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THE UNIVERSITY OF ALBERTA

SYNTHESIS AND PHARMACOLOGY OF SOME ESTERS RELATED TO ACETYLCHOLINE

BY

© DONNA BELLE HENDERSON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

EDMONTON, ALBERTA

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Synthesis and Pharmacology of Some Esters Related to Acetylcholine" submitted by Donna Belle Henderson in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

Methyl 3-dimethylaminopropionate methiodide (4), methyl 3-dimethylaminobutyrate methiodide (5) and methyl 3-dimethylamino-2-methylpropionate methiodide (6) have been synthesized and their muscarinic and nicotinic activities compared with acetylcholine (1), α-methylacetylcholine (2) and β-methylacetylcholine (3). Compounds 5 and 6 were more active at the muscarinic receptor than reported previously. The optical isomers of methyl 3-dimethylaminobutyrate methiodide (5a,5b) and methyl 3-dimethylamino-2-methylpropionate methiodide (6a,6b) were synthesized and their muscarinic and nicotinic activities compared with those of the optical isomers of α- and β-methylacetylcholine (2a,2b and 3a,3b). The pharmacological results showed that α-methylacetylcholine (2) was stereospecific in its interactions with guinea-pig cholinesterase but not stereospecific at the muscarinic receptors of this preparation. The α-methyl ester (5) was stereospecific at the muscarinic receptor, the L(-)S-isomer being nearly twice as active as the racemate. There was no evidence that the activity of this ester (5) was affected by cholinesterase. The isomers of the β-methyl ester (6a,6b) were equiactive at the muscarinic receptor unlike the isomers of β-methylacetylcholine (3a,3b). The relative activity of the isomers of

β-methylacetylcholine (3a,3b) was not altered by cholinesterase inhibition. Like the α-methyl ester (5) the β-methyl ester (6) did not appear to be hydrolyzed by cholinesterase. The results obtained are discussed in terms of the conformation and configuration of the agonists and their relative rates of hydrolysis by cholinesterase.

The structure-activity relationships of a series of N,N-dialkylamino-esters were investigated. None of the compounds exhibited significant muscarinic or nicotinic activity.

ACKNOWLEDGEMENTS

The author wishes to express her most sincere thanks to Dr. R.T. Coutts and Dr. D.F. Biggs for their supervision and encouragement throughout the course of this work.

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INTRODUCTION

Although acetylcholine(Ach) (1) was first synthesized by Baeyer in 1867 its pharmacological properties were not investigated for almost fifty years (Goodman and Gilman, 1965). Hunt (1899) reported that adrenal extracts, from which the adrenaline had been removed, caused a fall in blood pressure. He identified the presence of choline in these extracts and suggested that some derivative of choline might be the depressor agent. In 1906 Hunt and Taveau showed that this derivative was acetylcholine. The pharmacological properties of acetylcholine were investigated in detail by Dale (1914). He noted that acetylcholine mimicked the response to stimulation of the parasympathetic nerves and introduced the term "parasympathomimetic" to describe its properties.

Since these first experiments many attempts have been made to determine the structural features in a

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Note to the reader:

Each discrete compound in this thesis will be numbered consecutively as it appears in the text. Optical isomers will be designated by the letters a and b.

molecule which are necessary to stimulate the cholinergic receptor and the nature of the cholinergic receptor itself. Comparative pharmacological and biochemical studies (structure activity relationships - S.A.R.) have been extensively used in an attempt to define the nature of the receptor (Barlow, 1964; Bebbington and Brimblecombe, 1965; Burger, 1967; Triggle, 1971). In the past ten years S.A.R. studies have been replaced to some extent by physico-chemical techniques to determine the electronic and conformational properties of acetylcholine and other cholinergic molecules. The techniques used include x-ray crystallography, proton magnetic resonance spectroscopy, infrared spectroscopy and a number of theoretical calculations.

Recently a second field of research has emerged in the study of the cholinergic receptor. Workers are now engaged in the isolation of the actual receptor and the examination of its properties.

The intention of this introduction is to review the changes which have been made to the structure of the acetylcholine molecule, and to discuss the physico-chemical investigations undertaken in an attempt to determine the essential features for cholinergic activity and to map out the key features of the cholinergic receptor.

Particular attention will be paid to the work which has been done on α- and β-methylacetylcholine (α-MeAch and β-MeAch) (2 and 3) and the quaternary amino-esters; methyl 3-dimethylaminopropionate methiodide (4), methyl 3-dimethylaminobutyrate methiodide (5) and methyl 3-dimethylamino-2-methylpropionate methiodide (6). The last three compounds can be regarded as being the reversed esters of acetylcholine (RE Ach), α-methylacetylcholine (RE α-MeAch) and β-methylacetylcholine (RE β-MeAch) respectively.

$(CH_3)_3\overset{+}{N}CHRCHR^1OCOCH_3 \quad X^-$

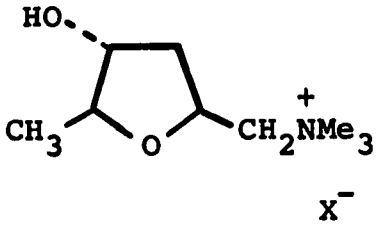
	R	R^1
1	H	H
2	CH_3	H
3	H	CH_3

$(CH_3)_3\overset{+}{N}CHRCHR^1COOCH_3 \quad I^-$

	R	R^1
4	H	H
5	CH_3	H
6	H	CH_3

Acetylcholine is the common transmitter substance at most efferent junctions in the peripheral nervous system. (The exception is the post-ganglionic sympathetic junction where the transmitter substance is usually noradrenaline.)[a] (See Figure 1.)

The pharmacological effects of acetylcholine may be divided into two types: a) muscarinic effects and b) nicotinic effects. This division is based on the observation that the activity of acetylcholine is mimicked by L(+)-muscarine (7)[b] at certain sites in the peripheral nervous system and by nicotine (8) at others. The nicotinic sites of action of acetylcholine are the



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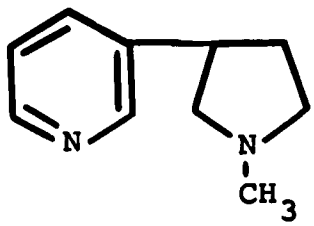

(8)

adrenal medulla, the motor end-plates of the somatic

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[a] Acetylcholine is the neurotransmitter at a few post-ganglionic sympathetic sites e.g. sweat glands

[b] L(+)-3(R)-hydroxy-2(S)-methyl-5(S)-trimethylammonium-methyltetrahydrofuran iodide

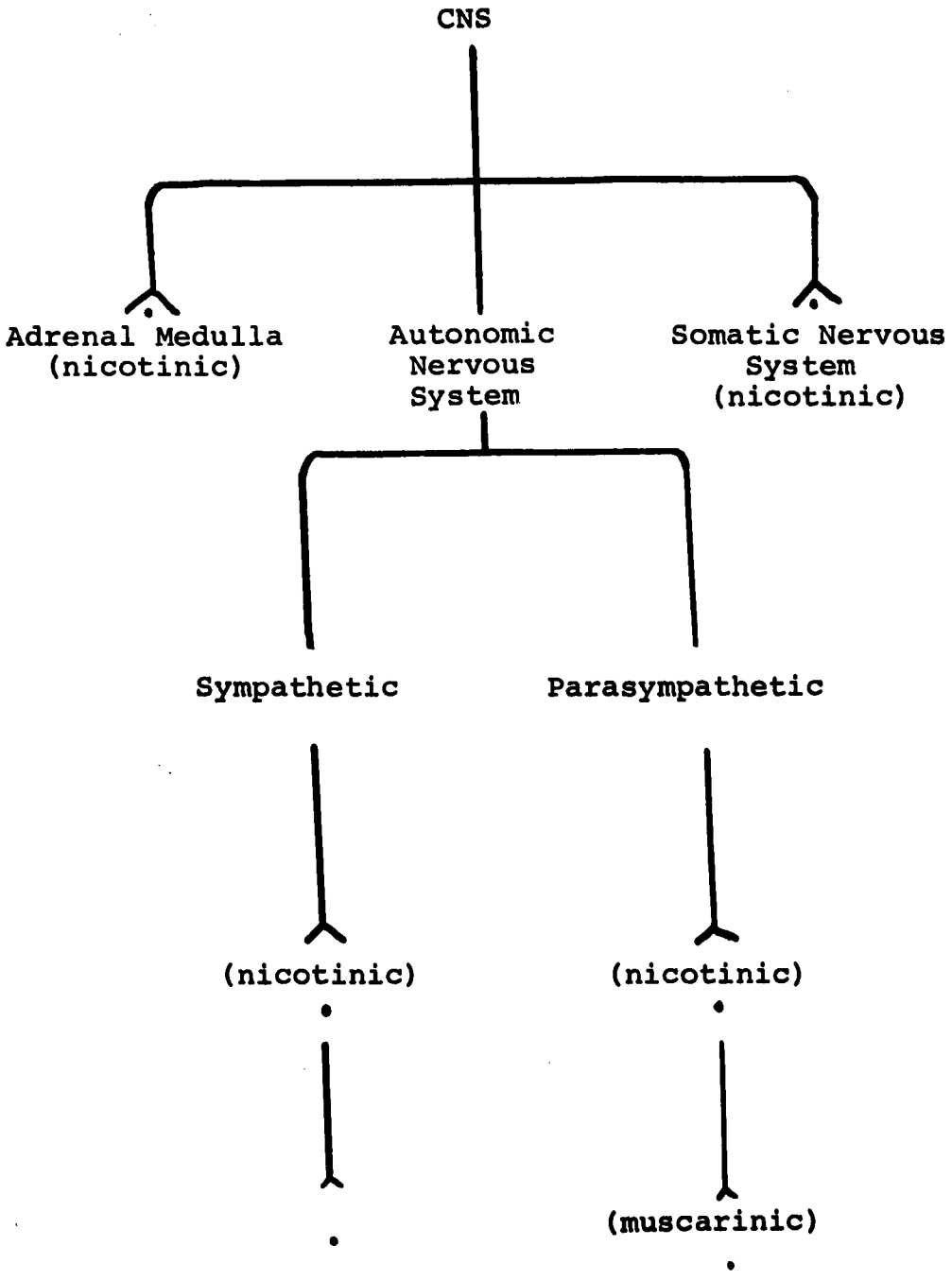
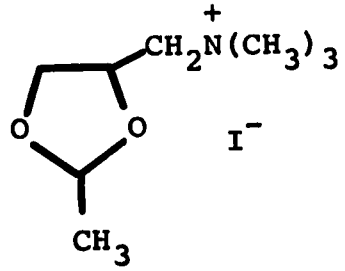


Figure 1: Sites of action of acetylcholine in the peripheral nervous system.

nervous system and the ganglia of the autonomic nervous system. The cholinomimetic effects of drugs, including acetylcholine, at autonomic effector cells are referred to as muscarinic.

From the many S.A.R. studies a series of criteria have been postulated for maximum cholinergic activity. These studies have been extensively reviewed by Barlow (1964), Burger (1967) Goldstein et al. (1969) and Triggle (1971). Most of the S.A.R. studies were performed using acyclic analogues of acetylcholine. However, there are a number of important cholinergic compounds which have a cyclic structure. These agents fall into three main categories: the derivatives of muscarine, the 1,3-dioxolanes and the furmethonium analogues. The most active compound in each series is L(+)-muscarine (7) (Waser, 1961), L(+)-cis-2(S)-methyl-4(R)-trimethylammoniummethyl-1,3-dioxolane iodide (9) (Belleau and Puranen, 1963) and 5-methyl-2-trimethylammoniummethylfuran iodide (10) (Ing et al., 1952).



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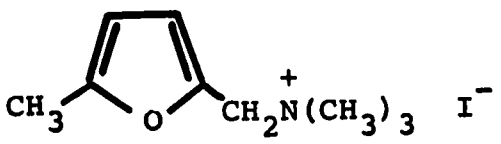
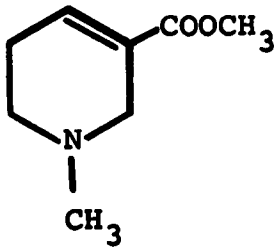


(10)

From a consideration of the S.A.R. studies Goldstein et al. (1969) summarized the requirements for maximum cholinergic activity as being:

1. a quaternary nitrogen atom bearing a positive charge and surrounded by three methyl groups
2. a two carbon methylene chain
3. an oxygen atom in the main axis of the molecule
4. a carbonyl oxygen atom about 7A° from the cationic charge
5. a two carbon acyl residue

Some significant exceptions exist to the requirements for a quaternary trimethylammonium structure. These are nicotine (8), arecoline (11) and oxotremorine (12).



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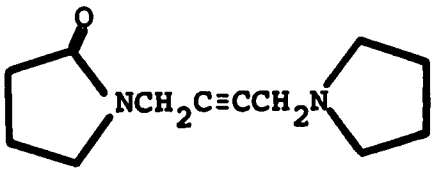


(12)

Many of the conclusions about the features necessary in a molecule for cholinergic activity and the nature of the cholinergic receptor have evolved

from the investigation of the pharmacological properties of α- and β-methylacetylcholine (2 and 3). The properties of α- and β-methylacetylcholine (2 and 3) were first reported by Hunt in 1914 but their activity was not extensively investigated until 1932. Simonart (1932) showed that α-methylacetylcholine (2) produced a fall in blood pressure at very low doses being about 1/20 as active as acetylcholine. On the cat's blood pressure after atropine its effect was quantitatively similar to acetylcholine, thus its nicotinic properties were quite marked. The author also investigated the activity of β-methylacetylcholine (3). This compound produced a fall in the cat's blood pressure in small doses. On the average it was slightly less active than acetylcholine. After the administration of atropine no nicotinic hypertension could be obtained.

The optical isomers of β-methylacetylcholine (3a and 3b) were synthesized and evaluated by Major and Bonnett (1935). These authors were the first to recognize that the muscarine-like activity of β-methylacetylcholine (3) resided primarily in the (+)-isomer (3a). (+)-β-Methylacetylcholine (3a) was somewhat more active than the racemate on blood pressure and on isolated tissue whereas the (-)-isomer (3b) had only

approximately 1/100 the activity of the racemate.

Since this initial study on the activity of the isomers of β-methylacetylcholine (3a and 3b) more extensive investigations have been carried out which have led to the postulation of hypothetical muscarinic and nicotinic receptors for acetylcholine. Ellenbroek and van Rossum (1960) established the absolute configuration of the enantiomers of β-methylacetylcholine (3a and 3b) by synthesis from L(+)S- and D(-)R-lactic acid respectively. They demonstrated that the more active isomer had the "L" configuration. The configuration of this more active isomer is designated as L(+)S-β-methylacetylcholine (3a). (See page 60 for a detailed description of the methods of designation of absolute configuration.) The results obtained from these experiments indicated that acetylcholine, β-methylacetylcholine (3) and the optical isomers of β-methylacetylcholine (3a and 3b) had different affinities for the muscarinic receptor but that their intrinsic activities were identical. The observation that L(+)S-β-methylacetylcholine (3a) had the same configuration as the active muscarine isomer (L(+)-muscarine (7)) led these authors to suggest that these parasympathomimetic agents are highly complimentary to their receptors.

The results of this work were confirmed by Ellenbroek et al. (1965).

Beckett et al. (1961, 1963, 1963a) also determined the absolute configuration of the enantiomers of β-methylacetylcholine (3a and 3b) and extended the study of acetylcholine analogues by determining the absolute configuration of the optical isomers of α-methylacetylcholine (2a and 2b) The isomers of α-methylacetylcholine (2a and 2b) were synthesized from D(-)R- and L(+)S-alanine respectively. The results of the pharmacological experiments conducted by Beckett et al., (1963a) are presented in Table I. From their work and a consideration of the isomers of muscarine prepared by Waser (1961), Beckett et al. (1961, 1963a) proposed a model for the muscarinic receptor (Figure 2).

The quaternary nitrogen group and the ether oxygen lie in the same plane and form the two main centres for drug-receptor association. The OH group of muscarine or C=O of acetylcholine were considered to act as a secondary site of association. The distance between the binding sites corresponds approximately to the distance between the active sites in the L(+)-muscarine (7) molecule (Jellinek, 1958).

Table I

The Muscarinic Activity and Rate of Hydrolysis by Acetylcholinesterase of α- and β-Methylacetylcholine (2 and 3) and Their Optical Isomers (2a, 2b and 3a, 3b) (According to Beckett et al., 1963a)

Compound [a]		Isomer	Guinea-pig Ileum Ach=1.0000	Ratio of Activity of Isomers	Cat Blood pressure Ach=1.0000	Ratio of Activity of Isomers	Rate of Hydrolysis Compared With Ach=100
α-MeAch	(2)	(±)	0.0204		0.0277		91.7
	(2a)	D(+)R-	0.0357		0.0400		78.0
	(2b)	L(-)S-	0.0043	8	0.0069	6	97.4
β-MeAch	(3)	(±)	0.6929		1.0310		46.2
	(3a)	L(+)S-	0.9900		1.4080		54.5
	(3b)	D(-)R-	0.0041	240	0.0049	280	weak inhibitor

a) complete structures of these compounds are recorded on pages 3,55,56

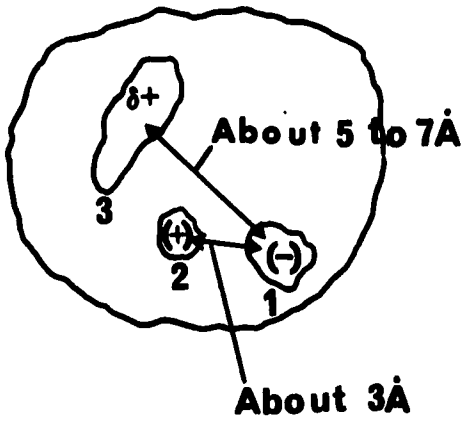


Figure 2: Diagramatic representation of muscarinic receptors. (Beckett et al., 1963a)

1. Anionic cavity negatively charged to accommodate quaternary nitrogen.
2. Positively charged point accommodating ether linkage of muscarine (7) or ester linkage of acetylcholine and its analogues.
3. Charged (+) area to accommodate OH of muscarine (7), C=O of acetylcholine and its analogues or double bond of furan analogues of muscarine.

A detailed evaluation of the series of muscarine analogues prepared and tested by Waser (1961) led Beckett et al. (1963a) to postulate that: a) steric factors were important in the interaction of the muscarines with the receptor b) it was important to have one methyl group in the 2-position c) a correctly orientated 3-OH group was necessary and d) planarity was important for muscarinic activity.

The authors assumed that acetylcholine and α- and β-methylacetylcholine (2 and 3) could adopt conformations similar to that of L(+)-muscarine (7) when acting at the muscarinic receptor. The L(+)S-isomer of β-methylacetylcholine (3a) can adopt a conformation complimentary to that of the depicted receptor but the D(-)R-isomer (3b) is unable to adopt this conformation. They suggested that both isomers could probably present surfaces complimentary to the receptor. Thus, when in the desired conformation the only difference between the two isomers is in the direction in which the β-methyl group projects. It was suggested that in the case of α-methylacetylcholine (2) the α-methyl group might exert a steric effect on the cationic head thereby preventing a close drug-receptor association and resulting in decreased muscarinic activity.

Beckett pointed out that the hydrolysis of the optical isomers of α- and β-methylacetylcholine (2a,2b and 3a,3b) was a complicating factor in the determination of their relative muscarinic activity. (See Table I.) The muscarine-like molecules are not susceptible to cholinesterase hydrolysis and their muscarinic potencies may be interpreted as being a measure of their relative interactions at the muscarinic receptor. It was suggested that the much slower rate of hydrolysis of L(+)S-β-methylacetylcholine (3a) compensates for the deleterious effect of the β-methyl group on the association of the molecule with the receptor. The L(-)S-isomer of α-methylacetylcholine (2b) probably presents a more favorable complementary conformation to the active site of acetylcholinesterase than does its enantiomorph. Beckett suggested that L(-)S-α-methylacetylcholine (2b) might also present a more favorable complimentary conformation to the muscarinic receptor than the D(+)R-isomer (2a) but, owing to the faster rate of hydrolysis and inactivation by acetylcholinesterase the D(+)R-isomer (2a) appears to exert greater muscarinic activity.

Lesser (1965) investigated the activity of D(+)R- and L(-)S-α-methylacetylcholine (2a and 2b) with

a view to demonstrate the stereospecificity of the nicotinic receptor. The compounds were tested on the frog rectus abdominis muscle, the innervated biventer cervicus muscle of the chick, the blood pressure of the atropinized cat and on the superior cervical ganglion of the cat. Little difference in potency was found between the isomers.

Cocolas et al. (1970) re-examined the muscarinic activity of the enantiomeric forms of α- and β-methylacetylcholine (2a, 2b and 3a, 3b together with α,α-dimethylacetylcholine (13) and β,β-dimethylacetylcholine (14) with the intention of demonstrating how the muscarinic receptor and the acetylcholinesterase receptor may interact with the choline fragment of acetylcholine. (See Table II.) They concluded that for optimal muscarinic activity the methyl group should not be juxtaposed between the receptor and the bulk of the molecule. The facet of the choline fragment which faces the receptor is the side which resembles acetylcholine. Steric requirements in the vicinity of the anionic site of the muscarinic receptor are more stringent but less stereospecific than those surrounding the esteratic site. The authors concluded that when considering hydrolysis by acetylcholinesterase, the

Table II

The Muscarinic Activity and Rate of Hydrolysis by Acetylcholinesterase of α- and β-Methylacetylcholine (2 and 3) Their Respective Optical Isomers (2a,2b and 3a,3b) α,α-Dimethylacetylcholine (13) and β,β-Dimethylacetylcholine (14) (According to Cocolas et al., 1970)

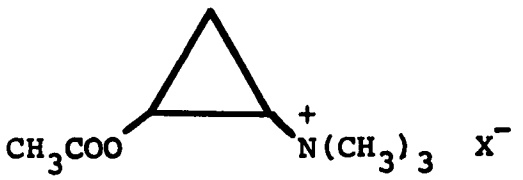
Compound [a]		Isomer	Guinea-pig Ileum Ach=1.0000	Rate of Hydrolysis Ach=100
α-MeAch	(2)	(±)	0.0339	76.9
	(2a)	D(+)R-	0.0240	61.3
	(2b)	L(-)S-	0.0045	87.7
α,α-diMeAch	(13)	(±)	0.0025	36.7
β-MeAch	(3)	(±)	0.622	27.2
	(3a)	L(+)S-	0.802	45.7
	(3b)	D(-)R-	0.090	15.9
β,β-diMeAch	(14)	(±)	0.001	22.0

a) complete structures of these compounds are recorded on pages 3,55,56

compounds that have the greatest affinity for the enzyme receptor area have the same configuration. Methyl substitution in the vicinity of the anionic site does not as profoundly affect the ability of the compound to be hydrolyzed as does substitution adjacent to the esteratic site.

Finally the authors concluded that the muscarinic receptor of the guinea-pig ileum and the enzyme receptor area of bovine erythrocyte acetylcholinesterase each best accomodate one isomer of an enantiomeric pair. The muscarinic site accommodates the R configuration at the α-carbon and the S configuration at the β-carbon of the choline moiety, while the enzymatic site will react more favorably with the S and R configuration respectively.

Two interesting rigid analogues of acetylcholine have been synthesized namely cis- and trans-2-acetoxycyclopropyltrimethylammonium iodide (15 and 16) (Armstrong et al., 1968; Cannon et al., 1972; Chiou et al., 1969).



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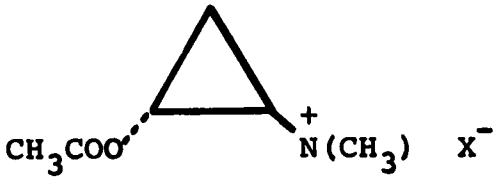


(16)

These compounds were regarded as being hybrid analogues of α- and β-methylacetylcholine (2 and 3). The trans-isomer (16) was primarily muscarinic in its activity and therefore was regarded more as an analogue of β-methylacetylcholine (3). The trans-isomer (16) showed marked stereospecificity the (+)-isomer being equipotent with acetylcholine. This stereospecificity was actually greater than that observed with β-methylacetylcholine (3) or L(+)-muscarine (7). The authors suggested that trans-2-acetoxycyclopropyltrimethylammonium iodide (16) served to define the geometry of the $-\overset{+}{N}Me_3$ and -O- groups (torsion angle approximately 180° and separated by 3.8A°) and a relative area for binding of the acetyl group in the bound conformation of acetylcholine.

The preceding discussions on the properties of α- and β-methylacetylcholine (2 and 3) and related compounds may be summarized as follows:

1. A three point muscarinic receptor may exist for acetylcholine (Figure 2). Sites 1 and 2 on the receptor form the two main sites for drug-receptor interaction. Site 3 is a secondary site of association.

2. The muscarinic activity of β-methylacetylcholine (3) resides almost exclusively in the L(+)S-isomer (3a). The D(+)R-isomer of α-methylacetylcholine (2a) is slightly more active than its enantiomorph in regards to muscarinic activity but this may be due to a difference in rates of hydrolysis by acetylcholinesterase.
3. Substitution on the β-carbon atom results in loss of nicotinic activity.
4. α-Methylacetylcholine (2) shows little stereospecificity in regards to its nicotinic activity.
5. The $-\overset{+}{N}Me_3$ group and -O- groups of acetylcholine may bear a trans (180°) relationship to each other and be separated by 3.8 A°.

A number of authors have investigated the cholinergic properties of alkyklamino-esters in order to define the structural requirements for interaction at the muscarinic and nicotinic receptors. In these compounds the positions of the carbonyl and ethereal oxygens atoms may be regarded as being 'reversed' when compared with the acetylcholine derivatives. Of particular interest is the early work in which the reversed esters of acetylcholine, α-methylacetylcholine and β-

methylacetylcholine, namely methyl 3-dimethylamino-propionate methiodide (4), methyl 3-dimethylamino-butyrate methiodide (5) and methyl 3-dimethylamino-2-methylpropionate methiodide (6), were investigated.

Bass et al. (1950) made an extensive pharmacological investigation of the reversed ester of acetylcholine (4) in an attempt to investigate the effect of reversing the position of the carboxyl group of acetylcholine. The ester (4) showed striking quantitative and qualitative similarities to acetylcholine in many of its effects. The results of acute toxicity studies in white mice indicated that death was apparently due to respiratory arrest. The mice exhibited extreme salivation, lacrimation, defecation and urination followed by muscular tremors which developed into clonic convulsions. The LD_{50} (oral) was 87 mg/kg; (intraperitoneal) 6.7 mg/kg. The effects on the circulatory system were evaluated on the blood pressure of anesthetized cats, and in situ on the perfused frog heart. The ester was equipotent with acetylcholine producing a brief and immediate fall in blood pressure when injected intravenously. The depressor effect was blocked by atropine, following which a nicotinic stimulant action quantitatively similar to acetylcholine

was noted . An injection of 0.1 μg of the ester (4) caused a decrease in amplitude and rate of heartbeat equivalent to the effect observed with 0.02 μg of acetylcholine.

In vitro experiments were carried out on the rabbit and guinea-pig ileum. Acetylcholine and its reversed ester (4) were equiactive and their contractile effects were blocked by atropine. The ester (4) appeared to have a less miotic activity than β-methylacetylcholine (3) when applied topically to the rabbit pupil. The ester (4) had no capacity to enhance or antagonize the effects of d-tubocuranine Enzymatic studies showed that methyl 3-dimethylaminopropionate methiodide (4) was not hydrolyzed to any appreciable extent by acetylcholinesterase and it did not inhibit the enzyme. These results were confirmed by Barrass et al. (1968). The authors concluded that reversing the position of the carboxyl group in acetylcholine did not materially affect potency with respect to the muscarinic receptor surfaces, but greatly altered activity as a substrate for the protein surfaces of acetylcholinesterase.

The same workers investigated the properties of the reversed esters of α- and β-methylacetylcholine (5

and 6) (Schueler et al., 1951, 1951a; Schueler and Keasling 1951). They reported that these compounds had no more than 0.0001 the activity of their respective acetylcholine analogues in regard to depressor, gut stimulant and salivary secretory properties. Neither of the esters (5 and 6) were shown to act as substrates for cholinesterase or to inhibit the hydrolysis of β-methylacetylcholine (3). However, our experiments (Biggs et al., 1971) showed that the reversed esters of α- and β-methylacetylcholine (5 and 6) had .01 the muscarinic activity of acetylcholine when evaluated on the rat blood pressure and guinea-pig ileum. The reversed ester of β-methylacetylcholine (6) had negligible nicotinic activity but, the reversed ester of α-methylacetylcholine (5) was equipotent with its acetylcholine analogue in regard to its nicotinic activity. (See Tables VIII, XI and X.)

A number of authors have studied the S.A.R. of several series of compounds related to the reversed esters of acetylcholine. Barrass et al. (1968) investigated the activity of two groups of compounds with the general structures $R_3\overset{+}{N}(CH_2)_nCO_2R^1\ X^-$ and $R_3\overset{+}{N}(CH_2)_nCONR_2^1\ X^-$ in order to provide additional

information on the relative spatial arrangements of sites 1,2 and 3 of the muscarinic receptor proposed by Beckett et al. (1963a). From pharmacological data and an examination of molecular models of the acetates and propionates (n=1 or 2) the authors postulated that interactions were at sites 1 and 2 of the muscarinic receptor, the quaternary nitrogen atom interacting at site 1 and either the carbonyl oxygen or ester oxygen at site 2. No interaction at site 3 appeared to be possible. Models of the butyrates (n=3) showed that the carbonyl oxygen could interact at site 2 or 3. The observation that no simultaneous interaction was possible at sites 1, 2 and 3 was assumed to be the reason for low muscarinic activity.

All the compounds investigated had relatively high nicotinic activity compared to acetylcholine. From models Barrass et al. (1968) proposed that the nicotinic receptor had at least two essential sites separated by 4-4.5 A°. Other studies conducted on the pharmacological activities of tertiary and quaternary amino-esters have been undertaken by Brimblecombe and Rowsell (1969), Coutts et al. (1969, 1971) and Porszasz et al. (1961). None of the compounds investigated showed any appreciable activity. Coutts et al.

(1969, 1971) also investigated the effect of converting the ester function to a hydroxamic acid. Only one of the compounds investigated showed noteworthy pharmacological activity, namely 2-methyl-3-[1-(4-phenylpiperidino)]propionohydroxamic acid hydrochloride (17) (Midha et al., 1970). This was found to be a ganglion blocking agent. A number of other compounds related to the reversed esters of acetylcholine have been prepared and tested pharmacologically by Selley (1971) and Towill (1971).

PHYSICO-CHEMICAL ANALYSES

X-ray Crystallography

X-ray crystallographic analysis has been performed on many muscarinic, nicotinic, anticholinergic, and ganglion blocking agents. The results of this work have been the subject of several reviews (Baker et al., 1971; Chothia, 1970; Chothia and Pauling, 1970a; Shefter, 1971). As pointed out by Shefter (1971) one of the most important considerations in the interpretation of this type of data is that, to date, there is no known correlation, simple or complex, between the molecular structure of acetylcholine in a crystal, in solution or at the receptor site. The structural data obtained from x-ray analysis studies are useful for understanding the conformational properties of cholinergic compounds. When combined with spectroscopic and biological data these studies provide insight into the conformational preferences of various acetylcholine analogues.

The spatial distributions of the atoms of a molecule are described in terms of torsion angles. A torsion angle relates the orientation of two atoms or groups of atoms (A and B) about a covalent bond (X-Y).

Angles of 0°, +60°, +120°, 180°, -120°, -60° are designated as synperiplanar (sp), +synclinal (+sc) (gauche), +anticlinal (+ac), antiperiplanar (ap) (trans), -anticlinal (-ac) and -synclinal (-sc) (gauche) respectively (Shefter, 1971). In making structural comparisons between analogues of acetylcholine it is sufficient to use four torsion angles. (See Figure 3.)

A summary of the cholinergic compounds whose crystalline structures have been determined by x-ray analysis and the type of activity which they exhibit is presented in Table III. It is not possible to discuss each structural analogue in this review. It has been shown that a gauche relationship exists between the $-\overset{+}{N}(CH_3)_3$ and $-OCOCH_3$ groups or their equivalent in the crystal state of these compounds (Shefter, 1971). The compounds which are of particular interest in these studies are acetylcholine, α-methylacetylcholine (2) and β-methylacetylcholine (3).

The acetylcholine molecule has been shown to have several stable conformations (Canepa et al., 1966; Chothia and Pauling, 1968; Liquori et al., 1968a; Herdklotz and Sass, 1970). The crystal structure of acetylcholine bromide was determined by Canepa et al. (1966). (See Figure 3.)

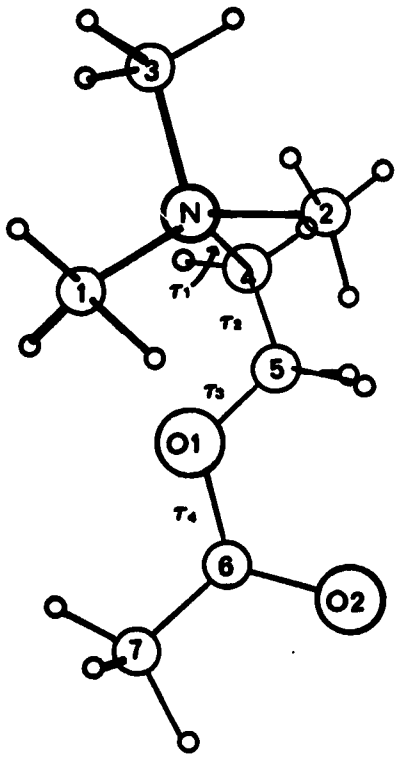


Figure 3: A perspective drawing of the molecule of acetylcholine showing numbering and the four torsion angles which are the four parameters of the conformation of the molecule. (The conformation shown is that proposed as relevant to the muscarinic receptor and is similar to that observed in crystals of the chloride.) (Baker et al., 1971)

Table III

Summary of the Cholinergic Agonists Investigated by X-Ray Crystallography

Compound	X-Ray Crystallography (Ref.)	Pharmacological Activity (Ref.)
A. Potent Muscarinic Agonists		
(1) acetylcholine bromide	Baker et al., 1971 Chothia and Pauling, 1968 Chothia, 1970 Chothia and Pauling, 1970	
(1) acetylcholine chloride	Canepa et al., 1966 Herdklotz and Sass, 1970	
(3a) L(+)S-β-methylacetylcholine iodide	Chothia and Pauling, 1969	Beckett et al., 1961, 1963a Ellenbroek and van Rossum, 1960 Ellenbroek et al., 1965 Smissman et al., 1966
(18) erythro-α(R)-β(S)-dimethylacetylcholine iodide	Brennan et al., 1970 Shefter, 1969 Shefter et al., 1970	

Table III Cont'd

(19)	carbamoyl-choline bromide	Barrans and Clastre, 1970	Barlow, 1964
(16)	(+)-*trans*-2(S)-acetoxycyclopropyl-1(S)-trimethylammonium iodide	Chothia and Pauling, 1970	Armstrong *et al.*, 1968 Chiou *et al.*, 1969 Cannon *et al.*, 1972
(7)	L(+)-muscarine	Jellinek, 1957	Waser, 1961
(11)	L(+)-*cis*-2(S)-methyl-4(R)-trimethylammonium-1, 3-dioxolane iodide	Pauling and Petcher, 1969, 1971	Belleau and Puranen, 1963
(12)	5-methylfurmethide iodide	Baker *et al.*, 1971	Ing *et al.*, 1952

Table III Cont'd

B.	Potent Nicotinic Agonists		
(1)	Acetylcholine bromide	Chothia and Pauling, 1968 Chothia and Pauling, 1970	
(2)	D(+)R- α-methyl-acetylcholine iodide	Chothia and Pauling, 1969a	Beckett et al., 1961, 1963a Lesser, 1965
(20)	L(+)-lactoyl-choline iodide	Chothia and Pauling, 1968	Sastry et al., 1960, 1968
(21)	1,1-dimethyl-4-phenylpiperazinium iodide	Chothia and Pauling, 1970 a	Chen et al., 1951 Herr and Gyermek, 1960
(8)	nicotine	Chothia and Pauling, 1970 a	Barlow, 1965 Barlow and Hamilton, 1965 Domino, 1965
C.	Weakly Active or Inactive Muscarinic Agents		
(22)	threo-α(R),β(R)-dimethylacetyl-choline iodide	Shefter, 1969 Shefter et al., 1970	Smissman et al., 1966

Table III Cont'd

(23)	acetylselenocholine iodide	Shefter and Kennard, 1966 Shefter, 1969	Mautner *et al.*, 1966 Scott and Mautner, 1964, 1967
(24)	acetylthiocholine iodide	Shefter, 1969 Shefter and Mautner, 1969	Mautner *et al.*, 1966 Scott and Mautner, 1964, 1967
(25)	acetylthionocholine iodide	Shefter, 1969	
(26)	choline bromide, chloride, iodide choline reineckate	Senko and Templeton, 1960 Conti *et al.*, 1971	Ariens, 1964 Barlow, 1964
(27)	L-α-glycerylphosphorylcholine L-α-glycerylphosphorylcholine cadmium chloride trihydrate	Abrahamsson and Pascher, 1966 Sundralingham and Jensen, 1965	
(28)	laurylcholine iodide	Stora, 1950	Barlow, 1964

Table III Cont'd

(29)	palmitylcholine iodide	Stora, 1949, 1949a	Barlow, 1964
(30)	stearylcholine bromide	Stora, 1949a	
	stearylcholine iodide	Stora, 1949	
(31)	succinylcholine iodide, perchlorate, picrate	Jensen, 1969	
(32)	2-(N,N-dimethyl-N-ethylammonium)-ethyl carbamate bromide	Barrans and Bideau, 1970	
(33)	2-(N,N-dimethyl-N-benzylammonium)-ethyl carbamate bromide	Barrans and Dangoumau, 1970	

Table III Cont'd

(34)	2(N,N-diethyl-N-benzylammonium)-ethyl carbamate bromide	Babeau and Barrans, 1970	
(9)	arecoline hydrobromide	Baker et al., 1971	Barlow, 1964
(35)	pilocarpine trichlorogermanate	Fregerslev and Rasmassen, 1968	Barlow, 1964
(36)	(-)R-3-acetoxyquinuclidine methiodide	Robinson et al., 1969 Belleau and Pauling, 1970	Robinson et al., 1969
(37)	2(S)-trimethylammonium-3(S)-acetoxy-trans-decalin iodide	Smissman et al., 1966	Shefter et al., 1969
(38)	1-methyl-3(S)-acetoxy-trans-decahydroquinoline methiodide	Shefter, 1971	Smissman and Chappell, 1969

The conformation of the potent muscarinic agonist L(+)S-β-methylacetylcholine iodide (3a) was determined by Chothia and Pauling (1969). (See Figure 4.) The conformation is the same as that determined in solution by p.m.r. studies (Casy et al., 1971). The conformation is synclinal at τ2 and τ3=147° because of the close contact of the β-methyl group and the carbonyl oxygen.

D(+)R-α-Methylacetylcholine iodide (2a) was shown to have two stable conformations (Chothia and Pauling, 1969a). (See Figure 5.) The major difference between the two conformations was that the torsion angle O(1)-C(5)-C(4)-N of molecule A was +90° and that of molecule B was -148°. The presence of two conformations in this compound in a single molecule, one synclinal and the other between anticlinal and antiplanar, is evidence of the lability of these molecules and their property of multiple stable conformations.

