THE SYNTHESIS AND PHARMACOLOGICAL PROPERTIES OF SOME ALICYCLIC COMPOUNDS RELATED TO ACETYLCHOLINE

by

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INTRODUCTION

There appear to be two ways in which a drug can affect cells; it may, in some way, modify the function of the whole cell by a physicochemical process, e,g, by an action at the cell surface; it may affect only certain vital processes (or even a single process), and in these circumstances, the drug may only interact with particular groups on or in the cell rather than on the whole cell. The idea that drugs might act in this second way was first suggested by Langley (1878, 1905) and was used extensively by Ehrlich (1913) to interpret his results in chemotherapy. This idea is important because of subsequent discoveries in biochemistry and physical chemistry (catalysis).

In enzymology, Michaelis and Menten (1913) considered the adsorption of the substrate at the "active spot" on an enzyme. Similarly, in physical chemistry, Langmuir (1916, 1918) regarded adsorption as due to the fact that a gas molecule, striking the surface of the solid, will tend to adhere to it for a period of time before evaporating.

It was Clark (1933) who collected and applied these ideas to the mode of action of drugs. He obtained evidence which showed that the molecules of some drugs cover only a fraction of the total area of cells and suggested,

therefore, that there were "active spots" on the surface of the cell; these "active spots", where the drug acts, are called "receptors".

If the reaction between drug and receptor is written

Drug + Receptor = Complex → Breakdown → Response

then the rate of formation of the complex will be $K_1[A](1-y)$, where [A] is the concentration of the drug and y the proportion of receptors combined with the drug. The rate of dissociation will be $K_2 y$.

At equilibrium, the rates will be equal. Therefore,

$$K_a[A] = \frac{y}{1 - y} \qquad (1)$$

or it can be written

$$y = \frac{K_a[A]}{1 + K_a[A]} \qquad (2)$$

where K_a is the association ("affinity") constant, i.e. K_1/K_2.

Michaelis and Menten and Langmuir had derived similar expressions.

i.e. $K_s = \frac{(e - p)[S]}{p}$ Michaelis - Menten (3)

where e is the enzyme concentration, p is the concentration of the complex, and [S] is the concentration of the substrate. (It should be noted that, whereas the enzymologist uses the dissociation constant $K_s = K_2/K_1$, the

pharmacologist uses the association ("affinity") constant $K_a = K_1/K_2$.

Langmuir's expression can be written as

$$\theta = \frac{\alpha u}{v + \alpha u} \qquad (4)$$

where θ is the fraction of the total available surface covered with gas molecules at any instant, α is the proportion which adhere, u is the number of gas molecules striking 1 sq.cm. of surface per second, and v is a constant for the given gas and surface.

Although the biochemist and physical chemist can verify their respective expressions, the pharmacologist cannot, because the steps between the formation of the complex and biological response are unknown. This led Clark to make further assumptions (which he pointed out were improbable ones in a pharmacological reaction, although they were true in enzymology) namely that the biological response was directly related to the proportion of receptors occupied, and that when all the receptors were occupied there was a maximal response; when only 50% of the receptors were occupied there would be a 50% response. Therefore in equation (1) when $y = 0.5$,

$$K_a = 1/[A] \qquad (5)$$

If this relationship were true, then

the affinity constant (K_a) would be the reciprocal of the concentration which would produce half the maximal response. Some workers (e.g. Ariëns *et al.* 1954 and 1957) have used this relationship in order to assess the affinity of an agonist for the receptors. (Other workers, e.g. Stephenson, 1956, have shown that there was no justification for this).

It may be argued that Clark's application of the Langmuir Adsorption Isotherm to drugs and receptors is valid because it can be successfully used in problems in physical chemistry and biochemistry (and the drug-receptor concept is similar to these). Although Clark used the Langmuir Adsorption Isotherm, he himself pointed out that the results can just as easily be interpreted by the Weber-Fechner law

$$Ky = \log(bA + 1) \qquad (6)$$

or by the Freundlich equation

$$y = KA^{\frac{1}{2}} \qquad (7)$$

In view of the empirical nature of equations (6) and (7), the Langmuir Adsorption Isotherm is preferred because of its validity in chemistry and biochemistry.

Clark and Raventos (1937) showed that equipotent doses of acetylcholine and tetramethylammonium on the rat intestine, frog auricle and frog rectus preparations could be antagonised

to the same extent by many antagonists. Further, although the concentration of tetramethylammonium required to produce the same effect as acetylcholine on the preparations was 1000 times that of acetylcholine, these two compounds acted additively, i.e. if a concentration a of acetylcholine produced the same effects as a concentration t of tetramethylammonium, then the same effects were obtained with a/2 + t/2. From the results of Clark and Raventos, it appeared that the lower activity of tetramethylammonium compared with acetylcholine could be abscribed simply to a lower affinity for the receptors. If the drug-receptor complex produced by tetramethylammonium was less effective than the complex produced by acetylcholine, it would be expected that the presence of tetramethylammonium would be reducing the number of receptors available for combination with acetylcholine, and so the effects of these two compounds would not be additive.

These results gave rise to the "all-or-none" theory of the drug-receptor complex, i.e. the idea that the drug-receptor complex was either completely effective or completely ineffective. If it were effective, then the drug would be an agonist; if it were ineffective, the

drug would be an antagonist. In either case the activity of the drug would depend only on its adsorbability (affinity).

Although the results obtained by Clark and Raventos for acetylcholine and tetramethylammonium appear to support the "all-or-none" theory, Ariëns and de Groot (1954) and Stephenson (1956) have shown that other compounds, e.g. some alkyltrimethylammonium salts, act like acetylcholine on the guinea-pig ileum or frog rectus preparations, but do not act additively with acetylcholine. These compounds are called "dualists" (Ariëns) and "partial agonists" (Stephenson) and in order to interpret this they introduced a new factor which the former called "intrinsic activity" and the latter, "efficacy". Thus, they suggested that the activity of a drug depends not only on its affinity (adsorbability) but also on this new factor.

Stephenson goes further and points out that there is no evidence that the response is linearly proportional to the number of receptors occupied, therefore he introduces the quantity S, the stimulus given to the tissue, which is some function of R, the response, i.e. $R = f(S)$. We do not know how the two are related. Stephenson defines the stimulus (S) as the product of efficacy and the proportion of receptors

occupied,

$$\text{i.e.} \quad S = ey \tag{8}$$

From equation (2),

$$S = \frac{eK[A]}{1 + K[A]} \tag{9}$$

To compare the activity of two agonists it is usual to measure the concentrations which produce comparable effects:

$$\text{i.e.} \quad S = e_1 y_1 = e_2 y_2$$

$$\text{or} \quad \frac{e_1 K_1[A_1]}{1 + K_1[A_1]} = \frac{e_2 K_2[A_2]}{1 + K_2[A_2]} \tag{10}$$

If the values of $[A_1]$, $[A_2]$, K_1 and K_2 were known, it would be possible to estimate the ratio e_1/e_2. The absolute values of the affinity constants of pure agonists are unobtainable since they depend on knowing e_1 and e_2, but they are obtainable for antagonists by using the Gaddum equation (1937).

$$\text{i.e.} \quad [A]/[a] = 1 + [B]K_b \tag{11}$$

where the response to a concentration, [A], of agonist in the presence of a concentration, [B], of antagonist, is the same as that to a concentration [a] of agonist alone: K_b is the affinity constant of the antagonist.

The derivation of this equation makes no assumptions about the relationship between biological stimulus and proportion of receptors occupied.

When the "dose ratio", ([A]/[a]) is 2, equation (11) becomes $[B] = 1/K_b$. Schild (1947) calls log. 1/[B] "pA_2", which is therefore log. K_b.

Recently, Paton (1961) has suggested that excitation by a stimulant drug is proportional to the rate of drug-receptor complex formation rather than to the proportion of receptors occupied.

This "rate" theory differs only from the "occupation" theory in that it uses k_2, the dissociation rate constant. In the "rate" theory, the idea of "efficacy" (as meant by Stephenson) is accounted for in the dissociation rate constant.

Thus the mathematical relationship relating dose and response is similar in both theories:

$$\text{Response} = f(S) = f(ey) = f\left[\frac{ex}{x + 1/K}\right] \text{ Stephenson}$$

and

$$\text{Response} = f\left[\frac{k_2 x}{x + k_2/k_1}\right] \text{ Paton}$$

where x is the dose, and K, k_1 and k_2 are constants.

Stephenson suggested that if two types of series of compounds were prepared, one antagonist and the other agonist, changes in affinity with structure could easily be measured

in the antagonist series, and might be applicable to the corresponding agonists. Accordingly, Scott (1963) prepared two types of series of compounds:

$RNMe_3^+$	$R'NMe_3^+$
$RNMe_2Et^+$	$R'NMe_2Et^+$
$RNMeEt_2^+$	$R'NMeEt_2^+$
$RNEt_3^+$	$R'NEt_3^+$

where R is a 2-(diphenylacetoxy)ethyl, 2-(benziloyloxy)ethyl, 2-(2,2-diphenylethoxy)ethyl, 3-(diphenylmethoxy)propyl, or 3,3-diphenylbutyrylmethyl group and R' is a 2-acetoxyethyl, 2-ethoxyethyl, 3-methoxypropyl or butyrylmethyl group. (R and R' are later referred to as the "body of the molecule). The compounds of the first type of series differ only from those of the second type in that they contain a diphenylmethyl (or a benziloyl) group in place of the methyl group. The former compounds are antagonists of acetylcholine whereas most of the latter were acetylcholine-like.

Scott measured the affinity constants of the antagonists and observed that similar alterations in the composition of the "onium" group produced similar changes in affinity in many series of compounds. Scott then measured the acetylcholine-like activity of the agonists and, if it is assumed that the same changes in

composition of the onium group produce similar changes in the affinity of these agonists, as they do in the antagonists, it then becomes possible to assess the effects of changes in chemical structure on the efficacy of these compounds. This assumption seems reasonable because, from the theory of Arrhenius, the relationship between the affinity (association) constant, K, for the drug and receptor, and the free energy change on adsorption is

$$\Delta F = -RT\log_e K$$

$$\text{or} \qquad \log_{10} K = \frac{-\Delta F}{2.3RT} \qquad (12)$$

If it is assumed that the change in free energy of adsorption depends only on the substitution in the onium group, i.e. any contribution from the "body" of the molecule is unaffected by changes in the onium group, and if we let the free energy of adsorption of $R\overset{+}{N}Me_3$ be F, and the free energy of adsorption of $R'\overset{+}{N}Me_3$ be F', then the free energy of adsorption of

$R\overset{+}{N}Me_2Et$	$= \Delta F + a$,	$R'\overset{+}{N}Me_2Et$	$= \Delta F' + a$
$R\overset{+}{N}MeEt_2$	$= \Delta F + b$,	$R'\overset{+}{N}MeEt_2$	$= \Delta F' + b$
$R\overset{+}{N}Et_3$	$= \Delta F + c$,	$R'\overset{+}{N}Et_3$	$= \Delta F' + c$

where "a" is the change in free energy brought about by replacing $\overset{+}{N}Me_3$ by $\overset{+}{N}Me_2Et$, "b" is the change for replacing $\overset{+}{N}Me_3$ by $\overset{+}{N}MeEt_2$ and "c" is the change for replacing $\overset{+}{N}Me_3$ by $\overset{+}{N}Et_3$. The affinity

constants for the antagonists can be obtained experimentally. For $R\overset{+}{N}Me_3$ let it be K, for $R\overset{+}{N}Me_2Et$ let it be K_a, for $R\overset{+}{N}MeEt_2$ let it be K_b, and for $R\overset{+}{N}Et_3$ let it be K_c.

$$\text{Hence log. } K = \frac{-\Delta F}{2.3\ RT}$$

$$\text{and log. } K_a = \frac{-(\Delta F + a)}{2.3\ RT}$$

$$\text{therefore log. } K_a/K = \frac{-a}{2.3\ RT}$$

$$\text{i.e.} \quad -a = 2.3\ RT \log.[K_a/K] \qquad (13)$$

$$\text{or} \quad K_a/K = 10^{-\left[\frac{a}{2.3\ RT}\right]} \qquad (14)$$

The values for -a should be the same and likewise those of -b and -c (similarly determined) regardless of the nature of the "body" of the molecule. With a few minor exceptions, which he attempted to explain, Scott (1963) found this to be true for all the series of antagonists he studied. Therefore, since the change in free energy of adsorption depended only on the substitution of the onium group and not at all on the body, the ratio K_a/K (obtained by experiment) for the antagonist series can be transferred to the corresponding agonist series.

Let us suppose that the two agonists $R'\overset{+}{N}Me_3$ and $R'\overset{+}{N}Me_2Et$ have affinity constants K' and K'_a respectively and that the equipotent molar

If the drug is a highly active agonist, the work of Nickerson (1956) on the histamine receptors of the guinea-pig ileum and of Ariëns *et al.* (1960) on the acetylcholine receptors of the guinea-pig ileum (and the adrenaline receptors of the rabbit aortic strip) indicates that the proportion of receptors occupied is small. In these circumstances it is justifiable to calculate the efficacy ratio as above. If, however, the compound has a much lower efficacy, the proportion of receptors occupied may be large, and the efficacy ratio cannot be calculated from these results. It may be apparent, if the efficacy is very low, because the compounds fail to produce a maximal contraction however much is given, but it is still possible that there are many compounds with low efficacy which are capable of producing maximal responses of the tissue and give dose-response curves parallel to those of acetylcholine. It is clearly not justifiable to use the approximation to calculate changes of efficacy with structure in these compounds.

If a compound is weakly active it is likely that it has a low efficacy rather than a low affinity (Scott *et al.*, 1963) and hence falls into this category. It is therefore unwise to attach too much importance to relationships between structure and apparent changes in efficacy of series of weak agonists. It does not follow that more active agonists have a higher efficacy and are not occupying a large proportion of receptors, though (again from the work of Scott *et al.*) it seems more likely that similar compounds are active because they have a reasonable efficacy than because they have a very high affinity. This present work depends upon the expectation that this is correct.

