overset{+}{N}R_3\,2X^- \qquad \text{(VI)}$$

$$Me_3\overset{+}{N}(CH_2)_2OCOCH_2CH_2COO(CH_2)_2\overset{+}{N}Me_3\,2X^- \qquad \text{(VII)}$$

$$Me_3\overset{+}{N}(CH_2)_2OCONH(CH_2)_6NHCOO(CH_2)_2\overset{+}{N}Me_3\,2X^- \qquad \text{(VIII)}$$

4. Dual block (Mixed block)

The differences between (+)-tubocurarine and decamethonium at first indicated that their mechanisms of action were diametrically opposed but it is now recognised that the block produced by the two compounds is not entirely unrelated. Man and cat are the only two mammalian species in which a pure acetylcholine-like action can be demonstrated for decamethonium and succinylcholine.[95] Zaimis[96] has demonstrated that these two blocking agents in the monkey, dog, rabbit and hare produce a type of block which, in addition to features of blockade of type 2 or 3 (vide supra), has features, such as tachyphyllaxis, which are representative neither of pure depolarisers nor pure non-depolarisers. Such activity is referred to as dual block. Whether (+)-tubocurarine-like or decamethonium-like activity predominates is a function not only of the muscle under examination but also of the drug since succinylcholine, compared with decamethonium, is a more faithful mimic of acetylcholine.[95] The biphasic action of decamethonium has been clearly

demonstrated in the isolated rabbit lumbrical muscle.[97]

Bovet[98] devised the terms pachycurare and leptocurare to distinguish the two main classes of neuromuscular blockers. The former were bulky molecules with a tubocurarine-like action whereas the latter were long thin molecules which caused depolarisation.

In an extension[99] of a general theory[100] of drug receptor interaction, neuromuscular blocking agents have been classified[101] using the parameters of affinity and intrinsic activity. Affinity defines the complex-forming ability of the drug with the receptor, the activity of this complex being the intrinsic activity. Cholinomimetics or depolarisers have affinity for acetylcholine receptors and have high intrinsic activity; cholinolytics or competitors have affinity for the same receptors but have a low intrinsic activity, non-competitors have affinity for other than acetylcholine receptors. This classification has the advantage of accomodating compounds with intermediate types of activity.

All methods of classification are complicated by variation in the response not only of different species but also of various muscles within a single species. Even such a reliable cornerstone as (+)-tubocurarine can cause depolarisation,[102] contracture and fibrillation[103]. Furthermore, a presynaptic site of action has been postulated[104,105] for this compound and thus blockade could be considered in part as due to deficiency of acetylcholine.

Of the large number of substances which have been tested for neuromuscular blocking activity, few have been examined beyond a simple estimate of potency often on a single preparation and even fewer have been adequately classified. The small number of compounds whose mode of action has been rigorously analysed have been instrumental

in developing present day concepts of neuromuscular blockade and in furthering research into the nature of the acetylcholine receptor.

The Cholinergic Receptor

The term receptor was invented by Ehrlich[106] and subsequently developed by Langley[107] and Lucas.[108] The receptor theory is now an integral part of pharmacology and its acceptance has aided the formulation of quantitative relationships[109,110] which have been used to establish a fundamental picture of drug action.

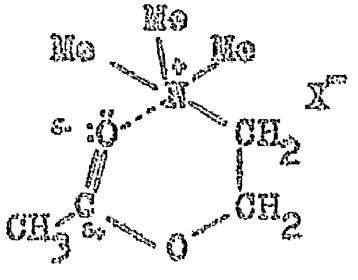
The precise nature of receptors is open to conjecture but some deductions have been made concerning their geometry and charge distribution by assuming the receptor will have shape and charge complementary to molecules with the highest biological activity.[111,112] This approach only provides unambiguous results when rigid molecules are employed since non-rigid molecules can exist in an infinite number of conformations. On the other hand, the receptor may alter at the demand of a drug molecule, in which event conclusions derived from rigid molecules are of doubtful value.[113] In some instances receptors are identified with proteins[114] and enzymes,[115] whereas in others a discrete physical entity is not envisaged.[116] The cholinergic receptor has been subjected to rigorous examination and a wealth of pertinent information has resulted.

Acetylcholine acts at many sites in the body and certain drugs can be classified by the manner in which they interfere with the normal functions of acetylcholine at the various sites, for example muscarinic and nicotinic drugs. The term nicotinic now tends to be replaced by ganglion stimulant, ganglion blocker, neuromuscular stimulant and neuromuscular blocker since the action of nicotine varies with dose level.

Analysis of the structural features associated with muscarinic and nicotinic activity has revealed that steric effects combined with a favourably disposed electron density at critical points in a molecule are more important than the presence of a particular chemical group.[8]

The fact that acetylcholine has different types of activity, and the ability of other compounds to elicit effects which are qualitatively similar, could be due to one of several factors. One is inability to cross certain permeability barriers and another could be attributed to divergence in the nature of the cholinergic receptor at various sites. Acetylcholine is a small molecule possessing rotational freedom, and thus conformational flexibility, and the predominant conformation will be that which permits most intimate contact with the receptors.[117] There will then be one conformation of acetylcholine complementary to each type of receptor.

Comparative kinetic and infrared measurements[118] were originally interpreted as meaning that acetylcholine exists in solution in the cyclic conformation (IX). The kinetic studies revealed for the acylation of hydroxylamine by acetylcholine a rate constant which was much higher than that observed with the homologous 3-dimethylaminopropyl acetate (X) and the 4-dimethylaminobutyl acetate (XI) methiodides. The infrared spectra of acetylcholine and



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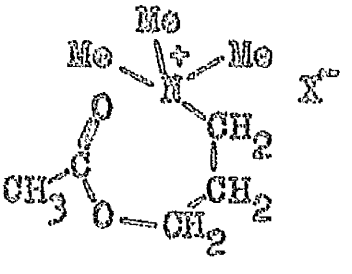

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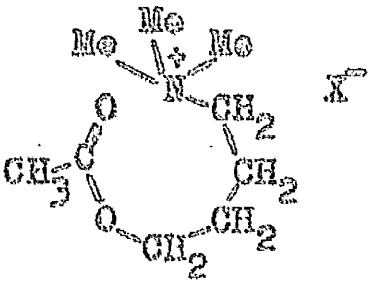
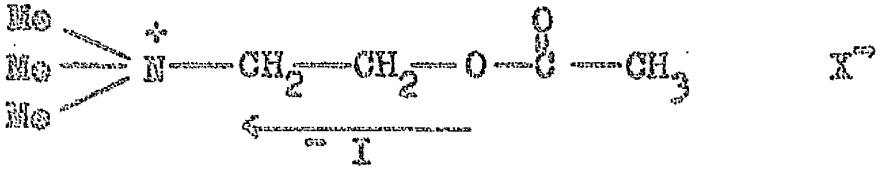


(XI)

the higher homologous methiodides, measured in ethanol, all exhibited two absorption bands attributable to the ester carbonyl functions. The lower frequency bands can be interpreted[119] as arising from intermolecular hydrogen bonding between the ester and the solvent, being some 20 cm^{-1} lower than the non-hydrogen bonded absorptions. The non-bonded absorption of acetylcholine which occurred at 1760 cm^{-1}, as compared with 1752 cm^{-1} for the absorption of the propyl acetate (X) and 1748 cm^{-1} for the butyl acetate (XI) was taken as evidence for the cyclic conformation (IX). However, this higher frequency observed for acetylcholine should have been taken to indicate a greater degree of double bond character[120,120a,120b] in the carbonyl group than was present in the other compounds, a result incompatible with the postulated conformation (IX) in which the carbonyl frequency would be decreased due to increased polarisation of the (C=O) group on bonding. In a later publication[120b] the cyclic structure (IX) was rejected and the kinetic and infrared results reinterpreted in terms of an inductive effect (XII) from the quaternary nitrogen which is compatible with the higher rate of acylation and increased carbonyl frequency in acetylcholine when compared with the higher homologous methiodides. The arguments

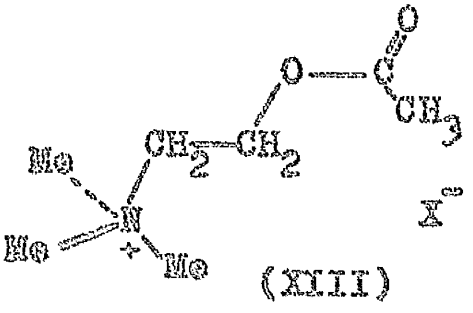


(XII)

presented to account for the higher frequency of the carbonyl group in terms of the inductive effect of the quaternary nitrogen atom have recently been criticised[120a] from infrared measurements of acetylcholine in various solvents by both

transmission and attenuated total reflection methods.

X-ray studies[121] have shown that the acetylcholine ion exists in the crystal lattice in two distinct conformations. One is the fully extended, fully staggered conformation (XIII), which had earlier been assumed to be the most likely conformation adopted by acetylcholine and the other is the quasi-ring conformation(XIV)in which the choline moiety forms an approximately planar ring having a "short" intramolecular distance[123,124] of ca. 3A° from the acyloxy oxygen atom to the methyl group.



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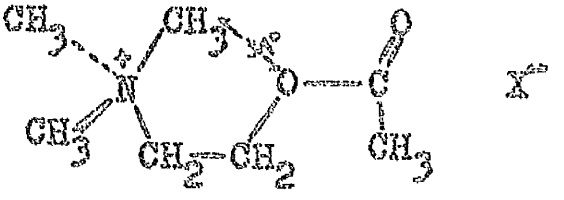
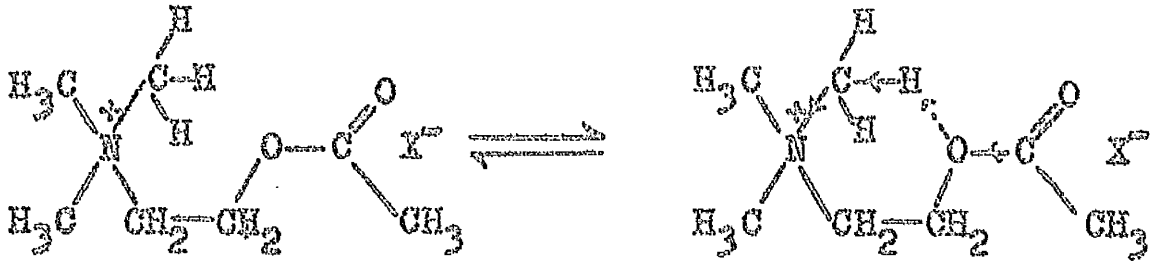


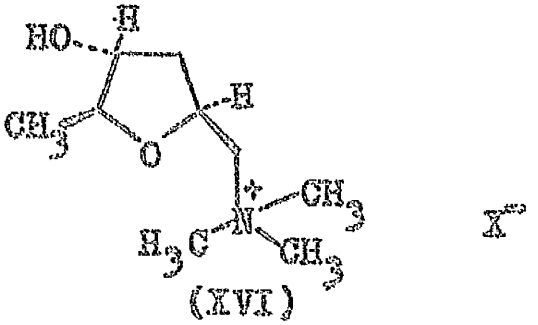
(XIV)

The unsuspected existence of this second conformation could be due to stabilisation by a series of inductive effects (XV). One of the N-methyl groups would, by the mechanism indicated, give rise to a quasi six-membered ring. These inductive effects, although not previously recognised have analogies in the literature. Thus the infrared C-H stretching of N-methyl groups is well known to occur at lower frequency than normal C-H stretching.[122] This lower bond order[120] must be due to inductive effects which arise solely as a result of the greater electronegativity of the tertiary nitrogen atom. When the nitrogen atom is quaternary a more pronounced effect would be expected. These inductive effects could be expected to give rise to a weak hydrogen bond to the alcoholic oxygen of the acetoxy moiety.



(XV)

This situation has an analogy in the recent formulation of C-H---O bonds in crystals.[123,124] X-ray crystallographic studies on muscarine[125,126] have revealed a similar ring structure (XVI) to that found in acetylcholine and in this instance C-H---O bonding has also been proposed.[123,124] Although it has not been conclusively proven, C-H---O hydrogen bonding has been postulated to explain the complex-forming properties of esters[127] and the adsorption of various organic solutes to chromatographic alumina.[128] As a result of hydrogen bonding the carbonyl carbon is rendered electron deficient through the inductive effect as shown (XV) and this will depress permanent polarisation of the carbonyl function. Thus both the kinetic studies and the infrared measurements (vide supra), although satisfactorily explained by the electronic effects shown in (XII), are in excellent agreement with (XV). Infrared spectral studies having direct bearing on these points are described in Part II of this thesis.



(XVI)

Sörum[121] has suggested that the quasi-ring conformation (XIV) of the acetylcholine molecule would predominate in

non-polar media whereas the extended form (XIII) would prevail in polar media. It is also suggested that the presence of two conformers could account for the behaviour of acetylcholine at lipid-water interfaces. The data used to explain the existence of the quasi-ring form of acetylcholine has recently been criticised but the above arguments still apply since muscarine iodide in the solid state exists solely in the quasi-ring form (XVI).

It would be intriguing should the two conformations of acetylcholine be involved at different receptors. Previously the different actions of acetylcholine have been attributed to differences in the various sites of action, mediated by permeability and other factors, and authors have largely neglected the possibility of conformational isomerism being involved. It is therefore suggested that the muscarinic receptors are complementary to the ring conformation of acetylcholine and that the nicotinic receptors are complementary to the extended form. Compounds possessing similar conformations or having the ability to adopt such conformations will then have either muscarinic or nicotinic properties. The validity of this hypothesis is supported in general by reference to many compounds possessing muscarinic and nicotinic activity (Part II) and in particular by muscarine which exists solely in the quasi-ring conformation (XVI). The reservation must be made however that even if XIII and XIV are the two most likely conformations of acetylcholine in solution then they are not necessarily the conformations involved in complexing with the receptor.

Recent studies by Waser[129] and Beckett[130] have indicated a three-point receptor attachment for muscarine and other compounds possessing high muscarinic activity and a complementary receptor has been tentatively proposed[130]

(Figure 1).

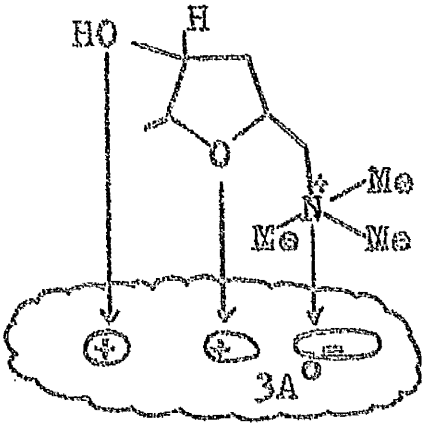


Figure 1.

The muscarinic receptor

Nicotinic activity has been associated with compounds possessing a cationic nitrogen atom and an atom bearing a partial positive charge three atoms removed from the nitrogen atom.[8] This has led to the classical representation (Figure 2) of the nicotinic receptor. The receptors affected by neuromuscular blocking agents are similar to those involved in the nicotine-like actions of acetylcholine[8] but the two cannot be absolutely identical since nicotine blocks the ganglia more than the neuromuscular junctions and also neuromuscular blocking agents have scarcely any effect on ganglia.

It has been agreed that a formal similarity exists between acetylcholinesterase and the cholinergic receptor although they have been shown to be independent entities.[131] Recently the cholinergic receptor has been postulated to be acetylated acetylcholinesterase.[131b] A difference between the enzymatic and muscarinic sites could perhaps explain the results of Friess and his colleagues[132] who found that epimuscarine is the most potent isomer in inhibiting acetylcholinesterase whereas muscarine is the most potent cholinergic isomer. Krupka and Laidler[133] have proposed that acetylcholine is bound to the enzyme by a three point attachment and have developed a picture of the active site in the enzyme

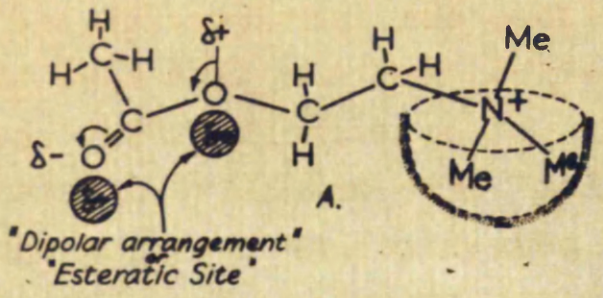


Figure 2. The nicotinic receptor.

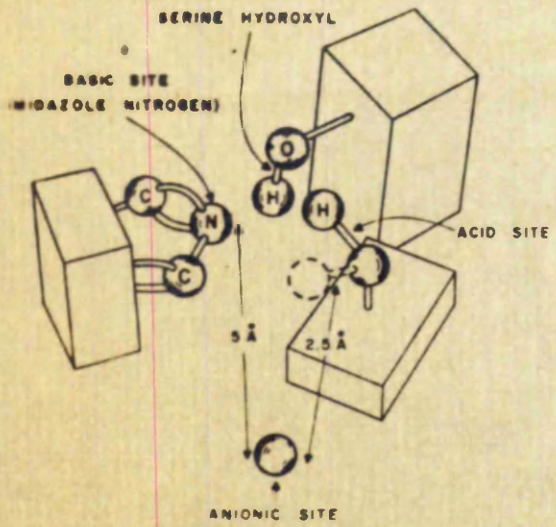


Fig. 3—Proposed arrangement of functional groups at the active centre of acetylcholinesterase.

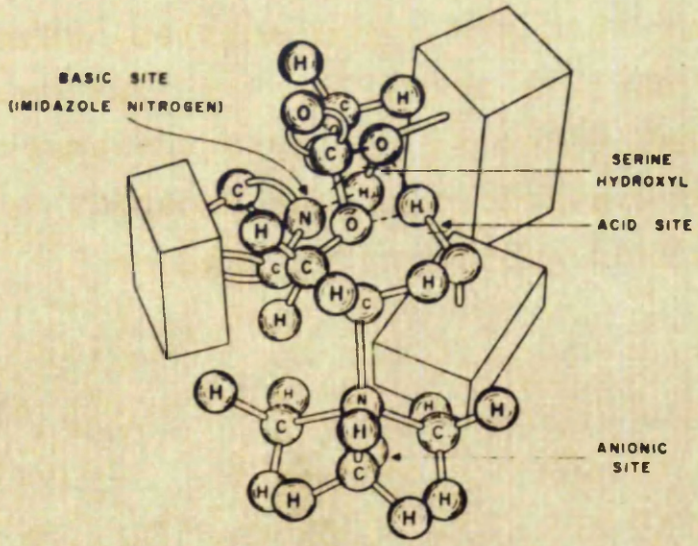
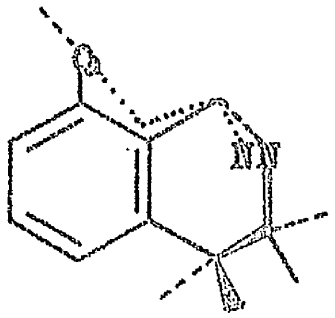


Fig. 4—A suggested structure for the Michaelis complex between acetyl choline and the enzyme; the dotted lines indicate electrostatic attractions and also bonds that are formed during acetylation. There is also electrostatic attraction between the imidazole nitrogen atom and the carbonyl carbon atom. The three bonds broken are indicated on the diagram.

(Figures 3 and 4). If a similar arrangement is present at the muscarinic receptor then, as Waser[134] suggested, these sites must have a three dimensional structure into which cholinergic molecules fit. By analogy a linear and surface distribution of sites, complementary to the extended conformation of acetylcholine, would appear more probable for the nicotinic receptor. The overall effect would be a system of anionic "cavities" with satellite dipolar structures. Indeed a study of neuromuscular blocking agents has strengthened the view that the receptor upon which these agents act possesses a mosaic of anionic sites. It is of interest that (+)-tubocurarine has a steric arrangement (XVIII) about one of the quaternary nitrogen and other functions similar to that of the extended conformation of acetylcholine, and this could be a part explanation of the non-depolarising action of (+)-tubocurarine, whilst the polymethylene bis-trimethylammonium quaternaries, lacking any functional groups other than the onium centres, cause depolarising block. The initial stimulatory effect seen with these compounds is probably due to the trimethylammonium cationic head. Two representations of the receptor involved in neuromuscular blockade are shown in Figures 5[135] and 6[136] and these



N——O = 4.2A°

N·····O = 3.85A°

Dreiding Models

(XVIII)

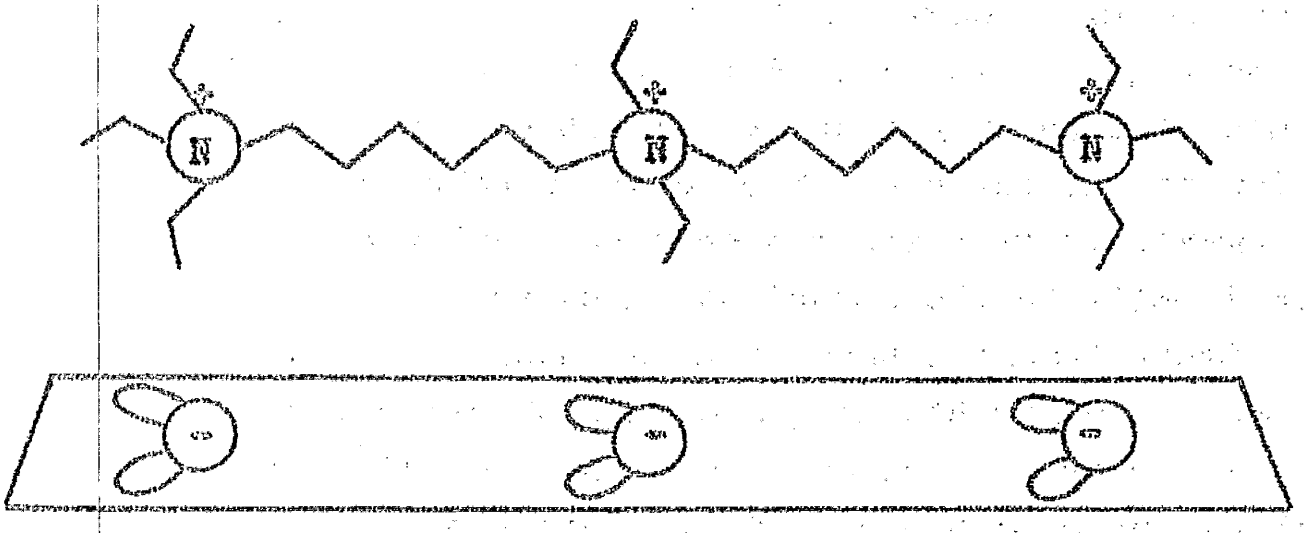


Figure 5

Representation of receptor involved in neuromuscular blockade

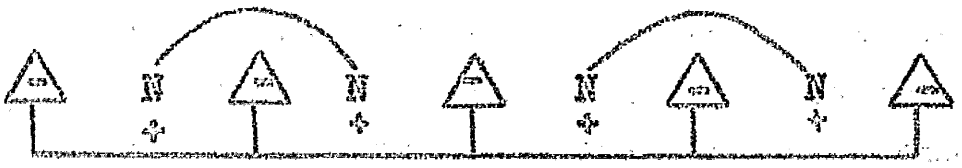


Figure 6

Representation of receptor involved in neuromuscular blockade

incorporate earlier theories of two point attachment to the receptor.[137]

An interonium distance of 14A° in bis-quaternaries was originally postulated for maximum curariform activity and this concept was extended to other molecules possessing neuromuscular blocking activity. The optimal distance is now regarded to be ca. 9-10A°, the speculated distance between the onium groups of (+)-tubocurarine, toxiferine I, gallamine and various other potent muscle relaxants, which is compatible with the distance from anionic site to anionic site in the postulated nicotinic receptor.

Other theories have been advanced to explain the action of neuromuscular blocking agents with receptors. One example is the one point attachment theory[138] in which the bulk of the molecule shields the receptor. The view that the receptor acts analogously with a cation exchange resin has also been forwarded.[97] Most theories involve at least one anionic site in the receptor. Cavallito[139] has suggested that this could act as a focus for the attachment of an onium group and that such bonding could induce configurational changes in the molecule resulting in the reorientation of other functional groups into positions suitable for multi-point attachment to the receptor.

The nature of the receptor

The exact physical nature of receptors is unknown although the concept of receptors is widely accepted. Chagas and his colleagues[140] attempted to bind radioactive gallamine to an extract of electric tissue from Electrophorus electricus and isolated, from the complex formed, a polysaccharide related to hyaluronic acid.[141] Experiments have indicated that the binding although strong is probably non-specific.[142] Reinvestigation of the polysaccharide material under a variety of conditions has recently been

undertaken.[143] Ehrenpreis isolated,[144,145] from a similar source, a protein which showed bonding characteristics remarkably similar to those of the cholinergic receptor *in vivo*. Isolation of a receptor protein from muscle has been claimed by Nistratova and Turpaev.[146]

The nature of the active sites is almost totally unknown but a number of suggestions have been tentatively forwarded. Thus the anionic site has been identified with phosphate[136,145] and carboxyl groups[136] and the esteratic site with an imidazolyl[134] group. Waser[131,134] has suggested that the receptor might be a pore in the postsynaptic membrane (Figure 7) and a similar approach has been adopted by Nachmansohn.[60] The action of acetylcholine may be to alter the configuration of the wall by introducing "local folding in a section of the protein which may remove a positively charged amino group from a strategically located point" thereby permitting the flow of sodium and potassium ions through the membrane. A very small shift of the charge may be sufficient. The

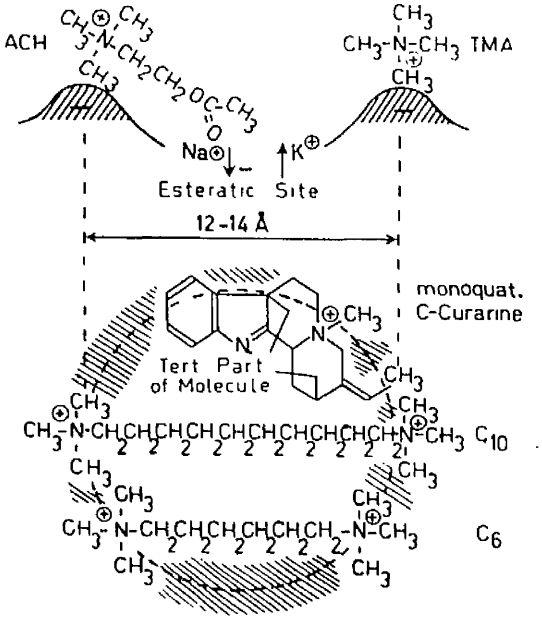


Fig. 7. Model of a receptor pore in section and from above. Anionic wall occupied by depolarizing acetylcholine (ACH) or tetramethylammonium (TMA), at the bottom permeation of sodium or potassium ions. The pore can be blocked by one bisquaternary or two monoquaternary curarine molecules, or by many smaller diamines such as hexamethonium (C_6). Depolarizing decamethonium (C_{10}) will leave enough room for ions to pass beside the molecule.

feasibility of such a scheme is demonstrated by recent work in an unrelated field where complexing of a protein molecule with traces of undissociated buffer acid produced subtle changes of structure which altered the net charge on the protein but not its molecular weight and frictional coefficient.[147]

Neuromuscular Blocking Agents in Clinical Practice

Crum Brown and Fraser[148] observed that the methiodides of strychnine, brucine, thebaine, codeine, morphine, atropine and coniine all produced paralysis similar to that of curare. These workers clearly established the association between neuromuscular blocking activity and quaternary ammonium compounds. It was subsequently demonstrated that not only tetraalkylammonium but also tetraalkylphosphonium, -stibonium, -arsonium and trialkylsulphonium salts all possessed blocking properties.[149,150] Neuromuscular blocking activity thus became associated with onium compounds as a class.

The knowledge that (+)-tubocurarine possessed two quaternary nitrogen functions, together with its successful introduction into anaesthetic practice, provided a stimulus to the search for simpler substitutes. In 1946 the first investigations into synthetic compounds modelled on (+)-tubocurarine led to gallamine.[87] Two years later, Barlow and Ing,[90] and simultaneously, Paton and Zaimis,[91] prepared compounds possessing two quaternary nitrogens separated by a polymethylene chain. Decamethonium was the most active and since then many similar compounds have been prepared. On the one hand the chain linking the two onium groups has been modified to include ether, ester, aromatic and hetero-cyclic functions. Alternatively the polymethylene chain has been retained and the onium centre altered either by higher alkyl or aralkyl substitution or by incorporation into a heterocyclic ring system. In yet other examples both

TABLE 2.[11]

Dose Levels and Potencies of Clinical Drugs

Agent	Initial dose in mg. N_2O/O_2/Barbiturate anaesthetic †		Comparative Potency On Weight Basis of		
	Average	Range	Cation	Salt	on Molar Basis
(+)-tubocurarine	17·2	10·0-30·0	1·0	1·0	1·0
Dimethyltubocurarine	8·9	4·0-18·0	3·2	2·9	3·4
Toxiferine I	2·0	0·5- 4·0	8·5	8·6	7·5
Gallamine	77·0	40·0-100	0·3	0·2	0·2
Benzoquinonium*	12·3	4·5-18·0	1·0	1·1	0·9
Laudexium	30·0	-	0·5	0·5	0·7
Decamethonium	2,4	1·0- 4·0	7·7	6·0	3·1
Succinylcholine	30·0	10·0-50·0	1·6	1·8	0·9
Carbolonium	3·5	-	5·7	5·0	3·4

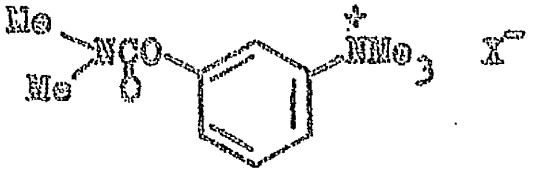
* Now seldom used[38]

† Marked reduction of dose of non-depolarisers with ether as anaesthetic since ether is itself a muscle relaxant.[154]

chain and end group have been modified. Compounds possessing more than two onium centres have also been prepared and although with the exception of gallamine, none have been used clinically, these polyonium compounds have been of considerable theoretical significance. At present, the search for better muscle relaxants has reverted to natural, as opposed to synthetic, materials. Certain alkaloids from calabash curare and Strychnos species,[151,152] and synthetic derivatives such as diallyl-nortoxiferine,[153] have yielded particularly promising results in this field. Table 2 lists the clinically useful blocking agents together with dose levels and relative potencies. It must be stressed that these figures apply to the anaesthetic system indicated. The synergism existing between certain anaesthetics and some of these drugs necessitates reduction of the dose often by sixty per cent.[38] The advantages and disadvantages of these compounds have been summarised in a recent review.[38]

Antagonists of Neuromuscular Blocking Agents[155]

The anticholinesterase drugs are successful antagonists of non-depolarising blocking agents and some of these are used clinically for this purpose. Neostigmine (XIX) and edrophonium (Tensilon)(XX) are particularly useful in this respect and their antagonism is not wholly due to their anticholinesterase activity. The depolarising drugs are not antagonised by these agents, a fact frequently utilised to distinguish the two main classes of neuromuscular blockers. Certain substances can antagonise the effects of decamethonium



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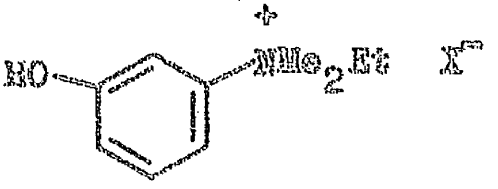


(XX)

and other depolarising drugs but have no clinical applications and cannot be used as a means of determining the type of action exhibited by a muscle relaxant.[8]

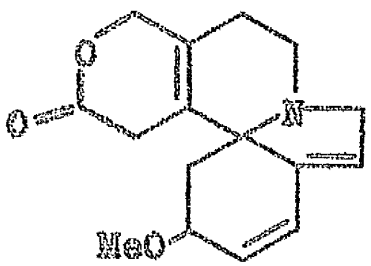
Chemical Considerations

Many reviews on various aspects of neuromuscular blockade have appeared in the last ten years. The mechanism of action of neuromuscular blocking agents[38] and their pharmacology in man[11] constitute the subject matter of two recent publications. The chemical aspects of neuromuscular blockade have very recently been analysed in an extensive review.[156] Similar aspects are relevant to the present work but discussion has been limited to compounds possessing other linkages.

NEUROMUSCULAR BLOCKING AGENTS WITH ETHER LINKAGES

1. TERTIARY BASES

The importance of an onium centre to neuromuscular blockade is amply illustrated by the almost complete absence of activity in the corresponding precursor derivatives.[156,157] In one or two instances, however, activity is associated with the tertiary base rather than the quaternary salt. The most notable compounds of this type are the Erythrina alkaloids,[158] in particular β-erythroidine (XXI) and its dihydro-derivative (XXII). These bases resemble (+)-tubocurarine in activity and quaternisation reduces potency.[159]



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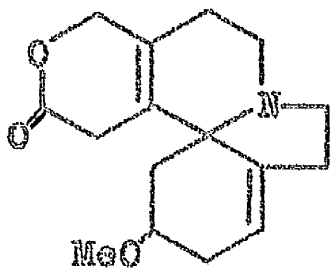
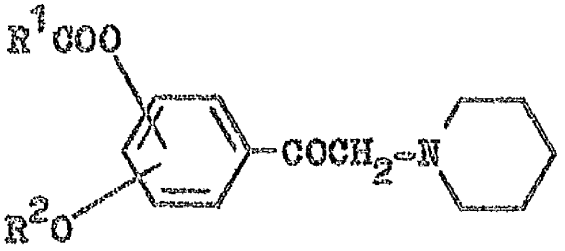


(XXII)

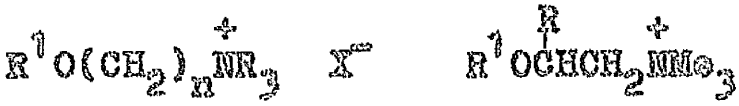
In a series of 1-(alkoxy-arylcarboxyphenacyl)-piperidines (XXIII; R^1=Ph, R^2=Me or Et) maximum activity is likewise associated with the tertiary compounds.[160]



(XXIII)

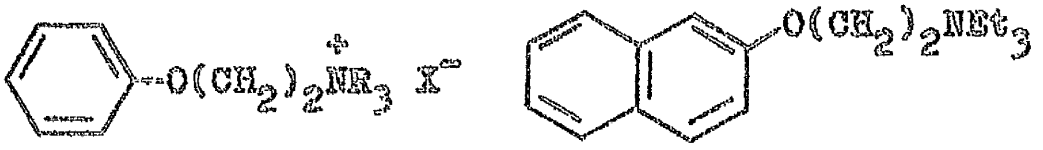
2. MONOQUATERNARY ETHERS

Simple alkyl ethers of formocholine,[161,162] (XXIV; n=1, R=Me, R^1=alkyl) choline[161,163] (XXIV; n=2, R=Me, R^1=alkyl), triethylcholine[164] (XXIV; n=2, R=Et, R^1=alkyl), homocholine[165] (XXIV; n=3, R=R^1=Me) and β-alkylcholines[166,167] (XXV; R=R^1=alkyl) all exhibit paralysing activity.



(XXIV) (XXV)

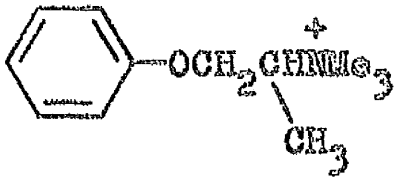
The various pharmacological actions of these compounds have been carefully tabulated by Craig[168] in an early review. Phenyl[87,169,170] (XXVI) and naphthyl[172] (XXVII) ethers of



(XXVI) (XXVII)

cholines show similar activity to the alkyl ethers. Choline phenyl ether (XXVI; n=2, R=Me) has a rabbit head drop dose (H .D.D.) of 2.5mg./kg.[170] whereas the triethyl analogue (XXVI; n=2, R=Et) requires a dose of 20mg./kg.

to produce a comparable effect.[87,173] Methyl substitution in the aromatic nucleus decreases potency and the block passes from mainly decamethonium-like to tubocurarine-like as more methyl groups are added to the aromatic nucleus.[169] The phenyl ether of α-methyl choline (XXVIII) blocks the



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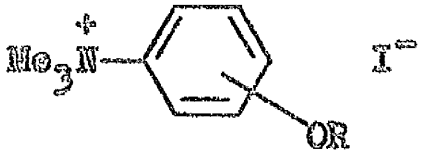
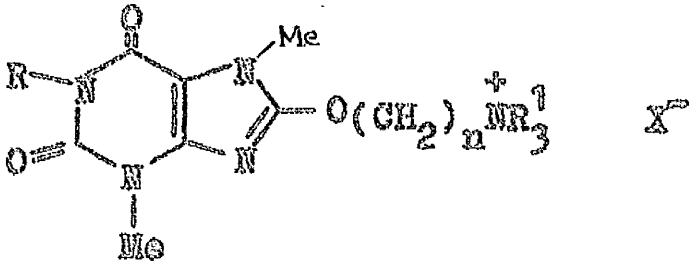


(XXIX)

cat anterior tibialis by depolarisation.[174] In the aminophenolic ethers[175] (XXIX; R=H or alkyl) the predominant effect was either "anticurare" or paralysing activity depending on the position of the phenolic function, the antagonistic effects being maximal with a free phenolic group in the meta position. Monoquaternary salts derived from 8-dialkylaminoalkoxycaffeine[176] (XXX; R=Me) showed potencies varying from one tenth to one hundredth of gallamine in rabbits and mice; with one exception the corresponding theobromine[177] (XXX; R=H) compounds were inactive.



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In general all of these monoquaternary salts are predominantly cholinergic in their actions and only at higher dose levels do they block conduction.

TABLE 3.

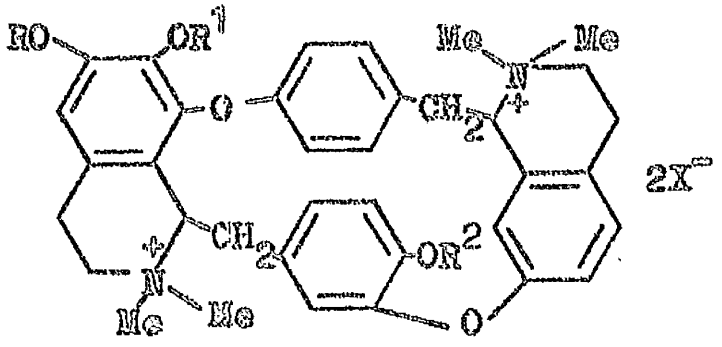
Comparative Potencies of *Chondodendron* Alkaloids and Derivatives

Compound	Structure	R	R^1	R^2	Potency (Rabbit)	Ref.
(+)-Tubocurarine	XXXI	Me	H	H	1·00	178
(−)-Tubocurarine	"	Me	H	H	0·06 in Rat	"
(+)-Curarine	"	Me	H	H	3·5	"
(−)-Curarine	"	Me	H	H	1·3	"
(+)-Chondocurarine	"	H	Me	H	2·9	"
N,N'-Diethylchondocurine	"	H	Me	H	0·9	"
N,N'-Dibenzylchondocurine	"	H	Me	H	0·17	"
N,N'-Dimethylisochondodendrine	XXXII	H	-	-	0·06	"
O,O'-Dimethyl-(+)-tubocurarine	XXXI	Me	Me	Me	6·7	"
O,O'-Di-n-butyl-(+)-tubocurarine	"	Me	Bu	Bu	0·09	"
O,O'-Dimethyl-(−)-curarine	"	Me	Me	Me	3·30	"
O,O'-Dimethyl-(+)-curarine	"	Me	Me	Me	1·6	179
O,O'-Dimethyl-N,N'-dimethylisochondendrine	XXXII	Me	-	-	0·26	178
O,O'-Diethyl-N,N'-diethylisochondodendrine	"	Et	-	-	Similar to (+)-tubocurarine	180
O,O'-Diamyl-N,N'-diamylisochondodendrine	"	Am	-	-	Similar to (+)-tubocurarine	180

3. BIS-QUATERNARY ETHERS

A. NATURAL PRODUCTS AND THEIR DERIVATIVES

Tubocurarine (XXXI; R=Me, R^1=R^2=H) contains two optical centres and the four stereoisomers show significant differences in potency. These and other quaternary salts from Chondodendron species are shown in Table 3. The position



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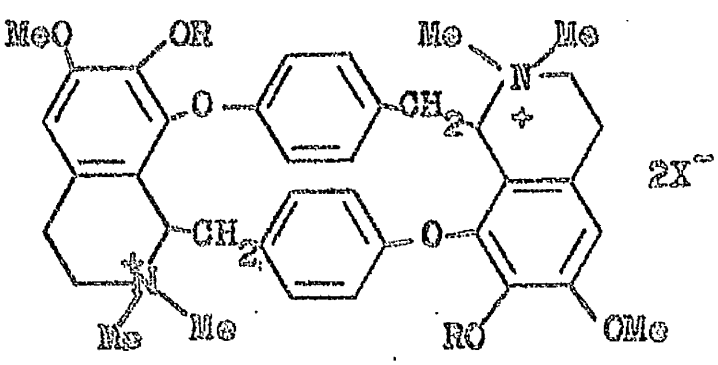
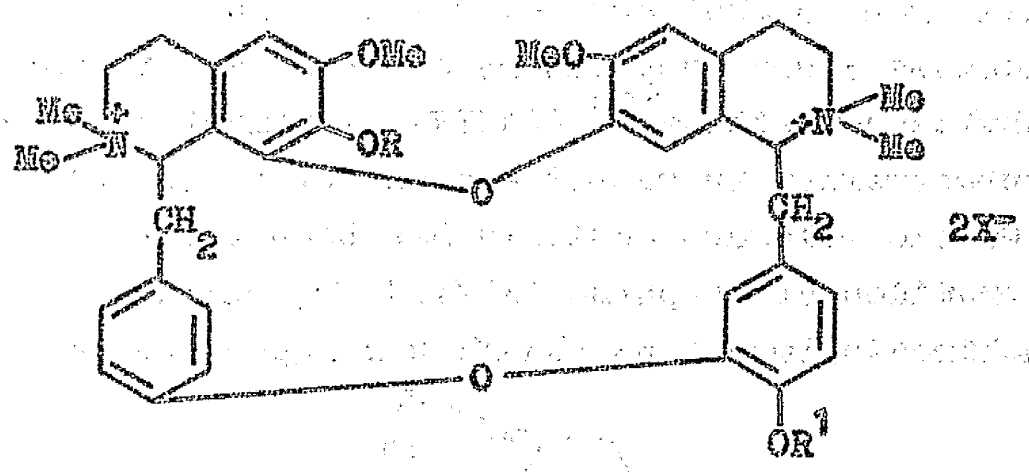


(XXXII)

of the methoxyl groups is obviously important since (+)-chondocurarine is approximately three times as potent as (+)-tubocurarine. Potency, in almost all cases, can be improved by methylation to yield the dimethyl ethers. The increase in activity on methylation has been associated with suppression of zwitterion formation[181,182] and with increase in lipoid solubility[145] but neither explanation accounts for the low potency of the dibutyl and dibenzyl ethers.

A large number of methiodides of other Menispermaceous alkaloids produce effects seen characteristically with (+)-tubocurarine. N-Methyloxyacanthine (XXXIIIA R=Me, R^1=H)[179] is almost equipotent with (+)-tubocurarine in rabbit and dog and the isomeric N-methylberbamine (XXXIII; R=Me; R^1=H) is 1·5 times as potent in rabbit and dog and equipotent in man.[1]



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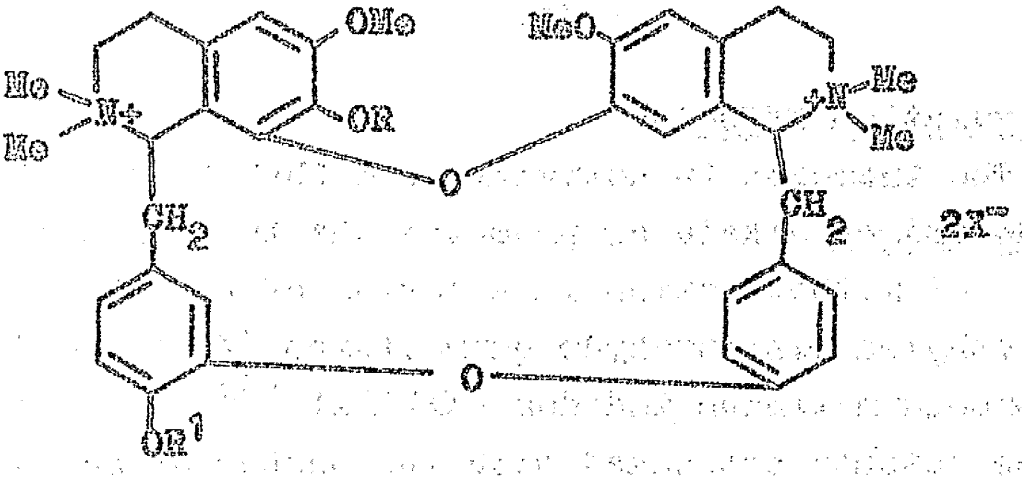

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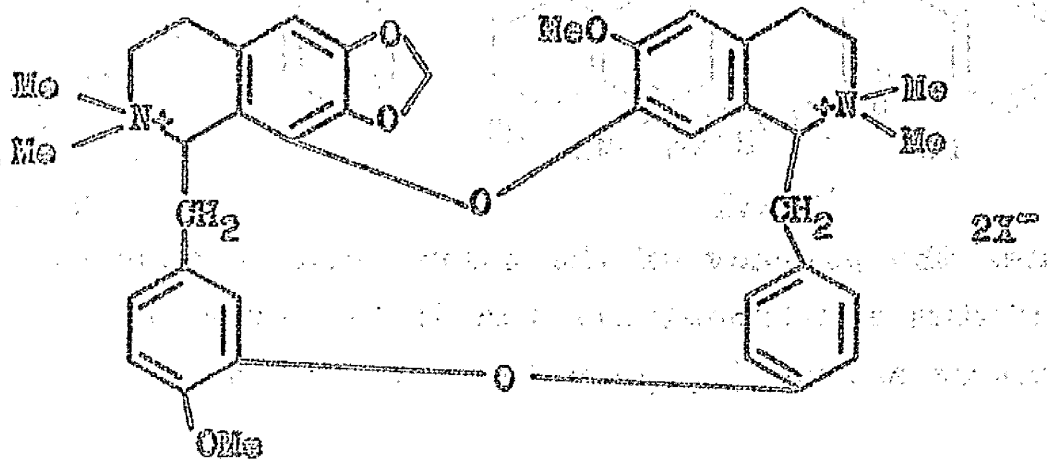
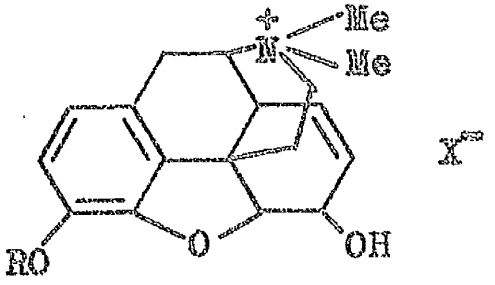


(XXXIV)

Methylation of the free hydroxyl group of N-methyloxyacanthine leads to a decrease in potency. Fangchinoline[183] (XXXIII; R=H, R^1=Me) and (+)-tetrandrine[183] (XXXIII; R=R^1=Me) methiodides cause respiratory embarassment in the cat. N-methylcepharanthine[179] (XXXIV) is almost as potent as (+)-tubocurarine in rabbit and slightly less potent in man.

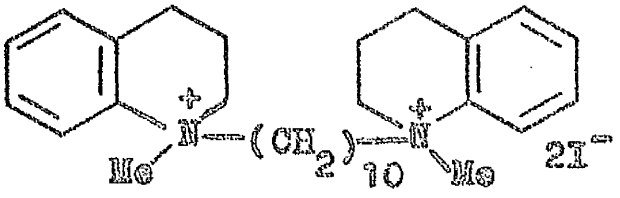
Muscle relaxant activity has been reported in the methobromides of morphine (XXXV; R=H), codeine (XXXV; R=Me) and morpholinoethylmorphine (XXXV; R=2-morpholinoethyl).[184]



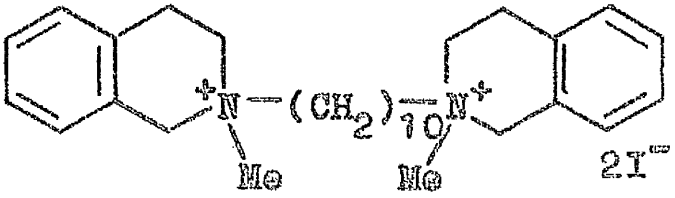
(XXXV)

Methoxylation Effects

The increase in activity on methylation of the Chondodendron alkaloids prompted the investigation of the effect of methoxylation in a series of N,N-dimethyl-1,10-decamethylenebistetrahydroquinolinium (XXXVI) and -tetrahydroisoquinolinium iodides (XXXVII).[185,186] In both series potency increased with the number of methoxyl groups (Table 4). Methoxylation has also been observed to



(XXXVI)



(XXXVII)

increase the potency of the corresponding decamethylene-quinolinium and -isoquinolinium derivatives and a series of quaternary salts of p-phenylene bisamines.[172] The elevation

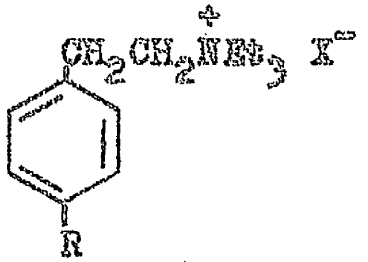
TABLE 4.

Effect of Methoxylation

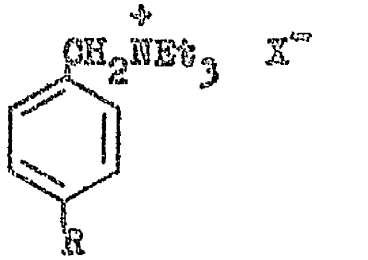
in Tetrahydroquinoliniums and -Isoquinoliniums

Methoxy Substituents	Structure	M.E.D. Rabbit mg/kg.
-	XXXVI	0.75
6-Methoxy	"	0.2
8-Methoxy	"	0.1
-	XXXVII	0.75
6-Methoxy	"	0.2
6,7-Dimethoxy	"	0.05
6,7,8-Trimethoxy	"	0.02
(+)-tubocurarine		0.1

of potency on methoxylation has been attributed to the increased lipophilic characteristics of the molecule and to greater electrostatic attraction between the drug molecule and the receptor as a result of the increased electron availability in the aromatic nucleus. Electronic effects would appear to be important since the position of the methoxyl groups is significant (Table 4) and furthermore potency increase parallels simple alkyl substitution which also increases electron availability in the aromatic ring in β-phenylethyl-[187] (XXXVIII) and benzyltriethyl-[172] ammonium iodides. (XXXIX)



(XXXVIII)



(XXXIX)

R	H.D.D.	R	H.D.D.
H	12mg./kg.	H	12mg./kg.
isoPr	6mg./kg.	Me	6mg./kg.

B. ALIPHATIC COMPOUNDS

a) One ether link.

The methonium compounds (VI; R=Me) fall broadly into two classes. The lower members, for example hexamethonium (VI; n=6, R =Me.), show high ganglion blocking activity and weak non-depolarising neuromuscular blockade. The higher homologues, for example decamethonium, are predominantly depolarising neuromuscular blocking agents.

In ether linked compounds (XL) precisely similar factors operate. Fakstorp and his colleagues[188,189] investigated the powerful ganglionic blocking activity of the lower members of the series (XL; n=m=2or3, R=R^1= lower alkyl) and proposed a one point attachment at the ganglion receptor. In the asymmetric compounds

$$R^1R_2\overset{+}{N}(CH_2)_nO(CH_2)_m\overset{+}{N}R_2R^1 \cdot 2X^-$$

(XL)

TABLE 5.

Neuromuscular Blocking Activity in Symmetrical and Asymmetrical Bis-onium Ethers

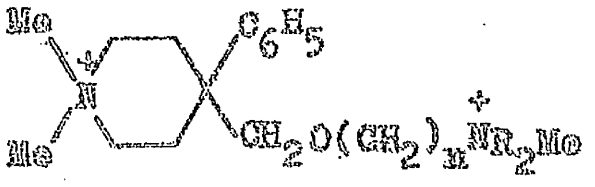
XL	R_2R^1	m	n	Type of Activity	Relative Molar Potency In Cat TC=100
	Me_3	2	3	Tubocurarine-like	0·08
	$MeEt_2$	2	3	"	0·13
	Et_3	2	3	"	0·45
	Me_3	3	3	"	No block at 25 mg/kg.
	Et_3	3	3	"	2·0
Hexamethonium				"	0·44

TABLE 6.

Neuromuscular Blocking Activity in Symmetrical and Asymmetrical Bis-onium Ethers

XL	R_2	R^1	n	m	Activity in Dog TC=1
	Et_2	Pr	3	3	0·025
	Piperidine	Bu	3	3	0·05
	"	Am	3	3	0·125
	"	Bz	3	3	0·17
	Me_2	Me	4	4	0·25
	Et_2	Me	4	4	0·08
	Pr_2	Pr	4	4	0·20
	Piperidine	Pr	4	4	0·33
	"	Bu	4	4	1
	"	B_2	4	4	0·25

(XL; n=2, m=3) the oxygen atom participated in the receptor interaction. Some of these compounds have been tested for neuromuscular blocking activity[190] (Table 5) but the block, although weak, is dependent neither upon one point attachment nor upon the position of the other oxygen. Higher alkyl substitution or incorporation of the onium centre into a heterocyclic ring increased potency in bis-oniumdipropyl ethers[191,192] (XL; n=m=3) but the effects are variable in the corresponding dibutyl ethers[193-196] (XL; n=m=4) (Table 6). This is probably due to a transitional type of block resulting from the increased chain length, although the authors account for the anomaly with the suggestion that receptor sites are irregular patches.[197] Basic ethers of piperidine alcohols (XLI; n=2 or 3, R_2=Me_2, Et_2, morpholino, piperidino) show weak curariform activity in mice.[198] Bis-laudanosinium-dipropyl and -dibutyl



(XLI)

ethers (V; n=m=4 and 5, X=O) show reduced potency when compared with the corresponding decamethylene compound (laudexium)(V; n=5, m=4, X=CH_2).[199]

Oxydipentonium[200] (Brevatonal) (XL; n=m=5, R=R^1=Me) is a depolariser which has been evaluated clinically[201] and resembles decamethonium in that replacement of methyl groups by ethyl changes the block to the non-depolarising type. The next higher homologue (XL; n=m=6, R=R^1=Me) is a non-depolarising blocker with approximately one fifth the activity of (+)-tubocurarine in the rabbit sciatic gastrocnemius preparation.[200] Marsak, Jacob and Guermont[202] have investigated the effect of introducing acetylenic and

TABLE 7.[202]

Comparative Potencies of Acetylenic Ethers

Compound	Dose→ 50% paralysis in ug/kg	Activity with respect to corresponding Saturated Derivative	Activity with respect to corresponding Comp^{d} with O replaced by CH_2	Activity with respect to corresponding Compound with one CH_2 less
$Me_3\overset{+}{N}(CH_2)_4\diagdown O$ $Me_3\overset{+}{N}(CH_2)_4\diagup$	80-160	-	0·25-0·5	-
$Me_3\overset{+}{N}(CH_2)_4\diagdown O$ $Me_3\overset{+}{N}(CH_2)_5\diagup$	60-160	-	0·25	1
$Me_3\overset{+}{N}CH_2C{\equiv}CCH_2$ $Me_3\overset{+}{N}CH_2C{\equiv}CCH_2$	40-80	2	1-2	-
$Me_3\overset{+}{N}CH_2C{\equiv}CCH_2\diagdown O$ $Me_3\overset{+}{N}CH_2C{\equiv}C(CH_2)_2\diagup$	60-160	1	2-5	0·5

TABLE 8.