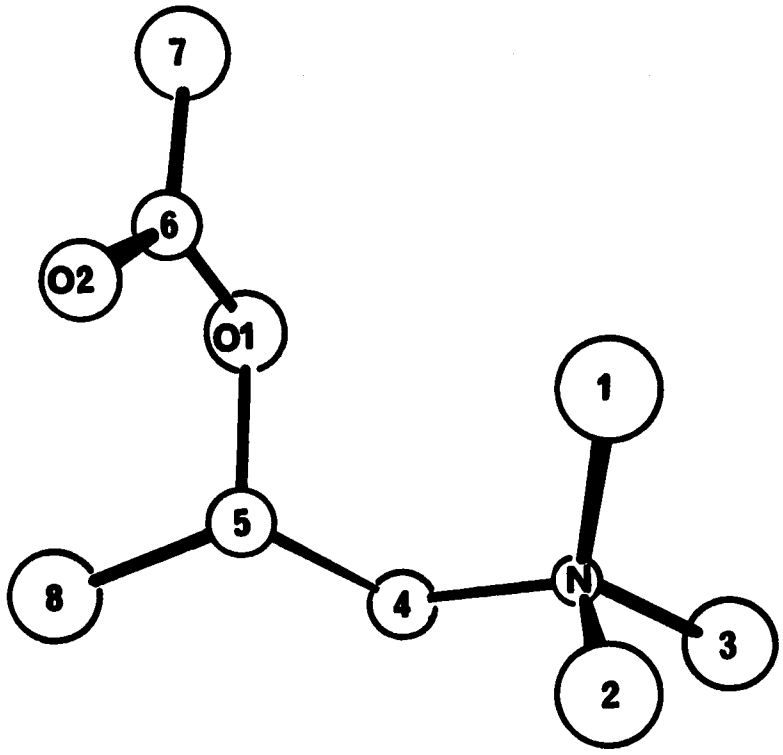


Figure 4: Crystal structure of L(+)S-β-methylacetylcholine iodide (3a). (Chothia and Pauling, 1969)

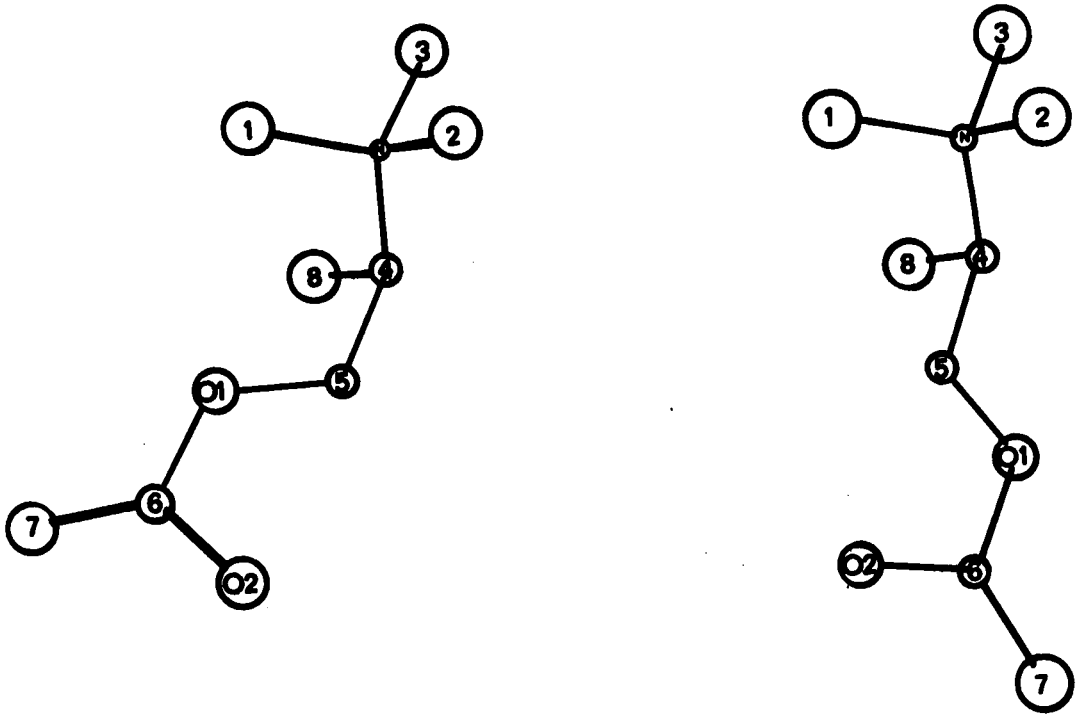


Figure 5: Diagramatic representation of the two conformations of D(+)R-α-methylacetylcholine (2a) in the crystal state. (Chothia and Pauling, 1969a)

From work on a series of muscarinic and nicotinic agonists Chothia (1970) arrived at a series of conclusions about the interaction of these drugs at the cholinergic receptors. Firstly, the conformations of acetylcholine relevant to the muscarinic and nicotinic receptors are similar (Chothia, 1970; Chothia and Pauling, 1968). (See Figure 6.)

Secondly, muscarinic receptors allow some variation in the conformation of agonists; observed values of τN-C4-C5-O1 are all between positive synclinal and positive anticlinal. The conformation of the muscarinic agonist carbamoylcholine bromide (19) (Barrans and Clastre, 1970) is the only known example of a potent muscarinic agonist where the torsion angle is approximately 180°. For nicotinic molecules the torsion angle τN-C4-C5-O1 or its equivalent is positive synclinal (∿+75°) and τC4-C5-O1-O6 is antiplanar (∿180°).

Thirdly, from a comparison of the molecular structure of acetylcholine with those agonists of high specific potency at nicotinic or muscarinic receptor sites he suggested that different groups of the acetylcholine molecule interact with these different types of receptors. A detailed analysis of the muscarinic agonists L(+)-muscarine (7) (Jellinek, 1957), L(+)S-β-

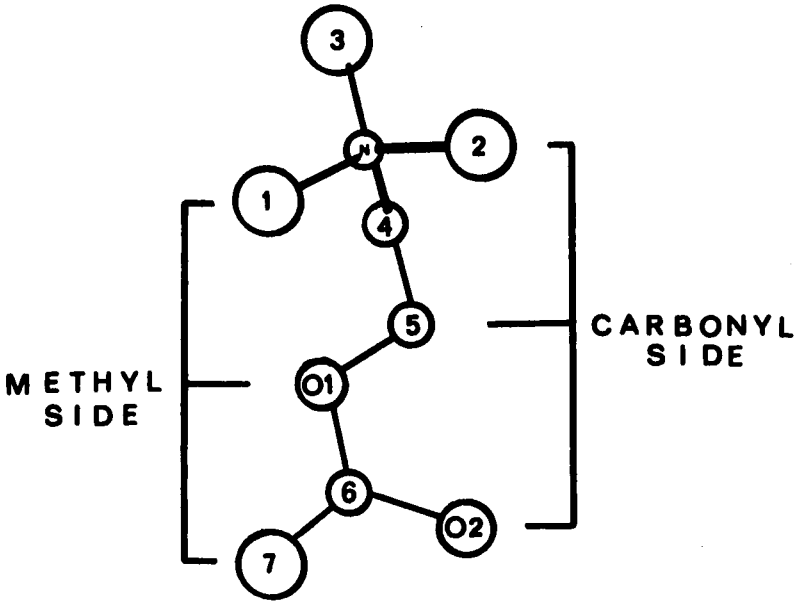


Figure 6: Acetylcholine in the conformation relevant to the muscarinic and nicotinic receptors. (Chothia, 1970)

methylacetylcholine (3a) (Chothia and Pauling, 1969) and trans-(+)-2-acetoxycyclopropyltrimethylammonium (ACTM) (16) (Chothia and Pauling, 1970) showed that preservation of the structural features of the methyl side of acetylcholine was necessary for activation of the muscarinic receptor by muscarinic agonists. (See Figure 7.)

In all these structures the methyl side of acetylcholine is preserved whilst the carbonyl side is blocked. Thus in L(+)S-β-methylacetylcholine (3a) the β-methyl group and in ACTM (16) the methylene group limit the possible interactions of the quaternary nitrogen group and the carbonyl oxygen. Similarly in L(+)-muscarine (7) the ring atoms block the interactions in the region between and around C5 and O2. However in this case muscarine does not contain a carbonyl group. Also it has been argued (Waser, 1961) that muscarine retains the carbonyl oxygen of acetylcholine in the form of the OH group. Since this hydroxyl oxygen is 1.4 A° further away from the N-O1 axis in muscarine than the carbonyl oxygen of acetylcholine itself, the two groups cannot be regarded as comparable.

Chothia, (1970) also compared the crystal structure of the potent nicotinic agonists DL-lactoylcholine iodide (20) (Chothia and Pauling, 1968),

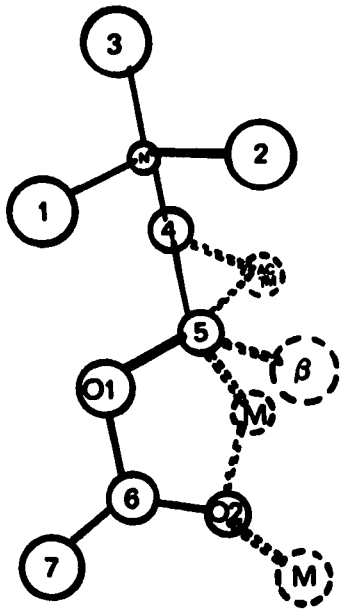
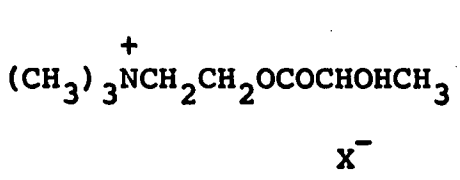
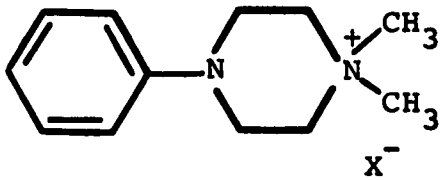


Figure 7: A schematic drawing of the superposition of the muscarinic agonists on the conformation of acetylcholine. (Chothia, 1970) Atoms common to the agonists and acetylcholine are numbered as in Figure 3. Atoms belonging to L(+)S-β-methylacetylcholine (3a) are labelled as β, to L(+)-muscarine (7), M and to trans-(+)-acetoxycyclopropyltrimethylammonium (16), ACTM.

D(+)R-α-methylacetylcholine (2a) (Chothia and Pauling, 1969a) and 1,1-dimethylphenylpiperazinium iodide (DMPP) (21 (Chothia, 1970; Chothia and Pauling, 1970b). (See Figure 8.)



(20)



(21)

Superposition of the acetylcholine residue of these structures revealed that the carbonyl side of acetylcholine is preserved and the methyl side is blocked. The α-methyl group of D(+)R-α-methylacetylcholine (2a) and the C6 of DMPP (21) severely limits the possible interactions of the ester oxygen O1 or the equivalent N2 in DMPP with the quaternary ammonium group. The hydroxyl and the methyl groups on C7 in lactoylcholine (20) block the possible interactions of C7. Blockade of the carbonyl side occurs in the muscarinic agonists and explains their lack of nicotinic activity. Similarly blockade of the methyl side in nicotinic agonists explains their lack of muscarinic activity.

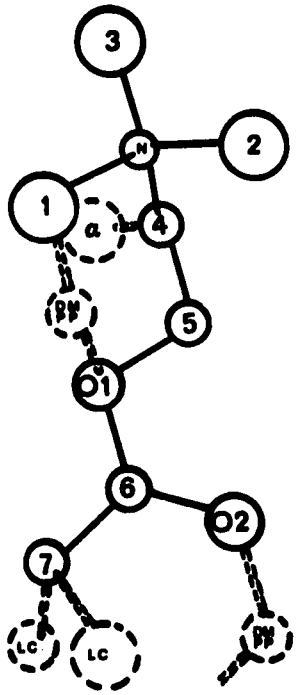


Figure 8: A drawing of the superposition of the nicotinic agonists. (Chothia, 1970) Atoms common to the agonists and acetylcholine are numbered as in Figure 3. Atoms belonging to lactoylcholine (20) are labelled as LC, to 1,1-dimethylphenylpiperazinium iodide (21), DMPP and to D(+)R-α-methylacetylcholine (2a), α.

Chothia (1970) concluded that the methyl side of nicotinic agonists or its equivalent and the carbonyl side of muscarinic agonists or its equivalent are only necessary for activity in so far as they hold the groups on the other side of the molecule in the correct three-dimensional arrangement. The essential structural features of a nicotinic agonist were a quaternary nitrogen group and a carbonyl group (or its equivalent). The essential structural features of muscarinic agonists included a quaternary nitrogen and a methyl group. Therefore an oxygen function was not necessary for muscarinic activity.

A comparison of the structural features of acetylselenocholine (23), acetylthiocholine (24), acetylthionocholine (25), erythro-α(R)-β(S)-dimethylacetylcholine (18) and threo-α(R)-β(R)-dimethylacetylcholine (22) with their relative potency as muscarinic agents led Shefter (1969) to suggest that the electronic make-up of the ester linkage as well as the N-C-C-O and C-C-O-C conformations is important in determining cholinergic activity.

There have been several attempts to determine the conformation of cholinergic compounds at the active site of acetylcholinesterase. Pauling (1968) and

Chothia and Pauling (1969b) examined the structures of L(-)S-α-methylacetylcholine iodide (2b), D(+)R-α-methylacetylcholine iodide (2a), L(+)S-β-methylacetylcholine iodide (3a), D(-)R-β-methylacetylcholine iodide (3b), acetylthiocholine bromide (24) and acetylselenocholine bromide (23). It was proposed that only one conformation of substrate at the enzyme surface was essential for hydrolysis to take place. The conformation of acetylcholine relative to the active site of acetylcholinesterase was proposed to be τC5-C4-N-C3=180°, τO1-C5-C4-N=150°, τC6-O1-C5-C4=-150° to -180° and τO2-C6-O1-C5=0°. The orientations of the methyl groups are approximately staggered in this conformation. It was also suggested that the synclinal conformation of the N-C-C-O group is stabilized by electrostatic interactions between the positive nitrogen atom and the slightly negative ester oxygen atom. The authors thought that it was necessary for this interaction to be broken for the ester to hydrolyze and that this was done by interaction with the enzyme in such a way that the torsion angle O1-C5-C4-N is +150°. This moves the ester oxygen O1 from a position 3.15A° distant from the nitrogen to a position approximately 3.6A° distant from the nitrogen.

Shefter et al. (1970) suggested that substrates having a value other than +150° can be hydrolyzed by acetylcholinesterase. They examined the structures of erythro- and threo-α,β-dimethylacetylcholine (18 and 22). The authors suggested that their hypothesis was confirmed by the work which has been performed on rigid analogues (Chiou et al., 1969; Robinson and Belleau, 1969; Smissman et al., 1966; Stephen et al., 1972) and also by recent studies on the muscarinic receptor which imply a dual mode of substrate binding, in which a common anionic site which is flanked by two loci for polar and nonpolar side chains (Belleau, 1970; Moran and Triggle, 1970). Shefter suggested that factors such as electronic features of the ester linkage and steric repulsion between the choline moiety and the enzyme surface are probably as important as the τ1 angle in influencing kinetic processes involved in acetylcholinesterase hydrolysis and cholinergic activity.

Theoretical Analyses

The conformations proposed from x-ray analyses have been confirmed using several theoretical approaches.

Liquori et al. (1968, 1968a) have calculated the minimum energy conformations of L(+)-muscarine (7) and acetylcholine. They showed that there are four minimum energy conformations for acetylcholine which are separated by an energy barrier of less than 1 kcal/mole. The conformation with the least energy was the one found in acetylcholine bromide crystals (Canepa et al., 1965). The conformation of L(+)-muscarine with the lowest energy was the one observed in the x-ray analysis (Jellinek, 1957).

The conformational and electronic properties of acetylcholine, L(+)-muscarine (7) and nicotine (8) have been investigated by the quantum mechanical method of perturbative configuration interaction using localized orbitals (Pullman et al., 1971). The results obtained were in satisfactory agreement with the experimental data found by x-ray analyses.

Extensive use has been made of molecular orbital calculations by Kier (1967, 1968, 1970, 1971). In this method the total energy of the molecule is evaluated by the linear combination of atomic orbitals. The calculation was carried through for all the combinations of torsion angles so that a plot of total molecular energy against the torsion angle was obtained.

The minimum energy corresponds to the preferred conformation. Kier calculated the preferred conformation of acetylcholine, L(+)-muscarine (7), oxotremorine (12), nicotine (8) and other molecules active in the cholinergic system. The calculation for L(+)-muscarine (7) and acetylcholine revealed a preferred conformation which was very similar to that reported in the literature based upon x-ray analyses. From this and other data, Kier (1967) proposed a receptor pattern for muscarinic agents which very closely ressembled that proposed by Beckett et al. (1963a) who used pharmacological data to reach their conclusions. (See Figure 9.) Kier (1970) also showed that the potent central acting cholinergic compound oxotremorine (12) fitted this proposed muscarinic 'chromophore'.

Using molecular orbital calculations two equally preferred conformations were shown to exist for nicotine (Kier, 1968). Kier proposed that there are two key features necessary for a nicotinic agonist. (See Figure 10.)

Beers and Reich (1970) examined Dreiding and CPK spacefilling models of compounds which affect nicotinic and muscarinic receptors. They suggested that the nicotinic and muscarinic specificities of cholin-

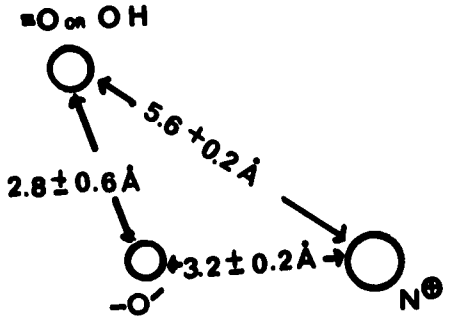


Figure 9: Calculated muscarinic chromophore based on the conformation calculated for acetylcholine and muscarine (7). (Kier, 1967)

Figure 10: Key features of nicotinic acting molecules. (Kier, 1968)

ergic receptors can be understood in terms of interactions based on two different combinations of functional groups in the transmitter substance, acetylcholine. The authors proposed that specific binding of nicotinic agents to their receptor is mediated by two elements: a) a coulombic interaction involving the alkylammonium moiety and b) a hydrogen bond which depends on an acceptor group in the drug and is formed approximately 5.9A° from the centre of the positive charge. In the muscarinic series of the functional groups which define interaction are: a) a quaternary ammonium group or its equivalent and b) an unshared pair of electrons which can mediate the formation of a hydrogen bond at approximately 4.4A° from the centre of the positive charge. In addition, the interaction at muscarinic sites is strongly reinforced by a suitably located alkyl residue. The position of this residue may correspond to that of the methyl group in the acetyl moiety of acetylcholine.

Spectroscopic Studies

Infrared and proton magnetic resonance studies have been used to determine the conformation of cholin-

ergic molecules, particularly in solution. Infrared data (Fellman and Fujita, 1962, 1963, 1966; Martin-Smith et al., 1967; Casy et al., 1971) provides additional evidence that acetylcholine shows a preference for a gauche $^{+}N/O$ conformation.

A p.m.r. study of the solute conformation of acetylcholine in D_2O has also provided good evidence for a preferred gauche conformation (Culvenor and Ham, 1966). Casy et al. (1971) reported some spectroscopic studies of solute conformations of α- and β-methylacetylcholine (2 and 3). They concluded that β-methylacetylcholine (3) displayed marked conformational preference in which a gauche $^{+}N/O$ interaction existed. α-Methylacetylcholine (2) did not exhibit any conformational preference in this study.

By combining the results of p.m.r. studies and molecular rotational data Inch et al. (197) have suggested that β-methylacetylcholine (3) has a similar conformation in solution to that found in the crystalline state. With α-methylacetylcholine (2) the p.m.r., molecular rotational and x-ray data indicate that two or more conformations are equally preferred and the preferred conformation in solution and crystal lattice may differ.

The preceding physico-chemical studies may be summarized as follows:

1. A gauche relationship exists between the $-\overset{+}{N}(CH_3)_3$ and $-OCOCH_3$ groups or their equivalent in the crystal state of most cholinergic compounds.
2. The methyl side of muscarinic agonists and the carbonyl side of nicotinic agonists must be preserved for activity.
3. β-methylacetylcholine (3) appears to have one preferred conformation in the crystal and solution state.
4. α-methylacetylcholine (2) may exist in more than one preferred conformation.

But it must be remembered that although the preceding studies have established the conformation of cholinergic compounds in the solid and solution state there is no direct evidence that these preferred conformations are the preferred conformation at the cholinergic receptor.

Isolation of the Cholinergic Receptor

Several attempts have been made at isolating and

determining the nature of the acetylcholine receptor however the results have not been conclusive. (See reviews by Beychok, 1965; Ehrenpreis, 1967; Garland and Durell, 1970; Nachmansohn, 1970; Smythies, 1970; Turpajev, 1963; Waser, 1963). There is evidence that the receptor for acetylcholine includes protein, calcium ions and phospholipid (Garland and Durell, 1970). De Robertis and De Plazas (1970) have established that in the electroplax membrane the acetylcholinesterase and the acetylcholine proteolipid are different macromolecules. A detailed discussion of this area of research is beyond the scope of this thesis.

The following study was undertaken to investigate further the nature and stereochemical requirements of the muscarinic and nicotinic receptors.

A comparison of 'Framework' molecular models of the reversed esters of acetylcholine and its α- and β-derivatives (4,5 and 6) with the models of their respective acetylcholine analogues (1,2 and 3) suggested that it was possible for all three of the reversed esters to fit at least two of the receptor sites of the muscarinic receptor proposed by Beckett et al (1963a). However, whilst the reversed ester 4 was known to be equipotent with acetylcholine (Bass et al., 1950) the

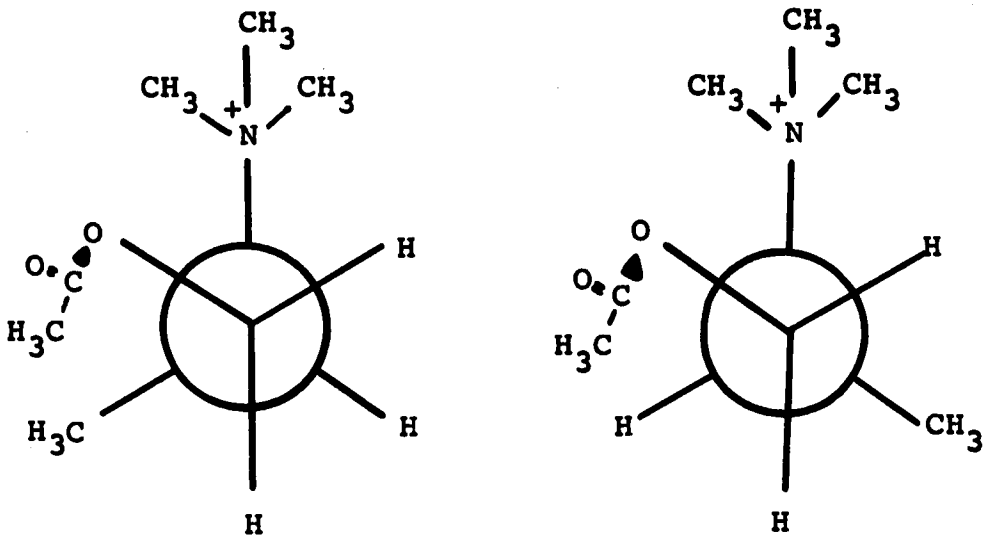
reversed esters of the α- and β-derivatives (5 and 6) had been reported (Schueler et al., 1951, 1951a; Schueler and Keasling, 1951) to have less than 0.0001 of the activity of their respective acetylcholine analogues. It was therefore decided to synthesize these esters (4,5,6) and investigate their pharmacological activity in an attempt to account for this discrepancy. The preliminary results showed that the reversed esters of α- and β-methylacetylcholine (5 and 6) were much more active than reported previously (Biggs et al., 1971).

In view of the activity shown by the reversed esters (5 and 6) it was decided to examine the activity of these compounds further and to synthesize and examine the activity of their optical isomers (5a,5b and 6a, 6b). It was hoped this would lead to a better understanding of the interactions of these compounds with the muscarinic and nicotinic receptors and provide more information about the stereospecificity of the respective receptors. In particular it was hoped to gain information on the importance of methyl substitution at the α- and β-carbon atoms of the reversed esters. The optical isomers of α- and β-methylacetylcholine (2a,2b and 3a,3b) were synthesized and evaluated pharmacologically for comparative purposes.

Newman projections of the compounds investigated are presented in Figures 11 and 12. Although there is no evidence to suggest that these isomers exist in the gauche conformation at the receptor sites, they have been presented in this conformation to make comparisons easier. From these diagramatic representations it was noted that the functional groups of D(+)R-methyl 3-dimethylaminobutyrate methiodide (5a) were in the same relative conformation as those of the more active isomer of α-methylacetylcholine (2a). Similarly L(-)S-methyl 3-dimethylamino-2-methylpropionate methiodide (6a) is related to the active isomer L(+)S-β-methylacetylcholine (3a). If the acetylcholine analogues and their reversed esters act in the same manner at the cholinergic receptors the activity and stereospecificity shown by the optically active esters (5a and 6a) should be related to the activity of the respective more active isomers of α- and β-methylacetylcholine (2a and 3a).

In addition to the above studies the structure activity relationships of a series of esters and derivatives related to the reversed acetylcholine analogues were investigated. The compounds synthesized had the general structures $RR^1R^2\overset{+}{N}CHR^3CHR^4COR^5$ X^- and

α-Methylacetylcholine (2)



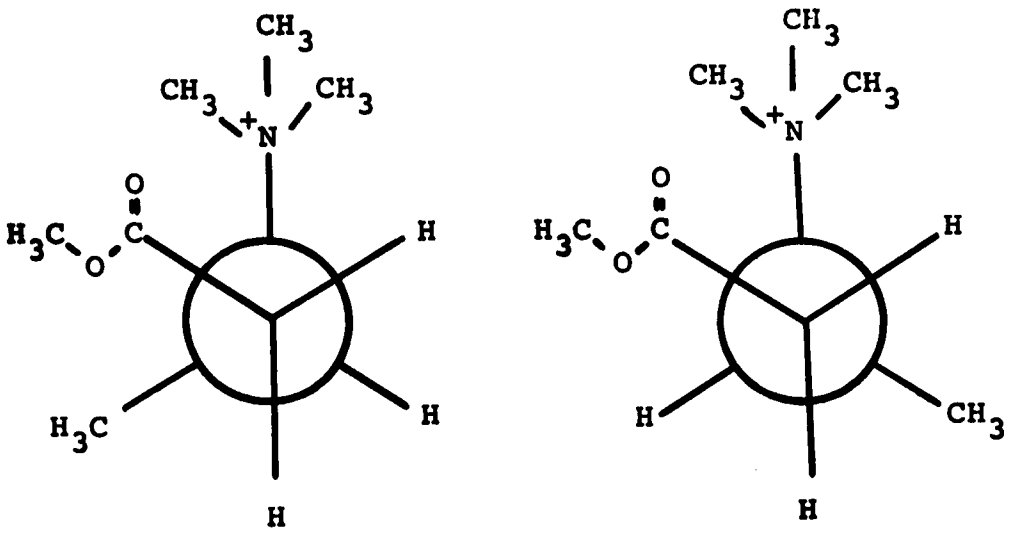
D(+)R-

(2a)

L(-)S-

(2b)

Methyl 3-dimethylaminobutyrate methiodide (5)



D(+)R-

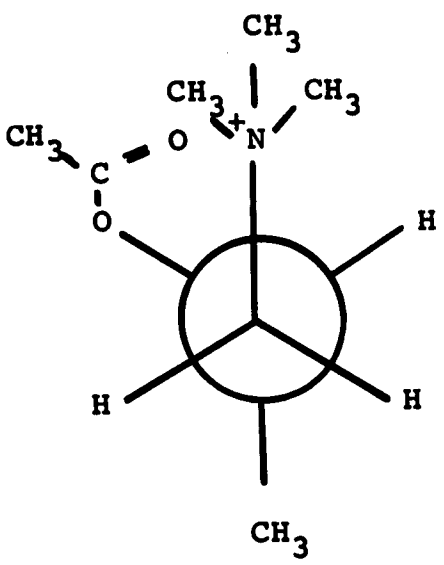
(5a)

L(-)S-

(5b)

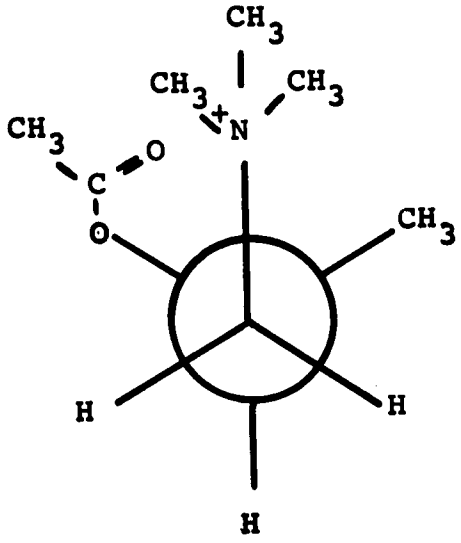
Figure 11: Newman projections of the optical isomers of α-methylacetylcholine (2a,2b) and methyl 3-dimethylamino-butyrate methiodide (5a,5b).

β-Methylacetylcholine (3)



L(+)S-

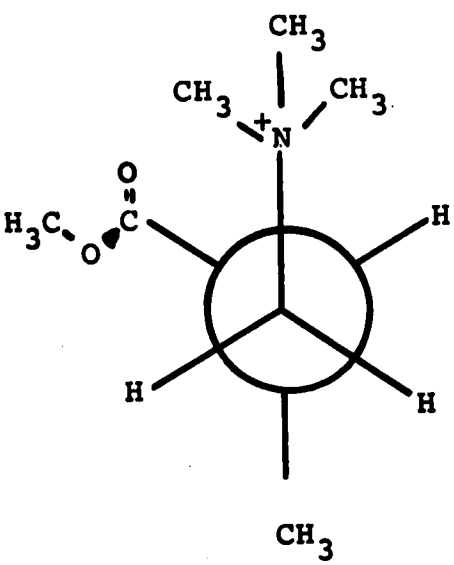
(3a)



D(-)R-

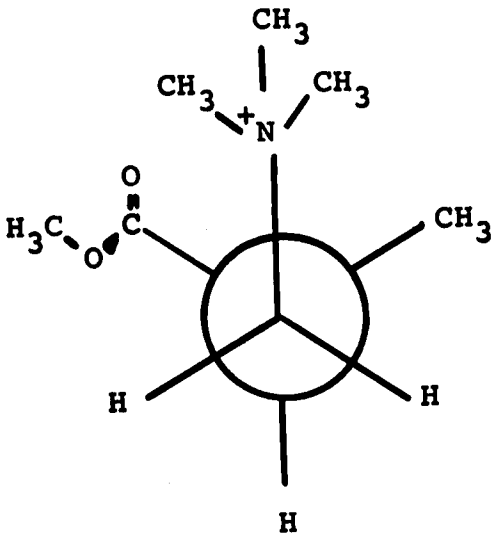
(3b)

Methyl 3-dimethylamino-2-methylpropionate methiodide (6)



L(-)S-

(6a)



D(+)R-

(6b)

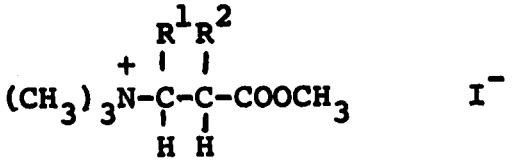
Figure 12: Newman projections of the optical isomers of β-methylacetylcholine (3a,3b) and methyl 3-dimethylamino-2-methylpropionate methiodide (6a,6b).

$RR^1R^2N^+(CH_2)_nCOOR^3$ X^-. Alteration of the 'R' substituents should provide information on the structural requirements for cholinergic activity in these compounds.

CHEMISTRY

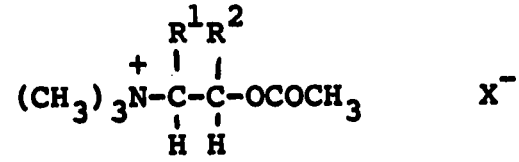
SYNTHESIS OF METHYL 3-DIMETHYLAMINO-PROPIONATE AND -BUTYRATE METHIODIDES

The tertiary amino-esters required for the synthesis of the racemic methiodides were prepared by condensation of anhydrous dimethylamine with methyl acrylate (Barrass et al., 1968), methyl crotonate (Adamson, 1950) or methyl methacrylate (Coutts et al., 1971) to give methyl 3-dimethylaminopropionate (39), methyl 3-dimethylaminobutyrate (40) and methyl 3-dimethylamino-2-methylpropionate (41) respectively. The mechanism of this addition will be discussed later. (See page 84 .) These tertiary amino-esters were then quaternized with methyl iodide (Barrass et al., 1968). The colorless crystalline salts; methyl 3-dimethylaminopropionate methiodide (4), methyl 3-dimethylaminobutyrate methiodide (5) and methyl 3-dimethylamino-2-methylpropionate methiodide (6), were found to be stable over a prolonged period of time.



	R^1	R^2
(4)	H	H
(5)	CH_3	H
(6)	H	CH_3

These quaternary amino-esters can be regarded as being the reversed esters (RE) of acetylcholine (1), α-methylacetylcholine (2) and β-methylacetylcholine (3) respectively. The structures of the racemates were



	R^1	R^2	X^-
(1)	H	H	Cl^-
(2)	CH_3	H	I^-
(3)	H	CH_3	Cl^-

verified by microanalysis, i.r., p.m.r. and mass spectrometry. The i.r. spectra of each compound had a carbonyl band within the range 1730-1745 cm^{-1} consistent with

that expected for an ester. The p.m.r. spectra were as predicted; the most diagnostic peaks being those due to the $-\overset{+}{N}(C\underline{H}_3)_3$ and $-COOC\underline{H}_3$ protons. The mass spectra of each compound was analyzed in detail.

(See page 92)

There are basically two different methods which can be employed in the synthesis of optical isomers. The first involves the stereospecific synthesis from optically pure material of known configuration (Mislow, 1966). The second method involves the combination of racemic material with a dissymmetric reagent producing diasteriomers which can usually be separated by fractional crystallization (Mislow, 1966a). Using this second method it is not possible to determine the absolute configuration of the isomers obtained. This must be done by relating the isomers through stereospecific reactions to compounds of known configuration or by using physical methods such as x-ray diffraction.

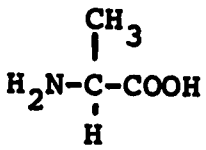
There are several systems of configurational nomenclature in use. The oldest one is applicable to molecules of the type $RCHXR^1$, where $R\text{-}C\text{-}R^1$ constitutes the main chain of the molecule in the sense of the International Union of Chemistry nomenclature. The molecule is so orientated that the No. 1 carbon of the

main chain is at the top in a Fischer projection formula. Then if X is on the right, the molecule is called "D", if X is on the left the molecule is called "L" (Eliel, 1962). This system has certain disadvantages since before a unique name can be established for a compound it is necessary to specify how its projected formula must be orientated. These difficulties are avoided by a system which is based on the actual three-dimensional formula of the compound to be named (Cahn, 1964). The symbols employed in this system are R (right) and S (left). In order to name a compound Xabcd (X - asymmetric atom) the groups are arranged in order of priority sequence according to the sequence rules (Cahn, 1964; Eliel, 1962). The molecule is then viewed from the side remote from d. If a→b→c traces a clockwise turn the configuration is R, if anticlockwise the configuration is S. Both systems will be used in naming the isomers synthesized.

D(+)R- and L(-)S-Methyl 3-dimethylaminobutyrate methiodide (5a and 5b)

The optical isomers of this reversed ester of α-methylacetylcholine were prepared by a stereospecific

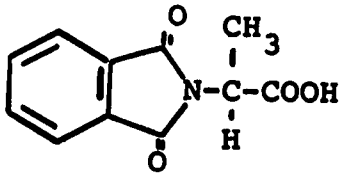
synthesis from D(-)- and L(+)-alanine (42a and 42b). (See Scheme 1.)



D(-)- and L(+)-alanine

(42a and 42b)

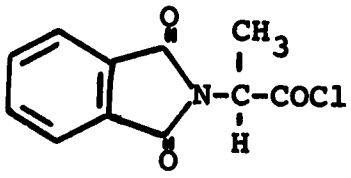
phthalic anhydride



D(+)- and L(-)-N-phthaloylalanine

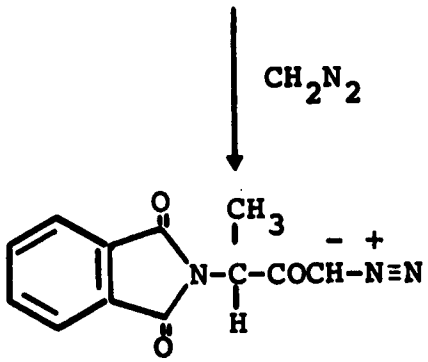
(43a and 43b)

$SOCl_2$



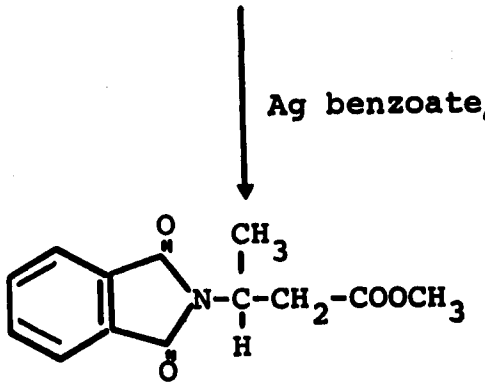
D(+)- and L(-)-N-phthaloylalanyl chloride

(44a and 44b)



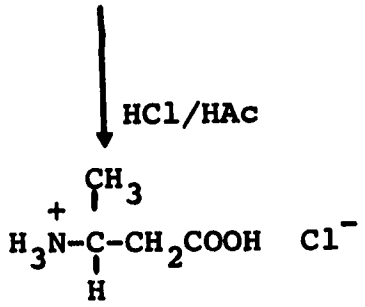
D(+)- and L(-)-1-diazo-3-phthalimidobutan-2-one

(45a and 45b)



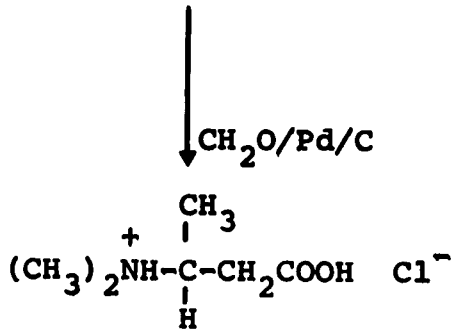
D(-)- and L(+)-methyl 3-phthalimidobutyrate

(46a and 46b)



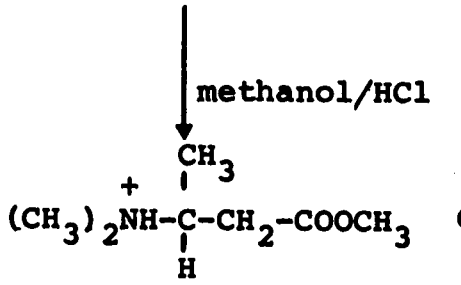
3-aminobutyric acid hydrochloride

(47a and 47b)



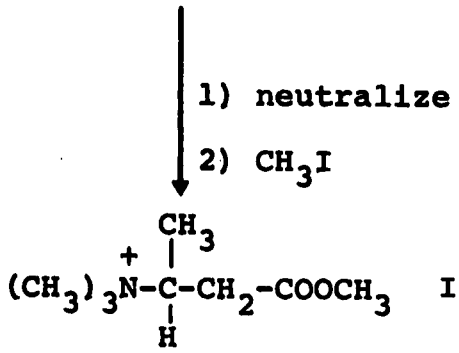
3-dimethylaminobutyric acid hydrochloride

(48a and 48b)



methyl 3-dimethylaminobutyrate hydrochloride

(49a and 49b)

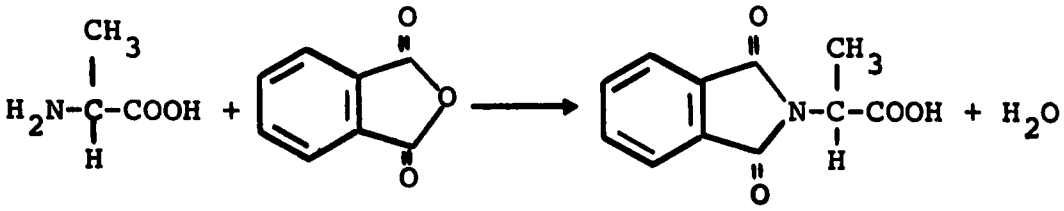


D(+)R- and L(-)S-methyl 3-dimethylaminobutyrate methiodide

(5a and 5b)

Scheme 1

3-Aminobutyric acid (15a and 15b) was synthesized from alanine (10a and 10b) by application of an Arndt-Eisert reaction essentially according to the methods developed by Balenovic et al. (1952) and Beckett and Casy (1955). The amino group of alanine was first protected by condensation with phthalic anhydride according to the method of Billman and Harting (1948). The amino group was thus protected from attack by the

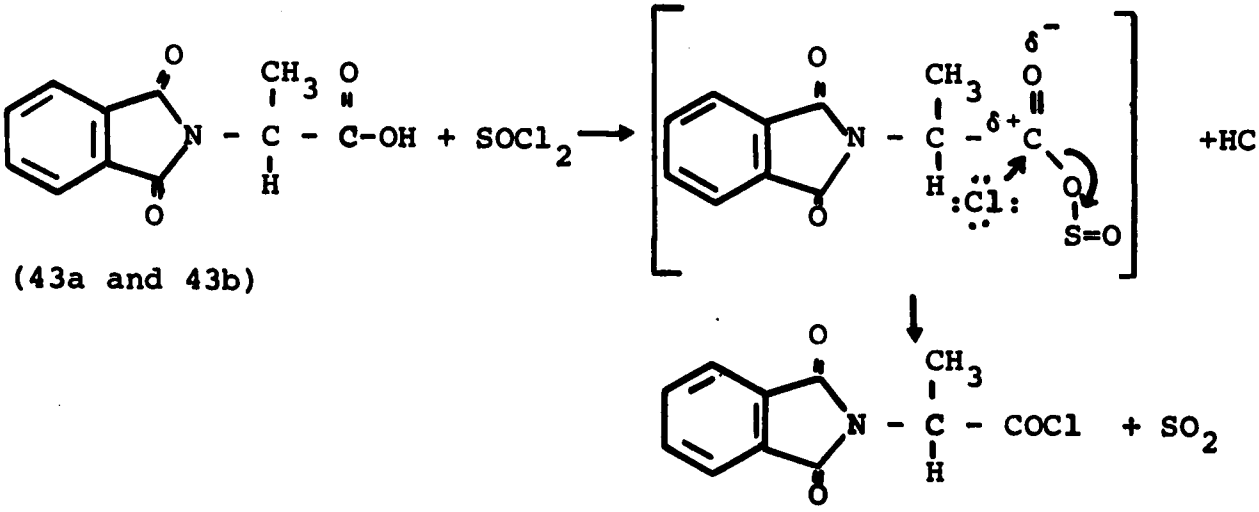


(42a and 42b) (43a and 43b)

reagents used in subsequent reactions. The basic function was suppressed and the derivative behaved like a normal carboxylic acid. It was found that this product must be thoroughly dried before proceeding to subsequent reactions.