In order to check this it would be necessary to repeat experiments such as those described by Ariëns *et al.* (1960) with the compounds being tested.

ratio of $R'\overset{+}{N}Me_2Et$ relative to $R'\overset{+}{N}Me_3$ is n, i.e. n molecules of the former are needed to produce the same response as the latter, then from equation (11) we have

$$\frac{eK'[A]}{1 + K'[A]} = \frac{e_aK'_a[A_a]}{1 + K'_a[A_a]} \qquad (15)$$

where e is the efficacy of $R'\overset{+}{N}Me_3$ and [A] is the concentration producing the response; e_a is the efficacy of $R'\overset{+}{N}Me_2Et$ and $[A_a]$ is the concentration producing the same response. Hence $[A_a]/[A] = n$.

If the proportion of receptors occupied by the drug is small, the expression $K'[A] = \frac{y}{y - 1}$ approximates to y, therefore the stimulus $S = ey = K'[A]$. Equation (15) then becomes,

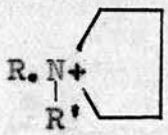
$$eK'[A] = e_aK'_a[A_a]$$

$$\text{i.e.} \quad e/e_a = \frac{K'_a[A_a]}{K'[A]} = \frac{K'_a}{K'} \cdot n \qquad (16)$$

If the assumption is correct then the ratio K_a/K (for the antagonists) will be the same as the ratio K'_a/K' (for the agonists). Although the efficacy cannot be calculated, the effect on efficacy of a change in composition of the onium group can be calculated from equation (16), since the affinity constants of the antagonists and n for the agonists can be determined experimentally.

The purpose of the present work is two-fold:

(i) To repeat some of Scott's work in order to check on the sort of errors which may be involved in the method, and to extend some of his series. The affinity constants of the antagonists in the diphenylacetoxyethyl and benziloyloxyethyl series were redetermined and these two series were extended by introducing ring structures into the onium group,

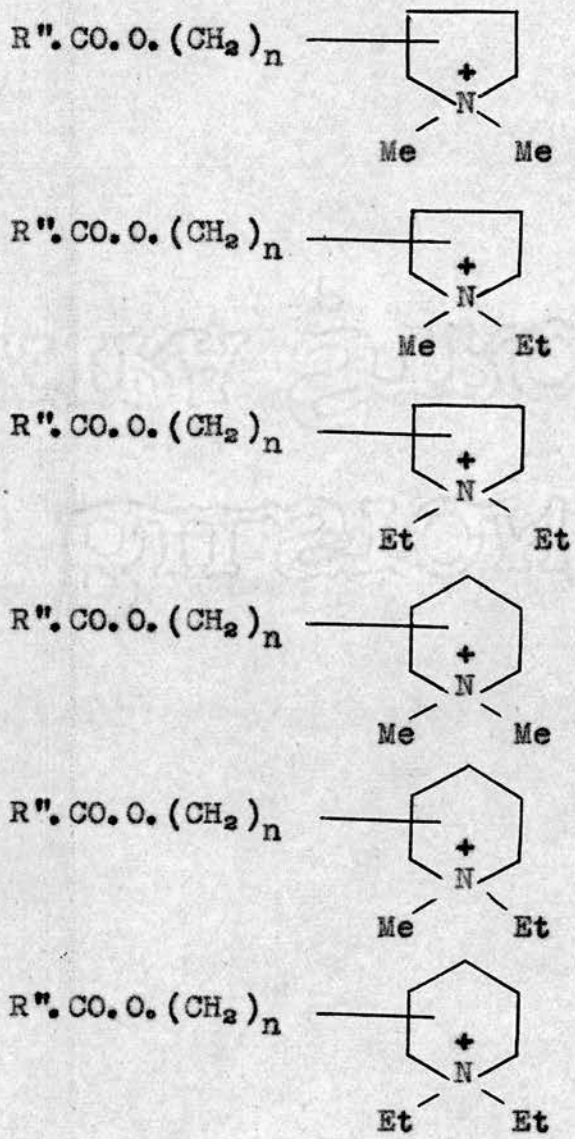
i.e.  and 

where R is diphenylacetoxyethyl ($Ph_2CH.CO.O.CH_2CH_2$-) or benziloyloxyethyl ($Ph_2C(OH).CO.O.CH_2CH_2$-) and R' is methyl or ethyl.

The corresponding agonists (where R = methyl) were also prepared.

(ii) To synthesise some series of cyclic compounds and study their pharmacological properties in a similar manner to that of Scott.

An attempt was made to prepare the following cyclic series:



When R" = diphenylmethyl the compounds are antagonists and when R" = methyl the compounds are agonists: n = 0 or 1.

These series can be written in another, more general, way,

i.e.	+ $RNMe_2$	+ $R'NMe_2$
	+ RNMeEt	+ R'NMeEt
	+ $RNEt_2$	+ $R'NEt_2$
	(antagonists)	(agonists)

The effects on affinity and efficacy of altering the onium group can be studied and hence, since there are several series of compounds, it should be possible to see whether similar changes in composition of the onium group produce similar changes in affinity.

Alternatively, the effects on affinity and efficacy of altering the "body" of the molecule can be studied although it is perhaps not justifiable to do this since, in addition to a change in size and shape of the ring (between five and six-membered rings), there are positional changes which have to be considered. This could lead to all kinds of complications from which it would be difficult to draw any genuine conclusions.

PHARMACOLOGY

EXPERIMENTAL

A stream of compressed air was blown through the bathing fluid whose pH was found to be 7.7 (at 37°C) and the pH of the Tyrode solution in the coils was between 7.7 and 7.8 (at 37°C).

Preparation:

All experiments were performed on the isolated guinea-pig ileum preparation.

A guinea-pig, weighing about 200-250g., was killed by a blow on the head and bled out. About 10cm. of ileum was carefully dissected out and placed in a dish of Tyrode solution (at about 25°C). The lumen of the gut was washed through; care was taken to distend the gut as little as possible. The terminal 2 cm., containing the Peyer's patch, was removed, and the adjacent 3cm. of the ileum was suspended in Tyrode solution in an organ bath of 2.5ml. capacity for agonist assays, and in one of 5.0ml. capacity for antagonist assays. The gut was attached to a light isotonic frontal writing lever giving a magnification of five and with a load of 0.5g.

The organ bath was connected to coils of glass tubing so that the fluid in the bath could be replaced by upward displacement and overflow, either by Tyrode solution alone or by Tyrode solution containing drugs at predetermined concentrations. The coils were kept at 37 ± 0.1°C in a thermostatically controlled bath.

The Tyrode solution used in all experiments contained double the normal concentration of potassium because it had been

found by Scott (1962) that the responses to a dose of drug were more regular in these conditions. In particular, in antagonist assays in which the same dose is given five successive times, the response became constant much more rapidly (e.g. after only 2 or 3 contractions) when the Tyrode solution contained double the normal concentration of potassium. The Tyrode solution also contained hexamethonium bromide (1.1×10^{-4} M) to ensure that the drugs were acting only on the postganglionic cholinergic receptors. In some experiments Mepyramine (4.0×10^{-7} M) was also present in the Tyrode solution in order to prevent a response caused by action on Histamine receptors. However, the presence of Mepyramine in the Tyrode solution appeared to have no effect whatever on the responses.

The antagonist assays were performed by an automatic apparatus similar to that described by Schild (1947), and the agonist assays were performed by an automatic apparatus described by Stephenson (1956).

Antagonist activity.

Antagonist activity was estimated by determining the affinity constant (K_b) of the drug for the acetylcholine receptors in the tissue.

A response was obtained with acetylcholine (usually 4.4×10^{-8} M applied for 10 or 15 seconds) and then washed out with about 20ml. of Tyrode solution. This was repeated at intervals of 60 or 90 seconds until the contractions became steady, usually after about an hour.

In all the experiments it was found that three concentrations of acetylcholine, that is, 2.2, 4.4, and 8.8×10^{-8} M, which produced between 20 and 80% of the maximal response, were always satisfactory. A group of five contractions was obtained with each concentration and the groups were repeated a number of times in a random order. When the responses to each concentration appeared to be regular (i.e. there was little variation between groups for a particular concentration) a final group of responses was obtained with the low concentration, then with the high concentration, then with the intermediate concentration, and lastly with the high concentration, this sequence was used by Scott for his antagonist assays. From these

results for low and intermediate concentrations, and from the last results for the high concentration, a graph of log. dose against response was plotted.

After the last response was obtained with the high concentration of acetylcholine. the Tyrode solution was replaced by Tyrode solution containing antagonist. The agonist solutions were replaced by another solution containing a much higher concentration of acetylcholine (of the order of 10^{-6} M) together with the same concentration of antagonist as was present in the washing Tyrode solution. The actual concentrations of antagonist and agonist were those which, from preliminary experiments, seemed likely to produce a response about the same as that given by the intermediate dose of acetylcholine alone. This solution (containing both agonist and antagonist) was applied repeatedly to the ileum, until such time as five contractions of the same height were obtained, indicating that equilibrium had been reached.

A higher concentration of antagonist, with a correspondingly increased concentration of acetylcholine was then used instead of the previous solutions, and the process repeated. The procedure was repeated a third time using still higher concentrations of agonist and

It was assumed that the sensitivity of the tissue did not alter during exposure to the antagonists. If it did, an incorrect dose ratio would be obtained. In all the experiments the graph of dose ratio - 1 against antagonist concentration was found to be linear and the values of K_b were reasonably consistant : if changes in the sensitivity did occur they must have occured to about the same extent in all the experiments. Though this could be true if all the experiments had taken the same length of time to perform it seems unlikely because some experiments were performed more rapidly than others and did not appear to give abnormal results.

antagonist.

A typical assay is shown in Figure (i) and the graph of the log. dose (agonist) against the response plotted, as shown in Figure (ii). When the graph was not linear a straight line was drawn joining the two points between which lay the response produced in the presence of the antagonist. The graph was used to estimate the concentration of agonist which alone would produce the same response as was obtained by the (higher) concentration of agonist in the presence of antagonist. The ratio of the two concentrations of agonist (i.e. the dose ratio) is a measure of the antagonism produced by the particular concentration of antagonist: the affinity constant could then be measured by substituting the observed value of this dose ratio into the Gaddum equation,

i.e. $[A]/[a] - 1 = BK_b$ (Equation 12)

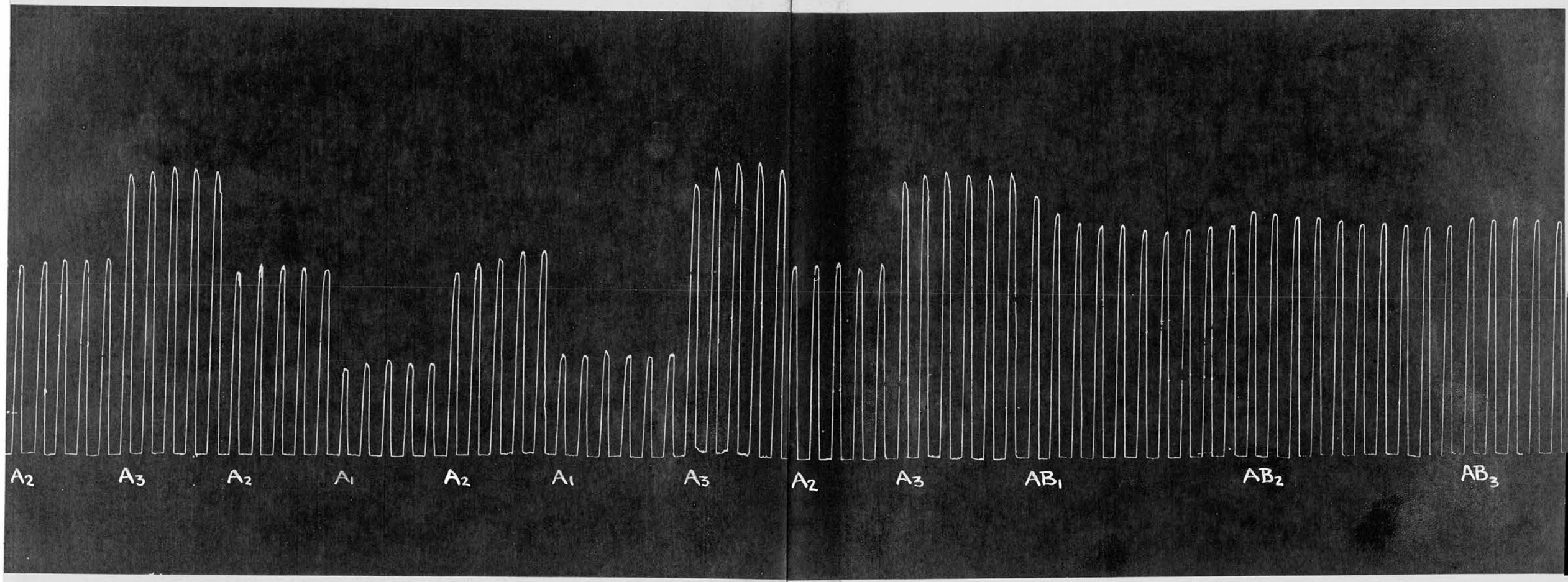
As three concentrations of antagonist were usually used, a graph of (dose-ratio - 1) against concentration of antagonist could be plotted (Figure iii) and this should be linear if the antagonism is competitive. This was always found to be so.

It was usually found that it was necessary to use about five preparations, each of

which afforded three results, in order to obtain a mean value with reasonably narrow fiducial limits.

Figure (i)

A typical assay used to determine the affinity constant of an antagonist.



A_1 = 2.2 x 10^{-8}M acetylcholine

A_2 = 4.4 x 10^{-8}M acetylcholine

A_3 = 8.8 x 10^{-8}M acetylcholine

AB_1 = 4.4 x 10^{-7}M acetylcholine + 6.0 x 10^{-8}M antagonist

AB_2 = 2.2 x 10^{-6}M acetylcholine + 3.0 x 10^{-7}M antagonist

AB_3 = 4.4 x 10^{-6}M acetylcholine + 6.0 x 10^{-7}M antagonist

The antagonist under test is

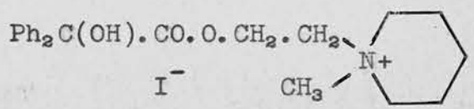
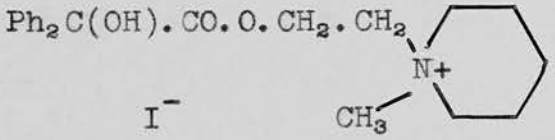


Figure (ii)

This shows the method employed for obtaining the dose ratios and the values for the affinity constant from the experimental data given in Figure (i).