Activity in Bis-choline Ethers.

$Me_3\overset{+}{N}(CH_2)_2O(CH_2)_nO(CH_2)_2\overset{+}{N}Me_3$ $2X^-$

n	Units in Chain	Rabbit HDD mg./kg.	Type of Activity	Reference
1	7	-	TC-like	203
2	8	40-60	"	203,204,205
3	9	2·9	Causes spasm	200
4	10	1·5	C10-like	204,206
5	11	0·7	C10-like	200
10	16	0·8	-	206

TABLE 9.

Effect of Onium Substitution on Potency

$R^1R_2\overset{+}{N}(CH_2)_2O(CH_2)_2O(CH_2)_2\overset{+}{N}R_2R^1$ $2X^-$

R_2R^1	Rabbit HDD mg./kg.[205]	Activity in Dog[192,195]
(+)-tubocurarine	0·2	1
Me_3	33·2	-
Me_2Et	22·5	-
$MeEt_2$	11·3	0·02
Et_3	7·2	0·13
Pr_3	-	0·3
Piperidine, Me	8	0·02
" Et	4	-
" Bu	-	0·17
Morpholine Me	49	-
" Et	23	-

ether functions into the interonium chain of decamethonium and its lower homologue. The simultaneous presence of an acetylenic bond and an ether oxygen resulted in derivatives which, although less active than the unsubstituted compound were more potent than those possessing only an acetylenic or only an ether group (Table 7).

b. Two ether links

Aliphatic compounds possessing two ether linkages can conveniontly be divided into ω-bis-ammoniumdioxo alkanes (XLII) and derivatives of bis-choline ethers (XLII; m=2) (Table 8). In the latter the effect of chain length upon type of activity closely parallels that of the polymethylenebistrimethylammonium salts. End group substitution[192,195,205,207] in bis-dialkylaminoethoxyethane quaternary salts (XLII; n=m=2) (Table 9) results in increase in potency

$$R_2R^1\overset{+}{N}(CH_2)_mO(CH_2)_nO(CH_2)_m\overset{+}{N}R_2R^1\ 2X^-$$

(XLII)

with increase in onium substitution. The high activity shown by bis-tripropylammoniumethoxyethane (XLII; n=m=2, $R=R^1=Pr$) is anomalous, but the low activity of the morpholinium compounds is to be expected due to delocalisation of the charge on the nitrogen. The higher activity of bis-N-butylpiperidiniumethoxyethane (XLII; n=m=2, R_2= piperidine, R^1=Bu) compared with the corresponding decamethylene derivative has been cited as evidence for an increase in potency on introduction of an ether linkage.[192] However due to the difference in interonium distance, the two, although undoubtedly both non-depolarisers, are not strictly comparable.

Only one series of ω-bisammoniumdioxoalkanes (XLII) has been extensively examined.[206] In this series it was

concluded that potency was determined primarily by the number of methylene groups separating the ether oxygen atoms. Thus activity falls in the three compounds (XLII; R=R^1=Et) where m=2, n=10; m=3, n=8; and m=4, n=6. However, it may be equally significant that the nitrogen-oxygen distance is increasing, a point which cannot be clarified from the compounds tested. Prodeconium bromide[208] (Prestonal) (XLII; n=10, m=2, R=Me, R^1=propylacetate) produces a non-competitive block and has been used clinically as a short acting muscle relaxant. Replacement of the polymethylene chain of the dioxoalkanes by a glycol ether linkage produces a compound (XLIII) with weak decamethonium-like activity in the rabbit.[200]

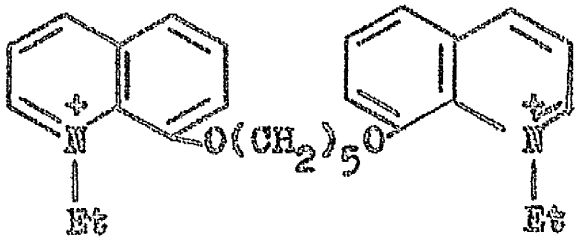
$$Me_3\overset{+}{N}(CH_2)_2O(CH_2)_2O(CH_2)_2O(CH_2)_2\overset{+}{N}Me_3\ 2I^-$$

(XLIII)

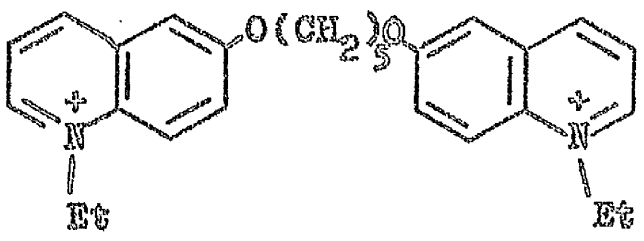
C. AROMATIC AND HETEROAROMATIC COMPOUNDS

a. Aliphatic Type.

In 1946, Bovet,[209] using (+)-tubocurarine as a model, prepared 1,5(8,8'-diquinolyloxy)-pentane bisethiodide (XLIV) which was the first synthetic compound with paralysing potency comparable to that of the natural alkaloid. Decrease or increase in the number of inter-oxygen



2I$^-$

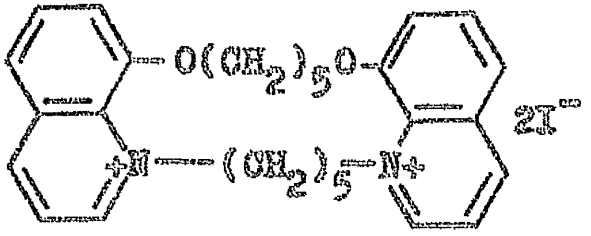


2I

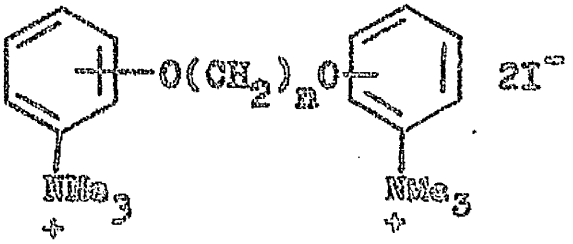
(XLIV) (XLV)

methylene groups, or alteration of the onium substituent

led to reduced potency.[210] The corresponding 6,6′-diquinolyloxy derivative[210] (XLV) and the related pentamethylene-bisquinolinium compound (XLVI) were respectively one third and one half as active.[210] That the quinoline nucleus was not indispensable for blocking activity was



(XLVI)



(XLVII)

shown by comparable potency in a series of 1,5-bis-(trimethylammoniumphenoxy)-pentanes (XLVI; n=5).[210,211] Potency varies with the position of substitution in the aromatic nucleus and with chain length. Although these compounds show no anticholinesterase activity, the lower homologues (XLVII; n=2 and 3) are effective inhibitors and show pronounced antagonism to non-depolarising blocking agents.[175] Whether the paralysing or anticholinesterase propensity predominates is, very generally, a function of the position of the phenolic groups in relation to the quaternary groups, the meta disposition being favourable for anticholinesterase activity. Similarly in the analogous p-phenylene ethers (XLVIII), only the meta derivative exhibits anticholinesterase activity, whereas the ortho and para isomers are respectively twelve and thirty times as active as the meta isomer when tested for neuromuscular blocking activity in the rabbit.[175]

TABLE 10.

Potency of Hydroquinone Ethers

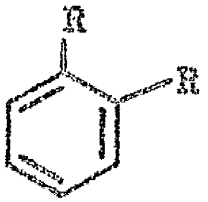
(I)

n	R=Me		R=Et	
	Rabbit HDD	Dog Sc/gn.	Rabbit HDD	Dog Sc/gn.
2	2 mg/kg.	10 mg/kg.	1·5mg/kg.	2mg/kg.
3	1·5 " "	1	0·5	0·5
4	0·25" "	0·005	0·4	0·4
5	0·25" "	0·5	0·3	0·5

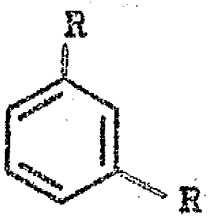
Sc/gn. = Sciatic-gastronecmius preparation

TABLE 11.

Effect of Position of Substitution in Aromatic Nucleus on Potency



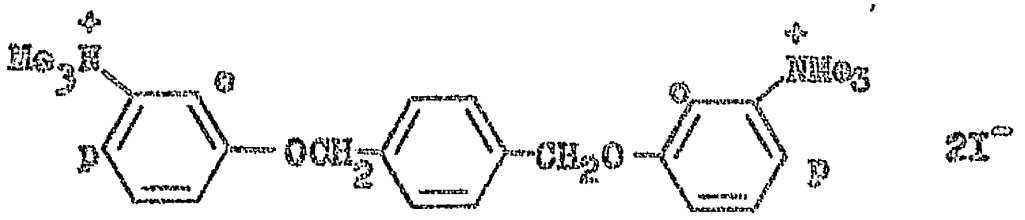
(LI)



(LII)

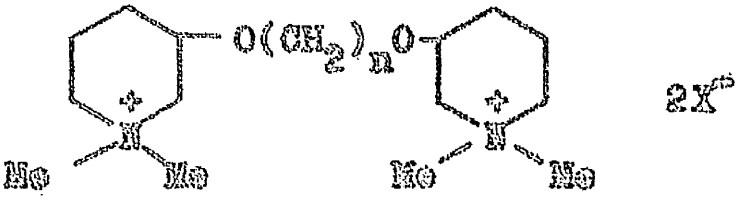
(LIII)

R	Rabbit HDD in mg/kg. (LI)	(LII)	(LIII)
$-OCH_2CH_2\overset{+}{N}Et_3$	1·5	1	3·5
$-CH_2\overset{+}{N}Et_3$	5	2	2·5
$-CH_2CH_2\overset{+}{N}Et_3$	-	1·5	2·5



(XLVIII)

A series of bis-(3-hydroxydimethylpiperidinium)-polymethylene ethers[212] (XLIX) bear at least a formal similarity to the compounds of this section. Maximum activity occurs in the dodecamethylene member (XLIX; n=12) which produces a mixed block by failing to contract avian muscle and yet not being antagonised by edrophonium.

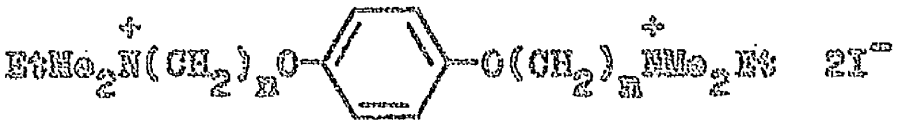


(XLIX)

b. Phenolic Type.

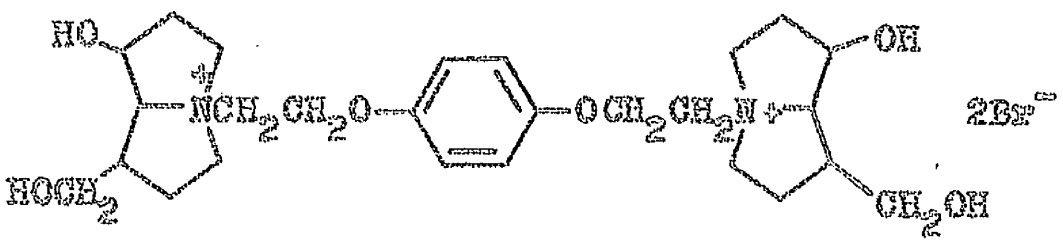
Basic ethers derived from hydroquinone[213] (Table 10) were among the early compounds tested for muscle relaxant activity. The influence upon activity of the relative position of the substituents in the aromatic nucleus has been considered in the β-triethylammoniumethyl ethers of dihydric phenols[87] (LI; LII; LIII; R=$OCH_2CH_2\overset{+}{N}Et_3$). The order of potency (Table 11), meta > ortho > para parallels the results in a series of non-ethers[172] (LI; LII; LIII; R=$CH_2\overset{+}{N}Et_3$) and (LII; LIII; R=$CH_2CH_2\overset{+}{N}Et_3$) and it would therefore appear that some factor, other than chain length,

is responsible for maximum activity. Bulbring and Depierre[214] have reported anticholinesterase activity in the meta isomer (LXI; R=$OCH_2CH_2\overset{+}{N}Et_3$), the disposition which normally favours this type of activity, but this cannot explain the observed results since this compound is probably, like the para isomer,[203] a non-depolariser. Paikan and Unna,[215] working with gallamine have proposed that repulsion of like charges in the 1,3-position in conjunction with restrictions imposed by the aromatic ring tend to maintain the quaternary groups at the optimal distance for neuromuscular blocking activity. Dipropamine (LIV; n=3) and the less potent diethamine



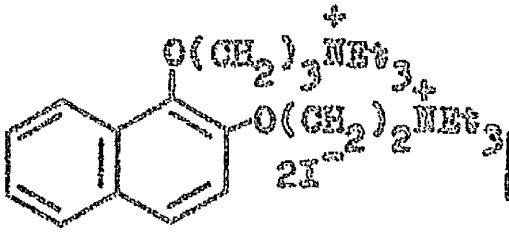
(LIV)

(LIV; n=2) produce a block which develops progressively, is of long duration and which is not reversed by neostigmine.[216] Dipropamine was found to have a greater affinity than neostigmine for some common receptor. Both compounds have been clinically evaluated.[217] Randall[218] has observed that introduction of a p-nitrobenzyl group into the onium centre of dipropamine and related hydroquinone ethers confers tensilon (XX)reversibility on the block. The platynecine derivative,[219,220] Diplacine (LV), illustrates another end group modified ether, derived from 1,4-dihydroxybenzene, which has paralysing activity.

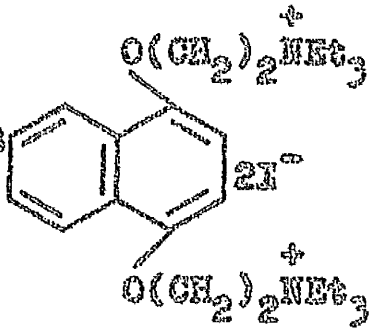


(LV)

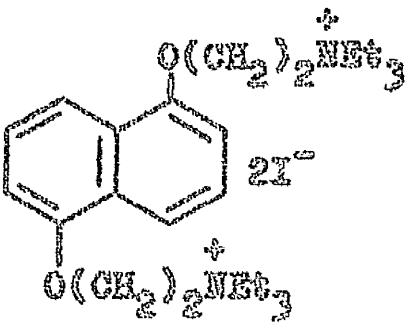
In a series of naphthyl ethers,[172] the 1,4-derivative (LVII) and the 1,5-derivative (LVIII) are more active than the 1,2- isomer (LVI) and thus differ from the corresponding



(LVI)



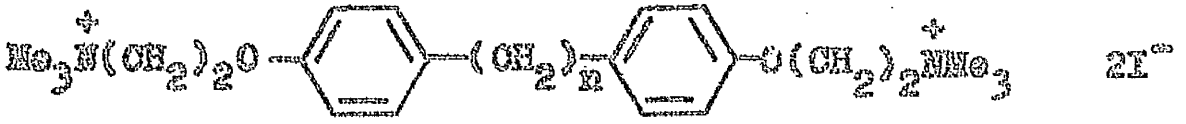
(LVII)



(LVIII)

H.D.D.	3mg./kg.	1mg./kg.	1mg./kg.

benzene derivatives (LI; LII; LIII) in which the ortho isomer was more potent than the para isomer. Comparable potency is found in 4,4''-(2''-trimethylammoniumethoxy)-diphenyl[170,200] (LIX; n=0) which has a rabbit head drop dose of 1mg/kg. and is decamethonium-like as judged by its ability to augment acetylcholine-induced spasm in the frog rectus, whereas the homologous diphenylmethane[200] (LIX; n=1)

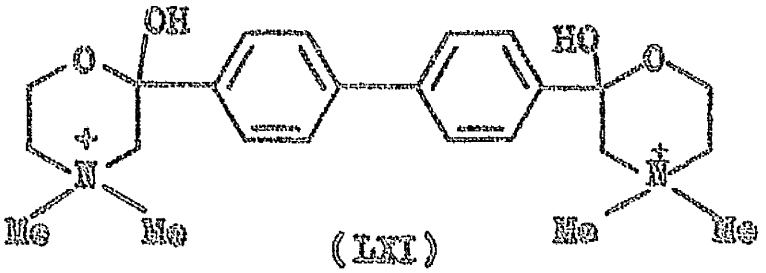


(LXI)

is tubocurarine-like in the same test.

The hemicholiniums[221] represent a group of diphenyl compounds with at least potential ether links. The most potent of these is α,α'-dimethylethanolamino-4,4''-diacetophenone (HC-3)(LX) which is capable of forming a hemiacetal (LXI). This compound is now known to be a

(LX)

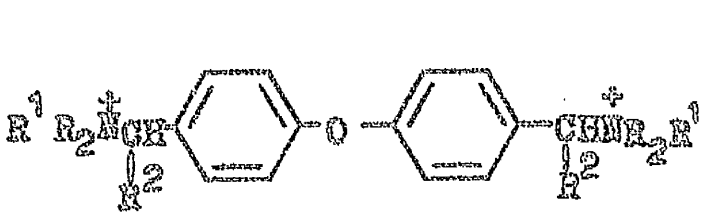


(LXI)

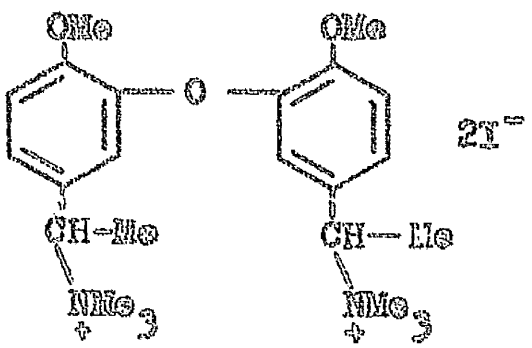
powerful, long-acting neuromuscular blocking agent[222,223] which acts by suppression of acetylcholine synthesis.[224] Choline chloride is an effective antagonist of the block. Introduction of an ether link between the two aromatic rings only slightly decreases potency.[225] Aliphatic analogues retain the characteristic activity of HC-3.[226]

D. DIPHENYL ETHERS

The presence of diphenyl ether linkages in tubocurarine prompted the preparation of a number of bis-onium diphenyl ethers. Compounds with a para (LXII) or a meta (LXIII) ether linkage, corresponding to the two halves of tubocurarine, have been prepared.[227] The most active p-diphenyl ether (LXII; R_2=piperidinium, R^1=Me, R^2=Am) was equipotent with



(LXII)

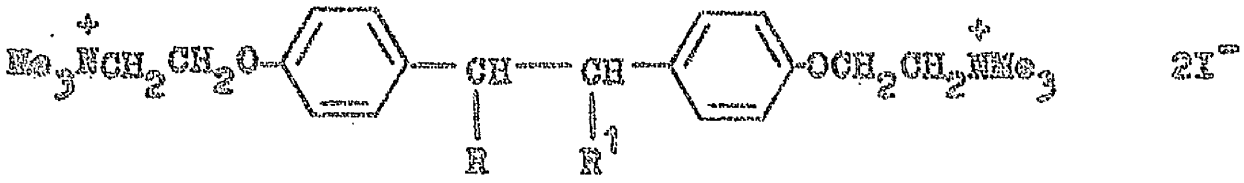


(LXIII)

(+)-tubocurarine in the rabbit and one third as active in the cat. The m-diphenyl ether was almost inactive.

E. DIPHENYLALKANES AND DIPHENYLALKENES

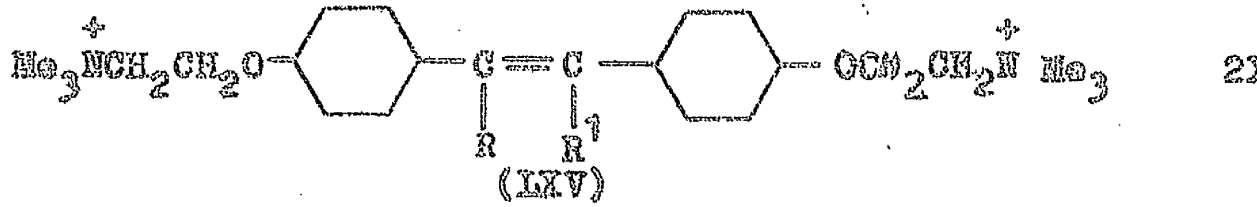
In the choline ethers of diphenylethane[200] (LXIV; R=R^1=H) the most potent compound, 1,2-[4,4'-(2''-trimethylammoniumethoxy)-diphenyl]-3-methylbutane (Mediational) (LXIV; R=H, R^1=isoPr), was as active as (+)-tubocurarine in the rabbit and has been used clinically. The corresponding hexoestrol[200,228] derivatives (LXIV; R=R^1=Et) are characterised by a long latent period and



(LXIV)

prolonged block of conduction. Higher alkyl end-group substitution decreases potency. All of these compounds produce flaccid paralysis in avian muscle but are not reversed by eserine.

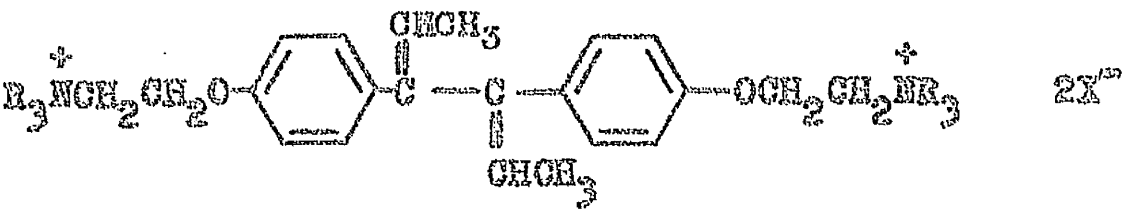
Choline ethers of dihydroxystilbene[200] (LXV; R=R^1=H) and its derivatives[200] (LXV; R=H, R^1=alkyl) cause prolonged block as do the corresponding cis[200] and trans[200,228] disubstituted compounds (LXV; R=R^1=Et.). The stilboestrol[228] derivatives have been used in the development of the



(LXV)

supporting moiety theory.[229]

Hazard and his colleagues[230] have investigated analogous dienoestrol ethers (LXVI) and found potency maximal in the

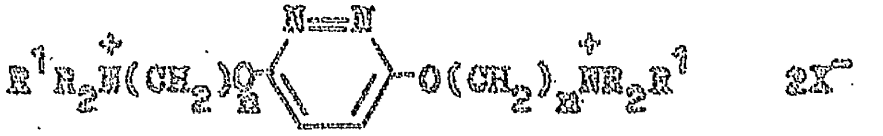


(LXVI)

triethyl analogue (LXVI; R=Et) in mice and rabbits. As in the hexoestrol and stilboestrol series these compounds are not reversed by eserine.

F. HETEROCYCLIC ETHERS

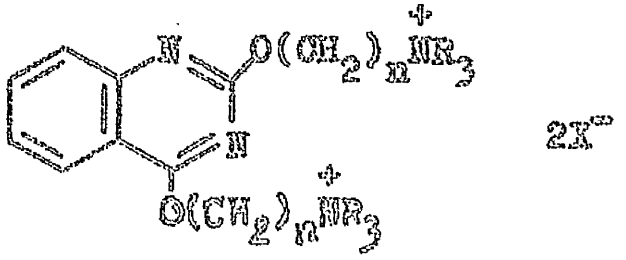
The bis-quaternary alkoxypyridazine structure (LXVII) resembles in many respects the bis-quaternary 1,4-dihydroxybenzene type of molecule (page 31). Gesler and Hoppe[231] and Biel[232] have synthesised a number of these derivatives.



(LXVII)

3,6-Bis(3'-trimethylammoniumpropoxy)-pyridazine dibromide (LXVII; n=3, R=R^1=Me) was equi-active with (+)-tubocurarine and was a depolariser. The benzyldiethyl analogue (LXVII; n=3, R=Et, R^1=Bz) was, on the other hand, reversed by edrophonium. The diethylmethyl derivative (LXVII; n=3, R=Et, R^1=Me) behaved as a typical hemicholinium[233].

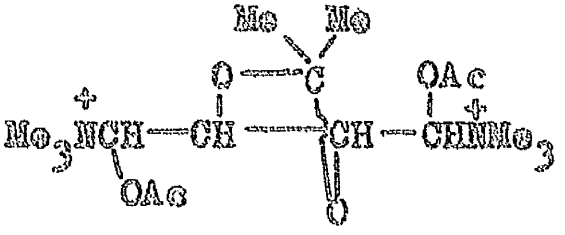
A series of 2,4-dialkylaminoalkoxyquinazolines (LXVIII)[234]



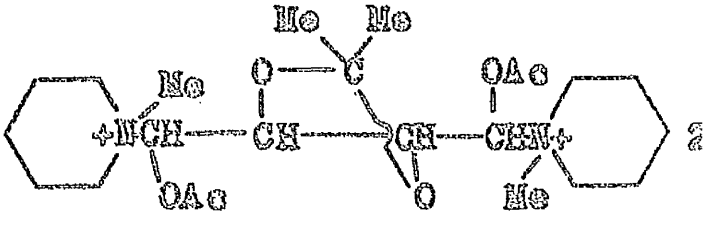
(LXVIII)

all produced flaccid paralysis in the chick. Potency increased with increase in chain length and on replacement of methyl by ethyl at the onium centres.

Vargha and Kasztreiner[235] have described weak blocking action in quaternary salts of nitrogenous derivatives of 1,2:5,6-dianhydro -3,4-O-isopropylidene-D-sorbitol. The trimethylammonium-(LXIX) and N-methylpiperidinium-(LXX) derivatives were about one tenth as active as (+)-tubocurarine.



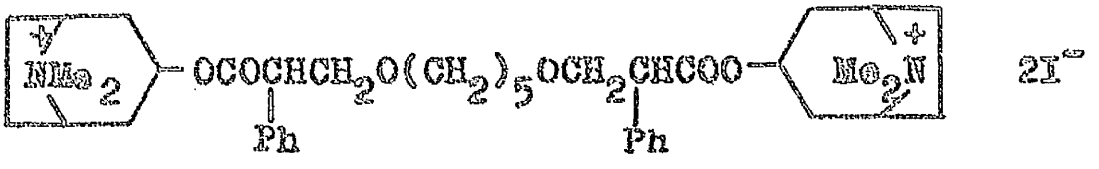
(LXIX)



(LXX)

G. MISCELLANEOUS ETHERS

Kimura, Unna and Pfeiffer,[236] recognising a similarity in structure in atropine and (+)-tubocurarine, prepared the bis-atropinium derivative (LXXI).

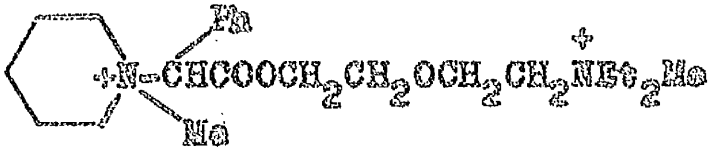


(LXXI)

The compound had a rabbit head drop dose of 0·325mg/kg. Ether esters of choline with p-hydroxy-aromatic and-arylaliphatic acids (LXXII; n=2 or 3, m=0, 1,2) show moderate blocking activity in rabbits.[237-239] Studies involving bis-quaternary ether esters have led to the development

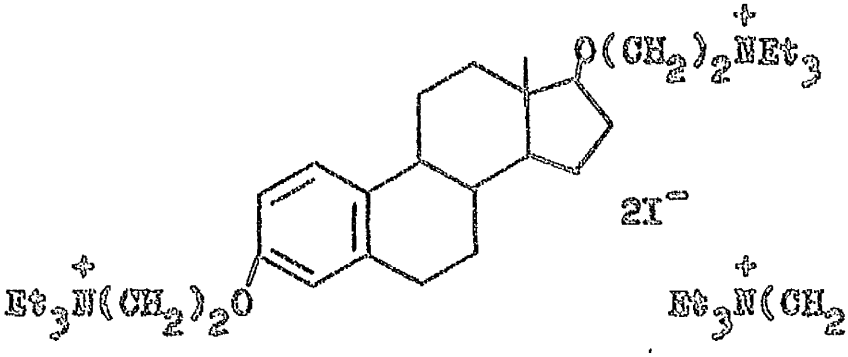
(LXXII)

of 2-diethylaminoethoxyethyl-α-phenyl-α-piperidino-acetate dimethiodide (LXXIII).[240] This compound is a short acting, non-depolarising muscle relaxant.

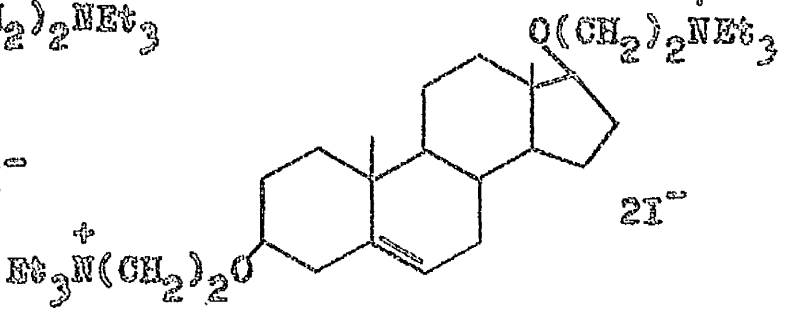


(LXXIII)

Muscle relaxant properties have also been found in bis-quaternary salts derived from β-diethylaminoethyl ethers of oestradiol (LXXIV) and 3,17-dihydroxyandrost-5-ene (LXXV).[241]



(LXXIV)



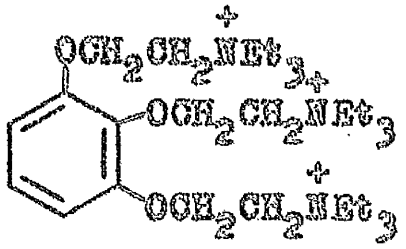
(LXXV)

4. TRIS-AND POLYQUATERNARY ETHERS

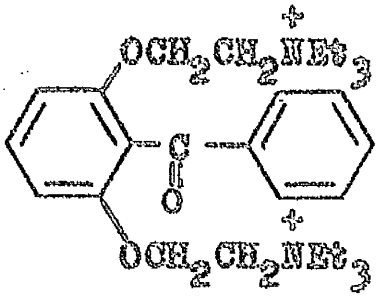
A. AROMATIC AND HETEROCYCLIC

Bovet and his co-workers synthesised a series of ethers of choline and homologous amino alcohols with mono-, di- and tri-hydric phenols and found that potency increased with increase in the number of onium centres. The most important

compound to emerge from this work was 1,2,3-(2′-triethylammoniumethoxy)-benzene (gallamine)[87] (LXXVI). Gallamine is widely used clinically and being a non-depolariser produces a block which is easily reversed. As in hexaethonium (VI; n=6, R=Et), successive replacement of ethyl substituents by methyl leads to diminished potency without alteration in the type of action.[242] Pelikan and Unna[215] regard the onium centres as lying at the apices



(LXXVI)

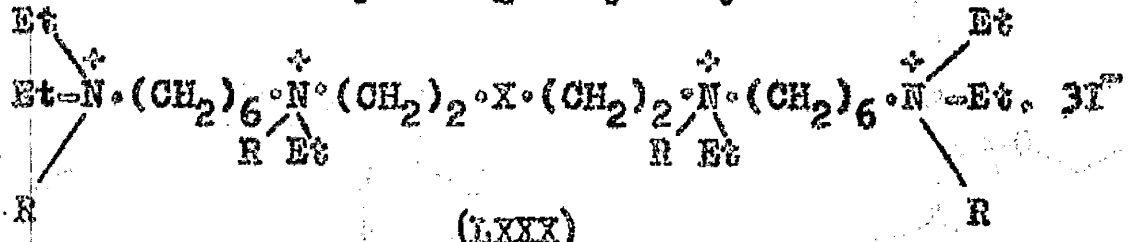


(LXXVII)

of a figure not far removed from a straight line. In this case the third onium centre only exerts a space occupying effect and that this may be so is shown by the high activity of 2,6-bis-(triethylammoniumethoxy)-benzophenone (LXXVII) in which the neutral arylcarbonyl moiety separates the two charged groups by some 15A°. On the other hand, a staggered orientation (LXXXVIII) in which the onium groups form the apices of an equilateral triangle of 9A° side, comparable to a fully extended hexaethonium molecule, has been proposed.[156] In the symmetrical triazines (LXXIX; n=2or3)[234] a similar disposition of onium centres at the apices of an equilateral triangle is envisaged. These compounds, all weak blocking agents, are not strictly comparable since the ether functions are on the 2,4,6- positions of the heteroaromatic ring.

TABLE 12

Blocking Activity in Linear Polyonium Ethers and Their Corresponding Polymethylene Derivatives

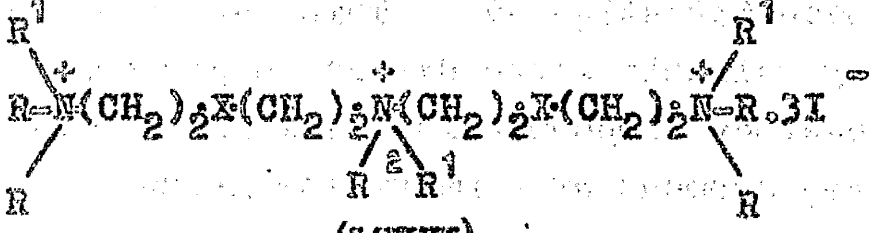


(LXXX)

Compound	(LXXX) R	X	Cat	Relative molar potencies Rabbit	Chick	Mouse	Frog
XV A	Et	O	278	108	139	165	375
XVI A	Et	CH_2	417	125	230	174	208
XV B	Me	O	87	66	110	132	77
XVI B	Me	CH_2	110	82	158	165	26
XV C	Pr	O	234	96	242	146	63
XVI C	Pr	CH_2	263	110	242	151	72

TABLE 13.

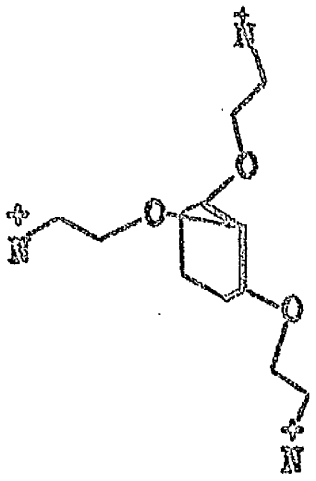
Blocking Activity in a Series of Linear Tris-onium Ethers and Their Corresponding Polymethylene Derivatives



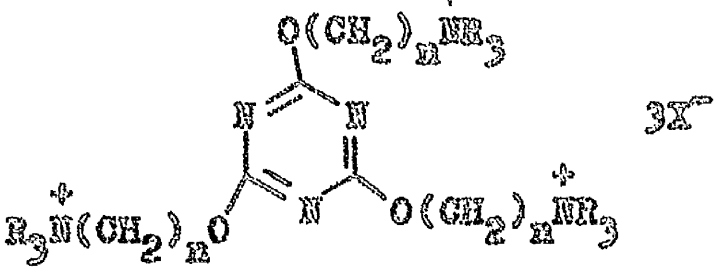
(LXXXI)

R	R^1	R^2	X	Cat	Relative Molar potencies TC=100 Rabbit	Hen	Frog	Type of Activity
Me	Me	Me	O	>30mg/kg	x	x	1.0	(+)-tubocurarine
Me	Et	Me	O	0.4	x	5.0	2.0	(+)TC-like
Me	Pr	Me	O	1.0	x	5.2	2.5	"
Et	Me	Me	O	0.7	x	15.0	4.0	"
Et	Me	Et	O	2mg/kg	x	16.0	x	"
Et	Me	Et	CH_2	6	x	52.0	x	"
Et	Et	Me	O	3.0	0.36	20.0	7.0	"
Et	Pr	Me	O	14.5	x	46.0	10.5	"
Et	Pr	Et	CH_2	2.0	x	88.0	x	"
Et	Et	Et	O	5.0	5.0	5mg/kg	x	"
Et	Et	Et	CH_2	50.0	x	101	x	"

x = Insufficient material



(LXXVIII)



(LXXIX)

B. ALIPHATIC COMPOUNDS

An extensive series of linear polyonium compounds have been shown[190,243] to resemble gallamine in many respects. The influence of introducing an ether linkage into the polymethylene chain of these compounds and its effect upon potency have been investigated. This series of compounds is particularly valuable since direct comparison between ether and non-ether compounds is possible (Tables 12 and 13). In all cases replacement of a methylene group of the polymethylene chain by an ether link reduces neuromuscular blocking potency. These results are similar to those of analogous bis-onium ethers (Table 5). In both series potency increases with increase in alkyl group size.

CONCLUSIONS

A number of very general conclusions can be drawn regarding the effect of chemical structure upon the type of activity and neuromuscular blocking potency exhibited by these compounds. Hard and fast rules cannot be formulated since it is often difficult to decide whether steric, electrostatic, polarisation, solubility or other effects, or a combination of any two or more of these effects, is

responsible for the observed alteration of potency or mode of action.

a) The interonium chain:

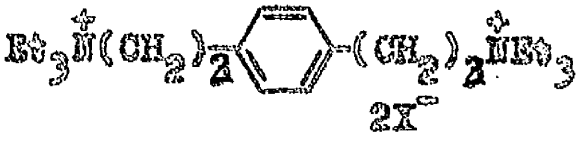
In the polymethylene bis-quaternary salts with lower alkyl substituted onium groups maximum activity is associated with a nine to eleven atom chain. Increase in the bulk of the onium head, whether by substitution with higher alkyl or aralkyl groups or by incorporation of the nitrogen atom into a heterocyclic ring system, renders chain length less critical and the hexamethylene member is often as active as the decamethylene derivative.[136] Introduction of an ether oxygen into the interonium chain in all cases reduces potency (e.g. Tables 6 and 12, and V; $n=m=5, X=O$) and furthermore maximum activity is found in an eleven to sixteen atom chain.[171] This is surprising since replacement of a -C-C-C by a -C-O-C- reduces chain length by a mere $0{\cdot}12A^{O}$ when fully extended molecules are considered and so the reduction in interonium distance will be even less significant in contracted molecules. When these molecules are in solution two factors which effect chain length operate in opposition to each other namely the repulsion of like charges tending to stretch the molecule and the high interfacial energy between the hydrocarbon portions and water which tend to make the molecule contract. A balance between these two probably exists and conductimetric measurements have shown that maximally extended forms do not exist in solution.[244,245] The reduction in activity associated with introduction of ether links into the polymethylene chain has been attributed to an increase in the polarity of the molecule which results in increased hydrophilic characteristics through potential hydrogen bonding with water.[117] The addition of polar groups can be compensated for by lipophilic substitution either at the onium centre (XLIV) or by increase in the number of atoms in the

chain and hence maximum activity becomes associated with the eleven to sixteen atom chain. An alternative explanation could be that the normal coiling tendency of hydrocarbon chains is increased by ether groups and thus a longer chain is necessary to maintain the onium groups at optimal separation. Thus it has been shown that the replacement of a $-CH_2-$ group in a chain by an oxygen atom leads to increased flexibility[246] and also rotation around a $-CH_2-O-$ bond appears to much easier than around a $-CH_2-CH_2$ bond.[247]

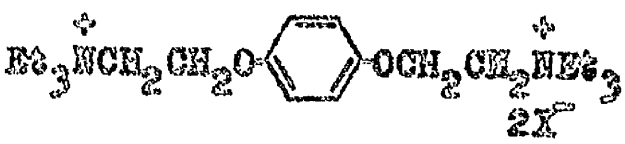
In asymmetrical ganglionic blocking agents (XL; x=2; n=3) which were more potent than hexamethonium (VI; x=6, R=Me) the higher activity was explained by an electron drift from the oxygen due to the proximity of the quaternary nitrogen resulting in a more stable attachment at the receptor (see page47). The availability of electrons on the oxygen atom of the ether linked polyonium compounds (LXXX and LXXXI) has been stated to perhaps exert an antibonding effect and to reduce the charge on the nitrogen.[190] This situation is not comparable with the influence of methoxyl substitution in (+)-tubocurarine and other structures (page 25) where the electron availability is confined to the aromatic rings and is without effect on the charge on the nitrogen. That both length and nature of the interonium chain are important to neuromuscular blocking activity has also been shown by Van Rossum[100] in decamethonium and tetramethylenebischoline ether (XLII; x=2, x=4, R=R^1=Me) where, although both are of approximately equal chain length, the latter has a lower intrinsic activity.

In compounds with an aromatic group as part of the interonium chain, introduction of ether linkages has similar effects on potency. Thus, 1,4-(2'-triethylammoniumethyl)-benzene (LXXXII) is six times as active as 1,4-(2'-triethyl-

-ammoniumethoxy)-benzene (LXXXIII) in rabbits and analogous compounds show simdlar tendencies.[172]

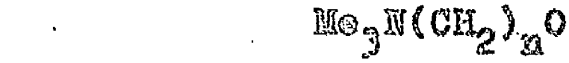


(LXXXII)



(LXXXIII)

The length of the chain also influences the type of activity. Thus in the series (LXXXIV) the homologues with n=2 or 3 are tubocurarine-like, compounds with n=4 are



LXXXIV

probably transitional but with n=5 are definitely decamethonium-like (oxydipentonium, LXXXIV; n=5) and when n=6 non-depolarising properties reappear. A similar pattern is found in the polymethylenebistrimethylammonium compounds. This variation in type of activity with variation in chain length has been related to the relative abilities of the homologues within either series to displace acetylcholine from a donor site.[117]

b) End group substitution:

The influence of charge density on the potency of neuromuscular blocking agents was recognised by Taylor,[248,249] a high concentration of charge on the nitrogen favouring high activity. Thus, in the frog, the tetramethylammonium ion is more potent than the tetrapropylammonium ion which in turn is more potent than the tetraethylammonium ion as would be

expected from this premise. Similarly morpholinium compounds are less active than their corresponding piperidinium derivatives (Table 9) due to the charge-reducing effect of the oxygen atom. Among analogues of decamethonium, hydrogenation of the heteroaromatic ring of the pyridinium, isoquinolinium and quinolinium derivatives to the corresponding piperidinium, tetrahydroisoquinolinium and tetrahydroquinolinium compounds increased activity. In this instance a greater charge density on the nitrogen arises since resonance stabilisation has been eliminated. A further example of the influence of charge density on activity is provided by the 8,8'-diquinolyloxypentane (XLIV) quaternary salts in which the bis-ethiodide was more potent than the bis-propiodide which in turn showed greater activity than the bis-methiodide. In this case the methyl group is most effective in reducing the charge on the nitrogen through hyperconjugation.

The effect of onium group substitution varies with the type of compound.[136] Generally in depolarisers a trimethyl cationic head is assumed to permit close approach to the receptor which, by analogy with monoquaternaries, accounts for the initial stimulatory properties which are a feature of this type of blocking agent. Progressive substitution with ethyl groups usually diminishes activity and changes the mode of action to the non-depolarising type.[203] Similar effects are produced by the introduction of a single benzyl or p-nitrobenzyl moiety into the end group.[218] In both these cases ethyl and aralkyl substitution restrict intimate contact between the onium centre and the receptor. In non-depolarisers maximum activity tends to be associated with the triethylammonium derivatives and replacement of ethyl by methyl groups lowers potency; the introduction of benzyl groups usually enhances activity. These compounds

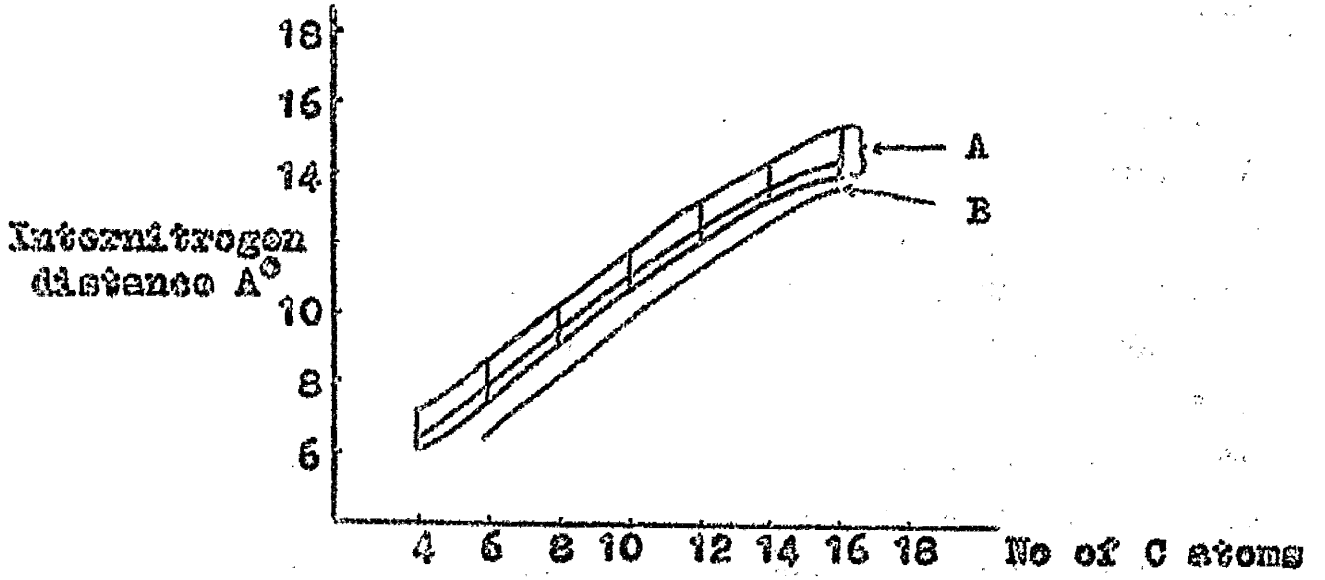


Figure 8. Internitrogen distance against number of carbon atoms separating the nitrogen atoms. A. Ethonium compounds - mean value from hatched area. B. Methonium compounds.

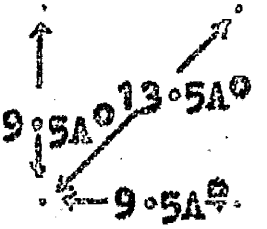


Figure 9. Geometrical arrangement of receptor points for neuromuscular blockade.

therefore have a "pure" blocking action and it has been suggested[117] that the depolarisation phenomenon of depolarisers is essentially a side reaction to neuromuscular blockade. Van Rossum (loc. cit.) has proposed that the intrinsic activity of neuromuscular blocking agents is directly concerned with the effectiveness of the onium centre in interfering with the electrical field of the receptor. The high intrinsic activity of trimethylammonium compounds is reduced by heavier onium substitution and this may be due partly to the increased distance between the cationic centre and the receptor and partly to a reduction or masking of the positive charge.

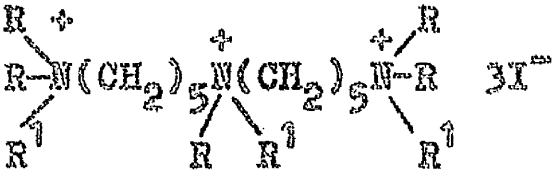
Recently Elworthy[245] has shown that there is a relationship between interonium distance and onium group substitution and further that it is possible to propose a geometrical arrangement of receptor points for neuromuscular blockade. In a series of polymethylenebistrimethylammonium compounds when ethyl groups are substituted for methyl groups on the onium centres the internitrogen distance increases (Figure 8); the geometry of the molecule is also changed and the fit on the receptor is consequently altered and so it follows that the type of action (or potency) of a molecule expanded by the introduction of ethyl groups cannot be expected to be the same as that of a longer chain methonium compound. If 9.5A° (decamethonium) is taken as the critical distance between receptor points and these are placed on the corners of a square then the hypotenuse is 13.6A° (Figure 9). This distance would then be expected to provide a second set of receptor points for attaching molecules to the surface and indeed the theory is substantiated by the high potency of the hexadecamethylene and octadecamethylene methonium compounds which were predicted to have the required internitrogen distance. Similarly the tridecamethylene member, the

longest chain compound tested, was the most potent member of the ethonium series.

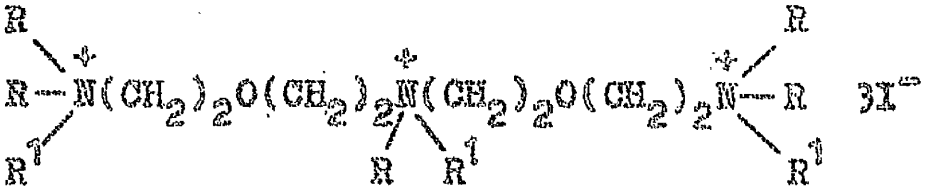
DISCUSSION

Introduction

In a series of polyonium compounds attention has been drawn to the changes in neuromuscular blocking potency observed on replacement of a methylene group in the inter-onium polymethylene chain by an ether linkage.[190,243,250] Thus, the polymethylene tris-onium compounds of general formula (LXXXV) have significantly greater muscle relaxant potency than the corresponding tris-onium ethers (LXXXVI). The relative importance of inter-oxygen or oxygen-nitrogen distances was not determined. Fakstorp and Pedersen[188,189] have, however, studied the influence of the position of an

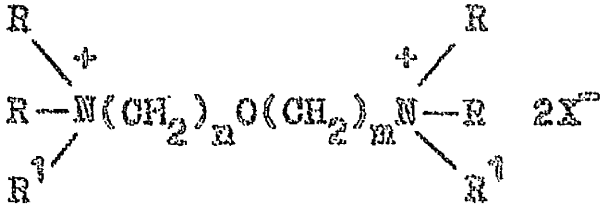


(LXXXV)



(LXXXVI)

ether oxygen atom on the ganglion-blocking activity of a series of bis-onium ethers of general formula (LXXXVII). The compounds (LXXXVII; n=2, m=3, R=R^1=Me; R=Me, R^1=Et; and R=Et, R^1=Me) were



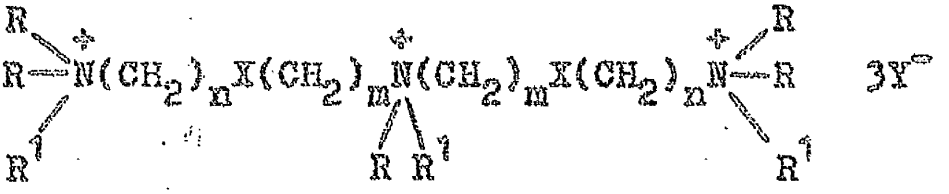
(LXXXVII)

more active than hexamethonium as ganglion blocking agents. The enhanced potency was ascribed to the proximity of the ether oxygen atom to the positively charged nitrogen atom. This was considered to cause an electron drift from the oxygen which would then acquire a partial positive charge thus permitting a more stable attachment to the negatively charged "berth" of the esteratic site in the conventional receptor model.[8] This hypothesis is strengthened by the reduced activity displayed by the analogous compounds of structure (LXXXVII; n=m=2; and n=m=3) in which the ether linkage is centrosymmetrically disposed with respect to the two onium groups. In both these compounds the electron drift will tend to be nullified and in the second case is probably absent since more than two carbon atoms separate the -O- and $\overset{+}{N}$ moieties.[243] However, since changes of chain length are also involved unequivocal conclusions are not possible.

The linear bis-onium ethers of Fakstorp and Pedersen have also been tested for neuromuscular blocking activity.[190] In this instance all of the compounds showed a very low potency and of more significance is the fact that when the compound (LXXXVII, n=2, m=3, R=R'=Me) is compared with hexamethonium there is a marked reduction in potency. This is apparently due to replacement of a methylene group by an ether oxygen.[190] This study also clearly demonstrated that the theory of a one point receptor attachment at the ganglion advanced by Fakstorp and Pedersen for these compounds does not hold for neuromuscular blockade.