N-phthaloylalanine (43a and 43b) was converted to N-phthaloylalanyl chloride (44a and 44b) by means of thionyl chloride. It has been proposed that the formation of the acid chloride occurs in two steps (Roberts and Caserio, 1964). The first step involves the formation of an unstable mixed anhydride which collapses

with the attack of the chloride ion at the carbonyl carbon. The acid chloride should be stored under vacuum

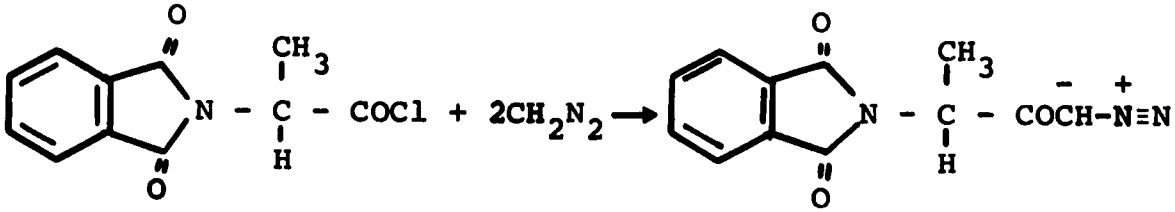


(43a and 43b)

(44a and 44b)

overnight to remove all traces of hydrogen chloride and sulfur dioxide and to prevent atmospheric moisture from decomposing the product to the starting acid.

The dried acid chloride (44a and 44b) was converted into the diazoketone (45a and 45b) using an ethanol-free solution of diazomethane. An excess of diazomethane was necessary for the reaction to proceed to

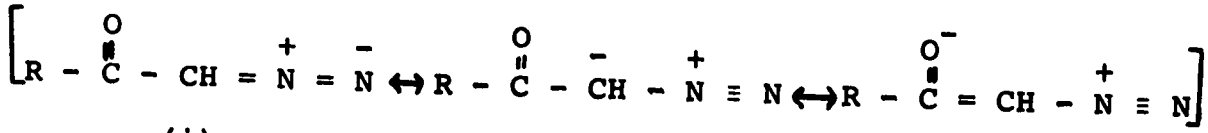


(44a and 44b)

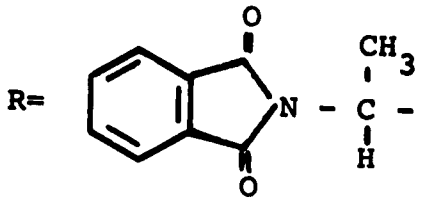
(45a and 45b)

$+ N_2 + CH_3Cl$

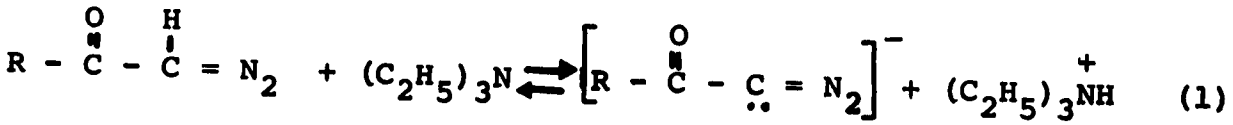
completion. The resulting diazoketone exists as a resonance hybrid (Cram and Hammond, 1964). Infrared evidence (strong band at 2100 cm^{-1}, due to N≡N, Dyer (1965)) indicated that (ii) was a major contributor to the



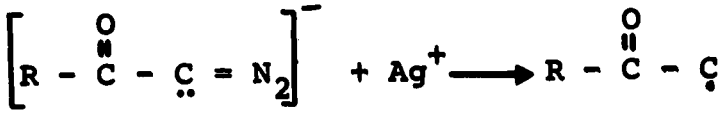
(i) (ii) (iii)



hybrid. Infrared evidence also indicated that the diazoketone decomposed after standing for several hours. The band at 2100 cm^{-1} in the infrared spectrum gradually disappeared. The ketone was therefore immediately rearranged without any attempt at purification using a modification of the Wolff rearrangement (Newman and Beal, 1950) to methyl 3-phthalimidobutyrate (46a and 46b). The reagent used in this reaction consists of a solution of silver benzoate in triethylamine. Newman and Beal proposed that the reaction occurs as follows:

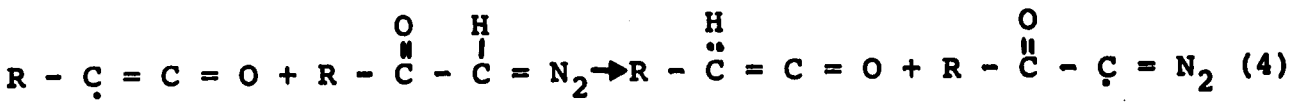


(45a and 45b) (1)

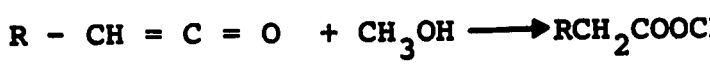


(2)

(3)

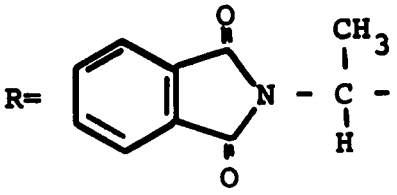


(4)

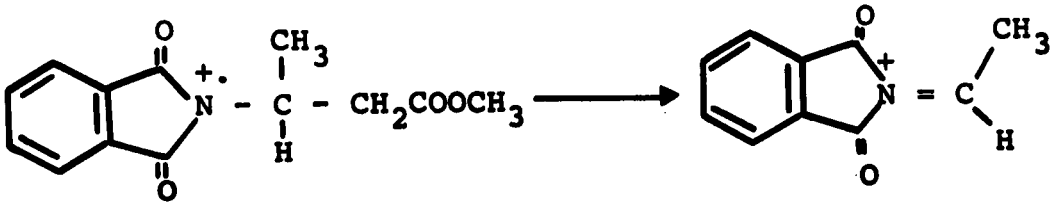


(5)

(46a and 46b)



The reaction has been shown to proceed with retention of configuration (Ingold, 1953). The structure of the ester was verified by mass spectrometry. The base peak in the mass spectrum was due to α-cleavage of the molecular ion.



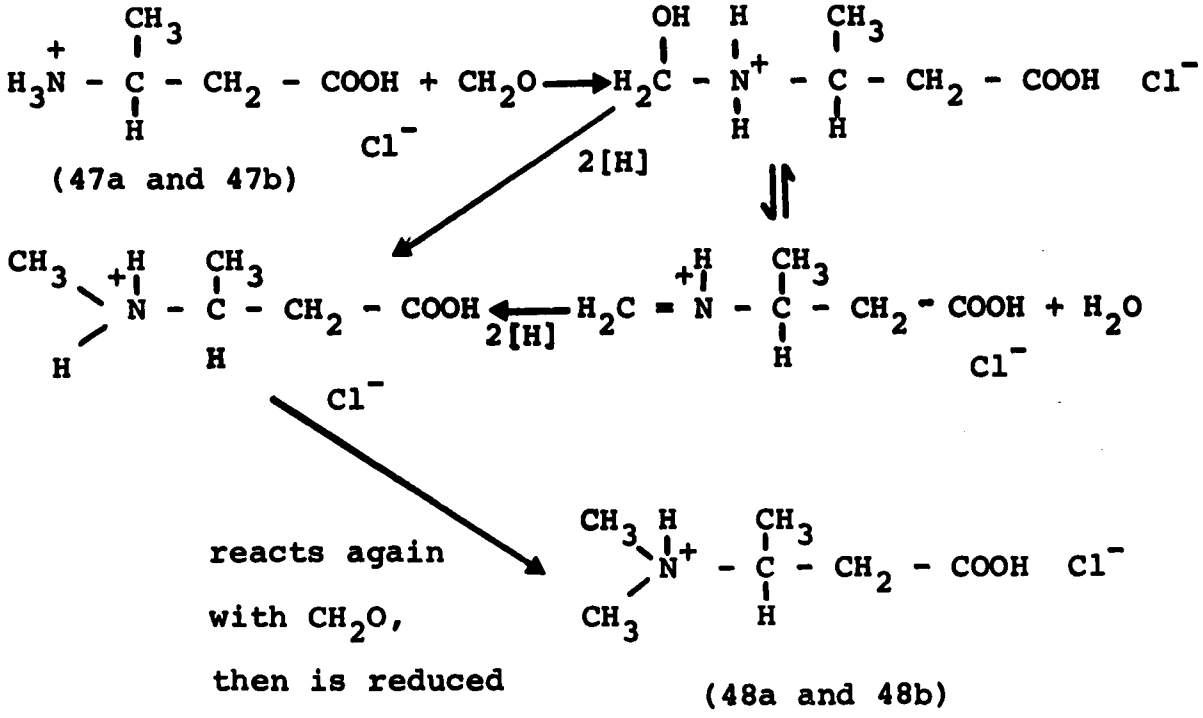
m/e 247

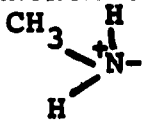
m/e 174

(46a and 46b)

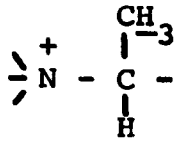
(50)

The ester (46a and 46b) was then hydrolyzed to 3-aminobutyric acid hydrochloride (47a and 47b) using a mixture of concentrated hydrochloric acid and glacial acetic acid. The acid hydrochloride was reductively methylated according to the method of Bowman and Stroud (1950). The mechanism of this reduction has been reviewed by Emerson (1948). It is probably the Schiffs base which is reduced.



As pointed out by Bowman and Stroud (1950), there was no evidence of formation of the monomethylated derivative, (no evidence of  structure in the p.m.r. spectrum).

Following established procedures the dimethylaminobutyric acid hydrochloride (48a and 48b) was then esterified, neutralized and quaternized to give the optically active D(+)R- and L(-)S-methyl 3-dimethylaminobutyrate methiodide (5a and 5b), $[\alpha]_D^{26.5}$ +7.33° (c 2.51 90% ethanol) and $[\alpha]_D^{26.5}$ -7.85° (c 5.15 90% ethanol) respectively. The limiting step in the synthesis appeared to be the extraction of methyl 3-dimethylaminobutyrate, obtained by neutralization of its hydrochloride salt (49a and 49b), from aqueous solution. Despite the fact that the aqueous solution was saturated with potassium carbonate the amino-ester was extracted in less than 50% yield. The structure of each optical isomer was verified by microanalysis, i.r., p.m.r. and mass spectrometry. The i.r spectra showed a carbonyl band at 1740 cm^{-1} as expected for an ester. The p.m.r. spectra were as predicted with the $-\overset{+}{N}(CH_3)_3$ and $-COOCH_3$ protons at 6.86 or 6.84 and 6.21τ respectively. The doublet at 8.51 and 8.50τ respectively due to

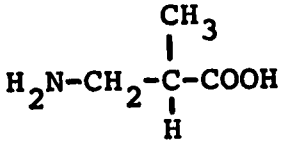


protons appeared as a doublet of triplets in the p.m.r. spectra as a result of additional coupling of the β-methyl protons with the ^{14}N (Kawazoe et al., 1967). The mass spectra exhibited a similar pattern to

that described in detail for the racemate. (See page 94.)

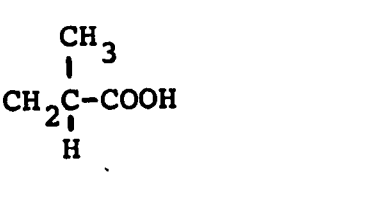
L(-)S- and (D(+)R-Methyl 3-dimethylamino-2-methylpropionate methiodide (6a and 6b)

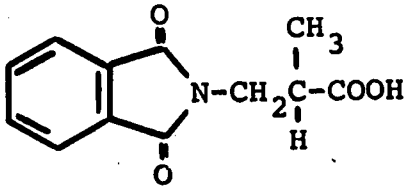
The isomers of this ester were prepared by resolution of racemic material with a dissymmetric reagent. (See Scheme 2.)



3-dimethylamino-2-methylpropionic acid

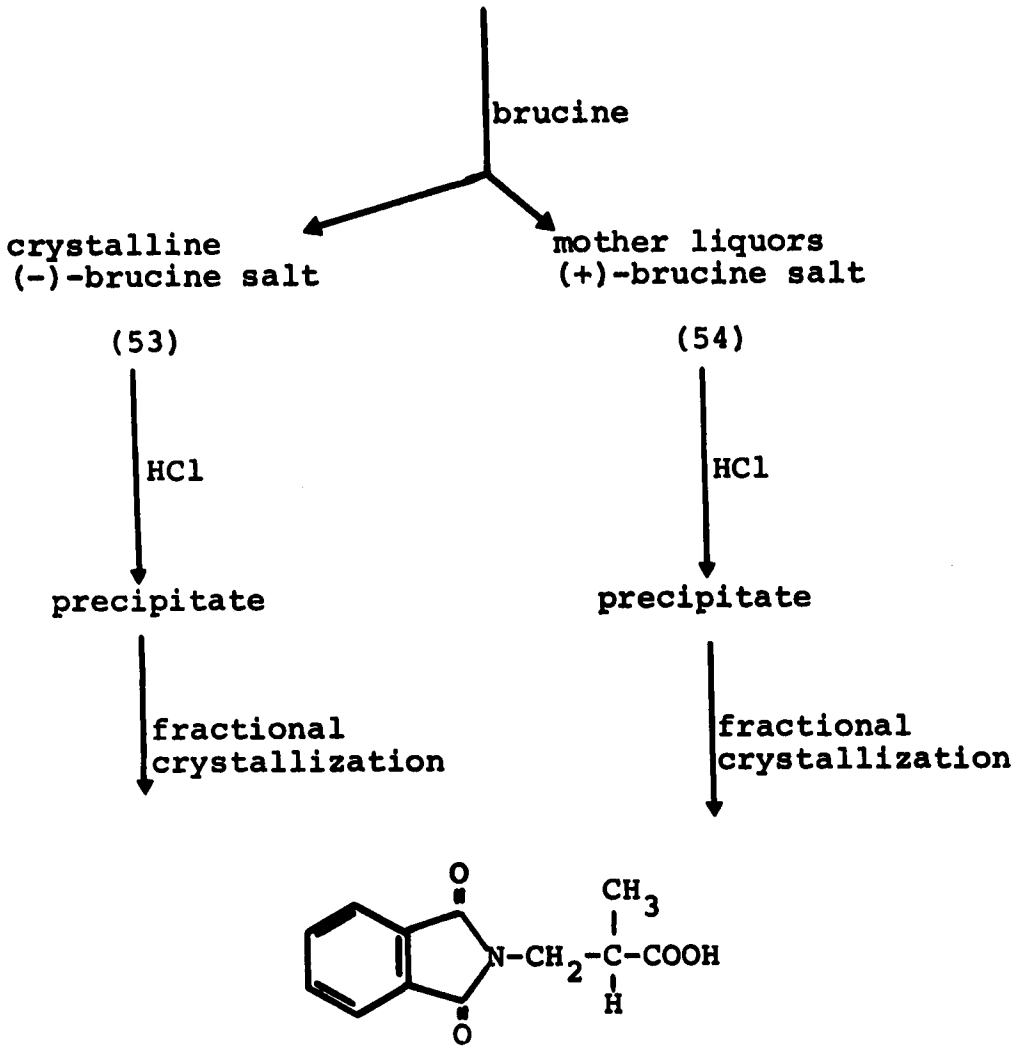
(51)





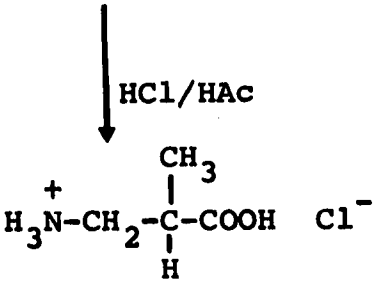
2-methyl-3-phthalimidopropionic acid

(52)



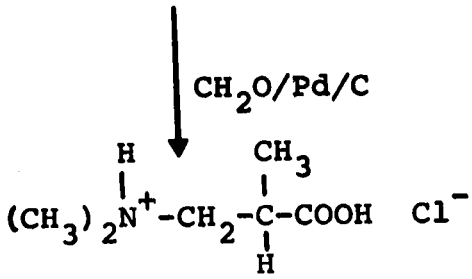
(-)- and (+)-2-methyl-3-phthalimidopropionic acid

(55a and 55b)



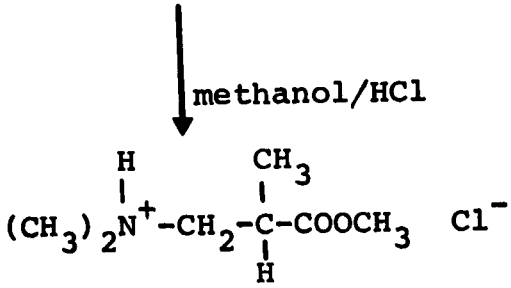
(-)- and (+)-3-amino-2-methylpropionic acid hydrochloride

(56a and 56b)



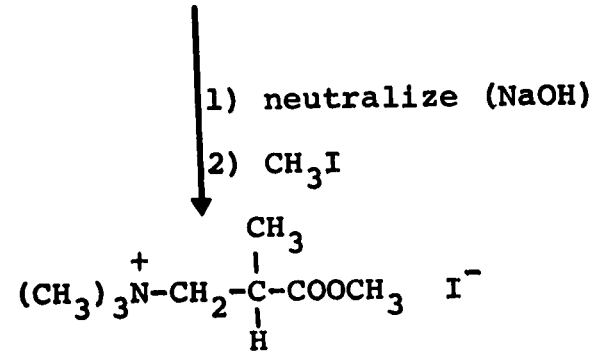
3-dimethylamino-2-methylpropionic acid hydrochloride

(57a and 57b)



methyl 3-dimethylamino-2-methylpropionate hydrochloride

(58a and 58b)



L(-)S- or D(+)R-methyl 3-dimethylamino-2-methylpropionate methiodide

(6a and 6b)

Scheme 2

2-Methyl-3-phthalimidopropionic acid (52) was resolved into its optical isomers using brucine. The (-)-brucine salt (53) was found to crystallize in almost 50% yield and had $[\alpha]_D^{25}$ -35.86° (c 2.20 chloroform). This result was in reasonable agreement with the literature values: $[\alpha]_D^{22}$ -39.7° (c 1.50 chloroform) (Beckett et al., 1962); [α] -44.2° (Balenovic and Bregant, 1959). The (-)-brucine salt (53) was neutralized with hydrochloric acid and the resulting precipitate fractionally crystallized three times to constant rotation. The resulting (-)-2-methyl-3-phthalimidopropionic acid (55a) had $[\alpha]_D^{25}$ -3.93° (c 1.37 chloroform). This rotation is much lower than that previously quoted in the literature: $[\alpha]_D^{21}$ -20.1° (c 1.5 chloroform) (Beckett et al., 1962); $[\alpha]_D^{17}$ -24.4° (c 0.98 chloroform) (Balenovic and Bregant, 1959). The product obtained by these workers may have been contaminated with brucine which has $[\alpha]_D$ -230° (chloroform).

When the mother liquors remaining after removal of the above (-)- brucine salt (53) were neutralized with hydrochloric acid, there resulted a precipitate of (+)-2-methyl-3-phthalimidopropionic acid (54) which when fractionally crystallized three times had $[\alpha]_D^{25}$ +3.36° (c 1.22 chloroform). It was therefore assumed that

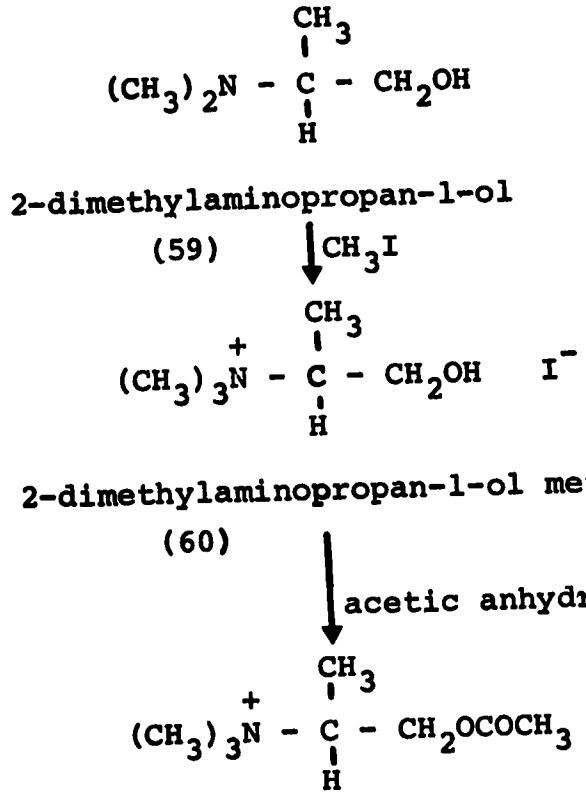
complete separation of the isomers had occurred.

The isomers of 2-methyl-3-phthalimidopropionic acid (55a and 55b) were treated in a similar manner to that described for methyl 3-phthalimidobutyrate (46). L(-)S- and D(+)R-Methyl 3-dimethylamino-2-methylpropionate methiodide (6a and 6b) had $[\alpha]_D^{25}$ -2.50° (c 1.92 90% ethanol) and $[\alpha]_D^{24.5}$ +2.25° (c 1.90 90% ethanol) respectively. The structures of the isomers were confirmed by microanalysis, i.r., p.m.r. and mass spectrometry. The i.r. spectra had two bands at 1715 and 1730 cm^{-1} (ester C=O) when recorded in Nujol. However, when the spectra were run again in DMSO there was only one band at 1730 cm^{-1}. It was therefore assumed that the ester had crystallized in two forms. The existence of two crystalline forms also explains the lower m.p. of 93-94° as compared to the racemate which had a m.p. 113-115°. The p.m.r. spectra were identical to that of the racemate. The mass spectra exhibited a similar pattern to that described for the racemate. (See page 94.)

Beckett et al. (1962) related (-)-3-dimethylamino-2-methylpropionic acid (57a) obtained from the (-)-brucine salt to S(-)-isomethadone and it was on this basis that the absolute configuration of the two isomers was assigned.

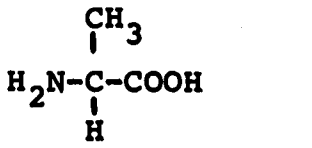
SYNTHESIS OF 2-DIMETHYLAMINO- ETHYL AND -PROPYL ACETATES

Acetylcholine chloride (2- dimethylaminoethyl acetate methochloride) (1) and β-methylacetylcholine chloride (1-dimethylaminoprop-2-yl acetate methochloride) (3) were obtained commercially. α-Methylacetylcholine (2) (2-dimethylaminopropyl acetate methiodide) was synthesized from 2-dimethylaminopropan-1-ol (59) according to the method of Beckett et al. (1963). (See Scheme 3.)



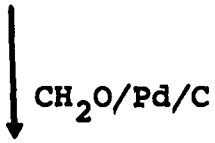
Scheme 3

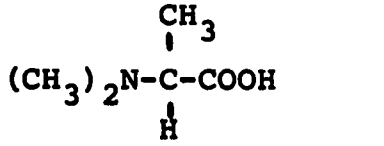
The optical isomers of α- and β-methylacetylcholine (2 and 3) were synthesized using literature procedures. The isomers of α-methylacetylcholine (2) were prepared from D(-)- or L(+)-alanine (42a and 42b) according to the method of Beckett et al. (1963) which is summarized in Scheme 4. The rotations of the two isomers were in good agreement with those reported in the literature. D(+)R- and L(-)S-2-Dimethylaminopropyl acetate (2a and 2b) had $[\alpha]_D^{22}$ +9.30° (c 5.14 90% ethanol) and $[\alpha]_D^{21}$ -9.60° (c 5.04 90% ethanol) respectively.



D(-)- and L(+)- alanine

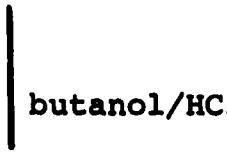
(42a and 42b)

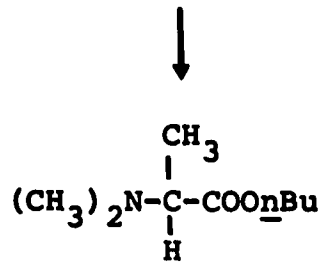




D(-)- L(+)-2-dimethylaminopropionic acid

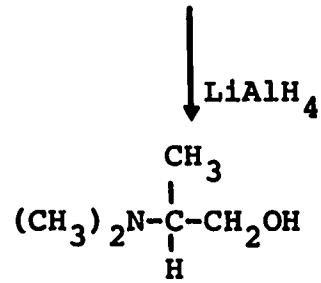
(61a and 61b)





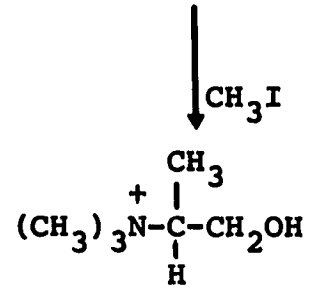
butyl 2-dimethylaminopropionate

(62a and 62b)



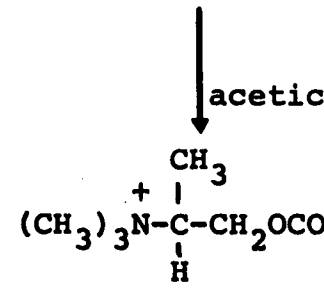
2-dimethylaminopropan-1-ol

(59a and 59b)



2-dimethylaminopropan-1-ol methiodide

(60a and 60b)

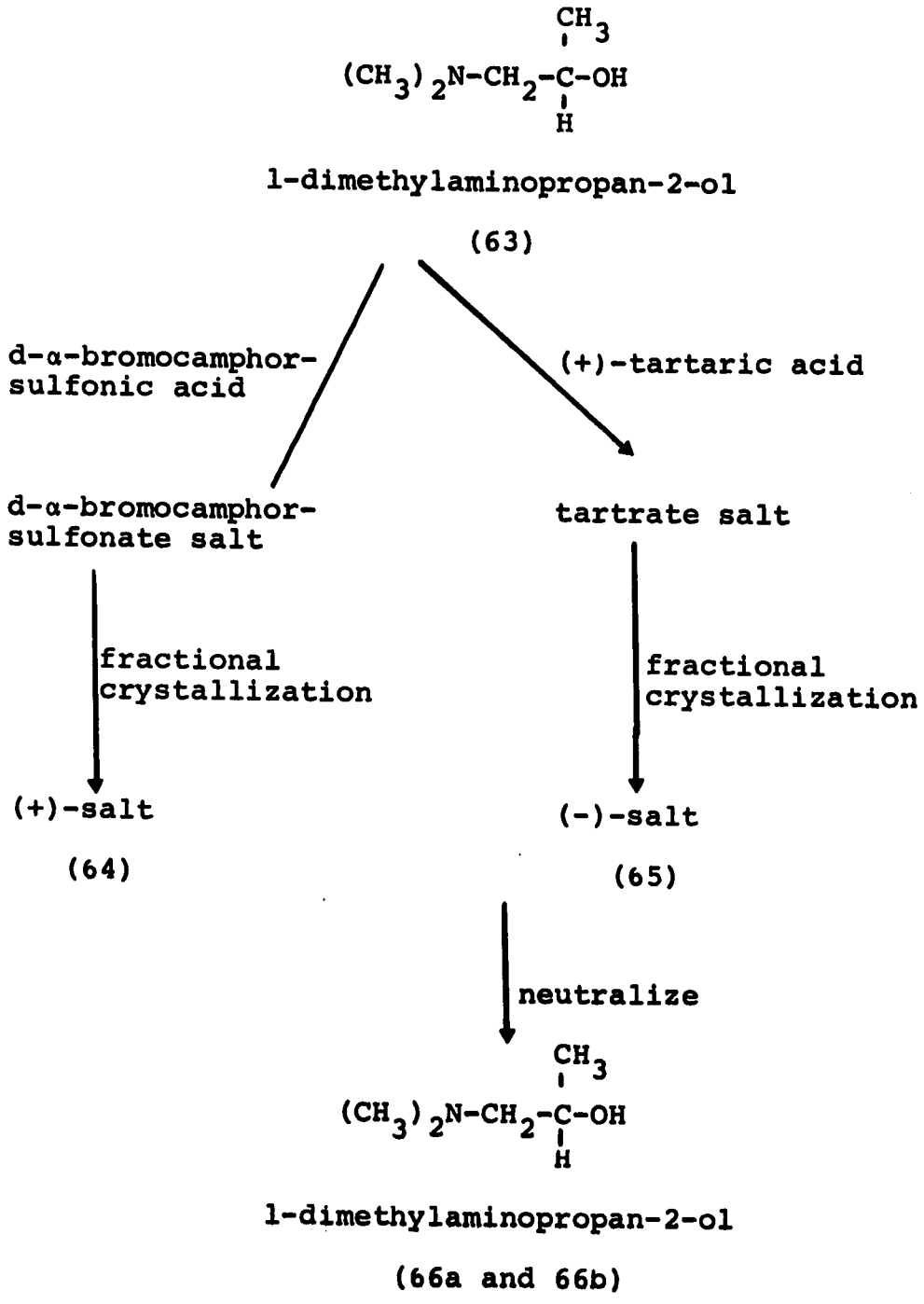


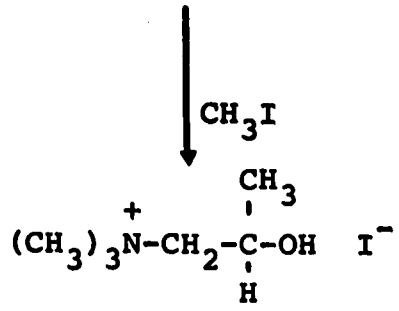
D(+)R- and L(-)S-2-dimethylaminopropyl acetate methiodide

(2a and 2b)

Scheme 4

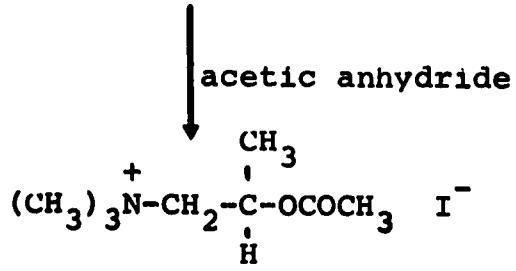
The isomers of β-methylacetylcholine (3) (1-dimethylaminoprop-2-yl acetate methiodides) were separated according to the method of Major and Bonnett (1935). (See Scheme 5.)





1-dimethylaminopropan-2-ol methiodide

(67a and 67b)



L(+)S- and D(+)R-1-dimethylaminoprop-2-yl acetate methiodide

(3a and 3b)

Scheme 5

The absolute configuration of these isomers was assigned on the basis of the work of Beckett et al., (1963) who correlated the L(+)S-isomer to S-lactic acid by a stereospecific synthesis. The rotation of the L(+)S-isomer (3a), $[\alpha]_D^{26.5}$ +25.85° (c 2.04 90% ethanol), was in good agreement with literature values. The rotation of the D(-)R-isomer (3b), $[\alpha]_D^{22.5}$ -26.60° (c 2.15 90% ethanol) was also in agreement with literature values despite the fact that the intermediate tartrate salt did not attain the specific rotation reported by Beckett et al. (1963). The tartrate salt (65) obtained in the present synthesis had $[\alpha]_D^{25}$ -3.15° (c 10.87 H_2O) whereas literature values were $[\alpha]_D^{22.5}$ -10.84° (c 4.8 H_2O) (Beckett et al., 1963); $[\alpha]_{589.3}^{25}$ +0.5° (c 10.0 H_2O) (Cocolas et al., 1971). In addition, Major and Bonnett (1935) have reported an $[\alpha]_D$ value = -10.7° without stating solvent or temperature.

The structures of all the acetylcholine analogues were verified by microanalysis, i.r., p.m.r. and mass spectrometry. Each isomer had a band 1730-1745 cm^{-1} in its i.r. spectrum due to the ester C=O. Although the resolution of the p.m.r. spectra was not good the spectra could be considered to be identical to those reported for the racemates (Casy et al., 1971).

PREPARATION OF AMINO-ESTERS

Most of the esters required for this study were prepared by one of two general methods i.e. condensation of the appropriate amine with the required acrylate or with ethyl 4-bromobutyrate.

The following esters with the general formula $R\text{-}CHR^1\text{-}CHR^2\text{-}COOCH_3$ were prepared in good yield by condensation of the appropriate amine with methyl acrylate, methyl crotonate or methyl methacrylate (Table IV).

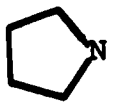
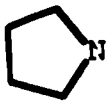
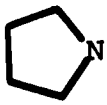
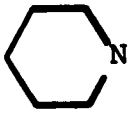
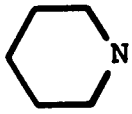
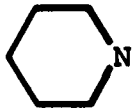
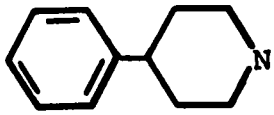
Table IV

Methyl 3-Amino-propionates and -butyrates

$R\text{-}CHR^1\text{-}CHR^2\text{-}COOCH_3$

Compound	R	R^1	R^2	% Yield
39	$(CH_3)_2N$	H	H	quantitative
40	$(CH_3)_2N$	CH_3	H	quantitative
41	$(CH_3)_2N$	H	CH_3	77.7
68	$(CH_3CH_2)_2N$	H	H	quantitative
69	$(CH_3CH_2)_2N$	CH_3	H	5.3
70	$(CH_3CH_2)_2N$	H	CH_3	43.4
71	$(CH_3CH_2CH_2)_2N$	CH_3	H	39.6

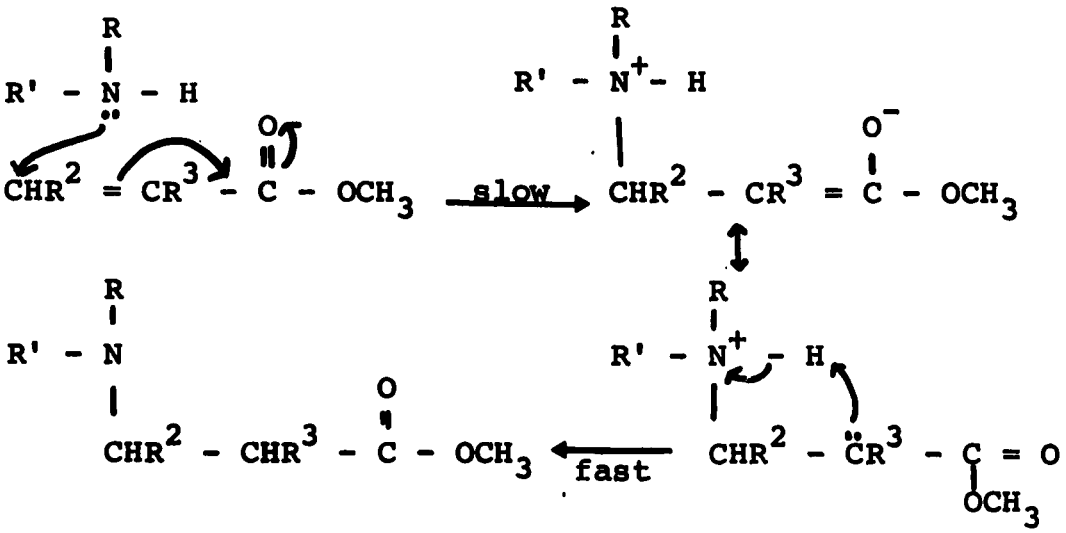
Table IV cont'd

72		H	H	82.7
73		CH_3	H	74.1
74		H	CH_3	88.8
75		H	H	61.2
76		CH_3	H	55.9
77		H	CH_3	59.6
78		CH_3	H	30.0

Methanol was used as the solvent for the reactions to prevent the possibility of transesterification of the ester occurring. In order to prevent amide formation the reactions were initially stirred at 0°. Where the reaction failed to proceed at this temperature the reaction mixture was heated under reflux for the minimum length of time. Bieber (1954) and Pfau (1967) showed that an increase in temperature increased the amount

of amide formation, and Coutts et al. (1971) showed that prolonging the reaction time also increased amide formation.

A mechanism of this nucleophilic addition was suggested by Pfau in 1967. Pfau indicated that the steric requirements of the amine are important in the



reaction. He was able to condense n-propylamine with ethyl acrylate but the reaction with isopropylamine failed to proceed. Similar results were obtained in our experiments, as the effective size of the quaternary amino group increased the yield decreased. The steric requirements of the acrylate ester are also important. Howton, in 1945, concluded that the esters of acylic acid exhibited a greater tendency to combine with primary and secondary bases than do the corres-

ponding esters of methacrylic acid. He was able to condense aniline with methyl acrylate but not with methyl methacrylate. Di-n-butylamine added to ethyl acrylate but not to ethyl methacrylate. Friedman and Wall (1966) made a more extensive study of the effect of introduction of methyl substituents near the reactive site of vinyl compounds and the subsequent reduction in their rate of reactivity with amino compounds. They concluded that this reduction might be due mainly to steric factors associated with the methyl groups. The lower rate of reaction of methyl crotonate as compared to methyl acrylate must be due to the additional steric requirements of the methyl group as well as to its inductive effect which results in an altered electron density at the reactive site. It has been suggested by these workers that the difference in rates of reactivity may also be due to a difference in the stability of the carbanion involved in the transition state of the reaction. (See Table V.)

Table V

Transition State Involved in the Reaction of Acrylates with Secondary Amines

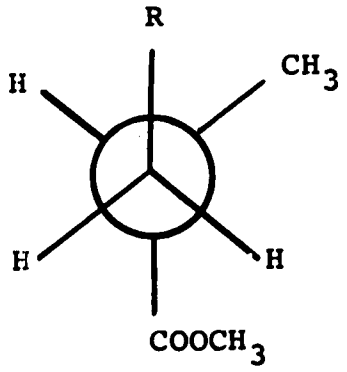
Acrylate	Transition State	Type of Carbanion
methyl acrylate	$RR'\overset{+}{N}H-CH_2-\overset{-}{C}H-COOCH_3$	secondary
methyl methacrylate	$RR'\overset{+}{N}H-CH_2-\overset{-}{C}(CH_3)-COOCH_3$	tertiary
methyl crotonate	$RR'\overset{+}{N}H-CHCH_3-\overset{-}{C}H-COOCH_3$	secondary

The transition state of methyl acrylate and methyl crotonate consists of a secondary carbanion whereas methyl methacrylate has an intermediate tertiary carbanion which is less stable. The slower rate of reaction of methyl methacrylate with the amine is due primarily to electronic effects. The steric factors of methyl methacrylate are less important than those of methyl crotonate. This was also shown in our experiments where condensation of the amines occurred more readily with methyl methacrylate than with methyl crotonate. (See Table IV.) The authors also showed that the polar and steric factors associated with the vinyl and amino compounds make independent contributions to observed reactivities of the acrylates.

The hydrochlorides of these amino-esters were prepared using a literature procedure (Coutts et al., 1971) by reacting the free amino bases with an ethereal solution of dry hydrogen chloride. All the salts were obtained in quantitative yield and were stable over an extended period of time.

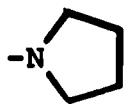
The methiodides were prepared according to the method of Barrass et al. (1968) by addition of an excess of methyl iodide in acetone to an acetone solution of the free amino-ester and subsequent crystallization of the salt from acetone/diethyl ether.

The methiodides were stable for a prolonged period of time if thoroughly dried and stored in a desiccator. The presence of moisture appeared to accelerate decomposition and the liberation of free iodine. It is noteworthy that it was not possible to form the methiodides of methyl 3-diethylamino-2-methyl-propionate (70) or methyl 2-methyl-3-(1-pyrrolidino) propionate (74) probably due to steric hindrance although the corresponding piperidino compound (77) readily formed a methiodide.



R

70 $-N(CH_2CH_3)_2$

74 

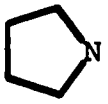
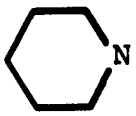
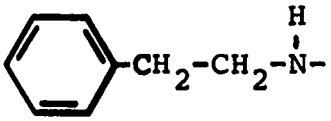
77 

The ethyl 4-aminobutyrates studied were prepared by condensation of the appropriate amine with ethyl 4-bromobutyrate. The following esters were prepared for this study (Table VI).