A = acetylcholine

B = antagonist, in this experiment it is



From Figure (i)

Dose	Response
A_1	2.61
A_2	4.98
A_3	7.30
AB_1	5.87
AB_2	5.89
AB_3	5.95

From Figure (ii)

1. AB_1 8.7575 5.72×10^{-8}
2. AB_2 8.7600 5.75×10^{-8}
3. AB_3 8.7700 5.89×10^{-8}

From the Gaddum equation, $K_b = [A/a - 1] / B$

Hence,

1. $\dfrac{4.4 \times 10^{-7}}{5.72 \times 10^{-8}} - 1 / 6 \times 10^{-8}$

$= \underline{1.12 \times 10^{8}}$

2. $\dfrac{2.2 \times 10^{-6}}{5.75 \times 10^{-8}} - 1 / 3 \times 10^{-7}$

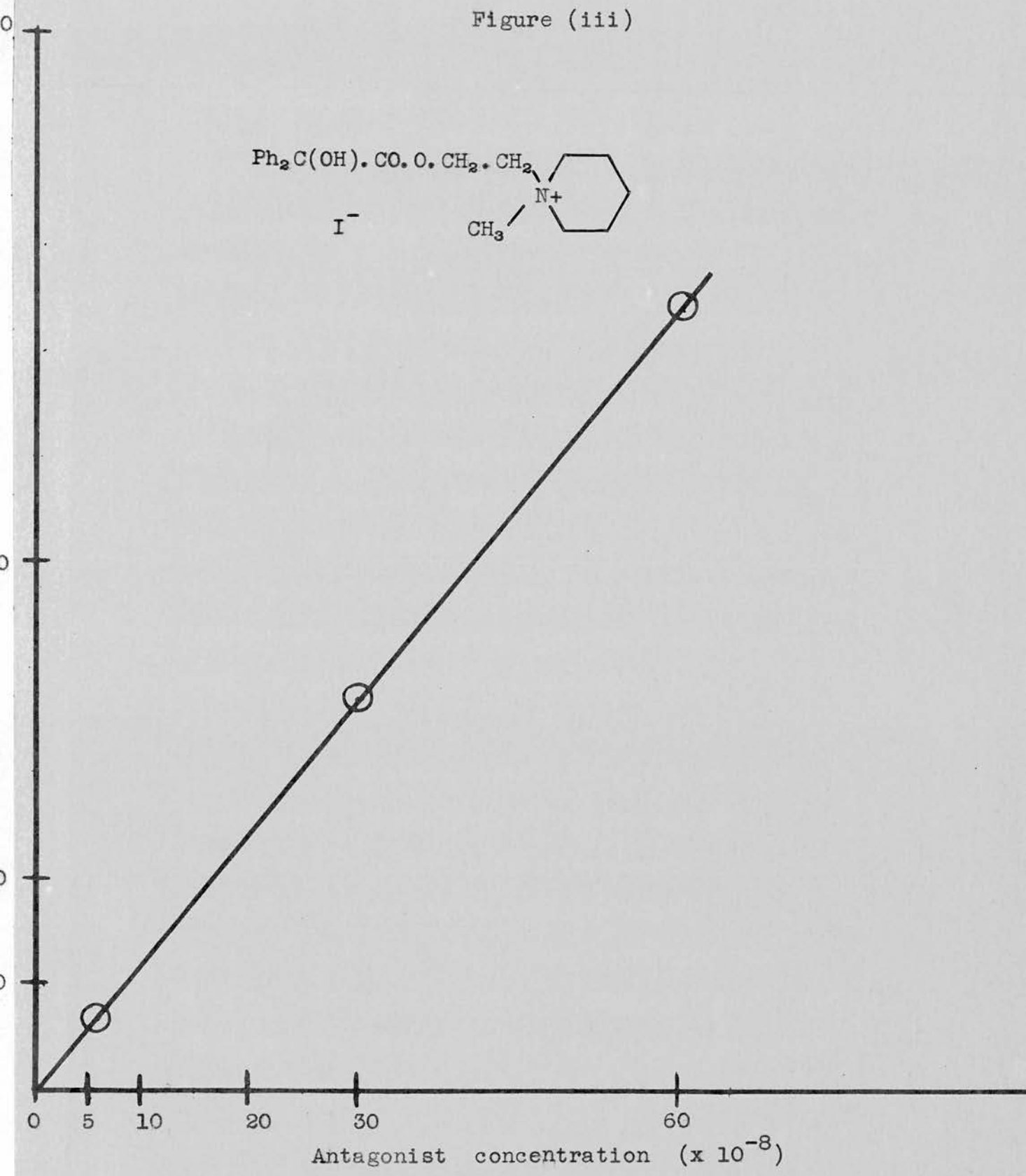
$= \underline{1.24 \times 10^{8}}$

3. $\dfrac{4.4 \times 10^{-6}}{5.89 \times 10^{-8}} - 1 / 6 \times 10^{-7}$

$= \underline{1.23 \times 10^{8}}$

Mean value of affinity constant is $\underline{1.20 \times 10^{8}}$

Figure (iii)



Graph of (dose ratio - 1) against antagonist concentration showing the linear relationship which is consistent with competitive antagonism.

Agonist activity.

Agonist activity was estimated by determining the equipotent molar ratios, relative to acetylcholine, as described by Stephenson (1956).

Preliminary experiments were performed by adding the agonist to the organ bath from a pipette, in order to determine what concentrations of drug were needed to produce between 20 and 80% maximal contraction of the ileum. Acetylcholine was added in a similar way and the log. dose - response curves for it and for the test compound were plotted: these were normally found to be parallel, e.g. Figure (iv).

From these experiments it was possible to calculate an approximate equipotent molar ratio for the agonist, relative to acetylcholine; hence 2 + 2 dose assays were performed.

The four different agonist solutions were used in varying order to produce forty-eight contractions (twelve groups of four, arranged in three Latin squares), i.e.

A B C D	D A B C	D C A B
B D A C	A C D B	C B D A
C A D B	B D C A	A D B C
D C B A	C B A D	B A C D

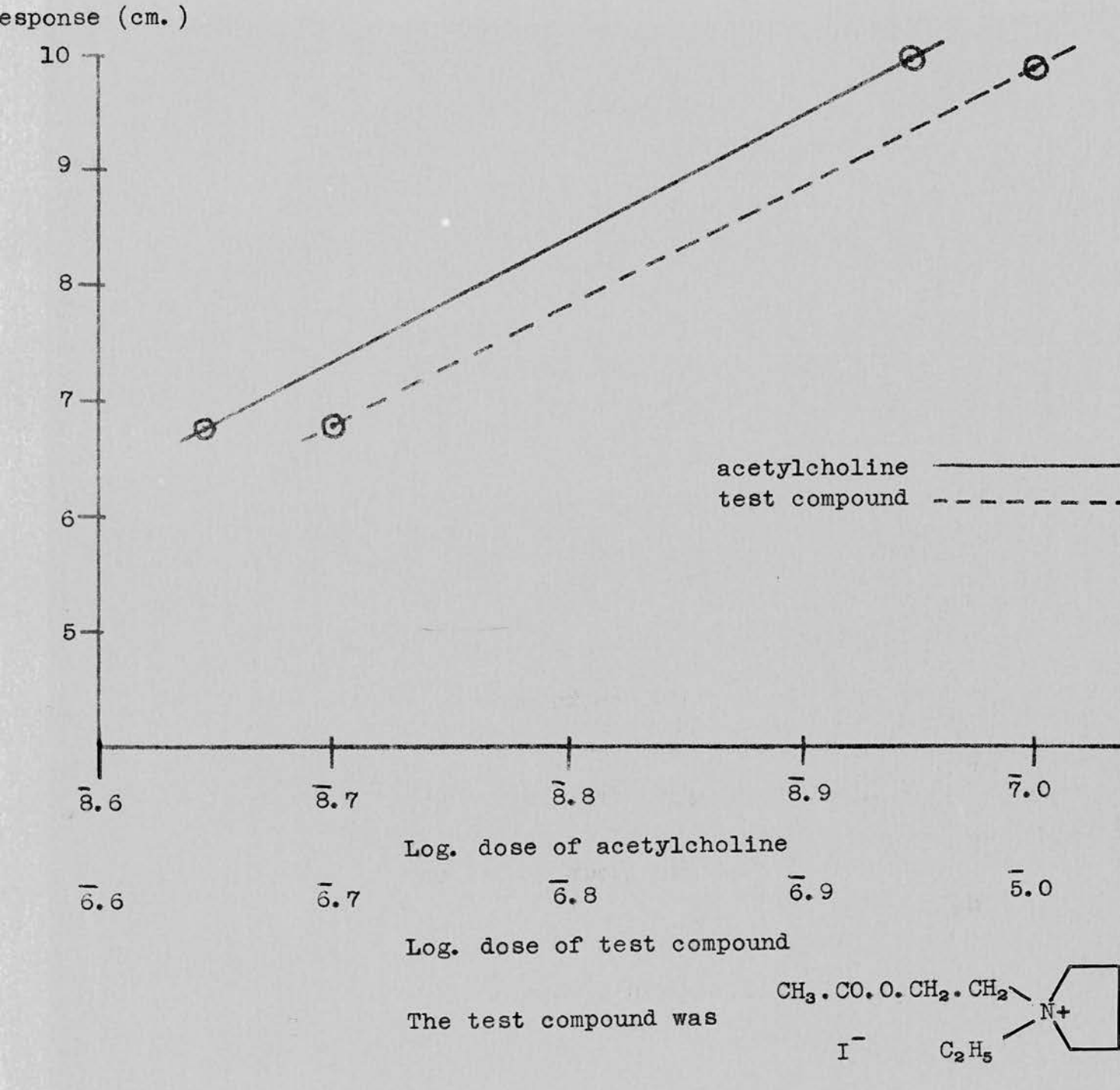
The dose order is so arranged that each

dose is preceded by all other doses the same number of times; thus it was hoped that the effect that a dose had, on the one following it, would not be likely to bias the results. However, it was observed that a large dose will usually increase the size of contraction of a following large dose, but diminish the size of contraction of a following small dose. In an attempt to modify this effect a third dose of acetylcholine was interpolated between each of the assay doses as described by Scott (1962). There did not seem to be any significant difference between an assay with an interpolated dose and an ordinary assay in my experiments, although Scott observed one in his experiments.

The two doses of acetylcholine used were usually 2.2 and 4.4 x 10^{-8} M although it was sometimes necessary to use higher or lower concentrations depending on the sensitivity of the preparation. The drugs were added every 60 or 90 seconds (this interval depended on the time taken for the ileum to relax after a wash with Tyrode solution, but it was the same interval for a complete assay) and they were left in contact with the ileum for 10 or 15 seconds.

A typical 2 + 2 assay is shown in Figure (v).

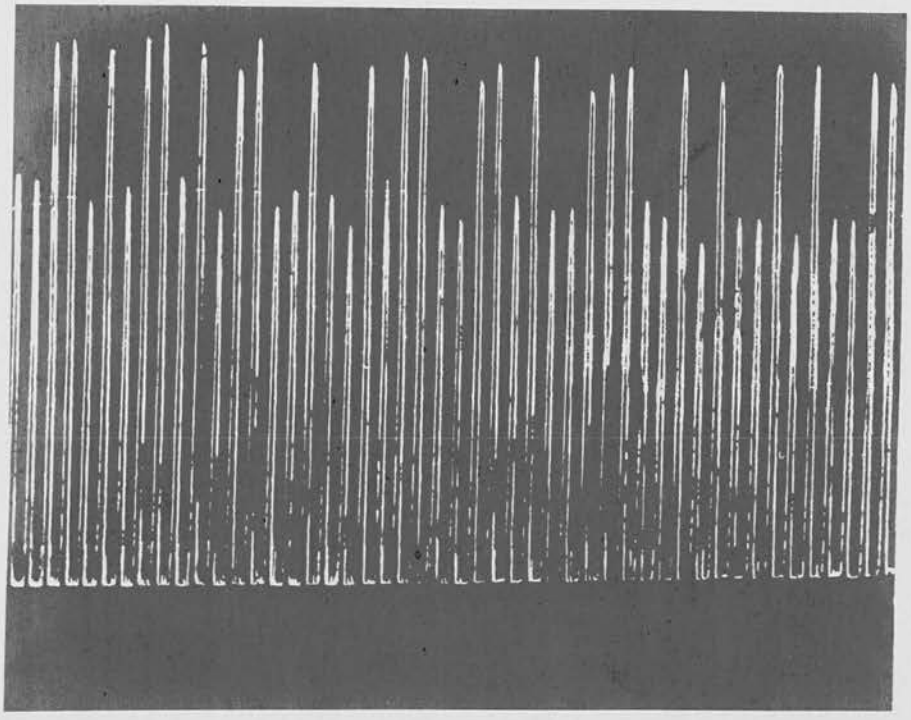
Figure (iv)



The test compound was

Figure (v)

A typical 2 + 2 dose assay



The four different agonist concentrations (two of acetylcholine and two of the test compound) were added in varying order to produce forty-eight contractions (twelve groups of four arranged in three Latin squares) as described on page 22.

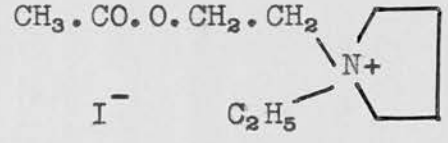
A = 0.5×10^{-5}M test compound

B = 4.4×10^{-8}M acetylcholine

C = 8.8×10^{-8}M acetylcholine

D = 1.0×10^{-5}M test compound

The test compound was



RESULTS

Antagonist activity.

The affinity constants of the antagonists are shown in Tables I to V.

Scott's results with the compounds of the diphenylacetoxyethyl and benziloyloxyethyl series are included in Tables I and II for comparison. I have also included results obtained by N.C. Scott along with my own in these two Tables.