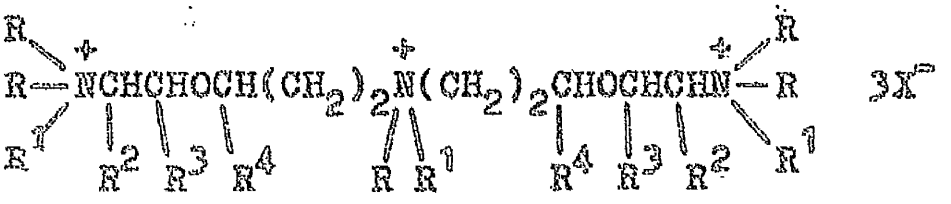
The reduction in neuromuscular blocking potency observed in the bis- and tris-onium series of ether linked compounds when compared with the corresponding polymethylene compounds has been tentatively ascribed to an anti-bonding effect arising from the availability of electrons on the symmetrically linked ether oxygen.[243,250] It therefore seemed of value

to synthesise a series of compounds of general structure (LXXXVIII; n=2, m=3, X=O) which would again, by a comparison with the corresponding polymethylene compounds[243] (LXXXVIII; n=2, m=3, X=CH_2) yield information on the role of the ether oxygen on neuromuscular blocking potency. Furthermore, a comparison between this new series (LXXXVIII; n=2, m=3, X=O) and the analogous compounds[190,243] (LXXXVIII; n=m=2, X=O)



(LXXXVIII)

which in contrast contain a symmetrical ether linkage, would afford an opportunity of assessing the anti-bonding effect of ether links since by analogy with the Fekstorp and Pedersen series (*vide supra*) an electron drift from the oxygen atoms mediated by the positively charged nitrogen atoms would now be expected. In addition, by means of methyl side-chains (LXXXIX; when R^2=Me, R^3=R^4=H; R^3=Me, R^2=R^4=H; R^4=Me, R^2=R^3=H), it should be possible to modify the electron density on the

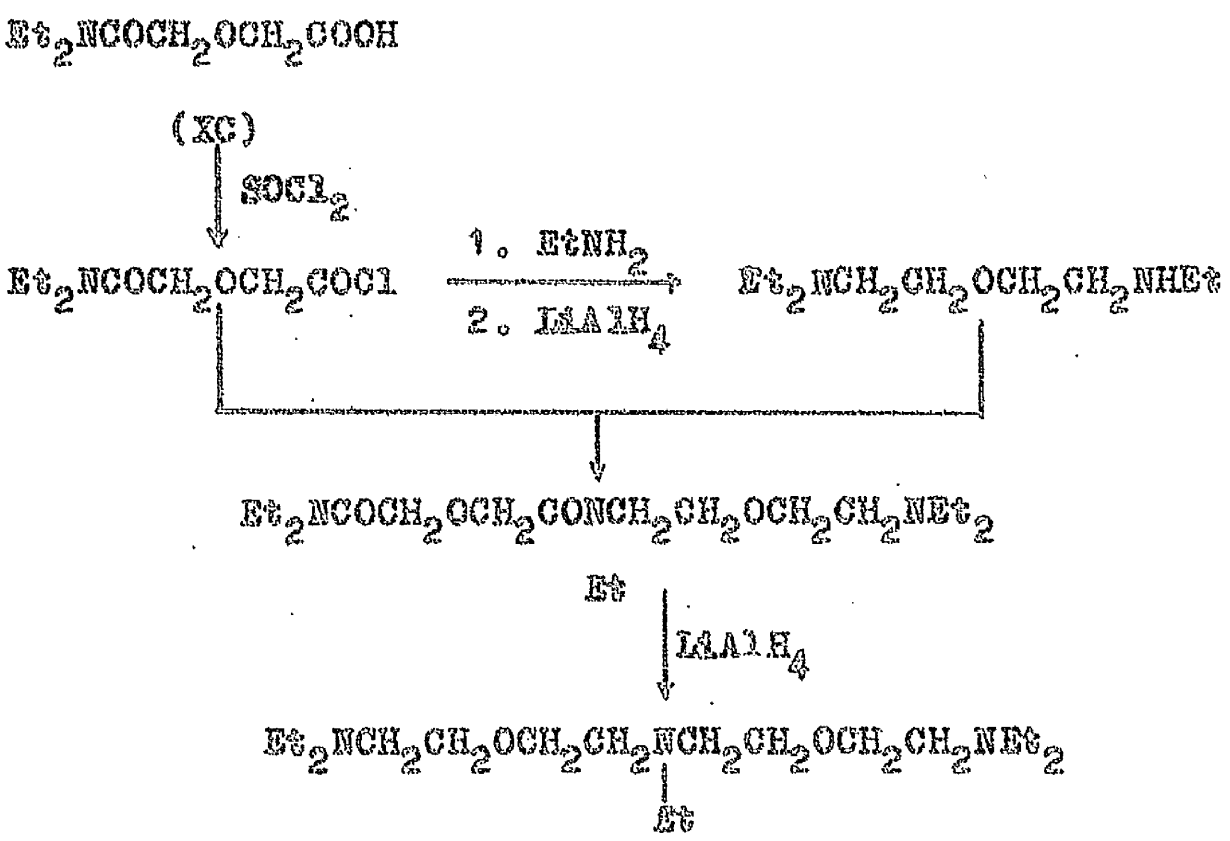


(LXXXIX)

ether oxygen atoms, the +I effect of methyl groups being well established.[251] This series of compounds was therefore synthesised with a view to effecting a balance between the -I effect of the onium groups and the +I effect of the methyl side-chains thereby affording an opportunity for a further evaluation of the role of electron availability on the ether oxygen atoms upon neuromuscular blocking potency.

Discussion of Experimental work

The triamines required for the synthesis of the ether linked tris-onium compounds (LXXXVI) prepared by Stenlake et al.[190,243] were obtained by two main routes. The first involved the assembly of the molecule in two portions with N,N-diethyldiglycollamic acid (XC), prepared from the readily available diglycollic acid, as the key intermediate (Scheme I) This route was not adopted in the projected syntheses on



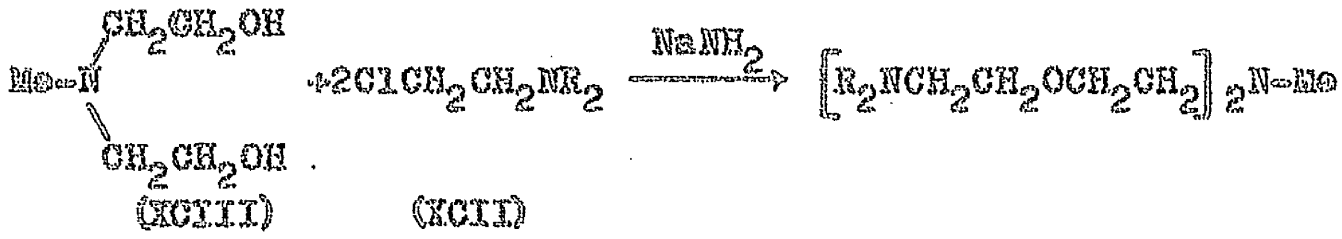
account of certain difficulties inherent in the method and also because the required 3-oxapentane-1,5-dicarboxylic acid (XCI) and its methyl branched derivatives were not readily available. The second route used by Stenlake et al.[190,243]

$$HOOCCH_2CH_2OCH_2CH_2COOH$$

(XCI)

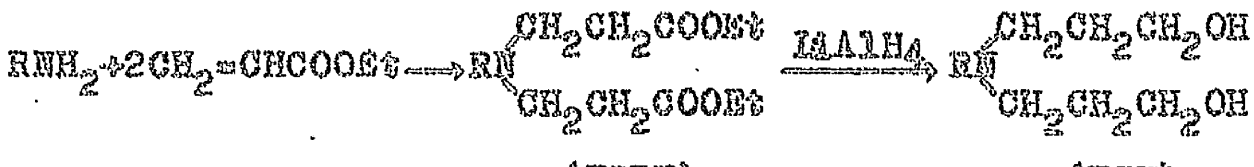
was essentially the method of Frotiva and Pliml[252] involving condensation of dialkylaminoethyl chlorides (XCII) with

N-methyldiethanolamine (XCIII) in the presence of sodamide.



(XCIII) (XCII)

An analogous scheme was considered in the present situation since monoalkylamines readily react with ethyl acrylate[253] to give bis-(ethoxycarbonylethyl) alkylamines (XCIV) and it seemed not unreasonable to expect that (XCIV) on reduction with lithium aluminium hydride would yield the corresponding N-alkyldipropan-1-olamines (XCV) required as starting materials. However difficulties were anticipated with the methyl branched

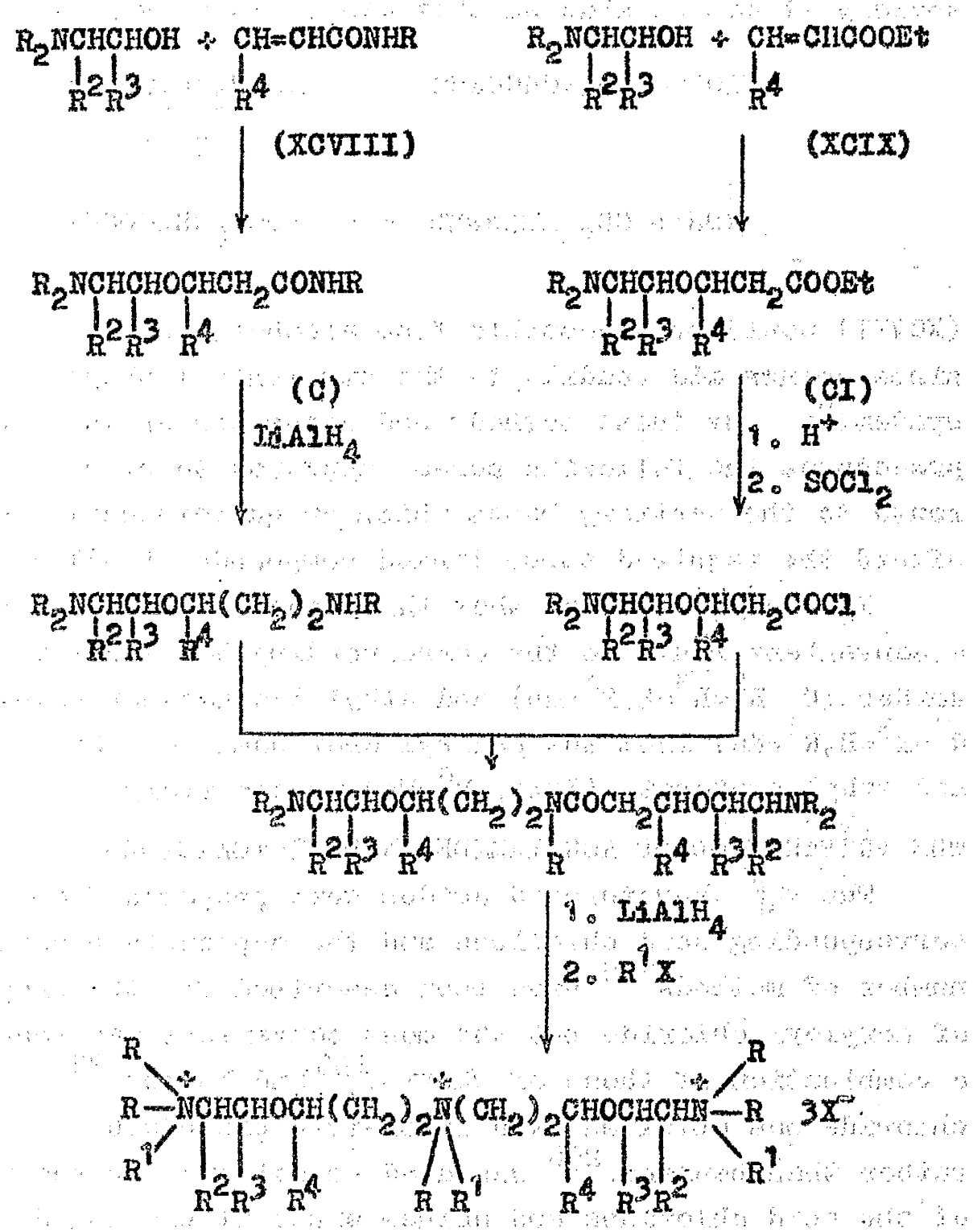


(XCIV) (XCV)

compounds (LXXXIX) which in one case (LXXXIX; R^4=Me, R^2=R^3=H) would have necessitated the use of ethyl crotonate and it is known that the addition of one mole of primary amine to two moles of crotonic ester is extremely difficult if not impossible.[253] On the other hand, the addition of one mole of amine to one mole of ester proceeds readily at room temperature.[253]

Bruson[254] has shown that a variety of organic and inorganic compounds possessing reactive hydrogen atoms add readily to acrylonitrile in the presence of an alkaline catalyst. This reaction, known as "cyanoethylation", closely resembles a Michael addition. Included among the typical compounds containing labile hydrogen atoms which have been added to acrylonitrile are alcohols, thereby providing a convenient

route to 3-alkoxypropionitriles. It therefore seemed not



R and R^1=alkyl; when R^2=Me, R^3=R^4=H; R^3=Me, R^2=R^4=H; R^4=Me, R^2=R^3=H; X=halogen

improbable that the similarly, albeit more weakly, activated double bond of N-substituted acrylamides would under similar conditions afford 3-alkoxypropion-N-alkylamides (XCVI). By analogy it was considered that ethyl 3-alkoxypropionates

$$ROH + CH_2{=}CHCONHR^1 \longrightarrow ROCH_2CH_2CONHR^1$$

(XCVI)

$$ROH + CH_2{=}CHCOOEt \longrightarrow ROCH_2CH_2COOEt$$

(XCVII)

(XCVII) would be accessible from alcohols and ethyl acrylate since amines add readily to the ethylenic linkage of this system.[253] By these methods and using conventional synthetic procedures the following scheme appeared to provide a feasible route to the tertiary bases which on quaternisation would afford the required ether linked compounds (LXXXIX).

It was anticipated that this scheme would also provide a convenient route to the corresponding 3-alkoxybutyr-N-alkyl-amides (C; $R^2{=}R^3{=}H, R^4{=}Me$) and ethyl 3-alkoxybutyrates (CI; $R^2{=}R^3{=}H, R^4{=}Me$) from the N-alkyl crotonamides (XCVIII; $R^4{=}Me$) and ethyl crotonate (XCIX; $R^4{=}Me$) respectively.

THE PREPARATION OF ACRYLAMIDES AND CROTONAMIDES

The α,β-unsaturated amides were prepared from the corresponding acid chlorides and the appropriate amine. A number of methods[255] have been described for the preparation of acryloyl chloride but the most convenient was found to be a combination of those of Moureu[256] and Kohler.[257] Crotonyl chloride was obtained by a literature procedure.[258] Ether, rather than benzene,[256] was used as solvent for the interaction of the acid chlorides and amines since it was found that it was difficult to remove the last traces of the precipitated amine hydrochloride with the latter solvent which resulted

in extensive polymerisation of the amide. The amides isolated from ether were of sufficient purity for use in subsequent stages thereby obviating distillation which likewise caused gross polymerisation. It was subsequently found that N-substituted acrylamides and crotonamides displayed interesting spectral features and so analytical samples of a number of these amides were prepared. The majority of these amides have been mentioned particularly in the patent literature but mainly without physical characteristics.

THE PREPARATION OF AMINO ALCOHOLS

The 2-dialkylaminoethanols (CII; R=Me, Et and Bu) were obtained commercially. 2-Diethylaminopropan-1-ol(CIII; R=Et, R^1=Me, R^2=H, (±)-2-dimethylaminopropan-1-ol(CIII; R=R^1=Me, R^2=H) and 1-diethylaminopropan-2-ol(CIII; R=Et, R^1=H, R^2=Me)

$R_2NCH_2CH_2OH$ (CII)

$R_2N\underset{R^1}{CH}\underset{R^2}{CH}OH$ (CIII)

were prepared by either literature or conventional synthetic procedures. L(-)-2-Dimethylaminopropan-1-ol(CIII; R=R^1=Me, R^2=H) has been prepared by Beckett et al.[259] by reduction of butyl L(-)-2-dimethylaminopropionate (CIV; R=Bu) but the final product was contaminated with butanol. In an effort to avoid

$Me_2N\underset{CH_3}{CH}COOR$ (CIV)

this, a modification of their method was adopted for the synthesis of this amino alcohol. L(+)-Alanine, prepared from racemic alanine,[259] was reductively methylated to L(+)-2-dimethylaminopropionic acid as described by Beckett et al.[259] but to accomodate the large volumes of hydrogen consumed in the process it was found that it was more convenient to generate the hydrogen by dropping an ethanolic solution of

sodium borohydride onto dilute acetic acid using the apparatus described by H.C. Brown.[260] The hydrogen was then led to a normal hydrogenation flask equipped with shaker and the volume of hydrogen used was readily calculated from the titre of borohydride solution. The L(+)-2-dimethylamino-propionic acid was converted to its methyl ester which, without further purification, was reduced to L(-)-2-dimethylamino-propan-1-ol with lithium aluminium hydride. Despite the use of the methyl ester, as opposed to the butyl ester of Beckett et al,[259] the distilled product still had a high equivalent titration indicative of contamination by methanol.

THE ADDITION OF ALCOHOLS TO N-MONOSUBSTITUTED ACRYLAMIDES AND CROTONAMIDES

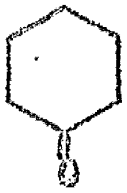
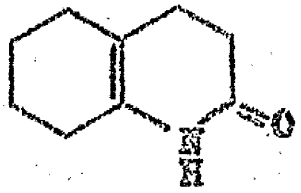
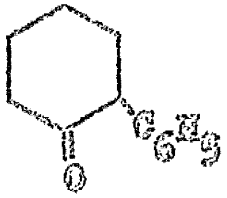
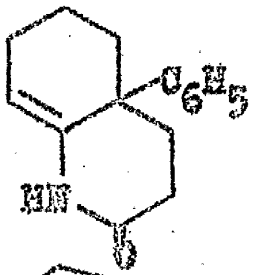
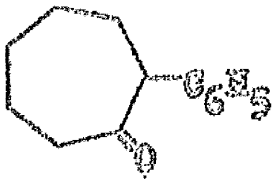
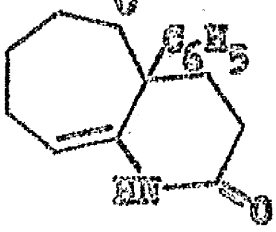
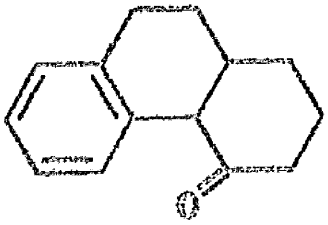
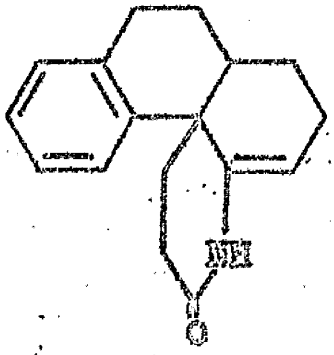
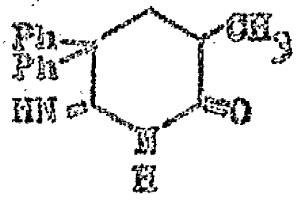
When work was initiated on the addition of alcohols to the ethylenic linkage of α,β-unsaturated amides a literature survey revealed few references to this type of reaction. The addition of compounds containing labile hydrogen atoms to acrylamide is termed carbamoylethylation[261] and is exemplified by the adducts formed with acrylamide and collagen,[262] cotton,[263] vinyl alcohol polymer fibres,[264] cellulose[265-267] and amines.[268,269] A paper by Carpenter and Davies[270] revealed that carbamoylethylations by acrylamide with compounds such as alcohols, mercaptans, sodium sulphide, sodium bisulphite and O,O-dialkyldithiophosphoric acids had been carried out in the Stamford Laboratories of the American Cyanamid Company but no details were published despite intentions to do so. A patent filed by Stade et al.[271] describes the preparation of 3-ethoxypropionamide (CV) from ethanol and acrylamide in the presence of 45 per cent sodium hydroxide and Kissinger and Schwartz[272] have prepared 4-nitro-4-azahexanamide (CVII)

$$C_2H_5OH + CH_2{=}CHCONH_2 \xrightarrow{45\%NaOH} C_2H_5OCH_2CH_2CONH_2$$

(CV)

TABLE 14.

Michael Condensations with Acrylamide and Methacrylamide

Reactant	Catalyst	Product
Acrylamide and		
	NaH	
$C_6H_5COCH_3$	KOC_4H_9-t	$C_6H_5COCH_2CH_2CH_2COOH$†
$C_6H_5CH_2COCH_2C_6H_5$	KOC_4H_9-t	$[C_6H_5CH(CH_2CH_2CONH_2)]_2CO$
	KOC_4H_9-t	
	KOC_4H_9-t	
	KOC_4H_9-t	
Methacrylamide and		
$(C_6H_5)_2CHCN$	$NaOEt$	

† This product was obtained after hydrolysis.

from N-nitritoethylamine (CVI) and acrylamide using Triton B (benzyltrimethylammonium hydroxide) as catalyst.

$$C_2H_5NHNO_2 + CH_2{=}CHCONH_2 \xrightarrow{\text{Triton B}} C_2H_5\underset{\underset{NO_2}{|}}{N}CH_2CH_2CONH_2$$

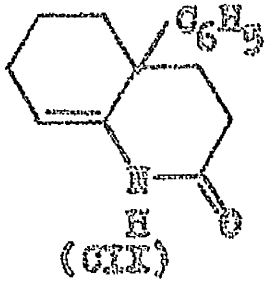
(CVI) (CVII)

A number of examples of the use of acrylamide as an acceptor in the Michael condensation have been recorded. Thus, acrylamide adds, in the presence of Triton B, one molecule of 2-nitropropane[273] to yield 4,4-dimethyl-4-nitrobutyramide (CVIII). The Michael condensation of acrylamide with a number of ketones has been achieved by Elad and Ginsburg[261] in yields ranging

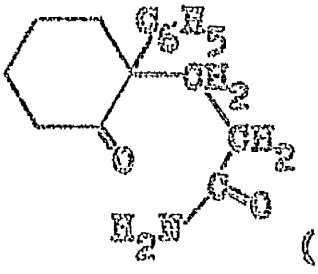
$$(CH_3)_2CHNO_2 + CH_2{=}CHCONH_2 \qquad (CH_3)_2\overset{\overset{NO_2}{|}}{C}CH_2CH_2CONH_2$$

(CVIII)

from 20 per cent to 70 per cent (Table 14). In most cases, the 2-carbamoylethyl derivative, which is undoubtedly the primary product, lost a mole of water to yield the corresponding lactam. For example, in the reaction of acrylamide with 2-phenylcyclohexanone, the lactam (CIX) rather than the 2-carbamoylethyl derivative (CX) is formed. The product resulting

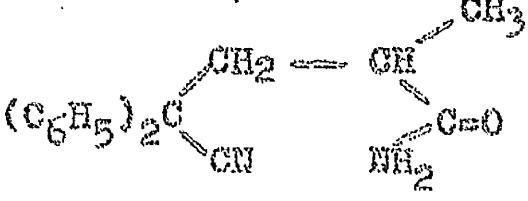


(CIX)



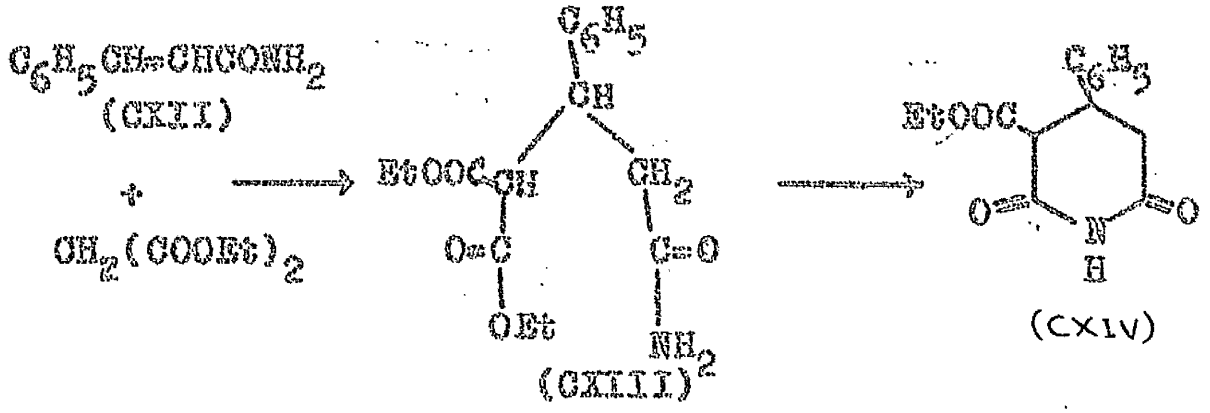
(CX)

from the condensation of methacrylamide and diphenylacetonitrile (Table 14) arises from an analogous intramolecular cyclisation of the intermediate 2-carbamoylpropyl derivative (CXI).[274] Another example of the Michael reaction involving an α,β-unsaturated amide is furnished by the condensation of cinnamamide (CXII) with diethyl sodiomalonate to give the



(CXI)

normal adduct (CXIII) which cyclises to yield ethyl 2,6-diketo-4-phenylpiperidine-3-carboxylate (CXIV).[275]



In the course of the present investigation additional examples of the formation of adducts with acrylamide and its N-substituted congeners have been described. The carbamoylethylation of the diols (CXV; n=2, 4 and 5) has been reported to proceed more readily and in higher yield using a Dowex anion exchange resin rather than a conventional base as catalyst.[276] Konigberg and Hartung[277] have prepared a number of adducts of various amines (CXVI) and malonic

$$HO(CH_2)_nOH + 2CH_2{=}CHCONH_2 \longrightarrow (CH_2)_n(OCH_2CH_2CONH_2)_2$$

(CXV)

ester derivatives (CXVII) with N-substituted acrylamides, additions of type 2 being classical Michael condensations.

1) $RNH_2 + CH_2{=}CHCONHR^1 \longrightarrow RNHCH_2CH_2CONHR^1$

(CXVI)

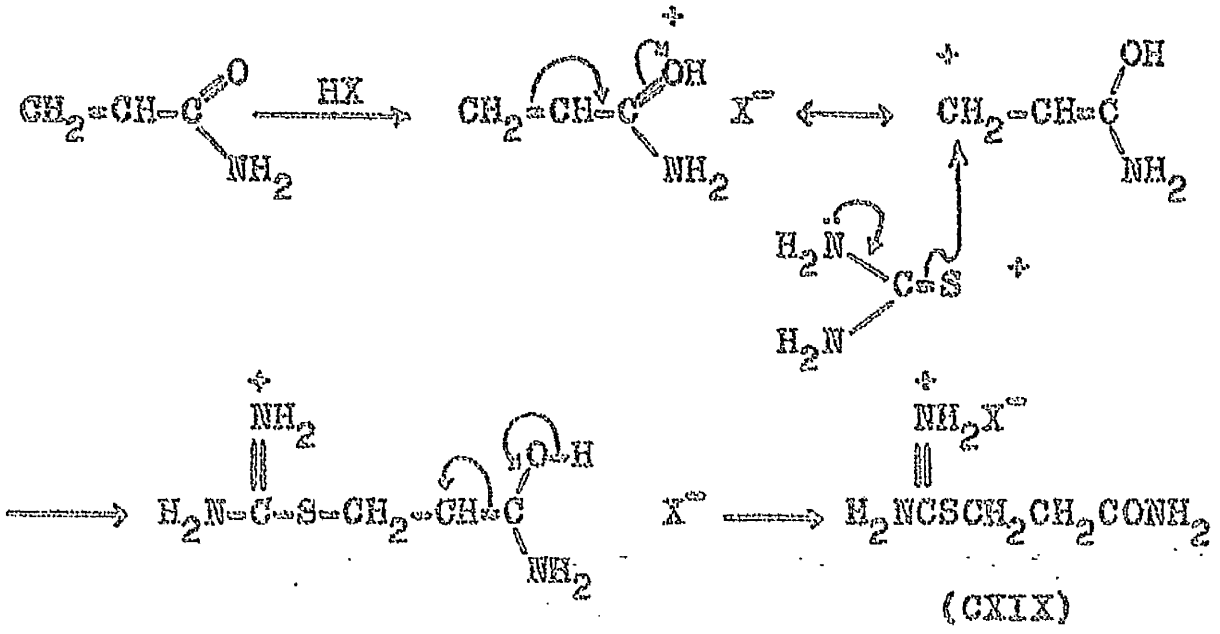
R=Benzyl; $R^1=C_6H_5$, $CH(COOEt)_2$ or CH_2COOEt

2) $RCH(COOEt)_2 + CH_2{=}CHCONHR^1 \longrightarrow RC(CH_2CH_2CONHR^1)(COOEt)_2$

(CXVII)

R^1=Ph; R=Et, $NHCOCH_3$, or

The acid catalysed addition of thiourea to N-substituted acrylamides and methacrylamides has been investigated by Bauer and Welsh.[278] The formation of the S-(2-carboxamido-ethyl)-isothiouronium salt (CXIX) from thiourea and acrylamide is typical of this class of reaction and the results are consistent with the assumption that the function of the acid catalyst is to convert the α,β-unsaturated carbonyl compound into its conjugate acid which may plausibly be supposed to be susceptible to nucleophilic attack by a thiourea molecule at the carbonium ion:-



(CXIX)

The Michael Reaction

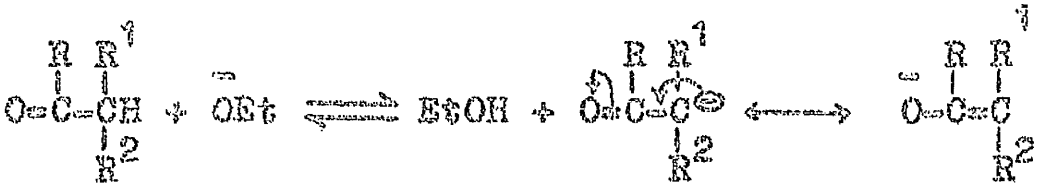
Cyanoethylation and carbamoylethylation (vide supra) both resemble closely a Michael type of addition. The Michael condensation,[279] stricto sensu, is the addition of a donor (D) containing an α-hydrogen atom in the system C-C-CH to an ethylenic double bond which forms part of the conjugated system C-C-C-O in an acceptor (A). By extension of the original scope the Michael condensation is now considered to embrace donors and acceptors activated by a variety of other functional groups including nitriles, nitro-compounds and sulphones.[279] The condensation is base catalysed, typically by alkali metal

alkoxides. The original method of Michael employed one molecular proportion of sodium ethoxide but the so-called "catalytic" method uses a much smaller amount of sodium alkoxide. The Knoevenagel method is exemplified by the

(D) (A)

use, not of sodium alkoxide, but of a secondary amine such as piperidine. A number of acid catalysed Michael-type condensations have been described and the kinetics and mechanism of the Michael reaction in non-alkaline media has recently been investigated.[280]

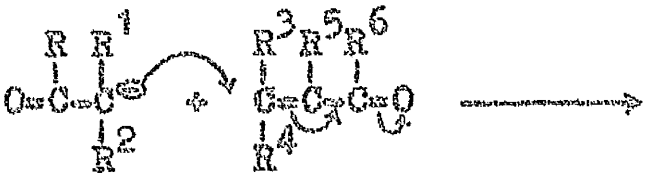
The alkaline reagents catalysing the Michael condensation are presumed to act by removing the α-hydrogen atom from the donor (D) as a proton. The carbanion (CXX), rather than



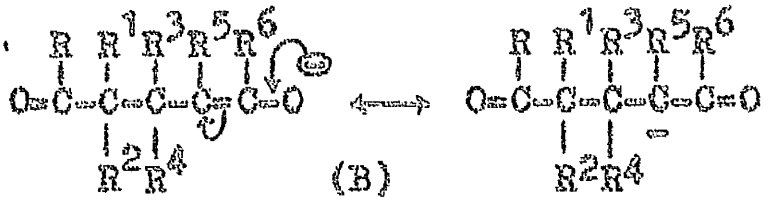
(D) (CXX) (CXXI)

the resonance enolate form (CXXI), is considered to be involved in the subsequent condensation. The new bond is formed between the carbanion (CXX) and the electron-deficient β-carbon atom of the acceptor (A).

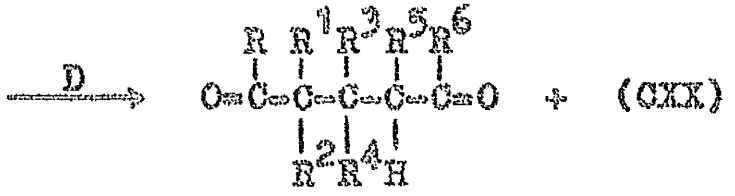
The carbanion product (B) is a resonance hybrid and it is noteworthy that the ability of acceptors (A) to serve in the Michael condensation is enhanced by polarising substituents (R^3,R^4,R^5) which stabilise the ions (B)[279] but steric effects must also be considered.[281] The proton which converts the ionised product (B) into the keto form isolated (P) may come from another donor molecule (D) thereby



(CXX) (A)



(B)



(F)

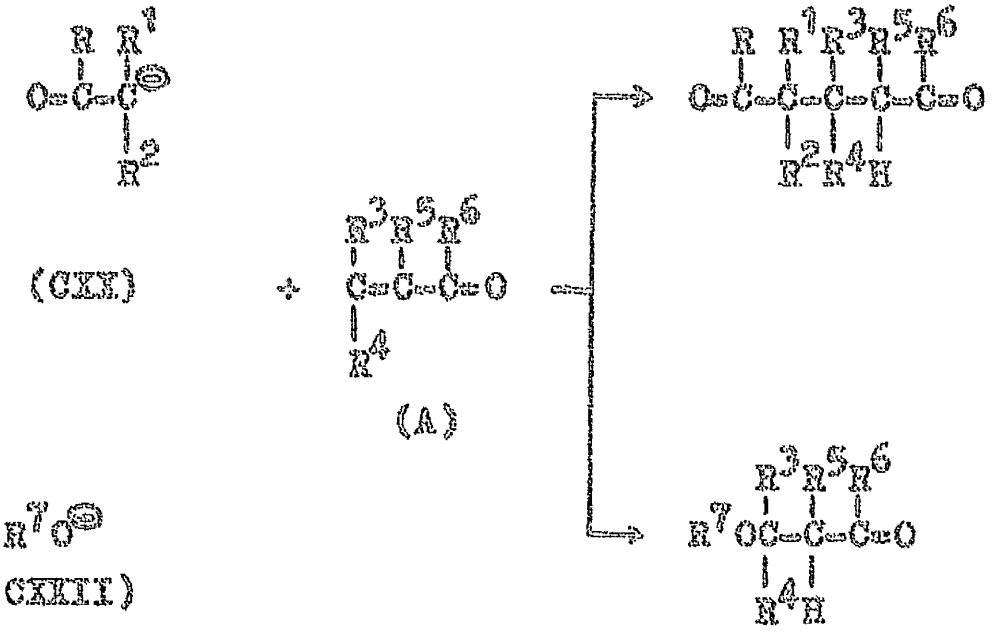
rationalising the efficacy of only catalytic amounts of base. The rate-determining stage is apparently the addition of the carbanion (CXX) to the β-carbon atom of the α,β-unsaturated molecule[281] although this is not always the case.[280]

The effect of structural changes in the donor and acceptor molecules on the rate of addition are theoretically similar to those advanced for the aldol condensation.[282] No simple polar effects of substituents (R, R^1 and R^2) in the donor (D) are expected since those which favour production of the anion (CXX) are just those which diminish the reactivity of the carbanion when produced. In the case of substituents at the double bonded carbon atoms (R^3, R^4 and R^5 in A) it would appear that + I substituents should impede nucleophilic addition to the ethylenic system, while - I substituents should accelerate it. These predictions will be invalidated where, for example, the substituents are unsaturated and conjugative effects are in evidence. Empirically it is recognised that β-alkyl groups

lower the rate of Michael additions whereas the rate of addition is greatly increased by a β-carbethoxy group as in fumaric ester (CXXI).[281]

(CXXI)

It is recognised that acceptors (A) in the Michael condensation undergo nucleophilic addition with anions in general, for example with the alkoxide anions which are commonly used as catalysts in the reaction.[279] In these instances the catalyst anion (CXXII) competes with the donor carbanion (CXX) for the acceptor molecule. This



competitive side reaction to the normal addition is especially prevalent in acceptors in which R^3-R^4-H and thus in the case of acrylates and acrylonitrile it is preferable to effect the condensation without solvent or in non-hydroxylic media.[283] However this competitive side reaction to a normal Michael condensation is the key to cyanoethylation and carbamoylethylation of alcohols which must proceed by a primary nucleophilic

attack by the alkoxide anion on the β-carbon of the ethylenic double bond. Ingold[281] has stated that it can hardly be doubted that this is the most probable mechanism and cites as an example the addition of methanol, in the presence of sodium methoxide, to methyl benzylidenemalonate (CXXIII). Thus, in the present work, the addition of 2-diethylamino-ethanol (CXXIV) to N-ethylacrylamide (CXXV) in the presence

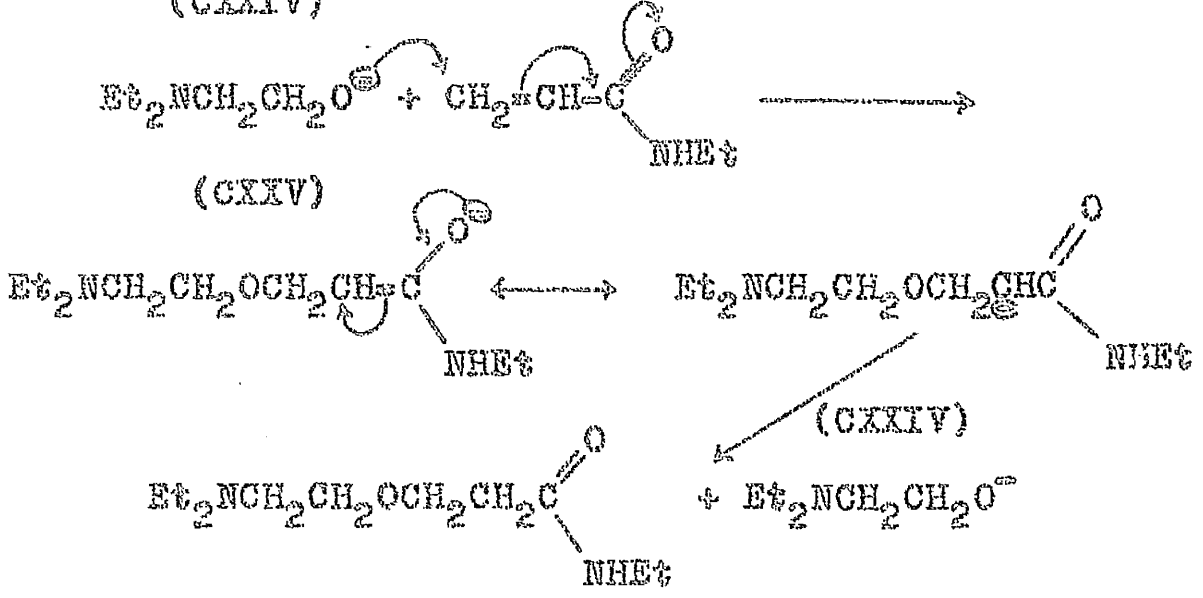
$$MeO^{\ominus} + C_6H_5CH{=}C(COOMe)_2 \longrightarrow C_6H_5CH(OMe)CH(COOMe)_2$$

(CXXIII)

of sodium methoxide must, by analogy with 'true' Michael condensations, conform to the following pathway and the factors influencing the Michael reaction would be expected to apply to these condensations:-

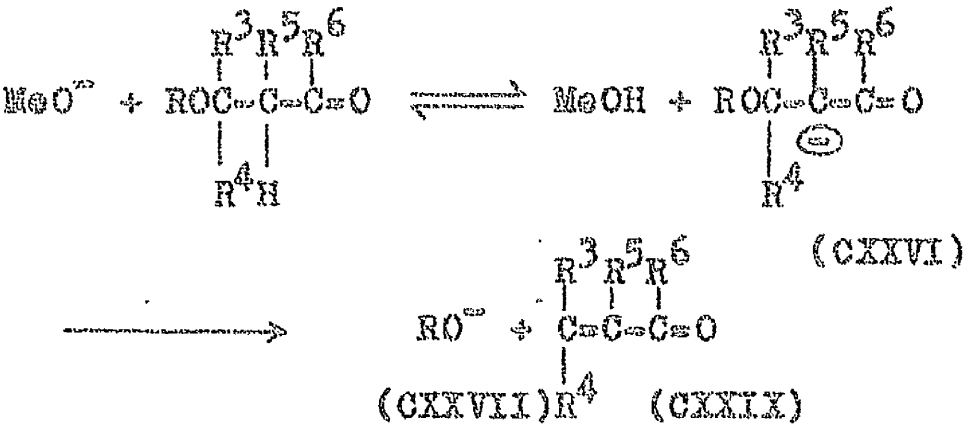
$$Et_2NCH_2CH_2OH + {}^{\ominus}OMe \longrightarrow Et_2NCH_2CH_2O^{\ominus} + MeOH$$

(CXXIV)



The Michael reaction is a reversible process and thus the addition products (P) can be split into the initial donor and acceptor moieties by the same catalysts used to effect condensation. Michael condensations as a rule are exothermic and so a larger yield of addition product results

at low temperatures. The tendency towards reversal can be counteracted by using an excess of one of the reactants. Retrogression is more likely to occur when the condensation is slow; the factors affecting the rate of condensation have been discussed above. The retrograde process is considered[281] to occur by loss of a proton from the decomposing molecule to give its anionic conjugate base (CXXVI) followed by unimolecular loss of an anion from the conjugate base (the ElcB elimination mechanism of Ingold) to yield the

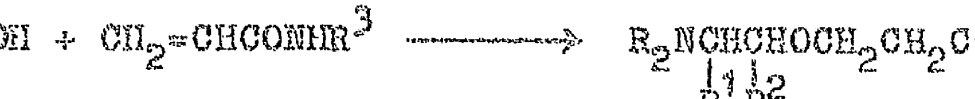


precursor donor anion (CXXVII) and acceptor (CXXIX)

The addition of aminoalcohols to α,β-unsaturated amides

Sodium methoxide (prepared by the addition of clean sodium metal to an excess of anhydrous methanol followed by prolonged evaporation to dryness at 100° under reduced pressure) was found to be the most satisfactory catalyst for the additions. The amount of catalyst used was 5 per cent w/w based on the quantity of amino alcohol.

1) Additions to acrylamides: the general method employed in these condensations is exemplified in the preparation of 3-(2-diethylaminoethoxy)propion-N-ethylamide (CXXX; R^1=R^2=H, R=R^3=Et) from 2-diethylaminoethanol and N-ethylacrylamide. The reactions were conducted at room temperature as far as possible but in the addition of 2-dibutylaminoethanol (CXXXI; R=Bu, R^1=R^2=H) to N-butylacrylamide (CXXXII; R^3=Bu) a temperature



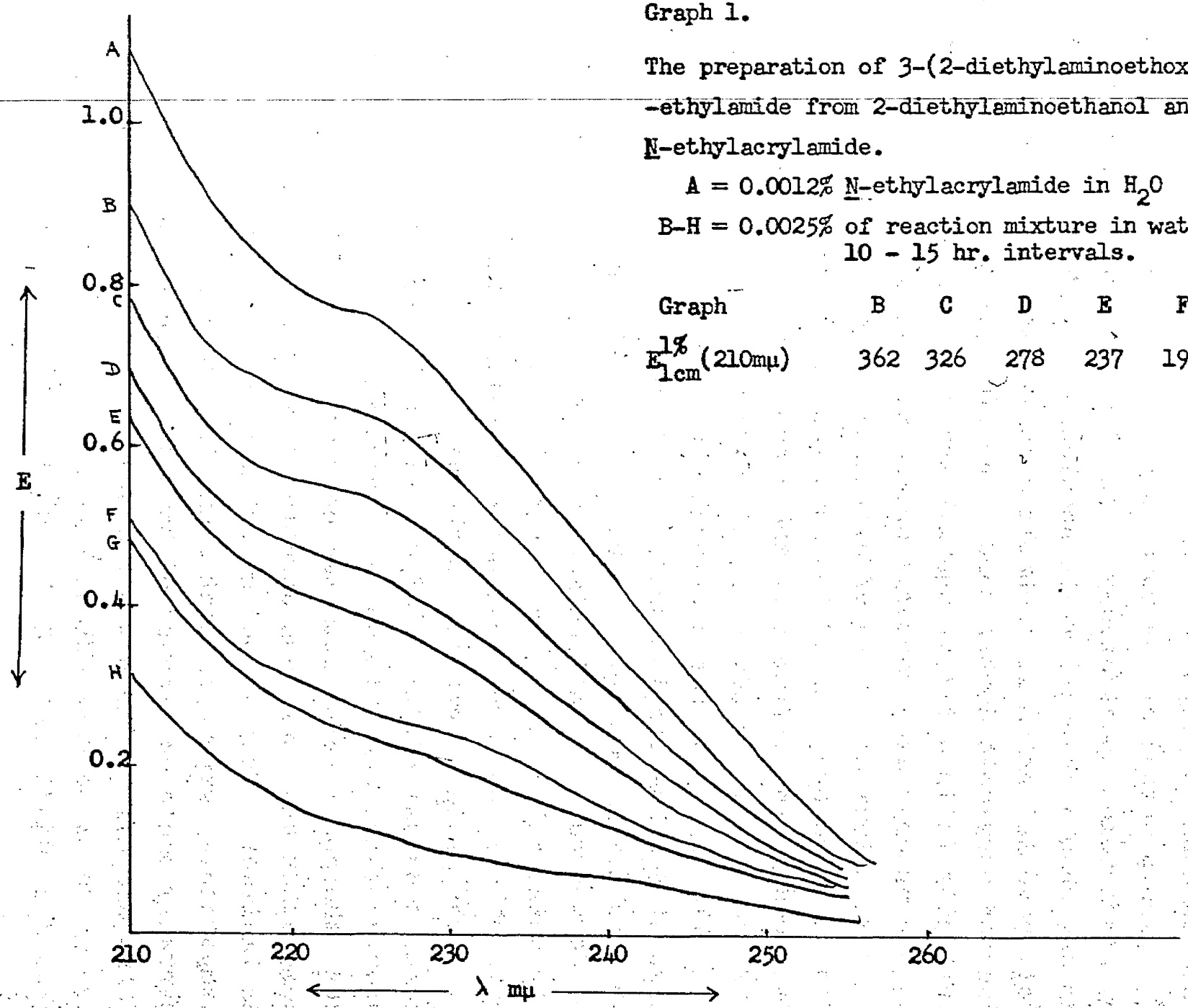
Graph 1.

The preparation of 3-(2-diethylaminoethoxy)propion-N--ethylamide from 2-diethylaminoethanol and N-ethylacrylamide.

A = 0.0012% N-ethylacrylamide in H_2O

B-H = 0.0025% of reaction mixture in water taken at 10 - 15 hr. intervals.

Graph	B	C	D	E	F	G	H
$E^{1\%}_{1cm}$ (210mμ)	362	326	278	237	198	166	130



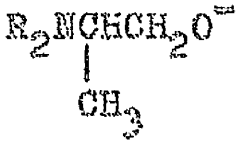
of 55° was employed. In the examples using N-methylacrylamide (CXXXII; R^3=Me), which is particularly susceptible to polymerisation, and N-butylacrylamide, which necessitated an elevated temperature, 0·1 per cent of hydroquinone was incorporated as polymerisation inhibitor.

The progress of the reactions was conveniently followed by ultraviolet spectroscopy. The N-monosubstituted acrylamides show a characteristic shoulder at ca. 220mμ ($\varepsilon \sim 0.5 \times 10^4$) (cf. page 82) which is probably a manifestation of the conjugated ethylenic double bond. As addition proceeds the intensity falls until at equilibrium only end-absorption due to the unconjugated carbonyl chromophore is observed. Graph 1 shows a typical series of ultraviolet spectroscopic measurements. The progress of the reaction may alternatively be followed in the infrared by the disappearance of the peak at 1624 cm^{-1}. The product in all cases was isolated by vacuum distillation. As has previously been observed[254] in the case of β-alkoxypropionitriles, caution must be exercised in the isolation procedure. The alkaline catalyst must first be destroyed by acidification since the products are readily dissociated by heat in the presence of alkalis into the original alcohol and a polymer of acrylonitrile.

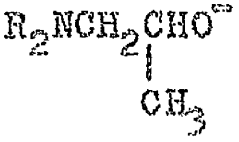
The yield of product in the case of the straight chain amino alcohols (CXXXI; R^1=R^2=H) was ca. 50 per cent. Spectroscopic measurements (Graph 1), however, in all cases indicated that the equilibrium attained corresponded to 75 per cent completion and thus considerable retrogression on distillation is implied. This is not surprising since the distillation temperatures were appreciable and the reaction mixture is markedly basic on account of both the product and the unreacted amino alcohol. These two factors provide just those conditions associated with retro-Michael reactions. The branched chain alcohols (CXXXI; R=Et, R^1=Me, R^2=H; R=R^1=Me, R^2=H; and R=Et,

(CXXXI)

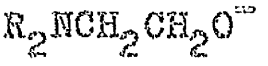
R^1=H, R^2=Me) added to N-ethylacrylamide much more rapidly (ca. 12 hours) than the homologous straight chain alcohols (CXXXI; R^1=R^2=H; ca. 3-5 days) but gave a lower yield of the addition product. This is, only in part, in accord with the findings in the Michael reaction since anions of type (CXXXII A and B) would be expected to be more nucleophilic than anions of type (CXXXIII) thereby facilitating addition to the ethenoid double bond of the amides. This is in



(CXXXIIA)

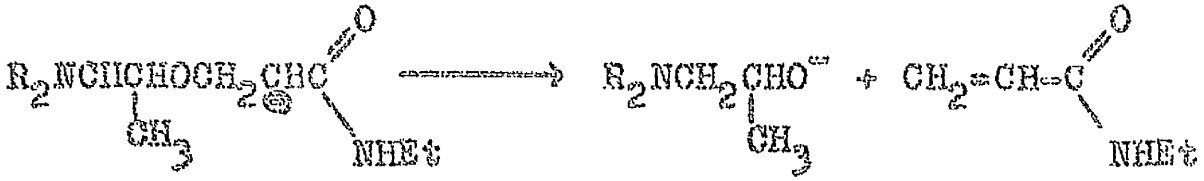


(CXXXIIB)



(CXXXIII)

agreement with the observed rate of addition. Conversely retrogression should be less favoured in these circumstances since it would now appear more difficult for the conjugate base of the addition product (eg CXXXIV) to eliminate the alkoxide anion. However it is virtually impossible to make



(CXXXIV) (CXXXIIB)

accurate predictions since other factors including the relative stability of the anions (CXXXIV) and (CXXXIIA and B) must be considered.[281] Nevertheless these reactions do not conform to the generalisation[279] that retrogression is more likely when the condensation is slow.

The condensation of 2-dimethylaminoethanol (CXXXI; R=Me, R^1=R^2=H) and N-methylacrylamide gave an impure product.

An analytical sample was obtained *via* the hydrochloride but it was surprising to find that the condensation product, 3-(2-dimethylaminoethoxy)propion-*N*-methylamide (CXXXV), formed a dihydrochloride. Amide hydrochlorides have been described

$$Me_2NCH_2CH_2OCH_2CH_2CONHMe$$

(CXXXV)

in a number of instances, but the possibility of an oxonium salt cannot be excluded.

The condensation of 2-diethylaminoethanol and *N*,*N*-diethylacrylamide (CXXXVI) to yield 3-(2-diethylaminoethoxy)propion-*N*,*N*-diethylamide (CXXXVII) is included in this section although it actually served as a model system for later work (page 84).

$$Et_2NCH_2CH_2OH + CH_2{=}CHCONEt_2 \longrightarrow Et_2NCH_2CH_2OCH_2CH_2CONEt_2$$

(CXXXVI) (CXXXVII)

2) Additions to crotonamides: an inhibitory effect from the β-methyl substituent in the crotonamide series was anticipated[279,281] and so two model systems were investigated involving the addition of methanol and ethanol respectively to *N*-isopropylcrotonamide (CXXXVIII). The reactions reached equilibrium after 12 hours at 90° and the 3-methoxy (CXXXIX)- and 3-ethoxybutyramides (CXL)

$$MeOH + CH_3CH{=}CHCONHiPr \longrightarrow MeOCH(CH_3)CH_2CONHiPr$$

(CXXXVIII) (CXXXIX)

$$EtOH + CH_3CH{=}CHCONHiPr \longrightarrow EtOCH(CH_3)CH_2CONHiPr$$

(CXL)

were isolated in good yield.

The addition of 2-dimethylaminoethanol to *N*-methylcrotonamide required a temperature of 120° for some 40 hours. Distillation gave an extremely poor yield of 3-(2-dimethylaminoethoxy)butyr-*N*-methylamide (CXLI; R=Me) which was contaminated with *N*-methylcrotonamide and a large polymeric residue remained in the distillation flask. Catalysis by sodium metal or

Triton B failed to improve the condensation. By comparison

$$R_2NCH_2CH_2OCH(CH_3)CH_2CONHR$$

(CXLI)

the preparation of 3-(2-diethylaminoethoxy)butyr-N-ethylamide (CXLI; R=Et) from the appropriate alcohol and amide was easily accomplished. This would seem to indicate that the diethylaminoethoxide anion is more nucleophilic than the dimethylaminoethoxide anion unless some subtle involvement of the N-alkyl substituent of the amide is invoked, an effect not apparent in the acrylamide series.

THE ATTEMPTED PREPARATION OF 2-DIETHYLAMINOETHOXY-PROPIONIC ACID

This acid was required for the second half of the projected syntheses (Scheme I, page 52.).

1) The Addition of 2-Diethylaminoethanol to Ethyl Acrylate

The use of α,β-unsaturated esters as acceptors in the Michael condensation is well documented[279] and the addition of amines to the olefinic linkage of acrylic esters has also been described.[284,285] The addition of an alcohol to an acrylic ester was first described by Purdie and Marshall.[286] Rohberg, Dixon and Fisher[287-290] showed that this type of addition provided a convenient route to 3-alkoxypropionates (CXLII; R=alkyl) and described the preparation and physical properties of a series of these compounds.

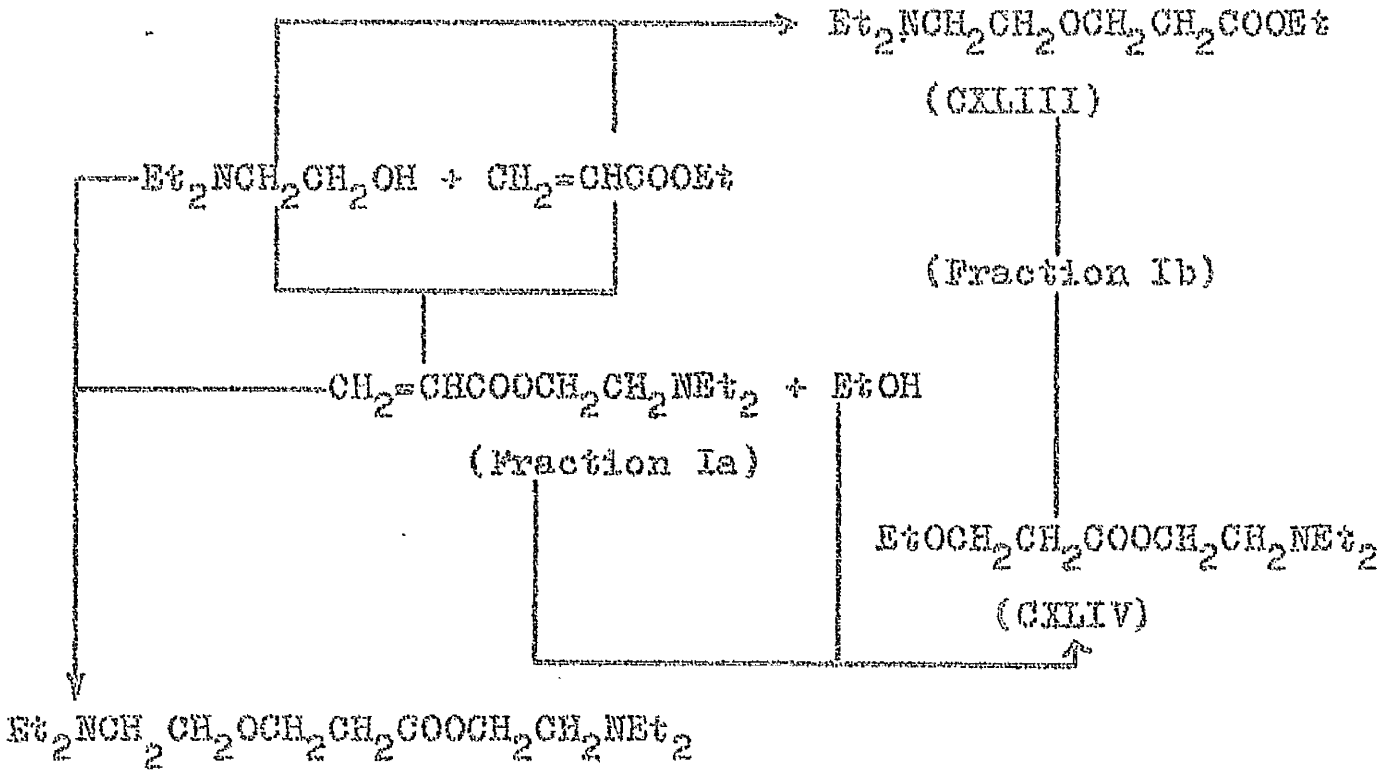
$$ROH + CH_2=CHCOOEt \longrightarrow ROCH_2CH_2COOEt$$

(CXLII)

The reaction is again analogous to the Michael condensation, cyanoethylation and carbamoylethylation (vide supra) and is base catalysed. Rohberg and colleagues[287] have shown that of the bases Triton B, concentrated sodium hydroxide, aluminium alcoholates and sodium alcoholates only the last mentioned are effective in catalysing the formation of an adduct and that

in general the order of reactivity of addenda[290] is phenols > primary alcohols > secondary alcohols; tertiary alcohols do not react. Furthermore, poor yields of adduct with methyl and ethyl acrylate were not improved by the use of an excess of alcohol and more vigorous conditions since this brought about addition to the unsaturated linkage and simultaneous alcoholysis of the ester function. Numerous intermediate and high boiling fractions were often obtained in distillation indicating that a complex mixture of by-products was produced.