Table VI

Ethyl 4-aminobutyrates

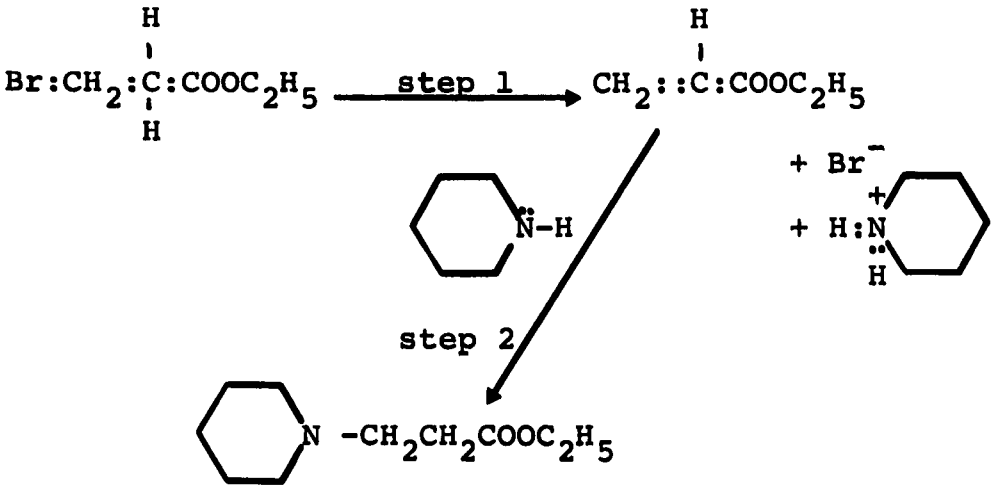
$R-(CH_2)_3-COOCH_2CH_3$

Compound	R	% Yield
79	$(CH_3)_2N-$	quantitative
80	$(CH_3CH_2)_2N-$	12.5
81	$(CH_3CH_2CH_2)_2N-$	26.7
82		11.2
83		77.8
84		49.2

It was found that the best results were obtained when three moles of the required amine were used per mole of ester. As the reaction proceeded the amine hydrobromide precipitated out of solution in quantitative yield.

This salt was filtered off before the required ethyl 4-aminobutyrate was isolated.

In 1934, Drake and colleagues proposed that the reaction of organic amines with bromo-esters occurred in two steps. These workers studied the reaction of piperidine with β-bromoesters. They envisaged the first step as removal of a proton from the α-carbon of the ester by the unshared electrons of the piperidine nitrogen followed by release of the bromide ion. This mechanism explains the observation that one mole of



amine hydrobromide was formed per mole of ester. The yield depends upon the basic strength of the amine used and on steric factors. Although diethylamine, pyrrolidine and piperidine have essentially the same basicity the pyrrolidine derivative was obtained in greater yield due to its lesser steric requirements.

Two dimethylaminoacetates were prepared in this study (Table VII). They were obtained in a similar manner to the 4-aminobutyrates.

Table VII

Dimethylaminoacetates

$(CH_3)_2N-CH_2-\overset{O}{\overset{\|}{C}}-OR$

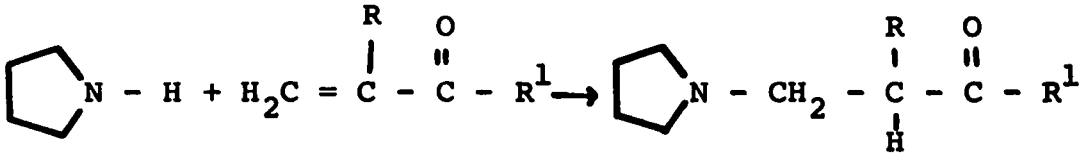
Compound	R	Yield
85	CH_3-	5.0
86	CH_3CH_2-	31.3

It is noteworthy that yields were much lower than with the ethyl 4-aminobutyrates. This may be due to lack of the α-hydrogen in these compounds. If more vigorous conditions were used in an attempt to increase the yield of the amino-ester, the corresponding amide was obtained as the sole product.

The amino-butyrates and -acetates formed stable hydrochlorides and methiodides in quantitative yield.

The two amides and the ketone required in this study were prepared by addition of pyrrolidine to the unsaturated amide of ketone. The addition of the amine to the amide or ketone proceeded at a much slower rate

than the similar addition to an unsaturated ester. The products were obtained in very poor yields, each one in fact being obtained in less than 5% yield.



Compound	R	R^1
87	H	NH_2
88	CH_3	NH_2
89	CH_3	CH_3

The hydroxamic acid, 2-methyl-3-(1-pyrrolidino)-propionohydroxamic acid hydrochloride (90), was prepared according to the method of Coutts et al. (1969). Ethyl 4-(N-methyl-N-phenethyl)aminobutyrate hydriodide (91) was obtained when an acetone solution of methyl iodide was added to an ethereal solution of 4-phenethylaminobutyrate (84).

MASS SPECTROMETRY

Since our experiments (see pharmacology section) showed that the esters methyl 3-dimethylaminobutyrate methiodide (5) and 3-dimethylamino-2-methylpropionate methiodide (6) were more potent than reported previously (Schueler and Kealing, 1951), it was considered necessary to verify the structures of our compounds (4, 5, 6) using mass spectrometry. (See Figure 13.)

Quaternary methyl iodides do not give a molecular ion (Budzikiewicz et al., 1967). Instead thermal decomposition occurs prior to fragmentation giving rise to the corresponding base and methyl iodide. None of the esters (4, 5, 6) analyzed gave a molecular ion but instead thermally decomposed to give 3-dimethylaminopropionate (39), 3-dimethylaminobutyrate (40) and 3-dimethylamino-2-methylpropionate (41) respectively plus methyl iodide. Subsequent fragmentations were consistent with those reported for tertiary amines and esters (Budzikiewicz et al., 1967). (See Scheme 6.) The structures of most of the fragments in Scheme 6 were verified by accurate mass determinations.

When the spectrum of the same sample of compound 5 was rerecorded for accurate mass measurements, the spectrum was not identical to the original one. Repeated attempts to reproduce the original spectrum of

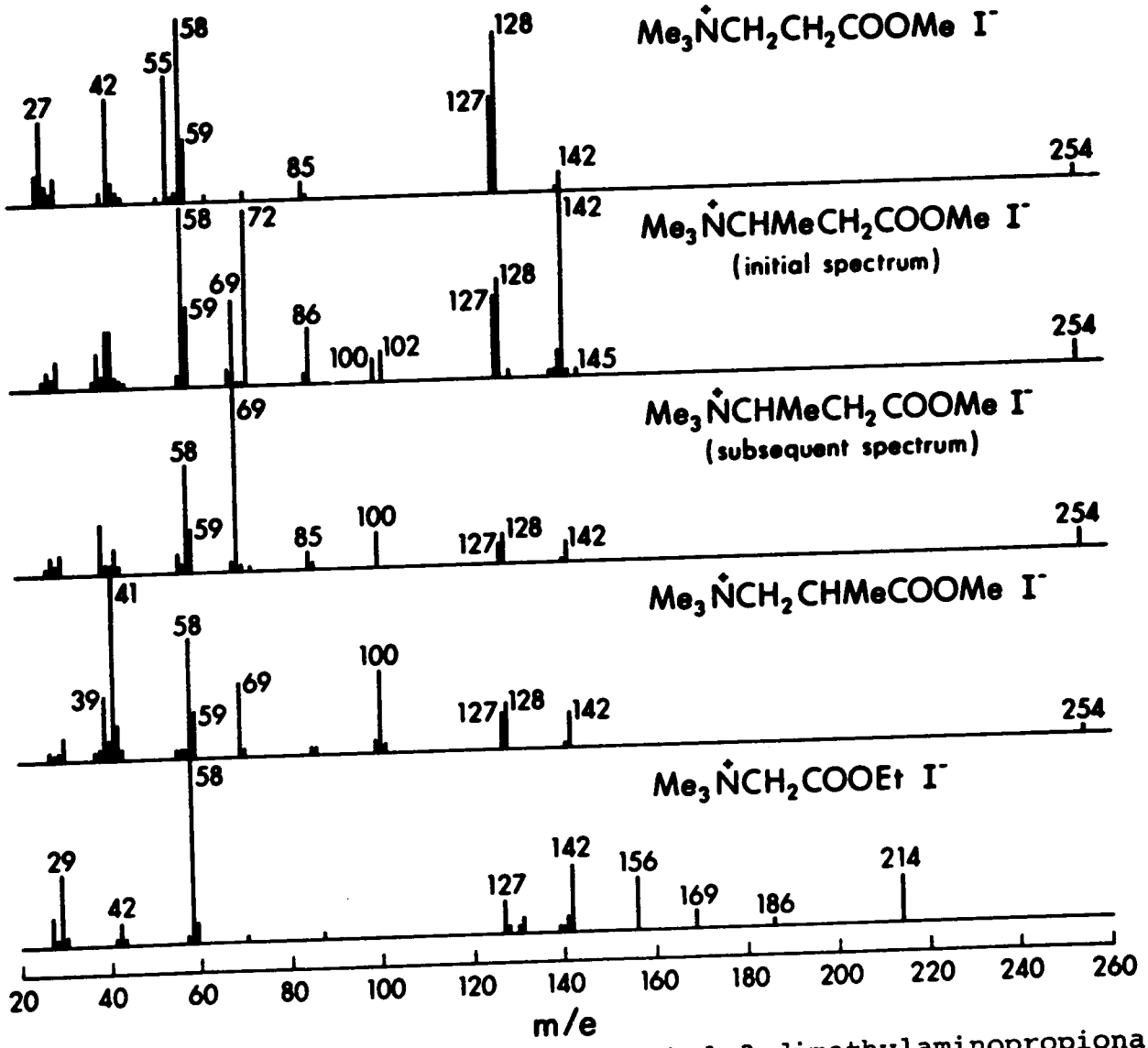
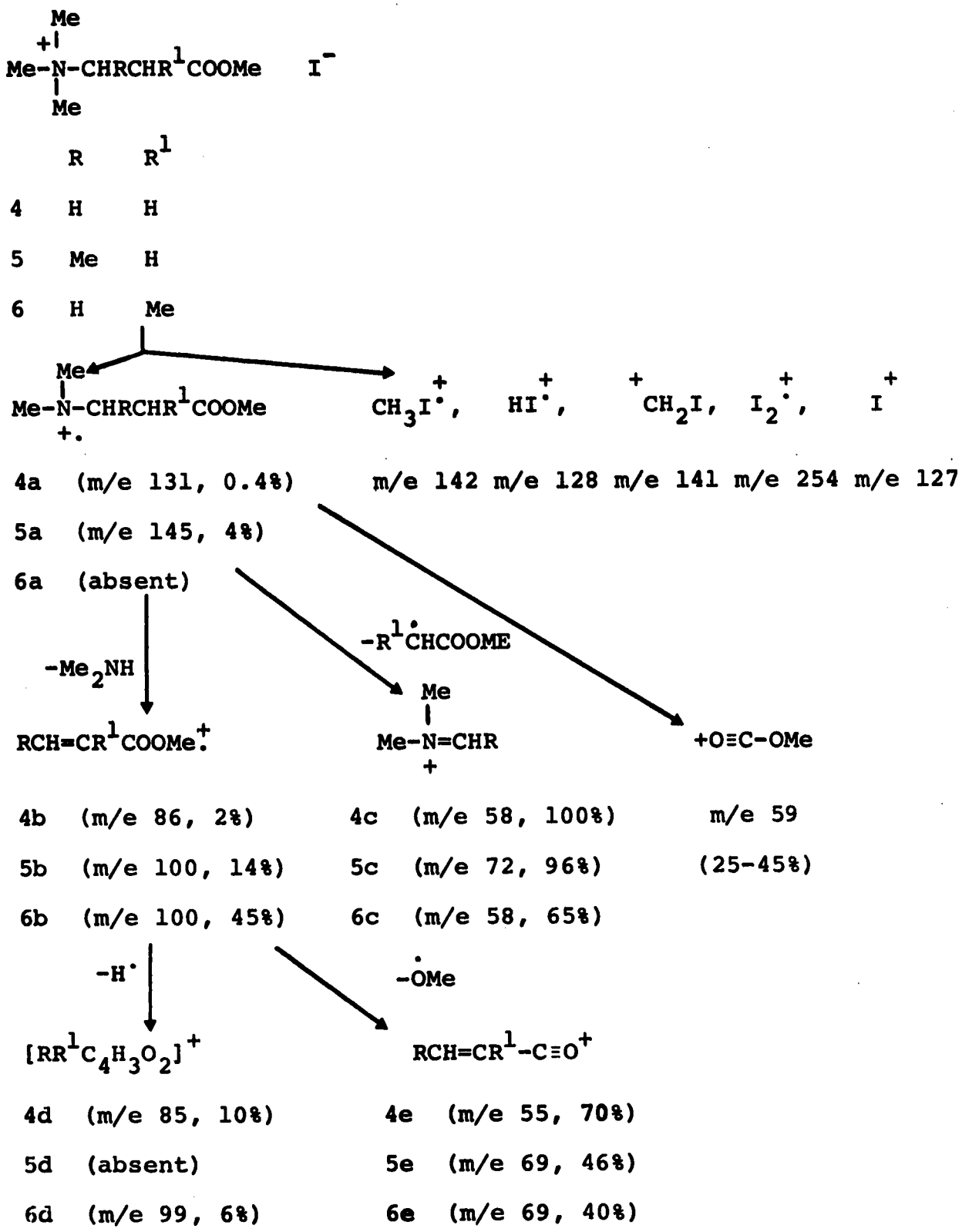


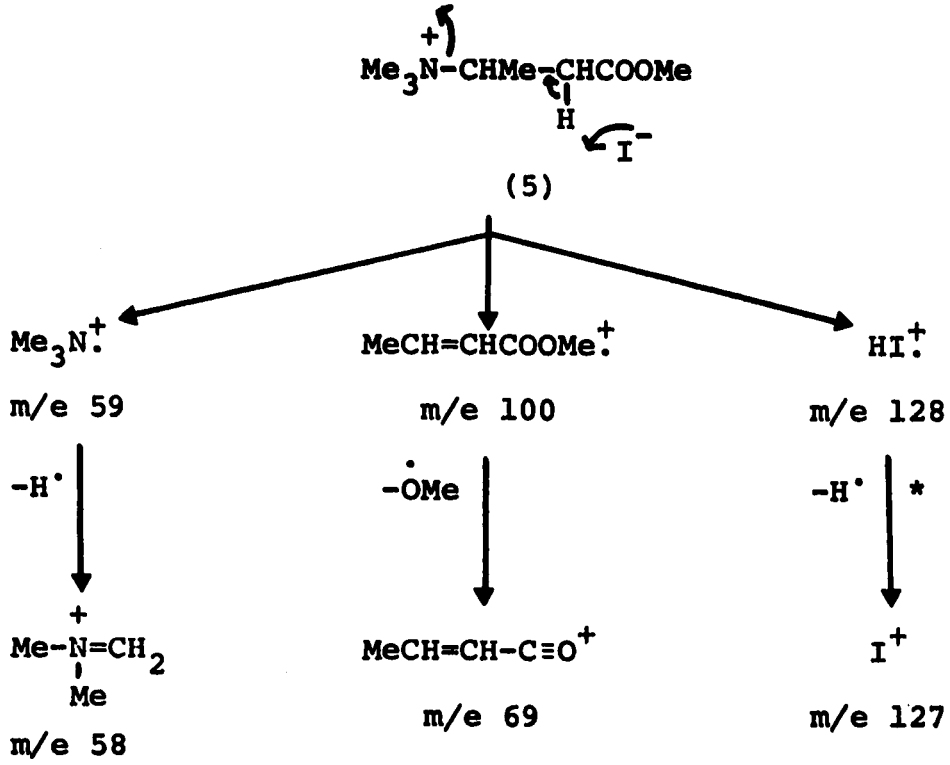
Figure 13: Representative mass spectra of methyl 3-dimethylaminopropionate methiodide (4), methyl 3-dimethylaminobutyrate methiodide (5), methyl 3-dimethylamino-2-methylpropionate methiodide (6) and ethyl dimethylamino-acetate methiodide (92).


Me
+
Me-N-CHRCHR1COOMe I-
Me
R R1
4 H H
5 Me H
6 H Me
Me
Me-N-CHRCHR1COOMe
+.
CH3I+., HI+., CH2I+, I2+., I+
4a (m/e 131, 0.4%) m/e 142 m/e 128 m/e 141 m/e 254 m/e 127
5a (m/e 145, 4%)
6a (absent)
-Me2NH
-R1•CHCOOME
Me
RCH=CR1COOMe+.
Me-N=CHR
+
+O≡C-OMe
4b (m/e 86, 2%)
4c (m/e 58, 100%)
m/e 59
5b (m/e 100, 14%)
5c (m/e 72, 96%)
(25-45%)
6b (m/e 100, 45%)
6c (m/e 58, 65%)
-H•
-•OMe
[RR1C4H3O2]+
RCH=CR1-C≡O+
4d (m/e 85, 10%)
4e (m/e 55, 70%)
5d (absent)
5e (m/e 69, 46%)
6d (m/e 99, 6%)
6e (m/e 69, 40%)

Scheme 6

compound 5 always failed, regardless of the ion source temperature employed. The major differences between the initial and all subsequent spectra were that whereas the former had peaks of appreciable intensities at m/e 102 (17%) (unidentified), 86 (31%) and 72 (96%) and the base peak at m/e 142, subsequent spectra lacked the peak at m/e 102, the peak at m/e 72 was almost absent (2%) and the peaks at m/e 142, 86, 58 and 41 were greatly reduced in intensity. The subsequent spectrum was still compatible with the structure of compound 5 but its formation indicated that the fragmentation pathway depicted in Scheme 7 was the preferred one with this compound. The composition of all fragment ions illustrated in Scheme 7 was confirmed by means of accurate mass measurements. In particular the m/e 59 peak was a triplet composed mainly of C_3H_9N (see Scheme 7), with minor contributions from the $C_2H_3O_2$ ions (see Scheme 6) and $^{13}CC_2H_8N$ (isotopic component of the m/e 58 ion in Scheme 6).

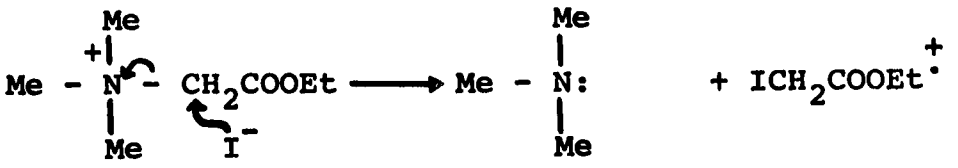
It can be concluded that the compounds 4 and 6 rearrange thermally in the mass spectrometer in predictable fashion whereas the chemically related ester 5 can give rise to two appreciably different spectra, both of which are compatible with the spectrum of 5.


Me3N-CHMe-CHCOOMe
H
I-
(5)
Me3N+.
m/e 59
-H·
Me-N+=CH2
Me
m/e 58
MeCH=CHCOOMe+.
m/e 100
-·OMe
MeCH=CH-C≡O+
m/e 69
HI+.
m/e 128
-H·
*
I+
m/e 127

Scheme 7

The mass spectra of the optical isomers (5a and 5b, 6a and 6b) of compounds 5 and 6 exhibited the same type of fragmentation pattern. Only the second pattern (Scheme 7) was observed in the optical isomers of 5.

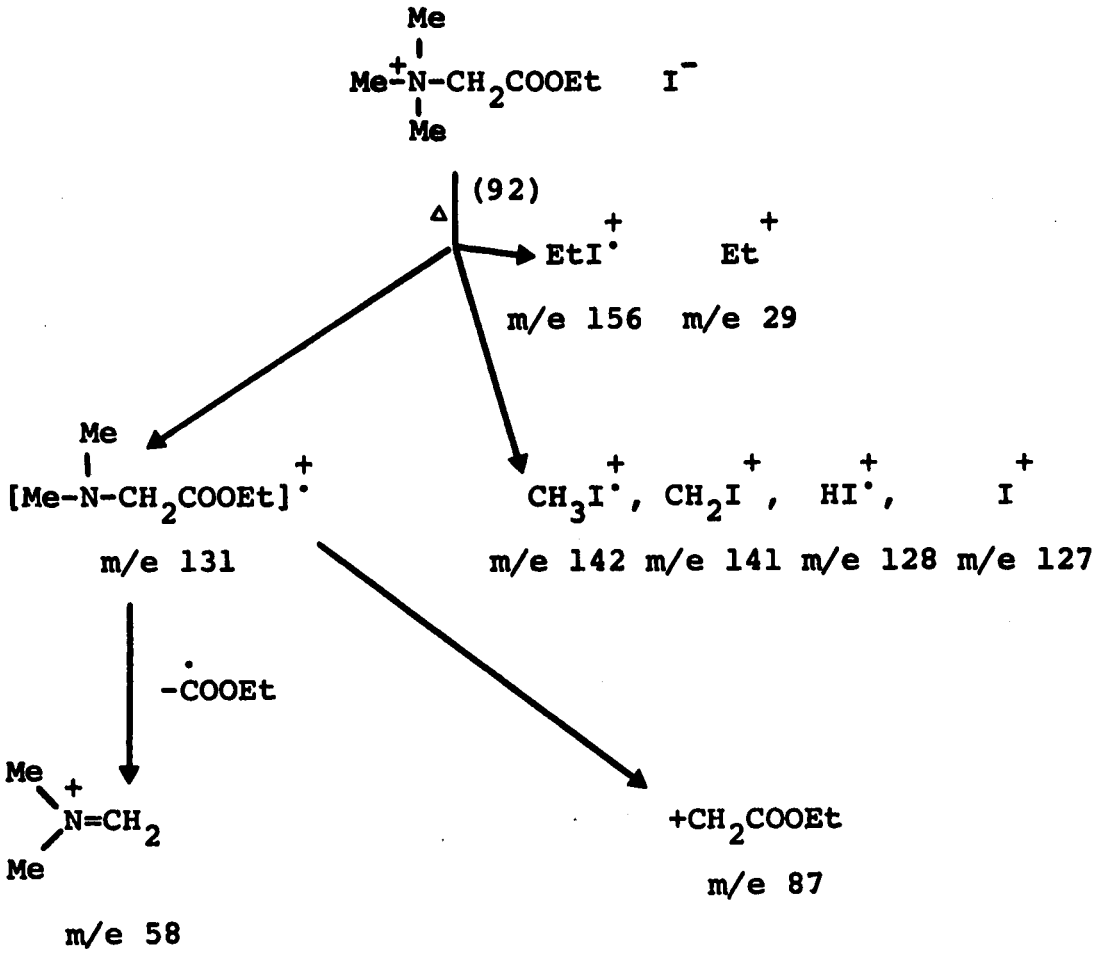
The mass spectrum of ethyl dimethylaminoacetate methiodide (92) was also recorded. Thermal decomposition again occurred prior to fragmentation and three fragment pathways were apparent, of which the major one was unexceptional and is depicted in Scheme 8. The second pathway was the expulsion of ethyl iodide from ethyl dimethylaminoacetate methiodide.(See also Scheme 8.) An alternative thermal rearrangement explains the presence of ions at m/e 214, 186, 169, 59 and 42 in the spectrum of 92. This rearrangement is apparently the result of attack of the iodide anion at the α-carbon of the ester and the subsequent expulsion of ethyl iodoacetate. Molecular ions corresponding to the molecules



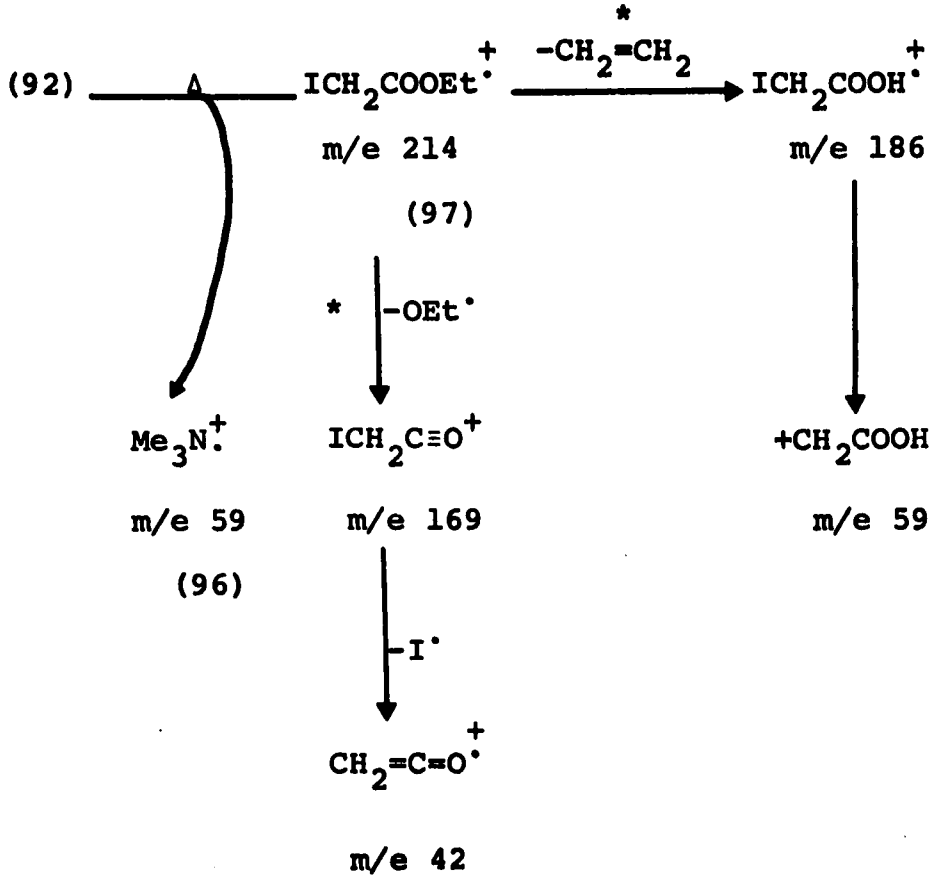
(92) (93) (94)

(93) and (94) were observed in the spectrum at m/e 59 in Scheme 9.

To confirm the validity of Schemes 8 and 9


Me
Me-N-CH2COOEt I-
Me
Δ (92)
EtI•+
Et+
m/e 156
m/e 29
Me
[Me-N-CH2COOEt]•+
m/e 131
CH3I•+, CH2I+, HI•+, I+
m/e 142 m/e 141 m/e 128 m/e 127
-•COOEt
Me
N=CH2+
Me
m/e 58
+CH2COOEt
m/e 87

Scheme 8


(92)
$ICH_2COOEt^{+\cdot}$
m/e 214
$-CH_2{=}CH_2$
$ICH_2COOH^{+\cdot}$
m/e 186
(97)
* -OEt·
$Me_3N^{+\cdot}$
m/e 59
(96)
$ICH_2C{\equiv}O^{+}$
m/e 169
$+CH_2COOH$
m/e 59
-I·
$CH_2{=}C{=}O^{+\cdot}$
m/e 42

Scheme 9

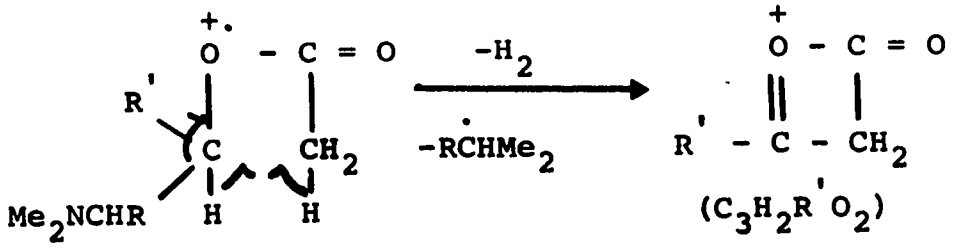
accurate mass measurements were made of all the ions depicted in both schemes and were compatible with the formulae shown. In addition, the mass spectra of authentic samples of ethyl dimethylaminoacetate (92) and ethyl iodoacetate (94) were examined. The spectrum of the former was simple and showed only six ions of relative abundance five percent or greater, viz 131 (6), 58 (100), 30 (9), 18 (27), 17 (6) and 15 (5), m/e (% relative abundance). In the spectrum of ethyl iodoacetate ions of m/e 214, 186, 169, 128, 127, 59 and 42 of significant abundance were all present (cf Scheme 9).

It was concluded that ethyl iodoacetate is a thermal fragmentation product of the methiodide of ethyl dimethylaminoacetate (92). Ethyl iodoacetate could not be an impurity in compound 92 since it was not employed in the preparation of 92.

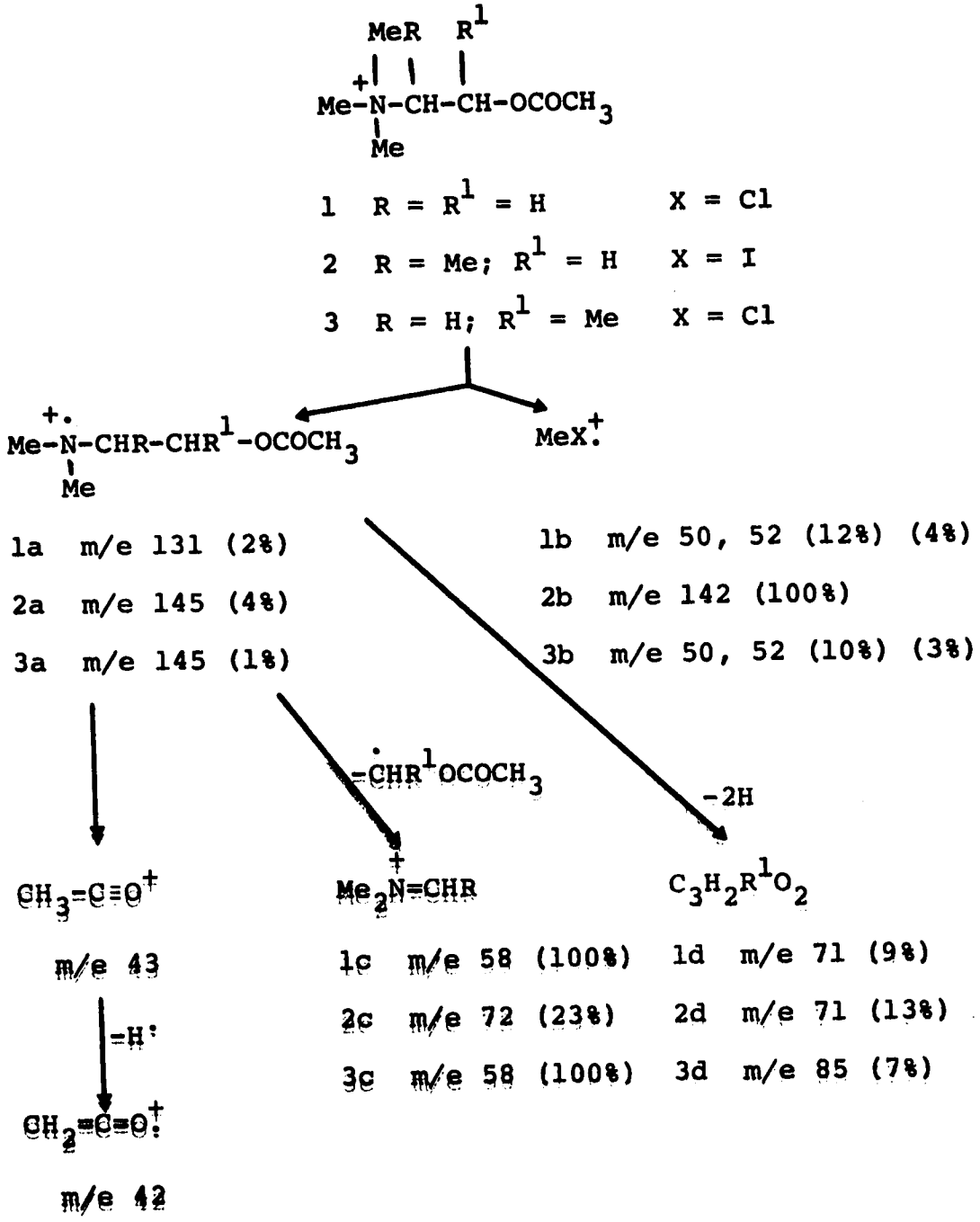
The mass spectra of acetylcholine (1), α- and β-methylacetylcholine (2 and 3) and their optical isomers were also recorded. Acetylcholine (1), α- and β-methylacetylcholine (3) gave similar spectra. (See Scheme 10.)

The mechanism of the formation of ions at m/e 71 (1d, 2d) and 85 (3d) is not known. The ion

$Me_2\overset{+\cdot}{N}CHRCHR^1OCOCH_3$ may fragment in the following manner, however, further studies which would include accurate mass measurements and isotopic labelling studies would be required as proof of this pathway.



The optical isomers of α- and β-methylacetylcholine (2a and 2b and 3a and 3b) exhibited a fragmentation pattern similar to their respective racemates.


MeR R1
Me-N-CH-CH-OCOCH3
Me
1 R = R1 = H X = Cl
2 R = Me; R1 = H X = I
3 R = H; R1 = Me X = Cl
Me-N-CHR-CHR1-OCOCH3
Me
MeX+.
1a m/e 131 (2%)
2a m/e 145 (4%)
3a m/e 145 (1%)
1b m/e 50, 52 (12%) (4%)
2b m/e 142 (100%)
3b m/e 50, 52 (10%) (3%)
-CHR1OCOCH3
-2H
CH3-C≡O+
m/e 43
-H·
CH2=C=O+·
m/e 42
Me2N=CHR
1c m/e 58 (100%)
2c m/e 72 (23%)
3c m/e 58 (100%)
C3H2R1O2
1d m/e 71 (9%)
2d m/e 71 (13%)
3d m/e 85 (7%)

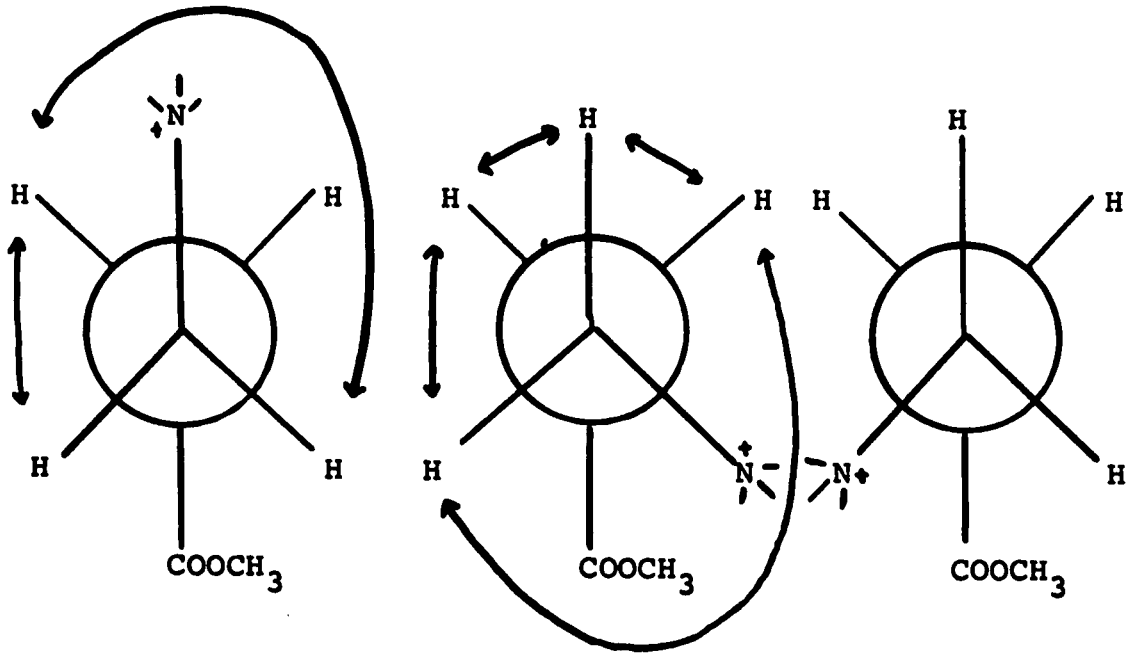
Scheme 10

CONFORMATIONAL ANALYSIS

Many molecules of pharmacological interest are 1, 2-disubstituted ethanes e.g. acetylcholine. P.m.r. spectroscopy of these molecules is important because it may provide information about their conformation which, in turn, may indicate the way in which the agonist interacts with the receptor. The conformations of acetylcholine, α-methylacetylcholine (2) and β-methylacetylcholine (3) in solution have been analyzed by Culvenor and Ham (1966), Casy et al. (1971) and Inch et al. (1970).

In this study a preliminary investigation of methyl 3-dimethylaminopropionate methiodide (4), methyl 3-dimethylaminobutyrate methiodide (5) and methyl 3-dimethylamino-2-methylpropionate methiodide (6), i.e. the reversed esters of the above acetylcholine analogues, was undertaken. An analysis was made of the 100MHz spectrum of each compound in D_2O using 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as the internal standard.

There are three possible staggered conformers for the molecule $(CH_3)_3\overset{+}{N}CH_2CH_2COOCH_3\ X^-$ (4), one trans (i) and two equivalent gauche forms (ii and iii) (Casy, 1971). In each conformer at least two distinct vicinal coupling constants are involved; in i the signal due to the methylene protons indicates the occurrence of one trans and one gauche coupling, and in ii and iii one



(i) (ii) (iii)

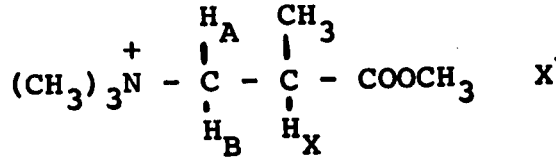
trans gauche

(4)

trans and three gauche couplings. If rapid interconversion of the conformers occurs by rotation about the C-C bond each geminal pair of protons is rendered chemically equivalent. An analysis of the 100MHz spectrum of the reversed ester of acetylcholine (4) suggested that there was no preferred conformation for this compound. The signal due to the $-\overset{+}{N}-\underline{CH_2}-CH_2-$ protons appeared as a distinct triplet centred at 4.12τ, J=7.5Hz.

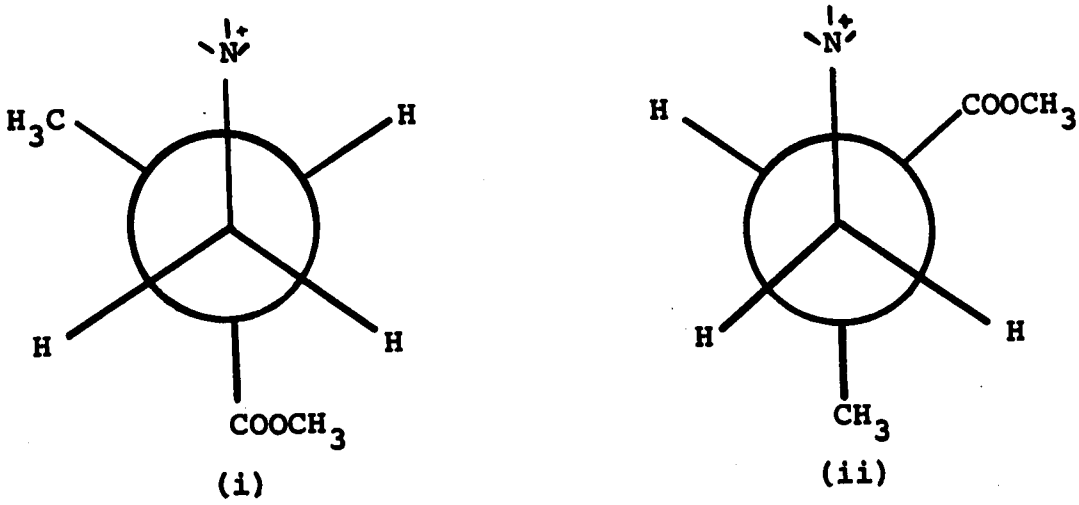
The β-protons ($-CH_2-\underline{C}H_2-CO-$) at 6.98τ were equivalent and appeared as a triplet of triplets as a consequence of coupling with the α-protons (J=7Hz) and with ^{14}N (J=2Hz). The value of J=7.5Hz is consistent with a rapidly equilibrating mixture of the conformers i, ii and iii. Therefore it may be concluded that methyl 3-dimethylaminopropionate methiodide (4) shows no preferred conformation in solution. In contrast acetylcholine shows a preference for the gauche conformation (Culvenor and Ham, 1966).

The p.m.r. spectra of the reversed esters of α- and β-methylacetylcholine (5 and 6) were recorded at a temperature of 30 and 90°. Inch et al. (1970) showed that better resolution of the signals due to methylene protons in compounds of this type was obtained when the spectra were recorded at elevated temperatures. The interactions between the protons were considered to be first order. The methylene signal of methyl 3-dimethylamino-2-methylpropionate methiodide (6) was treated as the AB part of an ABX system. The protons were clearly resolved and came to resonance at 6.18 and 6.64τ.



(6)

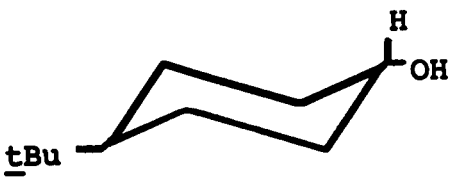
Measurement of the coupling constants gives J_{AX}=11.2 and J_{BX}=2.5Hz. The order of magnitude of these values indicates that the preferred rotomer(s) of this ester (6) involve(s) trans and gauche vicinal coupling constants (Casy et al., 1971). This conclusion supports either 6i or 6ii as the preferred conformer. The occurrence of a chemical shift difference between H_A and



(6)

H_B of 56Hz (at 100MHz) further indicates that the reversed ester of β-methylacetylcholine (6) displays a marked conformational preference but does not aid in the choice of the preferred conformation because the environments of the two protons differ in both conformers 6i and 6ii. In order to determine the preferred conformer it is necessary to use reference compounds. An initial study of the shielding influence of the -$COOCH_3$

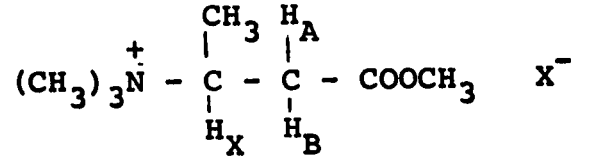
group on an α-C-H in compounds with the structures 95 and 96 together with the large chemical shift difference observed between the methylene protons, suggested that 6ii was the more likely conformer. Casy et al. (1971), using a similar argument, suggested that β-methylacetylcholine (3) adopted the same conformation as 6ii.