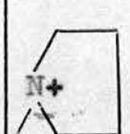
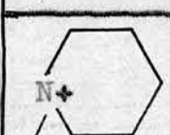
The affinity constants of the diphenylacetyl esters of α and β-hydroxymethyl-pyrrolidine (Table III), β-hydroxypiperidine (Table IV) and β-hydroxymethylpiperidine (Table V) derivatives are very similar to those of the diphenylacetoxyethyl series (Table I), i.e. of the order of 10^7. The affinity constants of the benziloyl esters (Table II) are much higher, being of the order of 10^8, but those of the diphenylacetyl esters of γ-hydroxypiperidine derivatives are higher still, and of the order of 10^9.

The standard errors attached to the estimates of the affinity constants of the antagonists lie between 8 and 10%.

TABLE I

DIPHENYLACETOXYETHYL SERIES

$Ph_2CH.CO.O.(CH_2)_2.\overset{+}{N}R$

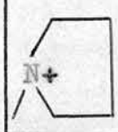
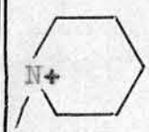
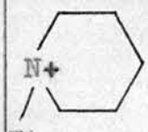
$\overset{+}{N}R$	Values of K_b obtained from each preparation	Mean K_b ($\pm$S.E.)	Mean K_b ($\pm$S.E.) Scott's results	No. of individual results on which mean is based
$\overset{+}{N}Me_3$	1.60×10^7 * 1.31 * 1.30 * 1.59 *	1.45×10^7 ($\pm$0.06)	1.48×10^7 ($\pm$0.02)	12
$\overset{+}{N}Me_2Et$	4.92×10^7 * 3.73 * 4.69 * 3.32 * 3.42 * 3.18 * 3.58 *	3.83×10^7 ($\pm$0.16)	4.40×10^7 ($\pm$0.16)	20
$\overset{+}{N}MeEt_2$	4.08×10^7 * 4.90 * 2.97 * 3.65 *	3.90×10^7 ($\pm$0.26)	3.09×10^7 ($\pm$0.12)	10
$\overset{+}{N}Et_3$	2.05×10^7 * 2.60 * 2.40 * 2.29 *	2.34×10^7 ($\pm$0.07)	$2.73 \times 10_7$ ($\pm$0.10)	12
	2.76×10^7 2.72 2.75 * 2.78 *	2.75×10^7 ($\pm$0.04)	-	11
	3.98×10^7 * 3.44 * 3.48 * 3.50 3.73 3.58	3.62×10^7 ($\pm$0.07)	-	18
	1.40×10^7 * 2.13 * 1.93 * 1.90 *	1.84×10^7 ($\pm$0.12)	-	12
	0.96×10^7 * 0.95 * 1.16 * 1.09 *	1.04×10^7 ($\pm$0.04)	-	12

* Results obtained by N.C. Scott.

TABLE II

BENZILOYLOXYETHYL SERIES

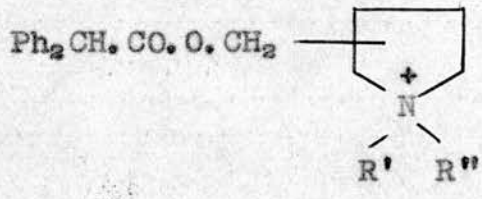
$Ph_2C(OH).CO.O.(CH_2)_2.\overset{+}{N}R$

$\overset{+}{N}R$	Values of K_b obtained from each preparation	Mean K_b ($\pm$S.E.)	Mean K_b ($\pm$S.E.) Scott's results	No. of individual results on which mean is based
$\overset{+}{N}Me_3$	3.38×10^8 * 3.24 3.27 3.09	3.25×10^8 ($\pm$0.05)	3.44×10^8 ($\pm$0.10)	12
$\overset{+}{N}Me_2Et$	8.28×10^8 9.04 8.59 8.19 8.30 8.91 8.92	8.60×10^8 ($\pm$0.11)	8.66×10^8 ($\pm$0.19)	21
$\overset{+}{N}MeEt_2$	9.80×10^8 * 9.01 8.79 9.51 8.68 8.67	9.08×10^8 ($\pm$0.13)	8.98×10^8 ($\pm$0.18)	18
$\overset{+}{N}Et_3$	4.82×10^8 * 4.64 4.82 4.94	4.81×10^8 ($\pm$0.09)	4.74×10^8 ($\pm$0.48)	12
	3.52×10^8 3.23 4.53 3.65 3.91 3.66 4.63	3.88×10^8 ($\pm$0.12)	-	21
	4.54×10^8 * 4.46 * 4.68 4.42 4.37	4.49×10^8 ($\pm$0.08)	-	14
	1.26×10^8 * 1.28 * 1.17 1.20 1.18 1.27	1.23×10^8 ($\pm$0.02)	-	18
	1.10×10^8 * 1.44 * 1.69 1.27 1.28	1.36×10^8 ($\pm$0.06)	-	14

* Results obtained by N.C. Scott

TABLE III

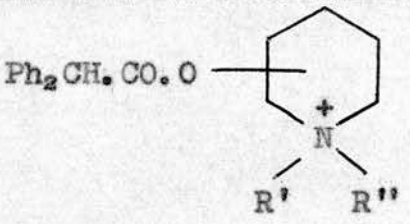
DIPHENYLACETOXYMETHYL-PYRROLIDINIUM SERIES



Point of attachment to ring	R'	R"	Values of K_b obtained from each preparation	Mean K_b (±S.E.)	No. of individual results on which mean is based
α	Me	Me	9.42×10^6 9.64 9.97 9.69 9.66 9.94 1.01×10^7 1.08	9.90×10^6 (±0.08)	23
α	Me	Et	1.43×10^7 1.73 1.47 1.55 1.48 1.58	1.54×10^7 (±0.05)	18
β	Me	Me	3.03×10^7 3.08 3.07 3.12 2.95 2.87	3.02×10^7 (±0.03)	18
β	Me	Et	4.56×10^7 4.45 4.69 4.56 4.73 4.47	4.58×10^7 (±0.06)	18

TABLE IV

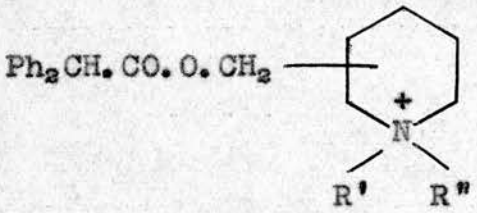
DIPHENYLACETOXY-PIPERIDINIUM SERIES



Point of attachment to ring	R'	R''	Values of K_b obtained for each preparation	Mean K_b ($\pm$S.E.)	No. of individual results on which mean is based
β	Me	Me	1.23×10^7 1.21 1.24 1.18 1.31 1.28	1.24×10^7 ($\pm$0.02)	18
β	Me	Et	1.69×10^7 1.75 1.72 1.57	1.68×10^7 ($\pm$0.04)	12
γ	Me	Me	1.07×10^9 1.13 1.28 1.17	1.16×10^9 ($\pm$0.03)	12
γ	Me	Et	1.15×10^9 1.30 1.17 1.31 1.07 1.32 1.12	1.21×10^9 ($\pm$0.03)	21
γ	Et	Et	8.25×10^8 7.58 8.13 8.38 8.17 7.65 7.76	7.99×10^8 ($\pm$0.08	21

TABLE V

DIPHENYLACETOXYMETHYL-PIPERIDINIUM SERIES



Point of attachment to ring	R'	R"	Values of K_b obtained from each preparation	Mean K_b ($\pm$S.E.)	No. of indivi Results on w mean is base
β	Me	Me	2.46×10^7 2.90 2.58 2.66	2.65×10^7 ($\pm$0.06)	12

From the values in the Tables it is possible to calculate either the effect on affinity of changing the composition of the onium group or of changing the composition of the body of the molecule; this is explained in the introduction (page 7).

The ratios of the affinity constants are shown in Tables VIa to IX. The fiducial limits for these ratios can be calculated by assuming a normal distribution of the values of the logarithm of the affinity constant (Gaddum 1939, 1953). The logarithm of the ratio K_a/K is the difference of the two means, i.e. $\overline{\log.K_a} - \overline{\log.K}$, where K_a is the affinity constant for the compound where the change has been made (e.g. replacement of methyl by ethyl in the onium group) and K, the affinity constant for the standard (e.g. the fully methylated compound).

The scatter of individual values of $\log.K_a$ about the mean, $\overline{\log.K_a}$, will be given by the variance $\frac{\sum d_1^2}{n_1 - 1}$ and similarly the scatter of individual results of log.K about the mean, $\overline{\log.K}$, will be given by the variance $\frac{\sum d_2^2}{n_2 - 1}$, where d_1 and d_2 are the deviations of values of $\log.K_a$ and log.K respectively, from their respective means (i.e. $\overline{\log.K_a}$ and $\overline{\log.K}$), and n_1 and n_2 are the number of experiments used to determine the mean values.

If it is also assumed that the variance arises from the same source in all experiments, it is reasonable to pool all the estimates of the variance. Hence the variance for individual results will be given by

$$S^2 = \frac{\Sigma d_1^2 + \Sigma d_2^2 + \Sigma d_3^2 + \ldots\ldots\ldots}{n_1 - 1 + n_2 - 1 + n_3 - 1 + \ldots\ldots}$$

where the denominator is the number of degrees of freedom for all the experiments.

Hence the variance of M (i.e. $\log. K_a/K$) is given by

$$V[M] = S^2 \left[\frac{1}{n_1} + \frac{1}{n_2}\right]$$

where n_1 is the number of results on which K_a is based and n_2 the number of results on which K is based.

Fiducial limits of M are obtained by applying the 't' distribution for the total number of degrees of freedom (the level of probability chosen was 95%), i.e.

$$M_L, M_U = M \pm t\sqrt{S^2 \left[\frac{1}{n_1} + \frac{1}{n_2}\right]}$$

In fact. the variance for the experiments with the diphenylacetoxyethyl and benzilyloxyethyl series was calculated separately from the other experiments.

In the former 237 experiments were performed with 16 compounds; hence there are 221 degrees of freedom. The sum of the (Σd^2) terms for these sixteen compounds was 0.616013 and thus

the estimate of the variance is

$$S^2 = \frac{0.616013}{221} = \underline{0.002787}$$

In the latter 173 experiments were performed with 10 compounds; hence there are 163 degrees of freedom. The sum of the (Σd^2) terms for these ten compounds was 0.195585 and thus the estimate of the variance is

$$S^2 = \frac{0.195585}{163} = \underline{0.0012}$$

The affinity constants of the diphenylacetoxyethyltrimethyl ammonium bromide (K) and diphenylacetoxyethyldimethylethyl ammonium bromide (K_a) may be obtained from Table I,

i.e. $K_a = 3.83 \times 10^7$ [20 results]

$K = 1.45 \times 10^7$ [12 results]

Thus, $\log. K_a = 7.577$ and $\log. K = 7.157$

and $M = \log.[K_a/K] = 0.420$

$$V[M] = S^2\left[\frac{1}{n_1} + \frac{1}{n_2}\right]$$

$$= 0.002787\ [0.83 + 0.050]$$

$$= [0.01925]^2$$

For 221 degrees of freedom, t (at the 0.05 level) has the limiting value of 1.96. Hence,

$$M_L, M_U = M \pm t\sqrt{S^2\left[\frac{1}{n_1} + \frac{1}{n_2}\right]}$$

$$= 0.420 \pm [1.96 \times 0.01925]$$

$$= 0.420 \pm 0.0377$$

In addition, the tables show the changes in free energy of adsorption of the compounds (with 95% confidence limits). These are obtained

by substituting the values of $\log. K_a/K$ into equation (13), i.e.

$$-f = -a = 2.3RT \log. [K_a/K]$$
$$= 1417 \log. [K_a/K]$$

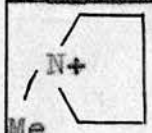
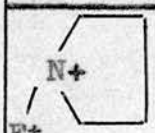
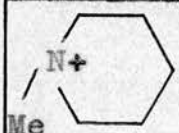
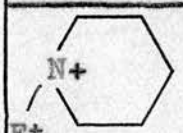
since R = 1.987 cal./deg./mole and T = 37°C = 310°A.

The effect on affinity of a change in composition of the onium group (replacement of methyl by ethyl) is shown in the following Tables.

TABLE VIa

DIPHENYLACETOXYETHYL SERIES

$Ph_2CH.CO.O.(CH_2)_2.\overset{+}{N}R$

$\overset{+}{N}R$	K_a/K	$\log.K_a/K$	$-f$
$\overset{+}{N}Me_2Et$	(2.41) 2.63 (2.87)	(0.382) 0.420 (0.458)	(541) 595 (649)
$\overset{+}{N}MeEt_2$	(2.36) 2.61 (2.88)	(0.372) 0.416 (0.460)	(527) 589 (652)
$\overset{+}{N}Et_3$	(1.47) 1.62 (1.78)	(0.167) 0.209 (0.251)	(237) 296 (356)
	(1.73) 1.91 (2.11)	(0.238) 0.281 (0.324)	(337) 398 (459)
	(2.30) 2.51 (2.74)	(0.362) 0.400 (0.438)	(513) 567 (621)
	(1.14) 1.25 (1.38)	(0.062) 0.104 (0.146)	(88) 147 (207)
	(0.65) 0.72 (0.79)	($\bar{1}$.816) $\bar{1}$.858 ($\bar{1}$.900)	(-261) -201 (-142)

Note : The variance used in calculating the results in this Table and in Tables VIb, VIIa and VIIb is 0.002787.

TABLE VIb

Effects on affinity of replacing N-methylpyrrolidinium (K) by N-ethylpyrrolidinium (K_x) and N-methylpiperidinium by N-ethylpiperidinium in the diphenylacetoxyethyl series.