Method A: The addition of 2-diethylaminoethanol containing ca. 2 per cent sodium methoxide to ethyl acrylate produced a strongly exothermic reaction. The temperature was maintained at 25° and the mixture then allowed to stand overnight. The distillation of the mixture, after destruction of the catalyst by concentrated sulphuric acid, proved to be extremely difficult on account of excessive frothing. Even under the best conditions, which were achieved by an extremely fine air leak, clear cut fractions could not be obtained but gave two main boiling ranges which were then separately distilled in vacuo, still with considerable difficulty. Molecular distillation failed to improve the fractionation and it was considered inadvisable to pack the distillation flask with glass wool, a technique which has been adopted for dealing with frothing, lest the alkalinity of the glass was sufficient to initiate retrogression. Distillation of the two main fractions afforded a number of sub-fractions from whose analyses the reaction pathway outlined below is tentatively proposed, all reactions occurring simultaneously. Whether this complexity of products arises under distillation conditions or at room temperature was not decided but probably both sets of conditions are involved.



It would appear that the primary reaction between 2-diethylaminoethanol and ethyl acrylate is alcoholysis to yield 2-diethylaminoethyl acrylate (Fraction Ia) and ethanol. Rehberg and colleagues[291-293] and Graves[294] have shown that the base (eg. aluminium isopropoxide) catalysed reaction of 2-dialkylaminoethanols (CXLV; R=R^1=Alkyl) with acrylic and methacrylic esters yields the corresponding 2-dialkylamino-alkyl esters by simple transesterification. Sims et al.[295,296] have similarly observed the ease of transesterification of acrylic and methacrylic esters with 2-alkylaminoethanols (CXLV; R=H, R^1=alkyl) but in this case the hydrogen of the secondary

$$RR^1NCH_2CH_2OH$$

(CXLV)

amino function introduces additional complicating factors by double bond addition and amide formation. In the literature examples cited, alcoholysis was effected at temperatures higher than those used in the present situation but this is

partly to enable azeotropic distillation of the simple alcohol (ethanol or methanol) produced and leaves undecided the possibility of at least some transesterification at lower temperatures.

The ethanol liberated by alcoholysis of the ethyl acrylate with 2-diethylaminoethanol adds across the double bond of the 2-diethylaminoethyl acrylate (Fraction Ia) to give the isomer (CXLIV) of the expected addition product (CXLIII) which was probably also obtained (Fraction Ib). Initially Fraction Ib was considered to be a single compound and in this context it should be noted that Rehberg et al.[287,288] have shown that compounds of general formula $ROCH_2CH_2COOR^1$ and $R^1OCH_2CH_2COOR$ have virtually identical boiling points, densities and refractive indices. Acid hydrolysis of fraction Ib gave 2-diethylaminoethanol hydrochloride and it was thus concluded that cleavage of the ether linkage of (CXLIII) had occurred. (compare page 70) but a later experiment revealed the presence of 3-ethoxypropionic acid in the hydrolysate thus strengthening the view that fraction Ib. was a mixture of the isomers (CXLIII) and (CXLIV).

The fourth ester (Fraction IIb) was also isolated but its acid hydrolysis yielded 2-diethylaminoethanol hydrochloride as the only identifiable product.

Method B: The addition of 2-diethylaminoethanol to ethyl acrylate was repeated using sodium 2-diethylaminoethoxide as catalyst.[287] A complex mixture of products similar to that described in Method A was suspected and the reaction was not further investigated.

The catalysts 50 per cent sodium hydroxide and Triton B failed to improve the condensation.

2) The Hydrolysis of 2-Diethylaminoethoxypropionitrile

The ease of hydrolysis of 3-alkoxypropionitriles[297-298]

to the corresponding 3-alkoxypropionic acids prompted the attempt to hydrolyse the readily accessible 3-(2-diethylamino ethoxy)propionitrile[254] (CXLV) to its derived acid (CXLVI).

ethoxy)propionitrile[254] (CXLV) to its derived acid (CXLVI).

Acid hydrolysis of the nitrile (CXLV) afforded acrylic acid,

$$Et_2NCH_2CH_2OCH_2CH_2CN \longrightarrow Et_2NCH_2CH_2OCH_2CH_2COOH$$

(CXLV) (CXLVI)

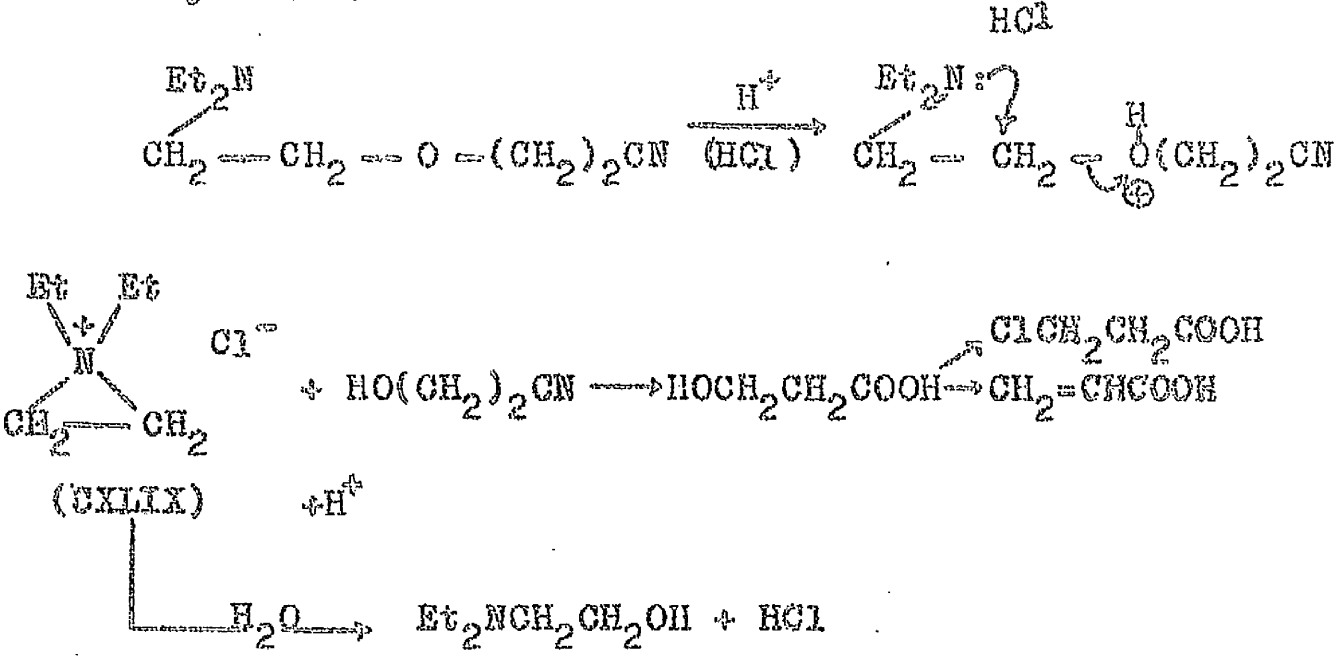
3-chloropropionic acid and 2-diethylaminoethanol all in low yield and a considerable quantity of an unidentified polymeric material, as a viscous yellow oil and an orange solid, both of which had identical infrared spectra. The recovery of products indicated that perhaps some of the desired amino acid (CXLVI) had remained in the aqueous phase left after extraction of the other products. A second hydrolysis of 3-(2-diethylamino-ethoxy)propionitrile similarly yielded acrylic acid. Evaporation of the acid hydrolysate to dryness followed by mild esterification gave a basic ester, which was identified as ethyl 3-(2-diethylaminoethoxy)propionate (CXLVII), and a viscous polymeric oil.

$Et_2NCH_2CH_2OCH_2CH_2COOEt$ $ROCH_2CH_2CN$

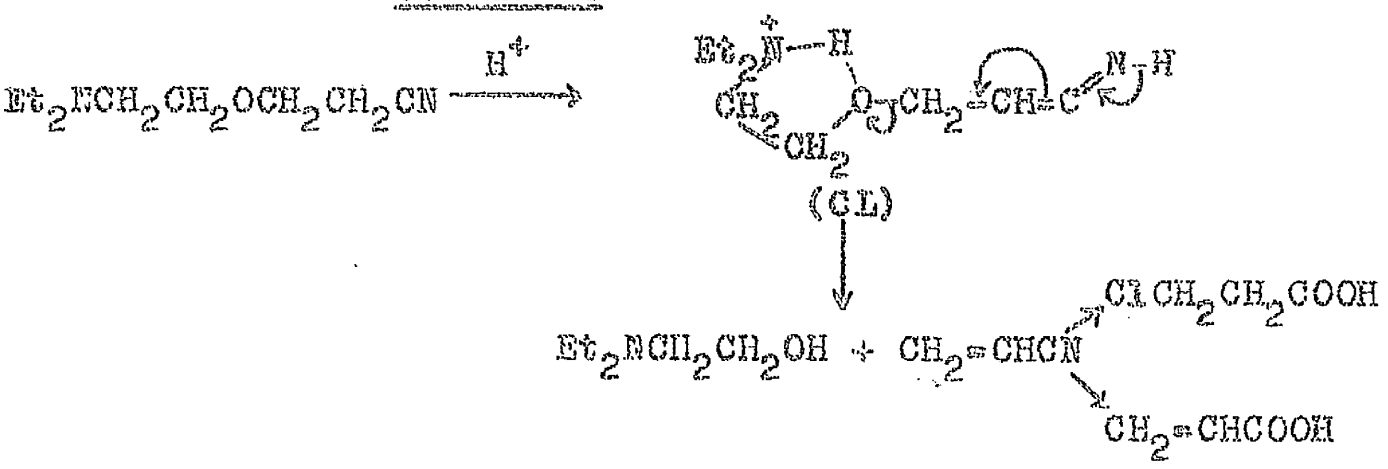
(CXLVII) (CXLVIII)

The acid hydrolysis of 3-(2-diethylaminoethoxy)propionitrile (CXLV) to the corresponding acid (CXLVI) must therefore occur to a limited extent and the isolation of acrylic acid, 3-chloropropionic acid and 2-diethylaminoethanol suggests that cleavage of the ether linkage is a competing reaction. Since 3-alkoxypropionitriles (CXLVIII; R=alkyl) hydrolyse in aqueous acid to the corresponding acids without difficulty,[297,298] neighbouring group participation by the diethylamino group would seem to be indicated to rationalise ether cleavage in the present example. Two possible mechanisms are shown but neither is particularly satisfactory. Scheme I invokes an ethylene-iminium ion (CXLIX) analogous to those shown in other situations[299]

but since the reaction was conducted in strong acid the internal nucleophilic attack by nitrogen would seem unlikely.[300] Scheme II involves the protonated amino group as an intramolecular acid catalyst (CL) and is somewhat similar to the effect

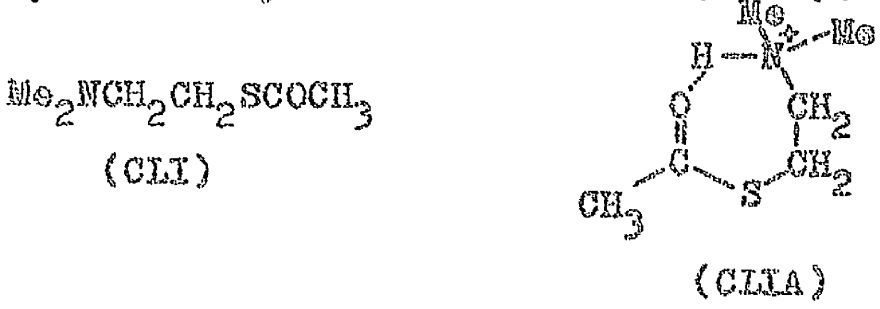


Scheme I.



Scheme II.

envisaged by Hansen[301] to explain the rapid acid hydrolysis of 2-dimethylaminoethyl thioacetate (CLI, CLIA). However it



(CLI) (CLIA)

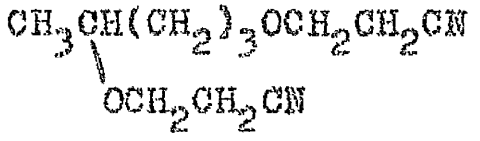
would be anticipated that protonation of the ether oxygen by the solvent (hydrochloric acid) would successfully compete with the proposed intramolecular effect in (CL).

A third explanation of the observed ether cleavage may be that the hydrolysis of 3-(2-diethylaminoethoxy)propionitrile (CXLV) proceeds readily to the corresponding acid (CXLVI) which in turn, in hot acid solution, cleaves to acrylic acid and 2-diethylaminoethanol. By analogy it is known that acid catalyses the addition of both alcohols and water to the double bond of α,β-unsaturated ketones and acids and similarly catalyses the retro-reaction:[281]

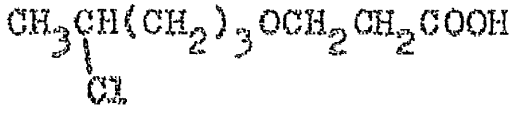
$$ROH + CH_2{=}CHCOOH \underset{H^+}{\overset{H^+}{\rightleftharpoons}} ROCH_2CH_2COOH$$

R=H or Alkyl

Christian and Hixon[297] have observed ether cleavage of an alkoxy-propionitrile during acid hydrolysis. 1,4-Di-(2-cyanoethoxy)pentane (CLI) on hydrolysis with concentrated acid yielded 3-(4-chloropentoxy)propionic acid (CLII; isolated as the ethyl ester); no attempt was made to explain the fission



(CLI)



(CLII)

of the cyanoethoxy group.

3) The Preparation of 3-(2-Diethylaminoethoxy)propionamide

A third attempted route to the required 3-(2-diethylamino-ethoxy)propionic acid (CXLVI) was by conversion of 3-(2-diethyl-aminoethoxy)propionitrile (CXLV) to the amide (CLIII) thence by nitrous acid[302,304] to (CXLVI). The nitrile (CXLV) reacted vigorously with alkaline hydrogen peroxide[305-308]

$$Et_2NCH_2CH_2OCH_2CH_2CN \xrightarrow[H_2O_2]{} Et_2NCH_2CH_2OCH_2CH_2CONH_2 \xrightarrow{HNO_2} Et_2NCH_2CH_2OCH_2CH_2COOH$$

(CXLV) (CLIII) (CXLVI)

but gave a poor yield of the amide (CLI) the remainder of the reaction mixture being an unidentified complex mixture.

4) Several other attempted routes, including metathesis,[309] to 3-(2-diethylaminoethoxy)propionic acid were unsuccessful.

THE LITHIUM ALUMINIUM HYDRIDE REDUCTIONS OF 3-(DIALKYLAMINOALKOXY)PROPION-*N*-ALKYLAMIDES AND RELATED PRODUCTS

The reduction of the 3-(dialkylaminoalkoxy)propion-*N* alkyl amides (page 54) of general formula (CLIV; R=alkyl, R_2 and R_3=Me or H, R_4=H) and of the homologous 3-(2-dialkylaminoethoxy)butyr-*N*-alkylamides (page 54) of formula (CLIV; R=MeorEt

$$R_2NCH(R^2)CH(R^3)OCH(R^4)CH_2CONHR \qquad R_2NCH(R^2)CH(R^3)OCH(R^4)(CH_2)_2NHR$$

(CLIV) (CLV)

R^2=R^3=H, R^4=Me) with lithium aluminium hydride in diethyl ether solution gave the corresponding amines of general formula (CLV) in 40-60 per cent yield. In all cases the reduction was accompanied by significant cleavage of the ether linkage as judged by the isolation of the amino alcohol and appreciable quantities of a polymeric amide. The reactions were usually conducted using an excess of lithium aluminium hydride (*ca*. 1·5-2moles) but ether cleavage was still comparable when ether a gross excess (4 moles) or the theoretical amount (0·75 moles) of hydride was employed.

The ability of lithium aluminium hydride to cleave ethers is well known[310-313] but in general ethers are resistant to attack by the reagent.[310] However it would appear from the work of Soffer and colleagues[314,315] and from the present investigation that 3-alkoxypropionitriles and 3-alkoxypropionamides present an unusual exception.

During the hydrolysis of the reaction mixture of a

3-alkoxypropionitrile (CLVI) and lithium aluminium hydride in ether Soffer and Parrotta[314] observed the formation of a volatile amine. The compound was n-propylamine (CLVII) the other products of reduction being the parent alcohol (CLVIII) and the desired 3-alkoxypropylamine (CLIX). Cleavage was in all cases accompanied by the evolution of hydrogen. The cleavage is general as shown in Table 15.

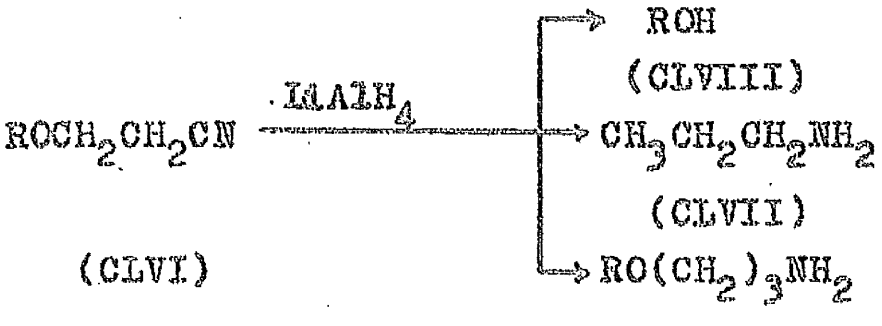


TABLE 15.

$LiAlH_4$ Cleavage of 3-Alkoxypropionitriles in Ether and THF[314,315]

3-Alkoxypropionitrile	Cleavage per cent in	
	Ether	THF
Isopropoxy	4·6	88·7*
n-Butoxy	7·6	-
n-Octyloxy	6·3	92†
Benzyloxy	11·3	81·8†
Phenoxy	20·7	99·3† 94·7†
2-Naphthoxy	Insoluble	92·6*

*at 65° †at 34°

When the reductions were performed in tetrahydrofuran the amount of cleavage was even more pronounced and that this is an effect of solvent rather than temperature is illustrated by the similar degree of cleavage of 3-phenoxypropionitrile in tetrahydrofuran at 34° and at 65° (Table 15).

Furthermore, when the 3-alkoxy- or 3-aryloxypropylamine (CLIX; R=alkyl or aryl) was subjected to the identical reaction procedure with lithium aluminium hydride in ether no significant amount of cleavage occurred, thus showing that cleavage must occur on the nitriles (CLVI) or one of the intermediate products of reduction and not on the propylamine ether. Soffer and colleagues[315] have also shown that as the molar ratios of hydride to nitrile were lowered below 1 the amount of cleavage increased and that direct addition (of nitrile to hydride) and reverse addition gave essentially similar results.

The most attractive mechanism for the cleavage of 3-alkoxy-(or aryloxy)-propionitriles appeared to involve the elimination and simultaneous reduction of acrylonitrile effected by lithium aluminium hydride.[314,315] Simultaneous reduction is envisaged since acrylonitrile, even at great dilution in ether, cannot be reduced to n-propylamine but instead yields polymeric materials.[314] It was also considered possible that the actual mechanism might involve a prior partial reduction of the propionitrile moiety, followed by its elimination or a simultaneous partial reduction and elimination. Soffer and Parrotta[314] expressed the reaction as

$$ROCH_2CH_2CN \xrightarrow{MAlH_4} ROCH_2\overset{\oplus}{C}HCN + AlH_3 + H_2$$

$$ROCH_2\overset{\oplus}{C}HCN \longrightarrow RO^{-} + CH_2{=}CHCN$$

which is analogous to the cleavage of ethers by organoalkali metal compounds,[316] for example propyl sodium.

In the reductions of the 3-(dialkylaminoalkoxy)-propion- and -butyr-N-alkylamides the action of lithium aluminium hydride on 3-(2-diethylaminoethoxy)propion-N-ethylamide (CLX) was chosen as typical example for further study. It was

realised that the scale of the reaction influenced the number of products isolated,[317] the largest scale reaction performed (being the most diagnostic of the course of the cleavage of the being the most diagnostic of the course of the cleavage of the ether linkage. That cleavage occurred during the actual reduction and not on subsequent distillation of the reduced material was shown by running some of ethereal solution of the reaction mixture on paper using butanol: acetic acid: water (4:1:5) as solvent. The reaction mixture clearly showed the presence of 2-diethylaminoethanol (R_F0·55) indicating that

$Et_2NCH_2CH_2OCH_2CH_2CONHEt$ (CLX)

$Et_2N(CH_2)_2O(CH_2)_3NHEt$ (CLXA)

cleavage had already taken place.

Distillation of the reaction mixture, after decomposition of the complexes and excess of lithium aluminium hydride by the method of Amunsden and Nelson,[318] gave two distinct fractions (A and B). A considerable quantity of a viscous polymeric residue remained in the distillation flask. The acetone / carbon dioxide trap on the vacuum pump also contained a small quantity of material. Paper chromatography of the two distillation fractions revealed the presence 2-diethylamino-ethanol and the required reduced amide 3-(2-diethylamino-ethoxy)propylethylamine (CLXA). In addition Fraction A had a third component with an R_F value comparable to that of diethylamine. This same component, together with 2-diethyl-aminoethanol, occurred in the "trap" fraction. The trap fraction also revealed the merest trace of an unidentified component R_F0·90.

An attempt to distil fraction A at atmospheric pressure through a column packed with glass helices gave one drop of distillate which in composition was identical to the "trap" fraction. Distillation of fraction A at the water pump gave a small forerun from which N,N'-diethyl-1,3-diamino-

-propane (CLXI) was isolated as the hydrochloride. The forerun had an equivalent of 82·5 and was thus impure N,N'-diethyl-1,3-diaminopropane which requires an equivalent of 62·5. The remainder of fraction A proved to be entirely composed of 2-diethylaminoethanol.

$$EtNH(CH_2)_3NHEt$$

(CLXI)

The viscous polymeric residue in the distillation flask showed amide absorption at ca. 1640cm^{-1} and some absorption at ca. 3400-3500cm^{-1} indicative of hydroxyl groups. Snyder and Putnam[319,320] have shown that the reduction of N,N-disubstituted α,β-unsaturated amides with lithium aluminium hydride gives products resulting from reductive coupling. Thus, for example, N,N-diethylacrylamide (CLXII; R=H) gives

$$RCH{=}CHCONEt_2 \longrightarrow [RCH_2CH_2CONEt_2] + RCH_2CHCONEt_2$$

(CLXII) (CLXIII) $RCHCH_2CONEt_2$

(CLXIV)

$$+ RCH_2CHCONEt_2$$

$$[RCHCHCONEt_2]_n$$

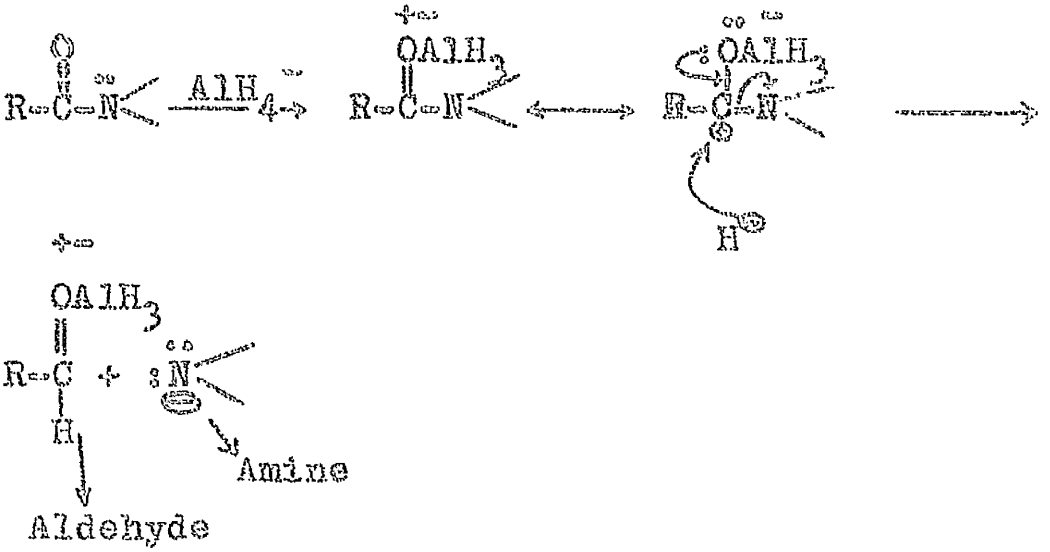
$$RCHCH_2CONEt_2$$

(CLXV; n ? 0)

a polymeric amide (CLXV; R=H) and N,N-diethylcrotonamide (CLXII; R=Me) gives the dimer (CLXIV; R=Me) and a low molecular weight polymer (CLXV; R=Me). Products akin to (CLXIII-CLXV) were isolated with other α,β-unsaturated amides. Basic products, assumed to arise from reduction of the amide groups, were also formed. Two essentially similar mechanisms, involving attack on the α,β-unsaturated amides by an aluminohydride ion were advanced to explain the reductive coupling.[320]

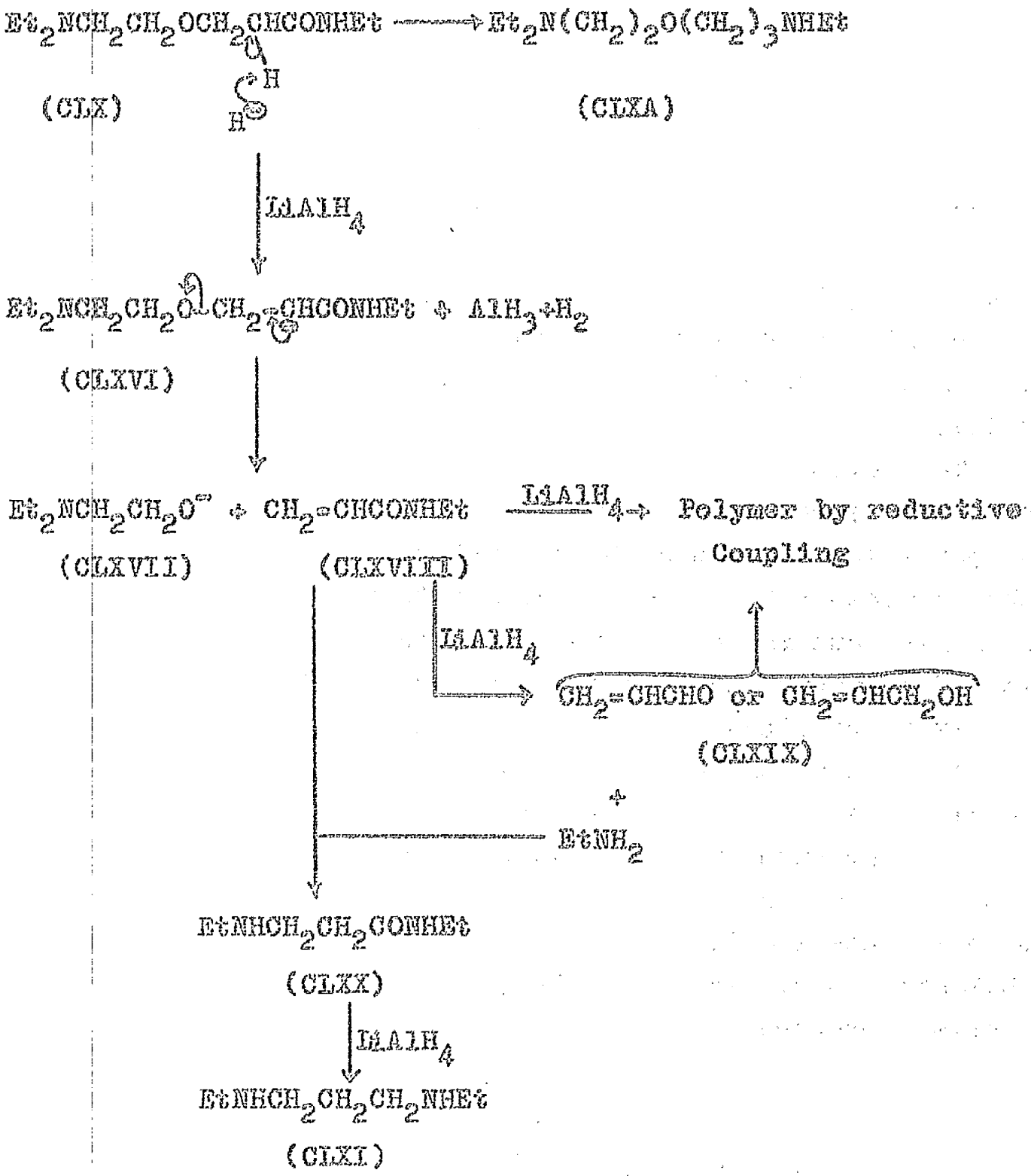
Snyder and Putnam[320] also observed that all reductions

of α,β-unsaturated amides gave rise to a volatile amine. This amine was shown to arise from cleavage of the nitrogen-carbon bond of the amide group to give an aldehyde (or alcohol) and the amine. The cleavage of amides to give these compounds has previously been observed in a number of instances[321] but is usually associated with steric hindrance on the nitrogen atom mediated by large substituents. A mechanism for this cleavage has been proposed by Paddock[322] and is shown below. The carbon fragment from the cleavage of the N-C bond of the



α,β-unsaturated amides then contributes to the polymeric product(s) formed by reductive coupling and the infrared spectra of these materials often showed hydroxyl and carbonyl absorptions.[320]

The isolation of N,N'-diethyl-1,3-diaminopropane (CLXI) in the lithium aluminium hydride reduction of 3-(2-diethylaminoethoxy)propion-N-ethylamide (CLX) together with the other products may now be rationalised by the pathways shown in Scheme III. The various eliminations and cleavages are shown on the unreduced products but may well occur on the partially reduced moieties. The action of lithium aluminium hydride on the propion-N-ethylamide (CLX) is considered, by

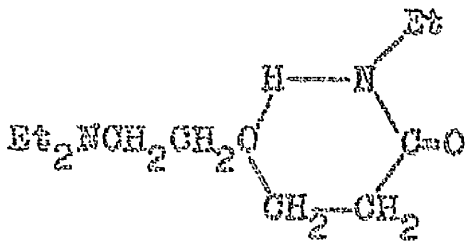

Et2NCH2CH2OCH2CHCONHEt
(CLX)
Et2N(CH2)2O(CH2)3NHEt
(CLXA)
LiAlH4
Et2NCH2CH2O CH2=CHCONHEt + AlH3 + H2
(CLXVI)
Et2NCH2CH2O⁻ + CH2=CHCONHEt
(CLXVII)
(CLXVIII)
LiAlH4
Polymer by reductive Coupling
LiAlH4
CH2=CHCHO or CH2=CHCH2OH
(CLXIX)
+
EtNH2
EtNHCH2CH2CONHEt
(CLXX)
LiAlH4
EtNHCH2CH2CH2NHEt
(CLXI)

Scheme XII.

analogy with the mechanism proposed by Soffer and Farrotta,[314,315] to give the reduced product (CLXA) and the anion (CLXVI). The anion then eliminates the alkoxide anion (CLXVII) to give N-ethylacrylamide which undergoes reductive coupling to give the polymer. The N-ethylacrylamide by C-N fission also gives rise to ethylamine and the carbon fragments (CLXIX), the latter contributing to the polymeric material. The ethylamine further reacts with the N-ethylacrylamide to give the adduct (CLXX) which reduces normally to N,N'-diethyl-1,3-diaminopropane (CLXI).

The polymeric residue in the distillation flask showed infrared absorption indicative of hydroxyl groups but it was not possible to exclude the possiblity that these arose from other sources (the polymer appeared to be hygroscopic). The polymeric material unlike that of Snyder and Putnam,[319,320] yielded no identifiable product on hydrolysis.

It is realised that other mechanisms may be used to explain the products of the above reduction. It is possible, for example, that some of the ethylamine may be liberated by lithium aluminium hydride from the propion-N-ethylamide (CLX). Thus, since C-N cleavage is usually only encountered in amides which are sterically hindered, it may be that the propion-N-ethylamide itself is sterically hindered by formation of a quasi-six membered hydrogen bonded ring as in (CLXXI).



(CLXXI)

The preparation of L (-)-3-(2-dimethylaminoethoxy)-propylethylamine (CLXXII) was attempted by reacting L (-)-2-dimethylaminopropan-1-ol with N-ethylacrylamide and reducing the product, without intermediate isolation, with lithium aluminium hydride. Distillation gave L (-)-2-dimethylamino-

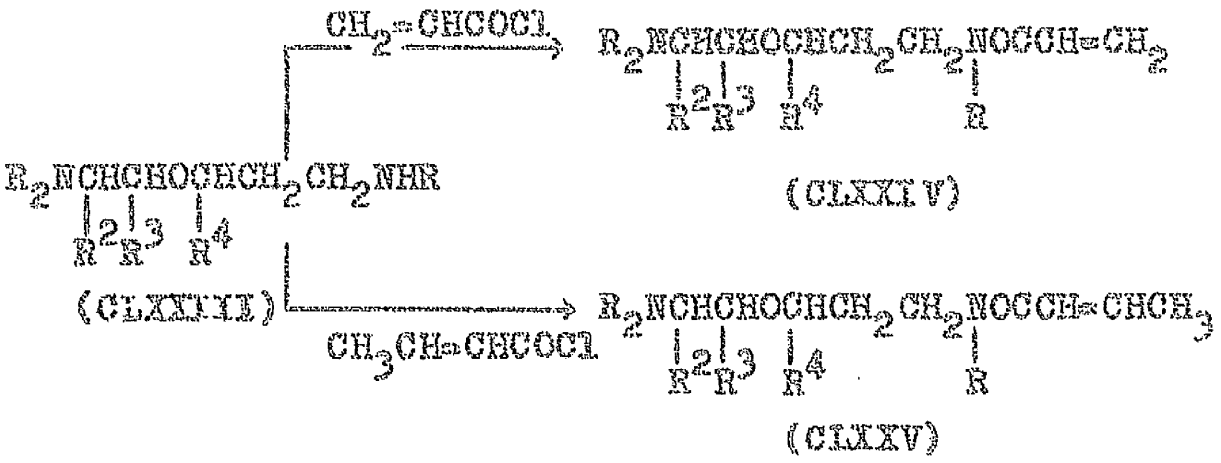
$$Me_2NCHCH_2OCH_2CH_2CH_2NHEt$$
$$\quad\quad|$$
$$\quad\quad CH_3$$

(CLXXII)

propan-1-ol[259] of $[\alpha]_D^{24}$ -2·13°. The distillation was stopped when none of the required amine (CLXXII) had come over at the expected temperature (ca. 74-76°/1mm). It was later confirmed from the results of other personel in the laboratory that the hydride used was of inferior quality and reduction had been minimal.

THE PREPARATION OF N,N-DISUBSTITUTED ACRYLAMIDES AND CROTONAMIDES FROM THE 3-ALKOXYPROPYLAMINES AND 3-ALKOXY-BUTYLAMINES

Since the 3-alkoxypropionic acids required in the projected syntheses (page52) could not be synthesised (page 66) it was decided to modify the route to the tris-ondium ethers. The addition of alcohols to α,β-unsaturated amides having been shown feasible, the logical step was to convert the 3-alkoxy-propylamines (CLXXIII;R^4=H) to the corresponding acrylamides (CLXXIV) and the 3-alkoxybutylamines (CLXXIII;R^4=Me) to the corresponding crotonamides (CLXXV). Addition of the appropriate amino alcohol across the double bond of the amides (CLXXIV)and (CLXXV) followed by reduction of the adducts would then afford the necessary tertiary bases (cf. page 52). A model experiment using 2-diethylaminoethanol and N,N-diethyl-acrylamide (CLXXVI) had shown that addition to a disubstituted amide was no different to those performed on the monosubstituted



(CLXXIV)

(CLXXIII)

(CLXXV)

α,β-unsaturated amides.

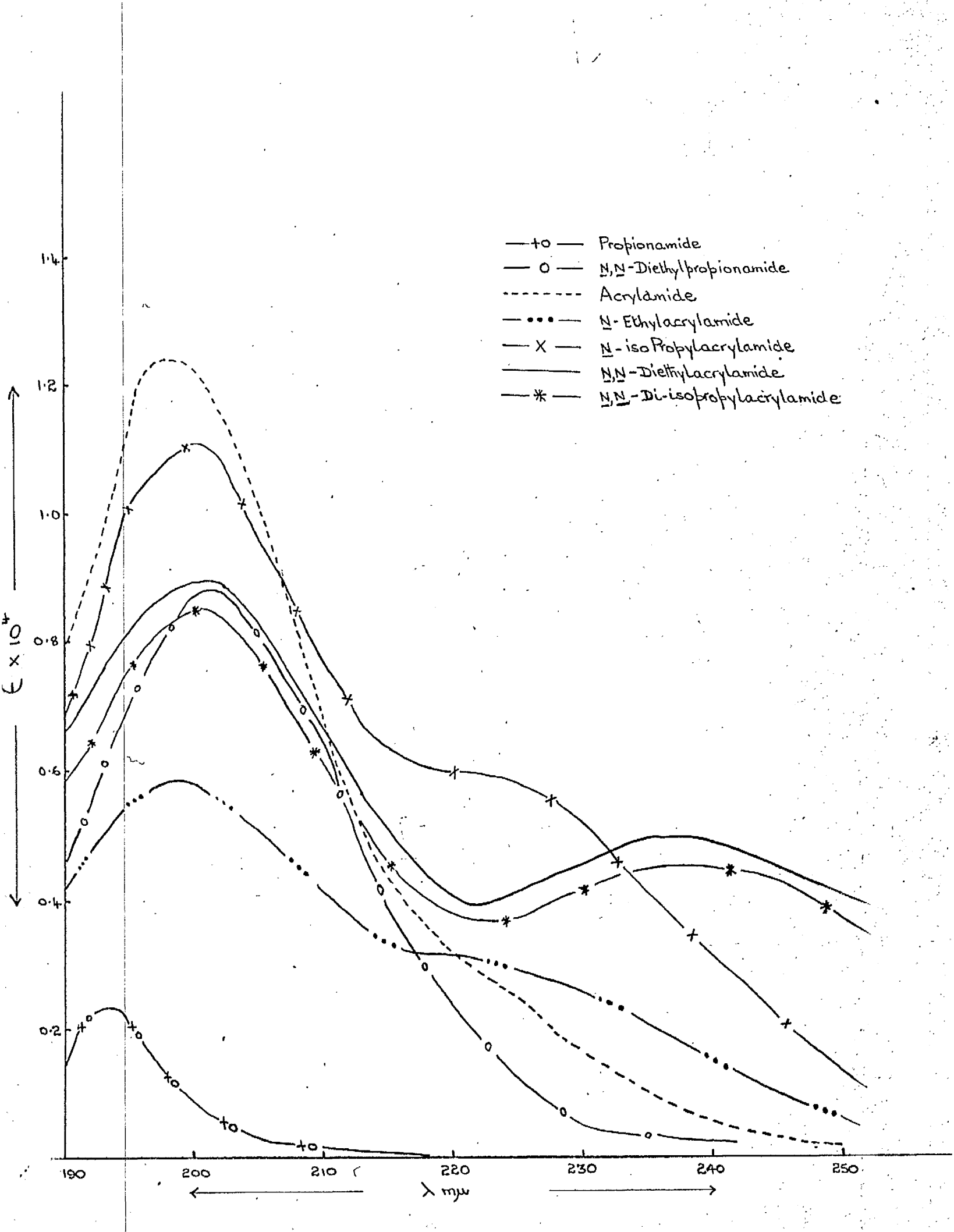
$$Et_2NCH_2CH_2OH + CH_2{=}CHCONEt_2 \longrightarrow Et_2N(CH_2)_2O(CH_2)_2CONEt_2$$

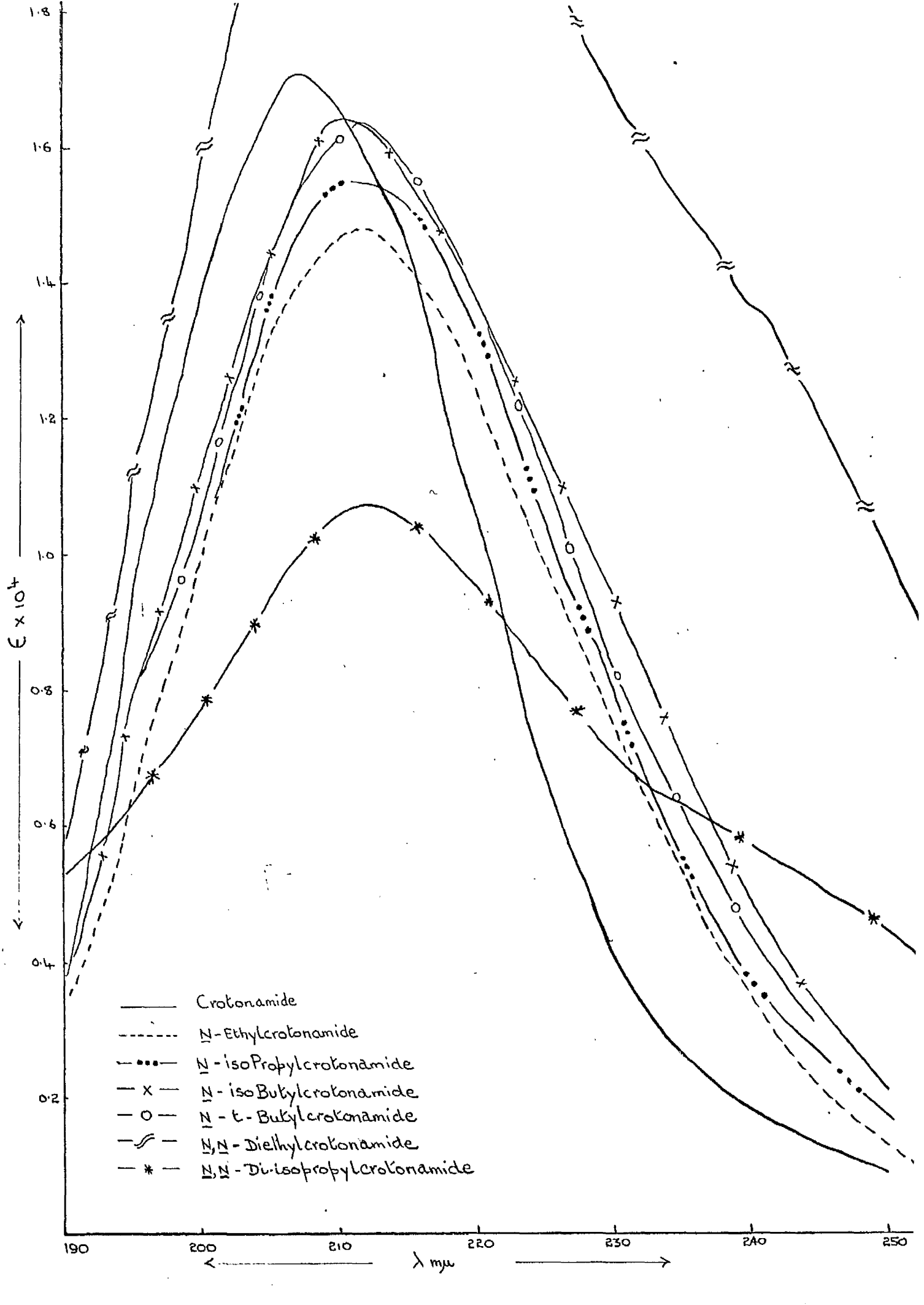
(CLXXVI)

The formation of the amides (CLXXIV and CLXXV) from the appropriate amine (CLXXIII) and acid chloride proceeded readily in ether solution. Initially the yields were in the region of 60 per cent probably because no acid scavenger was included in the reaction mixture. Triethylamine was not available for this purpose since it reacted with acryloyl chloride. However it was subsequently found that the addition of 1-2ml. of 20 per cent sodium hydroxide solution to the reaction mixture after all of the acid chloride had been incorporated led to improved yields. In all cases it was more convenient to use the impure products for the subsequent stage since they were high boiling compounds and being α,β-unsaturated amides subject to ready polymerisation. An analytical sample of one of them, N-3-(2-diethylaminoethoxy)propyl-N-ethylacrylamide (CLXXVII), was prepared by column chromatography on alumina but the separation was poor and the yield of pure product low.

$$Et_2NCH_2CH_2OCH_2CH_2CH_2N(Et)OCCH{=}CH_2$$

(CLXXVII)


—+o— Propionamide
— o — N,N-Diethylpropionamide
- - - - - - - Acrylamide
— • • • — N-Ethylacrylamide
— x — N-iso Propylacrylamide
——— N,N-Diethylacrylamide
— * — N,N-Di-isopropylacrylamide
ε x 10⁻⁴
1·4
1·2
1·0
0·8
0·6
0·4
0·2
190
200
210
220
230
240
250
λ mμ

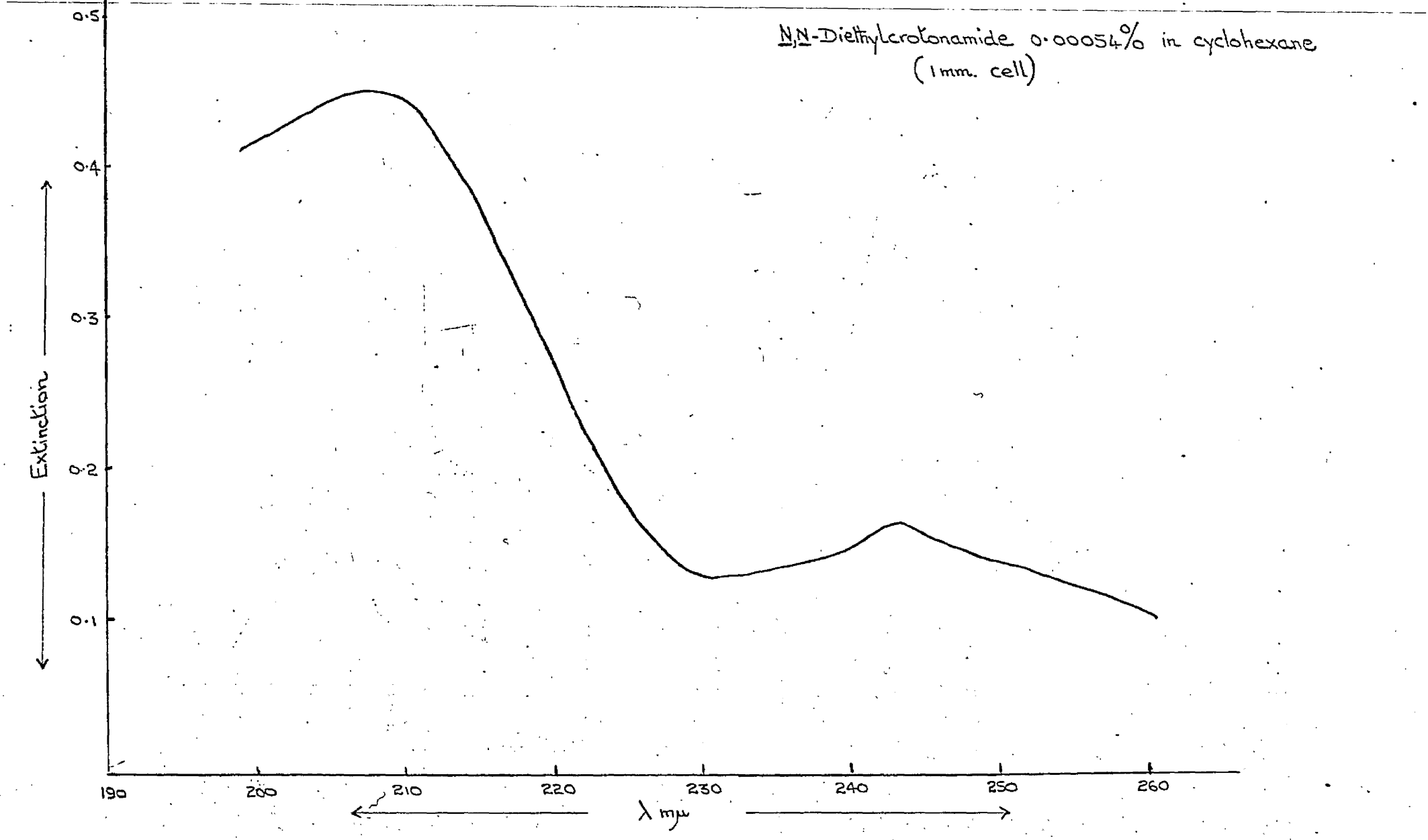

1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
ε x 10⁴
190
200
210
220
230
240
250
λ mμ
Crotonamide
N-Ethylcrotonamide
N-isoPropylcrotonamide
N-isoButylcrotonamide
N-t-Butylcrotonamide
N,N-Diethylcrotonamide
N,N-Di-isopropylcrotonamide

It was observed that these disubstituted α,β-unsaturated amides showed interesting spectral features and so a series of simple alkyl substituted acrylamides and crotonamides were examined by ultraviolet spectroscopy. As shown on the accompanying graphs of representative examples all of the N-monosubstituted acrylamides showed maximum absorption at ca. 200mμ with a pronounced inflexion at 220mμ. The N,N-disubstituted acrylamides all showed maximal absorption at ca. 202mμ and at 237mμ. N-Monosubstituted crotonamides absorb maximally at ca. 211mμ and the N,N-disubstituted derivatives at 211mμ with only a slight inflexion at 240mμ. All spectra were recorded on aqueous solutions of the amides using a Hilger Uvispek and 1mm. cells, the optical system being flushed with dry nitrogen.

The unsubstituted amides, acrylamide and crotonamide, show essentially similar absorption spectra when compared with the parent acids. The hypsochromic effect of an hydroxyl group on carbonyl absorption is well known.[323] Thus, ketones $\rangle C=O$ absorb at 270-285 mμ whereas carboxylic acids ($-C\overset{O}{\underset{OH}{=}}$) absorb at ca. 205 mμ. Similar effects are observed when α,β-unsaturated ketones ($)C=C-C=O$) which absorb at 220-235mμ ($\pi-\pi^*$ transition) and at ca. 310mμ ($n-\pi^*$ transition) are compared with α,β-unsaturated acids which show maximal absorption at 204-220mμ.[325] These effects are shown in Table 16 which compares the ultraviolet absorptions of an "acrylic" and a "crotonic" series.

TABLE 16.

	λ max mμ				
	R=H	R=CH_3	R=H	R=CH_3	Reference
RCH=CH-CHO	208	220	328	322	326
RCH=CH-$COCH_3$	212	224	324	315	326
RCH=CHCOOH	197	205			326
RCH=CHCONH$_2$	198	206			
$CH_3CH=CH-\overset{1}{C}=NBu^t_2$	220				327
$-CH=CH-\overset{1}{C}=\overset{+}{N}<$	274				328

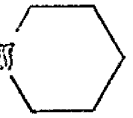
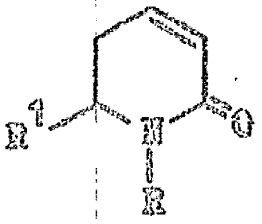

N,N-Diethylcrotonamide 0·00054% in cyclohexane
(1mm. cell)
Extinction
0·5
0·4
0·3
0·2
0·1
190
200
210
220
230
240
250
260
λ mμ

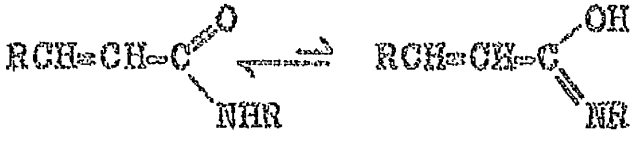
The present spectra are somewhat difficult to explain. It can be seen that acrylamide itself shows a very slight inflexion at ca. 225 mμ and whatever electronic transition is responsible for this absorption is intensified by substitution with an alkyl group as in the N-monosubstituted amides. Further substitution by an alkyl group on the amide nitrogen atom causes a bathochromic shift of this absorption to 237mμ. This bathochromic and hyperchromic effect of alkyl groups is amply illustrated by comparison of the spectra of propionamide and N,N-diethylpropionamide. In the acrylamide series the intensification of the absorption above 210mμ in both the N-monosubstituted and N,N-disubstituted derivatives is at the expense of the main absorption at ca. 200mμ. This effect is not so obvious in the crotonamide series since the bathochromic effect of N-disubstitution tends to be fused into the main absorption at ca. 210mμ. Nevertheless when N,N-diethylcrotonamide is examined in cyclohexane solution two distinct peaks are found. N,N-Diethylcrotonamide is somewhat anomalous in being the only substituted amide of either series which absorbs more strongly than the parent unsubstituted amide.

It is interesting to note that conjugated azomethines ( )C=C-C=N-) absorb at 220mμ whereas the protonated form ( )C=C-C=N$^+$) absorbs at 274mμ (Table 16). By analogy with α,β-unsaturated ketones ( )C=C-C=O) which undergo a hypsochromic shift of some 25mμ when an hydroxyl is added ( )C=C(OH)-C<O), it might be expected that the system ( )C=C-C=N$^+$) on addition of an hydroxyl group ( )C=C(OH)-C<N$^+$) would undergo a hypsochromic shift. It is possible, therefore, that in aqueous solution the N-monosubstituted amides exist in the tautomeric form (CLXXVIII) whereas the N,N-disubstituted amides exist in the mesomeric form (CLXXIX).

TABLE 17.