(95)

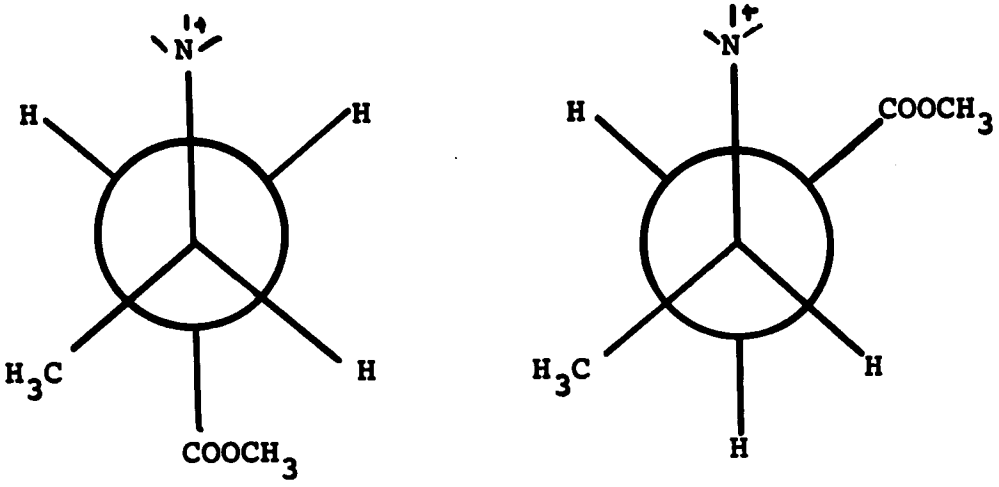
(96)

A similar analysis of the 100MHz spectrum of methyl 3-dimethylaminobutyrate methiodide (5) was carried out. One of the β-protons was resolved, 7.30τ.



(5)

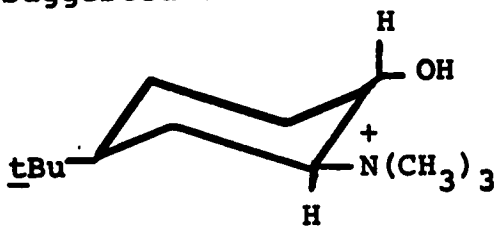
This signal was coupled to the α-proton (H_X) and to ^{14}N. The coupling constants were measured as J_{AX}=13.0 and J_{BX}=3.5Hz. Hence, this α-substituted ester (5) like the β-ester (6) has a preferred conformation, either 5i or 5ii. An investigation of the shielding influence of a $-\overset{+}{N}$ group on an adjacent proton in re-



(i) (ii)

(5)

ference compounds 95 and 97 together with chemical shift evidence, suggested that 5i was the preferred



(97)

conformer. In contrast, Casy et al. (1971) showed that α-methylacetylcholine (2) had no preferred conformation in solution.

In conclusion these preliminary studies suggest:

1. Methyl 3-dimethylaminopropionate methiodide (4) has no preferred conformation in contrast to the isomer, acetylcholine, which shows conformational preference (Culvenor and Ham, 1966).

2. Both β-methylacetylcholine (3) and its reversed ester (6) have a preferred conformation. In both compounds the $\overset{+}{N}-C_4-C_5-O_1$ bond angle is probably 60°.

3. The reversed ester of α-methylacetylcholine (5) has a preferred conformation. The evidence obtained so far suggests that the $\overset{+}{N}(CH_3)_3$ and $-COOCH_3$ groups are <u>trans</u> to eachother. In contrast Casy <u>et al</u>. (1971) reported that α-methylacetylcholine (2) had no preferred conformation in solution. Clearly further work is essential to clarify this situation. However, Culvenor and Ham (Chem. Commun. 1242, 1970) found, using p.m.r. spectroscopy, that acetylthiocholine (24) had a <u>trans</u> conformation in solution. In their computerized analysis of the spectra these workers found a J (N-C-$C\underline{H}_2$-) coupling constant of 0.75HZ. In the spectra of compound 5, the N-C-$C\underline{H}_3$ coupling constant was 0.7Hz, in close agreement with that observed by Culvenor and Ham (1970). This lends support to the suggestion that 5 exists in solution as the <u>trans</u> conformer 5i.

PHARMACOLOGY

Methods

A. Measurement of Muscarinic Activity *

1. Rat Blood Pressure

Male rats weighing approximately 200 g were anesthetized with urethane (1.875 g/kg) injected intraperitoneally. The trachea was cannulated and the arterial blood pressure recorded through a polythene cannula inserted in a carotid artery using an E & M pressure transducer, type P-1000A connected to an E & M physiograph type DMP-4A. Drugs were dissolved in 0.9% saline and injected through a polythene cannula inserted into a femoral vein. A dose response curve was obtained for acetylcholine as the standard drug and for each compound under investigation. Tests were carried out to check that the fall in blood pressure was blocked by hyoscine methiodide (5 mg/kg) which was injected intravenously several minutes before the test compound. The muscarinic activity was expressed as the molar potency relative to acetylcholine=1.00.

2. Guinea-pig Ileum

Guinea-pigs were killed by a blow on the head. Segments of ileum (2-3 cm) were suspended in Krebs solution, gassed with 95% O_2/5% CO_2 at 37°. Contractions were recorded isotonically using an ink-writing

* For further explanation see page 123

lever or isometrically using an E & M myograph Type A connected to an E & M physiograph model IV. Drugs were dissolved in Krebs solution. A dose-response line was obtained for acetylcholine and for each compound under investigation. The muscarinic potency was expressed as the molar potency relative to acetylcholine=1.00. Tests were carried out to check that the contractions were blocked by atropine (7 μg/ml). Experiments were also done in the presence of hexamethonium (25 μg/ml), eserine (2.5 μg/ml) plus morphine (25 μg/ml), at low Ca^{++} concentration, and at 25°.

B. Measurement of Nicotinic Activity

1. Rat Blood Pressure

The experiment was conducted as described previously. The pressor response to nicotine or DMPP as the standard drug and the compound under investigation was determined before and after the administration of a ganglion blocking agent. Hexamethonium, pentolinium or trimethaphan (5 mg/kg) were injected intravenously 5 minutes before the compound under investigation. The nicotinic activity was expressed as the molar potency relative to DMPP=1.00.

2. Frog Rectus Muscle

Frogs were stunned by a blow on the head and

pithed. The rectus abdominis muscles were removed and suspended in 70% Krebs solution, gassed with 95% O_2/5% CO_2 at 25°. Contractions were recorded isotonically or isometrically as described previously. A dose response line was obtained for acetylcholine as the standard drug and for each compound investigated. The nicotinic activity was expressed as the molar potency relative to acetylcholine = 1.00. A check was made that contractions were blocked by tubocurarine (7μg/ml). Experiments were also carried out in the presence of eserine (2.5 μg/ml).

C. Ganglion Blocking Activity

The pressor response on the rat blood pressure to DMPP (50-200 μg/kg) injected intravenously was determined immediately before and after the administration of the compounds under investigation.

Krebs Solution: NaCl, 6.9 g; KCl, 0.35 g; $CaCl_2.6H_2O$, 0.55g; KH_2PO_4, 0.16 g; $MgSO_4.7H_2O$, 0.29 g; dextrose, 1.0 g; distilled water to 1 l.

Drugs: acetylcholine chloride (Fluka), β-methylacetylcholine chloride (Aldrich), atropine sulfate (B.D.H.), carbachol chloride (Fluka), 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) (Fluka), eserine (Fluka), hexa-

methonium chloride (Fluka), hyoscine methiodide, morphine hydrochloride (B.D.H.), nicotine (Nutritional Biochemical Corporation), pentolinium tartrate (Poulenc), trimethaphan camphorsulfonate (Arfonad) (Roche), tubocurarine chloride (Fluka), urethane (B.D.H.). Doses refer to the appropriate salt of each compound. All solutions were prepared immediately before use.

Experimental Design:

Isolated Tissue Experiments

The following sequence was used to carry out the experiments:

1. A standard dose of acetylcholine was added at constant intervals to the tissue bath until uniform contractions were obtained.
2. A dose-response line for acetylcholine was obtained.
3. A dose-response line for the compound under investigation was obtained.
4. The dose-response line for acetylcholine was re-run.
5. A dose-response line for another compound was obtained.

Steps 4 and 5 were repeated to the end of the experiment then the appropriate antagonist was added to the Krebs

solution and an attempt was made to repeat the dose-response lines for acetylcholine and the compounds under investigation. Drugs were given in random order and in order of increasing concentration. The logarithm of the dose of the drug was plotted against contraction height and the best straight line calculated using the method of least squares.[a] In the case of the frog rectus abdominis experiments the average response to acetylcholine before and after administration of the compound under investigation was plotted since the preparation gradually lost sensitivity during the experiment. The molar potency relative to acetycholine was calculated. A two minute cycle was used for the guinea-pig ileum experiments: 0 sec - inject drug; 20 sec - wash for 10 sec period; 40 sec - wash for 5 sec period; 45-120 sec - rest period. The frog rectus abdominis experiments were run on a four minute cycle: 0 sec - inject drug; 90 sec - wash for 10 sec; 120 sec - wash for 5 sec; 125-240 sec - rest period.

Rat Blood Pressure Experiments

Steps 1-4 as outlined above were followed. Only one compound plus the standard drug was tested on each rat.

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[a]Only the middle portion of the dose-response curves was used in these calculations

Pharmacology of the Analogues of Acetylcholine and Their Reversed Esters

Results

Acetylcholine, its α- and β-methyl analogues (2 and 3) and their respective reversed esters (4, 5 and 6) were examined pharmacologically on the rat blood pressure, guinea-pig ileum and frog rectus abdominis muscle. The results have been summarized in Tables VIII, IX and X. The muscarinic and nicotinic activity of α- and β-methylacetylcholine (2 and 3) and their optical isomers (2a,2b and 3a,3b) agreed with literature values (Beckett et al., 1963a; Cocolas et al., 1970; Lesser, 1965). It is interesting to note that in the presence of a cholinesterase inhibitor, eserine (2.5 μg/ml), α-methylacetylcholine (2) and its optical isomers (2a,2b) were equiactive on the guinea-pig ileum in contrast to the experiments in normal Krebs solution in which D(+)R-α-methylacetylcholine (2a) had twice the activity of the racemate.

The reversed ester of acetylcholine (4) had 0.1 to 0.8 of the muscarinic activity, and 0.1 of the nicotinic activity of acetylcholine. These results agree

Table VIII

The Muscarinic and Nicotinic Activity of the Acetylcholine Analogues (2,3) and Related Reversed Esters (4,5,6) on the Rat Blood Pressure Relative to Acetylcholine = 1.00 (±S.E.)

Compound[a]		Untreated[a] n=4	Pretreated With Pentolinium (5 mg/kg)[b] n=2	Pretreated With Hyoscine Methiodide (5 mg/kg)[c,d] n=4
α-MeAch	(2)	0.021(±0.008)	0.041	0.80(±0.10)
β-MeAch	(3)	0.40(±0.11)	0.40	<0.010
RE-Ach	(4)	0.10(±0.03)	0.50	0.85(±0.07)
RE α-MeAch	(5)	0.014(±0.005)	0.030	0.75(±0.17)
RE β-MeAch	(6)	0.019(±0.010)	0.010	<0.010

a) complete structures of these compounds are recorded on pages 3,55,56

b) depressor response blocked by hyoscine methiodide (5 mg/kg)

c) potency relative to DMPP = 1.00 (±S.E.)

d) pressor response blocked by pentolinium (5 mg/kg)

Table IX

The Muscarinic Activity of the Acetylcholine Analogues (2,3) and Related Reversed Esters (4,5,6) on the Guinea-Pig Ileum Relative to Acetylcholine = 1.00 (±S.E.)[a]

Compound[b]		Isomer	Normal Conditions n=4	With Hexamethonium (25 µg/ml) n=4	at 25°[b] n=2	low Ca^{++}[b] n=2	With Eserine (2.5 µg/ml) and Morphine (25 µg/ml) n=2
α-MeAch	(2)	(±)	0.010(±0.002)	0.015(±0.002)	0.031	0.023	0.021
	(2a)	D(+)R-	0.026(±0.005)	0.037(±0.016)	0.046	0.036	0.025
	(2b)	L(-)S-	0.0047(±0.0012)	0.0063(±0.0011)	0.013	0.018	0.018
β-MeAch	(3)	(±)	0.86(±0.17)	0.78(±0.14)			1.05[d]
	(3a)	L(+)S-		1.43(±0.24)			1.77[d]
	(3b)	D(-)R-		0.052(±0.020)			0.047[d]
Re-Ach	(4)		0.30(±0.15)	0.30(±0.13)			0.15[d]

Table IX Cont'd

RE α-MeAch (5)	(±)	0.031(±0.014)	0.027(±0.005)	0.025	0.047	0.0028
(5a)	D(+)R-	0.013(±0.005)	0.016(±0.002)	0.010	0.037	0.0019
(5b)	L(-)S-	0.043(±0.015)	0.076(±0.018)	0.065	0.076	0.0049
RE β-MeAch (6)	(±)	0.025(±0.010)	0.027(±0.012)			0.0074[d]
(6a)	L(-)S-		0.032(±0.014)			0.0074[d]
(6b)	D(+)R-		0.045(±0.032)			0.0076[d]

a) all contractile responses blocked by atropine (7 μg/ml)

b) complete structures of these compounds are recorded on pages 3,55,56

c) hexamethonium (25 μg/ml) added to the Krebs solution

d) denervated preparation, Krebs solution with 0.1 Ca^{++}

Table X

The Nicotinic Activity of the Acetylcholine Analogues (2,3) and Related Reversed Esters (4,5,6) on the Frog Rectus Abdominis Muscle Relative to Acetylcholine = 1.00 (±S.E.)[a]

Compound		Isomer	Normal Conditions n=4	With Eserine (2.5 µg/ml) n=2
α-MeAch	(2)	(±)	0.82(±0.14)	0.17
	(2a)	D(+)R-	0.89(±0.13)	0.16
	(2b)	L(-)S-	0.52(±0.08)	0.16
β-MeAch	(3)	(±)	<0.001	
	(3a)	L(+)S-	<0.001	
	(3b)	D(-)R-	<0.001	
RE Ach	(4)		0.12(±0.03)	

Table X Cont'd

RE α-MeAch (5)	(±)	0.76(±0.22)	0.016
(5a)	D(+)R-	0.50(±0.15)	0.038
(5b)	L(-)S-	0.64(±0.13)	0.049
RE β-MeAch (6)	(±)	<0.001	
(6a)	L(+)S-	<0.001	
(6b)	D(-)R-	<0.001	

a) all contractile responses blocked by d-tubocurarine (7 µg/ml)

b) complete structures of these compounds are recorded on pages 3,55,56

with those of Bass et al. (1950) and Barrass et al. (1968). However, the muscarinic activity of the α- and β-esters (5 and 6) was significantly higher than reported previously. Schueler et al. (1951, 1951a) and Schueler and Keasling (1951) reported that these compounds had less than 0.0001 of the muscarinic activity of their respective acetylcholine analogues (2 and 3). In this work the reversed ester of α-methylacetylcholine (5) was approximately equipotent with its acetylcholine analogue (2), and the reversed ester of β-methylacetylcholine (6) had 0.02 of the muscarinic activity of its acetylcholine derivative (3). In all experiments L(-)S-methyl 3-dimethylaminobutyrate methiodide (5b) was ∿1.8x as active as the racemate (5) but, methyl 3-dimethylamino-2-methylpropionate methiodide (6) showed no stereospecificity in its interactions with the muscarinic receptor.

A possible explanation of our results is that previous workers may have been using impure compounds. The method of synthesis of the reversed esters of α- and β-methylacetylcholine (5 and 6) used by Schueler et al. (1951, 1951a) and Schueler and Featherstone (1951) has not been reported in the literature. Assuming that these compounds were synthesized in the same manner as was used to synthesize the reversed ester of acetyl-

choline (4), i.e. from trimethylamine and the appropriate bromo-ester, (Bass et al., 1950), the major contaminant would be expected to be trimethylamine hydrobromide. In the experiments undertaken for this thesis (see page 88) it was shown that when an amine was condensed with a bromo-ester the hydrobromide salt of the amine was obtained in quantitative yield.

The nicotinic activity of the reversed esters has not been studied previously. α-Methylacetylcholine (2) and its related ester (5) showed 0.1 - 0.8 of the nicotinic activity of acetylcholine, whereas β-methylacetylcholine (3) and its reversed ester (6) had less than 0.001 of the nicotinic activity of acetylcholine. None of the compounds showed stereospecificity in their interactions with the nicotinic receptor of the frog rectus abdominis muscle.

Discussion

In order to discuss the significance of the results obtained it is necessary first to discuss the pharmacology of the experiments. When considering the activity of the compounds it is important to know if the observed response is due to stimulation of the mus-

carinic or nicotinic receptor. If the drug is exerting its effect on the muscarinic receptor there are three possible mechanisms which must be considered. Is the observed response due to direct stimulation of the muscarinic receptor? Is it due to an indirect effect involving the stimulation of ganglia or the presynaptic release of acetylcholine? Lastly in considering the activity of compounds related to acetylcholine it is necessary to consider the effect of cholinesterase. Is the drug under investigation hydrolyzed by cholinesterase? If so, does the rate of hydrolysis affect the absolute activity of the compound or its activity relative to other substances?

In order to investigate if an observed response was due to stimulation of the muscarinic receptors in a preparation, an anticholinergic agent, atropine sulfate (7 μg/ml) or hyoscine methiodide (5 mg/kg), was administered after the response was obtained. The contractile response of the guinea-pig ileum and the depressor response on the blood pressure of the rat caused by all compounds investigated, including acetylcholine, were blocked by atropine sulfate (7 μg/ml) or hyoscine methiodide (5 mg/kg) respectively. Hyoscine methiodide was used in the rat experiments because it was found to

have a less depressant effect on respiration than the corresponding hydrobromide salt. It was concluded therefore that the response observed when α-methylacetylcholine (2), β-methylacetylcholine (3) and the reversed esters (4,5 and 6) were tested on these preparations was a result of stimulation of the muscarinic receptors of these preparations.

Next, the mechanism of this stimulation of the muscarinic receptor was investigated. The experiments on the blood pressure of the rat and on the guinea-pig ileum were performed in the presence of a ganglion blocking agent. Rats were predosed with pentolinium tartrate (5 mg/kg) or in the case of the guinea-pig ileum experiments hexamethonium (25 μg/ml) was added to the Krebs solution. The activity of the compounds was not altered in the presence of a ganglion blocking agent which suggested that no indirect component involving ganglia was affecting the muscarinic response. The possibility that the stimulation of the muscarinic receptor was a result of the presynaptic release of acetylcholine was investigated by determining the activity of the compounds on the guinea-pig ileum at 25° and when the Ca^{++} concentration was reduced to 0.05 of the normal amount. Johnson (1963) demonstrated that under

either of these conditions the release of acetylcholine from peripheral nerve endings was reduced to approximately 0.1 of the normal values. The relative activity of the α-methyl substituted compounds (2 and 5) and their optical isomers (2a,2b and 5a,5b) was not altered; although under these conditions the threshold dose of all compounds including acetylcholine was increased. (Compounds 3, 4 and 6 were not tested under these conditions.) It was concluded that compounds 2 and 5 were not acting indirectly by causing the release of acetylcholine. These results could be extended and verified by using tetrodotoxin (Gershon, 1967). It was therefore concluded that the activity of the compounds under investigation was due to direct stimulation of the muscarinic receptors and that no indirect component was present.

The nicotinic activity of the compounds under investigation was determined on the blood pressure of rats pretreated with hyoscine (5 mg/kg) and on the frog rectus abdominis muscle. The β-methyl analogues (3 and 6) had no activity (<0.001) on each of these preparations. The pressor response caused by the α-methyl analogues (2 and 5) on the rat blood pressure was blocked by the ganglion blocking agent pentolinium tartrate (5 mg/kg). Also the contractile effect on the

frog rectus abdominis muscle of these compounds and their optical isomers (2a,2b and 5a,5b) was blocked by d-tubocurarine (7 μg/ml). Therefore, it was concluded that the activity of the compounds on these preparations was due to stimulation of the nicotinic receptors.

Finally, what is the effect of acetyl- and/or butyrocholinesterase on the activity of the compounds on the guinea-pig ileum and frog rectus abdominis muscle? This question is of particular interest because of the statements made by previous workers. Beckett et al. (1963a) suggested that the relative rates of hydrolysis of the optical isomers of α-methylacetylcholine (2a,2b) was a complicating factor in the determination of their muscarinic activity. (See Table I.) Beckett proposed that the L(-)S-isomer of α-methylacetylcholine (2b) presented a more favourable complimentary conformation to the active site of cholinesterase and to the muscarinic receptor than did its enanthiomorph (2a). However, owing to the faster rate of hydrolysis and inactivation by acetylcholinesterase of the L(-)S-isomer (2b), the D(+)R-isomer (2a) appeared to exert greater muscarinic activity. Beckett also suggested that enzymatic hydrolysis was important in the interpretation of the activity of β-methylacetylcholine (3).

He postulated that the slower rate of hydrolysis of L(+)S-β-methylacetylcholine (3a) compensated for the deleterious effect of a β-methyl group on the association of this molecule with the muscarinic receptor, and that the weak inhibitory action of D(-)R-isomer (3b) on acetylcholinesterase slightly reinforced the muscarinic activity of the L(+)S-isomer (3a) when the activity of the racemate (3) was determined. Cocolas et al. (1970) proposed that the active site of acetylcholinesterase best accomodates compounds with the S configuration at the α-carbon atom and the R configuration at the β-carbon atom. The proposals of Beckett and Cocolas were based on in vitro experiments using bovine erythrocyte acetylcholinesterase. Lesser (1965) investigated the nicotinic activity of the isomers of α-methylacetylcholine (2a,2b) on the chick biventer preparation in the presence of eserine (0.025 μg/ml). His results suggested that tissue cholinesterase did not affect the relative potencies of the isomers on this preparation.

In these experiments an attempt was made to determine the influence of hydrolysis by cholinesterase on the muscarinic and nicotinic activity of the compounds under investigation. The muscarinic activity of

the compounds on the guinea-pig ileum and the nicotinic activity on the frog rectus abdominis muscle were determined in the presence of eserine (2.5 µg/ml). This concentration of eserine has been shown to be sufficient to inhibit the activity of acetyl- and butyrocholinesterase (Gershon, 1967) on these preparations. In preliminary experiments on the guinea-pig ileum it was found that the presence of eserine caused the tissue to exhibit rapid continuous contractions and to exhibit periods of prolonged spasms possibly due to the build up of acetylcholine in the bath (Johnson, 1963). Under these conditions it was not possible to obtain a dose response curve for the compounds under investigation. It was found necessary to depress the contractility of the tissue in order to determine the activity of the compounds. Morphine (25 µg/ml) was added to the bath, along with the eserine, to reduce the spontaneous contractions and allow easier observation of the response to the compounds (De La Lande and Porter, 1967). In some cases the addition of morphine alone was not found to be sufficient to prevent prolonged spasms of the tissue. In these experiments it was necessary to denervate the tissue by storage overnight at 4° (a modification of the method of Chiou and Long, 1969) and to re-

duce the Ca^{++} concentration in the bath to 0.1 of the normal amount. The conditions for these experiments needed to be determined for each individual case.

When cholinesterase is inhibited the activity of a compound whose action is limited by enzyme hydrolysis will be increased. This effect is seen in the form of a decreased threshold dose. If the compound is hydrolyzed at the same rate as acetylcholine its relative activity will be unchanged under these conditions. If a compound is not hydrolyzed by cholinesterase its threshold dose remains constant and its activity relative to acetylcholine decreases. The experiments on the guinea-pig ileum showed that α- and β-methylacetylcholine (2 and 3) were hydrolyzed by guinea-pig ileum cholinesterase but that the reversed esters (4,5 and 6) were not. In addition, α-methylacetylcholine (2) was hydrolyzed by frog cholinesterase but the reversed ester of acetylcholine (4) and the α-methyl reversed ester (5) were not.

One important observation must be made concerning these results. On the guinea-pig ileum in the presence of eserine the isomers of α-methylacetylcholine (2a,2b) and the racemate (2) were equipotent, whereas in the absence of eserine the D(+)R-isomer (2a) appeared to be twice as active as the racemate (2). The results

suggested that the L(-)S-isomer (2b) was, as proposed by other workers (Beckett et al., 1963a; Cocolas et al., 1970), hydrolyzed faster than the D(+)R-isomer (2a) and therefore appeared to have less muscarinic activity. The guinea-pig ileum experiments partially confirmed the proposals of Cocolas et al. (1971) that the active site of acetylcholinesterase best accommodates the S configuration at the α-carbon atom, but there was no evidence that the R configuration is preferred at the β-carbon atom. The results showed that both isomers of β-methylacetylcholine (3a, 3b) were hydrolyzed by guinea-pig cholinesterase, the L(+)S-isomer (3a) being hydrolyzed more rapidly as its activity was potentiated to a greater degree. It is unlikely that the suggestion of Beckett et al. (1963a) that the weak cholinesterase inhibitory action of D(-)R-β-methylacetylcholine (3b) slightly reinforces the muscarinic activity of the L(+)S-isomer (3a) in the racemate (3) is correct since in the presence of complete cholinesterase inhibition the relative activities of the compounds were not altered.

The relative nicotinic activity of the isomers of α-methylacetylcholine (2a,2b) and the racemate (2) on the frog rectus abdominis muscle in the presence of eserine was not altered. This suggests that their

rates of hydrolysis by frog cholinesterase were probably equal. These results on the frog rectus abdominis muscle agree with those of Lesser (1965) obtained on a different preparation. Lesser showed that tissue cholinesterase did not affect the relative nicotinic potency of the α-methylacetylcholine isomers (2a,2b) on the chick biventer cervicis preparation.

Since the nature of the activity shown by the acetylcholine analogues (2 and 3) and the reversed esters (4,5 and 6) has been established it is now possible to discuss the significance of the activity of these compounds. The reversed esters had the same spectrum of activity as their related acetylcholine analogues although there were differences in their activities relative to acetylcholine. There are three complicating factors which influence the activity of molecules at a receptor which were not investigated in this study. No attempt was made to determine if the compounds had the same intrinsic activity and/or efficacy as acetylcholine, or if the compounds had the same affinity for the receptor. In addition experiments were not performed to determine if all the compounds under investigation were pure agonists. However, as a maximal

response of the tissues could be obtained for both the acetylcholine derivatives (1,2 and 3) and the reversed esters (4,5 and 6) it is probably correct to assume that the compounds under investigation had similar intrinsic activity to acetylcholine. Also the dose response curves of all the compounds were parallel to those obtained for acetylcholine. For the purpose of this discussion it was assumed that the intrinsic activity of the compounds investigated was similar to that of acetylcholine. It was also assumed that all compounds investigated were pure agonists since all produced a maximal response of the tissue.

First, a consideration of the interaction of the compounds at the muscarinic receptor will be made. At the muscarinic receptor, with compounds 5 and 6, reversing the position of the ethereal and carbonyl oxygen atoms, as compared to acetylcholine, resulted in a reversal of the influence of methyl substitution at the α- or β-carbon atom on stereospecificity. In the acetylcholine series of compounds methyl substitution at the β-carbon atom resulted in a compound which showed stereospecificity in its interaction with the muscarinic receptor whereas in the case of the ester series methyl substi-

tution at the α-carbon atom had this effect. In order for a compound to exhibit stereospecificity at a receptor at least a three point interaction is necessary. Interaction at all three points may or may not be necessary in order to stimulate the receptor but the molecule must have the correct geometry at all three points in order to fit and stimulate the receptor. Only two points may be involved in the stimulation of the receptor and the third area may be one of zero interaction. It is also possible that two points on the receptor are stimulated and the third area is one of positive or negative interaction depending upon the geometry of the compound. Since L(+)S-β-methylacetylcholine (3a) and L(-)S-methyl 3-dimethylaminobutyrate methiodide (5b) show stereospecificity in their interaction with the muscarinic receptor it was assumed that they interact at three points on the receptor. As suggested by Beckett et al. (1963a) the decreased activity of the reversed α-methyl ester (5) compared to acetylcholine may be due to the steric influence of the α-methyl group on the cationic head which prevents a close drug-receptor interaction. The less active optical isomers D(+)R-β-methylacetylcholine (3b) and D(+)R-methyl 3-dimethylaminobutyrate methiodide (5a) can

probably only interact with one or two sites on the muscarinic receptor. The compounds (2 and 6) which exhibited no stereospecificity at the muscarinic receptor were all less active than acetylcholine. They probably also only interact with one or two sites on the muscarinic receptor. These results do not confirm the hypothesis of Cocolas et al. (1970) who suggested that the muscarinic receptor preferred the R configuration at the α-carbon atom and the S configuration at the β-carbon atom. Both compounds 3a and 5b have the S configuration at their asymmetric centre which suggests that the S configuration is favoured at both the α- and β-carbon atoms.

Stereospecificity was shown in these experiments to be a factor influencing the hydrolysis of α-methylacetylcholine (2) by cholinesterase. Beckett et al. (1963a) suggested that hydrolysis of the optical isomers of α-methylacetylcholine (2a, 2b) was a complicating factor in the determination of their muscarinic activity. α-Methylacetylcholine (2) appeared to show stereospecificity with guinea-pig cholinesterase but showed no stereospecificity towards the cholinesterase present in the frog rectus abdominis preparation. This may be due to species differences (Goldstein et al.,

1969a) or differences in the amounts of the various cholinesterases present in the muscle (Child and Zaimis, 1960). Butyrocholinesterase has not been shown to be stereospecific but, some observations can be made concerning the stereospecific requirements of the active site of acetylcholinesterase. Like the muscarinic receptor the active site of the enzyme appears to prefer the S configuration at both the α- and β-carbon atoms.

It appears that although α-methyl substitution may affect the close attachment of the cholinergic molecule to the muscarinic receptor and β-methyl substitution results in abolition of nicotinic activity the position of the substituent may not be the important factor in determining stereospecificity. Rather it appears that the conformation adopted by the molecules at the receptor is the factor which determines whether these compounds will show stereospecificity. Armstrong et al. (1968), Armstrong and Cannon (1970), Cannon et al. (1972) and Chiou et al. (1969) concluded from their studies on cis and trans-2-acetoxycyclopropyltrimethylammonium iodide (15 and 16) that for maximal muscarinic activity the $\overset{+}{N}$-C4-C5-O1 torsion angle of acetylcholine-like compounds should be 180° (trans). Trans-ACTM (16) was equiactive with acetylcholine and its muscarinic

activity resided primarily in the (+)-1(S)-2(S)-isomer. But, Casy et al. (1971), Chothia (1970) and Kier (1967) concluded from a consideration of the preferred conformation of a series of cholinergic molecules in solution and in the solid state that the $\overset{+}{N}$-C4-C5-O1 torsion angle of potent muscarinic agonists was 60° (gauche).

When comparing the results obtained in this study with those of previous workers it is necessary to assume that the reversed esters and the acetylcholine derivatives are acting at the same site on the muscarinic receptor. The pharmacological data obtained in this thesis combined with the results of conformational analysis (see page 103) supports the hypothesis of Cannon et al. (1972) (see above). Preliminary p.m.r. studies of the preferred conformation of methyl 3-dimethylaminobutyrate methiodide (5) in solution suggested that the compound had a preferred conformation and that the $\overset{+}{N}$-C4-C5-O1 torsion angle was 180° (trans). (See page 107.) This ester (5) showed stereospecificity in its interaction with the muscarinic receptor. (The decreased muscarinic activity is probably due to the steric influence exerted by the α-methyl substituent.) The reversed ester of β-methylacetylcholine (6) had a preferred conformation in which the $\overset{+}{N}$-C4-C5-O1 torsion

angle was 60° (gauche). (See page 106.) Although this compound had the necessary conformation suggested by Chothia (1970) for stimulation of the muscarinic receptor it showed no stereospecificity and was much less active than its corresponding acetylcholine analogue (3) at the muscarinic receptor. The preferred conformation of this compound is analogous to cis-ACTM (15) which exhibited negligible muscarinic activity on the guinea-pig ileum (Armstrong et al., 1968). However, it must be remembered that β-methylacetylcholine (3), a potent muscarinic agonist, has been shown to prefer a gauche (cis) conformation in solution. The reversed ester of acetylcholine (4) did not have a preferred conformation in solution. (See page 104.) In the presence of eserine it showed 0.15 of the muscarinic activity of acetylcholine.

Based upon the conformational preferences shown by the reversed esters (4,5 and 6) in solution it is possible to calculate the expected muscarinic activity of the compounds. If we assume interaction of the same sites in the case of the reversed ester of acetylcholine (4) there are three possible conformations, one trans and two equivalent gauche forms. (See page 104.) The p.m.r. spectrum of this compound showed that there was an equal amount of each conformer present. If the trans conformer is the active form at the receptor it would therefore be expected that the

compound would have 0.33 of the muscarinic activity of acetylcholine. If the gauche conformer were preferred the expected activity would be 0.66 of acetylcholine. The muscarinic activity of the ester (4) in the presence of eserine was lower than both these theoretical figures. It had 0.15 times the activity of acetylcholine. This figure is in closer agreement with the theoretical value for the trans conformer and for this reason it could be argued that the trans conformer is probably the active form at the muscarinic receptor. It is realized, however, that a gauche conformer which fitted imperfectly on the receptor would have an activity much reduced from the theoretical, and, therefore, a categorical conclusion that the trans conformer is the active conformer is being made. The observation that the ester (4) had less activity than expected suggests that reversing the positions of the carbonyl and ethereal oxygen atoms decreases the activity by a factor of approximately 0.5 (0.15/0.33). Similar calculations can be carried out for the reversed ester of α- and β-methylacetylcholine (5 and 6). The p.m.r. spectrum of the reversed α-ester (5) showed that the gauche form of this compound was present in less than 1%. (See page 107.) In calculating the expected activity of this compound the influence of an α-methyl substituent must be considered. An α-methyl substituent appears to reduce muscarinic

activity by a factor of 0.01 (cf activity of α-methylacetylcholine). If the gauche form is active at the receptor the expected activity of the reversed α-ester (5) would be 0.01 (contribution of the α-methyl group) X 0.01 (the contribution of the gauche conformer) X 0.5 (decrease in activity as a result of reversing the positions of the C=O and -O- groups) = 0.0005. If the trans conformer is active at the receptor the expected activity would be 0.01 X 1 X 0.5 = 0.005. The observed activity was approximately 0.003 in the presence of eserine. It can be concluded that the trans conformer is probably the active form at the receptor since for the gauche conformer to exhibit 0.003 the activity of acetylcholine the ester (5) would need to be 100 times as active as acetylcholine itself. Few compounds have been shown to be more active than acetylcholine.

The p.m.r. spectrum of the reversed β-ester (6) showed that it existed primarily in the gauche form. (See page 106.) The ester would therefore be expected to be equipotent with acetylcholine if the gauche conformer were preferred at the receptor (A β-methyl substituent does not reduce muscarinic activity.) However, this ester (6) showed 0.007 of the muscarinic activity of acetylcholine in the presence of eserine. This is the contribution expected from the 1% trans conformer present [0.01 (contribution of the trans conformer) X 0.5 reduction in activity

as a result of reversal of the positions of the C=O and -O- groups)]. Therefore these results suggest that the trans conformation is the active form at the muscarinic receptor.

But, how can the above results be interpreted in view of the known conformation and activity of α- and β-methylacetylcholine (2 and 3)? Casy et al. (1971) and Inch et al. (1971) showed that α-methylacetylcholine had no preferred conformation in solution. It would be expected that the activity of the gauche form would be 0.0066 that of acetylcholine (0.01 X 0.66) and the activity of the trans form would be 0.0033 (0.01 X 0.33). The activity shown by α-methylacetylcholine (2) was 0.02 that of acetylcholine a value far removed from either of the calculated figures. Therefore it was not possible, in this instance, to conclude which conformer is the active one. Similarly theoretical calculations suggest that the gauche form of β-methylacetylcholine (3) is active at the muscarinic receptor. The p.m.r. spectrum of 3 showed that the molecule was present almost exclusively in the gauche conformation. Since β-methylacetylcholine is approximately equipotent with acetylcholine it may be assumed that the gauche form is the active one. It is unlikely that the trans conformer is the active one at the receptor since this would indicate that β-methylacetylcholine (3) was at least 100 times as active as acetylcholine itself.

The results obtained with the reversed esters and the acetylcholine derivatives appear to be in opposition to each other. The reversed esters suggest that the trans conformer is active at the muscarinic receptor and the acetylcholine analogues suggest that the gauche conformer is preferred.

There are at least three possible explanations for this discrepancy. The first is that the compounds are acting at different areas on the receptor. It would be necessary to use highly selective antagonists in order to solve this problem. The second possibility is that after initial binding to the receptor occurs a conformational change is induced in the agonist. Since the quaternary nitrogen atom carries a full positive charge it is reasonable to assume that initial binding to the receptor takes place at this point in the molecule. The energy change which takes place as a result of this initial binding may be such that the trans conformer of the reversed ester is converted into the gauche conformation or the gauche conformer of the acetylcholine derivatives is converted into a trans conformation. Liquori et al. (1968a) showed that the energy difference between the conformers of acetylcholine was approximately only 1 kcal/mole. It appears to be more likely that the gauche conformation of the

acetylcholine derivatives is no longer favoured after initial binding takes place. Binding of the quaternary nitrogen to the receptor would be expected to reduce the positive charge on the nitrogen and therefore reduce the electrostatic attraction between the nitrogen and the carbonyl group. It has been suggested that this electrostatic attraction holds β-methylacetylcholine (3) and acetylcholine itself in the gauche conformation (Casy et al. 1971; Chothia and Pauling, 1969). It is highly unlikely that attachment to the receptor would increase the charge on the nitrogen and favour stronger gauche interaction between the $\overset{+}{N}$ and C=O groups of the acetylcholine derivatives or their reversed esters. With the reversed esters the possibility of induced fit brought about by a change in the charge distribution on the molecule is less likely. It is noteworthy that trans-ACTM (16) is active at the receptor whereas none of the rigid decalin or cyclohexyl derivatives which have a $\overset{+}{N}$-C4-C5-O1 torsion angle of 60° (gauche) are active. In the case of the rigid analogues there is no possibility of induced fit. However, it must be remembered that the bulk of these and other rigid analogues is a complicating factor in their activity.

A third possibility exists which would account for the differences observed in the preferred conformations of the reversed esters and the acetylcholine analogues. Recent studies on the muscarinic receptor imply a dual mode of substrate binding in which a common anionic site is flanked by two loci for polar and nonpolar side chains (Belleau, 1970; Moran and Triggle, 1970). The analogues of acetylcholine may bind at one side of the anionic site and the reversed esters on the other side. These two loci may have different conformational and stereochemical requirements.

From the studies undertaken it is possible to make certain observations concerning the structure activity relationships of the compounds under investigation. It has been established previously that a positively charged trimethylammonium group is necessary for maximal activity (Goldstein et al., 1969). Methyl substitution at the β-carbon atom of acetylcholine or its reversed ester (4) results in a loss of nicotinic activity but its muscarinic activity is not altered. Methyl substitution at the α-carbon atom results in reduction of muscarinic activity but does not alter nicotinic activity. Methyl substitution at the α- or β-carbon

atom has a different stereochemical result in the acetylcholine analogues as compared with the reversed esters. In the case of the acetylcholine analogues substitution at the β-carbon atom resulted in a stereospecific compound whereas substitution at the α-carbon atom of the reversed esters resulted in a compound which acts stereospecifically at the muscarinic receptor.

What can be concluded about the importance of the carbonyl and ethereal oxygen atoms? Reversal of their positions reduced the observed muscarinic activity by a factor of 0.5 suggesting that binding to the muscarinic receptor had been altered. (See page 138 .) A comparison of the relative positions of the functional groups of acetylcholine and its reversed ester (4) was made using 'Framework' molecular models. In order for this comparison to be valid it must be assumed that the compounds are acting at the same site on the muscarinic receptor. The positions of the ethereal and carbonyl groups were not superimposable when the compounds were in the gauche or trans conformation. This suggested that these two groups were not primarily involved in the interaction with the muscarinic receptor although they must play a role in interaction with the receptor since reversal of their relative positions results in

a decrease in activity. Next, the relative positions of the terminal methyl groups were examined. When both acetylcholine and its reversed ester were in the trans conformation the positions of the terminal methyl groups were superimposable. The methyl groups were not superimposable when the compounds were in the gauche conformation. This observation provides further support for the hypothesis that the trans conformer is favoured at the muscarinic receptor and supports the suggestion of Chothia (1970) and Beers and Reich (1971) that a charged nitrogen and terminal methyl group are essential for muscarinic activity. It has been shown that the formyl derivative of acetylcholine $(CH_3)_3\overset{+}{N}CH_2CH_2O\overset{O}{\overset{\|}{C}}H$ has decreased muscarinic activity. (Barlow, 1964).