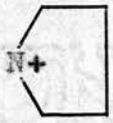
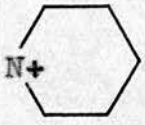
Ring	K_x/K	$\log. K_x/K$	-f
	(1.20) 1.32 (1.44)	(0.080) 0.119 (0.158)	(113) 169 (224)
	(0.51) 0.58 (0.62)	($\bar{1}$.719) $\bar{1}$.761 ($\bar{1}$.803)	(-398) -339 (-279)

TABLE VIIa

BENZILOYLOXYETHYL SERIES

$Ph_2C(OH)CO.O.(CH_2)_2.\overset{+}{N}R$

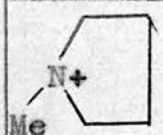
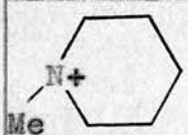
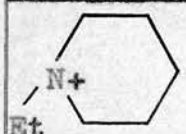
$\overset{+}{N}R$	K_a/K	$\log. K_a/K$	-f
$\overset{+}{N}Me_2Et$	(2.44) 2.65 (2.89)	(0.387) 0.424 (0.461)	(548) 601 (653)
$\overset{+}{N}MeEt_2$	(2.56) 2.80 (3.06)	(0.409) 0.447 (0.485)	(580) 633 (687)
$\overset{+}{N}Et_3$	(1.35) 1.48 (1.63)	(0.129) 0.171 (0.213)	(183) 242 (302)
	(1.09) 1.19 (1.29)	(0.037) 0.074 (0.111)	(52) 105 (157)
	(1.26) 1.39 (1.52)	(0.101) 0.142 (0.183)	(143) 201 (259)
	(0.35) 0.38 (0.42)	($\bar{1}$.540) $\bar{1}$.578 ($\bar{1}$.616)	(-652) -598 (-544)
	(0.38) 0.42 (0.46)	($\bar{1}$.581) $\bar{1}$.621 ($\bar{1}$.661)	(-594) -537 (-480)

TABLE VIIb

Effects on affinity of replacing N-methylpyrrolidinium (K) by N-ethylpyrrolidinium (K_x) and N-methylpiperidinium by N-ethylpiperidinium in the benziloyloxyethyl series.

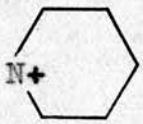
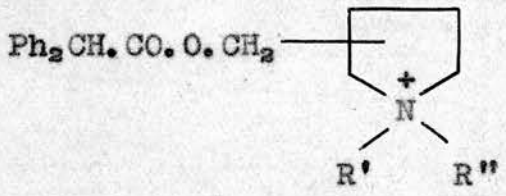
Ring	K_x/K	$\log. K_x/K$	-f
	(1.08) 1.17 (1.27)	(0.032) 0.068 (0.104)	(45) 96 (147)
	(1.02) 1.10 (1.19)	(0.007) 0.043 (0.079)	(10) 61 (112)

TABLE VIII

DIPHENYLACETOXYMETHYL-PYRROLIDINIUM SERIES

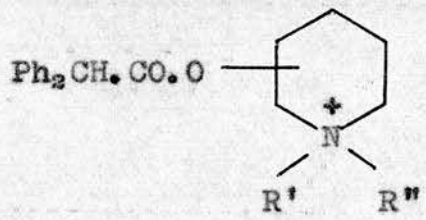


Point of attachment to ring	K_a/K	log. K_a/K	-f
α	(1.47) 1.54 (1.62)	(0.167) 0.188 (0.209)	(237) 266 (296)
β	(1.44) 1.52 (1.60)	(0.158) 0.181 (0.204)	(224) 256 (289)

Note : In this, and subsequent Tables, the results are calculated using a variance = 0.0012.

TABLE IX

DIPHENYLACETOXY-PIPERIDINIUM SERIES



Point of attachment to ring	K_a/K	$\log. K_a/K$	-f
β	(1.28) 1.35 (1.43)	(0.106) 0.131 (0.156)	(150) 186 (221)
γ	(0.98) 1.04 (1.09)	(1.991) 0.015 (0.039)	(-13) 21 (55)
γ*	(0.65) 0.69 (0.72)	($\bar{1}$.814) $\bar{1}$.838 ($\bar{1}$.862)	(-263) -229 (-195)

* This was the only instance where both methyl groups in the onium group were replaced; in all other instances the change in the onium group is replacement of one of the methyl groups by an ethyl group.

Agonist activity.

The equipotent molar ratios, relative to acetylcholine, are shown in Tables X to XII.

With the exception of acetoxyethyl-N-methyl pyrrolidinium iodide, the compounds are all less than one tenth as potent as acetylcholine.

The standard errors attached to the estimates of the equipotent molar ratios of the agonists vary from 1 to 3%.

TABLE X

ACETOXYETHYL SERIES

$CH_3.CO.O.(CH_2)_2.\overset{+}{N}R$

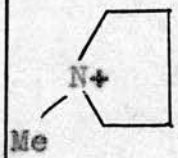
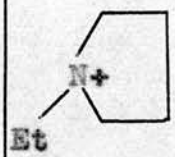
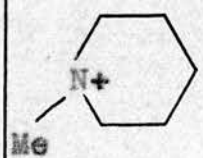
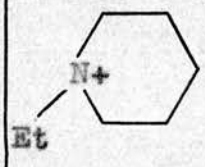
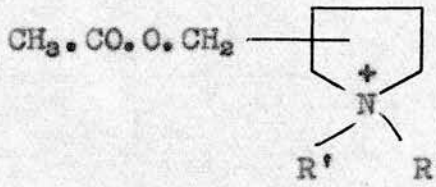
$\overset{+}{N}R$	Equipotent molar ratio (n) Ach. = 1	Mean value of (n) ($\pm$S.E.)	No. of experiments on which mean is based
	8.3 9.4 8.5 8.3 8.5 8.2	8.5 ($\pm$0.2)	6
	224 210 212 215 224 214 224 217 210	217 ($\pm$2)	9
	3,305 3,392 3,241 3,565 3,661 3,648 3,253 3,173 3,611 3,293	3,414 ($\pm$59)	10
	15,253 14,926 15,408	15,196 ($\pm$142)	3

TABLE XI

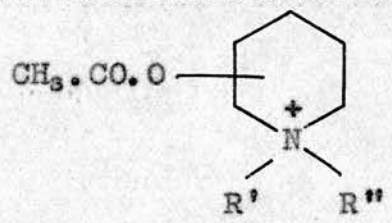
ACETOXYMETHYL-PYRROLIDINIUM SERIES



Point of attachment to ring	R'	R"	Equipotent molar ratio (n) Ach. = 1	Mean value of (n) (±S.E.)	No. of expts. on which mean is based
α	Me	Me	2,954 2,982 2,898 2,882 2,915	2,926 (±18)	5
α	Me	Et	10,888 10,786 11,424	11,033 (±198)	3
β	Me	Me	871	-	1
β	Me	Et	1,251	-	1

TABLE XII

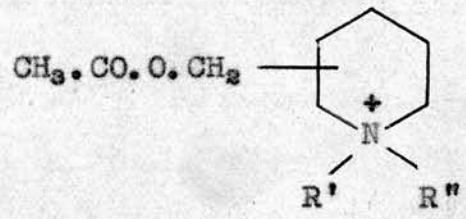
ACETOXY-PIPERIDINIUM SERIES



Point of attachment to ring	R'	R''	Equipotent molar ratio (n) Ach. = 1	Mean value of (n) (±S.E.)	No. of values on which mean is based
β	Me	Me	150 158 151 159 158 158	156 (±2)	6
β	Me	Et	8,760 8,955	8,857 (±97)	2
γ	Me	Me	74.7 84.7 74.1 74.3	77.0 (±2.5)	4
γ	Me	Et	4,719	-	1

TABLE XIII

ACETOXYMETHYL-PIPERIDINIUM SERIES



Point of attachment to ring	R'	R"	Equipotent molar ratio (n) Ach. = 1	Mean value of (n) (±S.E.)	No. of values on which mean is based
β	Me	Me	1,726 1,684 1,698	1,703 (±12)	3

From the equipotent molar ratios of the agonists and the affinity ratios of the antagonists (Tables VIa to IX) it is possible to calculate the effect on efficacy of changing the composition of the onium group or of changing the composition of the body of the molecule; this is explained in the introduction (equation (16), page 12).

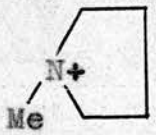
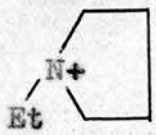
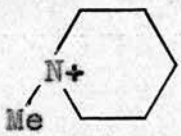
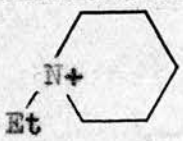
i.e. $e/e_a = n[K_a/K]$

The effect on efficacy of a change in composition of the onium group (replacement of methyl by ethyl) is shown in Tables XIVa to XVI.

TABLE XIVa

ACETOXYETHYL SERIES

$CH_3.CO.O.(CH_2)_2.\overset{+}{N}R$

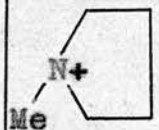
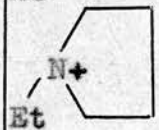
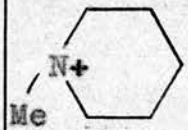
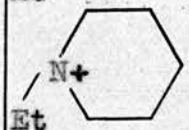
Agonist + NR	Equipotent molar ratio relative to Acetylcholine	K_a/K		e/e_a	
		a	b	a	b
	8.5	1.91	1.19	16.2	10.1
	217	2.51	1.39	545	302
	3,414	1.25	0.38	4,268	1,297
	15,196	0.72	0.42	10,941	6,382

Results in column a are derived from experiments with diphenylacetoxyethyl compounds and those in column b from the benziloyloxyethyl compounds.

TABLE XIVb

ACETOXYETHYL SERIES

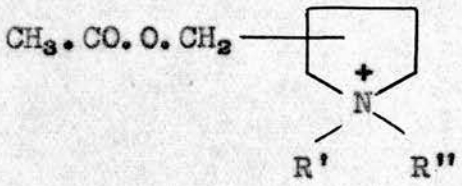
$CH_3.CO.O.(CH_2)_2.\overset{+}{N}R$

Agonist + NR	Equipotent molar ratio relative to methyl analogue	K_a/K		e/e_a	
		a	b	a	b
	1.0	1.0	1.0	1.0	1.0
	2.6	1.32	1.17	3.4	3.0
	1.0	1.0	1.0	1.0	1.0
	4.5	0.58	1.10	2.6	5.0

Results in columns a and b for K_a/K values are obtained from Tables VIb and VIIb.

TABLE XV

ACETOXYMETHYL-PYRROLIDINIUM SERIES

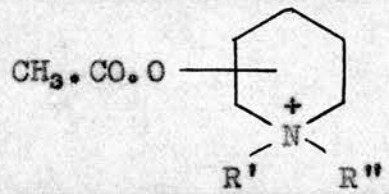


Point of attachment to ring	R'	R''	Equipotent molar ratio relative to		K_a/K	e/e_a
			Ach.	dimethyl analogue		
α	Me	Me	2,926	1.0	1.0	1.0
α	Me	Et	11,033	3.8	1.54	5.8
β	Me	Me	871	1.0	1.0	1.0
β	Me	Et	1,251	1.4	1.52	2.1

The values for the ratio K_a/K are obtained from Table VIII.

TABLE XVI

ACETOXY-PIPERIDINIUM SERIES



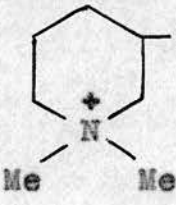
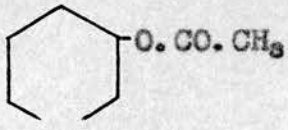
Point of attachment to ring	R'	R"	Equipotent molar ratio relative to		K_a/K	e/e_a
			Ach.	dimethyl analogue		
β	Me	Me	156	1.0	1.0	1.0
β	Me	Et	8,857	56.8	1.35	76.7
γ	Me	Me	77.0	1.0	1.0	1.0
γ	Me	Et	4,719	61.3	1.04	63.8

The values for the ratio K_a/K are obtained from Table IX.

DISCUSSION

*

As indicated on page 9 many of the compounds can be regarded as being made up of an onium nitrogen atom, with a number of simple alkyl substituents (methyl or ethyl groups), and a much more complicated fragment (shown as R or R' on page 9) such as a diphenylacetoxyethyl or benziloyloxyethyl group. This latter R or R' is referred to as the 'body' of the molecule.

The cyclic compounds can be divided into two groups. In the first, two of the simple alkyl groups (methyl or ethyl) on the onium atom can be regarded as having been transformed into a ring [e.g. ] and the more complicated part of the molecule, R, is again referred to as the 'body'. In the second type of cyclic structure the ring replaces only one of the simple alkyl substituents in the onium atom and the term 'body' is used to describe all the rest of the molecule apart from the two remaining alkyl groups and the onium nitrogen atom. The 'body' of the molecule in these molecules is thus attached to the onium atom at two points : for instance, in β-acetoxy-N-N-dimethyl piperidinium,  , the 'body' is 

This discussion will be divided into two sections.

In the first section I intend to discuss the effects that changes in the composition of the onium group have on affinity in the diphenylacetoxyethyl and benziloyloxyethyl series and then to consider the effects, on affinity, when the diphenylacetyl part of the molecule is replaced by benziloyl.

The activity of the corresponding agonists (only those with cyclic structures in the onium group) will then be discussed, and from the information obtained here, and from the results obtained above about changes in affinity in the antagonist series, deductions will be made about the efficacy of the agonists.

In the second section I intend to consider the effects on affinity, of changes in the "body" of the molecule* (i.e. changes in the size of the ring and the position of the substituents) and of changes in the onium group in the series of compounds (shown on page 14) in which the $-CH_2-CH_2-$ part of acetylcholine is incorporated in a cyclic structure or in the side chain of the ring.

From the conclusions obtained here and from results obtained about the activity of the corresponding agonists, deductions will be

made about the effects these changes have on efficacy.

It had been hoped to prepare the fully ethylated compounds in these series but unfortunately only one of them (N-diethyl-γ-[diphenylacetoxy]piperidinium iodide) could be induced to crystallise.

SECTION 1

Effects on affinity of changes in the composition of the onium group in the diphenylacetoxyethyl and benziloyloxyethyl series.