Ultraviolet Measurements of N-Substituted α,β-Unsaturated Amides Recorded in the Literature

Compound	Solvent	λ_{max} mμ	$\epsilon \times 10^4$	λ_{max} mμ	$\epsilon \times 10^4$	Ref.
$CH_3CH{=}CHCONEt_2$	Ethanol	215	1·1	242	0·65	329
$CH_3CH{=}CHCONHCH_2CH_2Bu$	Ethanol	227	0·85			330
$CH_3CH{=}CHCON$ 	Ethanol	215	1·1	235sh	0·66	331
						332
R=R^1=H	MeOH	204	0·84	240	0·12	
R=H, R^1=Me	MeOH			241	0·15	
R=Me, R^1=H	MeOH	204	0·65	250	0·10	
R=R^1=Me	MeOH			251	0·11	



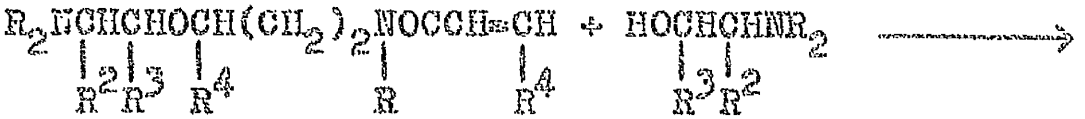
(CLXXVIII)

(CLXXIX)

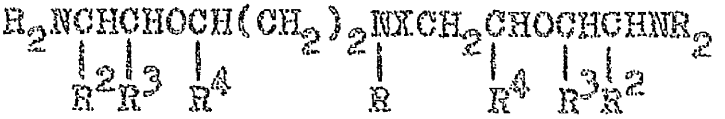
A literature survey revealed that few ultraviolet spectra of N-substituted α,β-unsaturated amides are recorded. Those that have been examined (Table 17) agree substantially with the present findings except in the case of N-isobutyl-crotonamide which is reported[330] to have λmax 227mμ as against λmax 211mμ in this study.

THE ADDITION OF AMINO ALCOHOLS TO THE N,N-DISUBSTITUTED ACRYLAMIDES AND CROTONAMIDES AND THE REDUCTION OF THE ADDUCTS WITH LITHIUM ALUMINIUM HYDRIDE

The addition of the amino alcohols of general formula (CLXXX) to the α,β-unsaturated amides of general formula (CLXXXI) to form the adducts (CLXXXII, X=O) was achieved by the method discussed on page 62. The rate and ease of addition varied considerably being particularly difficult in the butyl compounds (CLXXX, R=Bu, R^2=R^3=H; CLXXXI, R=Bu, R^2=R^3=R^4=H) and in the crotonamide compound (CLXXX, R=Et, R^2=R^3=H; CLXXXI, R=Et, R^2=R^3=H, R^4=Me). It was found that the



(CLXXXI) (CLXXX)



(CLXXXII)

adducts (CLXXXII;X=CO) were very high boiling oils and attempted distillation in one instance led to gross decomposition. In a number of cases, therefore, the adduct was not isolated but the total reaction mixture of (CLXXX), (CLXXXI) and (CLXXXII;X=CO) was reduced with lithium aluminium hydride and the amine (CLXXXII;X=CH_2) isolated by distillation. In those instances where the adduct (CLXXXII; X=CO) was isolated it was observed that the reduction to the amine (CLXXXII;X=CH_2) was accompanied by the liberation of small quantities of the amino alcohol (CLXXX) and thus, as in the previous examples (page 73), some other cleavage was effected by the reagent.

THE QUATERNISATION OF THE TRIAMINES

The triamine (CLXXXII;X=CH_2) was generally treated with 2-4 times its weight of alkyl halide with or without solvent. Only the quaternary salts of N,N-di-[3-(2-diethylaminoethoxy)-propyl] ethylamine could be recrystallised. In all other cases the quaternary salts were too hygroscopic to collect by filtration and were therefore centrifuged and washed by centrifuging with four successive portions of anhydrous ether. The products were finally dried *in vacuo* at room temperature. Gladych and Taylor[333] have used a similar technique for the hygroscopic quaternary salts of some tetrahydropapaverine derivatives. In a number of cases even this procedure failed to give a satisfactory product and in this event the quaternary halide was converted to the reineckate salt which was then recrystallised from aqueous acetone or acetone-ethanol-water. The melting points of the quaternary halides, which were all determined in sealed tubes, were very difficult to obtain because of the hygroscopic nature of the salts.

EXPERIMENTAL

All melting points are uncorrected. Ultraviolet spectra were recorded on a Hilger Uvispek H700.303 or on an Optica D.B.C.F4. Infrared spectra were recorded on a Perkin Elmer Infracord or Model 237.

Sodium Acrylate [334]

Acrylic acid (24g.) as a 60% aqueous solution was slowly added to a paste prepared from powdered sodium hydroxide (13g.) and ethanol. External cooling was applied and once the initial reaction had subsided the mixture was continuously shaken for 4-6 hours and then left overnight. The heavy crystalline mass was filtered off, washed with acetone and dried at room temperature. The acetone washings precipitated from the filtrate a further quantity of the acid salt which was similarly filtered washed and dried. Total yield 29g, (97%). Analysis: By sulphated ash a sample contained 98% $C_3H_3NaO_2$

Acryloyl Chloride

Phosphoryl chloride (34g.) was added dropwise to sodium acrylate (previously dried at 40°C for 2-4 hours; 40g.) using a calcium chloride guard tube. External cooling was applied when necessary and the hard crust which formed occasionally broken with a spatula. The mixture was heated under reflux on a steam bath for 30 minutes and then distilled collecting all the distillate to a bath temperature of 300°. The distillate was redistilled through a 9 inch Vigreux column to yield acryloyl chloride (30g., 79%), b.p. 74-76°. Mourèu[256] gives b.p. 74-76°.

Crotonyl Chloride was prepared in 82% yield from crotonic acid and thionyl chloride using the method of Maxim.[258]

PREPARATION OF ACRYLAMIDES AND CROTONAMIDES

N-Ethylacrylamide

Method A: Anhydrous ethylamine (40g.) in dry benzene (50ml.) was slowly added to an ice-cold solution of acryloyl chloride (33·7g.) in dry benzene (400ml.). The precipitate of ethylamine hydrochloride was removed by filtration, the benzene

removed under reduced pressure and the residue rapidly distilled to yield N-ethylacrylamide (24g.,65%), b.p. 94°/ 0·9mm. Mouren[256] gives b.p.128-130°/25mm.

Method B: Acryloyl chloride (20g.) in anhydrous ether (50ml.) was slowly added over 40 minutes to a stirred, ice-cold solution of anhydrous ethylamine (25g.) in anhydrous ether (200ml.). Filtration of the solution and removal of the ether under reduced pressure at room temperature yielded N-ethylacrylamide (20·2g.,92%) of sufficient purity for use at subsequent stages.

N-Methylacrylamide

Methylamine gas, displaced by nitrogen from an aqueous or alcoholic solution of the amine and dried by passage through solid potassium hydroxide, was bubbled through a solution of acryloyl chloride (49g.) in anhydrous ether (500ml.) until the ethereal solution was saturated (approximately 10 hours). Treatment of the reaction mixture as in method B (above) yielded N-methylacrylamide (40g.,87%) which was used without further purification.

N-n-Butylacrylamide was prepared from butylamine by the method described for N-ethylacrylamide (Method B).

N,N-Diethylacrylamide was prepared from diethylamine by the method described for N-ethylacrylamide (Method B).

N-Methylcrotonamide was prepared from crotonyl chloride by the method described for N-methylacrylamide using benzene as solvent. After filtration the benzene was evaporated to low volume and flooding with petroleum ether (b.p.40-60°) yielded N-methylcrotonamide (26g., 69%), m.p. 77-78°.

N-Ethylcrotonamide was prepared from ethylamine and crotonyl chloride by the method described for N-ethylacrylamide (method B) in 82% yield.

N-Isopropylcrotonamide was prepared from isopropylamine by the method described for N-ethylcrotonamide. The crude

	m.p.°	b.p.°/mm.Hg.	Formula	N Found
Acrylamide	84		C_3H_5NO	19·5
N-Ethylacryamide		94/0·9	C_5H_9NO	14·0
N-iso-Propylacryl-amide	82-83		$C_6H_{11}NO$	12·3
N-t-Butylacryl-amide	114		$C_7H_{13}NO$	10·85
N,N-Diethylacryl-amide		46-47/008	$C_7H_{13}NO$	10·9
N,N-Di-isopropyl-acrylamide		79/1	$C_9H_{17}NO$	9·2
Crotonamide	158		C_4H_7NO	16·2
N-Ethylcrotonamide	20-22	88-90/0·1	$C_6H_{11}NO$	12·3
N-iso-Propylcroton-amide	82		$C_7H_{13}NO$	11·0
N-iso-Butylcroton-amide	71·5		$C_8H_{15}NO$	9·9
N-t-Butylcroton-amide	148		$C_8H_{15}NO$	9·9
N,N-Diethylcroton-amide		83/1	$C_8H_{15}NO$	9·9
N,N-Di-isopropyl-crotonamide		74/0·15	$C_{10}H_{19}NO$	8·2
Propionamide				
N,N-Diethylpropion-amide				

% Calc.	λ_{max} (mμ)	$\epsilon \times 10^4$	λ_{sh} (mμ)	$\epsilon \times 10^4$
19·7	198	1·24		
14·1	199	0·59	219	0·32
12·4	200	1·11	220	0·60
11·0	202	0·94	222	0·58
11·0	201	0·90	-	
	237	0·50		
9·0	201	0·86		
	238	0·45	-	
16·5	207	1·71	-	
12·4	212	1·48	-	
11·0	211	1·58	-	
9·9	211	1·65	-	
9·9	212	1·64	-	
9·9	214	2·32	240	1·36
8·3	212	1·07	237	0·60
	194	0·23	-	
	201	0·88	-	

product was recrystallised from ether/petroleum ether (b.p.40-60°) to yield N-isopropylcrotonamide (13·7g.,91%), m.p.82°.

Found:		N,	11·0
$C_7H_{13}NO$	requires	N,	11·0%

Analytical samples of the above amides together with a number of other α,β-unsaturated amides were also prepared for spectrophotometric comparison. These compounds and their relevant absorption and other data are as shown.

THE PREPARATION OF AMINO ALCOHOLS

(±)-2-Dimethylaminopropan-1-ol

Ethyl (±)2-dimethylaminopropionate (90g.), prepared by the method of Beckett, Harper and Clitherow,[259] was reduced with lithium aluminium hydride (25g.) in ether (1l). After reduction, the crude reduced product was fractionated at atmospheric pressure to yield (±)-2-dimethylaminopropan-1-ol (25g.), b.p. 147-148°/760mm. (lit.[335] gives b.p. 140-141°/738mm. and[336] 54-58°/29mm.

Found:	equivalent (titration)	104·2
Calc. for $C_5H_{13}NO$	equivalent	103·3

Methiodide from ethanol-ether m.p.297-298°(decomp.) (lit.[259] gives 299-300°).

Resolution of Alanine

Alanine was resolved by the method of Beckett Harper and Clitherow[259] to give L(+)-alanine hydrochloride, $[\alpha]_D^{24}$ + 9·38°(c 12·5 in H_2O) and D(-)-alanine hydrochloride $[\alpha]_D^{24}$ -9·6°(c 12·75 in H_2O)

L (+)-2-Dimethylaminopropionic Acid Hydrochloride

L (+)-Alanine hydrochloride was reductively methylated by the method of Bowman and Stroud[337] and afforded L (+)-

-2-dimethylaminopropionic acid hydrochloride, m.p. 118°, $[\alpha]_D^{26}$ +14.46° (c 5.1 in H_2O). [lit.[259] gives m.p. 118.5-119.5°, $[\alpha]_D^{23.4}$ + 14.3° (c 5.1 in H_2O)].

Methyl L(-)-2-dimethylaminopropionate

L(+)-dimethylaminopropionic acid (7.5g.) was esterified with methanol by the method of Beckett, Harper and Clitherow[259] to give methyl L(-)-2-dimethylaminopropionate (5.9g.), $[\alpha]_D^{20}$ -23° (c 2 in $CHCl_3$) which was reduced without further purification.

L (-)-2-Dimethylaminopropan-1-ol

Methyl L(-)-2-dimethylaminopropionate (20g.) was reduced with lithium aluminium hydride (5g.) in ether (200ml.) to give a basic oil (10g.), b.p. 146-147°/760mm. having equivalent (titration) 113. [Calc. for $C_5H_{13}NO$ equivalent 103.2].
Methiodide from ethanol-ether m.p. 299°(decomp.), $[\alpha]_D^{21}$ -4.15° (c 0.8 in 90 per cent $^{w}/_{v}$ ethanol). [lit.[259] gives m.p. 299° (decomp.) $[\alpha]_D^{24.7}$ -4.14° (c 2.5 in 90 per cent $^{w}/_{v}$ ethanol)].

Ethyl 2-diethylaminopropionate was prepared by the method of Burnett and colleagues.[338] The ester was a colourless oil, b.p. 184-185°, n_D^{22} 1.4240. (lit.[338] gives b.p. 172-177°, n_D^{16} 1.4302).

2-Diethylaminopropan-1-ol

Ethyl 2-diethylaminopropionate (78g.) in anhydrous ether (100ml.) was added to a stirred hot suspension of lithium aluminium hydride (18g.) in anhydrous ether (300ml.) at a rate sufficient to keep the ether gently refluxing. The reaction mixture was allowed to stand for 2 hours and then decomposed with water and sodium hydroxide solution (20%) using the proportions described by Amundsen and Nelson.[318] The ether

was decanted, the granular precipitate washed copiously with ether (1x400ml., 2x100ml.) and the combined extracts dried (Na_2SO_4). Removal of solvent and distillation of the residue yielded 2-diethylaminopropan-1-ol (46·2g., 78%) b.p. 174-176°, n_D^{25} 1·4331. (lit.[338] gives b.p. 166-169°, n_D^{20} 1·4305).

1-Diethylaminopropan-2-ol was obtained in 64% yield from propylene oxide and diethylamine by the method described by Goldfarb.[339]

THE ADDITION OF ALCOHOLS TO N-MONOSUBSTITUTED ACRYLAMIDES AND CROTONAMIDES

3-(2-Diethylaminoethoxy)propion-N-ethylamide

Sodium methoxide (1·5g.) was dissolved in 2-diethylaminoethanol (28g.) at 60°, the solution cooled and added to N-ethylacrylamide (24g.). The reaction, after five days at 20°, reached an equilibrium corresponding to ca. 75% completion as judged from spectroscopic evidence. The mixture was acidified with concentrated sulphuric acid (1·4g) and distilled to yield 3-(2-diethylaminoethoxy)propion-N-ethylamide (34·2g., 66%), b.p. 132-134°/0·1mm.

Found:	equivalent(titration) 215·4;	N, 13·0
$C_{11}H_{24}N_2O_2$ requires	equivalent 216·3;	N, 12·95%

3-(2-Diethylaminoethoxy)propion-N,N-diethylamide was prepared by a method similar to that described for 3-(2-diethylaminoethoxy)propion-N-ethylamide from 2-diethylaminoethanol (6·4g.) and N,N-diethylacrylamide (7g.). The reaction reached equilibrium after 3 days and subsequent acidification and distillation yielded 3-(2-diethylaminoethoxy)propion-N,N-diethylamide (7·6g., 57%), b.p. 114-116/0·1mm.

Found:	equivalent (titration), 243;	N, 11·3
$C_{13}H_{28}N_2O_2$ requires	equivalent, 244·4;	N, 11·5%

3-(2-Dimethylaminoethoxy)propion-N-methylamide was prepared from 2-dimethylaminoethanol (11·6g.) and N-methylacrylamide (10·9g.) containing hydroquinone (0·1%) as polymerisation inhibitor by the method described for 3-(2-diethylamino-ethoxy)propion-N-ethylamide. The reaction reached equilibrium after 5 days and 3-(2-dimethylaminoethoxy)propion-N-methylamide (11·5g., 51·5%) was obtained as a colourless oil b.p. 121-126°/0·4mm.

Found:	equivalent (titration),	184·1
$C_8H_{18}N_2O_2$ requires	equivalent	174·2

A pure sample of the propion-N-methylamide was obtained by passing dry hydrogen chloride through a solution of the crude amide (5g.) in anhydrous ether (100ml.) until a granular crystalline mass was formed. The hydrochloride was collected, washed with ether and dissolved in water (15ml.). The aqueous solution was basified with sodium carbonate (10g.) and extracted with ether (2x100ml., 1x50ml.) and the combined extracts dried (Na_2SO_4). Evaporation of the solvent and distillation yielded 3-(2-dimethylaminoethoxy)propion-N-methylamide (2·3g., 46% recovery), b.p. 126-128°/0·3mm.

Found:	equivalent(titration),	178·2 ; N, 15·7
$C_8H_{18}N_2O_2$ requires	equivalent	174·2 ; N, 16·1%

Dihydrochloride (from ethanol-ether) m.p. 118-120°(sealed tube).

Found:	equivalent, 124 ;	N, 11·3
$C_8H_{20}Cl_2N_2O_2$ requires	equivalent, 123·6;	N, 11·3%

3-(2-Dibutylaminoethoxy)propion-N-butylamide was prepared by a method similar to that described for 3-(2-diethylaminoethoxy)-propion-N-ethylamide from 2-dibutylaminoethanol (90g.) and N-butylacrylamide (64g.) containing hydroquinone (0·1%) as polymerisation inhibitor. The reaction reached equilibrium after 70 hours at 55°. Subsequent acidification and distillation yielded 3-(2-dibutylaminoethoxy)propion-N-butylamide

(66g., 44%), b.p. 164-170°/0·6mm.

Found: equivalent (titration), 304·2 ; N, 9·3

$C_{17}H_{36}N_2O_2$ requires equivalent, 300·5 ; N, 9·3%

3-(2-Diethylaminopropoxy)propion-N-ethylamide was prepared by the method described for 3-(2-diethylaminoethoxy)propion-N-ethylamide from 2-diethylaminopropan-1-ol (46g.) and N-ethylacrylamide (34g.). The reaction reached equilibrium in 12 hours and 3-(2-diethylaminopropoxy)propion-N-ethylamide (27·8g., 35%), b.p. 152-162°/0·3mm. was obtained as a colourless oil.

Found: equivalent (titration) 231·2 ; N, 12·6

$C_{12}H_{26}N_2O_2$ requires equivalent 230·4 ; N, 12·2%

Reineckate (from acetone-ethanol-water) m.p. 129-131°(decomp.)

Found: equivalent (titration) 543·4 ; N, 20·3

$C_{16}H_{32}CrN_8O_2S_4$ requires equivalent 548·7 ; N, 20·4%

3-(1-Methyl-2-diethylaminoethoxy)propion-N-ethylamide was prepared from 1-diethylaminopropan-2-ol (26g.) and N-ethylacrylamide (20g.) by the method described for 3-(2-diethylaminoethoxy)propion-N-ethylamide. The reaction reached equilibrium in under 12 hours and 3-(1-methyl-2-diethylaminoethoxy)-propion-N-ethylamide (20g.,43%), b.p. 132-134°/0·06mm. was obtained as a colourless oil.

Found: equivalent (titration) 240·6

$C_{12}H_{26}N_2O_2$ requires equivalent 230·4

Reineckate (from acetone-ethanol-water) m.p. 136-137°(decomp.)

Found: equivalent (titration) 547·6 ; N, 20·5

$C_{16}H_{32}CrN_8O_2S_4$ requires equivalent 548·7 ; N, 20·4%

N-Isopropyl-3-methoxybutyramide

N-Isopropylcrotonamide (5g.) was added to a solution of sodium methoxide (0·2g.) in dry methanol (10g.) heating briefly at 60° to give a homogeneous solution. The mixture was heated for 12 hours at 90° in the presence of 0·1 per cent

of hydroquinone. Acidification and distillation yielded N-isopropyl-3-methoxybutyramide (4g., 63%)

Found: N, 9·3

$C_9H_{17}NO_2$ requires N, 8·8%

N-Isopropyl-3-ethoxybutyramide was prepared by the method described for N-isopropyl-3-methoxybutyramide from dry ethanol (10g.) and N-isopropylcrotonamide (10g.) and was obtained as a colourless oil (9·4g., 68%), b.p. 84-89°/0·03mm. An analytical sample was obtained by passing a solution of the oil (6g.) in petroleum ether (b.p. 40-60°; 80ml.) through a column of alumina (80g., column dimensions 30x2cm). Evaporation of the eluate (800ml.) and distillation yielded N-isopropyl-3-ethoxybutyramide (4·2g.), b.p.81°/0·03mm.

Found: N, 8·4

$C_9H_{19}NO_2$ requires N, 8·1%

3-(2-Dimethylaminoethoxy)butyr-N-methylamide was prepared by the method described for 3-(2-diethylaminoethoxy)propion-N-ethylamide from 2-dimethylaminoethanol (20g.) and N-methylcrotonamide (20g.). The reaction reached equilibrium after 44 hours at 120°. Acidification and distillation yielded the crude product (6g.),bp. 122-130°/0·09mm. Unreacted N-methylcrotonamide (10·5g.) was recycliised through the same process to yield a further 3g. of crude product b.p 126-132°/0·06mm. (Overall yield 9g., 46%).

Reineckate (from acetone-ethanol-water) m.p. 140-142° (decomp.)

Found: equivalent (titration) 509·7 ; N, 22·4

$C_{13}H_{26}CrN_8O_2S_4$ requires equivalent 506·7 ; N, 22·1%

3-(2-Diethylaminoethoxy)butyr-N-ethylamide was prepared by the method described for 3-(2-diethylaminoethoxy)propion-N-ethylamide from 2-diethylaminoethanol (38g.) and N-ethylcrotonamide (36g.). The reaction reached equilibrium after 40 hours at 90°. Subsequent acidification and distillation

yielded the crude product (20g.), b.p. 124-128°/0·3mm. Redistillation gave 3-(2-diethylaminoethoxy)butyr-N-ethylamide (17g.) as a colourless oil, b.p. 128-130/0·2mm. Unreacted N-ethylcrotonamide (20g.) was recycllised through the same process to yield a further 14·4g. of crude product. (overall yield of crude product 34·4g., 47%).

Found: equivalent (titration) 238·7 ; N, 11·7

$C_{12}H_{26}N_2O_2$ requires equivalent 230·4 ; N, 12·2%

Reineckate (from aqueous acetone as the monohydrate), m.p. 138-139° (decomp.).

Found: equivalent (titration) 564·4 ; N, 20·1

$C_{16}H_{34}CrN_8O_3S_4$ requires equivalent 566·8 ; N, 19·8%

Chloroplatinate m.p. 188-189° (softening at 180°).

Found: C, 33·7 ; H, 6·5

$C_{24}H_{54}Cl_6N_4O_4Pt$ requires C, 33·1 ; H, 6·25%

3-(2-(±)-Dimethylaminopropoxy)propion-N-ethylamide was prepared by the method described for 3-(2-diethylaminoethoxy)-propion-N-ethylamide from (±)-2-dimethylaminopropan-1-ol (15g.) and N-ethylacrylamide (14·5g.). The reaction reached equilibrium in ca. 12 hours and distillation yielded 3-(2-(±)-dimethylaminopropoxy)propion-N-ethylamide (14·7g., 50%), b.p. 136-140°/1·1mm.

Found: equivalent (titration) 205·9 ; N, 14·1

$C_{10}H_{22}N_2O_2$ requires equivalent 202·3 ; N, 13·9%

Reineckate (from acetone-ethanol-water) m.p. 138-139° (decomp.)

Found: equivalent (titration) 510·5 ; N, 20·7

$C_{14}H_{28}CrN_8O_2S_4$ requires equivalent 520·7 ; N, 21·5%

THE ATTEMPTED PREPARATION OF 3-(2-DIETHYLAMINOETHOXY)PROPIONIC ACID

1) The Addition of 2-Diethylaminoethanol to Ethyl Acrylate

Method A. Ethyl acrylate (17·1g.) was slowly added with

constant stirring to a solution of sodium methoxide (0·415g.) in 2-diethylaminoethanol (19·9g.) maintaining the temperature below 25°. The reaction mixture was allowed to stand overnight and the catalyst then destroyed by the careful addition of concentrated sulphuric acid (0·4g.). The unreacted ethyl acrylate (ca. 7g.) and 2-diethylaminoethanol (ca. 6g.) were removed under reduced pressure and the residue distilled to yield two main fractions- I (11·2g.), b.p. 37-100°/0·2mm. and II (6·2g.), b.p 140-170°/0·25mm. These two fractions were separately redistilled to yield the following sub-fractions

Fraction	b.p°(mm./Hg.)	Yield	Equivalent (titration)
I_a	50-60/0·2	2g.	178·2*
I_b	79-80/0·2	7g.	213·5†
I_c	84-90/0·2	1g.	173·3
I_d	undistilled residue	1g.	173·3
II_a	110-120/0·1	1·5g.	-
II_b	126-127/0·1	3·4g.	146·7†
II_c	undistilled residue	1g.	-

* ϵ^{210}_{max} 2872 Calc. for $C_9H_{17}NO_2$ equivalent 171·2

† Found: N, 6·8; $C_{11}H_{23}NO_3$ requires equivalent 217·3; N, 6·45%

† Found: N, 9·4; $C_{15}H_{32}N_2O_3$ requires equivalent 144·2; N, 9·7%

Method B.[287] Sodium metal (0·51g.) was dissolved in 2-diethylaminoethanol (5g.) and the solution added over 17 min. with constant stirring to a mixture of ethyl acrylate (17g.) and 2-diethylaminoethanol (17g.) maintaining the temperature ca.25°.

The mixture was allowed to stand overnight, the catalyst destroyed by the addition of concentrated sulphuric acid (1·18ml.) and distilled with difficulty to yield two main fractions - I (6g.), b.p. 38-48°/0·6mm. and II (2g.), b.p. 84-86°/0·6mm. together with a large forerun and polymeric residue. Both fractions were complex mixtures.

The Hydrolysis of the Product from the Michael Condensation of 2-Diethylaminoethanol and Ethyl Acrylate. (Fraction Ib)

A. The product (2g.) was refluxed with hydrochloric acid (5N, 10ml.) for 2 hours. The solution was evaporated to dryness under reduced pressure and the solid residue recrystallised from ethanol-acetone-ether to yield 2-diethylaminoethanol hydrochloride (0·8g.), m.p 120-122°.

B. The product (2g.) was refluxed with hydrochloric acid (5N, 10ml.) for 2 hours. The solution was cooled and extracted with ether (4x10ml.) and the combined extracts dried (Na_2SO_4). Evaporation of the ether afforded 3-ethoxypropionic acid (0·6g.).

S-Benzylthiouronium salt m.p. 126-129°. The S-benzylthiouronium salt of authentic 3-ethoxypropionic acid[298] had m.p. 128-130° and the infrared spectra were superimposable.

The acid layer on treatment as in A yielded 2-diethylaminoethanol hydrochloride (0·8g.), m.p. 120-122°.

2) The Hydrolysis of 3-(2-Diethylaminoethoxy)propionitrile

A) 3-(2-Diethylaminoethoxy)propionitrile[254] (10g.) in concentrated hydrochloric acid (40ml.) was refluxed for 1·5 hours. The acid solution was cooled and extracted with ether (4x100ml.) and the combined extracts dried (Na_2SO_4). Evaporation of the ether yielded 3-chloropropionic acid (0·25g.), m.p. 42° (lit.[340] gives m.p. 42°) and acrylic acid (0·5g.; identified by infrared comparison with authentic material). The aqueous acid solution was basified with sodium hydroxide

solution (20 per cent) and extracted with ether (4x100ml.) and the combined extracts dried (Na_2SO_4). Evaporation of the solvent afforded 2-diethylaminoethanol [1·3g., equivalent (titration) 115·6; calc. for $C_6H_{15}NO$ equivalent 117·2]. The aqueous layer was further basified by the addition of solid sodium hydroxide keeping the temperature at about 30°. The oily layer which separated was removed and washed with ether. The oily layer (ca. 5g.) was soluble in water, methanol and acetone but insoluble in chloroform, ether and benzene. Attempted distillation led only to a viscous polymeric product and an orange amorphous powder both of which had identical infrared spectra.

B. A mixture of 3-(2-diethylaminoethoxy)propionitrile (10g.) and concentrated hydrochloric acid (50ml.) was refluxed for 2 hours. The acid solution was cooled and continuously extracted with ether for 8 hours and the ethereal extract dried (Na_2SO_4). Evaporation of the solvent yielded acrylic acid (ca.1g.). The aqueous acid solution was evaporated to dryness under reduced pressure, the residue taken up in ethanol (100ml.) and the ethanolic solution filtered to remove ammonium chloride. The ethanolic solution was refluxed for 0·5hr. and evaporated to small volume, ethanol (50ml.) and acetone (20ml.) added and the solution filtered. Evaporation of the solvent yielded an oily residue which was treated with anhydrous sodium carbonate (20g.) and extracted with ether (2x100ml., 1x50ml.). The combined extracts were dried (Na_2SO_4) and evaporated to yield a basic fraction D (3·2g.). The aqueous alkaline solution was treated with solid potassium hydroxide and the viscous layer which formed was separated and dried *in vacuo*. The infrared spectrum of this oil (2·2g.) was similar to that of the oil obtained in Method A.

Fraction D

The infrared spectrum showed peaks at 2970-2800 cm^{-1},

1746 cm^{-1} and 1120 cm^{-1}. The fraction also reacted with hydroxylamine to give a hydroxamic acid. Thin layer chromatography on silica gel gave a single spot R_F 0·8 using ethanol : 10% aqueous ammonia 4:1.

Found:	equivalent (titration)	214·6;	N, 6·8
$C_{11}H_{23}NO_3$ requires	equivalent	217·3;	N, 6.45%

3.) 3-(2-Diethylaminoethoxy)propionamide.

Hydrogen peroxide (50ml., 10 per cent) made alkaline by the addition of sodium hydroxide (2ml., 20 per cent) was added to 3-(2-diethylaminoethoxy)propionitrile[254] (5g.) keeping the temperature at 40° and the mixture left overnight The reaction mixture, which was strongly ammoniacal, was extracted with chloroform (1x50ml., 4x25ml.), the combined extracts dried (Na_2SO_4) and evaporated to yield a pale yellow oil (ca. 0·35g.). The oil was dissolved in dry ethanol (5ml.) and dry hydrogen chloride passed through the solution from which the crude hydrochloride was precipitated by the addition of ether. The crude hydrochloride was recrystallised from ethanol-acetone-ether to give 3-(2-diethylaminoethoxy)-propionamide hydrochloride (0·3g., 5%), m.p 97-98°.

Found:	equivalent (titration)	219·5	N, 12·4
$C_9H_{21}ClN_2O_2$ requires	equivalent	224·8	N, 12·5%

4) The Attempted Preparation of 3-(2-Diethylaminoethoxy)-propionic Acid.

A) Sodium hydroxide (0·3g.) was dissolved in 2-diethylamino ethanol (5g.) and added to freshly prepared sodium acrylate (4g.). The mixture was maintained at 40° and examined spectrophotometrically at intervals. No reaction occurred.

B) A mixture of the sodium salt of 2-diethylaminoethanol (8g.) and 2-bromopropionic acid (4·2g) was heated at 120° for 1 hour with constant stirring. Acidification and extraction yielded 2-bromopropionic acid (4g.) unchanged.

c) Acrylic acid (3·1g.) was slowly added to 2-diethylaminoethanol (10g.) containing Amberlite IR-4B (OH form; 1g.) and the mixture stirred for 5 days at room temperature. The resin was filtered off and the filtrate flooded with dry chloroform to yield the gummy water-soluble polymer of acrylic acid.

THE LITHIUM ALUMINIUM HYDRIDE REDUCTIONS OF 3-(DIALKYLAMINOALKOXY)PROPION-N-ALKYLAMIDES AND RELATED COMPOUNDS

3-(2-Diethylaminoethoxy)propylethylamine.

A solution of 3-(2-diethylaminoethoxy)propion-N-ethylamide (10g.) in dry ether (10ml.) was slowly added to a stirred hot suspension of lithium aluminium hydride (3·5g.) in dry ether (100ml.) at a rate sufficient to keep the mixture gently refluxing. The mixture was stirred and refluxed a further 5 hours. The excess of lithium aluminium hydride and the complex were decomposed at 0° by the dropwise addition of water, and the mixture then made strongly alkaline with 20% sodium hydroxide solution (20ml.). The ethereal layer was decanted, the residual gel extracted with ether (2x100ml.; 1x50ml.) and the combined extracts dried (Na_2SO_4). Evaporation of the solvent and distillation yielded 3-(2-diethylaminoethoxy)propylethylamine (4·1g., 44%), b.p. 78-80°/0·1mm.

Found:	equivalent (titration)	101·3 ;	N, 13·8
$C_{11}H_{26}N_2O$ requires	equivalent	101·2 ;	N, 13·9%

The Lithium Aluminium Hydride Reduction of 3-(2-Diethylaminoethoxy)propion-N-ethylamide

3-(2-Diethylaminoethoxy)propion-N-ethylamide (50g.) in dry ether (50ml.) was slowly added to a stirred hot suspension of lithium aluminium hydride (17g.) in anhydrous ether (500ml.) over a period of 40 minutes. The mixture was refluxed a further 5 hours, decomposed, [318] and extracted with ether.

The combined extracts were dried (Na_2SO_4), the ether removed and the residue distilled to yield the following fractions:

Fraction	b.p. $^{\circ}$	Yield (g.)	Remarks
A	34-70/0·25mm	15	Equivalent ca. 90
B	85-86/0·1mm	11·3	
C	undistilled residue	ca. 15	
Acetone/CO_2 Trap		ca. 1·5	

Fractions 1 and 2 and the "trap" fraction were chromatographed on paper using butanol-acetic acid-water 4:1:5. 2-Diethylaminoethanol, diethylamine and 3-(2-diethylaminoethoxy)-propylethylamine were run as standards:

Fraction	R_F Values		
A	0·43	0·56	0·73
B	0·47	0·58	
Trap		0·55	0·71 0·90

2-Diethylaminoethanol 0·55; Diethylamine 0·70; 3-(2-Diethylaminoethoxy)propylethylamine 0·44.

Distillation of fraction A at atmospheric pressure through a 4 inch column packed with glass helices yielded 20mg. of a fraction b.p. 82°. Chromatography as above indicated 3 constituents R_F 0·90, 0·72, and 0·52. Fraction A was redistilled under reduced pressure to afford a small forerun (ca. 1·5g.) and 2-diethylaminoethanol (10g.) (Found: equivalent 118·1 n_D^{23} 1·4398; calc. for $C_6H_{15}NO$ equivalent 117·2; lit.[341] gives n_D^{25} 1·4400). The forerun (equivalent 82·5) on treatment with ethanolic hydrogen chloride yielded N,N'-diethyl-1,3-diaminepropane dihydrochloride m.p. 302-303° (decomp. and effervescence); Picrate m.p. 160-161° (decomp.)

Found: equivalent (titration) 102·7 & 101·7; N, 14·4

Calc. for $C_7H_{20}Cl_2N_2$ equivalent 101·6; N, 13·8%

An authentic sample of N,N'-diethyl-1,3-diaminepropane

dihydrochloride prepared from 1,3-dibromopropane and anhydrous ethylamine by a modification of the method of Boone[342] had m.p. 300-304° (decomp. and effervescence); picrate m.p. 160-161° (decomp.); the infrared spectra of the experimental and authentic hydrochlorides were superposable, and their chromatographic behaviour identical.

Fraction B was redistilled to yield 2-diethylaminoethanol (ca.1g.) and 3-(2-diethylaminoethoxy)propylethylamine (9.3g., 20%) b.p. 82-83°/0.05 mm.

An aliquot of the undistilled residue (7g.) was refluxed with concentrated hydrochloric acid (100ml.) for 2 hours. The acidic solution was cooled, extracted with ether (1x50ml., 2x25ml.) and the combined extracts dried (Na_2SO_4). Removal of solvent yielded no product. The acid aqueous layer was basified with sodium hydroxide solution and extracted with ether (2x50ml., 1x25ml.) and the combined extracts, after drying, evaporated to yield a viscous yellow polymer (~2g.).

3-(2-Dimethylaminoethoxy)propylmethylamine was prepared by the method described for 3-(2-diethylaminoethoxy)propylethylamine modified in that decomposition of the reaction mixture was effected with water and sodium hydroxide solution (20%) in the proportions described by Amundsen and Nelson.[318] 3-(2-Dimethylaminoethoxy)propylmethylamine (7.7g., 64%), prepared from 3-(2-dimethylaminoethoxy)propion-N-methylamide (13g.) and lithium aluminium hydride (4.5g.) was obtained as a colourless oil, b.p. 56-57°/0.02mm.

Found: equivalent (titration) 82.4; N, 16.1
$C_8H_{20}N_2O$ requires equivalent 80.1; N, 17.5%

Dihydrochloride (from ethanol-ether) m.p. 133° (sealed tube) softening at 125°.

Found: equivalent (titration) 117.1; N, 12.5
$C_8H_{22}Cl_2N_2O$ requires equivalent 116.6; N, 12.0%

N-Butyl-N-2′-dibutylaminoethoxy-3-propylamine was prepared by the method described for 3-(2-dimethylaminoethoxy)propyl-methylamine from 3-(2-dibutylaminoethoxy)propion-N-butylamide (26g.) and lithium aluminium hydride (6·5g.). Extraction and distillation yielded N-butyl-N-2′-dibutylaminoethoxy-3-propyl-amine (6·6g., 44%), b.p. 129-130°/0·6mm.

Found: equivalent (titration) 145·5 ; N, 9·2

$C_{17}H_{38}N_2O$ requires equivalent 143·25; N, 9·8%

3-(2-Diethylaminopropoxy)propylethylamine was prepared by the method described for 3-(2-dimethylaminoethoxy)propylmethylamine from 3-(2-diethylaminopropoxy)propion-N-ethylamide (27g.) and lithium aluminium hydride (8g.). 3-(2-Diethylaminopropoxy-propylethylamine (15·3g., 61%) was obtained as a colourless oil, b.p. 89-91°/0·1mm.

Found: equivalent (titration). 108·7 ; N, 13·2

$C_{12}H_{28}N_2O$ requires equivalent 108·2 ; N, 12·95%

3-(1-Methyl-2-diethylaminoethoxy)propylethylamine was prepared by the method described for 3-(2-dimethylaminoethoxy)propyl-methylamine from 3-(1-methyl-2-diethylaminoethoxy)propion-N-ethylamide (17g.) and lithium aluminium hydride (8g.). Decomp osition, extraction and distillation yielded 3-(1-methyl-2-diethylaminoethoxy)propylethylamine (9g., 56%) as a colourless oil, b.p. 80-82°/0·05mm.

Found: equivalent (titration) 109·1 ; N, 12·8

$C_{12}H_{28}N_2O$ requires equivalent 108·2 ; N, 12·95%

3-(2-Diethylaminoethoxy)butylethylamine was prepared by a method similar to that described for 3-(2-dimethylaminoethoxy)propyl-methylamine from 3-(2-diethylaminoethoxy)butyr-N-ethylamide (27·7g.) and lithium aluminium hydride (8g.), and was obtained as a colourless oil (14·5g., 56%), b.p. 95-97°/0·9mm.

Found: equivalent (titration) 109·1 ; N, 13·2

$C_{12}H_{28}N_2O$ requires equivalent 108·2 ; N, 12·95%

(±)-3-(2-Dimethylaminopropoxy)propylethylamine was prepared by the method described for 3-(2-dimethylaminoethoxy)propylmethylamine from (±)-3-(2-dimethylaminopropoxy)propion-N-ethylamide (14·4g.) and lithium aluminium hydride (4·5g.). Decomposition, extraction and distillation afforded (±)-3-(2-dimethylaminopropoxy)propylethylamine (4g., 30%), b.p. 74-76°/1mm.

Found: equivalent (titration) 94·9

$C_{10}H_{24}N_2O$ requires equivalent 94·2

Dihydrochloride from ethanol-acetone-ether m.p. 122-123°

Found: N, 10·5

$C_{10}H_{26}Cl_2N_2O$ requires N, 10·7%

The Attempted Preparation of L(-)-3-(2-Dimethylaminopropoxy)-propylethylamine.

L(-)-2-dimethylaminopropan-1-ol (7g.) containing sodium methoxide (500mg.) was added to N-ethylacrylamide (9·2g.). The reaction, after three days, reached an equilibrium as judged from spectroscopic evidence and the catalyst was destroyed by the careful addition of concentrated sulphuric acid (0·3ml.). The mixture was taken up in dry ether (ca. 55ml.), filtered and the filtrate reduced with lithium aluminium hydride (4g.). Decomposition and distillation yielded L(-)-2-dimethylaminopropan-1-ol (3g.), $[\alpha]_D^{24}$ -2·13° (c 0·8 in 90 per cent v/v ethanol).

THE PREPARATION OF N,N -DISUBSTITUTED ACRYLAMIDES AND CROTONAMIDES

N-3-(2-Diethylaminoethoxy)propyl-N-ethylacrylamide

A. Acryloyl chloride (1·8g.) in dry ether (25ml.) was slowly added to a cooled stirred solution of 3-(2-diethylaminoethoxy)-propylethylamine (4g.) in dry ether (50ml.). Sodium hydroxide solution (10ml., 20% w/v) was added, the ether layer decanted and the aqueous layer extracted with four further portions of

ether (3x50ml., 1x30ml.). The combined extracts were dried (Na_2SO_4) and the ether removed to yield a pale yellow oil (4·5g.) [Found: equivalent 219·6; $C_{14}H_{28}N_2O_2$ requires equivalent 256·4]. The crude material was distilled to yield a colourless oil (3g.), b.p. 140-144°/0·1mm. with equivalent 224. The oil (1g.) was chromatographed on alumina (30g., column dimensions 12cm.x1·5cm.) using 0·5 per cent ethanol in petroleum ether (b.p. 40-60°) as eluting solvent. The first 160ml. of eluate yielded N-3(2-diethylaminoethoxy)propyl-N-ethylacrylamide (0·32g.), λ max. 237mμ, ε 5238.

Found:	equivalent (titration)	255·3 ;	N,	10·7
$C_{14}H_{28}N_2O_2$	requires equivalent	256·4 ;	N,	10·9%

B. Acryloyl chloride (1·5g.) in dry ether (50ml.) was added to a cooled stirred solution of 3-(2-diethylaminoethoxy)propyl-ethylamine (3g.) in dry ether (100ml.) over a period of 15 minutes. The mixture was stirred a further hour at room temperature. Potassium carbonate (10g.) and water (5ml.) were then added and the mixture shaken vigorously. The ethereal layer was decanted and the residue extracted with ether (2x50ml.). The combined extracts were dried (Na_2SO_4) and removal of the ether under reduced pressure yielded N-3-(2-diethylaminoethoxy)-propyl-N-ethylacrylamide (2·5g., 66%) as a yellow oil, λmax. 237 mμ, ε ca. 5000, of sufficient purity for use in subsequent stages.

Found:	equivalent (titration)	254·6 ;	N,	10·5
$C_{14}H_{28}N_2O_2$	requires equivalent	256·4 ;	N,	10·9%

N-3-(2-Dimethylaminoethoxy)propyl-N-methylacrylamide was prepared by the method described for N-3-(2-diethylaminoethoxy)propyl-N-ethylacrylamide (Method B) from 3-(2-dimethylaminoethoxy)-propylmethylamine (7·7g.) and acryloyl chloride (5g.). The method was modified in that basification was effected with 20% sodium hydroxide solution (20ml.). Extraction and removal

of the solvent yielded N-3-(2-dimethylaminoethoxy)propyl-N-methylacrylamide (5·4g., 52%) as a yellow oil with characteristic ultraviolet and infrared absorption spectra.

N-3-(2-Dibutylaminoethoxy)propyl-N-butylacrylamide was prepared by a further modification of the method described for N-3-(2-diethylaminoethoxy)propyl-N-ethylacrylamide (method B) from N-butyl-N-2′-dibutylaminoethoxy-3-propylamine (10g.) and acryloyl chloride (4·5g.). When the addition of the acid chloride was complete sodium hydroxide (1-2ml.,20%) was added and the mixture stirred another 0·5hr. at room temperature. Basification was effected by 20% sodium hydroxide solution (30ml.), the ether layer decanted and the aqueous layer further extracted with ether (2x200ml.). Anhydrous sodium carbonate (ca. 30g.) was added to the aqueous layer to form a pasty mass which was then extracted with ether (2x50ml.). Drying of the combined extracts and evaporation of the solvent yielded N-3-(2-dibutylaminoethoxy)propyl-N-butylacrylamide (11g., 92%) as a yellow oil. The product was again characterised by ultraviolet and infrared absorption spectra.

N-3-(2-Diethylaminopropoxy)propyl-N-ethylacrylamide was prepared from 3-(2-diethylaminopropoxy)propylethylamine (15·3g.) and acryoyl chloride (7·5g.) by the method described for N-3-(2-dibutylaminoethoxy)propyl-N-butylacrylamide and was obtained as a yellow oil (18g., 95%) with the expected ultraviolet and infrared spectra.

N-3-(1-Methyl-2-diethylaminoethoxy)propyl-N-ethylacrylamide was prepared by the method described for N-3-(2-dibutylaminoethoxy) propyl-N-butylacrylamide from 3-(1-methyl-2-diethylaminoethoxy) propylethylamine (13·7g.) and acryoyl chloride (6·3g.) and was obtained as a yellow oil (13.8g., 80%) with characteristic ultraviolet and infrared absorption spectra.

N-3-(2-Dimethylaminopropoxy)propyl-N-ethylacrylamide was prepared from 3-(2-dimethylaminopropoxy)propylethylamine (4g.) and acryloyl chloride (2·2g.) by the method described for N-3-(2-dibutylaminoethoxy)propyl-N-butylacrylamide and the product (4·1g., 81%) showed the expected infrared and ultraviolet spectra.

N-3-(2-Diethylaminoethoxy)butyl-N-ethylcrotonamide was prepared from 3-(2-diethylaminoethoxy)butylethylamine (14·5g.) and crotonyl chloride (9g.) by a modification of the method described for N-3-(2-dimethylaminoethoxy)propyl-N-methylacrylamide. When the initial addition of the acid chloride to the amine was complete the reaction mixture was refluxed for 20 minutes and cooled. N-3-(2-diethylaminoethoxy)butyl-N-ethylcrotonamide (16·3g., 85%) was obtained as a yellow oil with absorption spectra similar to those of model N,N-disubstituted crotonamides.

THE ADDITION OF ALCOHOLS TO NN-DISUBSTITUTED ACRYLAMIDES AND CROTONAMIDES.

N-3'-(2-Diethylaminoethoxy)propyl-N-ethyl-3-(2-diethylaminoethoxy)propionamide was prepared by the method described for 3-(2-diethylaminoethoxy)propion-N-ethylamide from 2-diethylaminoethanol (2·5g.) and N-3-(2-diethylaminoethoxy)propyl-N-ethylacrylamide (5g.). The reaction reached equilibrium after 3 days and saponification and distillation yielded the product (3·6g., 50%), b.p. 196-200°/0·025mm.

Found: equivalent (titration) 185·9 ; N, 11·2

$C_{20}H_{43}N_3O_3$ requires equivalent 186·8 ; N, 11·25%

N-3'-(2-Dimethylaminoethoxy)propyl-N-methyl-3-(2-dimethylaminoethoxy)propionamide was prepared from 2-dimethylaminoethanol (9g.) and N-3-(2-dimethylaminoethoxy)propyl-N-methylacrylamide (12g.) using hydroquinone (0·1%) as polymerisation inhibitor by the method described for 3-(2-diethylaminoethoxy)propion-N-ethylamide. The reaction reached equilibrium after 4 days at

65° and the product (9·4g., 55%), b.p. 188-190°/0·12mm. was obtained as a pale yellow oil.

Found: equivalent (titration) 150·9 ; N, 14·2

$C_{15}H_{33}N_3O_3$ requires equivalent 151·7 ; N, 13·85%

N-3′-(2-Dibutylaminoethoxy)propyl-N-butyl-3-(2-dibutylaminoethoxy propionamide was prepared by the method described for 3-(2-diethylaminoethoxy)propion-N-ethylamide from 2-dibutylamino-ethanol (9·5g.) and N-3-(2-dibutylaminoethoxy)propyl-N-butyl-acrylamide (11g.) using hydroquinone (0·1%) as polymerisation inhibitor. The reaction reached equilibrium after 100 hours at 95° and the product (3·75g.), b.p. 230-240°/0·04mm. was obtained as a yellow oil. The recovered N-3-(2-dibutylamino-ethoxy)propyl-N-butylacrylamide (6·3g.) was recycllised to yield a further 2·6g. of product (overall yield 6·35g., 39%).

Found: equivalent (titration) 256·4 ; N, 8·2

$C_{30}H_{63}N_3O_3$ requires equivalent 256·9 ; N, 8·2%

N-3′-(2-Diethylaminopropoxy)propyl-N-ethyl-3-(2-diethylamino-propoxy)propionamide was prepared from N-3-(2-diethylamino-propoxy)propyl-N-ethylacrylamide (18g.) and 2-diethylamino-propan-1-ol (10g.) by the method described for 3-(2-diethylamino-ethoxy)propion-N-ethylamide. The reaction reached equilibrium in less than 6 hours and subsequent solidification and distill-ation yielded the product (8·6g., 32%), b.p. 235-250°/0·3mm.

Found: equivalent (titration) 200·6 ; N, 10·7

$C_{22}H_{47}N_3O_3$ requires equivalent 200·8 ; N, 10·5%

N-3′-(1-Methyl-2-diethylaminoethoxy)propyl-N-ethyl-3-(1-methyl-2-diethylaminoethoxy)propionamide.

N-3-(1-methyl-2-diethylaminoethoxy)propyl-N-ethylacryl-amide (13·8g.) and 1-diethylaminopropan-2-ol were treated as described in the preparation of 3-(2-diethylaminoethoxy)propion-N-ethylamide. In less than six hours the reaction reached

equilibrium. Distillation was accompanied by gross decomposition and unreacted N-3-(1-methyl-2-diethylamino-ethoxy)propyl-N-ethylacrylamide (3g.) was recovered over the range 130-190°/0.03mm. with a forerun of 1-diethylamino-propan-2-ol. The residue in the distillation flask was extracted with ether, filtered to remove carbon, chercoaled and again filtered. Evaporation of the ether left only a viscous residue (2.1g.)

N-3'-(2-Diethylaminoethoxy)butyl-N-ethyl-3-(2-diethylamino-ethoxy)butyramide was prepared by the method described for 3-(2-diethylaminoethoxy)propion-N-ethylamide from N-3-(2-diethylaminoethoxy)butyl-N-ethylcrotonamide (16.25g.) and 2-diethylaminoethanol (8g.). The reaction reached equilib-rium after 90 hours at 150° and the product (2.1g.), b.p. 194-204°/0.05mm. was obtained as a yellow oil. Unreacted N-3-(2-diethylaminoethoxy)butyl-N-ethylcrotonamide (10.8g.) was recycliced to yield, after 96 hours at 120°, a further 21g. of product. The unreacted substituted crotonamide (7g.) from this second condensation was again recycliced and the process repeated on one more occasion to yield a further 1.8g. of product (overall yield 6g., 26%).

Found: equivalent (titration) 200.1 ; N, 10.6

$C_{22}H_{47}N_3O_3$ requires equivalent 200.8 ; N, 10.5%

THE REDUCTION OF THE ADDITION PRODUCTS WITH LITHIUM ALUMINIUM HYDRIDE.

N,N-Di-[3-(2-diethylaminoethoxy)propyl]ethylamine was prepared from N-3'-(2-diethylaminoethoxy)propyl-N-ethyl-3-(2-diethyl-aminoethoxy)propionamide (9g.) and lithium aluminium hydride (1g.) by the method described for 3-(2-diethylaminoethoxy)-propylethylamine. Decomposition, extraction and distillation yielded N,N-di-[3-(2-diethylaminoethoxy)propyl]ethylamine (5.2g., 60%) as a colourless oil, b.p 158-160°/0.04mm.

Found: equivalent (titration) 119 ; N, 11·7
$C_{20}H_{45}N_3O_2$ requires equivalent 119·9 ; N, 11·7%

N,N-Di-[3-(2-dimethylaminoethoxy)propyl]methylamine was prepared by the method described for 3-(2-dimethylaminoethoxy) propylmethylamine from N-3'-(2-dimethylaminoethoxy)propyl-N-methyl-3-(2-dimethylaminoethoxy)propionamide (9·3g.) and lithium aluminium hydride (1·48g.) and was obtained as a colourless oil (5·7g., 63%), b.p. 148-152°/0·09mm.

Found: equivalent (titration) 100·2 ; N, 14·4
$C_{15}H_{35}N_3O_2$ requires equivalent 96·5 ; N, 14·5%

N,N-Di-[3-(2-dibutylaminoethoxy)propyl]butylamine was prepared from N-3'-(2-dibutylaminoethoxy)propyl-N-butyl-3-(2-dibutylaminoethoxy)propionamide (6·35g.) and lithium aluminium hydride (1g.) by the method described for 3-(2-dimethylaminoethoxy)propylmethylamine and was obtained as a yellow oil (3·9g. 63%), b.p. 206-212°/0·03mm.

Found: equivalent (titration) 167·3 ; N, 8·3
$C_{30}H_{65}N_3O_2$ requires equivalent 166·6 ; N, 8·4%

N,N-Di-[3-(2-diethylaminopropoxy)propyl]ethylamine was prepared from N-3'-(2-diethylaminopropoxy)propyl-N-ethyl-3-(2-diethylaminopropoxy)propionamide (8g.) and lithium aluminium hydride (1g.) by the method described for 3-(2-dimethylaminoethoxy)-propylmethylamine and was obtained as a colourless oil (4·3g., 56%), b.p. 230-231°/0·1mm.

Found: equivalent (titration) 131·7 ; N, 10·8
$C_{22}H_{49}N_3O_2$ requires equivalent 129·2 ; N, 10·8%

N,N-Di-[3-(1-methyl-2-diethylaminoethoxy)propyl]ethylamine

N-3-(1-methyl-2-diethylaminoethoxy)propyl-N-ethylacrylamide (8g.) and 1-diethylaminopropan-1-ol were reacted together as described in the preparation of 3-(2-diethylaminoethoxy)-propion-N-ethylamide. Spectroscopic evidence indicated the

reaction had reached equilibrium in less than six hours. The reaction mixture was dissolved in ether (50ml.) and filtered to remove the sodium methoxide and the filtrate was added to a stirred hot suspension of lithium aluminium hydride (3·6g.) in anhydrous ether (200ml.) at a rate sufficient to keep the solvent gently refluxing. The reaction mixture was refluxed for five hours and then decomposed and extracted. Removal of the solvent and distillation yielded a low boiling forerun of amino alcohol and amine followed by N,N-Di-[3-(1-methyl-2-diethylaminoethoxy)-propyl]ethylamine (3·9g., 34%) as a colourless oil, b.p. 162-170°/0·02mm.

Found: equivalent (titration) 129·4 ; N, 10·8

$C_{22}H_{49}N_3O_2$ requires equivalent 129·2 ; N, 10·8%

N,N-Di-[3-(2-diethylaminopropoxy)propyl]ethylamine (cf. page 109) was prepared from N-3-(2-diethylaminopropoxy)propyl-N-ethyl-acrylamide (9·6g.) and 2-diethylaminopropan-1-ol (5g.) by the method described for N,N-di-[3-(1-methyl-2-diethylaminoethoxy)-propyl]ethylamine. Reduction was effected by lithium aluminium hydride (4g.) and the product (6·1g., 44%) obtained as a colourless oil, b.p. 180-186°/0·06mm.