The preceding observations along with those of other workers suggest that the muscarinic receptor has the form represented in Figure 14. This is essentially the shape for the muscarinic receptor outlined by Cocolas et al. (1970). The sites of interaction with the receptor appear to be at the quaternary nitrogen and the terminal methyl groups. The ethereal and carbonyl oxygens may aid in binding to the receptor and are therefore considered to be secondary sites of inter-

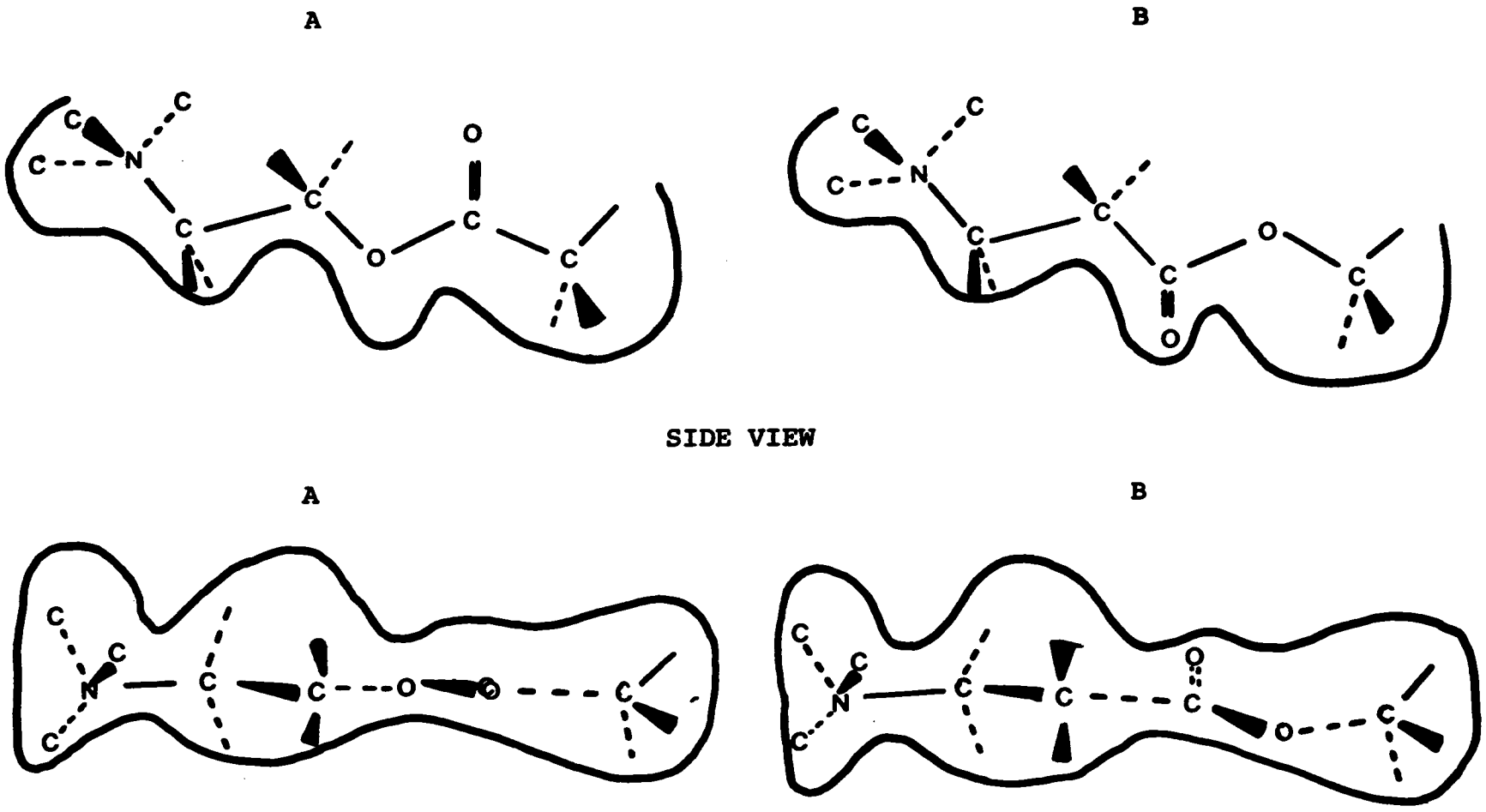


Figure 14: Suggested form of the muscarinic receptor: A. conformation of acetylcholine B. conformation of methyl 3-dimethylaminopropionate methiodide (4). (Interaction may take place with the receptor on any side of the molecules.)

action. It appears evident that the methylene side chain fits into a very narrow area since all the rigid and more bulky analogues with the exception of trans-ACTM (16) are inactive. The observation that α,α-dimethyl- and β,β-dimethylacetylcholine (13,14) have no muscarinic activity also supports this hypothesis. (Cocolas et al., 1971). It is probably in this narrow area that stereochemical requirements come into play. The methyl substituents on the choline chain of both compounds which exhibited stereospecificity, L(+)S-β-methylacetylcholine (3a) and L(-)S-methyl 3-dimethyl-aminobutyrate methiodide (5b) project on the same side of the choline chain. The methylene group of the cyclopropyl ring of the active isomer of trans-ACTM (16) also projects on this side of the chain suggesting that one side of the narrow area of the receptor must be slightly distorted in order to accommodate these groups. Therefore it can be suggested that only two areas of the cholinergic molecules (N^+ and terminal methyl groups) are involved in stimulation of the receptor and the third area which results in stereospecificity in the interaction at the receptor is one of zero interaction (but, positive fit to this area is necessary for activity).

Do the results obtained throw any light upon the requirements for nicotinic activity? Archer et al. (1962) proposed that the nicotinic action of acetylcholine was due to the cis conformation. However, the studies reported here showed that the reversed α-ester (5), which was a potent nicotinic stimulant, preferred a trans conformation, and the β-ester (6), which had negligible nicotinic activity, preferred the cis conformation. These results along with those of Armstrong et al. (1968), where cis-ACTM (15) was shown to have negligible nicotinic activity, cast doubt upon the proposal of Archer et al. (1962). The lack of stereospecificity of the cholinergic agonists at the nicotinic receptor tends to support the hypothesis that only a two point interaction is necessary for stimulation of the nicotinic receptor (Barrass et al., 1968; Kier, 1968). The antagonist tubocurarine does show stereospecificity at the nicotinic receptor (King, 1947).

Lastly, is it possible to draw any conclusions concerning interactions at the active site of acetylcholinesterase? As shown by the results obtained with α-methylacetylcholine (2) it is important to consider cholinesterase hydrolysis when evaluating the cholinergic

activity of a compound. Although Chothia and Pauling (1969b) suggested that the conformer of α-methylacetylcholine (2) which had a $\overset{+}{N}$-C4-C5-O1 torsion angle of 180° (trans) was the active conformer at the enzymatic site, this study has yielded no evidence that the trans conformer is preferred at this site.

In conclusion these studies have suggested that the trans conformer of cholinergic molecules is the active form at the muscarinic receptor. A study of molecular models has led to the hypothesis that the $\overset{+}{N}$- and terminal methyl groups are involved in the interaction with the muscarinic receptor and the carbonyl and ethereal oxygen groups provide additional binding sites. A diagramatic representation of the muscarinic receptor has been presented in Figure 14. The nicotinic receptor appeared to have no stereospecific requirements. The active site of cholinesterase was stereospecific in its interactions with α-methylacetylcholine but to date it is not possible to relate this stereospecificity to conformational preference. Further experiments will be necessary to assure that the acetylcholine derivatives (1,2 and 3) and their reversed esters (4,5 and 6) are acting at the same receptor and have the same affinity

and intrinsic activity. The results obtained support the statement of Shefter (1971) that the observed conformation of cholinergic compounds in the solid state or in solution is not necessarily the active conformer at the receptor. It is necessary to combine pharmacological data with theoretical calculations when evaluating the interactions of cholinergic molecules with their receptors.

N,N-Dialkylamino-propionic, -butyric and -acetic Acid Esters

The pharmacological activity of the series of N,N-dialkylamino-propionic, -butyric and -acetic acid esters and several of their derivatives was evaluated on the blood pressure of anesthetized rats. The compounds were tested as their hydrochloride salts (Table XI) and in most cases as their methiodides (Table XII). Only two of this series of compounds showed any appreciable muscarinic activity. These were the methiodides of methyl and ethyl 4-dimethylaminobutyrate (85 and 86) which had 0.01 of the muscarinic activity of acetylcholine (see Table XII) on the rat blood pressure. These results are comparable with those reported for cats and the guinea-pig ileum (Barrass et al., 1968). As predicted from previous work (Coutts et al., 1971) and from experiments with acetylcholine analogues (Barlow, 1964), an increase in the size of the basic substituent above that of a trimethylammonium group greatly reduced muscarinic activity. See Table VIII-X for the activity of the methyl 3-dimethylamino-propionate and -butyrate methiodides). Branching and lengthening or shortening of the alkyl chain joining the $\overset{+}{N}$ atom and the C=O group

Table XI

Pharmacological Activity of the Hydrochloride Salts of Some N,N-Dialykylamino-propionic, -butyric and -acetic Acid Esters and Several of Their Derivatives on the Rat Blood Pressure

Ester ** (or related compound)	Dose mg/kg (No. of Expt.)	Relative Molar Potency Ach= 1.00	Relative Molar Potency DMPP = 1.00	Mechanism	Ref. ***
68	5-20(2)	<0.0001	---		
69	5-20(2)	<0.0001	---		
70	5-20(2)	<0.0001	---		
71	5-20(2)	<0.0001	---	non-specific[a]	Coutts, et al., 1971
72	5-20(4)	0.0050	0.0050	mixed muscarinic[b] and nicotinic[c]	Barrass et al., 1968; Matkovics, et al., 1961; Porszasz, et al., 1961

73	5-20(2)	<0.0001	---		
74	5-20(4)	<0.0001	---	muscarinic[b]	
75	5-20(2)	---	0.0500	nicotinic[d]	Matkovics et al., 1961; Philips, 1950; Porszasz et al., 1961
76	5-20(2)	<0.0001	---		
77	5-20(2)	---	0.0050	nicotinic[d]	
78	5-20(4)	<0.0001	---	ganglion blocker[e]	
79	5(2)	<0.0001	---		Barrass et al., 1968; Lightlowler and MacLean 1963
80	5-20(2)	<0.0001	---		
81	5-20(2)	<0.0001	---		
82	5-20(2)	<0.0001			Barrass et al., 1968

83	5-20(2)	<0.0001	---	non-specific[a]
84	0.5-2(2)	<0.0001	---	non-specific[a]
86	5-20(4)	<0.0001	---	
87	10-20(3)	<0.0001	---	ganglion blocker[e]
88	5-20(2)	<0.0001	---	
89	5-20(2)	<0.0001	---	
90	5-20(2)	<0.0001	---	muscarinic[c]
91[f]	2,500(2)	---	0.0050	nicotinic[d]

a) depressor response not blocked by hyoscine methiodide and compound does not reduce response to DMPP

b) depressor response blocked by hyoscine methiodide

c) pressor response blocked by hexamethonium

d) pressor response blocked by pentolinium

e) reduces response to DMPP

f) obtained as hydriodide salt

**) complete structures of these compounds are recorded on pages 82,88,90,91

***) see references for pharmacological results of other workers

Table XII

Pharmacological Activity of the Methiodides of Some N,N-Dialkylamino-propionic, -butyric and -acetic Acid Esters and Several of Their Derivatives on the Rat Pressure

Ester** (or related compound)	Dose mg/kg (No. of Expt.)	Relative Molar Potency Ach= 1.00	Relative Molar Potency DMPP = 1.00	Mechanism	Ref.***
68	5-20(2)	---	0.00050	nicotinic[a]	
69	5-20(2)	<0.0001	---		
71	5-20(2)	<0.0001	---		
72	0.1(4)	<0.0001	---	muscarinic[b]	Barrass et al., 1968; Matkovics et al., 1961; Porszasz et al., 1961
	1.0(4)	---	0.8500	nicotinic[c]	
73	5-20(4)	---	0.0500	nicotinic[d]	

75	5-20(2)	---	0.0500	nicotinic[a]	Matkovics et al., 1961; Porszasz et al., 1961
76	5-20(2)	<0.0001	---		
77	5-20(2)	<0.0001	---		
79	5-20(4)	0.0100	---	muscarinic[b]	Barrass et al., 1968; Lightlowler and MacLean, 1963
80	5-20(2)	<0.0001	---		
81	5-20(2)	<0.001	---		Barrass et al., 1968
82	5-20(2)	<0.0001	---		
83	5-20(2)	<0.0001	---		
85	5-20(8)	0.0100	---	muscarinic[b]	Barrass et al., 1968
86	5-20(8)	0.0100	---	muscarinic[b]	Barrass et al., 1968

87	5-20(2)	<0.0001	<0.0001

a) pressor response blocked by hexamethonium

b) depressor response blocked by hyoscine methiodide

c) pressor response blocked by hexamethonium

d) pressor response blocked by trimethaphan

**) complete structures of these compounds are recorded on pages 82,88,90,91

***) see references for pharmacological results of other workers

resulted in muscarinic activity lower than that displayed by compounds with an unsubstituted two carbon alkyl chain.

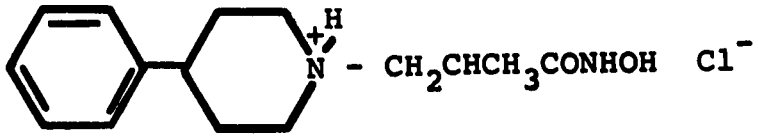
A number of the compounds showed some degree of nicotinic activity. Barrass et al. (1968) proposed that compounds with the general structure $RR^1N(CH_2)_nCOOR^2$ showed primarily nicotinic activity when tested as their methiodides. These results appear to support this observation.

Alteration of the ester grouping to an esterase resistant amide, ketone or hydroxamate (87, 88, 89, 90) had no effect on muscarinic activity with the exception of the hydrochloride salt of the amide 87, namely 3-dimethylaminopropionamide hydrochloride, which in a limited number of experiments showed ganglion blocking activity unlike the corresponding ester 72 which showed mixed muscarinic and nicotinic properties.

With the exception of the methiodide of ethyl 4-dimethylaminobutyrate (79) which had 0.01 the muscarinic activity of acetylcholine, compounds with the general structure $RR^1R^2\overset{+}{N}(CH_2)_3COOCH_2CH_3\ I^-$ can be considered to be essentially inactive. The slight depressor effect of these compounds relative molar potency <0.001, appeared to be nonspecific since it was not altered by

the prior administration of hyoscine methiodide or a ganglion blocking agent such as hexamethonium, pentolinium or trimethaphan.

Methyl 3-[1-(4-phenylpiperidino)]butyrate hydrochloride (78) showed ganglion blocking activity in four experiments. This compound is structurally related to 2-methyl-3-[1-(4-phenylpiperidino)]propionohydroxamic acid hydrochloride (17) which was reported to be a ganglion blocking agent (Midha et al., 1970). None of the compounds synthesized showed sufficient activity to



(17)

warrant further investigation.

EXPERIMENTAL

Melting points were determined on a Thomas Hoover capillary melting point apparatus. All melting points and boiling points quoted are uncorrected. Infrared (i.r.) spectra were recorded on a Beckman IR10 Spectrophotometer; and proton magnetic resonance (p.m.r.) spectra were taken on a Varian A-60D Spectrophotometer using tetramethylsilane (TMS) as the internal standard. Mass spectra were run on either an AEI MS-9 or MS-12 mass spectrometer by Dr. A.M. Hogg and his associates. The direct insertion probe was used in all cases. The electron beam energy was 70 eV and the ion source temperature ranged from 120 to 205° depending on the compound. Accurate mass measurements were made by the peak matching method. Elemental analyses were determined by Mr. W. Dylke of the Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta. All the chemicals obtained from commercial sources were used without further purification.

GENERAL METHODS

Preparation of 3-amino-esters

Most of the esters required in this study were prepared by mixing anhydrous methanolic solutions of an appropriate amine and an α,β-unsaturated ester (methyl acrylate, methyl methacrylate and methyl crotonate), in equimolar quantities. The cooled amine solution was slowly added with stirring to the cooled solution of the ester and the resulting mixture maintained at 0° for 2 h, allowed to come to room temperature then, in some instances heated under reflux for an appropriate length of time. The methanol was removed and the residual oil was distilled under reduced pressure.

Each of the esters was characterized by means of microanalysis and its p.m.r. and i.r. spectra. The absence of a C=C stretching band near 1630 cm^{-1} in an aliquot portion (due to acrylate starting material) and/or the absence of a band within the range 3320-3500 cm^{-1} (due to secondary amine >N-H) were considered to be positive indications that the reaction had gone to completion.

Preparation of ethyl 4-aminobutyrates

Separate portions of ethyl 4-bromobutyrate (1 mole) and the appropriate amine (3 mole) were dissolved in dry benzene. The cooled amine solution was slowly added with stirring to the cooled solution of the ester and the resulting mixture was maintained at 0° for 2 h, allowed to come to room temperature then, in some instances heated under reflux for an appropriate length of time. The hydrobromide salt of the starting amine was filtered off, the benzene removed under reduced pressure and the residual oil was distilled under reduced pressure.

Preparation of hydrochloride salts of amino-esters

The hydrochloride salts of the amino-esters were prepared by passing dry hydrogen chloride through an ethereal solution of the appropriate ester. The hydrochlorides, obtained as colorless crystals, were recrystallized from hot acetone/diethyl ether and were characterized by microanalysis and their p.m.r. and i.r. spectra. All showed strong $\rangle\overset{+}{N}$-H stretching bands in the 2350-2710 cm^{-1} region of their i.r. spectra (Thompson *et al.*, 1965).

Preparation of methiodides of amino-esters

The methiodides of the amino-esters were prepared by dissolving each amino-ester in acetone and adding an excess of methyl iodide in acetone. The methiodides, obtained as colorless crystals, were recrystallized from hot acetone/diethyl ether and characterized by microanalysis and their p.m.r. and i.r. spectra. The $\geq\overset{+}{N}-CH_3$ protons characteristically came to resonance 6-7τ downfield from TMS (Culvenor and Ham, 1966) in each p.m.r. spectrum.

Methyl 3-dimethylamino-propionates and -butyrates

Methyl 3-dimethylaminopropionate

The title compound was prepared in quantitative yield from dimethylamine and methyl acrylate using the general method for the preparation of 3-amino-esters. The reaction mixture was kept at room temperature for 24 h prior to fractional distillation. The compound had b.p. 29-31°/0.07 mm. Literature b.p. 60°/30 mm (Barrass et al., 1968). The i.r. spectrum (thin film): 1740 cm^{-1} (ester C=O). The p.m.r. spectrum (CCl_4): 6.42τ (3H, s, $-OCH_3$), 7.30-7.70τ (4H, m, $-CH_2-CH_2-$), 7.80τ (6H, s, $-N(CH_3)_2$).

Anal. Calcd. for $C_6H_{13}NO_2$: C, 54.91; H, 9.99; N, 10.68. Found: C, 54.85; H, 9.76; N, 10.55.

The hydrochloride had m.p. 120°. The i.r. spectrum (Nujol): 1740 cm^{-1} (ester C=O); 2480, 2520, 2580 cm^{-1} ($\overset{+}{\rangle}N-H$). The p.m.r. spectrum ($CDCl_3$): -2.30τ (1H, s, $\overset{+}{\rangle}N-H$, exchanged with D_2O).

Anal. Calcd. for $C_6H_{14}ClNO_2$: C, 42.95; H, 8.42; N, 8.33. Found C, 42.81; H, 8.37; N, 8.33.

The methiodide had m.p. 201-202°. Literature m.p. 208-209° (Barrass et al., 1968). The i.r. spectrum (Nujol): 1740 cm^{-1} (ester C=O). The p.m.r. spectrum

(D_2O): 5.60-6.35τ (2H, m, $\overset{+}{\underset{}{\gtrless}}N-\underline{CH}_2-CH_2-$) 6.22τ (3H, s, $-O\underline{CH}_3$), 6.83τ (9H, s, $-\overset{+}{N}(\underline{CH}_3)_3$), 6.60-7.18τ (2H, m, $-CH_2-\underline{CH}_2-CO-$). The mass spectrum: 254 (2.94), 142 [$CH_3I^{+\cdot}$] (10.6), 131 [$(CH_3)_2\overset{+\cdot}{N}CH_2CH_2COOCH_3$] (0.35), 128 (88.3), 127 (53.0), 86 (16.5), 85 (10.0), 72 (5.9), 68 (2.0), 63.5 (4.1), 59 (35.2), 58 [$(CH_3)_2\overset{+}{N}=CH_2$] (100.0), 57 (7.6), 56 (5.3), 55 (70.6), 53 (2.8), 45 (3.5), 44 (7.6), 43 (11.6), 42 (58.8), 40 (5.9), 39 (2.4), 30 (15.3), 29 (7.1), 28 (9.5), 27 (46.0), 26 (17.0), m/e (% relative abundance).

Anal. Calcd. for $C_7H_{16}INO_2$: C, 30.77; H, 5.91; N, 5.13. Found: C, 31.00; H, 5.74; N, 5.31.

(±)-Methyl 3-dimethylaminobutyrate

The title compound was prepared in quantitative yield from dimethylamine and methyl crotonate using the general method for the preparation of 3-amino-esters. The reaction mixture was heated under reflux for 23 h prior to fractional distillation. The compound had b.p. 50°/10 mm. Literature b.p. 66°/17 mm (Adamson, 1950). The i.r. spectrum (thin film): 1735 cm^{-1} (ester C=O). The p.m.r. spectrum (CCl_4): 6.42τ (3H, s, $-O\underline{CH}_3$), 6.68-7.50τ (1H, m, $>N-\underset{\underline{H}}{\overset{CH_3}{\underset{|}{\overset{|}{C}}}}-CH_2-$), 7.59-8.17τ

(2H, m, $\rangle N-\underset{H}{\overset{CH_3}{C}}-CH_2-$), 7.85τ (6H, s, $-N(CH_3)_2$), 9.03τ (3H, d, J=7Hz, $H-\underset{}{C}-CH_3$).

Anal. Calcd. for $C_7H_{15}NO_2$: C, 57.93; H, 10.34; N, 9.66. Found: C, 58.00; H, 10.28; N, 9.91.

The hydrochloride had m.p. 105-106°. The i.r. spectrum (Nujol): 1735 cm^{-1} (ester C=O); 2350-2700 cm^{-1} ($\overset{+}{\rangle}N-H$). The p.m.r. spectrum ($CDCl_3$): -1.23τ (1H, s, $\overset{+}{\rangle}N-H$, exchanged with D_2O).

Anal. Calcd. for $C_7H_{16}ClNO_2$: C, 46.28; H, 8.81; N, 7.71. Found: C, 46.04; H, 9.07; N, 7.44.

The methiodide had m.p. 183-184°. The i.r. spectrum (Nujol); 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 5.50-6.40τ (1H, m, $\overset{+}{\rangle}N-\underset{H}{\overset{CH_3}{C}}-CH_2-$). 6.20τ (3H, s, $-OCH_3$), 6.55-7.50τ (2H, m, $-\underset{H}{\overset{CH_3}{C}}-CH_2-$), 6.76τ (9H, s, $-\overset{+}{N}(CH_3)_3$), 8.50τ (3H, doublet of triplets, J=7 and 2Hz, $\overset{+}{\rangle}N-\underset{H}{\overset{CH_3}{C}}-CH_2-$). The mass spectrum: A) Initial spectrum: 254 (11.6), 145 $[(CH_3)_2\overset{+\cdot}{N}CH(CH_3)CH_2COOCH_3]$

(3.8), 143 (3.8), 142 [$CH_3I^{+\cdot}$] (100.0), 141 (15.1), 140 (3.6), 139 (3.7), 128 (54.6), 127 (45.6), 102 (17.7), 100 (13.8), 86 (31.0), 85 (7.2), 82 (2.2), 72 (96.0), 71 (2.5), 69 (46.6), 68 (8.5), 64 (2.6), 63.5 (2.9), 59 (43.9), 58 (98.9), 57 (7.0), 56 (2.7), 45 (3.3), 44 (3.5), 43 (6.5), 42 (31.0), 41 (31.5), 40 (6.1), 39 (20.6), 38 (3.3), 30 (15.0), 29 (6.3), 28 (8.1), 27 (3.7), 20 (2.6), 18 (16.8), 17 (3.4), 15 (5.8), m/e (% relative abundance). B) Subsequent spectrum: 254 (7.7), 142 [$CH_3I^{+\cdot}$] (45.5), 141 (6.4), 128 [$HI^{+\cdot}$] (100.0), 127 (59.0), 100 (5.3), 86 (15.0), 85 (3.5), 72 (2.0), 69 (20.4), 68 (3.2), 64 (3.4), 63.5 (3.9), 59 (3.4), 58 (86.3), 57 (6.4), 43 (4.6), 42 (14.5), 41 (12.3), 40 (2.6), 39 (4.5), 30 (11.8), 29 (2.9), 28 (2.8), 27 (2.1), 18 (44.5), 17 (9.6), 15 (8.6), m/e (% relative abundance).

Anal. Calcd. for $C_8H_{18}INO_2$: C, 33.44; H, 6.32; N, 4.88. Found C, 33.33; H, 6.55; N, 5.10.

D(+)R-Methyl 3-dimethylaminobutyrate methiodide

D(-)-Alanine (B.D.H., $[\alpha]_D^{25.2}$ -1.94° (c 5.31 H_2O)) (10 g) and phthalic anhydride (16.6 g) were thoroughly mixed then heated at 160-180° for 15 min. The resulting oil was poured while still hot into water and

allowed to crystallize. The resulting compound (+)-N-phthaloyl-D-alanine, recrystallized twice from water and thoroughly air dried, had m.p. 145-146°, yield 86.2%, $[\alpha]_D^{23.8}$+32.51° (c 1.59 95% ethanol). Literature m.p. 149-150°, $[\alpha]_D^{20}$+23.4 ± 0.5° (c 1.8 ethanol) (Beckett and Casy, 1955). The i.r. spectrum (Nujol): 1690, 1745, 1760, 1780 cm^{-1} (C=O), 2400-3400 cm^{-1} (acid OH). The p.m.r. spectrum (deuterated acetone): 2.12τ (4H, s, $C_6\underline{H}_4$-), 2.98τ (1H, s, -COO<u>H</u>, exchanged with D_2O), 4.98τ (1H, q, J=7Hz, $H_3C-\underset{|}{\overset{|}{C}}-\underline{H}$), 8.31τ (3H, d, J=7Hz, $H-\underset{|}{\overset{|}{C}}-C\underline{H}_3$).

Anal. Calcd. for $C_{11}H_9NO_4$: C, 60.25; H, 4.14; N, 6.34. Found C, 60.40; H, 4.09; N, 6.41.

(+)-N-phthaloyl-D-alanine (15 g) was heated with redistilled thionyl chloride (15 ml) at 60° for 30 min. then the excess thionyl chloride removed under vacuum. The residue, (+)-N-phthaloyl-D-alanyl chloride, obtained in quantitative yield, solidified when stored under vacuum for 24 h and had m.p. 52-53°, $[\alpha]_D^{23.8}$+32.51° (c 1.59 benzene). The i.r. spectrum (thin film): 1720, 1780, 1795, 1830 cm^{-1} (C=O). The p.m.r. spectrum (CCl_4): 2.14τ (4H, s, $C_6\underline{H}_4$-), 4.89τ (1H, q, J=7Hz, $H_3C-\underset{|}{\overset{|}{C}}-\underline{H}$), 8.21τ (3H, d, J=7Hz, $H-\underset{|}{\overset{|}{C}}-C\underline{H}_3$).

Anal. Calcd. for $C_{11}H_8ClNO_3$: C, 55.56; H, 3.39;

N, 5.90. Found: C, 55.41; H, 3.44; N, 5.78.

The (+)-N-phthaloyl-D-alanyl chloride (15 g) was then dissolved in dry benzene (200 ml) and added slowly to an ice cooled ethanol-free solution of diazomethane[a] in diethyl ether (400 ml containing approximately 6 g of diazomethane; two equivalents of diazomethane are required for each equivalent of acid chloride). The reaction mixture was stirred at room temperature for 1 h then allowed to stand for 2 h. The solvent and excess diazomethane were removed under vacuum to give the yellow oil, D(+)-1-diazo-3-phthalimidobutan-2-one,

[a]Preparation of diazomethane:

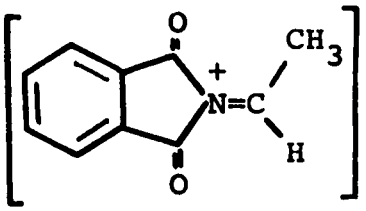
Ethylene glycol monomethylether (35 ml) and diethyl ether (20 ml) were added to a solution of potassium hydroxide (6 g) in water (10 ml). The solution was placed in a 100 ml long-necked distilling flask with dropping funnel, efficient condenser, and water bath at 70°. As the distillation of the ether started, a solution of Diazald[R] (N-methyl-N-nitroso-p-toluenesulfonamide) (21.5 g) in about 200 ml of diethyl ether was added through the dropping funnel over 20 min. The ethereal solution was collected in a receiving flask containing diethyl ether (20-30 ml) which had been cooled to 0°. Yield: approximately 3 g.

in quantitative yield, $[\alpha]_D^{23.8}$+87.14° (c 0.61 ethyl acetate). Literature $[\alpha]_D^{17}$+86.7 ± 0.3° (c 0.48 ethyl acetate) (Beckett and Casy, 1955). The oil was not purified. The i.r. spectrum (thin film): 1640, 1720, 1780 cm^{-1} (C=O); 2100 cm^{-1} (N≡N). The p.m.r. spectrum (CCl_4): 2.24τ (4H, s, $C_6\underline{H}_4$-), 2.72τ (1H, s, $\equiv N-\overset{+}{\underset{|}{C}}-\underline{H}$), 5.22τ (1H, q, J=7Hz, $H_3C-\underset{|}{C}-\underline{H}$), 8.42τ (3H, d, J=7Hz, $H-\underset{|}{C}-C\underline{H}_3$), (unidentified singlets at 4.56τ and 5.84τ were attributed to impurities).

The diazoketone (15 g) was immediately dissolved in anhydrous methanol (200 ml) and a 7% solution of silver benzoate in triethylamine (30 ml) added dropwise over 2 h. The reaction mixture was then filtered, the filtrate was heated under reflux for 5 min. with activated charcoal, filtered and the solvent removed under vacuum. The resulting residue was extracted with diethyl ether (3 X 20 ml) and the ether extract evaporated to dryness to give D(-)-methyl 3-phthalimidobutyrate in 70.4% yield, $[\alpha]_D^{23.8}$-20.54° (c 0.51 benzene). Literature b.p. 140-144°/0.7 mm, $[\alpha]_D^{23}$-33.1 ± 0.5° (c 0.5 benzene) (Beckett and Casy, 1955). The resulting orange oil was not purified. The i.r. spectrum (thin film): 1710, 1730, 1770 cm^{-1} (C=O). The p.m.r. spectrum (CCl_4): 2.28τ (4H, s, $C_6\underline{H}_4$), 5.37τ (1H, q, J=7Hz, $H_3C-\underset{|}{C}-\underline{H}$),

6.42τ (3H, s, $-OCH_3$), 7.00-7.50τ (2H, m, $-\overset{\overset{CH_3}{|}}{\underset{\underset{H}{|}}{C}}-CH_2-$),

8.54τ (3H, d, J=7Hz, $H-\overset{|}{\underset{|}{C}}-CH_3$). The mass spectrum:

247 $[C_{13}H_{13}NO_4]^{+\cdot}$ (2.44), 174  (100), m/e (% relative abundance).

D(-)-Methyl 3-phthalimidobutyrate (13 g) was then heated under reflux with glacial acetic acid (32 ml) and hydrochloric acid (40 ml) for 8 h. The reaction mixture was allowed to stand overnight and then the precipitated phthalic acid was filtered off. The filtrate was heated under reflux with activated charcoal for 10 min., filtered then evaporated to dryness under vacuum. The 3-aminobutyric acid hydrochloride, obtained in quantitative yield, m.p.>300° (decomp.), was not purified. The i.r. spectrum (thin film): 1700 cm^{-1} (acid C=O).

The crude amino acid hydrochloride (7 g) was dissolved in water (200 ml) to which 10% palladium/charcoal and an aqueous solution of formaldehyde (40% w/v, 10 ml) were added. The reaction mixture was stirred under an atmosphere of hydrogen at room temperature and

pressure until the reaction ceased to consume hydrogen and the theoretical amount of hydrogen had been taken up. The mixture was filtered and the filtrate evaporated to dryness. The paraformaldehyde formed was removed by redistillation from water. The impure 3-dimethylaminobutyric acid hydrochloride was obtained in quantitative yield. The p.m.r. spectrum (DMSO.d_6): 3.80-5.00τ (2H, s, $\overset{+}{\rangle}$N-H and -COOH, exchanged with D_2O), 7.06τ (6H, s, $\overset{+}{N}(CH_3)_2$), 8.58τ (3H, d, J=7Hz, H-$\overset{|}{\underset{|}{C}}$-$CH_3$), the remainder of the spectrum could not be interpreted.

The crude dimethylamino acid hydrochloride (8.4 g) was next dissolved in methanol/3% HCl (200 ml) and heated under reflux for 24 h. The solvent was removed under vacuum to yield a light yellow oil, methyl 3-dimethylaminobutyrate hydrochloride. The i.r. spectrum (thin film): 1730 cm^{-1} (ester C=O); 2220-2600 cm^{-1} ($\overset{+}{\rangle}$N-H).

The above oil was dissolved in water (5 ml) and neutralized with a cold aqueous solution of 20% sodium hydroxide. Potassium hydroxide was added to the aqueous solution to make a slurry and the solution extracted with diethyl ether (3 X 20 ml). The ether extract was dried over anhydrous sodium sulfate then concentrated to 10 ml and diluted with acetone (10 ml).

A cooled solution of excess methyl iodide in acetone was added to the ether/acetone solution and the resulting D(+)-methyl 3-dimethylaminobutyrate methiodide allowed to precipitate. The precipitate was crystallized from acetone/diethyl ether and had m.p. 184-185°, yield 34.0%, $[\alpha]_D^{26.5}$+7.33° (c 2.51 90% ethanol). The i.r. spectrum (Nujol): 1740 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 6.21τ (3H, s, $-OC\underline{H}_3$), 6.86τ (9H, s, $-\overset{+}{N}(C\underline{H}_3)_3$), 6.70-7.50τ (2H, m, $-\overset{CH_3}{\overset{|}{C}}-C\underline{H}_2-$), 5.70-6.30τ (1H, m, $\overset{+}{N}-\overset{CH_3}{\overset{|}{C}}-CH_2-$), 8.51τ (3H, doublet of triplets, J=7 and 2Hz $\overset{+}{N}-\overset{C\underline{H}_3}{\overset{|}{C}}-CH_2-$). The mass spectrum: 254 (0.75), 145 $[(CH_3)_2\overset{+.}{N}CH(CH_3)CH_3COOCH_3]$ (0.24), 142 $[CH_3I^{+.}]$ (8.5), 128 (13.6), 127 (8.5), 100 (24.6), 86 (3.0), 85 (18.8), 72 (4.3), 70 (4.6), 69 $[CH_3CH{=}CH{-}C{\equiv}O^+]$ (100.0), 68 (5.8), 59 (20.8), 58 (51.5), 57 (3.9), 43 (3.6), 42 (11.8), 41 (42.5), 40 (4.9), 39 (19.1), 38 (3.3), 36 (2.1), 30 (8.8), 29 (3.0), 28 (2.4), 27 (2.4), 15 (10.3), m/e (% relative abundance).

Anal. Calcd. for $C_8H_{18}INO_2$: C, 33.44; H, 6.32; N, 4.88. Found: C, 33.67; H, 6.60; N, 4.86.

L(-)S-Methyl 3-dimethylaminobutyrate methiodide

The title compound was prepared in a manner identical to that described for the D(+)-isomer. (-)-N-Phthaloyl-L-alanine was prepared from L(+)-alanine (Aldrich, $[\alpha]_D^{24.5}$+1.78° (c 4.90 H_2O)) (25 g) and had m.p. 145-146°, yield 65.8%, $[\alpha]_D^{24.5}$-17.06° (c 1.58 95% ethanol). Literature $[\alpha]_D^{18}$-17.5° (Balenovic et al., 1952); m.p. 149-150°, $[\alpha]_D^{20}$-23.6° (c 1.8 ethanol) (Beckett and Casy, 1955); m.p. 150-151° (correc.), $[\alpha]_D^{20}$-17.85° (c 3.04 absolute alcohol), -17.62° (c 3.33 absolute alcohol) (Fischer, 1907). The i.r. spectrum (Nujol): 1700, 1770, 1790, 1810 cm^{-1} (C=O); 2400-3300 cm^{-1} (acid OH). The p.m.r. spectrum (deuterated acetone): 2.08τ (4H, s, $C_6\underline{H}_4$-), 3.21τ (1H, s, -COO<u>H</u>, exchanged with D_2O), 4.92τ (1H, q, J=7Hz, $H_3C-\underset{|}{C}-\underline{H}$), 8.3τ (3H, d, J=7Hz, $H-\underset{|}{C}-C\underline{H}_3$).

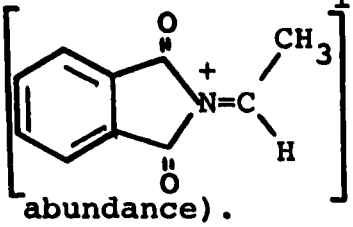
(-)-N-Phthaloyl-L-alanyl chloride obtained in quantitative yield from the (-)-N-phthaloyl-L-alanine had m.p. 50°, $[\alpha]_D^{24.5}$-28.83° (c 1.46 benzene). Literature m.p. 38°, $[\alpha]_D^{17}$-36.4 ± 0.5° (c 0.2 benzene) (Balenovic et al., 1952). The i.r. spectrum (thin film): 1720, 1780, 1795, 1830 cm^{-1} (C=O). The p.m.r. spectrum (CCl_4): 2.10τ (4H, s, $C_6\underline{H}_4$), 4.88τ (1H, q, J=7Hz, $H_3C-\underset{|}{C}-\underline{H}$), 8.20τ (3H, d, J=7Hz, $H-\underset{|}{C}-C\underline{H}_3$).

Anal. Calcd. for $C_{11}H_{18}ClNO_3$: C, 55.56; H, 3.39; N, 5.90 Found: C, 55.77; H, 3.69; N, 5.79.

After storing under vacuum for 24 h the (-)-N-phthaloyl-L-alanyl chloride was reacted with diazomethane in the usual manner to give (-)-1-diazo-3-phthalimido-butan-2-one as a light yellow oil, yield 99.3%. The diazoketone was not purified, $[\alpha]_D^{24.5}$-70.53° (c 0.42 ethyl acetate). Literature $[\alpha]_D^{18}$-69.3 ± 1° (c 0.48 ethyl acetate) (Balenovic et al., 1952); $[\alpha]_D^{24}$-88.5 ± 1° (c 0.5 ethyl acetate) (Beckett and Casy, 1955). The i.r. spectrum (thin film): 1640, 1710, 1780 cm^{-1} (C=O); 2100 cm^{-1} (N≡N). The p.m.r. spectrum (CCl_4): 2.20τ (4H, s, $C_6\underline{H}_4$), 2.66τ (1H, s, $\equiv\overset{+}{N}-\overset{-}{C}(H)-\underline{H}$), 5.26τ (1H, q, J=7Hz, $H_3C-C(H)-\underline{H}$), 8.42τ (3H, d, J=7Hz, $H-C(H)-C\underline{H}_3$), (unidentified singlets at 4.56τ and 5.84τ were attributed to impurities.

The diazoketone was immediately converted into (+)-methyl 3-phthalimidobutyrate, b.p. 160°/0.9 mm, yield 63.4%, pale yellow oil, $[\alpha]_D^{24.5}$+20.82° (c 1.36 benzene). Literature m.p. 38°, $[\alpha]_D^{16}$+26.3 ± 1° (c 0.26 benzene) (Beckett and Casy, 1955). The i.r. spectrum (thin film): 1710, 1740, 1780 cm^{-1} (C=O). The p.m.r. spectrum (CCl_4): 2.22τ (4H, s, $C_6\underline{H}_4$-), 5.30τ (1H, q, J=7Hz, $H_3C-C(H)-\underline{H}$), 6.42τ (3H s, $-OC\underline{H}_3$), 6.50-7.70τ (2H, m,

CH_3
|
$-C-CH_2-$), 8.56τ (3H, d, J=7Hz, $H-C-CH_3$). The mass
H
spectrum: 247 $[C_{13}H_{13}NO_4]^+$ (1.74), 174

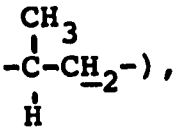
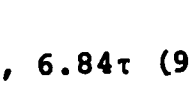


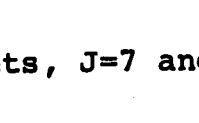
(100), m/e (% relative abundance).