At a level of probability of 1 in 100 there is no significant difference between Scott's (1963) results and mine (which include N.C. Scott's results) for the diphenylacetoxyethyl and benziloyloxyethyl series (Tables I and II), but at a level of probability of 1 in 20 the results for the dimethylethyl and diethylmethyl compounds in the diphenylacetoxyethyl series are significantly different. Apart from these two minor differences it can be concluded that Scott's results are reproduceable.

In both these series the affinity constant of the dimethylethyl compounds was about 2.5 times that of the fully methylated compound. Further replacement of a methyl group by an ethyl group in the onium group caused a slight increase in the affinity constant, but the value of the affinity constant of the fully ethylated compound was only slightly greater than that of the fully methylated compound. Scott found that this sequence was reproduceable in other series that he tested. It was therefore thought that further changes in the composition of the onium group would produce similar changes in the affinity constants of

compounds in the diphenylacetoxyethyl and benziloyloxyethyl series. The changes that were made were the introduction of $\overset{+}{N}$-methyl pyrrolidinium, $\overset{+}{N}$-ethyl pyrrolidinium, $\overset{+}{N}$-methyl piperidinium and $\overset{+}{N}$-ethyl piperidinium groups respectively.

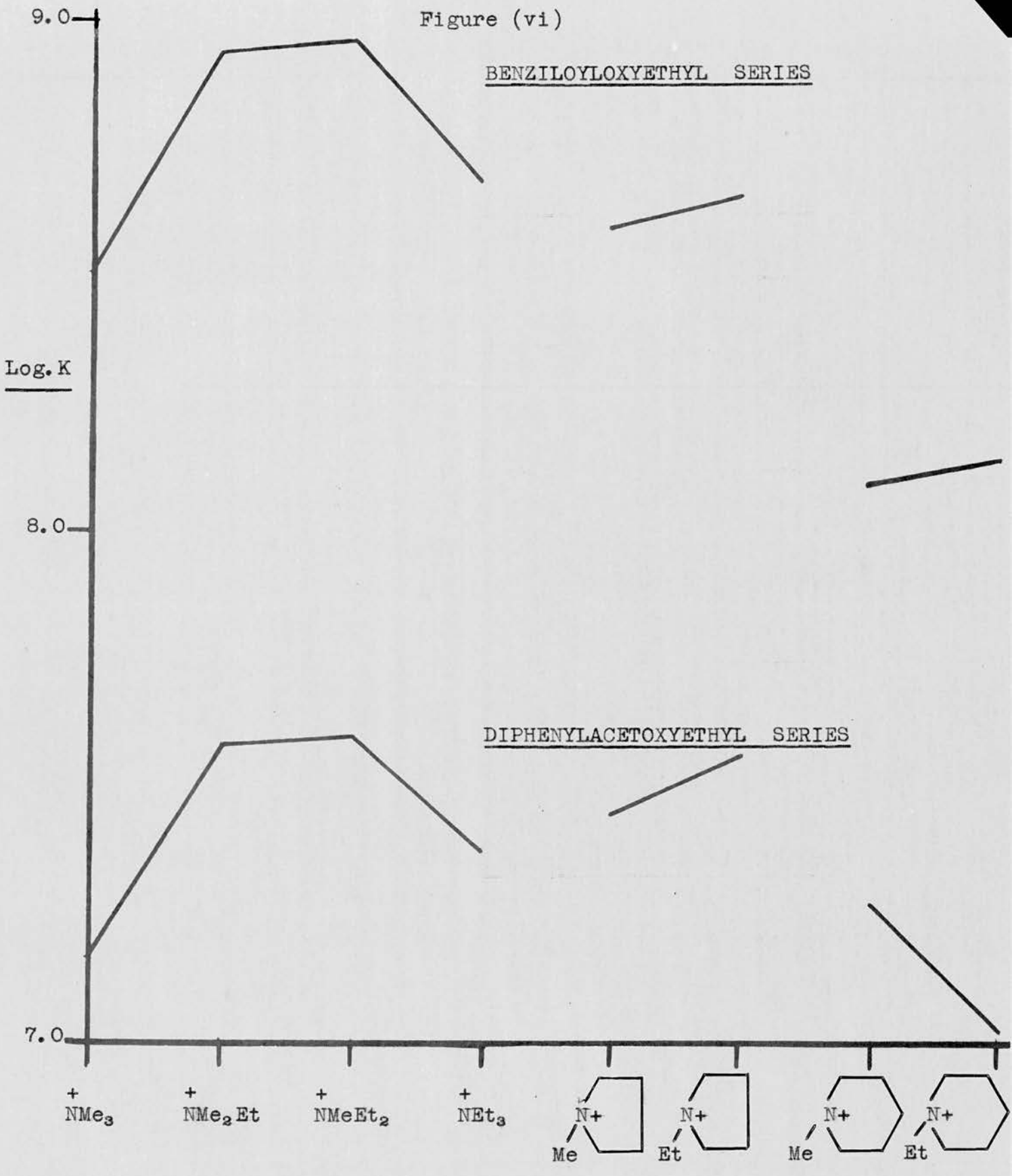
The pyrrolidinium compounds have quite a considerable affinity which is increased by replacement of methyl by ethyl. On the other hand the piperidinium compounds have a lower affinity, which in the diphenylacetoxyethyl series is decreased by replacement of methyl by ethyl, whereas in the benziloyloxyethyl series there is a slight increase (not significant at a level of probability = 0.05).

The results for both these series are shown pictorially in Figure (vi) where the logarithms of the affinity constants of the members of the two series are plotted against the composition of the onium group.

If the effect of change in composition in the onium group is really independent of the nature of the "body" of the molecule, then these lines for the two series should be parallel.

This is quite strikingly true for the compounds studied by Scott (and which have been repeated) but for the cyclic compounds there are discrepancies which are far too large to be

Figure (vi)



Composition of the Onium group

ascribed to experimental error. These discrepancies imply that by introducing the ring into the onium group there is some interference with the binding of the molecule to the receptor.

This could be brought about by :

a) Reduced flexibility of the molecule

b) Intramolecular forces

c) Decreased binding of onium group because it is bigger.

a) Reduced flexibility of the molecule.

It might be that the ring reduces the flexibility of the molecule hence making it more difficult for the groups within the molecule to combine with the receptor groups. As might be expected this effect was greater with the bulky six-membered ring than with the five-membered ring. It might be possible to detect this effect by studying the infra-red spectra of these compounds, since adsorption depends on the energy required to produce rotation and vibration of groups within the molecule, and, in this case the adsorption will be reduced if the groups are less flexible.

b) Intramolecular forces.

There may also be intramolecular forces between certain groups in certain molecules which would reduce the binding of the molecule to the receptor (e.g. there might be an interaction

between the cationic head and the hydroxyl group in the benziloyloxyethyl series).

c) Decreased binding of onium group because it is bigger.

When the cationic head is very large (as in the cyclic compounds) the electrostatic force binding it to a negative centre on the receptor will be much weaker if it obeys the inverse square law.

Apart from these discrepancies the changes of free energy of adsorption brought about by replacing the fully methylated onium group by the dimethylethyl or diethylmethyl onium group in both series are of the order of 0.6Kcalories; this suggests that the increased affinity involves van der Waal's binding of a methylene group, in the onium part of the molecule, to the receptor.

Effects on affinity of replacing the diphenylacetyl part of the molecule by benziloyl.

The benziloyl esters all have a greater affinity for the receptors than the diphenylacetyl esters, this is in agreement with Acred et al. (1957). This difference in affinity between the benziloyl esters and the diphenylacetyl esters corresponds to a difference in free energy of adsorption of about 2 Kcalories for the compounds with simple alkyl groups substituted in the onium group and about 1 Kcalorie to 1.6 Kcalories for the compounds in which there is a cyclic structure in the onium group. This suggests that there is hydrogen bonding between the hydroxyl group in the benziloyl part of the molecule and the receptor with the compounds with simple alkyl groups substituted in the onium group. When the onium group contains the bulky cyclic structure the binding is much weaker for reasons already discussed on page 54.

Effects of introducing cyclic structures into the onium group on the activity of members of the acetoxyethyl series.

It is possible that the agonist activity of the compounds tested could arise from actions at the parasympathetic ganglia or at receptors other than those affected by acetylcholine (such as histamine or 5-hydroxytryptamine receptors). An action at ganglia, however, is unlikely because hexamethonium was present throughout the experiments. The responses of the compounds were not antagonised by mepyramine and so are unlikely to be caused by actions at the histamine receptors, but they were antagonised by atropine which is consistent with an action at acetylcholine receptors (though it does not entirely rule out an action at 5-hydroxytryptamine receptors.)

As the size of the cyclic onium group increases, the equipotent molar ratio (relative to acetylcholine) increases ; that is, the activity of the agonist decreases. The N-methyl pyrrolidinium compound has some activity which is reduced by replacement of methyl by ethyl, while the piperidinium compounds are only feebly active.

Effects on efficacy of changes in composition of the cyclic onium group.

As I have already pointed out (page 53) the effects on affinity of replacing $\overset{+}{N}Me_3$ by $\overset{+}{N}Me_2Et$, $\overset{+}{N}MeEt_2$ or $\overset{+}{N}Et_3$ were similar in both the diphenylacetoxyethyl and benziloyloxyethyl series, but the effects on affinity of replacing $\overset{+}{N}Me_3$ by N-methyl pyrrolidinium, N-ethyl pyrrolidinium, N-methyl piperidinium or N-ethyl piperidinium are significantly different in the different series of antagonists, and so it is not really known what effect these latter changes have on the affinity of the agonists.

If the changes in the affinity constants for these antagonists can be applied to the agonist series the results (Table XIVa) suggest that the replacement of $\overset{+}{N}Me_3$ by N-methyl pyrrolidinium reduces the efficacy to somewhere about 1/10th to 1/16th that of acetylcholine and the replacement of $\overset{+}{N}Me_3$ by N-ethyl pyrrolidinium reduces the efficacy to somewhere about 1/300th to 1/550th. Although it might be questioned whether the ratio of the affinity constants is the same in the agonists as it is in the antagonists, unless it is wildly different, it is clear that the presence of a five-membered ring in the onium group lowers efficacy considerably and that the presence of a

six-membered ring has a drastic effect on efficacy, because, although the antagonists have a considerable affinity, the agonists have virtually no activity.

SECTION 2

Effects on affinity of changes in the size of the ring and position of the substituents in the cyclic series.

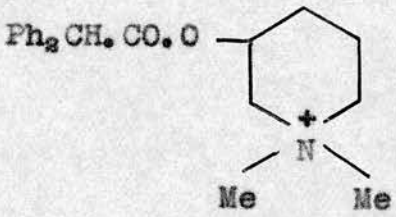
The quaternary salts of the diphenylacetyl esters in the α and β-hydroxypyrrolidine, β-hydroxypiperidine and β-hydroxymethylpiperidine series all have affinities of the same order as that of diphenylacetylcholine, though the affinity of the β-substituted compounds is slightly higher than that of the α-substituted compounds. On the other hand the quaternary salts of the diphenylacetyl esters of γ-hydroxypiperidine have a quite outstanding affinity (about 200 times that of diphenylacetylcholine) and comparable with that of atropine. Perhaps this might have been anticipated when one considers that they are structurally similar to atropine. (In fact, if the benziloyl esters of the quaternary salts of γ-hydroxypiperidine were prepared, then it would be expected that they would have an extremely high affinity).

If one of the series is chosen as a standard and all the other series compared with it, then it is found that the effect on affinity of a change in composition of the "body" of the molecule is similar within each series but there is a

significant difference between each series. This is shown in the following Table.

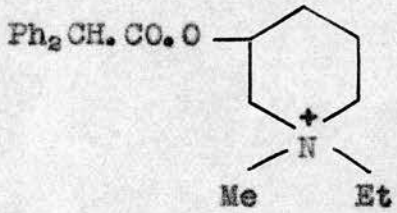
TABLE XVII

Standards chosen were

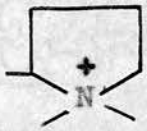
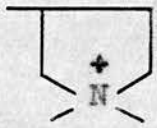
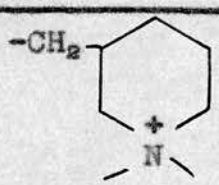
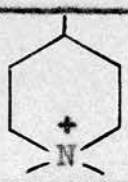


K = 1.24 x 10^7

and



K = 1.68 x 10^7

"Body"	K_z	K_z/K	-f
	9.90 x 10^6 1.54 x 10^7	0.80 0.92	-137 -51
	3.02 x 10^7 4.58 x 10^7	2.43 2.73	547 618
	2.65 x 10^7 -	2.13 -	464 -
	1.16 x 10^9 1.21 x 10^9	93 72	2,788 2,631

In the column headed K_z the "upper" number in each series is the affinity constant for the $\overset{+}{N}Me_2$ compound while the "lower" number is the affinity constant of the $\overset{+}{N}MeEt$ compound

The β-substituted hydroxypiperidine series were chosen as the standard because the affinity constants of the members of this series were very similar to that of diphenylacetylcholine. It might be unjustifiable to draw any conclusions from the changes in free energy of adsorption associated with the change in the "body" of the molecule (TableXVII) because the series do differ from the standard series not only in the constitution of the "body" of the molecule but also (to some extent) in the orientation of the substituents of the onium group relative to the rest of the molecule: consequently, the changes in free energy might be due either to the different orientation of the onium group, or to the different constitution of the "body" of the molecule. If it is assumed that the contribution to the change in free energy from the different orientation of the onium group is small compared with the change in free energy involved in the change of constitution of the body of the molecule then it appears that in the γ-hydroxypiperidine derivatives there might be hydrogen bonding (about 3 Kcalories) between the carbonyl group in the molecule and the receptor. This might be due to the onium group and the ester group being in a better position relative to each other than the same groups are in the other series for binding with the receptor.

Effects on affinity of changes in the composition of the onium group in the cyclic series.