Found: equivalent (titration) 128·3

$C_{22}H_{49}N_3O_2$ requires equivalent 129·2

N,N-Di-[3-(2-diethylaminoethoxy)butyl]ethylamine was prepared from N-3'-(2-diethylaminoethoxy)butyl-N-ethyl-3-(2-diethylamino-ethoxy)butyramide (6g.) and lithium aluminium hydride (2g.) by the method described for 3-(2-dimethylaminoethoxy)propylmethyl-amine and was obtained as a colourless oil (1·9g., 22%), b.p. 162-164°/0·04mm.

Found: equivalent (titration) 134·5 ; N, 11·0

$C_{22}H_{49}N_3O_2$ requires equivalent 129·2 ; N, 10·8%

N,N-Di-[3-(2-dimethylaminopropoxy)propyl]ethylamine was obtained from N-3-(2-dimethylaminopropoxy)propyl-N-ethylacrylamide (4g.)

and (±)-2-dimethylaminopropan-1-ol (2g.) followed by reduction with lithium aluminium hydride (1·5g.) as described in the preparation of N,N-di-[3-(1-methyl-2-diethylaminoethoxy)propyl]-ethylamine. The product (1·43g., 27%), b.p. 182-184°/0·3mm., was obtained as a colourless oil.

Found:	equivalent (titration)	112·4	; N,	12·4
$C_{18}H_{41}N_3O_2$ requires	equivalent	110·5	; N,	12·7%

The Preparation of the Quaternary Ammonium Salts

The quaternary ammonium salts of the various tertiary bases were prepared by three methods.

Method A: The base in dry ethanol (1-5ml.) was treated with the appropriate alkyl halide and the solution refluxed if required. The solution was evaporated to dryness and the residue recrystallised from a suitable solvent.

Method B: The base was treated with the appropriate alkyl halide in the absence of solvent or in ether solution and the mixture set aside till reaction was complete. The mixture was then washed copiously with ether, centrifuging the precipitate between each washing, and the product finally dried *in vacuo* over phosphorus pentoxide.

Method C: Where either method A or method B failed to produce a product capable of being easily characterised the quaternary ammonium halide was converted to the corresponding reineckate which was recrystallised from either aqueous acetone or acetone-ethanol-water.

7,7-Dimethyl-3,11-dioxa-7-azoniatridecylenebis-(trimethylammonium) tri-iodide

N,N-Di-[3-(2-dimethylaminoethoxy)propyl]methylamine (0·68g.) in anhydrous ether (22ml.) was treated with methyl iodide (1·5ml. and the product (1·25g., 73%) isolated using method B, m.p. 238-240°(decomp.).

Found:	I,	52·95	; N,	5·8
$C_{18}H_{44}I_3N_3O_2$ requires	I,	53·2	; N,	5·9%

7,7-Dimethyl-3,11-dioxa-7-azoniatridecylenebis-(trimethylammonium) tri-reineckate

N,N-Di-[3-(2-dimethylaminoethoxy)propyl]methylamine (0·25g.) was treated with methyl iodide (1·0ml.) (Method A). The oily product was converted to the reineckate (0·83g., 75%) m.p. 220-225° (decomp.) from aqueous acetone.

Found:	equivalent (titration)	440·2 ;	N, 22·3
$C_{30}H_{62}Cr_3N_{21}O_2S_{12}$	requires equivalent	429·9 ;	N, 22·8%

7-Ethyl-7-methyl-3,11-dioxa-7-azoniatridecylenebis-(dimethylethylammonium) tri-reineckate

N,N-Di-[3-(2-dimethylaminoethoxy)propyl]methylamine (0·48g.) and ethyl iodide (0·5ml.) (method A) afforded an oily product which was converted to the reineckate (1·19g., 54%), m.p. 215-220° (decomp.) from aqueous acetone.

Found:	equivalent (titration)	446·4 ;	N, 22·3
$C_{33}H_{68}Cr_3N_{21}O_2S_{12}$	requires equivalent	443·9 ;	N, 22·1%

7-Ethyl-7-methyl-3,11-dioxa-7-azoniatridecylenebis-(diethylmethylammonium) tri-iodide

N,N-Di-[3-(2-diethylaminoethoxy)propyl]ethylamine (0·68g.) in dry ethanol (1ml.) was refluxed with methyl iodide (2·5ml.) for 15 mins. to give (Method A) the product (0·84g., 57%), m.p. 228-229° (decomp.) from n-propanol-ether.

Found:		I, 48·4 ;	N, 5·4
$C_{23}H_{54}I_3N_3O_2$	requires	I, 48·5 ;	N, 5·35%

7,7-Diethyl-3,11-dioxa-7-azoniatridecylenebis-(triethylammonium) tri-iodide

N,N-Di-[3-(2-diethylaminoethoxy)propyl]ethylamine (0·4g.) in dry ethanol (1ml.) was refluxed with ethyl iodide (2·5ml.) for 15 mins. to yield (Method A) the product (0·64g., 71%), m.p. 231° (decomp.) from ethanol.

Found: I, 45·7 ; N, 5·0

$C_{26}H_{60}I_3N_3O_2$ requires I, 46·0 ; N, 5·1%

7-Ethyl-7-propyl-3,11-dioxa-7-azoniatridecylenebis-(diethylpropylammonium) tri-iodide

N,N-Di-[3-(2-diethylaminoethoxy)propyl]ethylamine (0·9g.) in dry ethanol (1ml.) and propyl iodide (3ml.) were refluxed 75 mins. to afford (method A) the product (1·85g., 84%), m.p. 145-146° (decomp.) from ethanol-acetone-ether.

Found: I, 43·4; N, 4·8

$C_{29}H_{66}I_3N_3O_2$ requires I, 43·8; N, 4·8%

7-Butyl-7-methyl-3,11-dioxa-7-azoniatridecylenebis-(dibutylmethylammonium) trireineckate

N,N-Di-[3-(2-dibutylaminoethoxy)propyl]butylamine (0·53g.) and methyl iodide (2·5ml.) (Method A) afforded an oily product which was converted to the reineckate (0·5g., 29%), m.p. 95-98° (decomp.) from acetone-ethanol-water as the triethanolate.

Found: C, 37·7; H, 6·4; N, 18·5

$C_{51}H_{110}Cr_3N_{21}O_5S_{12}$ requires C, 37·4; H, 6·8; N, 18·0%

7-Butyl-7-ethyl-3,11-dioxa-7-azoniatridecylenebis-(dibutylethylammonium) trireineckate

N,N-Di-[3-(2-dibutylaminoethoxy)propyl]butylamine (0·5g.) and ethyl iodide (2·5ml.) (Method A) yielded an oily product which was converted to the reineckate (1·45g., 86%), m.p. 93-96° (decomp.) from acetone-ethanol-water as the triethanolate.

Found: C, 38·45; H, 6·2 N, 17·9

$C_{54}H_{116}Cr_3N_{21}O_5S_{12}$ requires C, 38·6 ; H, 6·95 N, 17·5%

7-Ethyl-7-methyl-3,11-dioxa-4,10-dimethyl-7-azoniatridecylene-bis-(diethylmethylammonium) tri-iodide

N,N-Di-[3-(2-diethylaminoethoxy)butyl]ethylamine (0·5g.) was treated with methyl iodide (2ml.) and the product (0·8g., 76%) isolated using method B, m.p. 208-209° (decomp.).

Found: I, 46·85; N, 4·9

$C_{25}H_{58}I_3N_3O_2$ requires I, 46·8 ; N, 5·2%

7,7-Diethyl-3,11-dioxa-4,10-dimethyl-7-azoniatridecylenebis-(triethylammonium) trireineckate.

N,N-Di-[3-(2-diethylaminoethoxy)butyl]ethylamine (0·7g.) and ethyl iodide (3ml.) (Method A) gave a gummy product which was converted to the reineckate (1·2g., 45%), m.p. 139-141° (decomp.) from aqueous acetone as the trihydrate.

Found: equivalent (titration) 504·0; N, 19·4

$C_{40}H_{88}Cr_3N_{21}O_5S_{12}$ requires equivalent 494·7; N, 19·9%

7-Ethyl-7-methyl-1,13-dimethyl-3,11-dioxa-7-azoniatridecylenebis-(diethylmethylammonium) tri-iodide

N,N-Di-[3-(2-diethylaminopropoxy)propyl]ethylamine (0·3g.) and methyl iodide (2ml.) (method B) yielded the product (0·53g., 84%), m.p. 204-205° (decomp.).

Found: I, 46·9; N, 5·0

$C_{25}H_{58}I_3N_3O_2$ requires I, 46·8; N, 5·2%

7,7-Diethyl-1,13-dimethyl-3,11-dioxa-7-azoniatridecylenebis-(triethylammonium) tri-iodide

N,N-Di-[3-(2-diethylaminopropoxy)propyl]ethylamine (0·58g.) and ethyl iodide (2·4ml.) (method B) gave the product (1·12g., 88%), m.p. 226-227° (decomp.).

Found: I, 44·9; N, 5·1

$C_{28}H_{64}I_3N_3O_2$ requires I, 44·5; N, 4·9%

7-Ethyl-7-methyl-2,12-dimethyl-3,11-dioxa-7-azoniatridecylene-bis-(diethylmethylammonium) tri-iodide

N,N-Di-[3-(1-methyl-2-diethylaminoethoxy)propyl] ethylamine (0·51g.) in dry ether (22ml.) was treated with methyl iodide (2ml.) and the product (0·86g., 80%) isolated using Method B, m.p. 214-215° (decomp.).

Found: I, 46·4; N, 5·0

$C_{25}H_{58}I_3N_3O_2$ requires I, 46·8; N, 5·2%

7,7-Diethyl-2,12-dimethyl-3,11-dioxa-7-azoniatridecylenebis-(triethylammonium) tri-iodide

N,N-Di-[3-(1-methyl-2-diethylaminoethoxy)propyl]ethyl-amine (0·9g.) and ethyl iodide (3ml.) (Method B) afforded the product (1·8g., 90%), m.p. 169-170° (decomp.).

Found: I, 44·1; N, 4·5

$C_{28}H_{64}I_3N_3O_2$ requires I, 44·5; N, 4·9%

7-Ethyl-7-methyl-1,13-dimethyl-3,11-dioxa-7-azoniatridecylene-bis-(trimethylammonium) tri-iodide

N,N-Di-[3-(2-dimethylaminopropoxy)propyl]ethylamine (0·5g.) in anhydrous ether (20ml.) was treated with methyl iodide (2ml.) and the product (0·85g., 75%) isolated using Method B, m.p. 244-246° (decomp.).

Found: I, 49·7; N, 5·3

$C_{21}H_{50}I_3N_3O_2$ requires I, 50·3; N, 5·5%

7,7-Diethyl-1,13-dimethyl-3,11-dioxa-7-azoniatridecylenebis-(dimethylethylammonium) tri-iodide

N,N-Di-[3-(2-dimethylaminopropoxy)propyl]ethylamine (0.42g. and ethyl iodide (3ml.) (Method B) yielded the product (0·97g. 95%), m.p. 247-248° (decomp.).

Found: I, 47·8; N, 5·25

$C_{24}H_{56}I_3N_3O_2$ requires I, 47·6; N, 5·3%

REFERENCES

1.	Foldes et al.,	J. Amer. Med. Assoc.,	1952,	150,	1559
2.	Griffith,	Ann. N.Y. Acad. Sci.,	1951,	54,	493
3.	Burman,	J. Bone and Joint Surg.,	1938,	20,	754
4.	Idem.,	Arch. Neurol and Psychiat.,	1939,	41,	307
5.	Bennet,	J. Amer. Med. Assoc.,	1940,	114,	322
6.	Griffith & Johnson,	Anesthesiology,	1942,	3,	418
7.	Cullen,	Surgery,	1943,	14,	261
8.	Barlow,	Introduction to Chemical Pharmacology, Methuen, London, 1955.			
9.	Beecher & Todd,	Ann. Surg.,	1954,	140,	2
10.	Cullen,	Anesthesiology,	1959,	20,	530
11.	Foldes,	Clin. Pharmacol. et Therap.,	1960,	1,	345
12.a)	McIntyre, Curare,	University of Chicago Press, Chicago, 1947.			
b)	Bovet & Bovet-Nitti,	Experientia,	1948,	4,	325
c)	McIntyre,	Anesthesiology,	1959,	20,	409
13.	Boehm,	Curare and Curaroalkaloide in A. Heffter, Handbuch der experimentellen Pharmakologie. Springer, Berlin, 1920. (Cited by 12a).			
14.	Marsh,	Ann. N.Y. Acad. Sci.,	1951,	54,	307
15.a)	Boehm,	Abhandl. Kgl. sachs. Ges. Wissensch.,	1895,	22,	201
b)	Idem.,	ibid.,	1897,	24,	1
16.	Späth, Leithe & Ladek,	Ber.,	1928,	61,	1698
17.a.	King,	J. Chem. Soc.,	1935,	1381	
	Idem.,	ibid.,	1948,	265	
18.	Wintersteiner & Dutcher,	Science,	1943,	97,	467
19.a)	King,	Nature,	1946,	158,	515
b)	Idem.,	J. Chem. Soc.,	1947,	936	

20.	Dutcher,	J. Amer. Chem. Soc.,	1952,	74,	2221
21.	Idem.,	Ann. N.Y.Acad. Sci.,	1951,	54,	326
22.	Winterstein,	in Curare and Curare-like Agents ed. by Bovet, Bovet-Nitti and Marini-Bettolo, Elsevier, London, 1959. p. 153.			
23.	Wieland & Aster,	Annalen,	1938,	536,	68
24.	Schmid, Kebrle & Karrer,	Helv. Chim. Acta,	1952,	35,	1864
25.	Battersby et al.,	J. Chem. Soc.,	1960,	1848	
26.	Von Philipsborn, Schmid & Karrer,	Helv. Chim. Acta,	1956,	29,	913
27.	Wieland, Konz & Sonderhoff,	Annalen,	1937,	527,	160
28.	Nagyvary et al.,	Tetrahedron,	1961,	14,	138
29.	Battersby & Hodson,	Proc. Chem. Soc.,	1958,	287	
30.	Bernauer et al.,	Helv. Chim. Acta,	1958,	41,	2293
31.	Proos,	See reference 22 p.83			
32.	Battersby & Hodson,	Quart. Rev.,	1960,	14,	77
33.	Bernard & Polonovo,	Compt. rend.,	1950,	31,	533
34.	Idem.,	Union Med.,	1950,	4,	505
35.	Bernard,	Compt. rend.,	1956,	43,	825
36.	Whittaker & Gray,	Brit. Med. Bull.,	1962,	18,	223
37.	Grundfest,	Ann. N.Y. Acad. Sci.,	1957,	66,	537
38.	Bowman,	Progress in Medicinal Chemistry Vol. II, ed. by Ellis and West, Butterworths, London, 1962.			
39.	Von Muralt,	Significant Trends in Medical Research, Ciba Foundation Symposium, Churchill, London, 1959. p. 105.			
40.	Wood-Smith & Stewart,	Drugs in Anaesthetic Practice, Butterworths London. 1962, 313.			

41.	Dale, Feldberg & Vogt.	J. Physiol.,	1936,	86,	353
42.	del Castillo & Katz,	Progr. Biophys. Chem.,	1956,	6,	121
43.	Palade & Palay,	J. Biophys. Biochem. Cytol.,	1955,	1,	69
44.	de Robertis & Bennett,	ibid.,	1955,	1,	47
45.	Fernandez-Moran,	Metabolism of the Nervous System, ed. Richter, Pergamon, London, 1957.			
46.	del Castillo & Katz,	J. Physiol.,	1955,	128,	396
47.	Sjöstrand,	J. Appl. Phys.,	1953,	24,	1422
48.	Palay & Palade,	Anat. Rec.,	1954,	118,	336
49.	de Robertis & Bennet,	Fed. Proc.,	1954,	13,	35
50.	Nastuk,	J. Cell. Comp. Physiol.,	1953,	42,	249
51.	del Castillo & Katz,	J. Physiol.,	1955,	128,	157
52.	idem.,	Proc. Roy. Soc. B.,	1957,	146,	339
53.	idem.,	J. Physiol.,	1954,	125,	546
54.	Eccles, Katz & Kuffler,	J. Neurophysiol.,	1941,	4,	362
55.	Spencer & Worthington,	Nature,	1960,	187,	388
56.	Weker & Portzehl,	Progr. Biophys.,	1954,	4,	66
57.	Singh,	Nature,	1962,	196,	172
58.	Marnay & Nachmansohn,	J. Physiol.,	1938,	92,	37
59.	Koelle,	J. Pharm. Pharmacol.,	1962,	14,	65
60.	Nachmansohn,	Science,	1961,	134,	1962
61.	idem.,	J. Amer. Med. Assoc.,	1962,	179,	639
62.	idem.,	Chemical & Molecular Basis of Nerve Activity, Academic Press, London, 1959			
63.	idem.,	Anesthesiology,	1959,	20,	416

64.	Idem.,	Structure and Function of Muscle Ed. by Bourne, Academic Press, London, 1960.			
65.	Barnett,	J. Cell. Biol.,	1962,	12,	247
66.	Bullock, Nachmansohn & Rothenberg,	J. Neurophysiol.,	1946,	9,	9
67.	Rothenberg, Sprinson & Nachmansohn,	ibid.,	1948,	11,	111
68.	Walsh & Deal,	Amer. J. Physiol.,	1959,	197,	547
69.	Rosenberg & Ehrenpreis,	Biochem. Pharmacol.,	1961,	8,	192
70.	Idem.,	Nature,	1961,	190,	726
71.	Rosenberg & Podleski,	J. Pharmacol.,	1962,	137,	249
72.	Dettbarn,	Nature,	1960,	186,	891
73.	Ottolenghi,	See ref. 22, p. 442.			
74.	McIntosh,	Canad. J. Biochem. Physiol.,	1959,	37,	343
75.	Nachmansohn,	"Choline Acetylase" in Cholinesterases and Anticholinesterases, Ed. Koelle, Heffter-Heubner Handb. d. exp. Pharmakol Suppl. 15, Heidelberg, Springer-Verlag, Berlin, 1963.			
76.	Bowman & Rand,	Brit. J. Pharmacol.,	1961,	17,	176
77.	Brown & Harvey,	J. Physiol.,	1940,	97,	330
78.	del Castillo & Engbaek,	ibid.,	1954,	124,	370
79.	Brown & Dias,	Anais. Acad. brasil Ciens.,	1947,	19,	359
		cited by de Carvalho, see ref. 22 p. 181			
80.	Harvey,	Bull Johns Hopkins Hosp.,	1939,	65,	223
81.	Bergen, Dickens & Zatman,	J. Physiol.,	1949,	109,	10
82.	Thesleff,	J. Physiol.,	1960,	151,	598
83.	Loew, Achenbach, & Metcalf,	J. Pharmacol.,	1949,	97,	441

84.	Riker,	Pharmacol. Rev.,	1953,	5,	1
85.	Theoleff,	Acta Physiol. Scand.,	1955,	34,	218
86.	Collier & Hall,	Brit. Med. J.,	1950,	1,	1293
87.	Bovet, Depierre & Lestrange,	Compt. rend.,	1947,	225,	74
88.	Taylor & Collier,	Nature,	1951,	167,	692
89.	Bacq & Brown,	J. Physiol.,	1937,	89,	45
90.	Barlow & Ing,	Nature,	1948,	161,	718
91.	Paton & Zaimis,	Nature,	1948,	161,	718
92.	Fusco et al.,	Gazzette,	1949,	79,	836
93.	Choymol et al.,	Compt. rend.,	1952,	235,	1711
94.	Robson & Keele,	Recent Advances in Pharmacology, Churchill London, 1956, p. 45.			
95.	Zaimis,	See ref. 22. p. 201.			
96.	Idem.,	J. Physiol.,	1953,	122,	238
97.	Holmes, Jenden & Taylor,	J. Pharmacol.,	1954,	111,	230
98.	Bovet,	Ann. N.Y. Acad. Sci.,	1951,	54,	407
99.	Ariens,	Arch. int. Pharmacodyn.,	1954,	99,	32
100.	Van Rossum & Ariens,	ibid.,	1959,	119,	394
101.	Van Rossum, Ariens & Hinsson,	Biochem. Pharmacol.,	1958,	1,	193
102.	Buchtal,	Arch. Soc. Physiol.,	1949,	3,	603
103.	Jarcho et al.,	Ann. N.Y. Acad. Sci.,	1951,	54,	337
104.	Abdon & Bjarke,	Acta pharm. tox. Kbh.,	1945,	1,	1
105.	Millehoil & Naess,	Acta Physiol. Scand.,	1961,	52,	120
106.	Heymann,	Klin. Woch.,	1928,	7,	1305
107.	Langley,	Proc. Roy. Soc. B.,	1906,	78,	170
108.	Lucas,	J. Physiol.,	1907,	36,	113
109.	Ariens,	Pharmacol. Rev.,	1957,	9,	218
110.	Paton,	Proc. Roy. Soc. B.,	1961,	154,	21

111. Pfeiffer, Science, 1948, 107, 94

112. Ing, Ibid., 1949, 109, 264

113. Koshland, Proc. Nat. Acad. Sci. U.S.A., 1958, 44, 98

114. Welch, Bull. Johns Hopkins Hosp., 1949, 83, 568

115. Wescoe, Pharmacology in Medicine, 2nd Ed. by V.A. Drill, McGraw Hill, New York, 1958, p.342.

116. Martin-Smith & Reid, J. Med. Pharm. Chem., 1959, 1, 507

117. Cavallito & Grey, Drug Research, Vol. II, Ed. by Jucker Birkhauser, Basle, 1960, p. 135.

118. Fellman & Fujita, Biochim. Biophys. Acta, 1962, 56, 227

119. Brooks, Eglinton & Morman, J. Chem. Soc., 1961, 105

120. Jones & Sandorfy, Technique of Organic Chemistry, Vol. IX, Ed. by A. Weissberger, Interscience, New York, 1956, p. 247.

120a. Canepa & Mooney, Nature, 1965, 207, 78

120b. Fellman & Fujita, Biochim. Biophys Acta, 1963, 71, 701

121. Sörum, Acta Chem. Scand., 1959, 13, 345

122. Cross, Introduction to Practical Infrared Spectroscopy, Butterworths, London, 1960.

123. Sutor, Nature, 1962, 195, 68

124. Sutor, J. Chem. Soc., 1963, 1105

125. Kögl et al., Rec. Trav. Chim., 1957, 76, 109

126. Jellinek, Acta Cryst., 1957, 10, 277

127. Arshid, Giles & Jain, J. Chem. Soc., 1956, 1272

128. Cummings et al., J. Chem. Soc., 1959, 535

129. Vasey, Experientia, 1958, 14, 356

130. Beckett et al., Nature, 1961, 189, 671

131. Vasey, Enzymes in Drug Action, Ciba Symposium Churchill, London, 1962

131b. Belleau, J. Med. Chem., 1964, 7, 776

132. Preiss et al.,	Arch. Biochem. Biophys.,	1962,	96,	158
133. Krupka & Laidler,	J. Amer. Chem. Soc.,	1961,	83,	1458
134. Waser,	J. Pharm. Pharmacol.,	1960,	12,	577
135. Carey et al.,	ibid.,	1959,	11,	707
136. Cavallito,	Curare & Curare-like Agents, Ciba Foundation Study Group No. 12, Churchill, London, 1962, p.55.			
137. Paton & Zaimis,	Brit. J. Pharmacol.,	1951,	6,	155
138. Loewe & Harvey,	Arch. exp. Path. Pharmakol.,	1952,	214,	214
139. Cavallito,	See ref. 22. p. 296			
140. Chagas,	See ref. 22. p. 327			
141. Hasson & Chagas,	Comparative Bioelectrogenesis, Elsevier, Amsterdam, 1961, p. 362. (cf. Chem. Abs., 1963, 59, 7769^{e})			
142. Chagas,	Ann. N.Y. Acad. Sci.,	1959,	81,	345
143. Hasson,	Biochim. Biophys. Acta,	1962,	56,	275
144. Ehrenpreis,	Science,	1959,	129,	1613
145. Idem.,	Biochim. Biophys. Acta.	1960,	44,	561
146. Mistretova & Turpaev,	Biokhimiya,	1961,	26,	952
147. Cass,	J. Amer. Chem. Soc.,	1961,	83,	4784
148. Crum Brown & Fraser,	Trans. Roy Soc. Edinburgh,	1869,	23,	151, 693
149. Ing & Wright,	Proc. Roy. Soc., B,	1931,	109,	337
150. Idem.,	ibid.,	1933,	114,	48
151. Waser & Harbeck,	Der. Anaesthetist,	1959,	8,	193
152. Seeger,	Anaesthetist,	1961,	10,	325
153. Waser & Harbeck,	ibid.,	1962,	11,	33
154a) Auer & Meltzer,	J. Pharmacol.,	1914,	5,	521
b) Haass,	Acta Physiol. Scand.,	1950,	20,	241
c) Secher,	Acta pharm. tox. Kbh.,	1949,	5,	196
155. Lewis,	Introduction to Pharmacology, 2nd Edition, Livingston, Edinburgh, 1962, p. 150.			

156. Stenlake,	Progress in Medicinal Chemistry, Ed. by Ellis and West, Butterworths, London, 1962.			
157. Roberts, Elkor & Roy,	Fed. Proc.,	1953,	12,	361
158. Doulofou,	See ref. 22 p. 163			
159. Salama & Wright,	Brit. J. Pharmacol.,	1951,	6,	459
160. Copp & Wilkinson,	British Patent, 617, 709, 1949.			
161. Ewins,	Biochem. J.,	1914,	8,	366
162. Hunt & Renshaw,	J. Pharmacol.,	1932,	44,	151
163. Simonart,	ibid.,	1932,	46,	157
164. Hunt & Renshaw	J. Pharmacol.,	1933,	48,	105
165. Simonart,	Arch. int. Pharmacodyn.,	1928,	34,	375
166. Idem.,	J. Pharmacol.,	1934,	50,	1
167. Idem.,	Arch. int. Pharmacodyn.,	1934,	48,	328
168. Craig,	Chem. Rev.,	1948,	42,	285
169. Edge, Mason & Wylie,	Brit. J. Pharmacol.,	1957,	12,	312
170. Bovet et al.,	Arch. int. Pharmacodyn.,	1949,	80,	172
171. Bovet,	See ref. 22 p. 252.			
172. Funke & Engelor,	Bull. Soc. chim. France,	1950,	17,	340
173. Bovet et al.,	Rend. ist. sup. Sanità,	1949,	12,	81
174. Polonovski, Schmitt & Polon,	Arch. int. Pharmacodyn.,	1953,	94,	127
175. Funke, Krueker & Dapierre,	Compt. rend.,	1950,	231,	498
176. Chakravarty & Jones,	J. Amer. Pharmaceut. Assoc. (Sci. Edn.),	1958,	47,	233
177. Idem.,	ibid.,	1959,	48,	607

178. Holaday & Vmraey,	cited by 22.			
179. Marsh & Herring,	Experientia,	1950,	6,	31
180. Ramos,	Rev. brasil. Biol.,	1961,	21,	65
	through Chem. Abs.,	1962,	56,	1670[f]
181. Cavallito,	See ref. 139, p. 292.			
182. Kalow,	J. Pharmacol.,	1954,	110,	433
183. Kupchan, Yoko Yama & Thyagarajan,	J. Pharm. Sci.,	1961,	50,	164
184. Chabrier, Giudicelli & Kvlstenssen,	Compt. rend.,	1950,	231,	289
185. Collier & Taylor,	Nature,	1949,	164,	491
186. Taylor & Collier,	ibid.,	1950,	165,	602
187. Funke et al.,	Compt. rend.,	1949,	228,	716
188. Fakstorp et al.,	Acta pharm. tox. Kbh.,	1957,	13,	52
189. Fakstorp & Pedersen,	ibid.,	1957,	13,	359
190. Lewis et al.,	J. Pharm. Pharmacol.,	1961,	13,	543
191. Khanna & Dhar,	J. Sci. Ind. Res., India,	1953,	12B,	532
192. Pradhan et al.,	ibid.,	1954,	13B,	122
193. Khanna & Dhar,	ibid.,	1955,	14B,	214
194. Rastogi, Khanna & Dhar,	ibid.,	1956,	15C,	177
195. Ray, Kohli & De,	ibid.,	1957,	16C,	33
196. Maier,	German patent 921, 267.			
197. Collier,	Brit. J. Pharmacol.,	1952,	7,	392
198. Elpern,	J. Amer. Chem. Soc.,	1954,	76,	281
199. Taylor,	J. Chem. Soc.,	1952,	142	
200. Levis, Preat & Dauby,	Arch. int. Pharmacodyn,	1953,	93,	46
201. Mundeleer & Levis,	Acta Anaesth. Belg.,	1952,	3,	110
202. Marszak, Jacob & Guermont,	Compt. rend.,	1953,	237,	1184
203. Thesleff & Umna,	J. Pharmacol.,	1954,	111,	99

204.	Bovet et al.,	Arch. int. Pharmacodyn.,	1951,	88,	1
205.	Hazard et al.,	Arch. int. Pharmacodyn.,	1953,	94,	1
206.	Girod & Häfliger,	Experientia,	1952,	8,	233
207.	Vanecek & Votava,	Physiology Bohemoslovon,	1955,	4,	220
208.	Frey,	Proc. World Congr. Anaesth. (Schevingen)	1955,	262	
209.	Bovet et al.,	Compt. rend.,	1946,	223,	597
210.	idem.,	Arch. int. Pharmacodyn.,	1949,	80,	137
211.	idem.,	Compt. rend.,	1947,	224,	1733
212.	Fisher & Keasling,	J. Amer. Pharmaceut. Assoc., (Sci. Edn.)	1958,	47,	438
213.	Rosnati, Angelini-Kothny & Bovet,	Gazzetta,	1958,	88,	1293
214.	Bulbring & Depierre,	Brit. J. Pharmacol.,	1949,	4,	22
215.	Pelikan & Unna,	J. Pharmacol.,	1952,	104,	354
216.	Winter & Lehman,	J. Pharmacol.,	1950,	100,	489
217.	Hunter,	Anaesthesia,	1953,	8,	90
218.	Randall,	J. Pharmacol.,	1952,	105,	16
219.	Kusovkov et al.,	Doklady Akad. Nauk. S.S.S.R.,	1955,	103,	251
220.	Menshikov et al.,	Russian Patent, 118, 205, 1959 Through Chem. Abs., 1959, 53, 19313[b]			
221.	Long & Schueler,	J. Amer. Pharmaceut. Assoc. (Sci. Edn.)	1954,	43,	79
222.	Longo,	Arch. int. Pharmacodyn.,	1959,	119,	1
223.	Reitzel & Long,	ibid.,	1959,	119,	20
224.	Gardner,	Biochem. J.,	1961,	81,	297
225.	Marshall & Long,	J. Pharmacol.,	1959,	127,	236
226.	Powers, Kruger & Schueler,	J. Pharm. Sci.,	1962,	51,	27
227.	Copp & Hogey,	Nature,	1950,	165,	601
228.	Cavallini et al.,	Arch. int. Pharmacodyn.,	1954,	99,	283

229. Cavallini,	Farmaco,	1955,	10,	644
230. Hazard et al.,	Arch. int. Pharmacodyn,	1954,	96,	462
231. Gesler & Hoppe,	J. Pharmacol.,	1956,	118,	388
232. Biel,	U.S. Patent 2,830,050,	1958.		
233. Gesler et al.,	J. Pharmacol.,	1959,	125,	323
234. Hohmann & Jones,	J. Amer. Pharmaceut Assoc., (Sci. Edn.)	1954,	43,	453
235. Vargha & Kasztreiner,	Chem. Ber.,	1959,	92,	2506
236. Kimura, Unna & Pfeiffer,	J. Pharmacol.,	1949,	95,	149
237. Rosnati,	Gazzetta,	1957,	87,	228
238. Rosnati & Puschner,	ibid.,	1957,	87,	586
239. Scognamiglio,	Rend. ist. super. Sanità,	1959,	22,	48
240.a) Cheymol et al.,	Compt. rend.,	1959,	248,	1723
b) idem.,	Arch. int. Pharmacodyn.,	1960,	125,	121
241. Cavallini & Massarini,	J. Med. Pharm. Chem.,	1959,	1,	365
242. Riker & Vescoe,	Ann. N.Y. Acad. Sci.,	1951,	54,	373
243. Edwards et al.,	J. Pharm. Pharmacol.,	1960,	12,	137T
244. Rice,	J. Amer. Chem. Soc.,	1958,	80,	3207
245. Elworthy,	J. Pharm. Pharmacol.,	1963,	15,	137T and 1964, 16, 375.
246. Bunn & Holmes,	Discuss. Faraday Soc.,	1958,	No.25,	95
247. Bunn,	J. Polymer Sci.,	1955,	16,	323
248. Holmes, Jenden & Taylor,	Nature,	1947,	159,	86
249. Taylor,	Pharmacol. Rev.,	1957,	3,	412
250. Lewis, Martin-Smith & Muir,	Brit. J. Pharmacol.,	1963,	20,	307
251. Ingold,	Structure & Mechanism in Organic Chemistry, G. Bell & Sons, London, 1953. p.70.			
252. Protiva & Fliml,	Coll. Czech. Chem. Comm.,	1953,	18,	636

253. Williams, personal communication.

254. Bruson, Organic Reactions, Vol. V, Ed. by R. Adams et al., John Wiley, New York, 1949, p.79.

255. Huntress, Organic Chlorine Compounds, John Wiley & Sons, New York, 1948, 3:7153.

256. Moureu, Ann. Chimie et de Physique, 1894, 2, 179

257. Kohler, Amer. Chem. J., 1909, 42, 380

258. Maxim, Bull. Soc. Chim. Romania, 1928, 10, 97

259. Beckett, Clitherow & Harper, J. Pharm. Pharmacol., 1963, 15, 349,362

260. Brown & Brown, J. Amer. Chem. Soc., 1962, 84, 2829

261. Elad & Ginsburg, J. Chem. Soc., 1953, 4137

262. Seligsberger & Clayton, J. Amer. Leather Chemists' Assoc., 1958, 53, 90

263. Dorset, Textile Mfr., 1958, 84, 90

264. Ito, Sen-i Gakkaishi, 1960, 16, 381

265. Bock & Houk, U.S. Patent 2,338,681

266. Vaughan, U.S. Patent 2,618,633

267. Bikales & Stephens, U.S. Patent 3,029,232

268. Erickson, J. Amer. Chem. Soc., 1952, 74, 6281

269. Buess, ibid., 1955, 77, 6613

270. Carpenter & Davies, J. Appl. Chem., 1957, 7, 671

271. Stade, Flemming & Glaser, German Patent 842, 194.

272. Kissinger & Schwartz, J. Org. Chem., 1958, 23, 1342

273. Bruson, U.S. Patent 2,484,683

274. Austrian Patent 176,845 Through Chem. Abs. 1954, 48, 10772

275. Herrmann & Vorlaender, Chem. Zentr., 1899, 1, 730

276. Hsia Chen, J. Org. Chem., 1962, 27, 1920

277. Honigberg & Hartung, ibid., 1960, 25, 1822

278. Bauer & Welsh, ibid., 1961, 26, 1443

279. Bergmann, Ginsburg & Pappo, Organic Reactions, Vol. X, Ed. by R. Adams et al., John Wiley and Sons, New York, 1959, p. 179.

280. Kaplan & Glover, J. Amer. Chem. Soc., 1966, 88, 84

281. Ingold, Structure & Mechanism in Organic Chemistry, G. Bell and Sons, London, 1953, p. 690-696.

282. idem., ibid., p. 680-684.

283. Koelsch, J. Amer. Chem. Soc., 1943, 65, 437

284. Luskin et al., J. Amer. Chem. Soc., 1956, 78, 4042

285. Howton, J. Org. Chem., 1945, 10, 277

286. Purdie & Marshall, J. Chem. Soc., 1891, 59, 468

287. Rohberg, Dixon & Fisher, J. Amer. Chem. Soc., 1946, 68, 544

288. idem., ibid., 1947, 69, 2966

289. Dixon, Rohberg & Fisher, ibid., 1948, 70, 37 33

290. Rohberg & Dixon, ibid., 1950, 72, 2205

291. Rohberg & Fisher, ibid., 1944, 66, 1203

292. Rohberg, Faucette & Fisher, ibid., 1944, 66, 1723

293. Rohberg & Faucette, ibid., 1949, 71, 3164

294. Graves, U.S. Patent 2,138,763.

295. Sims, De Bonneville & Kresge, J. Org. Chem., 1957, 22, 787

296. De Bonneville, Luskin & Sims, ibid., 1958, 23, 1355

297. Christian & Hixon, J. Amer. Chem. Soc., 1948, 70, 1333

298. McGregor & Pugh, J. Chem. Soc., 1945, 535

299. Capon,	Quart. Rev.,	1964,	18,	45
300. Chapman & Triggle,	J. Chem. Soc.,	1963,	1385	
301. Hansen,	Acta Chem. Scand.,	1958,	12,	324
302. Zaugg & Horrom,	J. Amer. Chem. Soc.,	1950,	72,	3006
303. Whitmore & Langlois,	ibid.,	1932,	54,	3438
304. Fuson, Bertelli, & Ross,	ibid.,	1932,	54,	4381
305. Terent'ev	J. Gen. Chem. (U.S.S.R.),	1956,	26,	719
306. Payne et al.,	J. Org. Chem.,	1961,	26,	651
307. West,	J. Amer. Chem. Soc.,	1920,	42,	1662
308. Noller,	Organic Synthesis, Collective Vol. II p. 586.			
309. Jones & Powers,	J. Amer. Chem. Soc.,	1924,	46,	2518
310. Gaylord,	Reduction with Complex Metal Hydrides, Interscience, New York, 1956. Chapter 11, p. 643.			
311. Awe & Wiegrebe,	Arch. Pharm.,	1962,	295,	817
312. Latif & Mishriky,	J. Org. Chem.,	1962,	27,	846
313. Bailey & Marktscheffel,	ibid.,	1960,	25,	1797
314. Soffer & Parrotta,	J. Amer. Chem. Soc.,	1954,	76,	3580
315. Soffer, Katz, & Parrotta,	J. Amer. Chem. Soc.,	1956,	78,	6120
316. Letsinger & Pollart,	ibid.,	1956,	78,	6079
317. Ayres & Powwels,	J. Chem. Soc.,	1962,	5025	
318. Amunsden & Nelson,	J. Amer. Chem. Soc.,	1951,	73,	242
319. Snyder & Putnam,	ibid.,	1954,	76,	33
320. idem.,	ibid.,	1954,	76,	1893
321. Gaylord,	Reduction with Complex Metal Hydrides, Interscience, New York, 1956. Chapter 10, p. 544 and p. 807.			

322. Paddock, Nature, 1951, 167, 1070

323. Mey & Arends, (cited by 324), Z. phys. Chem., 1932, B17, 177

324. Gillam & Stern, Electronic Absorption Spectroscopy, Edward Arnold, London, 1957, p. 61.

325. Idem., ibid., Chapter 7, p. 93.

326. Bladon, Physical Methods in Organic Chemistry, Ed. by J.C.P. Schwarz, Oliver & Boyd, Edinburgh, 1964. Chapter 4, p. 126.

327. Barany, Braude & Planka, J. Chem. Soc., 1949, 1898

328. Johnson et al., J. Amer. Chem. Soc., 1956, 78, 430

329. Bowden et al., J. Chem. Soc., 1946, 45

330. Crombie, ibid., 1952, 2997

331. Edwards & Singh, Canad. J. Chem., 1954, 32, 683

332. Mazur, J. Amer. Chem. Soc., 1959, 81, 1454

333. Gladych & Taylor, J. Chem. Soc., 1962, 1481

334. Jones et al., J. Org. Chem., 1944, 9, 506

335. Karrer et al., Helv. Chim. Acta, 1922, 5, 469

336. Clitherow, Personal Communication.

337. Bowman & Stroud, J. Chem. Soc., 1950, 1342 and 1346

338. Burnett et al., J. Amer. Chem. Soc., 1937, 59, 2248

339. Goldfarb, ibid., 1941, 63, 2280

340. Heilbron, Dictionary of Organic Compounds, Vol. I, p. 556.

341. Idem., ibid., Vol. II, p. 180

342. Boone, J. Chem. Soc., 1947, 307

PART II.

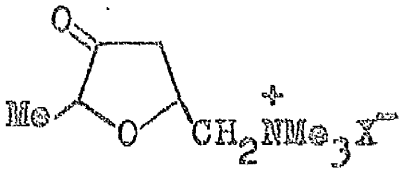
SPECTRAL STUDIES BEARING ON THE POSSIBLE ROLE OF CONFORMATIONAL ISOMERISM IN THE BIOLOGICAL ACTIONS OF ACETYLCHOLINE

INTRODUCTION

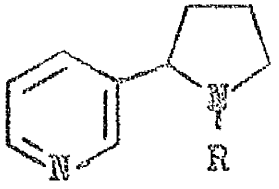
The neurohormone, acetylcholine, appears to play an essentially similar role in the generation of bioelectric potentials in all types of neurons and muscle cells[1] and consequently it is involved in a number of distinct physiological situations. Thus it plays a vital role at the sympathetic and parasympathetic ganglionic synapses,[2-5] at the neuromuscular synapse[6-8] and at those autonomic nerve endings which in consequence have been termed cholinergic.[9-12] It may also play a role at the termination of sensory nerves[13-17] and is involved in the release of adrenaline and noradrenaline from the modified ganglion cells constituting the adrenal medulla.[18,19] More recently it has been shown to be implicated in autonomic adrenergic transmission.[20-22] New emphasis[1,23,24] has been given to older views[25-30] that it may participate in the conduction of nerve impulses along the axons; conversely it has equally recently been argued that the available evidence does not support the theory of a physiological role of acetylcholine in axonal conduction.[31] The occurrence of acetylcholine within the central nervous system suggests yet other roles for this compound but its exact functions in this location are still inconclusively established[32] although it is known to be concerned in the release of antidiuretic hormone from the neurohypophysis[33-38] and to have a transmitter role at the Renshaw cells in the spinal cord.[39] Further speculations concerning the biological significance of acetylcholine are to be found in the various comprehensive reviews concerned with the many drugs which exert their effects by mimicking or antagonising the neurohormone.[40-47] Yet with some exceptions, at normal dose levels, drugs mimicking or antagonising the actions of acetylcholine tend to do so at a limited number of sites only, thus giving

rise to the well accepted pharmacological classifications as "nicotinic" or "muscarinic" agents in the case of the mimetics, and as ganglion-blocking agents, neuromuscular blocking agents or anti-spasmodic drugs in the case of the antagonists. In part this relative selectivity with respect to site of action may have its origin in permeability factors - absence of lipid barriers at the neuromuscular synapse[47a] explaining the ready access to this site of polyonium salts which are normally unable to penetrate such barriers.[48-52] Certainly the central actions of neuromuscular blocking agents on intrathecal, intracisternal or intraventricular injection[53] serve to make apparent the role of the "blood-brain barrier "where these compounds are administered by other routes, while studies with nerve fibre preparations lacking protective lipids show these agents to be capable of exhibiting activity at sites not affected in the intact animal.[31,51,52,54,55] Consideration of permeability factors alone, however, fails to provide a full explanation as to why atropine-like drugs show little or no action at "nicotinic" sites (especially in view of the activity of atropine at certain ganglia which has led to a distinction between "muscarinic" and "nicotinic" receptors within the ganglion[56,57]), or why local anaesthetics which appear to occupy certain acetylcholine[58] receptors exhibit no activity at normal "muscarinic" and "nicotinic" receptors. Other factors are obviously involved, and that the conformational flexibility of the acetylcholine molecule could make possible the existence of several distinct types of acetylcholine receptor has been implied by several workers.[59-61] This would provide a fundamental basis for the observed selectivities of the different groups of acetylcholine mimetics and acetylcholine

antagonists. Furthermore, such a situation would not be inconsistent with the somewhat different pictures of the "muscarinic" and "nicotinic" receptors emerging from other considerations[45,62-74] especially since "nicotinic" activity, in contrast to "muscarinic" activity[74-78] does not appear to be greatly influenced by changes in stereochemistry. This is illustrated by the high "nicotinic" potency present in both enantiomorphs of compounds such as muscarone[70] (I),



(I)



(III)

$$Me_3\overset{+}{N}CH_2CH_2OCOCH(OH)CH_3 \quad X^-$$

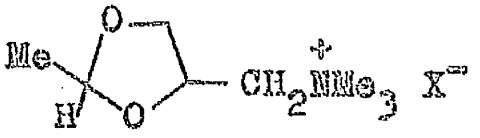
(II)

lactoylcholine[79] (II) and nicotine(III;R=Me) or nornicotine[80,81] (III;R=H), and by the retention of activity in acylcholines where the acid radical has considerable steric bulk.[82,83] Similarly the conformational flexibility of the acetylcholine molecule could rationalise the apparent differences between these "muscarinic" and "nicotinic" receptors and the acetylcholinesterase surface.[84-91] On the other hand recent evidence would suggest near identity of the two proteins considered to represent the muscarinic receptor and the enzyme acetylcholinesterase.[92,93]

Unfortunately detailed consideration of the molecular

features of the many drugs now known which act as mimetics or antagonists of acetylcholine sheds little light on the possible biological importance of conformational isomerism in the neurohormone for three main reasons. 1. The majority of these drugs are themselves conformationally flexible and there is no way of rigorously establishing the different situations which can presumably exist — such as where precise mimickry of only a single biologically - significant acetylcholine conformer is possible on account of unfavourable non-bonded interactions within the drug molecule or between the drug molecule and acetylcholine receptors at which no action occurs, or where subtle variations in the case of conformational interconversion within a series of drugs has significant effects on their relative selectivity of site of action. 2. Since there is usually no clear distinction between the molecular features primarily determining the binding of a drug to a receptor (affinity) and those determining its ability to produce the stimulus necessary to evoke a positive biological response when receptor occupation has been achieved (intrinsic activity)[94], it is difficult to assess the significance of conformational phenomena in drugs mimicking the actions of acetylcholine against the significance of conformational phenomena in drugs antagonising acetylcholine at a given site. However it would seem in the case of the antagonists that shielding of the receptor, rather than an exact fit with it, could be the significant factor. Thus many antispasmodic drugs possess somewhat bulky molecules and increase in the size of the cationic substituents of depolarising neuromuscular blocking agents (which would be expected to sterically hinder approach to the receptor) is accompanied by a change to non-depolarising activity. Recently the attractive

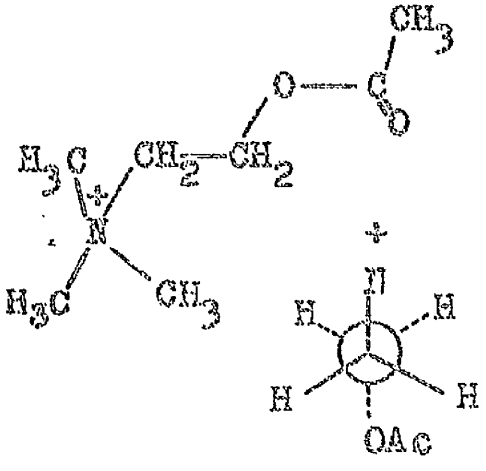
hypothesis has been advanced[93] that affinity may be ascribed to hydrophobic forces serving to transfer a drug from the aqueous phase to non-polar sites on the receptor protein whilst intrinsic activity can be regarded as a reflection of the degree to which specific conformational perturbations in the tertiary structure of the receptor protein, requiring the intercession of highly specific van der Waals' forces, are possible. The operation of hydrophobic forces alone while affording non-specific conformational perturbations in the tertiary structure of the protein will not initiate a physiological stimulus and in this situation a drug can only exhibit antagonistic activity. Where specific perturbations produced by a drug are favourable to the initiation of a physiological stimulus, agonistic activity will result, the degree of which will depend upon the precise favourability of the specific perturbations. The phenomenon of ambivalence, as shown by heptyltrimethylammonium iodide, may be rationalised by assuming that these molecules induce the production of equilibrium mixtures of receptor protein transformed by specific and non-specific conformational perturbations. It is to be noted that in the 1,3 - dioxolane analogues (eg. IIIA) of acetylcholine upon which Belleau[93] bases his deductions concerning the conformations adopted by acetylcholine at the muscarinic receptor and during complex formation with acetylcholinesterase, only the equivalent of the $CH_3C(=O)-O-CH_2$ fragment of the acetyl-choline molecule is locked in a rigid conformation and free rotations about

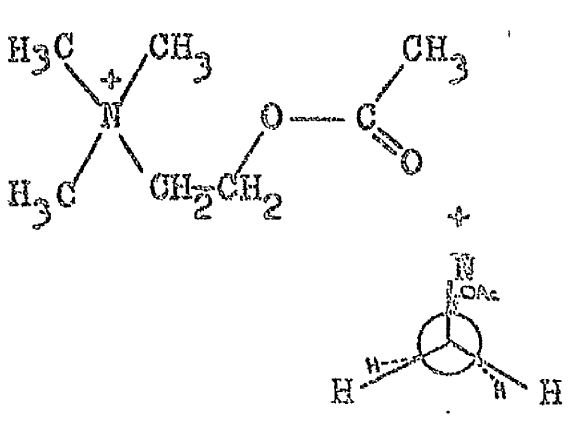


(IIIA)

the remaining bonds still permits of conformational heterogeneity. Thus the 2-methyl-4-trimethylammonium-methyl-1,3-dioxolane iodides, of which the cis-L-(+)-isomer shows maximal cholinomimetic potency on the guinea pig ileum and a high affinity for acetylcholinesterase, can exist in conformations corresponding to IV and V with respect to the $Me_3\overset{+}{N}CH_2CHO$-moiety. 3. The activity displayed by a compound such as tetramethylammonium, which formally represents a portion of the acetylcholine molecule divorced from conformational significance with respect to the acetoxy-ethyl moiety, indicates that conformational isomerism within this radical cannot exert a crucial influence at all acetylcholine receptors. This raises serious doubts as to its exact significance at other centres, and it would therefore seem necessary to look elsewhere for evidence bearing on the possible role of conformational isomerism in the biological actions of acetylcholine.

X-ray studies on crystalline acetylcholine bromide[95] have shown that in the solid state acetylcholine exists in two favoured conformations — the not unexpected fully staggered conformation (IV) and the quasi-ring conformation (V). This work, however, has recently been criticised and the X-ray data are currently being reinvestigated.[95a] The stability of the latter conformation has been attributed to intramolecular N-C-H·····O hydrogen bonding.[96,97,98]

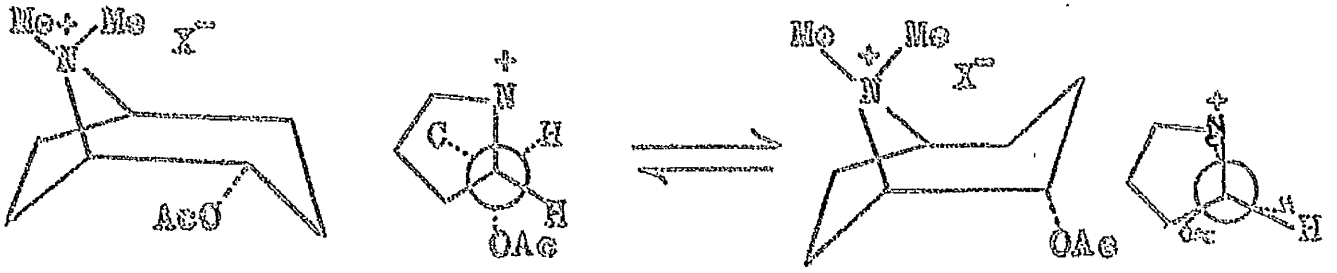




(IV) (V)

The existence of two conformations for the acetylcholine cation has given rise to conjecture[60,61,95] that these two conformers may separately be of biological significance. Should the X-ray evidence for the quasi-ring form of acetylcholine prove unfounded the following discussion is not invalidated since muscarine in the solid state exists solely in an analogous quasi-ring form (X).

Archer, Lands and Lewis[61] have recently advanced the hypothesis that "nicotinic" activity depends on conformation (V) of the acetylcholine molecule and "muscarinic" activity on conformation (IV) of the acetylcholine molecule since the two 2-acetoxytropine methiodides (VI and VII) showed the appropriate though weak acetylcholine - like actions. This hypothesis would



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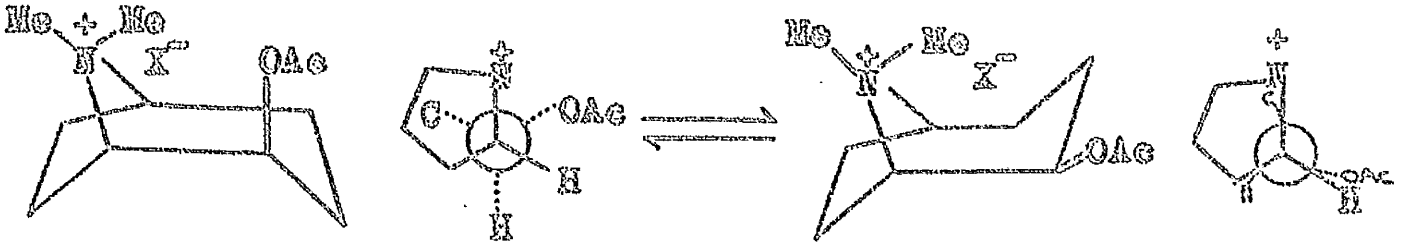
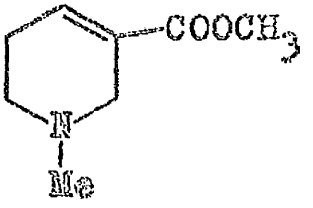
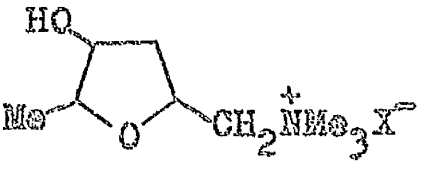


(VII)

also accomodate the pronounced muscarinic properties of arecoline (VIII) which can assume a conformation in which



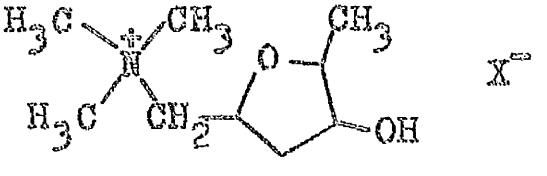
(VIII)



(IX)

the acyloxy oxygen atom and the tertiary nitrogen atom are spatially disposed in a manner somewhat akin to the corresponding functional groups in conformation (IV) of acetylcholine. Arecoline, however also exhibits significant nicotine-like actions.