The ester was heated under reflux with glacial acetic acid and hydrochloric acid as described for the D-isomer, to give 3-aminobutyric acid hydrochloride, m.p.>300°. The i.r. spectrum (Nujol): 1710 cm^{-1} (acid C=O). 3-Dimethylaminobutyric acid hydrochloride had i.r. spectrum (thin film): 1710 cm^{-1} (acid C=O). Methyl 3-dimethylaminobutyrate hydrochloride had i.r. spectrum (thin film): 1720 cm^{-1} (ester C=O).

The final product L(-)-methyl 3-dimethylamino-butyrate methiodide had m.p. 183-184° (acetone/diethyl ether), yield 30.1% $[\alpha]_D^{26.5}$ -7.85° (c 5.15 90% ethanol). A second crop of crystals obtained from the mother liquors had $[\alpha]_D^{25}$ -6.09° (c 1.99 90% ethanol). The i.r. spectrum (Nujol): 1740 cm^{-1} (ester C=O). The p.m.r. spectrum

CH_3
|
(D_2O): 5.80-6.30τ (1H, m, $\overset{+}{N}-C-CH_2-$), 6.21τ (3H, s, $-OCH_3$),
H

6.50-7.50τ (2H, m, ), 6.84τ (9H, s, ),

8.50τ (3H doublet of triplets, J=7 and 2Hz, ).

The mass spectrum: 254 (trace), 145 [$(CH_3)_2\overset{+\cdot}{N}CH(CH_3)CH_2COOCH_3$] (.02), 142 [$CH_3I^{+\cdot}$] (37.4), 141 (3.9), 128 (13.4), 122 (2.5), 105 (3.4), 100 (18.7), 86 (3.0), 85 (29.6), 77 (3.3), 72 (10.9), 70 (5.3), 69 [$CHCH_3{=}CH{-}C{\equiv}O^{+}$] (100.0), 68 (9.4), 59 (29.6), 58 (64.0), 57 (6.3), 56 (3.1), 51 (2.3), 45 (2.7), 44 (3.1), 43 (6.3), 42 (3.1), 41 (78.0), 40 (12.5), 39 (68.5), 38 (6.3), 37 (4.7), 30 (17.2), 29 (7.8), 28 (14.0), 27 (10.9), 26 (6.3), 18 (4.5), 15 (46.8), 14 (4.7), m/e (% relative abundance).

Anal. Calcd. for $C_8H_{18}INO_2$: C, 33.44; H, 6.32; N, 4.88. Found: C, 33.69; H, 6.38; N, 4.96.

(±)-Methyl 3-dimethylamino-2-methylpropionate

The title compound was prepared in 77.7% yield from dimethylamine and methyl methacrylate using the general method for the preparation of 3-amino-esters. The reaction mixture was heated under reflux for 24 h prior to fractional distillation. The compound had

b.p. 26°/0.15 mm. Literature b.p. 66-68°/30 mm (Coutts et al., 1971); b.p. 70-71°/24 mm (Traynelis and Dadura, 1961). The i.r. spectrum (thin film): 1740 cm^{-1} (ester C=O). The p.m.r. spectrum (CCl_4): 6.37τ (3H, s, $-OC\underline{H}_3$), 7.25-7.77τ (1H, m, $-CH_2-\overset{CH_3}{\overset{|}{C}}-\underline{H}$), 7.84τ (6H, s, $-N(C\underline{H}_3)_2$), 8.92τ (3H, d, J=7Hz, $H-\overset{|}{C}-C\underline{H}_3$).

Anal. Calcd. for $C_7H_{15}NO_2$: C, 57.90; H, 10.41; N, 9.70. Found: C, 58.10; H, 10.42; N, 9.29.

The hydrochloride had m.p. 128-129°. Literature m.p. 123-125° (Coutts et al., 1971), m.p. 94-96° (Spickett et al., 1966). The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O): 2460-2640 cm^{-1} ($\overset{+}{N}-H$). The p.m.r. spectrum ($CDCl_3$): - 1.23τ (1H, s, $\overset{+}{N}-\underline{H}$, exchanged with D_2O).

Anal. Calcd. for $C_7H_{16}ClNO_2$: C, 46.25; H, 8.88; N, 7.71. Found: C, 46.12; H, 8.66; N, 7.47.

The methiodide had m.p. 113-115°. The i.r. spectrum (Nujol): 1725 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 5.54-7.00τ (1H, m, $-CH_2-\overset{CH_3}{\overset{|}{C}}-\underline{H}$), 6.16τ (3H, s, $-OC\underline{H}_3$), 6.82τ (9H, s, $-\overset{+}{N}(C\underline{H}_3)_3$), 8.65τ (3H, d, J=7Hz $H-\overset{|}{C}-C\underline{H}_3$).

The mass spectrum: 254 (4.0), 145 [$(CH_3)_2\overset{+\cdot}{N}CH_2CH(CH_3)COOCH_3$] (trace), 142 [$CH_3\underline{I}^{+\cdot}$] (2.0), 141 (2.0), 128 (25.0),

127 (20.0), 101 (4.5), 100 (45.0), 99 (5.5), 86 (5.0), 85 (5.0), 82 (2.3), 69 (4.5), 68 (40.0), 59 (25.0), 58 (65.0), 57 (5.0), 56 (5.5), 54 (3.5), 43 (5.5), 42 (20.0), 41 [$C_3H_5^+$] (100.0), 40 (10.0), 37 (5.5), 36 (3.0), 30 (12.5), 29 (4.0), 28 (3.5), 27 (4.0), 26 (2.0), 15 (30.0), m/e (% relative abundance).

Anal. Calcd. for $C_8H_{18}INO_2$: C, 33.44; H, 6.32; N, 4.88. Found: C, 33.23; H, 6.30; H, 4.77.

L(-)S-Methyl 3-dimethylamino-2-methylpropionate methiodide

3-Amino-2-methylpropionic acid (20 g), phthalic anhydride (28.4 g) and sodium acetate (48 g) were heated under reflux for 2 h in glacial acetic acid (100 ml). The reaction mixture was then diluted with water and 2-methyl-3-phthalimidopropionic acid allowed to crystallize. The product was recrystallized from ethanol/water (1:3) and had m.p. 161-163°, yield 84.0%. Literature m.p. 161° (Balenovic and Bregant, 1959); m.p. 162° (Beckett *et al.*, 1962). The i.r. spectrum (Nujol): 1710, 1770 cm^{-1} (C=O); 2200-3200 cm^{-1} (acid OH). The p.m.r. spectrum (100.1 mHz) (deuterated acetone): 2.22τ (4H, s, $C_6\underline{H}_4$-), 5.98τ (1H, s, -COO<u>H</u>, exchanged with D_2O),

6.32τ (2H, doublet, J=7Hz, $-C\underline{H}_2-\overset{\overset{CH_3}{|}}{\underset{\underset{H}{|}}{C}}-$), 7.15τ (1H, sextet, J=7Hz, $-H_2C-\overset{\overset{CH_3}{|}}{\underset{|}{C}}-\underline{H}$), 8.99τ (3H, d, J=7Hz, $H-\overset{|}{\underset{|}{C}}-C\underline{H}_3$).

Anal. Calcd. for $C_{12}H_{11}NO_4$: C, 61.78; H, 4.76; N, 6.00. Found: C, 61.41; H, 4.76; N, 5.83.

2-Methyl-3-phthalimidopropionic acid (39.4 g) and brucine (66.7 g) were dissolved in 95% ethanol (333 ml) and the product allowed to crystallize for 7 days. The first crop of crystals had $[\alpha]_D^{24.5}$-25.30° (c 1.54 chloroform). The brucine salt was recrystallized twice from hot absolute ethanol to constant rotation, yield 47.6 g, $[\alpha]_D^{25}$-35.86° (c 2.20 chloroform). Literature $[\alpha]_D$ - 44.2° (Balenovic and Bregant, 1959); $[\alpha]_D^{22}$-39.7° (c 1.50 chloroform) (Beckett et al., 1962).

The brucine salt was suspended in water (1500 ml) and 4N hydrochloric acid (850 ml). The precipitated free acid, (-)-2-methyl-3-phthalimidopropionic acid, was fractionally crystallized three times from ethanol/water (1:3) and had m.p. 160-161°, yield 12.85 g, $[\alpha]_D^{25}$-3.93° (c 1.37 chloroform). Literature m.p. 145-146°, $[\alpha]_D^{17}$ -24.4° (c 0.98 chloroform) (Balenovic and Bregant, 1959); m.p. 145-146°, $[\alpha]_D^{21}$-20.1° (c 1.5 chloroform) (Beckett et al., 1962).

Anal. Calcd. for $C_{12}H_{11}NO_4$: C, 61.78, H, 4.76; N, 6.00. Found: 61.68; H, 4.77; N, 5.85.

The free acid (12.8 g) was heated under reflux in glacial acetic acid (28 ml) and hydrochloric acid (35 ml) for 8 h then allowed to stand overnight. The precipitated phthalic acid was filtered off, the filtrate extracted with diethyl ether (3 X 20 ml) and the acid extract then evaporated to dryness under vacuum. The resulting (-)-3-amino-2-methylpropionic acid hydrochloride, yield 7.5 g, had $[\alpha]_D^{25.2}$ -1.55° (c 1.35 H_2O).

The crude amino acid hydrochloride (7.5 g) was dissolved in water (200 ml) to which 10% palladium/charcoal (7.5 g) and an aqueous solution of formaldehyde (40% w/v, 20 ml) were added. The solution was stirred under an atmosphere of hydrogen at room temperature and pressure until the theoretical amount of hydrogen had been consumed and the reaction mixture ceased to take up hydrogen. The reaction mixture was filtered and the solvent removed under reduced pressure. The paraformaldehyde formed was removed by redistillation from water. The i.r. spectrum of 3-dimethylamino-2-methylpropionic acid hydrochloride (thin film): 1710 cm^{-1} (acid C=O).

The crude dimethylamino acid hydrochloride was heated under reflux in methanol/3% HCl (200 ml) for 24 h. The solvent was then removed under vacuum to yield methyl 3-dimethylamino-2-methylpropionate hydrochloride. The i.r. spectrum (thin film): 1720 cm^{-1} (ester C=O).

The ester was dissolved in water (5 ml) and neutralized with cold 20% sodium hydroxide. The aqueous solution was made into a slurry with potassium carbonate and extracted with diethyl ether (3 X 20 ml). The ether extract was then dried over anhydrous sodium sulfate, concentrated to 10 ml and diluted with 10 ml of acetone. A cooled solution of an excess of methyl iodide in acetone was added to the ether/acetone solution and S(-)-methyl 3-dimethylamino-2-methylpropionate methiodide was allowed to precipitate. The compound was recrystallized from acetone/diethyl ether, m.p. 93°, yield 0.6 g, $[\alpha]_D^{25}$-2.50° (c 1.92 90% ethanol). The i.r. spectrum (Nujol): 1715, 1730 cm^{-1} (C=O). The p.m.r. spectrum (D_2O): 5.54-6.30τ (1H, m, $-H_2C-\underset{\underset{H}{|}}{\overset{\overset{CH_3}{|}}{C}}-\underline{H}$), 6.16τ (3H, s, $-OC\underline{H}_3$), 6.34-7.30τ (2H, m, $-C\underline{H}_2-\underset{\underset{H}{|}}{\overset{\overset{CH_3}{|}}{C}}-$), 6.82τ

(9H, s, $-\overset{+}{N}(CH_3)_3$), 8.66τ (3H, d, J=7Hz, $H-\overset{|}{\underset{|}{C}}-CH_3$). The mass spectrum: 254 (0.3), 145 [$(CH_3)_2\overset{+\cdot}{N}CH_2CH(CH_3)COOCH_3$] (0.5), 142 [$CH_3I^{+\cdot}$] (43.5), 128 (31.1), 127 (27.7), 101 (2.6), 100 (17.4), 99 (5.2), 85 (3.5), 70 (2.6), 69 (38.2), 68 (3.5), 59 (44.8), 58 [$(CH_3)_2\overset{+}{N}=CH_2$] (100), 57 (9.5), 56 (6.1), 55 (5.2), 45 (4.4), 44 (5.2), 43 (12.2), 42 (37.4), 41 (95.5), 40 (14.7), 39 (39.2), 38 (6.1), 30 (32.2), 29 (8.7), 28 (8.7), 27 (6.9), 26 (3.5), 18 (3.5), 15 (76.5), 14 (41.7), 13 (10.4), 12 (6.1), m/e (% relative abundance).

Anal. Calcd. for $C_8H_{18}INO_2$: C, 33.44; H, 6.32; N, 4.88. Found: C, 33.41; H, 6.48; N, 4.86.

The absolute configuration of this compound can be deduced from the work of Beckett et al., 1962 (see chemistry discussion).

D(+)R-Methyl 3-dimethylamino-2-methylpropionate methiodide

The mother liquors from the brucine salt of the S(-)-isomer (see page 180) were combined and evaporated to dryness, yield 53.6 g. The salt was suspended in water (1325 ml) and 4N hydrochloric acid (935 ml). (+)-2-methyl-3-phthalimidopropionic acid was allowed to precipitate. The compound was fractionally crystallized

from ethanol/water (1:3) and had m.p. 161-162°, yield 12.2 g, $[\alpha]_D^{25}$ +3.36° (c 122 chloroform).

Anal. Calcd. for $C_{12}H_{11}NO_4$: C, 61.78; H, 4.76; N, 6.00. Found: C, 61.40; H, 5.00; N, 6.28.

The acid was hydrolyzed and esterified as described for the S(-)-isomer. (+)-3-Amino-2-methylpropionic acid, yield 6.4 g, had $[\alpha]_D^{25.2}$ + 1.33° (c 0.98 H_2O). The i.r. spectrum (Nujol): 1720 cm^{-1} (acid C=O).

R(+)-Methyl 3-dimethylamino-2-methylpropionate methiodide had m.p. 94-95°, yield 1.9 g, $[\alpha]_D^{24.5}$ +2.25° (c 1.90 90% ethanol). The i.r. spectrum (Nujol): 1715, 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 5.70-6.30τ (1H, m, $-CH_2-\underset{\underset{H}{|}}{\overset{\overset{CH_3}{|}}{C}}-H$), 6.20τ (3H, s, $-OCH_3$), 6.40-7.00τ (2H, m, $-CH_2-\underset{\underset{H}{|}}{\overset{\overset{CH_3}{|}}{C}}-$), 6.84τ (9H, s, $-\overset{+}{N}(CH_3)_3$), 8.69τ (3H, d, J=7Hz, $H-\overset{|}{\underset{|}{C}}-CH_3$). The mass spectrum: 145 $[(CH_3)_2\overset{+\cdot}{N}CH_2CH(CH_3)COOCH_3]$ (0.25), 142 $[CH_3I^{+\cdot}]$ (29.0), 141 (4.5), 128 (21.5), 127 (20.8), 100 (8.0), 99 (2.0), 86 (18.5), 69 (20.5), 68 (2.5), 59 (30.0), 58 $[(CH_3)\overset{+}{N}=CH_2]$ (100.0), 57 (6.5), 56 (3.0), 45 (4.5), 44 (3.5), 43 (7.5), 42 (26.5), 41 (65.0), 40 (1.0), 39 (31.5), 38 (6.0), 37 (3.5), 30 (18.5), 29 (6.0), 27 (5.5), 26 (2.5), 15 (45.0),

14 (2.5), m/e (% relative abundance).

Anal. Calcd. for $C_8H_{18}INO_2$: C, 33.44; H, 6.32; N, 4.88. Found: C, 33.42; H, 6.47; N, 4.96.

2-Dimethylamino-ethyl and -butyl acetates

2-Dimethylaminoethyl acetate methochloride (Acetylcholine-Fluka)

The commercially obtained sample had m.p. 151-151.5°. The i.r. spectrum (Nujol): 1735 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 5.28-5.56τ (2H, m, $-CH_2-\underline{CH}_2-O-$), 6.10-6.34τ (2H, m, $-\overset{+}{N}-\underline{CH}_2-CH_2-$), 6.77τ (9H, s, $-\overset{+}{N}(\underline{CH}_3)_3$), 7.83τ (3H, s, $-COCH_3$). The mass spectrum: 132 (0.1), 131 [$(CH_3)_2\overset{+\cdot}{N}CH_2CH_2OCOCH_3$] (0.8), 72 (2.2), 71 (8.7), 59 (3.3), 58 [$(CH_3)_2\overset{+}{N}=CH_2$] (100.0), 57 (3.3), 56 (2.4), 52 (3.9), 50 (12.1), 44 (3.5), 43 (12.1), 42 (8.6), 30 (5.0), m/e (% relative abundance).

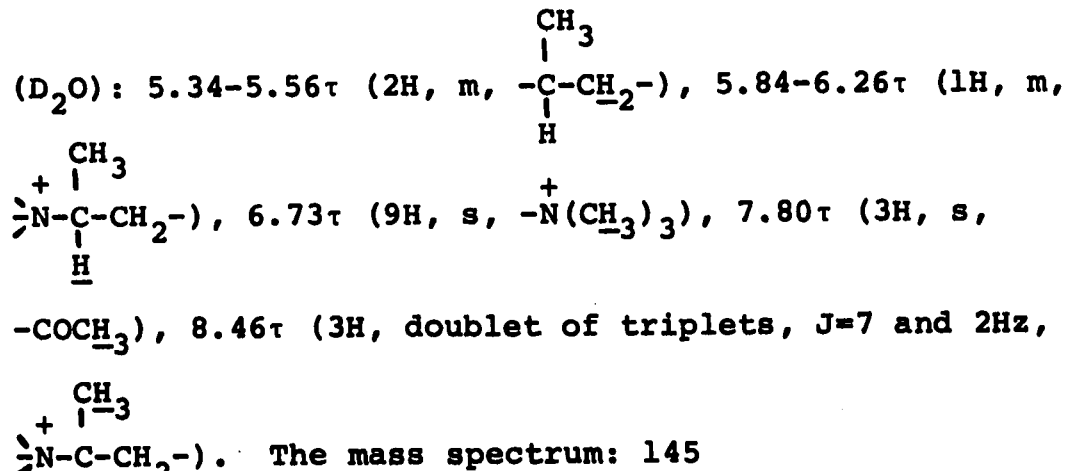
Anal. Calcd. for $C_7H_{16}ClNO_2$: C, 46.24; H, 9.36; N, 7.71. Found: C, 46.19; H, 9.14; N, 7.54.

(±)-2-Dimethylaminopropyl acetate methiodide

(α-Methylacetylcholine)

2-Dimethylaminopropan-1-ol (0.52 g) was dissolved in absolute ethanol (50 ml) and an excess of methyl iodide

in absolute ethanol added. Diethyl ether was added to initiate crystallization and the resulting 2-dimethylaminopropan-1-ol methiodide allowed to crystallize overnight, yield 0.94 g. The 2-dimethylaminopropan-1-ol methiodide (0.9 g) was then heated under reflux with acetic anhydride (25 ml) for 30 min. Diethyl ether was added to the cooled reaction mixture and the resulting 2-dimethylaminopropyl acetate methiodide allowed to crystallize. The compound was recrystallized twice from ethanol/diethyl ether and had m.p. 129-130°, yield 54.3%. Literature m.p. 131-132° (Beckett et al., 1963); m.p. 137-138° (Cocolas et al., 1970). The i.r. spectrum (Nujol): 1740 cm^{-1} (ester C=O). The p.m.r. spectrum



(D_2O): 5.34-5.56τ (2H, m, $-\underset{\underset{H}{|}}{\overset{\overset{CH_3}{|}}{C}}-\underline{CH_2}-$), 5.84-6.26τ (1H, m, $\overset{+}{\underset{\underline{H}}{\overset{\overset{CH_3}{|}}{N}}}-\underset{|}{C}-CH_2-$), 6.73τ (9H, s, $-\overset{+}{N}(\underline{CH_3})_3$), 7.80τ (3H, s, $-COC\underline{H_3}$), 8.46τ (3H, doublet of triplets, J=7 and 2Hz, $\overset{+}{\underset{\underline{H}}{\overset{\overset{CH_3}{|}}{N}}}-\underset{|}{C}-CH_2-$). The mass spectrum: 145 $[(CH_3)_2\overset{+.}{N}CH(CH_3)CH_2OCOCH_3]$ (4.5), 142 $[CH_3\underline{I}^{+.}]$ (100.0), 127 (32.0), 88 (10.5), 73 (16.0), 72 (22.5), 70 (15.5), 56 (14.0), 44 (42.0), 43 (32.5), m/e (% relative abundance).

Anal. Calcd. for $C_8H_{18}INO_2$: C, 33.44; H, 6.32; N, 4.88. Found: C, 33.23; H, 6.30; N, 4.77.

D(+)R-2-Dimethylaminopropyl acetate methiodide

D(-)-Alanine (B.D.H., $[\alpha]_D^{25.2}$ -1.94° (c 5.31 H_2O)) (10 g) was dissolved in water (400 ml) to which 10% palladium/charcoal (10 g) and an aqueous solution of formaldehyde (40% w/v, 15 ml) were added. The reaction mixture was stirred under an atmosphere of hydrogen at room temperature and pressure until the theoretical amount of hydrogen had been taken up and the reaction ceased to consume hydrogen. The mixture was then heated to boiling for several minutes, filtered and the filtrate evaporated to dryness under vacuum. The paraformaldehyde formed was removed by redistillation from water. The residue was crystallized from ethanol/acetone to give D(-)-2-dimethylaminopropionic acid, m.p. 186-186.5°, yield, 50.5%, $[\alpha]_D^{25.2}$ -8.43° (c 5.67 H_2O). Literature m.p. 184°, $[\alpha]_D^{17.5}$ -8.73° (c 5.12 H_2O), $[\alpha]_D^{16.5}$ -3.5° (c 5.1 ethanol) (Bowman and Stroud, 1950). The i.r. spectrum (Nujol): 1600 cm^{-1} (acid C=O). The p.m.r. spectrum (D_2O): 6.28τ (1H, q, J=7Hz, $CH_3-\underset{|}{\overset{|}{C}}-\underline{H}$), 7.16τ (6H, s, $-N(C\underline{H}_3)_2$), 8.52τ (3H, d, J=7Hz, $H-\underset{|}{\overset{|}{C}}-C\underline{H}_3$).

Anal. Calcd. for $C_4H_{11}NO_2$: C, 51.24; H, 9.47;

N, 11.96. Found: C, 51.06; H, 9.18; N, 11.73.

D(-)-Dimethylaminopropionic acid (5.0 g) was heated under reflux with butanol/3% HCl (50 ml) for 49 h. The excess solvent was removed under vacuum and the residue dissolved in water then neutralized with 10% sodium carbonate. The aqueous solution was extracted with diethyl ether (3 X 20 ml), the ether extract dried over anhydrous sodium sulfate and evaporated to dryness. No attempt was made to purify the resulting oil, D-butyl dimethylaminopropionate, yield 83.9%. Literature b.p. 94-96°/23mm, n_D^{18} 1.4264, $[\alpha]_D^{24}$+25.1° (c 1.16 ethanol) (Beckett et al., 1963). The i.r. spectrum (thin film): 1730 cm^{-1} (ester C=O).

D-Butyl dimethylaminopropionate (5.8 g) was dissolved in anhydrous ether (50 ml) and added slowly to a vigorously stirred solution of lithium aluminum hydride (2 g) in anhydrous diethyl ether (200 ml). When the reaction mixture stopped boiling it was heated under reflux for 2 h then stirred at room temperature for 18 h. Water was added to quench the reaction and the reaction mixture made alkaline with 10N sodium hydroxide then extracted with diethyl ether (3 X 20 ml). The ether extract was dried over anhydrous sodium sulfate and evaporated to dryness to give

D-2-dimethylaminopropan-1-ol, yield 40.0%. Literature $[\alpha]_D^{23.5}$-3.83° (Beckett et al., 1963). The i.r. of the crude oil (thin film): 3200-3500 cm^{-1} (primary alcohol OH).

D-2-Dimethylaminopropan-1-ol (1.4 g) was dissolved in cold acetone (50 ml) and a cooled solution of an excess of methyl iodide in acetone added. The resultant precipitate of D-2-dimethylaminopropan-1-ol methiodide was recrystallized from ethanol/diethyl ether and had m.p.> 250° (decomp.) yield 59.5%. The product was not purified further. Literature: m.p. 298.5-299.5° (decomp.), $[\alpha]_D^{22.5}$+4.15° (c 2.5 90% ethanol) (Beckett et al., 1963). The i.r. spectrum (Nujol): 3315 cm^{-1} (primary alcohol OH).

D-2-Dimethylaminopropan-1-ol methiodide (1.9 g) was heated under reflux for 2 h with acetic anhydride (950 ml). The excess acetic anhydride was removed under vacuum and the residual oil crystallized from ethanol/diethyl ether to give the product D(+)-2-dimethylaminopropyl acetate methiodide, m.p. 109-110°, yield, 76.5%, $[\alpha]_D^{22}$+9.30° (c 5.14 90% ethanol). Literature m.p. 107-108°, $[\alpha]_D^{20.7}$+8.61° (c 5.0 90% ethanol) (Beckett et al., 1963); m.p. 106-107°, $[\alpha]_{589.3}^{30}$+6.55± 0.5° (c 1.00 H_2O) (Cocolas et al., 1970). The i.r.

spectrum (Nujol): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 5.35-5.56τ (2H, m, $-\overset{\overset{CH_3}{|}}{\underset{\underset{H}{|}}{C}}-CH_2-$), 5.84-6.28τ (1H, sextet, J=7Hz, $\overset{\diagup}{\underset{\diagdown}{}}N-\overset{\overset{CH_3}{|}}{\underset{\underset{H}{|}}{C}}-CH_2-$), 6.77τ (9H, s, $-\overset{+}{N}(CH_3)_3$), 7.80τ (3H, s, $-COCH_3$), 8.47τ (3H, doublet of triplets, J=7 and 2 Hz, $\overset{\diagup}{\underset{\diagdown}{}}\overset{+}{N}-\overset{\overset{CH_3}{|}}{\underset{\underset{H}{|}}{C}}-CH_2-$). The mass spectrum: 145 $[(CH_3)_2\overset{+.}{N}CH(CH_3)CH_2OCOCH_3]$ (2.8), 142 $[CH_3I^{+.}]$ (58.0), 141 (7.6), 140 (1.6), 139 (2.4), 127 (8.6), 88 (5.8), 86 (5.6), 73 (7.6), 72 $[(CH_3)_2\overset{+}{N}=CHCH_3]$ (100.0), 71 (7.4), 70 (5.0), 58 (2.2), 57 (4.2), 56 (5.2), 45 (2.0), 44 (10.8), 43 (15.4), 42 (8.6), 41 (2.6), 15 (14.4), m/e (% relative abundance).

Anal. Calcd. for $C_8H_{18}INO_2$: C, 33.44; H, 6.32; N, 4.88. Found: C, 33.18; H, 6.48; N, 4.78.

L(-)S-Dimethylaminopropyl acetate methiodide

The title compound was prepared in a manner identical to that described for the D(+)-isomer. L(+)-2-dimethylaminopropionic acid was prepared from L(+)-

alanine (Aldrich, $[\alpha]_D^{25.2}$+1.78° (c 4.90 H_2O)) (10 g) and had m.p. 187-188°, yield 78.0%, $[\alpha]_D^{25.2}$+9.18° (c 4.87 H_2O). The i.r. spectrum (Nujol): 1600 cm^{-1} (acid C=O). The p.m.r. spectrum (D_2O): 6.22τ (1H, q, J=7Hz $CH_3-\underset{|}{\overset{|}{C}}-\underline{H}$), 7.14τ (6H, s, $-N(\underline{CH}_3)_2$), 8.52τ (3H, d, J=7Hz, $H-\underset{|}{\overset{|}{C}}-\underline{CH}_3$).

Anal. Calcd. for $C_4H_{11}NO_2$: C, 51.24; H, 9.47; N, 11.96. Found: C, 51.36; H, 9.55; N, 12.10.

L(+)-Dimethylaminopropionic acid (7.3 g) was esterified with butanol/3% HCl to give L-butyl dimethylaminopropionate, yield 45.7%. The ester was not purified. Literature b.p. 91-92°/19 mm, n_D^{22} 1.41411, $[\alpha]_D^{26}$-26.2° (c 1.1 ethanol) (Beckett et al., 1963). The i.r. spectrum (thin film): 1730 cm^{-1} (ester C=O).

L-2-Dimethylaminopropan-1-ol was prepared by reduction of L-butyl dimethylaminopropionate (4.75 g) with lithium aluminum hydride, yield 41.3%. No attempt was made to purify the product. Literature $[\alpha]_D^{24.6}$+2.26° (ethanol) (Beckett et al., 1963). The i.r. spectrum (thin film): 3330-3500 cm^{-1} (primary alcohol OH).

L-2-Dimethylaminopropan-1-ol methiodide was prepared in the usual manner from L-2-dimethylaminopropan-1-ol (1.1 g), m.p.> 250° (decomp.), yield 44.4%. Literature m.p.> 299° (decomp.), $[\alpha]_D^{24.7}$-4.14° (c 2.5

90% ethanol) (Beckett et al., 1963). The i.r. spectrum (Nujol): 3315 cm^{-1} (primary alcohol OH).

Anal. Calcd. for $C_6H_{16}INO$: C, 29.38; H, 6.58; N, 5.72. Found: C, 29.12; H, 6.77; N, 6.05.

The above compound was then heated under reflux with acetic anhydride as described previously to give L(-)-2-dimethylaminopropyl acetate methiodide in quantitative yield, m.p. 109-110°. The compound was recrystallized three times from ethanol/diethyl ether. Two crops of crystals were obtained, the first had $[\alpha]_D^{21}$ -9.07° (c 5.04 90% ethanol); the second crop of crystals obtained from the mother liquors had $[\alpha]_D^{21}$-8.04° (c 5.08 90% ethanol). Literature m.p. 108-109°, $[\alpha]_D^{27}$-9.07° (c 5.0 90% ethanol) (Beckett et al., 1963). The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 5.34-5.60τ (2H, m, $-\overset{\overset{CH_3}{|}}{\underset{\underset{H}{|}}{C}}-C\underline{H}_2-$), 5.84-6.26τ (1H, m, $\overset{+}{\underset{\underset{\underline{H}}{|}}{N}}-\overset{\overset{CH_3}{|}}{\underset{\underset{H}{|}}{C}}-CH_2-$), 6.80τ (9H, s, $-\overset{+}{N}(C\underline{H}_3)_3$), 7.81τ (3H, s, $-COC\underline{H}_3$), 8.47τ (3H, doublet of triplets, J=7 and 2Hz, $-\overset{+}{N}-\overset{\overset{C\underline{H}_3}{|}}{\underset{\underset{H}{|}}{C}}-CH_2-$). The mass spectrum: 145

[$(CH_3)_2\overset{+\cdot}{N}CH(CH_3)CH_2OCOCH_3$] (3.8), 142 [$CH_3\overset{+}{I}\cdot$] (56.0), 141 (7.6), 140 (2.2), 139 (2.6), 128 (1.2), 127 (14.0), 88 (7.8), 86 (5.0), 85 (2.0), 73 (10.0), 72 [$(CH_3)_2\overset{+}{N}=CHCH_3$] (100.0), 71 (6.4), 70 (7.5), 58 (5.0), 57 (4.0), 56 (6.0), 45 (2.6), 44 (15.2), 43 (16.2), 42 (10.4), 41 (3.6), 30 (2.4), 29 (2.4), 28 (3.2), 18 (7.6), 15 (16.2), m/e (% relative abundance).

Anal. Calcd. for $C_8H_{18}INO_2$: C, 33.44; H, 6.32; N, 4.88. Found: C, 33.29; H, 6.37; N, 4.86.

(±)-1-Dimethylaminoprop-2-yl acetate methochloride

(β-Methylacetylcholine-Aldrich)

The commercially obtained sample was recrystallized from ethanol/diethyl ether and had m.p. 168-169°. The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 6.26-6.88τ (1H, m, $-CH_2-\overset{CH_3}{\overset{|}{C}}-\underline{H}$), 5.50-6.46τ (2H, m, $-\underline{CH_2}-\overset{CH_3}{\overset{|}{\underset{H}{\overset{|}{C}}}}-$), 6.80τ (9H, s, $\overset{+}{N}(\underline{CH_3})_3$), 7.87τ (3H, s, $-COC\underline{H_3}$), 8.70τ (3H, d, J=7Hz, $H-\overset{|}{C}-C\underline{H_3}$). The mass spectrum: 145 [$(CH_3)_2\overset{+\cdot}{N}CH_2CH(CH_3)OCOCH_3$] (0.36), 88 (2.2), 86 (3.6), 85 (7.3), 58 [$(CH_3)_2\overset{+}{N}=CH_2$] (100.0), 52 (3.6), 50 (10.9), 44 (3.6), 43 (9.1), 42 (8.7), 30 (8.5), m/e (% relative abundance).

Anal. Calcd. for $C_8H_{18}ClNO_2$: C, 49.07; H, 9.28; N, 7.16. Found: C, 49.00; H, 8.99; N, 7.08.

L(+)S-1-Dimethylaminoprop-2-yl acetate methiodide

The ammonium salt of d-α-bromocamphorsulfonic acid (Aldrich) (25 g) was suspended in ethyl acetate (100 ml) and concentrated hydrochloric acid (10 ml). The ammonium chloride formed was filtered off and the filtrate evaporated to dryness yielding the free α-bromocamphorsulfonic acid as a viscous light yellow oil. The free acid and racemic 1-dimethylaminopropan-2-ol were dissolved in 95% ethanol (45 ml). The neutral solution was evaporated to dryness under vacuum, then ethyl acetate (45 ml) was added to the residue and the salt allowed to crystallize. The initial crop of crystals of (+)-1-dimethylaminopropan-2-ol hydrogen α-camphorsulfonate, yield 33.79 g had $[\alpha]_D^{24.5}$+68.45° (c 1.00 H_2O). Literature $[\alpha]_{589.3}^{25}$+69° (c 1.0 H_2O). (Cocolas et al., 1971). The salt was recrystallized from ethyl acetate/ethanol (10:1) (6 ml/g of salt) to constant rotation. The compound was allowed to crystallize at room temperature for 24 h each time. After six recrystallization the pure (+)-salt, yield 11.3 g, had $[\alpha]_D^{25.5}$+79.30° (c 1.22

H_2O). Literature $[\alpha]_D^{24.5}$+80.6° (c 1.0 H_2O) (Beckett et al., 1963); $[\alpha]_{589.3}^{25}$+74.0° (c 1.0 H_2O) (Cocolas et al., 1971); $[\alpha]_D$+83.5° (Major and Bonnett, 1935).

The (+)-salt (11 g) was dissolved in water (5 ml) and neutralized with cold 20% sodium hydroxide. The aqueous solution was made into a slurry with potassium carbonate and extracted with diethyl ether (3 X 20 ml). The ether extract was dried over anhydrous sodium sulfate, concentrated to 10 ml, then diluted with acetone (10 ml). A cooled solution of an excess of methyl iodide in acetone was added to the ether/acetone solution and crystalline (+)-1-dimethylaminopropan-2-ol methiodide separated. The compound had m.p. 174°, yield 1.6 g, $[\alpha]_D^{25}$+24.70° (c 1.85 90% ethanol). Literature m.p. 174-175°, $[\alpha]_D^{24.5}$+27.27° (c 2.2 90% ethanol) (synthesis from L(+)-lactic acid), m.p. 177-178°, $[\alpha]_D^{24}$+29.7° (c 2 90% ethanol) (Beckett et al., 1963); m.p. 176-177°, [α]+24.7° (Major and Bonnett, 1935).

S(+)-1-Dimethylaminoprop-2-yl acetate methiodide was obtained by heating (+)-1-dimethylaminopropan-2-ol methiodide (1.6 g) under reflux in acetic anhydride (25 ml) for 45 min. The excess acetic anhydride was removed under vacuum and the residue crystallized from

ethanol/diethyl ether. The compound had m.p. 176-176.5°, yield 1.5 g, $[\alpha]_D^{26.5}$ +25.85° (c 2.04 90% ethanol). Literature m.p. 177-178.5°, $[\alpha]_D^{22.5}$ +27.0° (c 2.0 90% ethanol) (Beckett et al., 1963); $[\alpha]_{589.3}^{30}$ +27.0 ± 0.5° (c 1.00 H_2O) (Cocolas et al., 1970). The i.r. spectrum (Nujol): 1735 cm^{-1} (ester C=O).

The p.m.r. spectrum (D_2O): 6.10-6.70τ (3H, m, $-C\underline{H}_2-\overset{CH_3}{\overset{|}{C}}-\underline{H}$), 6.76τ (9H, s, $-\overset{+}{N}(C\underline{H}_3)_3$), 7.82τ (3H, s, $-COC\underline{H}_3$), 8.66τ (3H, d, J=7Hz, $H-\overset{|}{C}-C\underline{H}_3$). The mass spectrum: 145 $[(CH_3)_2\overset{+\cdot}{N}CH_2CH(CH_3)OCOCH_3]$ (0.76), 142 $[CH_3I^{+\cdot}]$ (42.0), 141 (11.8), 139 (2.0), 127 (7.0), 102 (5.4), 88 (3.6), 86 (4.0), 85 (6.8), 71 (3.6), 59 (4.8), 58 $[(CH_3)_2\overset{+}{N}=CH_2]$ (100.0), 57 (2.8), 43 (18.0), 42 (8.0), 41 (2.0), 30 (4.4), 28 (2.2), 18 (2.0), 15 (42.0), m/e (% relative abundance).

Anal. Calcd. for $C_8H_{18}INO_2$: C, 33.44; H, 6.32; N, 4.88. Found: C, 33.74; H, 6.38; N, 5.26.

The absolute configuration of this compound was determined by Beckett et al., 1963.

D(-)R-1-Dimethylaminoprop-2-yl acetate methiodide

Racemic 1-dimethylaminopropan-2-ol (40 g) and

(+)-tartaric acid (52.5 g) were dissolved in 96% ethanol (555 ml) and allowed to crystallize. The initial crop of crystals, yield 76.5 g, had $[\alpha]_D^{22.5}$ +13.10° (c 9.70 H_2O). The salt was recrystallized fifteen times from 96% ethanol (6 ml/g salt) to constant rotation. The compound was allowed to crystallize each time for 24 h at room temperature. The pure (-)-1-dimethylaminopropan-2-ol hydrogen tartrate, yield 14.0 g, had $[\alpha]_D^{25}$-3.15° (c 10.84 H_2O). Literature $[\alpha]_D^{22.5}$-10.84° (c 4.8 H_2O) (Beckett et al., 1963); $[\alpha]_{589.3}^{25}$+0.5° (c 10.0 H_2O) Cocolas et al., 1971). [α]-10.7° (Major and Bonnett, 1935).

The salt was then converted to the acetate methiodide in manner identical to that described for the (+)-isomer. (-)-1-Dimethylaminopropan-2-ol methiodide had m.p. 172-173°, yield 5 g, $[\alpha]_D^{25}$-21.40° (c 1.97 90% ethanol). Literature m.p. 175.5-176.5°, $[\alpha]_D^{23}$-29.04° (c 2 90% ethanol) (Beckett et al., 1963); m.p. 176.5-177.5°, [α]-24.7° (Major and Bonnett, 1935).

R(-)-1-Dimethylaminoprop-2yl acetate methiodide had m.p. 174-175°, yield 4.4 g, $[\alpha]_D^{25}$-20.29° (c 1.70 90% ethanol), $[\alpha]_D^{22.5}$-26.60° (c 2.15 95% ethanol). Literature m.p. 176-178°, $[\alpha]_D^{23}$-27.38° (c 2.0 90% ethanol) (Beckett et al., 1963); $[\alpha]_{589.3}^{30}$-26.8°

(c 1.0 H_2O) (Cocolas et al., 1970). The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 6.10-6.70τ (3H, m, $-C\underline{H}_2-\overset{CH_3}{\overset{|}{C}}-\underline{H}$), 6.78τ (9H, s, $-\overset{+}{N}(C\underline{H}_3)_3$), 7.84τ (3H, s, $-COC\underline{H}_3$) 8.64τ (3H, d, J=7Hz, $H-\overset{|}{C}-C\underline{H}_3$). The mass spectrum: 145 $[(CH_3)_2NCH_2CH(CH_3)OCOCH_3]$ (0.74), 142 $[CH_3I^{+\cdot}]$ (25.1), 141 (4.1), 127 (6.7), 88 (3.0), 86 (3.7), 85 (5.6), 59 (4.5), 58 $[(CH_3)_2\overset{+}{N}=CH_2]$ (100.0), 44 (3.4), 43 (8.9), 42 (6.3), 41 (2.2), 30 (3.4), 15 (3.7), m/e (% relative abundance).

Anal. Calcd. for $C_8H_{18}INO_2$: C, 33.44; H, 6.32; N, 4.88. Found: C, 33.16; H, 6.22; N, 4.78.

Methyl 3-diethylamino-propionates and -butyrates

Methyl 3-diethylaminopropionate

The title compound was prepared from diethylamine and methyl acrylate in quantitative yield using the general method for the preparation of 3-amino-esters. The reaction mixture was heated under reflux for 48 h prior to fractional distillation. The compound had b.p. 50-53°/5 mm. The i.r. spectrum (thin film): 1740 cm^{-1} (ester C=O). The p.m.r. spectrum

(CCl_4): 6.46τ (3H, s, $-OC\underline{H}_3$), 7.15-7.90τ (8H, m, $-C\underline{H}_2\diagdown$ / $-C\underline{H}_2\diagup N-C\underline{H}_2-C\underline{H}_2-$), 9.08τ (6H, t, J=7Hz, $-N(-CH_2-C\underline{H}_3)_2$).