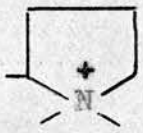
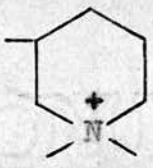
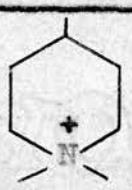
Replacement of methyl by ethyl in the onium group causes an increase in affinity. The change in free energy brought about by replacing $\overset{+}{N}Me_2$ by $\overset{+}{N}MeEt$ is about 0.2 Kcalories to 0.3 Kcalories in the quaternary salts of the diphenylacetyl esters of α and β-hydroxymethyl-pyrrolidine and β-hydroxypiperidine which suggests that the methylene group in the onium group is being bound by a similar force in these series. On the other hand the change in free energy brought about by replacement of $\overset{+}{N}Me_2$ by $\overset{+}{N}MeEt$ in the quaternary salts of the diphenylacetyl esters of the γ-hydroxypiperidine series is very small and suggests that the binding after substitution in these molecules differs from what it is in the other series possibly because of the orientation of the groups within in the molecule (page63).

The affinity constant of N-diethyl-γ-(diphenylacetoxy)piperidinium iodide has also been determined and it was found that although the affinity constant had increased when $\overset{+}{N}Me_2$ was replaced by $\overset{+}{N}MeEt$, it decreased when $\overset{+}{N}Me_2$ was replaced by $\overset{+}{N}Et_2$.

From this work it appears that the effects on affinity of a chemical change in the

structure of the onium group are different in the cyclic series from what they are in the series studied by Scott (1963). Moreover, the effects on affinity are different in some of the cyclic series from what they are in others. This is shown in the following Table, where the chemical change in the onium group is replacement of methyl by ethyl.

TABLE XVIII

Cyclic series	Effect of the change on affinity	-f
	1.54	266
	1.52	256
	1.35	186
	1.04	21

In Scott's series a chemical change in the onium group produced roughly the same change in affinity in five series of antagonists. The results obtained in this work (TableXVIII) can be explained by supposing that the orientation of the substituents in the onium group relative to the ester group is likely to be quite different in a rigid ring structure (more so when it is a piperidinium ring than when it is a pyrrolidinium ring) from what it is in a flexible straight-chain compound (Scott's series).

Effects of changes in the size of the ring and position of substituents on the activity of agonists in the cyclic series.

The equipotent molar ratios of the agonists in the five and six-membered ring series are all relatively high compared with acetylcholine, i.e. the compounds are only feebly active. The γ-substituted compounds appear to be the most active, the β-substituted compounds less active, while the α-substituted compounds appear to have virtually no activity.

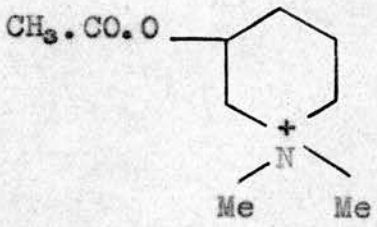
Effects on efficacy of changes in the size of the ring and position of substituents in the cyclic series.

From the equipotent molar ratios of the N-dimethyl and N-methyl-N-ethyl derivatives of the cyclic compounds relative to N-dimethyl-β-(acetoxy)piperidinium iodide and N-methyl-N-ethyl-β-(acetoxy)piperidinium iodide respectively (i.e. the corresponding agonists of the antagonists chosen as standards for Table XVII), and from the ratio of the affinity constants obtained in Table XVII, it is possible to assess the effects on efficacy of changes in the size of the ring and

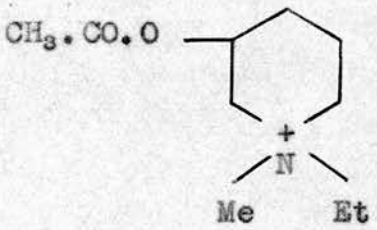
and position of substituents in the cyclic series. This is shown in the following Table.

TABLE XIX

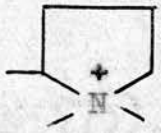
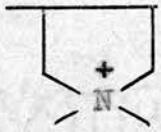
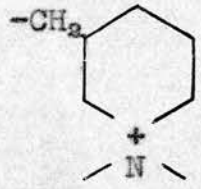
Standards chosen were



and



n = 156 n = 8,857

"Body"	Equipotent molar ratio (n) relative to standard	e/e_z
	18.7 1.2	14.9 1.1
	5.6 0.1	13.6 0.3
	10.9 -	23.2 -
	0.5 0.5	46.5 36.0

From these results it appears that a six-membered ring is associated with a lower degree of efficacy than a five-membered ring. It is particularly interesting to find that a high affinity, e.g. as in the γ-hydroxypiperidine series, is not associated with a high degree of efficacy.

Effects of changes in composition of the onium group on the activity of the agonists in the cyclic series.

The replacement of methyl by ethyl in the onium group decreases the activity of the agonists considerably.

Effects on efficacy of changes in composition of the onium group in the cyclic series.

Although there may be no justification for transferring the ratio of the affinity constants from the antagonist series to the corresponding agonist series, it appears that, unless there is some very considerable difference, replacement of methyl by ethyl in the onium group markedly reduces efficacy.

In the α-substituted pyrrolidinium series the change in the onium group causes a six-fold decline in efficacy, whereas in the β-substituted pyrrolidinium series the decline is two-fold; in the piperidinium series the decline is about seventy-fold.

Conclusion

Although I have made a number of agonists which appear likely to have an affinity comparable with that of acetylcholine, none of these appear likely to have an efficacy anywhere near that of acetylcholine.

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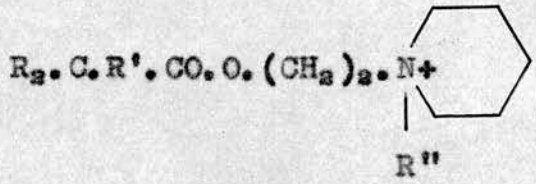
CHEMISTRY

Introduction: The following series of compounds were synthesised.

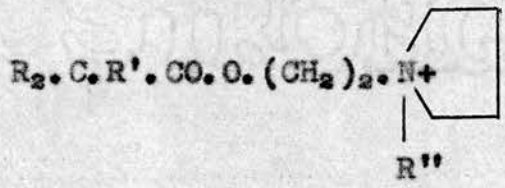
SERIES [1]



I^-

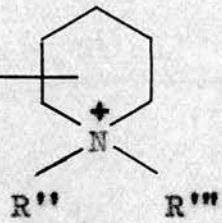
R	R'	R''
H	H	Me
H	H	Et
Ph	H	Me
Ph	H	Et
Ph	OH	Me
Ph	OH	Et

SERIES [2]

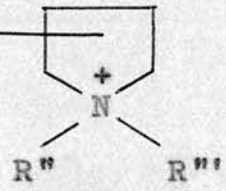


X^-

R	R'	R''	X^-
H	H	Me	I
H	H	Et	I
Ph	H	Me	I
Ph	H	Et	I
Ph	OH	Me	Br
Ph	OH	Et	Br

SERIES [3] $R_2.C.R'.CO.O.(CH_2)_n$  I^-

R	R'	R''	R'''	Point of attachment to ring	n
H	H	Me	Me	β	0
H	H	Me	Et	β	0
Ph	H	Me	Me	β	0
Ph	H	Me	Et	β	0
H	H	Me	Me	γ	0
H	H	Me	Et	γ	0
Ph	H	Me	Me	γ	0
Ph	H	Me	Et	γ	0
Ph	H	Et	Et	γ	0
H	H	Me	Me	β	1
Ph	H	Me	Me	β	1

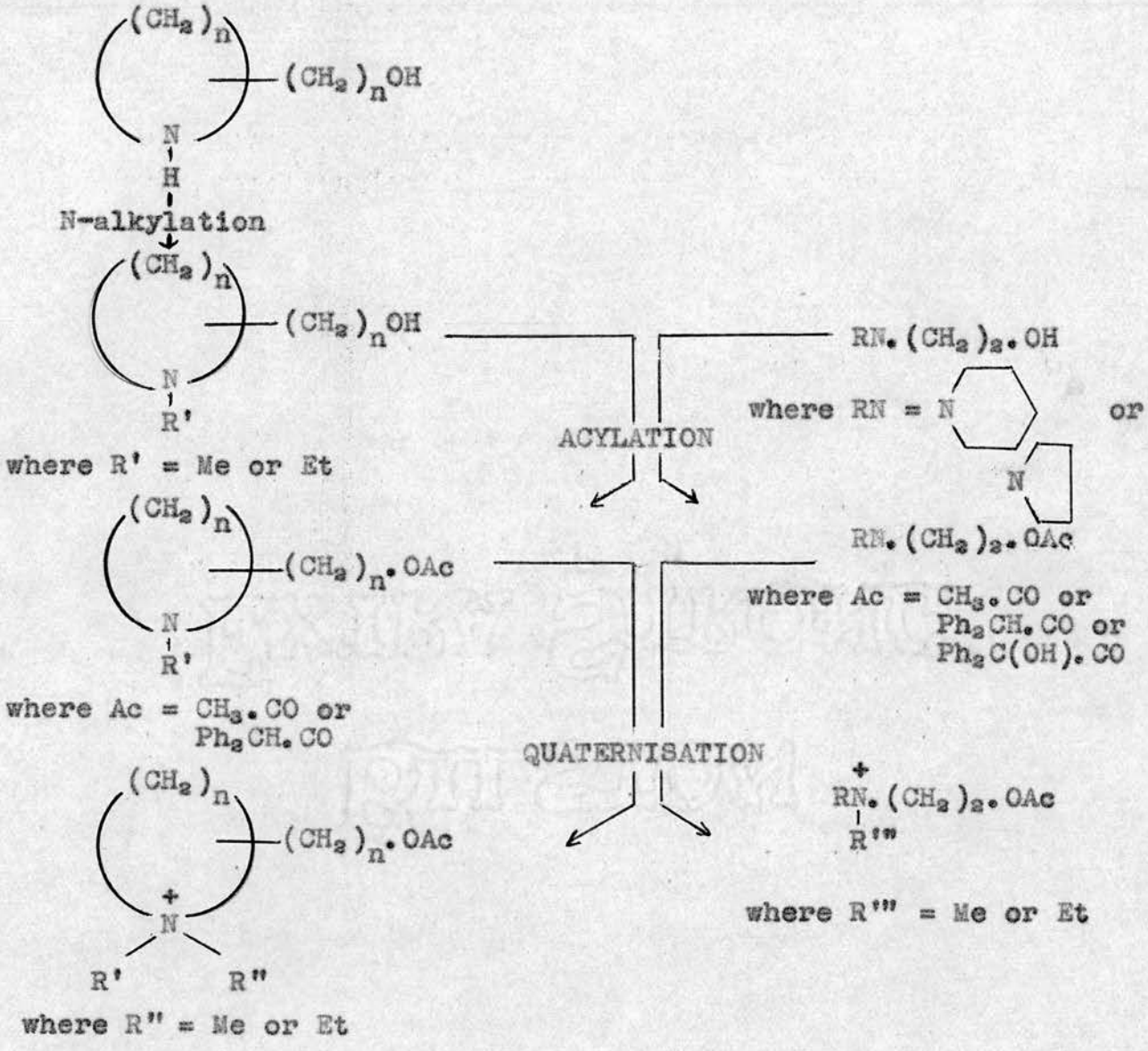
SERIES [4] $R_2.C.R'.CO.O.(CH_2)_n$  X^-

R	R'	R''	R'''	X^-	Point of attachment to ring	n
H	H	Me	Me	I	α	1
H	H	Me	Et	I	α	1
Ph	H	Me	Me	I	α	1
Ph	H	Me	Et	I	α	1
H	H	Me	Me	I	β	1
H	H	Me	Et	I	β	1
Ph	H	Me	Me	Br	β	1
Ph	H	Me	Et	Br	β	1

With one exception, series [3] and [4] are unfortunately incomplete because the diethyl compounds could not be obtained crystalline.

Originally it was planned to make more compounds in series [3] in which n was 1 or 2, but it was not found possible to make sufficient amounts of the α and γ- hydroxymethylpiperidines to do this.

The methods for making the agonists (R = R' = H) and the antagonists (R = Ph, R = H or OH) were very similar and are outlined in the following scheme.


(CH2)n
(CH2)nOH
N
H
N-alkylation
(CH2)n
(CH2)nOH
N
R'
where R' = Me or Et
ACYLATION
RN.(CH2)2.OH
where RN = N or N
RN.(CH2)2.OAc
where Ac = CH3.CO or Ph2CH.CO or Ph2C(OH).CO
(CH2)n
(CH2)n.OAc
N
R'
where Ac = CH3.CO or Ph2CH.CO
QUATERNISATION
(CH2)n
(CH2)n.OAc
+
N
R'
R''
where R'' = Me or Et
+
RN.(CH2)2.OAc
R'''
where R''' = Me or Et

The work is divided into three sections:

I. The synthesis of the appropriate aminoalcohols.

II. Acylation of the aminoalcohols.

III. Quaternisation of the acyl esters.

I. Aminoalcohols.

a) N-piperidinoethanol.

b) N-pyrrolidinoethanol.

c) N-methyl-β-hydroxypiperidine.

d) N-ethyl-β-hydroxypiperidine.

e) N-methyl-γ-hydroxypiperidine.

f) N-ethyl-γ-hydroxypiperidine.

g) N-methyl-β-hydroxymethylpiperidine.

h) N-ethyl-β-hydroxymethylpiperidine.

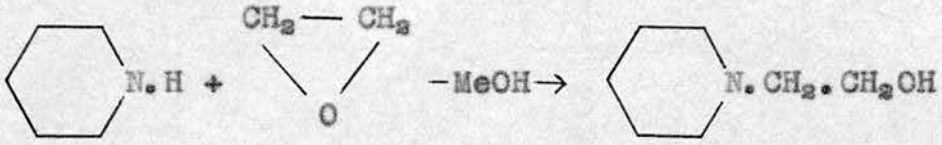
i) N-methyl-α-hydroxymethylpyrrolidine.

j) N-ethyl-α-hydroxymethylpyrrolidine.

k) N-methyl-β-hydroxymethylpyrrolidine.

l) N-ethyl-β-hydroxymethylpyrrolidine.

a) N-piperidinoethanol



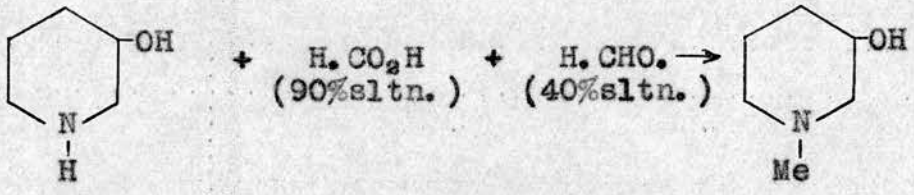
To piperidine (255 g., 3 mole) dissolved in methanol (500 ml.) and cooled in an ice-bath, ethylene oxide (132 g., 3 mole) was added at such a rate that the temperature did not rise above 40°. The mixture was then refluxed for eight hours and the methanol distilled off. The residual oil was distilled under reduced pressure.