The fact that muscarine (IX) in the solid state exists solely in the quasi-ring conformation[99] (X) which again involves a short N-C-H---O bond might suggest that conformation V of the acetylcholine molecule, rather than the fully staggered conformation IV, is more



(X)

likely to be the "muscarinic" conformation. Furthermore, should the molecules of both acetylcholine and nicotine have a common two-point attachment at the nicotinic

receptors, evidence is available that conformation IV of the acetylcholine molecule may be the nicotinic conformation since examination of molecular models of nicotine shows that this compound can adopt a conformation having certain features in common with IV but that it cannot adopt a conformation at all closely related to V. Thus, in one of the two conformations in which the pyridine and pyrollidine rings are co-planar, the inter-nitrogen distance of 3.85A° coincides exactly with the distance in IV, between the nitrogen atom and the acetoxyl oxygen atom (Figure 1). Since

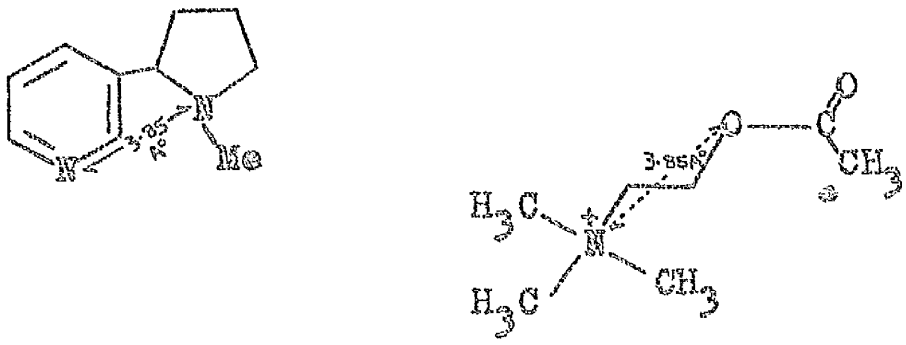
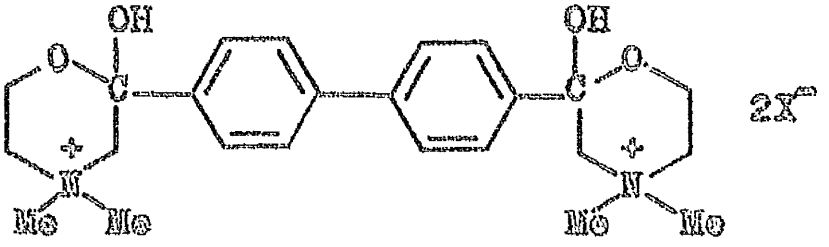


Figure 1.

Comparison of inter-nitrogen distance in nicotine with N-O (acyloxy) distance in the extended conformation of acetylcholine.

the pyrollidine nitrogen atom of nicotine is generally assumed to be in cationic form at physiological pH[100-102] and the pyridine nitrogen atom can be regarded as being somewhat analogous electronically to the acetoxy oxygen atom in acetylcholine, the purely nicotinic actions of nicotine are fully rationalised in terms of a receptor accepting conformation IV of acetylcholine, provided both molecules share a common two point attachment involving the atoms just indicated. It is perhaps of some interest that the homicholiniums [103a](XI), which

possess morpholine rings in which the nitrogen and oxygen atoms are constrained in a steric relationship akin to that pertaining in conformation V of acetylcholine do not act at the myoneural synaptic receptors in the same manner as other muscle relaxants, but exert their effect by preventing



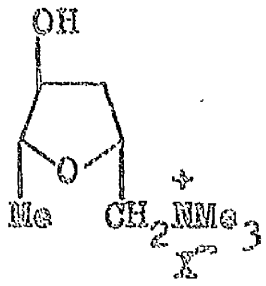
(XI)

the synthesis of acetylcholine[103] and this could perhaps be taken as evidence that conformation V is not accepted by the "nicotinic" receptors. Similarly a number of morpholine and isoxazolidine compounds synthesised by Eugster[104] exhibit high muscarinic activity[45] although in this case the situation is complicated by the simultaneous presence of nicotinic activity.

That a three-point or multipoint attachment is not involved in production of an acetylcholine-like response at the nicotinic receptors would seem to be strongly indicated by the high "nicotinic" activity of both enantiomorphs of lactoylcholine, muscarone, nicotine and nor-nicotine (page133). This clearly demonstrates that the three-point projected asymmetry associated with optical isomerism is of little significance at nicotinic sites (compare Beckett[105]). Moreover it is impossible for the muscarones to achieve a three-point correspondence with any conformation of acetylcholine as is apparent from

inspection of molecular models.

Conformational isomerism in the acetylcholine molecule may indeed be an important factor in determining its ease of access to a sterically protected anionic receptor where only a one-point attachment is involved, but the operation of such a one-point attachment does not explain the interpretation of "nicotinic" activity in other molecules, through the absence of a second point of reference. However, the muscarinic receptor, in contrast to the "nicotinic" receptor, is obviously highly stereoselective as evidenced by the pronounced differences in activity of L(+)- and D(-)- muscarine(XII);[75,77] of the enantiomorphs of acetyl-β-methylcholine (XIII)[76,106,107] of the different stereoisomers of muscarine (XII-XIIC), 4,5-dehydromuscarine[78,115] (XIV) and 2-methyl-4-trimethylammonium-methyl-1,3-dioxolane iodide[72,74] (XV) and by the marked effect of the size of the acyl group of the acylcholines upon affinity for the muscarinic receptor.[10,109] The activity of the n-butyl-



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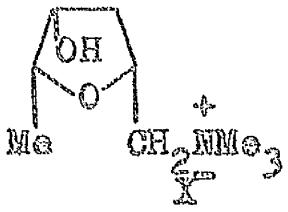

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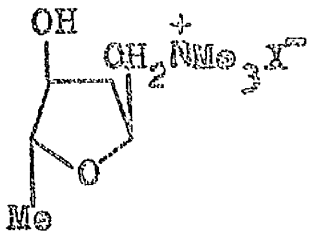

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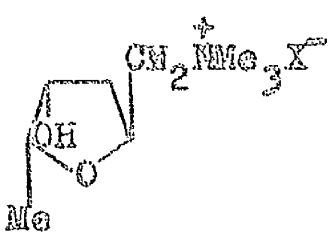
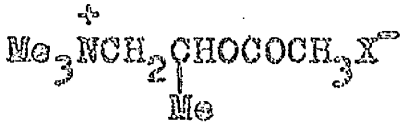


(XIIC)



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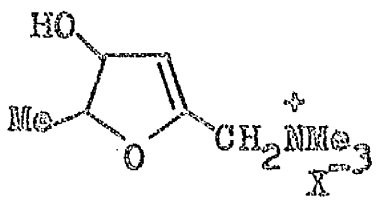

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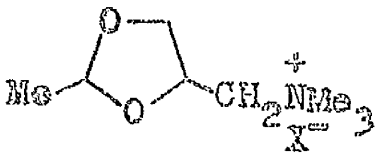
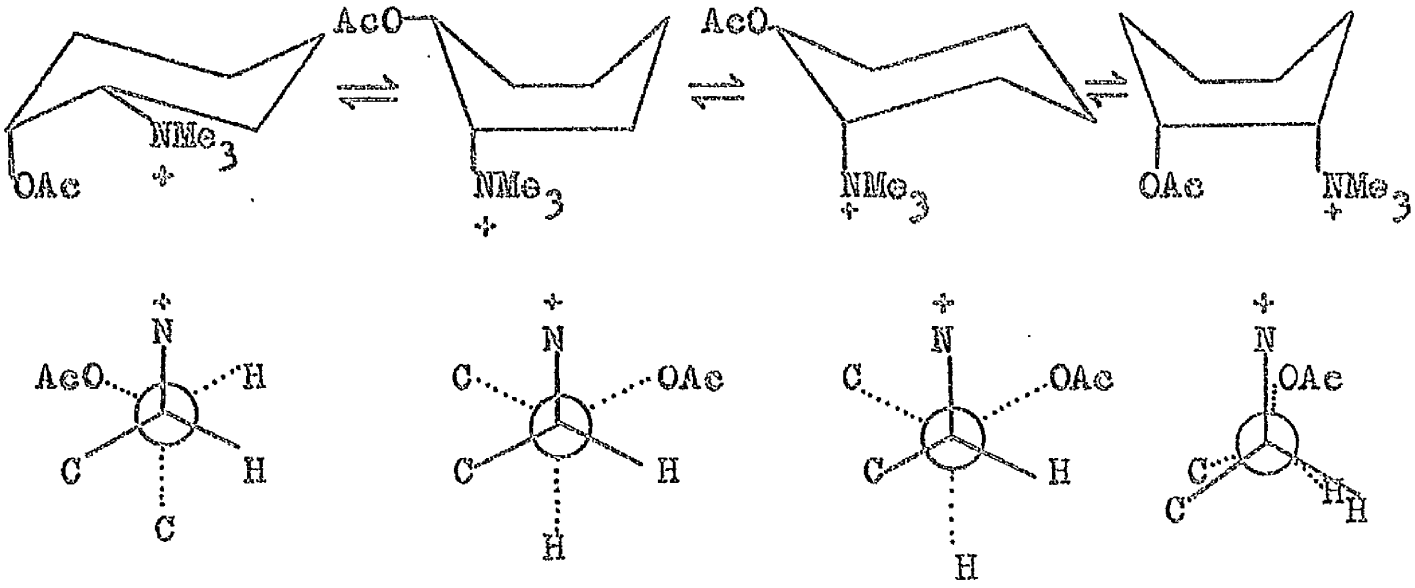


(XV)

trimethylammonium ion and the n-amyltrimethylammonium ion on certain heart preparations[65,65a] represents something of an enigma but could result from coiling of the C4 and C5 alkyl substituents with decrease in volume. The stereoselectivity of the muscarinic site has been further emphasised in various attempts to give a pictorial representation of the receptor[45,73,74,76] but this in no way invalidates any conformational considerations.

It would appear therefore that a comprehensive study of conformationally-rigid acetylcholine-like molecules in which the quaternary nitrogen atom and the acetoxyl group are spatially held in mimickry of IV and V with respect to "muscarinic" activity is more likely to yield definite information concerning the role of conformational isomerism in determining the biological actions of acetylcholine than is any study of "nicotinic activity" although the situation is complicated by the possibility that two acetylcholine molecules might react with one receptor.[91] The appropriate absence of nicotinic actions might give supporting evidence - provided that unfavourable solubility, permeability, transport or biotransformation do not prevent such drugs reaching the receptors, and provided that Gill's contention[110] that conformationally rigid molecules will prove inactive is unfounded.

Few such rigid molecules have so far been examined. The 2-acetoxytropine methiodides (VI and VII) examined by Archer, Lands and Lewis[61] are conformationally non-rigid as are the cyclohexane derivatives[111] XVI and XVII



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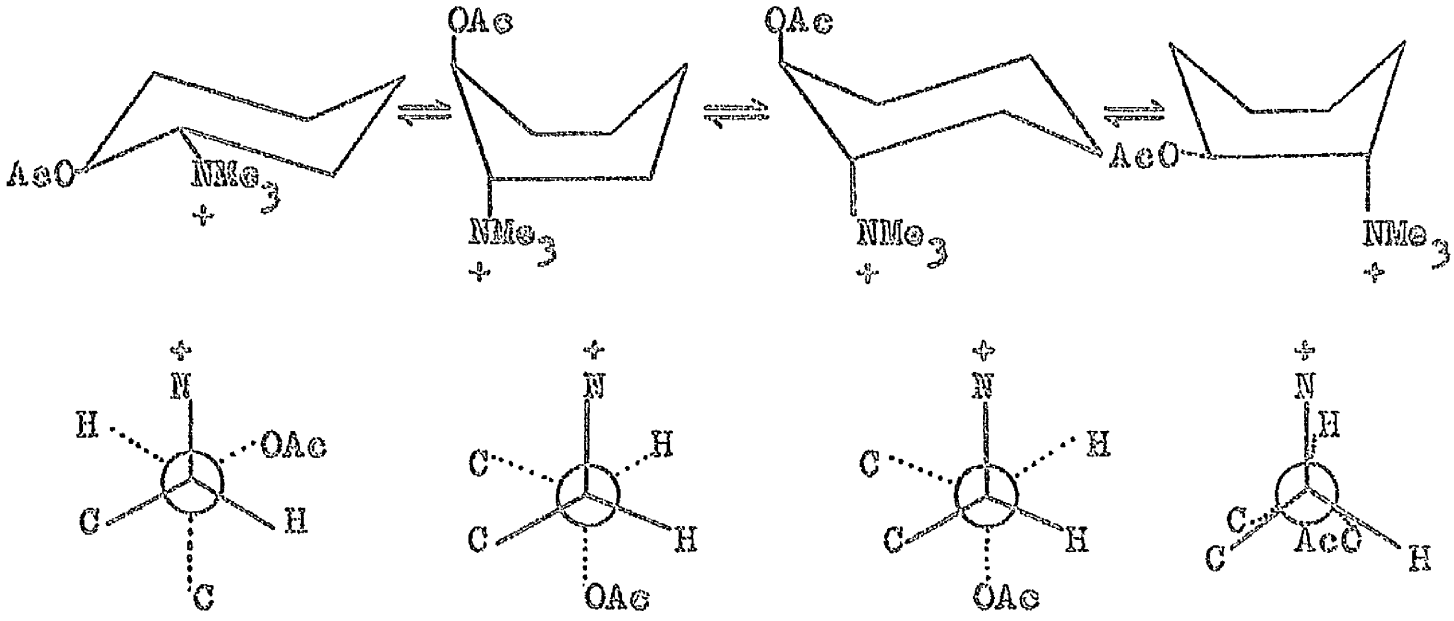
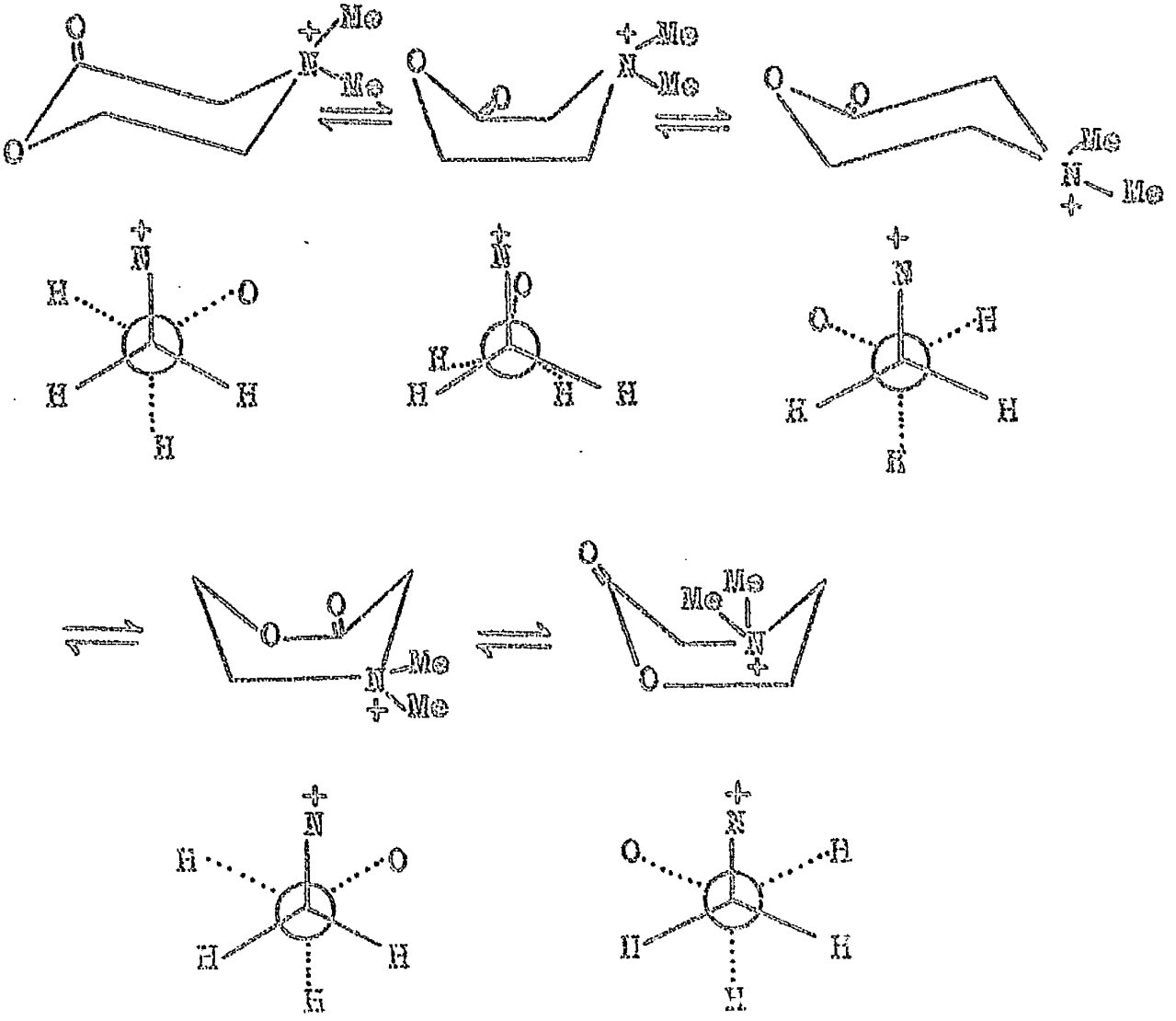


(XVII)

and the cyclic derivatives[59] XVIII and XIX. These compounds may not necessarily complex with the receptors in the thermodynamically most stable conformation. Unfortunately compounds XVI and XVII, although tested

for their ability to function as substrates of acetylcholinesterase and for their activity on the kitten phrenic nerve diaphragm preparation[112] have not been investigated for muscarinic actions.[113] Compounds XVIII and XIX both show comparable muscarinic activity.[59] The cyclopentane derivatives[86] XX and XXI are more nearly rigid as the cyclopentane ring permits only of limited conformational isomerism[114-116] but in these compounds none of the possible conformers with their partial eclipsing can be expected to exactly correspond to IV although XX is very close to V.



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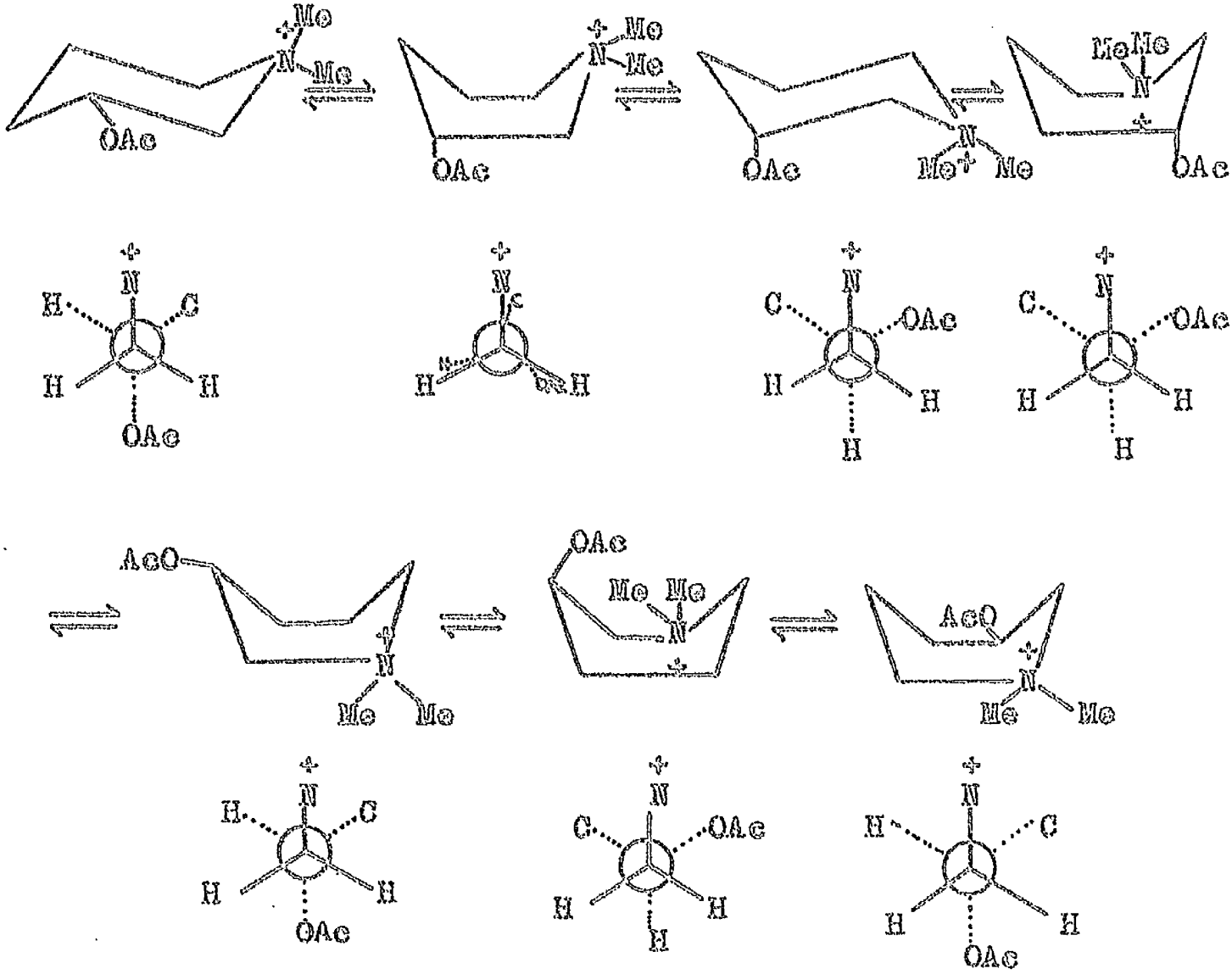

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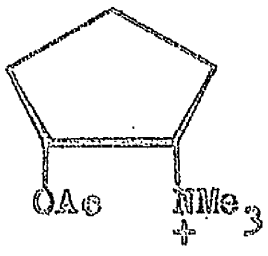

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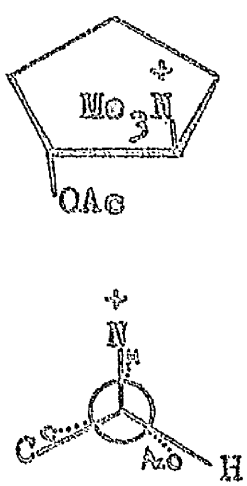
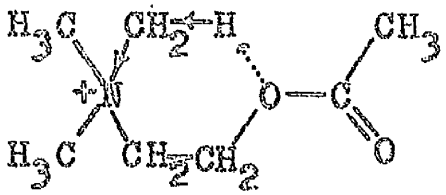


(XXI)

Further support that IV may be the "nicotinic" conformation and V the "muscarinic" conformation of acetylcholine is perhaps available if it could be conclusively established that the stability of the quasi-ring conformations V and X has its origin either in intramolecular coulombic attraction between the quaternary nitrogen and the ether oxygen or in intramolecular N-C-H---O hydrogen bonding as suggested by Sutor.[96,97] These factors all of which tend to reduce the electron density on the ether oxygen atom (or its equivalent) — and hence also inhibit hydrogen bonding — would appear to favour "nicotinic" properties, whilst factors tending to increase electron density at this point, and so favour quasi-ring formation would appear to favour "muscarinic" activity.

The proposed N-C-H---O bonding could perhaps be rationalised in terms of the inductive effects shown in XXII. The primary C---N inductive effect is unexceptional and is even paralleled in amines other than quaternary salts where electronegativity differences alone are operating, as evidenced by the increase in nucleophilicity of the nitrogen atom on replacement of hydrogen atoms

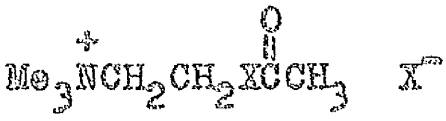


(XXII)

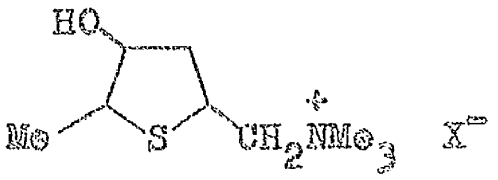
in the ammonia molecule by one and then two methyl groups.[117] The weakening of the NC-H bond responsible for the hydrogen bonding, would also seem a normal phenomenon in N-methyl compounds as is perhaps evidenced by the low N-methyl C-H stretching frequency in the infrared spectrum of these compounds[118] and by analogy with ylide formation.[119]

Since sulphur and selenium are less electronegative

than oxygen[120] and do not as readily enter hydrogen bonding, or possess the same high point electron charge density as does oxygen it is instructive to compare the acetylcholine - like properties of the sulphur and selenium isosteres of such compounds as acetylcholine,[19] acetyl-β-methylcholine and muscarine, in which quasi-ring conformations analogous to V and X are less likely to be favoured, with those of their prototypes. Indeed acetylthiocholine (XXIII, X=S) and acetylselenocholine (XXIII, X=Se) are reported to be weaker "muscarinic" agents and stronger "nicotinic" agents than acetylcholine[121,122] whilst thiomuscarine (XXIV) gives rise to a decrease in "muscarinic" activity without the production of any marked "nicotinic" properties.[45] In the latter case steric hindrance akin to

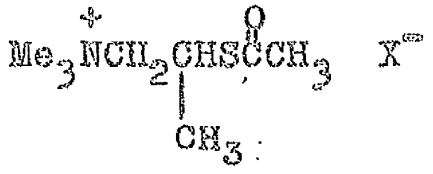


(XXIII)

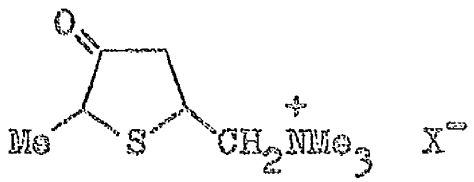


(XXIV)

that proposed for acetyl-β-methylcholine would readily explain the absence of "nicotinic" potency, although anomalies exist in acetyl-β-methylthiocholine[121] (XXV) and in thiomuscarone[45,123] (XXVI) which show nicotinic activity. These considerations then,

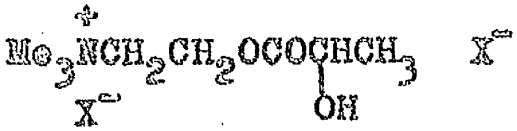


(XXV)

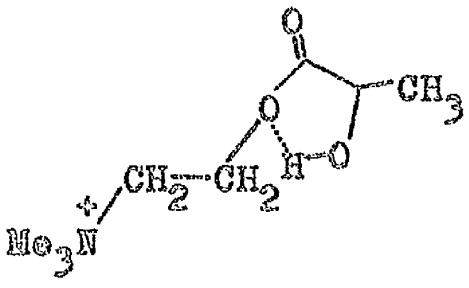


(XXVI)

might suggest that conformation IV could be the nicotinic conformer of acetylcholine, a view reinforced by the virtual absence of muscarinic activity[79] in the (+)- and (-)- lactoylcholines (XXVII) where hydrogen bonding from the hydroxyl group to the acyloxy oxygen atom (XXVIIA) would stabilise the extended conformer. Further instances



(XXVII)

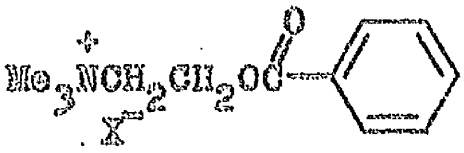


(XXVIIA)

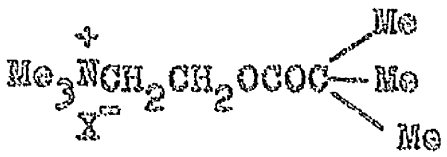
of expected destabilisation of quasi-ring conformations akin to V may be sought in compounds where the thermodynamically favoured 6-membered ring present in XXII is not possible or where the electron density on the acyloxy oxygen atom is decreased. The former situation is encountered in higher homologues of acetylcholine possessing more than two methylene groups between the trimethylammonium group and the acyloxy function and indeed such compounds show markedly reduced "muscarinic" properties.[124]

With respect to the second situation it is of some interest that stress has been laid by other workers[73] upon the importance of the ether oxygen of the muscarine molecule in terms of a primary binding site in the receptor, whereas the function of this atom with its high electron density could in fact be more concerned in conformational stabilisation. Decreased electron density on this atom with consequent increase in favourability of an open chain conformer would be expected in benzoylcholine (XXVIII) where the benzene ring can act as an electron sink and this compound in fact shows little or no "muscarinic" activity.[82]

although the concurrent operation



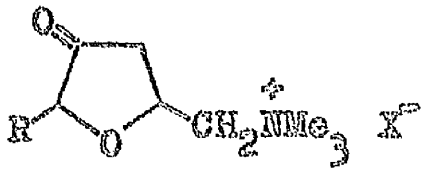
(XXVIII)



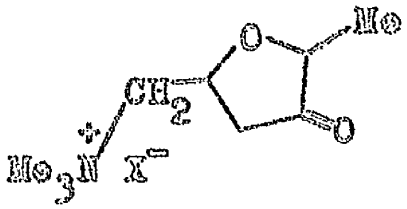
(XXIX)

of steric factors can not be overlooked as is clear from the pronounced "nicotinic" properties of trimethylacetylcholine[83] (XXIX) where the inductive effect is in the opposite direction.

The appearance of "nicotinic" as well as "muscarinic" activity in the muscarones (XXX, R=CH_3) can perhaps also

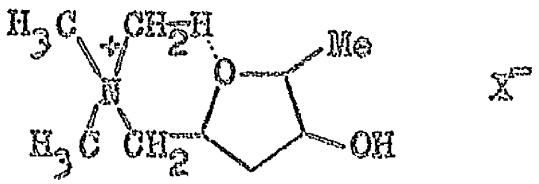


(XXX)

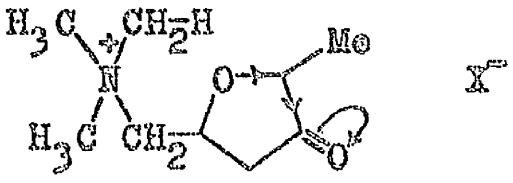


(XXXA)

be attributed to an increased favourability of the extended conformation as compared to the situation with muscarine and it will be interesting to learn whether an X-ray study of crystalline muscarone iodide (XXX, R=CH_3) will demonstrate the existence of such a conformation (XXXA) rather than the sole presence of a quasi-ring conformation as is characteristic of muscarine iodide (X). If indeed stabilisation of the quasi-ring conformation of muscarine is due to intramolecular N-C-H---O bonding[96,97] (XXXI) then the polarisation of

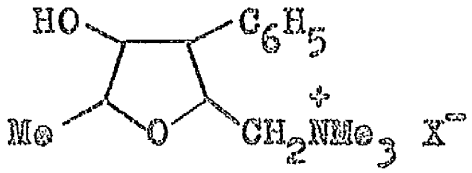


(XXXI)



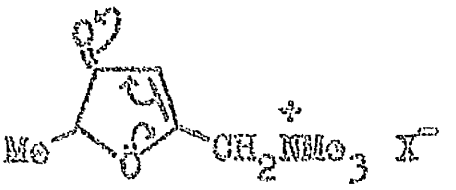
(XXXIA)

the oxo group could perhaps destabilise the quasi-ring form through relayed inductive effects (XXXIA), but complexities are introduced by the possibility of enol formation.[45] Similar relayed inductive effects might explain the dual "nicotinic" and "muscarinic" properties of 3-phenylmuscarine (XXXII), in which a delicate balance between the extended and quasi-ring conformers could be envisaged, but once again such



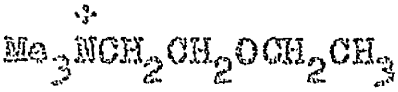
(XXXII)

electronic considerations can not be divorced from steric arguments.[45,73,74] In the case of 4,5- dehydromuscarine (XIV) resonance between the π electrons of the double bond and the p electrons of the furan oxygen would serve to lower the electron density on the latter, again explaining the dual "nicotinic" and "muscarinic" properties in terms of the coexistence of an extended conformer and a quasi-ring conformer. The extended conjugation present in 4,5-dehydromusearone (XXXIII) would presumably have a similar effect.

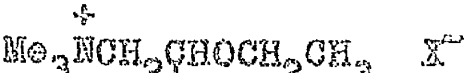


(XXXIII)

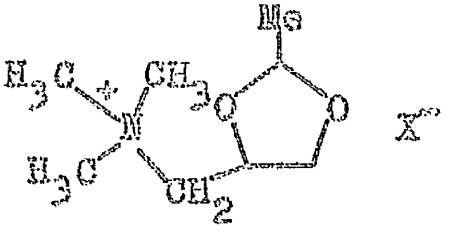
On the other hand, factors tending to increase the electron density of the acyloxy oxygen atom of acetylcholine should favour the quasi-ring conformation V, if it is due to coulombic attractions or hydrogen bonding of the types discussed. Such a situation would be expected to pertain in choline ethyl ether[125] (XXXIV) and β-methylcholine ethyl ether[126] (XXXV) which are potent "muscarinic" agents and in ketals[72,127] (eg. XXXVI) many of which are also potent "muscarinic" agents.



(XXXIV)



(XXXV)



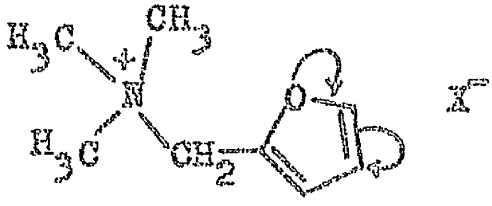
(XXXVI)

This is in contrast to the aryl ethers of choline (XXXVII, R=aryl) in which the electron density on the acyloxy oxygen atom will be decreased and which are well established as "nicotinic" agents.[128-130]

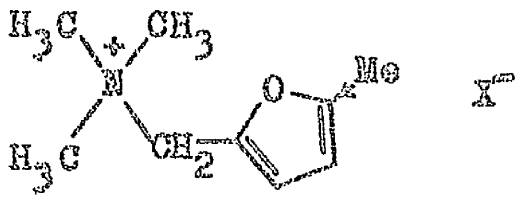
$Me_3\overset{+}{N}CH_2CH_2OR$

(XXXVII)

If the stability of quasi-ring conformations akin to V is crucially influenced by quite minor changes in the electron density of the ether oxygen atom it becomes possible to reinterpret the Five-Atom Rule[63,131,132] of "muscarinic" activity. For instance, as Beckett and others[76] have proposed, the pronounced differences in "muscarinic" potency between furfuryltrimethylammonium (XXXVIII) and 5- methylfurfuryltrimethylammonium (XXXIX) could be explained on the grounds that the inductive effect of the 5-methyl substituent in the latter may induce a crucial restoration of electron density on the furan oxygen[132a] which in the former will be depleted through resonance interaction with the furan ring. The net result is restoration of the stability of the quasi-ring conformation.



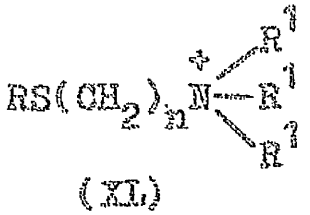
(XXXVIII)



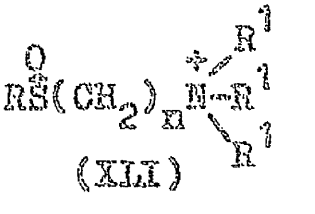
(XXXIX)

The "muscarinic" properties exhibited by a series of ω-alkylmercaptopolymethylene quaternary ammonium salts[133] (XL;R^1=Me) might also be a reflection of the existence of a higher electron density on the sulphur atom than is possible on the sulphur atom of thioesters or even on the ether oxygen of esters because of the influence of the adjacent carbonyl group. Under these circumstances a quasi-ring conformation might again be expected to be

favoured. Further support is given by the fact that the corresponding sulphoxidee[133] (XLI;R^1=Me) are relatively inactive.



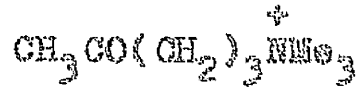
(XL)



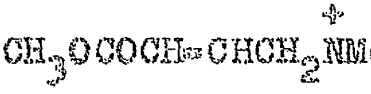
(XLI)

However, the fact that the most active member of the series was 3-(methylmercapto)-propyltrimethylammonium iodide (XL;R=R^1= CH_3, n =3) appears to indicate that in this case a quasi five-membered ring was formed by coulombic attractions rather than a quasi seven-membered ring being formed through hydrogen bonding.

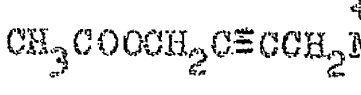
Certain complications to the interpretation of "muscarinic" and "nicotinic" properties in terms of conformational analysis of the acetylcholine mimetics are associated with the accomodation of the potent "nicotinic" properties of such chemically diverse compounds as the 4-oxoalkyltrimethylammonium salts[134,135] (eg XLII), and various double[136,137] (eg. XLIII) and triple bonded[137] (eg XLIV) quaternary ammonium salts in terms of a common two-point



(XLII)



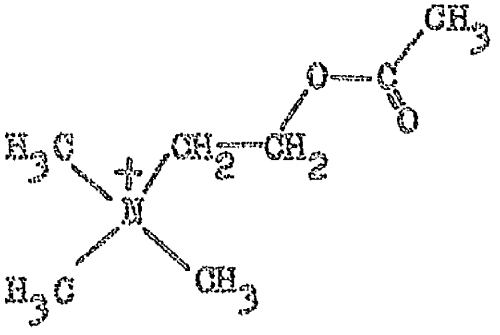
(XLIII)



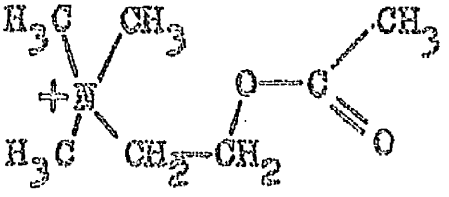
(XLIV)

attachment to the receptor, although the possibility that different two-point attachments involving alternative subsidiary sites of interaction between drug and receptor can not be ruled out. Examination of models shows that a *cis* arrangement of the acetoxy and methylene ammonium groups about the double bond of XLIII does permit a conformation somewhat akin to the quasi-ring form of acetylcholine; a *trans* configuration, on the other hand, does not.

Although there is no *a priori* reason for assuming that the fully staggered conformation (XLV) and the quasi-ring conformation (XLVI) of the acetylcholine molecule are actually involved at the receptors, the supposition that they could be would be considerably strengthened if it were demonstrated that both are simultaneously present in solution as well as in the solid state.



(XLV)



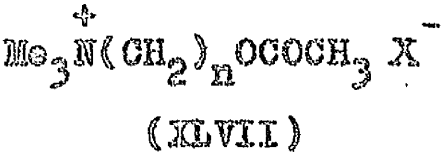
(XLVI)

RESULTS AND DISCUSSION

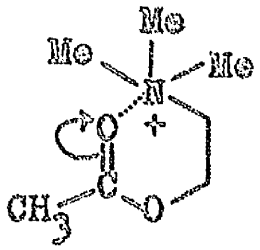
PUBLISHED AND DISTRIBUTED

INTRODUCTION

Fellman and Fujita[138] interpreted the results of infrared and kinetic studies with the homologous series of acetylcholine analogues (XLVII; n=2,3 and 4) as providing support for the existence in solution of conformation XLVIII of the acetylcholine molecule, the cyclic character being ascribed to

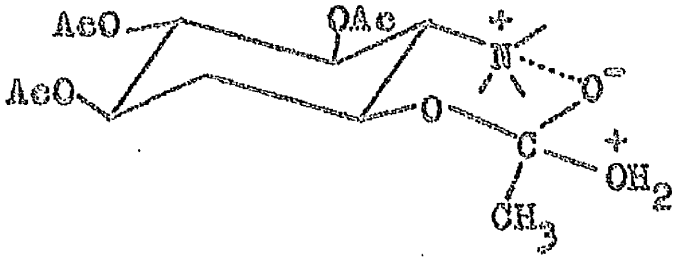


(XLVII)

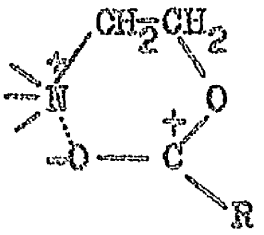


(XLVIII)

an electrostatic attraction between the polarised carbonyl group and the quaternary nitrogen atom. Indeed the fact that of the homologous series acetylcholine shows the fastest rate of acylation of hydroxylamine in basic aqueous solution would be consistent with XLVIII in view of the facilitation of nucleophilic attack at the carbonyl carbon atom through the polar effects shown if it can be assumed that analogous quasi-ring conformations for the higher members of the series are improbable on steric grounds - the favoured nature of six-membered quasi-rings being well established. The inductive effects present in conformation XLVIII likewise fully explain the observation that on comparison with simple esters the hydrolysis of acetylcholine is slower in acid solution but faster in basic solution. Indeed very similar cyclic intermediates have been postulated to explain the labilisation of ester bonds in aminocyclitol derivatives[139] (eg. XLIX) and to explain the facilitated hydrolysis of the quaternary salts derived from aryl-2-halogenoalkylamine esters[140] (eg. L).



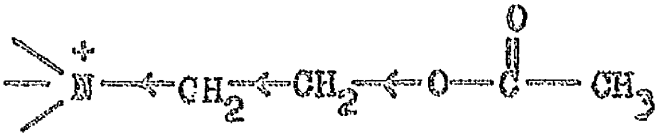
(XLIX)



(L)

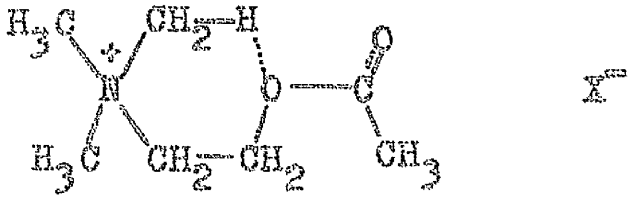
The infrared measurements of Fellman and Fujita, were, however, wrongly interpreted. Thus the well resolved split carbonyl absorptions of the homologues (XLVII, n=3 and 4) occurred at lower frequency than the corresponding carbonyl absorption of acetylcholine (XLVII, n=2). Conformation XLVIII with its decreased carbonyl double bond character as compared to its higher homologues not existing in analogous quasi-ring conformations would require that acetylcholine absorb at the lowest frequency of the series since a lower bond order gives rise to a lower absorption frequency.[158]

Fellman and Fujita[141] in a later publication, however, indicated that the shift toward higher energy of the carbonyl absorption observed in the infrared was indeed evidence for a decrease in the polarisation of the carbonyl group which would argue against the proposed cyclic conformation (XLVIII). It was concluded that the high carbonyl stretching frequency and the high electrophilicity of the ester carbonyl carbon atom in acetylcholine are best interpreted in terms of the influence of the inductive effect from the quaternary ammonium group (LI).



(LI)

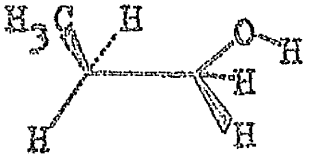
The infrared results of Fellman and Fujita[138,141] are not, however, incompatible with the existence of conformation XLVI of the acetylcholine molecule provided that the stability of this conformation does result from intramolecular N-C-H---O hydrogen bonding (LIIA). The inductive effect of the acyloxy oxygen atom would serve to depress the permanent polarisation of the carbonyl group thus increasing its double bond character with consequent rise in absorption frequency as compared with the higher homologues of acetylcholine. Indeed, intramolecular bonding to the alcoholic oxygen atom of esters is well known[142-145] to raise the carbonyl absorption frequency. The kinetic results on the rate of acylation of hydroxylamine in basic aqueous solution are equally well accomodated by LIIA in which nucleophilic attack at the carbonyl carbon atom is also facilitated by electron withdrawal via the alcoholic oxygen atom.



(LIIA)

Hydrogen bonding involving C-H groups as proton donors is not without parallel and considerable evidence is available for the existence of intramolecular C-H---O bonds in the crystal structures of a number of compounds other than acetylcholine.[96,97] Hydrogen bonding involving the C-H group has been suspected in other instances[146,147] and the various aspects have been reviewed.[148] A recent infrared survey[149] has shown that the ability of C-H groups to act as proton donors in intermolecular hydrogen bonds depends upon the hybridisation of the carbon atom

($sp > sp^2 > sp^3$) and increases with the number of electron withdrawing groups. It was concluded that the C-H groups of an sp^3-hybridised carbon atom will only display proton donor propensities in those instances where that carbon atom is attached to at least two strongly activating groups. At variance with these generalisations and more pertinent to the present situation is the recent infrared study by Krueger and Mettee[150] of the conformational heterogeneity of propan-1-ol (LIII) in dilute carbon tetrachloride solution; evidence is adduced for the existence of a weak intramolecular C-H---O bond by interaction of



(LIII)

the C-H of the methyl group with one of the oxygen lone-pair orbitals. The favourable geometry of the n-propanol molecule is considered to be responsible for the detection of the C-H---O bond. In the quasi-ring conformation of acetylcholine (XLVI) analogous features pertain in the favourable disposition for C-H---O bonding of one of the N-methyl groups and the acyloxy oxygen atom. This, together with the reinforcing effect of an electron withdrawing substituent, namely the quaternary nitrogen atom, attached to the carbon atom lends credence to the stabilisation of conformation XLVI by the proposed intramolecular mechanism.

Unfortunately however, although the infrared results are consistent with conformation LIIA, they by no means afford unequivocal proof of its existence in solution.

Dubieties arise from the fact that Fellman and Fujita[138,141] took their infrared measurements in ethanolic solution giving rise to difficulty in determining the exact role played by intermolecular hydrogen bonding between solvent molecules and the quaternary ammonium ester molecules. Indeed the observed split carbonyl absorptions could arise through such hydrogen bonding, although they could arise from other causes including Fermi resonance, dipole interactions and "hot transitions"[151] as well as the coexistence of two or more conformers. This aspect is further complicated by the second report[141] of these authors in which acetylcholine is stated to exhibit a single carbonyl absorption peak whereas the earlier communication shows the carbonyl absorption as a peak bearing a shoulder despite the fact that both determinations were conducted under similar experimental conditions.

The further application of infrared spectroscopy appeared to offer a promising method of establishing whether or not the two conformations of acetylcholine were indeed present in solution. If the quasi-ring conformation of acetylcholine does exist in solution and, further, if the stability of this conformation does result from intramolecular C-H---O bonding then it would be expected that the effect of such C-H---O bonding would be reflected in the nature of the C-H stretching frequencies as well as in the nature of the ester carbonyl absorption. Accordingly a infrared study of these absorptions in acetylcholine and several other selected trimethylammonium salts was undertaken. In addition a nuclear magnetic resonance study of the N-methyl protons in certain of these compounds was made. A series of acetoxyalkylethyl

$$CH_3CH_2SO_2(CH_2)_nOCOCH_3$$

(LIV)

sulphones (LIV; n=1,2 or 3) were examined in dioxan solution to gain information, uncomplicated by intermolecular hydrogen bond formation, about the influence of inductive effects on the carbonyl stretching frequency of simple esters formally related to acetylcholine and its homologues.

The Examination of the NC-H Absorptions

The choice of solvents for the infrared study of the NC-H stretching and deformation absorptions is extremely limited owing to the insolubility of quaternary ammonium salts in the solvents routinely employed in infrared spectroscopy and in this instance the choice of solvent is further restricted to those incapable of accepting a hydrogen bridge proton in order to ensure the absence of intermolecular hydrogen bonding between the N-methyl hydrogen atom and a solvent molecule. No solvent meeting this latter requirement and capable of dissolving the quaternary salts could be found. Infrared spectra were accordingly measured in the solid state for a number of quaternary salts and these were compared with the spectra of the corresponding tertiary dimethylamino-bases measured in the liquid state. It was anticipated that a comparison between a trimethylammonium salt and its corresponding tertiary base would give an indication of the influence of the positively charged nitrogen atom on the N-methyl C-H stretching frequency and further that a comparison of the spectra of quaternary salts theoretically capable[36] of forming a C-H---O hydrogen bonded quasi-ring with those incapable of doing so, would then give an indication as to whether hydrogen bonding was indeed occurring. It was appreciated, however, that any conclusions were not above unambiguous interpretation for two main reasons:

(i) the possibility of intermolecular as opposed to intramolecular hydrogen bonding, and (ii) the well-recognised difficulty in the interpretation of solid state spectra.

The results of a comparison of the solid state spectra of acetylcholine, acetyl-β-methylcholine and succinylcholine in the 3100-2700 cm^{-1} region with a number of quaternary ammonium salts and their corresponding tertiary bases in the solid and liquid state respectively are shown in Tables I and II. No obvious effect attributable to the influence of C-H---O bonding on the NC-H stretching frequencies was apparent. As is readily seen from Table I, which lists the absorption frequencies in the region 3100-2700 cm^{-1} for seven quaternary ammonium salts, the NC-H stretching region presents a particularly complex picture. The simplest compound, tetramethylammonium iodide, exhibits some ten well-resolved peaks -- presumably a manifestation of dipole interactions, orientation, reflectivity and scattering effects in the crystals together with absorptions arising from combination and overtone effects. Attempts to simplify the interpretation of the spectra by the deletion of absorptions taken as overtones of other absorption modes were unsuccessful on account of the uncertainty of conclusively establishing any peak as an overtone. It was therefore difficult to identify C-H stretching absorptions originating solely from the N-methyl carbon atoms of the selected compounds and it was virtually impossible to confirm unambiguously the existence or otherwise of any type of NC-H---O hydrogen bonding in solid acetylcholine from a study of the 3100-2700cm^{-1} region of the spectrum.

Recently Ebsworth and Sheppard[152] have made the assignments νCH_3 asymm. 3005 cm^{-1} and νCH_3 sym. 2925 cm^{-1}

TABLE I

Solid State Spectra (KCl Disc)

(Representative spectra are shown in Appendix I)

	Compound	Absorption Frequencies in 3100-2700 cm^{-1} Region cm^{-1}				
1.	Tetramethylammonium Iodide	3100 3054	3010	3005sh		
2.	Trimethyloctylammonium Iodide	3024	3010			
3.	Choline Iodide	3026		3005	2985	
4.	Acetylcholine Chloride	3023	3010	3000sh	2977	2960
5.	Acetyl-β-methylcholine Chloride	3043	3010sh	3000	2982	2965
6.	Succinylcholine Chloride	3048 3024sh 3019			2973	2957
7.	Acetonyltrimethylammonium Iodide	3055 3046	3009		2952	

sh denotes shoulder

TABLE II

Thin Liquid Film Spectra

8.	Dimethyloctylamine	2952	2925	2068	2853	2810
9.	2-Dimethylamino-ethanol	2967sh	2944		2855	2820
10.	Dimethylamino-acetone	2970	2944	2873		2823

							Carbonyl Stretching Frequency cm
1.	2949	2928 2905	2880	2830		2780 2730	-
2.	2940	2917	2867 2851			2781	-
3.	2950	2930	2881	2820		2770	-
4.	2946	2920	2852	2825	2807	2790	1737
5.	2938	2920				2796	1738
6.	2933						1740
7.		2915			2806	2776 2740	1734
8.	2778	2760					-
9.	2777						-
10.	2776						1725sh 1715

for the tetramethylammonium ion and indeed in all of the quaternary ammonium salts described in Table I strong absorption was observed at 3010-3000 cm^{-1} (3019 cm^{-1} in succinylcholine) corresponding to νCH_3 asymm. No similar consistency was evident however in the 2925 cm^{-1} region but consistency was observed at 2952-2940 cm^{-1} (2957 cm^{-1} in succinylcholine). The weak bonds in the region 2800-2400 cm^{-1} have been interpreted as combination frequencies involving probably both infrared and Raman-active fundamentals.[152] It is also to be noted that compounds 3-7, in which NC-H---O hydrogen bonding is possible, are characterised by absorptions between 3000 and 2950 cm^{-1} which are lacking in compounds 1 and 2 where no such hydrogen bonding can occur. These peaks could represent lowered absorption frequencies corresponding to those observed just above 3000 cm^{-1} (i.e. νCH_3 asymm.), which result from hydrogen bonding, although absorption at ca. 2985 cm^{-1} common to the solid state spectra of all choline esters so far examined has been tentatively ascribed to superposition of C-H stretching from the acyl group on weak C-H stretching originating in the choline moiety.[98] It is also of some interest that whereas choline, acetylcholine, acetyl-β-methylcholine and succinylcholine show well defined bands at 2985, 2977, 2982 and 2973 cm^{-1} respectively the methiodide of dimethylaminoacetone shows absorption at 2952 cm^{-1}, which would not be inconsistent with NC-H---O hydrogen bonding since the greater inherent proton-accepting propensity of a carbonyl oxygen as compared with an hydroxylic or an acyloxy oxygen[153, 154] would be expected to give a greater decrease in the bond order of the C-H bond concerned, with consequent lowering of the absorption frequency.

Table II lists the absorption frequencies in the 3100-2700 cm^{-1} region, measured in liquid film, for the tertiary

amines corresponding to the quaternary compounds 2,3 and 7 of Table I. These compounds were examined with a view to securing information concerning the influence of the positively charged quaternary nitrogen atom on the N-methyl C-H stretching frequencies but once again the spectra are complex and no simple correlation in the terms sought is immediately apparent. The situation in this instance is further complicated by the fact that the hybridisation of the nitrogen in amines and quaternary salts is not identical. Thus the C-H stretching in N-methyl compounds is well known[118] to occur at lower frequency than in C-methyl compounds due to the greater electronegativity of the nitrogen atom with consequent lowering of the bond order whereas in quaternary ammonium compounds the C-H stretching occurs at higher frequency than in tertiary amines[155] despite the still greater inductive effect of the positively charged nitrogen. It is of interest that dimethylaminoacetone in contrast to its methiodide shows a split carbonyl absorption at 1725 cm^{-1} (sh) and 1715 cm^{-1}. The fundamental C-H stretching absorptions of the dimethylamino - compounds (Table II) were observed at 2823-2810 cm^{-1} and 2778-2776 cm^{-1} in very close agreement with the literature assignments[155,156] of 2825-2810 cm^{-1} and 2775-2765 cm^{-1} for the $-N-(CH_3)_2$ group in amines.

The 1st. overtone C-H stretching absorptions of the tertiary amines 2-dimethylammoethanol, dimethyloctylamine and dimethylaminoacetone were also examined but the solid state spectra of the corresponding quaternary salts in this region required for comparative purposes could not be obtained for technical reasons. The 1st. overtone C-H stretching absorptions of acetylcholine and acetyl-β-methylcholine in D_2O solution also failed to afford any evidence of NC-H---O bonding.