Anal. Calcd. for $C_8H_{17}NO_2$: C, 61.73; H, 10.76; N, 8.80. Found: C, 61.60; H, 10.99; N, 8.85.

The hydrochloride had m.p. 128°. The i.r. spectrum (Nujol): 1740 cm^{-1} (ester C=O); 2460-2540 cm^{-1} ($\overset{+}{\rangle}N-H$). The p.m.r. spectrum ($CDCl_3$): -1.50τ (1H, s, $\overset{+}{\rangle}N-\underline{H}$, exchanged with D_2O).

Anal. Calcd. for $C_8H_{18}ClNO_2$: C, 49.07; H, 9.27; N, 7.16. Found: C, 49.36; H, 9.47; N, 7.06.

The <u>methiodide</u> had m.p. 138°. The i.r. spectrum (Nujol): 1740 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 5.00τ (3H, s, $\overset{+}{\rangle}N-C\underline{H}_3$).

Anal. Calcd. for $C_9H_{20}INO_2$: C, 35.87; H, 6.69; N, 4.65. Found: C, 35.99; H, 6.70; N, 4.65.

<u>Methyl 3-diethylaminobutyrate</u>

The title compound was prepared in 5.3% yield from diethylamine and methyl crotonate using the general method for the preparation of 3-amino-esters. The reaction mixture was heated under reflux for 48 h prior to fractional distillation. The compound had

b.p. 40°/10mm. Literature b.p. 84°/18 mm (Adamson, 1950). The i.r. spectrum (thin film): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (CCl_4): 6.32τ (3H, s, $-OC\underline{H}_3$), 6.50-7.10τ (1H, m, $-\overset{CH_3}{\underset{\underline{H}}{C}}-CH_2-$), 7.35-8.15τ (6H, m, $(CH_3-C\underline{H}_2)_2N-\overset{CH_3}{\underset{H}{C}}-C\underline{H}_2-$), 8.80-9.15τ (9H, m, $(C\underline{H}_3-CH_2)_2N-\overset{C\underline{H}_3}{\underset{H}{C}}-$).

Anal. Calcd. for $C_9H_{19}NO_2$: C, 62.36; H, 11.06; N, 8.09. Found: C, 62.19; H, 10.67; N, 8.19.

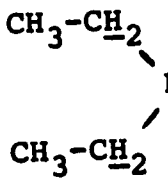
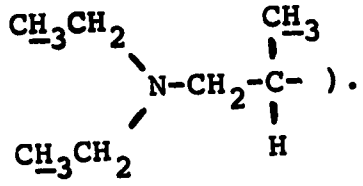
The hydrochloride had m.p. 101-103°. The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O); 2470, 2510, 2550, 2590 cm^{-1} ($\geq\overset{+}{N}-H$). The p.m.r. spectrum ($CDCl_3$): -1.17τ (1H, s, $\geq\overset{+}{N}-\underline{H}$, exchanges with D_2O).

Anal. Calcd. for $C_9H_{20}ClNO_2$: C, 51.54; H, 9.61; N, 6.68. Found: C, 51.77; H, 9.58; N, 6.57.

The methiodide had m.p. 105°. The i.r. spectrum (Nujol): 1740 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 7.08τ (3H, s, $\geq\overset{+}{N}-C\underline{H}_3$).

Anal. Calcd. for $C_{10}H_{20}INO_2$: C, 37.85; H, 6.94; N, 4.42. Found: C, 38.34; H, 7.24; N, 4.62.

Methyl 3-diethylamino-2-methylpropionate

The title compound was prepared from diethylamine and methyl methacrylate in 43.4% yield using the general method for the preparation of 3-amino-esters. The reaction mixture was heated under reflux for 48 h prior to fractional distillation. The compound had b.p. 48°/5 mm. Literature b.p. 77°/15 mm (Bieber, 1950). The i.r. spectrum (thin film): 1740 cm^{-1} (ester C=O). The p.m.r. spectrum (CCl_4): 6.44τ (3H, s, $-OC\underline{H}_3$), 7.28-7.88τ (7H, m, ), 8.84-9.26τ (9H, m, ).

Anal. Calcd. for $C_9H_{19}NO_2$: C, 62.36; H, 11.06; N, 8.09. Found: C, 62.46; H, 11.04; N, 8.23.

The hydrochloride had m.p. 70-72°. The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum ($CDCl_3$): -2.20τ (1H, s, $\rangle\overset{+}{N}-\underline{H}$, exchanged with D_2O).

Anal. Calcd. for $C_9H_{20}ClNO_2$: C, 51.51; H, 9.62; N, 6.68. Found: C, 51.57; H, 9.63; H, 6.63.

Methyl 3-dipropylaminobutyrates

Methyl 3-dipropylaminobutyrate

The title compound was prepared in 39.6% yield from dipropylamine and methyl crotonate using the general method for the preparation of 3-amino-esters. The reaction mixture was heated under reflux for 116 h prior to fractional distillation. The compound had b.p. 78°/6 mm. Literature b.p. 116-118°/15 mm (Adamson, 1950). The i.r. spectrum (thin film): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (CCl_4): 6.32τ (3H, s, $-OC\underline{H}_3$), 6.55-7.00τ (1H, m, $-\underset{\underline{H}}{\overset{\overset{CH_3}{|}}{C}}-CH_2-$), 7.35-8.15τ (6H, m, $-CH_2-C\underline{H}_2-N(-C(CH_3)-C\underline{H}_2-)-CH_2-C\underline{H}_2$), 8.28-8.82τ (4H, m, $CH_3-C\underline{H}_2-CH_2-N(-)-CH_2-C\underline{H}_2-CH_3$), 8.95-9.35τ (9H, m, $C\underline{H}_3-CH_2-CH_2-N(-C(C\underline{H}_3)H)-CH_2-CH_2-C\underline{H}_3$).

Anal. Calcd. for $C_{11}H_{23}NO_2$: C, 65.43; H, 11.40; N, 6.94. Found: C, 65.09; H, 11.21; N, 6.77.

The hydrochloride had m.p. 92-94°. The i.r. spectrum (Nujol): 1735 cm^{-1} (ester C=O); 2500-2700 cm^{-1}

($\overset{+}{\rangle}$N-H). The p.m.r. spectrum ($CDCl_3$): -1.18τ (1H, s, $\overset{+}{\rangle}$N-H, exchanged with D_2O).

Anal. Calcd. for $C_{11}H_{24}ClNO_2$: C, 55.59; H, 10.10; N, 5.90. Found: C, 55.31; H, 10.23; N, 6.02.

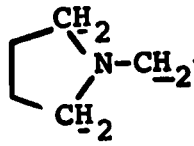
The methiodide had m.p. 105°. The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 6.88τ (3H, s, $\overset{+}{\rangle}$N-CH_3).

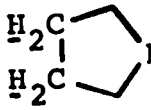
Anal. Calcd. for $C_{12}H_{26}INO_2$: C, 41.75; H, 7.54; N, 4.06. Found: C, 41.65; H, 7.83; N, 4.21.

Methyl 3-pyrrolidino-propionates and -butyrates and their derivatives

Methyl 3-(1-pyrrolidino)propionate

The title compound was prepared from pyrrolidine and methyl acrylate in 82.7% yield using the general method for the preparation of 3-amino-esters. The reaction mixture was heated under reflux for 22 h prior to fractional distillation. The compound had b.p. 90°/18 mm. Literature b.p. 101°/22 mm (Barrass et al., 1968); 76°/5 mm (Matkovics et al., 1961). The i.r. spectrum (thin film): 1735 cm^{-1} (ester C=O). The p.m.r. spectrum (CCl_4): 6.42τ (3H, s, -OCH_3),

7.17-7.80τ (8H, m, ), 8.15-8.50τ

(4H, m, ).

Anal. Calcd. for $C_8H_{15}NO_2$: C, 61.15; H, 9.55; N, 8.92. Found: C, 61.21; H, 9.39; N, 8.61.

The hydrochloride had m.p. 123-126°. Literature m.p. 128° (Matkovics et al., 1961). The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O); 2460, 2560, 2650 cm^{-1} ($\overset{+}{\text{N}}$-H). The p.m.r. spectrum ($CDCl_3$): -1.20τ (1H, s, $\overset{+}{\text{N}}$-H, exchanged with D_2O).

Anal. Calcd. for $C_8H_{16}ClNO_2$: C, 49.61; H, 8.26; N, 7.23. Found: C, 49.62; H, 8.33; N, 7.24.

The methiodide had m.p. 167°. Literature m.p. 167° (Barrass et al., 1968); m.p. 166° (Matkovics et al., 1961). The i.r. spectrum (KBr): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 6.42τ (3H, s, $\overset{+}{\text{N}}$-CH_3).

Anal. Calcd. for $C_9H_{18}INO_2$: C, 35.88; H, 5.98; N, 4.65. Found. C, 36.01; H, 6.35; N, 4.28.

3-(1-Pyrrolidino)propionamide hydrochloride

Pyrrolidine (10 g) and acrylamide (10 g) were dissolved in separate 50 ml portions of anhydrous methanol. The cooled amine solution was added slowly with stirring to the solution of acrylamide. The reaction mixture was heated under reflux for 48 h then diluted with diethyl ether and extracted with 2N hydrochloric acid. The acid extract was then neutralized with 2N potassium hydroxide and extracted with diethyl ether (3 X 20 ml). The ether extract was dried over anhydrous sodium sulfate and then evaporated to dryness to give an oil which was dissolved in absolute ethanol. When this solution was saturated with dry HCl, needle crystals were obtained and recrystallized from hot ethanol/diethyl ether. The compound was obtained in 1.0% yield, m.p. 141-143°. The i.r. spectrum (Nujol): 1680 cm^{-1} (amide C=O); 2480-2640 cm^{-1} ($\overset{+}{\text{N}}$-H).

Anal. Calcd. for $C_7H_{15}ClN_2O$: C, 47.06; H, 8.40; N, 15.63. Found: C, 47.22; H, 8.32; N, 15.81.

3-(1-Pyrrolidino)propionamide methiodide

Impure 3-(1-pyrrolidino)propionamide (1 g) (see above) was dissolved in ethanol (10 ml) and an excess of methyl iodide in ethanol added. Diethyl

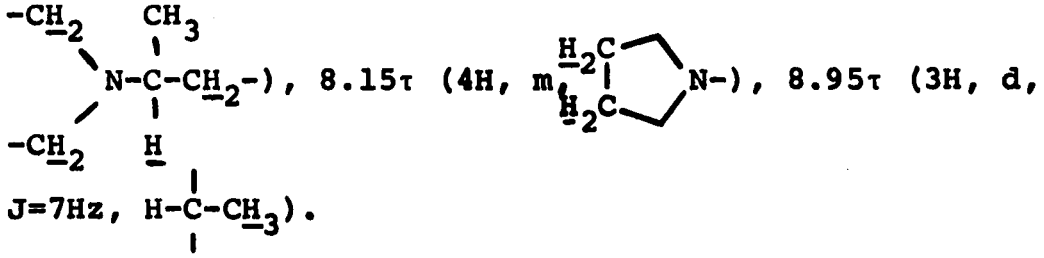
ether was added to cloud point. The resulting precipitate was recrystallized from hot ethanol/diethyl ether. The resulting compound obtained in quantitative yield had m.p. 81-83°. The i.r. spectrum (Nujol): 1660 cm^{-1} (amide C=O). The p.m.r. spectrum (D_2O): 6.92τ (3H, s, $\overset{+}{N}-CH_3$).

Anal. Calcd. for $C_8H_{17}IN_2O$: C, 33.79; H, 6.03; N, 9.86. Found: C, 34.02; H, 6.29; N, 9.99.

Methyl 3-(1-pyrrolidino)butyrate

The title compound was prepared from pyrrolidine and methyl crotonate in 74.1% yield using the general method for the preparation of 3-amino-esters. The reaction mixture was heated under reflux for 44 h prior to fractional distillation.

The compound had b.p. 41-42°/0.4 mm. Literature b.p. 100-102°/23 mm (Adamson, 1950). The i.r. spectrum (thin film): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (CCl_4): 6.40τ (3H, s, $-OCH_3$), 6.90-7.95τ (7H, m,



), 8.15τ (4H, m,

), 8.95τ (3H, d, J=7Hz, $H-\underset{}{\overset{}{C}}-CH_3$).

Anal. Calcd. for $C_9H_{17}NO_2$: C, 63.16; H, 9.94; N, 8.19. Found: C, 63.05; H, 9.98; N, 7.89.

The hydrochloride had m.p. 140-141°. The i.r. spectrum (Nujol): 1735 cm^{-1} (ester C=O): 2460, 2530, 2660 cm^{-1} ($\overset{+}{N}$-H). The p.m.r. spectrum ($CDCl_3$): -1.17τ (1H, s, $\overset{+}{N}$-H, exchanged with D_2O).

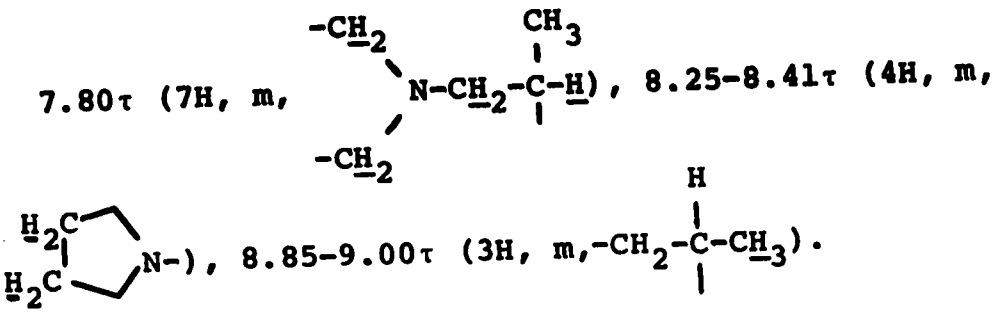
Anal. Calcd. for $C_9H_{18}ClNO_2$: C, 52.01; H, 9.03; N, 6.77. Found: C, 51.83; H, 9.03; N, 6.70.

The methiodide had 90-91°. The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 7.08τ (3H, s, $\overset{+}{N}$-CH_3).

Anal. Calcd. for $C_{10}H_{20}INO_2$: C, 38.33; H, 6.42; N, 4.01. Found: C 38.68; H, 6.42; N, 4.01.

Methyl 2-methyl-3-(1-pyrrolidino)propionate

The title compound was prepared in 88.8% yield from pyrrolidine and methyl methacrylate using the general method for the preparation of 3-amino-esters. The reaction mixture was heated under reflux for 8 h prior to fractional distillation. The compound had b.p. 86°/14 mm. Literature b.p. 53°/0.2 mm (Barron et al., 1968); b.p. 86°/13 mm (Moffett, 1949). The i.r. spectrum (thin film): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (DMSO.d_6): 6.42τ (3H, s, -OCH_3), 7.15-



Anal. Calcd. for $C_9H_{17}NO_2$: C, 63.16; H, 9.94; N, 8.19. Found C, 63.02; H, 9.96; N, 8.16.

The hydrochloride had m.p. 146-148°. The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O); 2460, 2590, 2660 cm^{-1} ($\overset{+}{N}$-H). The p.m.r. spectrum ($CDCl_3$): -1.20τ (1H, s, $\overset{+}{N}$-H, exchanged with D_2O).

Anal. Calcd. for $C_9H_{18}ClNO_2$: C, 52.04; H, 8.67; N, 6.74. Found: C, 52.35; H, 8.81; N, 6.50.

2-Methyl-3-(1-pyrrolidino)propionamide hydrochloride

Pyrrolidine (17 g) and methacrylamide (24 g) were dissolved in separate portions of absolute ethanol (50 ml). The cooled amine solution was added slowly with stirring to the solution of the amide and after the addition was complete the reaction mixture was heated under reflux for 26 h. The crude reaction mixture was cooled, diluted with diethyl ether and shaken with 2N hydrochloric acid. The acid extract was then neutralized

with 10N sodium hydroxide and extracted with diethyl ether (3 X 20 ml). This ether extract was dried over anhydrous sodium sulfate then saturated with dry HCl and the resulting white precipitate was crystallized from absolute ethanol/diethyl ether and had m.p. 173-177°. The i.r. spectrum (Nujol): 1680 cm^{-1} (amide C=O); 2460-2660 cm^{-1} ($\overset{+}{N}$-H).

Anal. Calcd. for $C_8H_{17}ClN_2O$: C, 49.87; H, 8.83; N, 14.54. Found: C, 49.50; H, 9.05; N, 13.96.

2-Methyl-3-(1-pyrrolidino)propionohydroxamic acid hydrochloride

Methyl 2-methyl-3-(1-pyrrolidino)propionate (5 g) and hydroxylamine hydrochloride (2 g) were dissolved in separate portions of anhydrous methanol. The cooled solution of hydroxylamine hydrochloride was added slowly with stirring to the solution of the ester. The reaction mixture was stirred at room temperature for 24 h then heated at 30° for 11 days. The methanol was removed under reduced pressure and the residual oil (30% yield) crystallized from acetone and had m.p. 153°. The i.r. spectrum (Nujol); 1655 cm^{-1} (hydroxamate C=O); 2480-2650 cm^{-1} ($\overset{+}{N}$-H). The p.m.r. spectrum (DMSO.d_6): -0.7τ (1H, s, -N-O$\underline{H}$, exchanged with D_2O).

Anal. Calcd. for $C_8H_{17}ClN_2O_2$: C, 46.04; H, 8.15; N, 13.43. Found: C, 45.77; H, 8.24; N, 13.84.

4-(1-Pyrrolidino)pentan-2-one hydrochloride

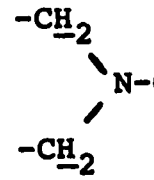
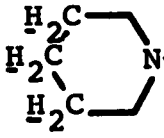
Pyrrolidine (8.5 g) and 3-penten-2-one (10 g) were dissolved in separate portions of dry benzene. The cooled solution of pyrrolidine was added slowly with stirring to the solution of the ketone. The reaction mixture was stirred at 0° for 5 h then at room temperature for 48 h. The benzene was removed under reduced pressure and the residual oil distilled under reduced pressure to give a compound, b.p. 72°/5 mm. This oil was dissolved in diethyl ether and the solution was saturated with dry HCl. The resulting precipitate was recrystallized from acetone/diethyl ether and had m.p. 179-180°. The i.r. spectrum (Nujol): 1715 cm^{-1} (ketone C=O); 2470-2660 cm^{-1} ($\overset{+}{N}$-H).

Anal. Calcd. for $C_9H_{18}ClNO$: C, 56.36; H, 9.47; N, 7.31. Found: C, 56.27; H, 9.30; N, 7.34.

Methyl 3-piperidino-propionates and -butyrates

Methyl 3-(1-piperidino)propionate

The title compound was prepared in 61.2% yield

from piperidine and methyl acrylate using the general method for the preparation of 3-amino-esters. The reaction mixture was heated under reflux for 24 h prior to fractional distillation. The compound had b.p. 42°/0.35 mm. Literature b.p. 72°/2 mm (Matkovics et al., 1961). The i.r. spectrum (thin film): 1735 cm^{-1} (ester C=O). The p.m.r. spectrum (CCl_4): 6.42τ (3H, s, $-OCH_3$), 7.32-7.90τ (8H, m, ), 8.25-8.70τ (6H, m, ).

Anal. Calcd. for $C_7H_{17}NO_2$: C, 63.11; H, 10.01; N, 8.19. Found: C, 62.73; H, 9.96; N, 8.14.

The hydrochloride had m.p. 160-161°. Literature m.p. 190° (Matkovics et al., 1961); m.p. 195-196° (Phillips, 1950). The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O); 2400-2700 cm^{-1} ($\overset{+}{N}$-H). The p.m.r. spectrum ($CDCl_3$): -1.92τ (1H, s, $\overset{+}{N}$-H, exchanged with D_2O).

Anal. Calcd. for $C_9H_{18}ClNO_2$: C, 52.01; H, 8.74; N, 6.76. Found: C, 51.95; H, 8.53; N, 6.75.

The methiodide had m.p. 157°. Literature m.p. 147-148° (Matkovics et al., 1961). The i.r. spectrum (Nujol): 1735 cm^{-1} (ester C=O). The p.m.r. spectrum

(D_2O): 6.58τ (3H, s, $\overset{+}{\underset{/}{\overset{\diagdown}{}}}N-\underline{C}H_3$).

Anal. Calcd. for $C_{10}H_{20}INO_2$: C, 38.01; H, 6.39; N, 4.44. Found: C, 38.31; H, 6.55; N, 3.93.

Methyl 3-(1-piperidino)butyrate

The title compound was prepared from piperidine and methyl crotonate in 55.9% yield using the general method for the preparation of 3-amino-esters. The reaction mixture was heated under reflux for 24 h prior to fractional distillation. The compound had b.p. 54°/0.4 mm. Literature b.p. 100-102°/23 mm (Adamson, 1954). The i.r. spectrum (thin film): 1740 cm^{-1} (ester C=O). The p.m.r. spectrum (CCl_4): 6.43τ (3H, s, $-OC\underline{H}_3$), 7.20-7.92τ (7H, m, $-C\underline{H}_2$, CH_3, $N-C-C\underline{H}_2-$, $-C\underline{H}_2$, $\underline{H}$), 8.25-8.65τ (6H, m, $\underline{H}_2C$, $\underline{H}_2C$, $\underline{H}_2C$, N-), 9.03τ (3H, d, J=7Hz, $H-C-C\underline{H}_3$).

Anal. Calcd. for $C_{10}H_{19}NO_2$: C, 64.81; H, 10.34; N, 7.57. Found: C, 64.98; H, 10.50; N, 7.81.

The hydrochloride had m.p. 159-161°. The i.r.

spectrum (Nujol): 1740 cm^{-1} (ester C=O); 2400, 2490, 2580, 2620 cm^{-1} ($\overset{+}{N}$-H). The p.m.r. spectrum ($CDCl_3$): -1.17τ (1H, s, $\overset{+}{N}$-H, exchanged with D_2O).

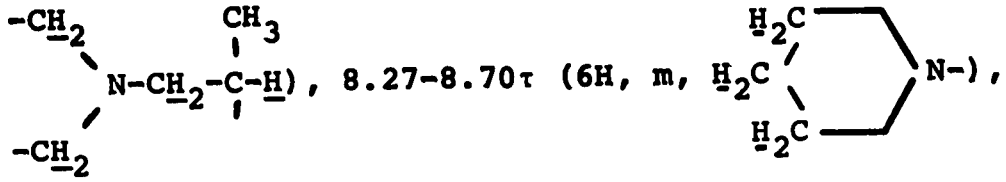
Anal. Calcd. for $C_{10}H_{20}ClNO_2$: C, 54.14; H, 9.91; N, 6.31. Found: C, 53.98; H, 9.91; N, 6.41.

The methiodide had m.p. 151°. The i.r. spectrum (Nujol): 1735 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 7.05τ (3H, s, $\overset{+}{N}$-CH_3).

Anal. Calcd. for $C_{11}H_{22}INO_2$: C, 40.02; H, 6.72; N, 4.25. Found: C, 40.40; H, 6.92; N, 3.91.

Methyl 2-methyl-3-(1-piperidino)propionate

The title compound was prepared in 59.6% yield from piperidine and methyl methacrylate using the general method for the preparation of 3-amino-esters. The reaction mixture was heated under reflux for 24 h prior to fractional distillation. The compound had b.p. 40°/0.3 mm. Literature b.p. 96-98°/14 mm (Adamson, 1949); b.p. 56°/0.5 mm (Barron et al., 1968), b.p. 102-103/18 mm (Bieber, 1950); b.p. 99-100°/18 mm (Bieber, 1954); b.p. 107-108°/ 18 mm (Coutts et al., 1969); b.p. 56°/0.5 mm (Spickett et al., 1966). The i.r. spectrum (thin film): 1735 cm^{-1} (ester C=O). The p.m.r. spectrum (CCl_4): 6.42τ (3H, s, -OCH_3), 7.20-7.95τ (7H, m,



(3H, d, J=7Hz, H-C-CH_3).

Anal. Calcd. for $C_{10}H_{19}NO_2$: C, 64.81; H, 10.34; N, 7.57. Found: C, 65.19; H, 10.20; N, 7.89.

The hydrochloride had m.p. 208°. Literature m.p. 156° (Bieber, 1950), m.p. 156° (Bieber, 1954). The i.r. spectrum (Nujol): 1720 cm^{-1} (ester C=O); 2540, 2660, 2690 cm^{-1} ($\overset{+}{N}$-H). The p.m.r. spectrum ($CDCl_3$): -1.80τ (1H, s, $\overset{+}{N}$-H, exchanged with D_2O).

Anal. Calcd. for $C_{10}H_{20}ClNO_2$: C, 54.14; H, 9.09; N, 6.32. Found: C, 53.98; H, 8.90; N, 6.27.

The methiodide liquified at room temperature. The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 6.20τ (3H, s, $\overset{+}{N}$-CH_3).

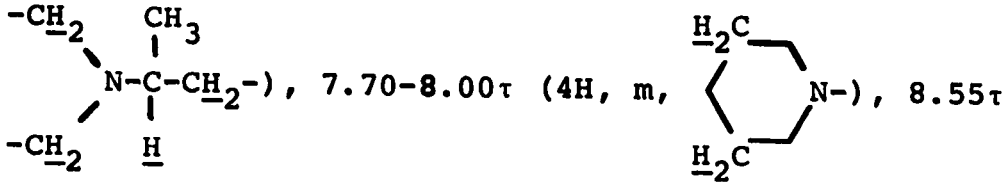
Methyl 3-[1-(4-phenylpiperidino)] butyrates

Methyl 3-[1-(4-phenylpiperidino)]butyrate

The title compound was prepared from 4-phenylpiperidine and methyl crotonate in 30.0% yield using the general method for the preparation of 3-amino-esters. The reaction mixture was heated under reflux

for 24 h prior to fractional distillation. The solvent was then removed under vacuum. The crude product was not further purified. The i.r. spectrum (thin film): 1735 cm^{-1} (ester C=O).

The hydrochloride had m.p. 207-210°. The i.r. spectrum (Nujol): 1740 cm^{-1} (ester C=O); 2410-2620 cm^{-1} ($-\overset{+}{N}-H$). The p.m.r. spectrum (D_2O): 2.62τ (5H, s, $C_6\underline{H}_5-$), 6.21τ (3H, s, $-OC\underline{H}_3$), 6.42-7.33τ (7H, m,



(3H, d, J=7Hz, $H-\underset{\underline{H}}{\overset{|}{C}}-C\underline{H}_3$).

Anal. Calcd. for $C_{16}H_{24}ClNO_2$: C, 64.54; H, 8.07; N, 4.71. Found: C, 64.81; H, 8.12; N, 4.81.

Dialkylamino-acetates and -butyrates

Methyl 2-dimethylaminoacetate methiodide

The title compound was prepared in 5% yield. Methyl bromoacetate (25 g) and anhydrous dimethylamine (20 g) were dissolved in separate portions of dry benzene (50 ml). The cooled amine solution was added slowly with stirring to the solution of methyl bromo-acetate. The reaction mixture was maintained at 0°

for 24 h then stirred at room temperature for 24 h. The dimethylamine hydrobromide formed was filtered off. The benzene was removed under reduced pressure and the residual oil fractionally distilled under reduced pressure. The compound had b.p. 26°/5 mm. Literature b.p. 38°/13 mm (Barrass et al., 1968). The distilled oil was dissolved in acetone and the solution cooled to 0°, then an excess of methyl iodide in acetone was added. The resulting precipitate was crystallized from hot acetone and had m.p. 154-156°. Literature m.p. 152-154° (Barrass et al., 1968). The i.r. spectrum (Nujol): 1750 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 6.63τ (9H, s, $-\overset{+}{N}(CH_3)_3$).

Anal. Calcd. for $C_6H_{14}INO_2$: C, 27.80; H, 5.54; N, 5.41. Found: C, 27.79; H, 5.15; N, 5.54.

Ethyl 2-dimethylaminoacetate

Ethyl bromoacetate (20 g) and anhydrous dimethylamine (16.5 g) were dissolved in separate portions of dry benzene (50 ml). The cooled amine solution was added slowly with stirring to the solution of ethyl bromoacetate. The reaction mixture was maintained at 0° for 4 h then stirred at room temperature for 16 h. After the precipitated dimethylamine hydro-

bromide was filtered off, and the benzene removed from the filtrate under reduced pressure, the residual oil was fractionally distilled under reduced pressure. The title compound had b.p. 28°/1 mm, yield 31.3%. Literature b.p. 55°/20 mm (Barrass et al., 1968); b.p. 48-49°/12 mm (Viscontini and Meier, 1950). The i.r. spectrum (thin film): 1740 cm^{-1} (ester C=O). The p.m.r. spectrum (CCl_4): 5.92τ (2H, q, J=7Hz, $-OC\underline{H}_2-CH_3$), 6.94τ (2H, s, $-C\underline{H}_2-$), 7.72τ (6H, s, $-N(C\underline{H}_3)_2$), 8.77τ (3H, t, J=7Hz, $-O-CH_2-C\underline{H}_3$).

Anal. Calcd. for $C_6H_{13}NO_2$: C, 54.91; H, 9.99; N, 10.68. Found: C, 55.20; H, 9.83; N, 10.42.

The hydrochloride had m.p. 85-87°, the compound liquified on standing at room temperature. The i.r. spectrum (Nujol):1740 cm^{-1} (ester C=O); 2450-2700 cm^{-1} ($\overset{+}{\text{N}}$-H).

The methiodide had m.p. 178-181°. Literature m.p. 176-177 (Barrass et al., 1968). The i.r. spectrum (Nujol): 1740 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 6.65τ (9H, s, $-\overset{+}{\text{N}}(C\underline{H}_3)_3$). The mass spectrum: 214 $[ICH_2COOCH_2CH_3]^{+\cdot}$ (10.7), 186 (5.0), 169 (6.4), 156 (30.0), 142 (36.5), 141 (10.5), 140 (2.0), 139 (2.0), 131 (9.0), 130 (3.4), 128 (3.3), 127 (18.0), 87 (4.0), 59 (6.7), 58 $[(CH_3)_2\overset{+}{\text{N}}=CH_2]$ (100.0), 57 (3.7),

43 (2.7), 42 (11.5), 41 (2.4), 30 (6.2), 20 (40.0), 28 (4.3), 27 (15.5), 18 (25.0), 17 (4.3), 15 (12.5), m/e (% relative abundance).

Anal. Calcd. for $C_7H_{16}INO_2$: C, 30.80; H, 6.16; N, 5.17. Found: C, 30.77; H, 5.91; N, 5.13.

Ethyl 4-dimethylaminobutyrate

The title compound was prepared in quantitative yield from dimethylamine and ethyl 4-bromobutyrate using the general method for the preparation of ethyl 4-aminobutyrates. The reaction mixture was stirred at room temperature for 8 h then heated under reflux for 24 h prior to fractional distillation. The compound had b.p. 48°/5 mm. Literature b.p. 56°/5 mm (Barrass et al., 1968). The i.r. spectrum (thin film): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (CCl_4): 5.97τ (2H, q, J=7Hz, $-OC\underline{H}_2-CH_3$), 7.40-8.50τ (6H, m, $-C\underline{H}_2C\underline{H}_2C\underline{H}_2-$), 7.78τ (6H, s, $-N(C\underline{H}_3)_2$), 8.72τ (3H, t, J=7Hz, $-O-CH_2-C\underline{H}_3$).

Anal. Calcd. for $C_8H_{17}NO_2$: C, 60.38; H, 10.60; N, 8.81. Found: C, 59.99; H, 10.82; N, 8.66.

The hydrochloride had m.p. 104-106°. Literature m.p. 98-100° (Lightowler and MacLean, 1963). The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O); 2460-2660 cm^{-1} ($\overset{+}{N}$-H). The p.m.r. spectrum ($CDCl_3$): -2.00τ

(1H, s, $\overset{+}{N}$-H, exchanged with D_2O).

Anal. Calcd. for $C_8H_{18}ClNO_2$: C, 49.08; H, 9.28; N, 7.16. Found: C, 49.23; H, 9.06; N, 6.86.

The methiodide had m.p. 152-153°. Literature m.p. 156-158° (Barrass et al., 1968). The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 6.92τ (9H, s, $-\overset{+}{N}(CH_3)_3$).

Anal. Calcd. for $C_9H_{20}INO_2$: C, 35.64; H, 6.60; N, 4.62. Found: C, 35.88; H, 6.64; N, 4.31.

Ethyl 4-diethylaminobutyrate

The title compound was prepared in 12.5% yield from diethylamine and ethyl 4-bromobutyrate using the general method for the preparation of ethyl 4-aminobutyrates. The reaction mixture was stirred at room temperature for 24 h then heated under reflux for 24 h prior to fractional distillation. The compound had b.p. 74°/10 mm. The i.r. spectrum (thin film): 1740 cm^{-1} (ester C=O). The p.m.r. spectrum (CCl_4): 5.93τ (2H, q, J=7Hz, $-OCH_2-CH_3$), 7.35-7.90τ (8H, m, $CH_3CH_2\diagdown N-CH_2-\underset{|}{C}-CH_2-$), 8.10-8.55τ (2H, m, $-H_2C-CH_2-CH_2-$), $CH_3CH_2\diagup$

8.60-9.15τ (9H, m, $(C\underline{H}_3-CH_2)_2N-$ and $-O-CH_2-C\underline{H}_3$).

Anal. Calcd. for $C_{10}H_{21}NO_2$: C, 64.1; H, 11.31; N, 7.48. Found: C, 63.98; H, 11.49; N, 7.29.

The <u>hydrochloride</u> had m.p. 115-118°. The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O); 2440-2660 cm^{-1} ($\overset{+}{N}-H$). The p.m.r. spectrum ($CDCl_3$): -2.80τ (1H, s, $\overset{+}{N}-H$, exchanged with D_2O).

Anal. Calcd. for $C_{10}H_{22}ClNO_2$: C, 53.69; H, 9.84; N, 5.95. Found: C, 53.38; H, 9.66; N, 5.97.

The <u>methiodide</u> had m.p. 132°. The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 7.00τ (3H, s, $\overset{+}{N}-C\underline{H}_3$).

Anal. Calcd. for $C_{11}H_{24}INO_2$: C, 40.13; H, 7.35; N, 4.26. Found: C, 40.11; H, 7.23; N, 4.37.

<u>Ethyl 4-dipropylaminobutyrate</u>

The title compound was prepared in 26.7% yield from dipropylamine and ethyl 4-bromobutyrate using the general method for the preparation of ethyl 4-aminobutyrates. The reaction mixture was heated under reflux for 24 h prior to fractional distillation. The compound had b.p. 88°/5 mm. The i.r. spectrum (thin film): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (CCl_4): 6.00τ (2H, q, J=7Hz, $-OC\underline{H}_2-CH_3$), the remainder

of the spectrum was difficult to interpret due to overlap of the signals.

Anal. Calcd. for $C_{12}H_{25}NO_2$: C, 66.91; H, 11.71; N, 6.51. Found: C, 66.51; H, 11.44; N, 6.36.

The hydrochloride had m.p. 74°. The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O); 2470-2620 cm^{-1} ($\overset{+}{\rangle}N-H$). The p.m.r. spectrum ($CDCl_3$): -1.80τ (1H, s, $\overset{+}{\rangle}N-\underline{H}$, exchanged with D_2O).

Anal. Calcd. for $C_{12}H_{26}ClNO_2$: C, 57.26; H, 10.34; N, 5.57. Found: C, 57.54; H, 10.44; N, 5.27.

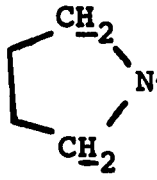
The methiodide had m.p. 77-78°. The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 6.94τ (3H, s, $\overset{+}{\rangle}N-C\underline{H}_3$).

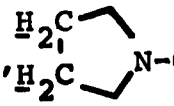
Anal. Calcd. for $C_{13}H_{28}INO_2$: C, 43.68; H, 7.90; N, 3.92. Found: C, 43.54; H, 7.75; N, 3.22.

Ethyl 4-(1-pyrrolidino)butyrate

The title compound was prepared in 11.2% yield from pyrrolidine and ethyl 4-bromobutyrate using the general method for the preparation of ethyl 4-aminobutyrates. The reaction mixture was heated under reflux for 48 h prior to fractional distillation. The compound had b.p. 56-57°/0.5 mm. The i.r. spectrum (thin film): 1730 cm^{-1} (ester C=O). The p.m.r.

spectrum (CCl_4): 5.95τ (2H, q, J=7Hz, $-OC\underline{H}_2-CH_3$),

7.40-7.95τ (8H, m, ), 8.05-

8.50τ (6H, m, ), 8.80τ (3H, t, J=7Hz,

$-O-CH_2-C\underline{H}_3$).

Anal. Calcd. for $C_{10}H_{19}NO_2$: C, 64.81; H, 10.34; N, 7.57. Found: C, 65.13; H, 10.20; N, 7.79.

The hydrochloride liquified at room temperature. The i.r. spectrum (Nujol): 1725 cm^{-1} (ester C=O); 2470-2700 cm^{-1} ($\overset{+}{\rangle}N-H$). The p.m.r. spectrum ($CDCl_3$): -1.30 (1H, s, $\overset{+}{\rangle}N-\underline{H}$, exchanged with D_2O).

The methiodide had m.p. 133-134°. Literature m.p. 132-133° (Barrass et al., 1968). The i.r. spectrum (Nujol): 1735 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 6.78τ (3H, s, $\overset{+}{\rangle}N-C\underline{H}_3$).

Anal. Calcd. for $C_{11}N_{22}INO_2$: C, 40.36; H, 6.78; N, 4.28. Found: C, 40.65; H, 6.78; N, 4.23.

Ethyl 4-(1-piperidino)butyrate

The title compound was prepared in 77.8% yield

from piperidine and ethyl 4-bromobutyrate using the general method for the preparation of ethyl 4-amino-esters. The reaction mixture was heated under reflux for 16 h prior to fractional distillation. The compound had b.p. 86°/10 mm. The i.r. spectrum (thin film): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (CCl_4): 5.95τ (2H, q, J=7Hz, $-OC\underline{H}_2-CH_3$), the remainder of the spectrum was difficult to interpret due to overlap of the signals.

Anal. Calcd. for $C_{11}H_{21}NO_2$: C, 66.27; H, 10.63; N, 7.03. Found: C, 66.27; H, 10.63; N, 7.05.

The <u>hydrochloride</u> had m.p. 129-129.5°. The i.r. spectrum (Nujol): 1725 cm^{-1} (ester C=O); 2400, 2520, 2580, 2630 cm^{-1} ($\overset{+}{\text{N}}$-H). The p.m.r. spectrum could not be interpreted due to overlap of the signals.

Anal. Calcd. for $C_{11}H_{22}ClNO_2$: C, 56.01; H, 9.41; N, 5.94. Found: C, 55.72; H, 9.32; N, 6.21.

The methiodide had m.p. 160-161°. The i.r. spectrum (Nujol): 1730 cm^{-1} (ester C=O). The p.m.r. spectrum (D_2O): 6.90τ (3H, s, $\overset{+}{\text{N}}-C\underline{H}_3$).

Anal. Calcd. for $C_{12}H_{24}INO_2$: C, 42.22; H, 7.09; N, 4.11. Found: C, 42.02; H, 6.90; N, 4.40.

Ethyl 4-phenethylaminobutyrate

The title compound was prepared in 49.2% yield from phenethylamine and ethyl 4-bromobutyrate using the general method for the preparation of ethyl 4-aminobutyrates. The reaction mixture was stirred at room temperature for 24 h then heated under reflux for 48 h prior to fractional distillation. After the phenethylamine hydrobromide was filtered off the benzene extract was extracted with 5% hydrochloric acid, the acid layer was then neutralized with 5% sodium hydroxide and extracted with diethyl ether (3 X 20 ml). This ether extract was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residual oil was not purified. The i.r. spectrum (thin film): 1735 cm^{-1} (ester C=O); 1590 cm^{-1} (C=C).

Anal. Calcd. for $C_{14}H_{21}NO_2$: C, 71.45; H, 8.94; N, 5.96. Found: C, 71.23; H, 8.54; N, 6.02.

The hydrochloride had m.p. 171-174°. The i.r. spectrum (Nujol): 1725 cm^{-1} (ester C=O); 2500-2660 cm^{-1} ($\overset{+}{\text{N}}$-H). The p.m.r. spectrum ($CDCl_3$): -0.32τ (1H, s, $\overset{+}{\text{N}}$-H, exchanged with D_2O).

Anal. Calcd. for $C_{14}H_{22}ClNO_2$: C, 61.88; H, 8.10; N, 5.16. Found: C, 61.43; H, 8.06; N, 5.07.

Ethyl 4-(N-methyl-N-phenethyl)aminobutyrate hydriodide

The title compound was prepared by dissolving ethyl 4-phenethylaminobutyrate (1 g) in acetone and adding an excess of methyl iodide in acetone. The resulting precipitate was crystallized from acetone/diethyl ether. The compound had m.p. 160-164°. The i.r. spectrum (Nujol): 1725 cm^{-1} (ester C=O).

Anal. Calcd. for $C_{15}H_{24}INO_2$: C, 47.74; H, 6.42; N, 3.72. Found: C, 47.65; H, 6.64; N, 3.93.

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