If NC-H---O hydrogen bonding is involved in the stabilisation of the proposed quasi-ring conformation of acetylcholine then the effect of such bonding might also be reflected in the nature of the C-H deformation frequencies.[157] The frequency shift in this instance is upwards due to an increase in the restoring force tending to keep the C-H bond directed towards the oxygen atom.[157] The relative upward shift is smaller than the relative downward shift of the corresponding stretching modes. Only in a very few instances, however, have the effects of H-bonding on deformation modes been well substantiated. Ebsworth and Sheppard[152] have made the assignments δCH_3 asymm. 1483 cm^{-1} and δCH_3 sym. 1403 + 1397 cm^{-1} for the tetramethylammonium ion. The results of a comparison of the solid state spectra of acetylcholine, acetyl-β-methylcholine and succinylcholine in the 1500-1390 cm^{-1} region with a number of quaternary ammonium salts are shown in Table III. Thus tetramethylammonium iodide shows only three well defined peaks at 1484 cm^{-1}, 1403 and 1396 cm^{-1} in complete agreement with the literature assignment. Octyltrimethylammonium iodide shows well defined peaks at comparable frequencies although in this instance considerable broadening and a series of shoulders are in evidence on the lower frequency side of the 1484 cm^{-1} peak undoubtedly a manifestation of the 8 carbon chain. In the remaining compounds of Table III it is impossible to identify with certainty the δCH_3 sym. modes but in all cases a well defined peak is observed at 1485± 2 cm^{-1} corresponding to δCH_3 asymm. and it is interesting to note that in the three choline esters broadening has occurred on the high frequency side of this mode: thus succinylcholine shows a second peak at 1497 cm^{-1} whereas acetylcholine and acetyl-β-methylcholine show shoulders at 1492 and 1495 cm^{-1} respectively

TABLE III

Solid State Spectra (KCl disc)

(Representative spectra are shown in Appendix II)

Compound	Absorption Frequencies in 1500-1390 cm^{-1} Region				
1. Tetramethylammonium Iodide		1484			
2. Octyltrimethylammonium Iodide		1484	1478sh 1470sh	1462sh	
3. Choline Iodide		1485		1466sh	1455
4. Acetylcholine Chloride	1492sh	1487	1475		1455
5. Acetyl-β-methylcholine Chloride	1495sh	1483		1465	
6. Succinylcholine Chloride	1497	1483			1455
7. Acetonyltrimethylammonium Iodide		1485	1470		

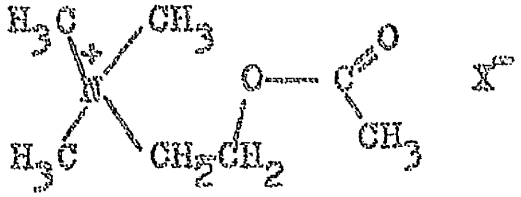
sh denotes shoulder

1.					1403	1396
2.		1433			1408	1395
3.			1423	1410		
4.	1445	1435	1425	1412		
5.	1442				1409	1390sh
6.	1448		1429 1421sh	1415		
7.	1449	1435		1416	1401	

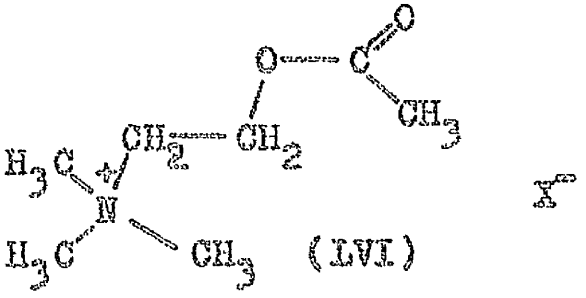
Although this could be regarded as evidence for NC-H---O bonding it is impossible to be definitive since this region is not sufficiently well-documented[157] and it is also recognised that deformation modes frequently couple with other vibrational modes in the lower finger print region of the spectrum.[157] Furthermore the other two compounds of Table III, choline[43] iodide and acetonyltrimethylammonium iodide, although theoretically capable of NC-H---O bonding show no similar characteristics on the high frequency side of the absorption at 1485 cm^{-1}.

The Examination of the C=O Stretching Absorptions

The coexistence of the two possible conformers (LV,LVI) of acetylcholine in solution would also be expected to be reflected in the nature of the ester carbonyl absorption. Hydrogen bonding is well known[158] to lower the frequency of the carbonyl stretching mode



(LV)



(LVI)

where the hydrogen bond is to the carbonyl oxygen atom but, in the present circumstance where the intramolecular hydrogen bond will occur at the acyloxy oxygen atom the frequency of the carbonyl stretching absorption will be raised due to depression of the permanent polarisation of the carbonyl group and consequent increase in its double bond character. The choice of solvent for such studies is again extremely limited. Solvents containing hydrogen atoms capable of hydrogen bond formation were unavailable

for the proposed investigation as they would introduce additional complications through the existence of intermolecular hydrogen bonding to the carbonyl oxygen atom. Dimethylsulphoxide proved too hygroscopic for use in the projected studies and so the infrared spectra were measured in dry dioxan.

Acetylcholine and acetyl-β-methylcholine proved sparingly soluble in dry dioxan and both showed split ester carbonyl absorptions at 1753 cm^{-1} and 1732 cm^{-1} which might be attributable to the coexistence of conformations of types LV and LVI but as low intensity absorption at ca. 1720 cm^{-1} was also present which could be attributable to the presence of traces of water or free acetic acid, despite rigorous precautions against this, firm conclusions are not possible. Ethyl succinate, used as a standard of reference, showed a single ester carbonyl band in dioxan solution at 1737 cm^{-1}, and ethyl acetate a single band at 1743 cm^{-1}.

The Infrared Study of the Sulphones

Table IV shows the carbonyl stretching frequencies of the sulphones formally related to acetylcholine and its homologues studied in dry dioxan solution. It is seen that all showed a higher ester carbonyl stretching frequency than diethyl succinate and that only acetoxymethyl ethyl sulphone showed any indication of a split carbonyl absorption-showing a shoulder at 1775 cm^{-1} on the high frequency side of th main absorption at 1764 cm^{-1}. The progressive decrease in the

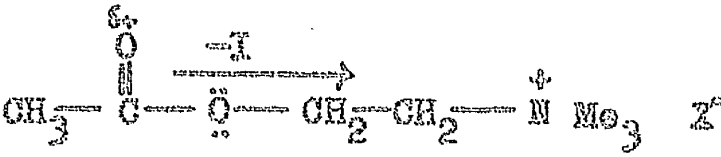
TABLE IV

Carbonyl Stretching Frequencies in Dioxan Solution.

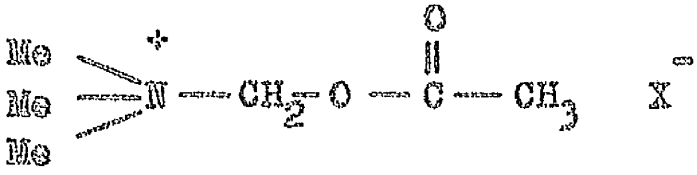
Sulphone $EtSO_2(CH_2)_nOCOCH_3$	Carbonyl Stretching Frequency cm^{-1}	
n=1	1775sh	1764
n=2		1751
n=3		1745
Ethyl succinate		1737
Ethyl acetate		1743

ester carbonyl stretching frequency as n increases from 1 to 3 is in complete agreement with the expected decrease in the influence of the inductive effect of the sulphone group[159] and serves to confirm the conclusions of Fellman and Fujita[141] (page 156) in a situation uncomplicated by the existence of intermolecular hydrogen bonding involving solvent molecules. It also provides an analogy for the observations of Nakamoto et al.[160] who showed that protonation of the nitrogen atom of the iminodiacetic acid di-anion produced a shift to higher frequency of the carboxylate carbonyl absorption.

Very recently it has been stated[161] that the arguments presented by Fellman and Fujita to account for the higher frequency of the carbonyl group in acetylcholine in terms of the inductive effect of the quaternary nitrogen atom are unsatisfactory. From earlier infrared studies[162] of substitution patterns in monosubstituted benzene derivatives Canepa and Mooney[161] argue that the contribution from LVI is not expected



(LVI)



(LVII)

to be important and proceed to show by attentuated total reflexion and transmission measurements that the carbonyl frequency in acetylcholine is of the order expected for aliphatic esters. The high frequency carbonyl absorptions observed by Fellman and Fujita[141] (and omitted by Canepa and Mooney[161] in their criticism) at 1780 cm^{-1} for trimethylaminomethylacetate (LVII) and observed at 1775cm^{-1} and 1764 cm^{-1} for acetoxymethyl ethyl sulphone (Table IV) are difficult to rationalise assuming the conclusions of Canepa and Mooney[161] that the mesomeric and/or inductomeric interaction between alkoxy and acyl groups are negligible.

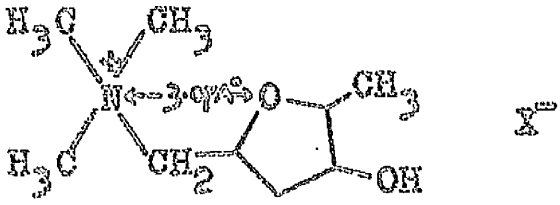
Nuclear Magnetic Resonance Studies

Examination of the N.M.R. spectra of tetramethylammonium iodide, octyltrimethylammonium iodide, acetylcholine chloride and acetyl-β-methylcholine in D_2O solution failed to reveal any evidence of NC-H---O hydrogen bonding. The tetramethylammonium ion[163a] showed a 1:1:1 triplet structure, similar to that observed in the tetraethylammonium ion[163] due to coupling of the protons to the ^{14}N nucleus (I=1). In this particular case the symmetrical structure of the ion prevents the broadening due to quadrupole relaxation. The other three compounds all showed unresolved N-methyl proton absorptions at 6·88τ, 6·76τ and 6·81τ respectively. Thus if NC-H---O hydrogen bonding is present in acetylcholine or acetyl-β-methyl choline in D_2O, interchange between

hydrogen-bonded and non-hydrogen bonded forms is too fast for it to be detected in the N.M.R spectrum, comparable to the situation in the investigation of certain cases of tautomeric equilibrium by nuclear magnetic resonance spectroscopy[164].

Conclusions

From the above results it is apparent that little information on the possible role of conformational isomerism in acetylcholine has been forthcoming from the spectral studies. Ring structures do however exist in the crystalline state the electrostatic interaction being between the quaternary nitrogen atom and the acyloxy oxygen and not the acyl oxygen. Thus it has been calculated[99,165] that the $\overset{+}{N}$------ring ether oxygen distance is only 3·07A^{o} in crystalline muscarine iodide (LVIII). When compared with the $\overset{+}{N}$---I^{-} distance of 4·49A^{o} the $\overset{+}{N}$---O distance of 3·07A^{o} is of great significance. From the stability of choline cations to irradiation in aqueous solution as compared to the marked sensitivity of crystalline orthorombic choline chloride to ionising radiation



(LVIII)

Canepa[165] has deduced that the $\overset{+}{N}$---O distance in both choline and acetylcholine in aqueous solution is shortened to at least 3·07A^{o}. The $\overset{+}{N}$---O conformation in acetylcholine in aqueous solution is relevant to its absorption at the cholinergic receptor and to its hydrolysis.[165]

EXPERIMENTAL

All melting points are uncorrected.

Instruments

Infrared spectra were measured in dioxan solution, liquid film and solid state (KCl disc) employing a Unicam SP100 double beam spectrophotometer equipped with an SP100 sodium chloride prism - grating double monochromator operated under vacuum conditions.

The 1st. overtone C-H stretching absorptions of acetylcholine and acetyl-β-methylcholine in D_2O solution were recorded on a Perkin-Elmer Model 125 spectrophotometer.

Nuclear magnetic resonance spectra were recorded in D_2O solution employing a Perkin-Elmer R10 spectrometer operating at 40 Mcs/sec. Trimethylsilane propionsulphonic acid was used as internal standard.

Solvents

Dry dioxan was prepared by the method described by Vogel[166] (decomposition of the acetal with dilute acid followed by fractionation).

Dry dimethylsulphoxide was prepared[167] by refluxing the commercial solvent with calcium hydride followed by distillation from calcium hydride in vacuo.

Tertiary Bases

Dimethyloctylamine

Dimethyloctylamine was prepared from an ethereal solution of anhydrous dimethylamine and octyl bromide, b.p. 189-190°/760mm. (lit.[168,169] 189-190°/760mm. and 191°/760mm.)

Dimethylaminoacetone

Dimethylaminoacetone was prepared from an ethereal solution of anhydrous dimethylamine and monochloroacetone, b.p. 123-124°/760mm. (lit.[170,171] 123°/760mm. and 124-125°/

/760mm.). Dimethylaminoacetone darkens rapidly on exposure to light and, as far as possible, infrared spectra were determined on freshly distilled samples.

2-Dimethylaminoethanol

2-Dimethylaminoethanol was obtained by redistillation of a commercial sample through a 9 in. Vigreux Column, b.p. 134.5°/760mm.

Quaternary Salts

Choline iodide

The crude product obtained from 2-dimethylaminoethanol and methyl iodide was recrystallised three times from ethanol-acetone-ether, m.p. 261-262° [lit.[172] 258°(decomp.)]

Octyltrimethylammonium iodide

Octyltrimethylammonium iodide was prepared in almost quantitative yield from dimethyloctylamine and methyl iodide. The product after three recrystallisations from ethanol-acetone-ether was obtained as waxy flakes, m.p. 135-136°.

Found:	C, 44.0;	H, 8.6;	N, 4.3
$C_{11}H_{26}IN$ requires	C, 44.1;	H, 8.8;	N, 4.7%

Acetonyltrimethylammonium iodide

Dimethylaminoacetone was quaternised with methyl iodide and the crude product was recrystallised three times from ethanol-acetone-ether to give fine white needles, m.p. 172-173° [lit.[170,171] 171-172° and 168° and records[171] ν max 1728 cm^{-1} (Nujol and Chloroform)]

Tetramethylammonium iodide

Tetramethylammonium iodide was prepared from trimethyl-

-amine and methyl iodide. Several recrystallisations from aqueous ethanol gave long needles, m.p. 350° (lit.[173] 230°).

Acetylcholine chloride, Acetyl-β-methylcholine chloride and Succinylcholine chloride

Commercial samples of acetylcholine chloride, acetyl-β-methylcholine chloride, and succinylcholine chloride were each recrystallised three times from ethanol-acetone-ether and dried *in vacuo* over phosphorus pentoxide.

Acetoxyalkyl Ethyl Sulphones

Chloromethyl ethyl sulphide

Chloromethyl ethyl sulphide was obtained by the method described by Böhme *et al.*[174] for the preparation of the homologous chloromethyl methyl sulphide. A stirred mixture of ethyl mercaptan (46g.) and paraformaldehyde (24g.) was cooled to -15°. Dry hydrogen chloride was bubbled through the mixture at such a rate that the temperature was maintained at about -5°. When the reaction, as shown by the disappearance of the solid paraformaldehyde and the cessation of uptake of hydrogen chloride (*ca.* 3hr.), was complete, calcium chloride (15g.) was added and the mixture kept in a sealed flask for 24 hr. The mixture was extracted with dichloromethane (2x200ml.), the combined extracts dried ($CaCl_2$) and distilled to yield the product (36g., 44%) as a colourless fuming evil-smelling liquid, b.p. 128-129°/760mm. (lit.[175,176] 127-129°/760mm).

Chloromethyl ethyl sulphone

Chloromethyl ethyl sulphide (36g) was treated with an ethereal solution of monoperphthalic acid (12g in 200ml) and

the mixture allowed to stand for 3 days at room temperature. The ethereal solution was cautiously evaporated to dryness under reduced pressure at room temperature and the solid residue extracted with dry chloroform (2x100ml.). The chloroform was removed under reduced pressure to yield the crude sulphone (3·3g., 69%). A portion of the crude material was recrystallised from ether-petroleum ether (b.p 40-60°) to give white plates, m.p. 29-30° (lit.[175] 33°).

Found:	C, 25·4;	H, 5·05;	S, 22·3
Calc. for $C_3H_7ClO_2S$	C, 25·45;	H, 5·0;	S, 22·65%

Acetoxymethyl ethyl sulphide

Chloromethyl ethyl sulphide (16g) and anhydrous sodium acetate (16g) were heated at 160° for 6 hr., 190° for 0·5hr., and finally at 175° for 3hr. and the product (15g., 80%; b.p 104-106°/40mm.) isolated according to the method of Behme et al.[174] who record b.p 65-67°/16mm.

Acetoxymethyl ethyl sulphone

a) From chloromethyl ethyl sulphone: chloromethyl ethyl sulphone (3·2g.) and anhydrous sodium acetate (2·2g.) were heated at 160° for 18hr. and the reaction mixture was then extracted with chloroform (2x100ml). Evaporation of the chloroform extract yielded only unreacted chloromethyl ethyl sulphone. The chloroform insoluble residue contained no halogen.

b) By oxidation of acetoxymethyl ethyl sulphide: acetoxymethyl ethyl sulphide (5g.) was treated with an ethereal solution of monoperphthalic acid (18g. in 200 ml). A white precipitate of phthalic acid began to separate within 5 min. and the mixture was left standing for 3 days. The ethereal solution was evaporated to dryness under reduced pressure at

room temperature and the residue extracted with dry chloroform (2x100ml., 1x50ml.). The solvent was removed *in vacuo* and the residue distilled to yield acetoxymethyl ethyl sulphone (2.2g., 35%) as a pale yellow oil, b.p 98-100°/0·5mm. (lit.[174] 107°/0·7mm. and 148°/18mm.).

Found:	C, 36·4;	H, 6·0;	S, 19·1
Calc. for $C_5H_{10}O_4S$	C, 36·1;	H, 6·1;	S, 19·3%

Ethyl 2-hydroxyethyl sulphide

The method used was essentially that described by Garner and Bowman[177] for the preparation of 2-propylthio-ethan-1-ol.

Aqueous sodium hydroxide (25%, 52ml.) was slowly added to ethyl mercaptan (20·7g) cooled in ice. Ethylene chlorohydrin (27g.) was added over 0·5hr. with constant stirring, the temperature being maintained at 0°. The mixture was stirred until the ice in the cooling bath had melted and for a further 0·5 hr. at room temperature. The lower aqueous layer was separated from the upper organic layer and the latter taken up in benzene (*ca.* 70ml.). The benzene solution was washed successively with 25% aqueous sodium hydroxide (6ml.), water (6ml.), 1% hydrochloric acid (6ml.) and water (6ml.). The benzene extract was dried (Na_2SO_4), the benzene removed, and the residue distilled to give ethyl 2-hydroxyethyl sulphide (18g., 51%), b.p 78-80°/15-20mm. (lit.[178] 81°/20mm.).

2-Acetoxyethyl ethyl sulphone

Ethyl 2-hydroxyethyl sulphone was prepared from ethyl 2-hydroxyethyl sulphide according to the method of Exner.[179]

Method A.

Ethyl 2-hydroxyethylsulphone (6·9g.), acetic anhydride

(12·5ml), and concentrated sulphuric acid (3ml) were heated for 1hr. at 100°. The reaction mixture was cooled, crushed ice (6g.) added, and extracted with chloroform (2x100ml., 2x50ml.). The combined chloroform extracts were dried (Na_2SO_4), the chloroform removed, and the residue distilled to yield the crude product (4g.,44%), b.p. 149-150°/1.1mm. The impure product was redistilled to give 2-acetoxyethyl ethyl sulphone (1·5g.), as an almost colourless oil, b.p. 126-127°/0.2mm.

Found:	C, 40·2;	H, 6·8;	S, 17·5
$C_6H_{12}O_4S$ requires	C, 40·0;	H, 6·7;	S, 17·8%

Method B.

Ethyl 2-hydroxyethyl sulphone (6g.) in ether-chloroform (ca. 1:3, 150ml.) was treated for 3·5hr. with a vigorous stream of freshly generated ketene. The solvents were removed and the residue distilled. The fraction (1g.,13%) boiling at 130°/0·3mm. corresponded to pure 2-acetoxyethyl ethyl sulphone. The remaining fractions (2·7g.) were impure product.

Found:	C, 40·9;	H, 6·8;	S, 17·4
$C_6H_{12}O_4S$ requires	C, 40·0;	H, 6·7;	S, 17·8%

Ethyl 3-hydroxypropylsulphone

Ethyl 3-hydroxypropyl sulphide was prepared according to the method of Rothstein[180]. The ethyl 3-hydroxypropyl sulphide (10g.) in glacial acetic acid (25ml.) was cautiously treated with aqueous hydrogen peroxide (30% w/v, 18ml.) at such a rate that the temperature did not rise above 40°, and allowed to stand overnight. The solvent was removed *in vacuo* and the residue distilled to give ethyl 3-hydroxypropylsulphone (7g, 60%), b.p. 161-163°/0·7mm., m.p. 36-37° (from chloroform-carbon tetrachloride and cooling at

ca. - 10°).

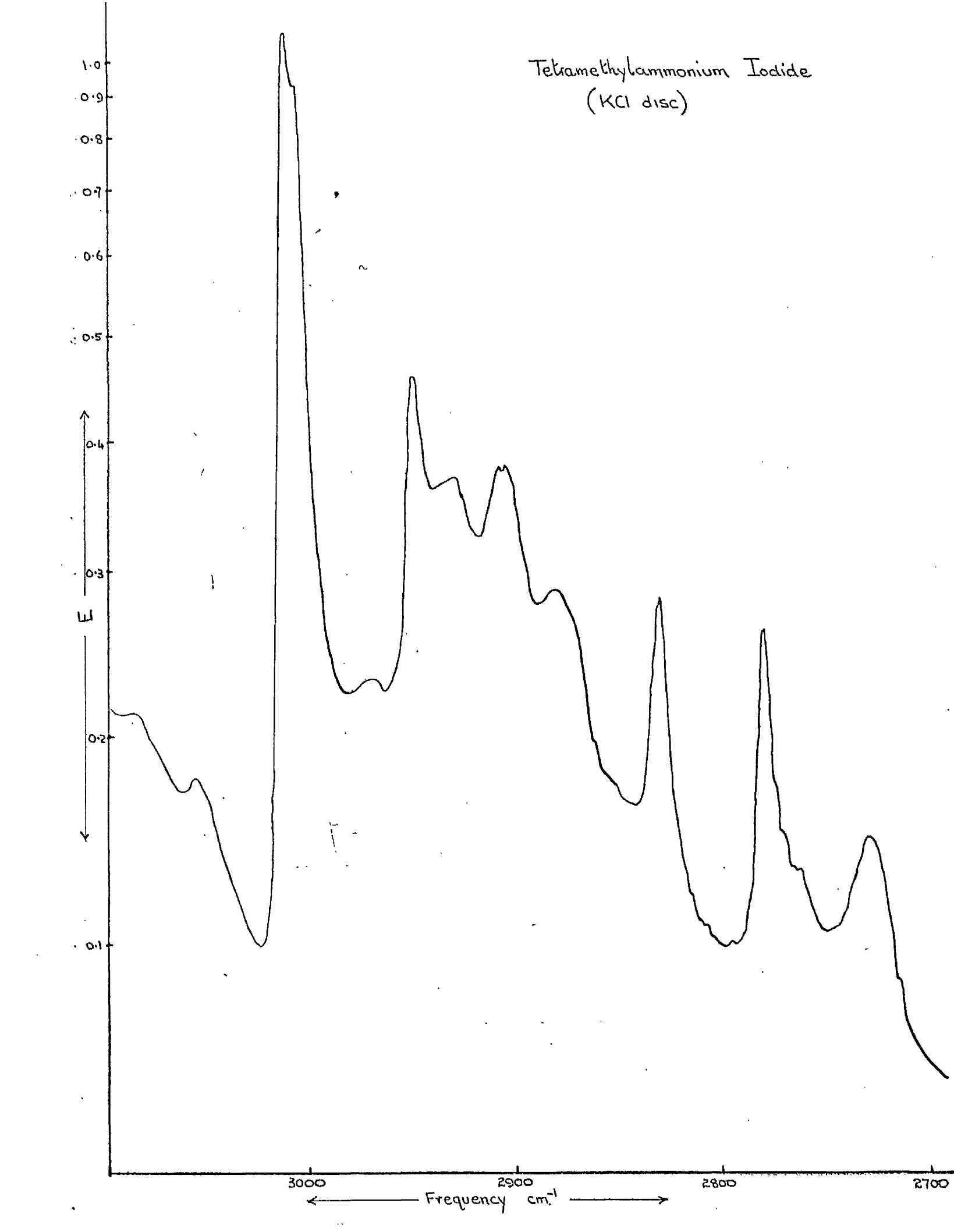
Found:	S, 21·8
$C_5H_{12}O_3S$ requires	S, 21·1%

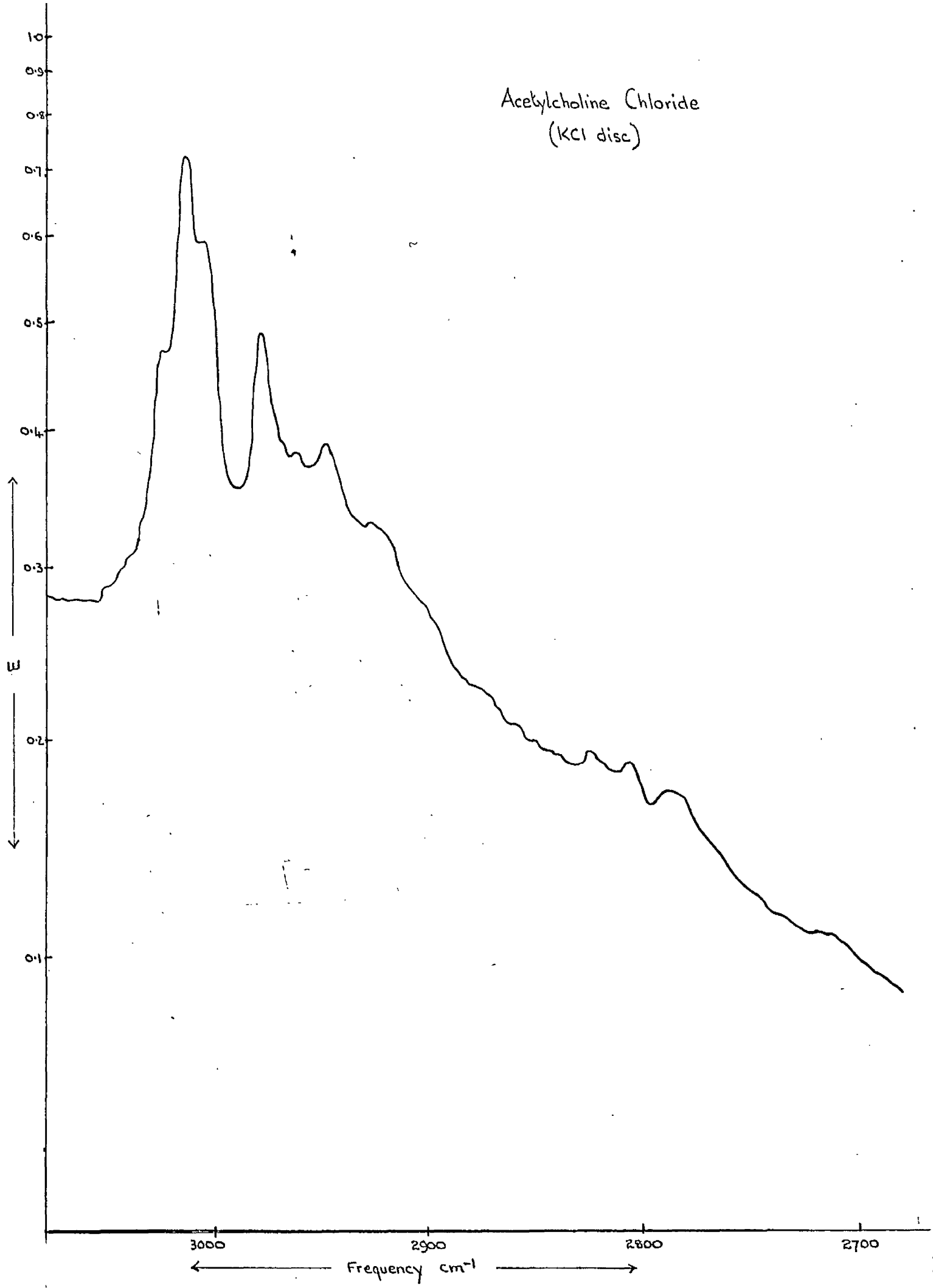
3-Acetoxypropyl ethyl sulphone

3-Acetoxypropyl ethyl sulphone was obtained from ethyl 3-hydroxypropylsulphone (7g.), acetic anhydride (14ml.) and concentrated sulphuric acid (3ml.) by a method similar to that described for the preparation of 2-acetoxy-ethyl ethyl sulphone (Method A). Distillation gave the product (2g, 22%) as a pale yellow oil, b.p. 147-149°/0·2mm.

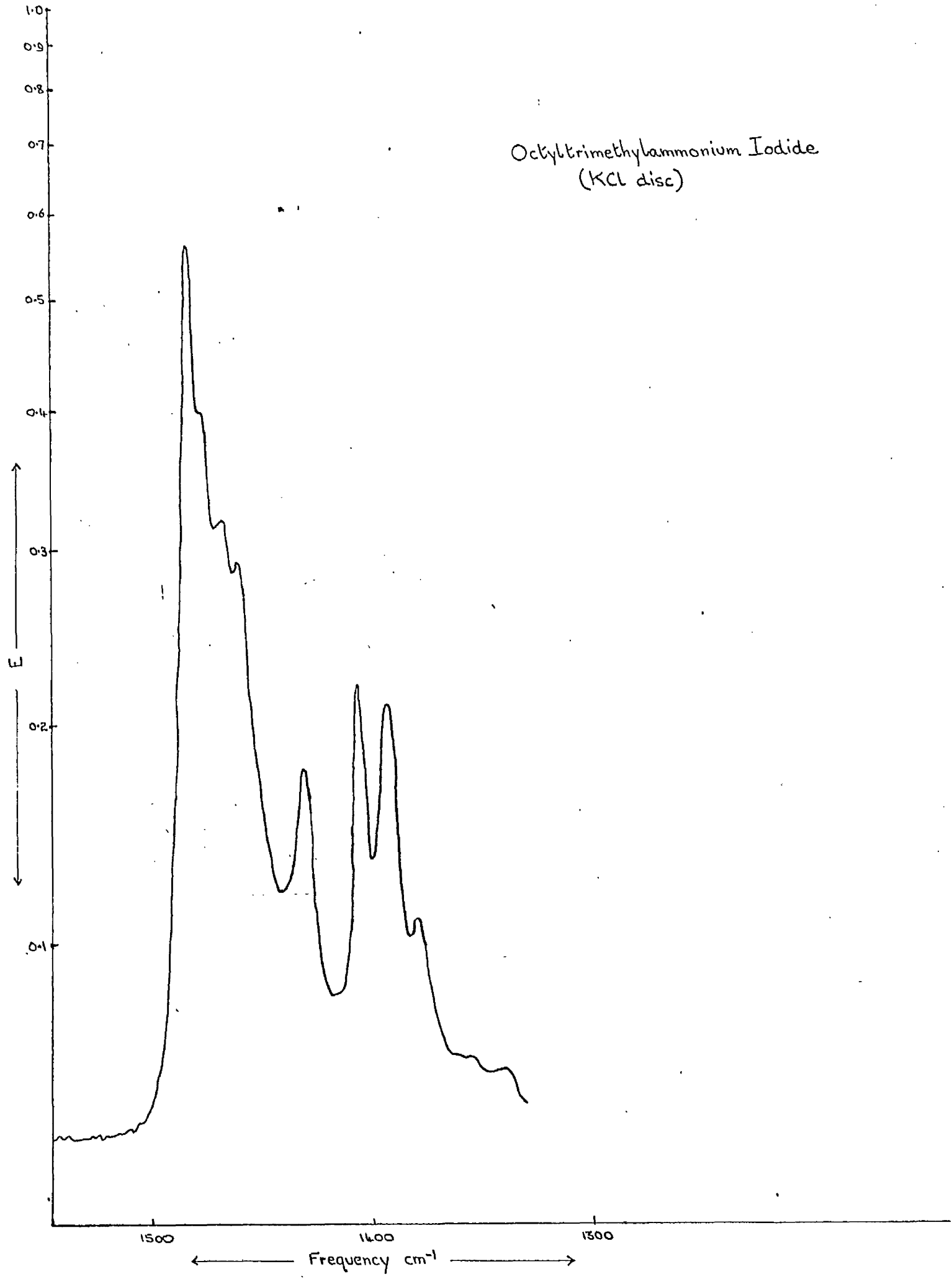
Found:	C, 43·5;	H, 7·3
$C_7H_{14}O_4S$ requires	C, 43·3;	H, 7·3%

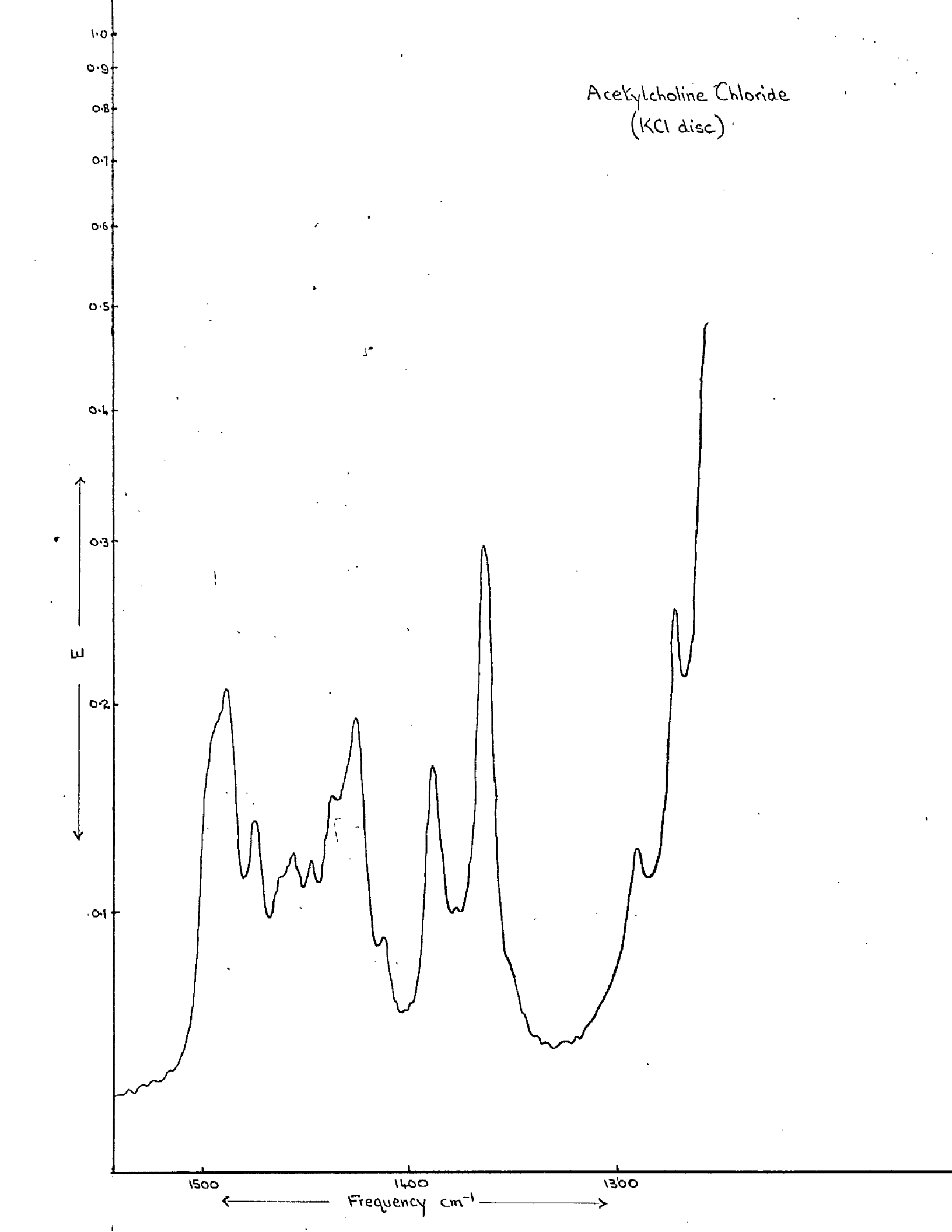
APPENDIX I

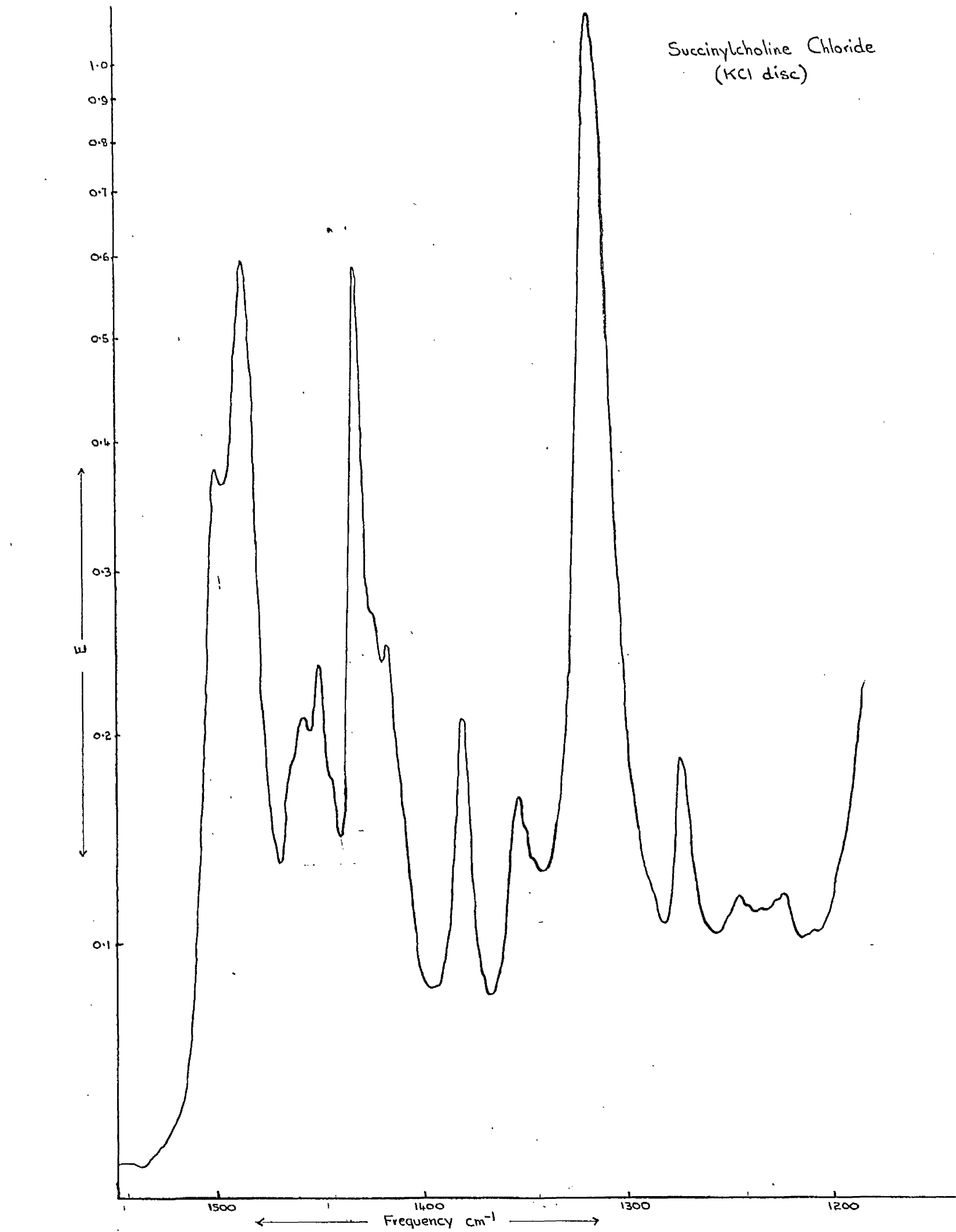

Tetramethylammonium Iodide
(KCl disc)
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
E
3000
2900
2800
2700
Frequency cm.⁻¹


Acetylcholine Chloride
(KCl disc)
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
T
3000
2900
2800
2700
Frequency cm⁻¹

APPENDIX II


Octyltrimethylammonium Iodide
(KCl disc)
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
E
1500
1400
1300
Frequency cm⁻¹


Acetylcholine Chloride
(KCl disc)
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
E
1500
1400
1300
Frequency cm⁻¹


Succinylcholine Chloride
(KCl disc)
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
I
1500
1400
1300
1200
Frequency cm⁻¹

REFERENCES.

1.	Nachmansohn,	Chemical and Molecular Basis of Nerve Activity. Academic Press, New York, 1959.			
2.	Kibjakow,	Pflugers Arch. ges. Physiol.,	1933,	232,	432
3.	Feldberg and Gaddum,	J. Physiol.,	1934,	81,	305
4.	Feldberg and Vartiainen,	J. Physiol.,	1935,	83,	103
5.	Grundfest,	Ann. N.Y. Acad. Sci.,	1957,	66,	537
6.	Dale, Feldberg and Vogt,	J. Physiol.,	1936,	86,	353
7.	Brown, Dale and Feldberg,	J. Physiol.,	1936,	87,	394
8.	Brown,	J. Physiol.,	1937,	89,	220
9.	Loewi,	Pflugers Arch. ges. Physiol.,	1921,	189,	239
10.	Chang and Gaddum,	J. Physiol.,	1933,	79,	255
11.	Dale and Feldberg,	J. Physiol.,	1934,	82,	121
12.	Gaddum,	Gefasserweiternde Stoffe der Gewebe, Thieme, Leipzig, 1936.			
13.	Brown and Gray,	J. Physiol.,	1948,	107,	306
14.	Douglas and Gray,	ibid.,	1953,	119,	118
15.	Davis,	Physiol. Rev.,	1961,	41,	391
16.	Keelle,	Nature, Lond.,	1961,	190,	208
17.	Keelle,	J. Pharm. Pharmacol.,	1962,	14,	65
18.	Feldberg and Minz,	Pflugers Arch. ges. Physiol.,	1933,	233,	657
19.	Feldberg, Minz and Tsudzimura,	J. Physiol.,	1934,	81,	286
20.	Burn,	Brit. Med. J.,	1961,	(1)	1623
21.	Burn and Froede,	Brit. J. Pharmacol.,	1963,	20,	378
22.	Burn, Rand and Vlen,	ibid.,	1963,	20,	83

23. Nachmansohn, Science, 1961, 134, 1962

24. Nachmansohn, J. Amer. Med. Ass., 1962, 179, 639

25. Nachmansohn, Ann. N.Y. Acad. Sci., 1946, 47, 395

26. Lorente De Nó, J. Cell. comp. Physiol. (Suppl.), 1949, 33, 1

27. Hodgkin, Biol. Rev., 1951, 26, 339

28. Toman, Pharmacol. Rev., 1952, 4, 168

29. Eccles, The Neurophysiological Basis of Mind, Clarendon, Oxford, 1953.

30. Tasaki, Nervous Transmission, Charles C. Thomas, Springfield, Illinois, 1953.

31. Ritchie and Armett, J. Pharmacol., 1963, 139, 201

32. Crossland, J. Pharm. Pharmacol., 1960, 12, 1

33. Pickford, Physiol. Rev., 1945, 25, 573

34. Idem., J. Physiol., 1947, 106, 264

35. Verney, Proc. Roy. Soc., 1947, 135B, 25

36. Duke, Pickford and Watt, J. Physiol., 1950, 111, 81

37. Harris, Brit. Med. J., 1951(ii) 559

38. Jewell, J. Physiol., 1953, 121, 167

39. Eccles, Eccles and Fatt, J. Physiol., 1956, 131, 154

40. Goodman and Gilman, The Pharmacological Basis of Therapeutics, 2nd. Edn. McMillan, New York, 1955.

41. Del Castillo and Katz, Progr. Biophys., 1956, 6, 121

42. Bovet, Bovet-Nitti and Marini-Bettolo, Curare and Curare-like Agents, Elsevier, Amsterdam, 1959.

43. Cavallito and Grey, Progress in Drug Research, Vol. 2, ed. E. Jucker, Birkhauser, Basle 1960. p. 135

44. Waser, J. Pharm. Pharmacol., 1960, 12, 577

45. Waser, Pharmacol. Rev., 1961, 13, 465

46. Bowman, Progress in Medicinal Chemistry, Vol. 2, ed. by Ellis and West, Butterworths, London, 1962, p.88.

47. Stenlake, ibid., Vol. 3, 1963 p.1.

47a. Couteaux, Rev. Canad. Biol., 1947, 6, 563

48. Brodie and Hogben, J. Pharm. Pharmacol., 1957, 9, 345

49. Walsh and Deal, Amer. J. Physiol., 1959, 197, 547

50. Rosenberg and Podleski, J. Pharmacol., 1962, 137, 249

51. Rosenberg and Ehrenpreis, Biochem. Pharmacol., 1961, 8, 192

52. idem., Nature, Lond., 1961, 190, 728

53. Wisliki, Atti XI Congresso Societa Italiana Di Anestesiologia, 1958 p.53.

54. Armett and Ritchie, J. Physiol., 1960, 152, 141

55. Dettbarn, Nature, Lond., 1960, 186, 891

56. Takeshige and Volle, J. Pharmacol., 1963, 141, 206

57. Jones, ibid., 1963, 141, 195

58. Higman and Bartels, Biochim. Biophys. Acta., 1960, 54, 543

59. Schueler, J. Amer. Pharm. Ass. (Sci. Ed.), 1956, 45, 197

60. Kennard, Biochem. Soc. Symposia No. 19, Steric Aspects of the Chemistry and Biochemistry of Natural Products, University Press, Cambridge, 1960. p.29.

61. Archer, Lands and Lewis, J. Med. Pharm. Chem., 1962, 5, 423

62. Pfeiffer, Science, 1948, 107, 94

63. Ing, ibid., 1949, 109, 264

64. Kimura, Unna and Pfeiffer, J. Pharmacol., 1949, 95, 149

65. Lands, Ibid., 1951, 102, 219

65a. Raventos, Quart. J. exp. Physiol., 1937, 27, 99

66. Nachod and Lands, Trans N.Y. Acad. Sci 1953, 16, 2

67. Schueler, Arch. int. Pharmacodyn., 1953, 93, 417

68. Long, Ludueña, Tullar and Lands, J. Pharmacol., 1956, 117, 29

69. Kögl et al., Rec. Trav. chim. Pays-Bas., 1957, 76, 109

70. Waser, Pflügers Arch. ges. Physiol., 1962, 274, 431

71. Cavallito, Ciba Foundation Study Group, No. 12, Curare and Curare-Like Agents ed. A.V.S. de Reuck, Churchill, London, 1962 p.55.

72. Triggle and Belleau, Can. J. Chem., 1962, 40, 1201

73. Beckett, Harper and Clitherow, J. Pharm. Pharmacol., 1963, 15, 349 and 362

74. Belleau and Puranen, J. Med. Chem., 1963, 6, 325

75. Hardegger and Lohse, Helv. Chim. Acta, 1957, 40, 2383

76. Beckett et al, Nature, Lond., 1961, 189, 671

77. Gyermek and Unna, Proc. Soc. Exp. Biol. Med., 1958, 98, 882

78. idem., J. Pharmacol., 1960, 128, 30 and 37

79. Rama-Sastry and Auditore, Proceedings of the First International Pharmacological Meeting, Vol. 7, Ed. by K.J. Brunings, Pergamon, London, 1963. p. 323.

80. Hicks et al., Arch. int. Pharmacodyn., 1935, 51, 335

81. Hicks et al., Australian J. Exp. Biol. Med. Sci., 1947, 25, 363

82. Akcasu, Sinha and West, Brit. J. Pharmacol., 1952, 7, 331

83. Bergel, J. Pharm. Pharmacol., 1951, 3, 385

84. Nachmansohn and Wilson, Adv. Enzymology, 1959, 12, 259

85. Wilson, The Enzymes, 2nd ed., Vol. 4, Ed. by Myrback et al., Academic Press, New York, 1963, p. 501.

86. Friess and Baldridge, J. Amer. Chem. Soc., 1956, 78, 2482

87. Thomas, J. Med. Pharm. Chem., 1961, 3, 309

88. Krupka and Laidler, J. Amer. Chem. Soc., 1961, 83, 1445

89. Friess et al., Arch. Biochem. Biophys., 1962, 96, 158

90. Belleau and Lacasse, Abstr. Papers of A.C.S. 145th meeting, 1963, 27-O.

91. Turpajev et al., Proceedings of the First International Pharmacological Meeting, Vol. 7, Ed. by K.J. Brunings and P. Lindgren, Pergamon, London, 1963, p. 145.

92. Belleau and Lacasse, J. Med. Chem., 1964, 7, 768

93. Belleau, ibid., 1964, 7, 776

94. Ariens and Simonis, J. Pharm. Pharmacol., 1964, 16, 137

95. Sorum, Acta Chem. Scand., 1959, 13, 345

95a. Dunitz, ibid., 1963, 17, 1471

96. Sutor, Nature, Lond., 1962, 195, 68

97. Sutor, J. Chem. Soc., 1963, 1105

98. Whittaker, Hefter's Handbuch der Experimentellen Pharmakologie, Sub. Ed. G.B. Koelle, Springer, Berlin, 1963, p.1.

99.	Jellinek,	Acta Cryst.,	1957,	10,	277
100.	Taylor,	Pharmacol. Rev.,	1951,	3,	412
101.	Barlow and Dobson,	J. Pharm. Pharmacol.,	1955,	7,	27
102.	Gillis and Lewis,	ibid.,	1956,	8,	46
103.	Reitzel and Long,	Arch. int. Pharmacodyn.,	1959,	119,	20
103a.	Schueler,	J. Pharmacol.,	1955,	115,	127
104.	Eugster et al.,	Helv. Chim. Acta,	1958,	41,	205, 583 and 705

105. Beckett, Progress in Drug Research, Vol. I, Ed. by E. Jucker, Birkhauser, Basle, 1959, p. 455.

106.	Major and Bonnet,	J. Amer. Chem. Soc.,	1935,	57,	2125
107.	Major and Cline,	U.S. Patent, 2,040,145;	1936.		
108.	Van Rossum,	Science,	1960,	132,	954
109.	idem.,	J. Pharm. Pharmacol.,	1963,	15,	285
110.	Gill,	Proc. Roy. Soc.,	1959,	150B,	381
111.	Baldridge et al,	J. Amer. Chem. Soc.,	1955,	77,	739
112.	Standaert and Friess,	J. Pharmacol.,	1960,	128,	55
113.	Friess,	Personal Communication.			
114.	Pitzer and Donath,	J. Amer. Chem. Soc.,	1959,	81,	3213
115.	Brutcher et al.,	ibid.,	1959,	81,	4915
116.	McCullough et al.,	ibid.,	1959,	81,	5880

117. Sykes, A Guidebook to Mechanism in Organic Chemistry, Longmans, London, 1961, p.48-50.

118. Cross, Introduction to Practical Infrared Spectroscopy, Butterworths, London.

119.	Cope et al.,	J. Amer. Chem. Soc.,	1961,	83,	3861

120. Pauling, The Nature of the Chemical Bond, Cornell University Press, Ithaca, 1944, p. 64.

121. Renshaw et al., J. Amer. Chem. Soc., 1938, 60, 1765

122. Gunther and Mautner, Abstr. Papers of A.C.S. 145th Meeting, 1963. p. 26-O.

123. Waser, Experientia, 1961, 17, 300

124. Barlow, Introduction to Chemical Pharmacology, 1st Ed., Methuen and Co., London, 1955.

125. Simonart, J. Pharmacol., 1932, 46, 157

126. Holton and Ing, Brit. J. Pharmacol., 1949, 4, 190

127. Fourneau et al., Bull. Soc. Chim. Biol., Paris, 1944, 26, 134 & 576

128. Hey, J. Physiol., 1949, 110, 28P

129. idem., Brit. J. Pharmacol., 1952, 7, 117

130. Fukui et al., Science, 1960, 132, 87

131. Alles and Knoefel, Univ. Calif. Publ. Pharmacol., 1939, 1, 187

132. Ing et al., Brit. J. Pharmacol., 1952, 7, 103

132a. Acheson, Introduction to the Chemistry of Heterocyclic Compounds, Interscience, New York, 1960, p. 89.

133. Lands and Cavallito, J. Pharmacol., 1954, 110, 369

134. Edwards and Marsh, ibid., 1951, 103, 196

135. Welsh and Taub, ibid., 1951, 103, 62

136. Burger and Hobbinger, Brit. J. Pharmacol., 1949, 4, 229

137. Jacob et al., Arch. int. Pharmacodyn., 1952, 91, 303

138. Fellman and Fujita, Biochim. Biophys. Acta, 1962, 56, 227

139. Holland et al., J. Amer. Chem. Soc., 1958, 80, 6031

140. Davis and Ross, J. Chem. Soc., 1950, 3056

141. Follman and Fujita, Biochim. Biophys. Acta, 1963, 71, 701

142. Henbest and Lovell, J. Chem. Soc., 1957, 1965

143. West et al., J. Org. Chem., 1960, 25, 1976

144. Bruice and Fife, J. Amer. Chem. Soc., 1962, 84, 1973

145. Biggins et al., J. Chem. Soc., 1963, 1750

146. Arshid, Giles and Jain, ibid., 1956, 1272

147. Cummings et al., ibid., 1959, 535

148. Pimentel and Maclellan, The Hydrogen Bond, Freeman and Co., San Francisco, 1960. Chapter 6.

149. Allerhand and Schleyer, J. Amer. Chem. Soc., 1963, 85, 1715

150. Krueger and Mateo, Can. J. Chem., 1964, 42, 347

151. Allen et al., J. Chem. Soc., 1960, 1909

152. Ebsworth and Sheppard, Spectrochim Acta, 1958-59, 13, 261

153. Jaffé, Chem. Rev. 1953, 53, 191

154. Stewart and Yates, J. Amer. Chem. Soc., 1960, 82, 4059

155. Braunholtz et al., J. Chem. Soc., 1958, 2780

156. Hill and Meakins, J. Chem. Soc., 1958, 760

157. Hallam, Hydrogen Bonding and Solvent Effects in Infrared Spectroscopy and Molecular Structure ed. by Mansel Davies, Elsevier, Amsterdam, 1963. p. 405.

158. Jones and Sandorfy, Technique of Organic Chemistry, Vol.IX ed. by A. Weissberger, Interscience, New York, 1956. p. 247

159. Ingold, Structure and Mechanism in Organic Chemistry, Bell and Sons Ltd., London 1953 p. 69 and p. 235.

160. Nakamoto et al., J. Amer. Chem. Soc., 1962, 84, 2081

161. Canepa and Mooney, Nature, Lond., 1965, 207, 78

162. Gerrard et al., J. Chem. Soc., 1961, 3153

163a Ogg and Ray, J. Chem. Phys., 1957, 26, 1339

163. Bullock, Tuck and Woodhouse, ibid., 1963, 38, 2318

164. Rople et al., High Resolution Nuclear Magnetic Resonance, Reinhold, New York, 1962 p. 418 and 433.

165. Canepa, Nature, Lond., 1965, 207, 1152

166. Vogel, Practical Organic Chemistry, Longmans Green and Co., London, 1948. p.175

167. Knox, Personal Communication.

168. Gautier et al., Bull. Soc. Chim. France, 1957, 1014

169. Clarke, J. Chem. Soc., 1913, 1689

170. Stoermer and Dzimski, Ber., 1895, 28, 2223

171. Craig et al., Australian J. Chem., 1961, 14, 84

172. Rug and Luenberger, Helv. Chim. Acta, 1946, 29, 1048

173. Heilbron, Dictionary of Organic Compounds, Eyre and Spottiswoode, London, 1953.

174. Böhme et al., Ann., 1949, 563, 54

175. Böhme, Ber., 1936, 69B, 1610

176. Walter et al., J. Amer. Chem. Soc., 1945, 67, 655

177. Garner and Bowman, British Patent 662, 656, 1951

178. Culvenor et al., J. Chem. Soc., 1949, 278

179. Exner, Coll. Czech. Chem. Comm., 1954, 19, 1191

180. Rothstein, J. Chem. Soc., 1937, 309