B.p. 87°/15 mm., n_D^{20} 1.4802, yield 70% (270 g.)

b) N-pyrrolidinoethanol was prepared in exactly the same way using pyrrolidine (213 g., 3 mole) in place of piperidine.

B.p. 78-81°/15 mm., n_D^{20} 1.4710, yield 84% (294 g.)

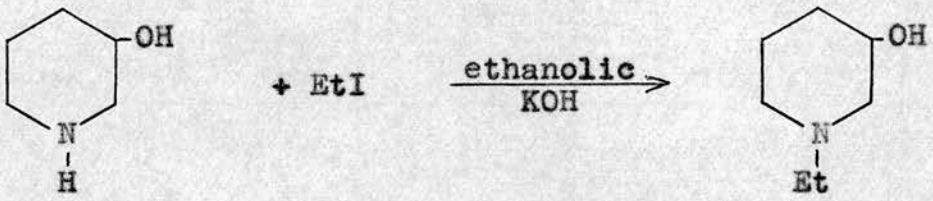
c) N-methyl-β-hydroxypiperidine.



β-Hydroxypiperidine (10.1 g., 0.1 mole) was dissolved in a 90% solution of formic acid (13.0 ml.) and, after the initial exothermic reaction had subsided, 40% formaldehyde solution (10.0 ml.) was added [Clarke et al. 1933]. The mixture was refluxed for three hours and then cooled; concentrated hydrochloric acid (10 ml.) was added and the excess formic acid - formaldehyde solution was removed by distillation under slightly reduced pressure. A small quantity of water was added to the residue and this was then made alkaline with 25% sodium hydroxide, extracted with ether, dried, and distilled.

B.p. 83°/17 mm., n_D^{20} 1.4746, yield 87% (10 g.) [Biel et al. (1952), B.p. 81°/15 mm., Paul and Tchelitcheff (1945), B.p. 79°/15 mm., n_D^{16} 1.4695]

d) N-ethyl-β-hydroxypiperidine.



β-Hydroxypiperidine (3.3 g., 0.3 mole) dissolved in ethanol (10 ml.) was refluxed with potassium hydroxide (2.3 g. Analar) and ethyl iodide (5.2 g., 0.3 mole) for eighteen hours. The potassium iodide formed was filtered off and the filtrate acidified with concentrated hydrochloric acid, and concentrated to dryness in vacuo [Biel et al. 1952].

The residue was taken up in the minimum of water, extracted with ether, dried and distilled.

B.p. 91-2°/12 mm., n_D^{14}1.4771, yield 70% (3 g.) [Biel et al. 1952), B.p. 93-5°/15 mm., Paul and Tchelitcheff (1945), B.p. 93°/15 mm., n_D^{14}1.4769]

e) N-methyl-γ-hydroxypiperidine.

B.p. 90-2°/15 mm., n_D^{20}1.4795, yield 53% (2 g.)

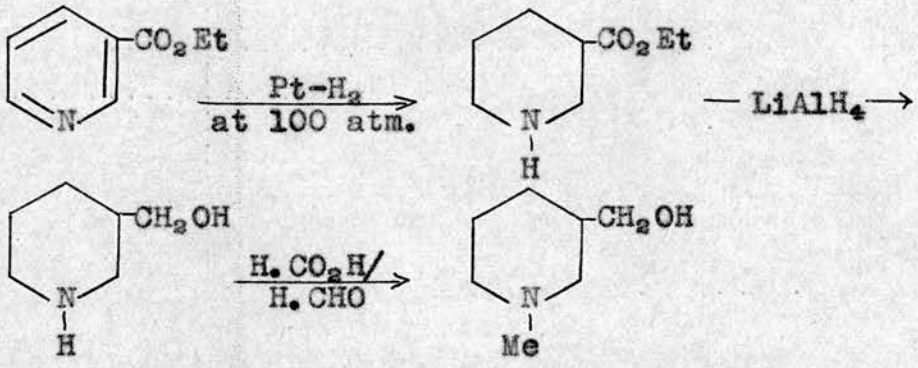
and

f) N-ethyl-γ-hydroxypiperidine.

B.p. 106°/14 mm., n_D^{14}1.4813, yield 58% (2.5 g.),

were prepared by the same methods as the corresponding N-alkyl-β-hydroxypiperidines.

g) N-methyl-β-hydroxymethylpiperidine.



Beecham's Research Laboratories kindly prepared some ethyl nipecotate (B.p. 100-2°/11 mm., n_D^{19} 1.4609) by reducing ethyl nicotinate catalytically.

Lithium aluminium hydride (7.6 g., 0.2 mole) suspended in anhydrous ether (250 ml.) was refluxed for five minutes. The heating was switched off and ethyl nipecotate (31.4 g., 0.2 mole) in anhydrous ether (350 ml.) added at such a rate that refluxing continued spontaneously. The mixture was then refluxed for six hours.

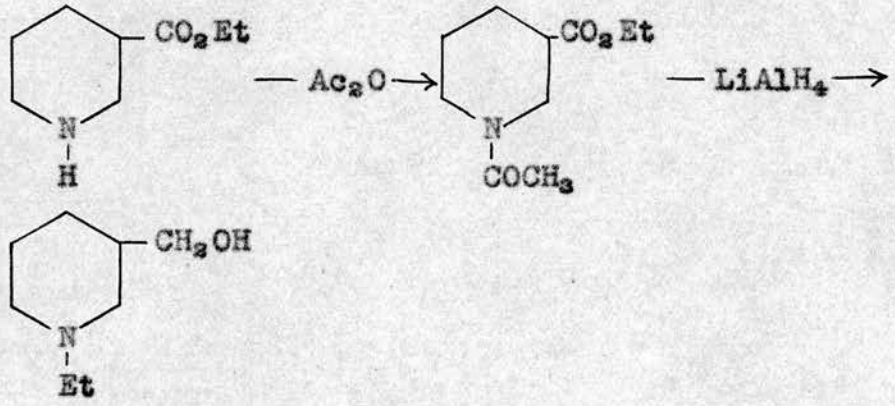
Water (7 ml.), 10% sodium hydroxide (20 ml.) then more water (5 ml.) was added carefully in this order to the well cooled mixture [Mićović and Mihailovic, 1953]. The residue was removed and the filtrate dried over anhydrous potassium carbonate. The ether was removed by distillation and the residue distilled under reduced pressure.

B.p. 97-100°/0.7 mm., n_D^{20} 1.4960, yield 65% (15 g.) [Doyle et al. (1962), B.p. 93-6°/0.6 mm.]

N-methyl-β-hydroxymethylpiperidine was obtained from the β-hydroxymethylpiperidine by the method described on page 84.

B.p. 112°/14 mm., n_D^{20} 1.4775, yield 53% (9 g.) [Doyle et al. (1962), B.p. 106°/9 mm., n_D^{20} 1.4775]

h) N-ethyl-β-hydroxymethylpiperidine.



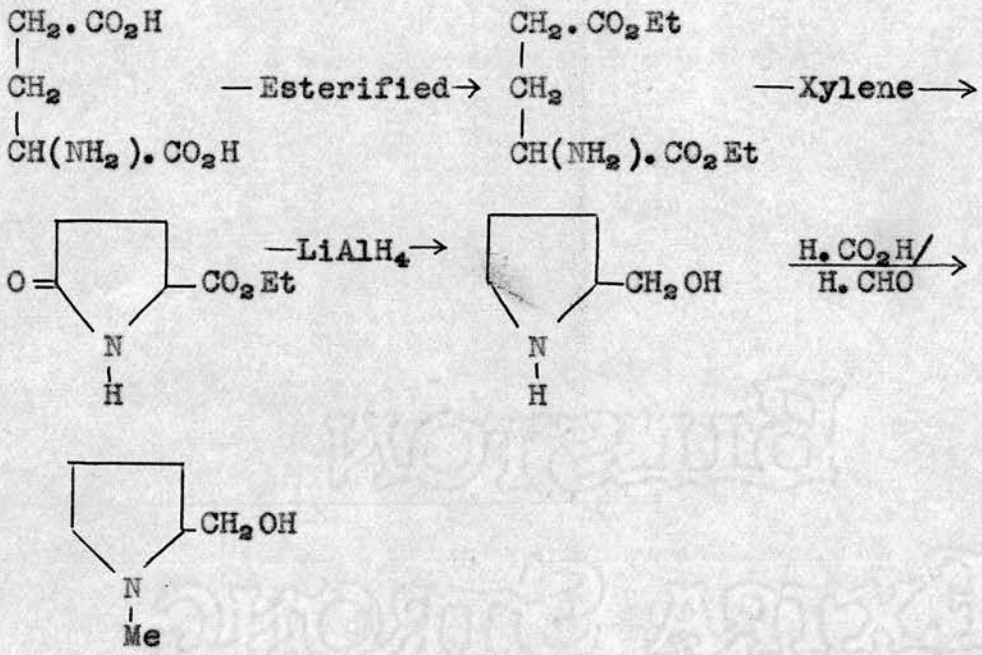
Ethyl nipecotate (15.7 g., 0.1 mole) and acetic anhydride (15.3 g., 0.15 mole) were refluxed for three hours. The excess reagent was removed and the residue distilled under reduced pressure.

B.p. 126°/0.2 mm., n_D^{20} 1.4800, yield 85% (17 g.) [Doyle et al. (1962), B.p. 110°/0.05 mm., n_D^{20} 1.4808]

The product, N-acetyl ethyl nipecotate (19.9 g., 0.1 mole), in anhydrous ether (250 ml.) was reduced with lithium aluminium hydride (11.4 g., 0.3 mole) by the method described on page 87, whereby N-ethyl-β-hydroxymethylpiperidine was obtained.

B.p. 117°/13 mm., n_D^{20} 1.4806, yield 72% (10 g.) [Doyle et al. (1962), B.p. 120-5°/11 mm., $n_D^{20.5}$ 1.4808]

i) N-methyl-α-hydroxymethylpyrrolidine.



Dry hydrogen chloride was bubbled through a slurry of L-glutamic acid (147 g., 1 mole) in ethanol (500 ml.), until all the L-glutamic acid had dissolved. The solution was then heated under reflux for an hour. (This is a modification of the method described by Angier and Smith, 1956).

The ethanol was distilled under reduced pressure and water (50 ml.) added before it had time to solidify, then cooled and ether (50 ml.) added. Ammonia (S.G.=0.88) was added, with vigorous stirring, until the pH was 9.6; during the addition, the temperature was not allowed to rise above 15°.

The solution was extracted successively

with portions of ether (5 x 100 ml.), and the ether extracts combined, dried, and the ether removed under reduced pressure. The residue, diethyl glutamate, was refluxed with xylene (500 ml.) for twelve hours, then the xylene was removed under reduced pressure and the residue was distilled.

B.p. 126-30°/0.5 mm. (m.p. 53°) yield 69% (108 g.) [Fischer and Boehner (1911), m.p. 54°]

The produce, 2-carboxyethyl-pyrrolid-5-one, was reduced to α-hydroxymethylpyrrolidine with lithium aluminium hydride according to the method described by Karrer and Portman (1948).

B.p. 96-9°/14 mm., n_D^{20} 1.4880, yield 52% (36 g.) [Blicke and Lu (1955), B.p. 96-8°/14 mm.]

N-methylation of α-hydroxymethyl-pyrrolidine with formic acid - formaldehyde was performed by the method on page 84, yielding N-methyl-α-hydroxymethylpyrrolidine.

B.p. 71°/14 mm., n_D^{20} 1.4690, yield 34% (14 g.) [Blicke and Lu (1955), B.p. 67-9°/12 mm.]

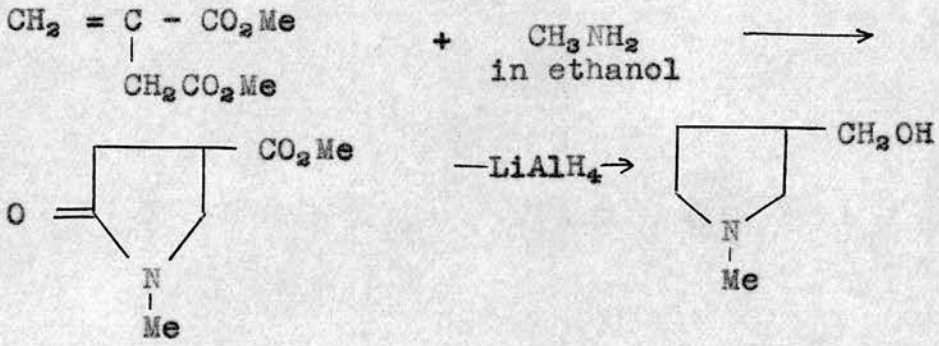
j) N-ethyl-α-hydroxymethylpyrrolidine.

Ethylation of α-hydroxymethyl-pyrrolidine, using ethyl iodide in ethanolic

potassium hydroxide, was performed as previously described on page 85.

B.p. 74°/12 mm., n_D^{18} 1.4726, yield 5 g. (48%)

k) N-methyl-β-hydroxymethylpyrrolidine.



To a solution of methylamine in ethanol (100 ml., 33% sltn.), cooled in an ice-bath, dimethyl itaconate (158 g., 1 mole) was added at such a rate that the temperature remained between 5 - 10°. With constant stirring the mixture was then left for twelve hours at room temperature. The solvent was removed by distillation and the residual oil distilled under reduced pressure.

B.p. 160-1°/18 mm., n_D^{25} 1.4740, yield 81% (127 g.) [Wu and Feldkamp (1961), B.p. 160-1°/18 mm., n_D^{25} 1.4742]

This product, N-methyl-3-carboxymethyl-pyrrolid-5-one, was then reduced with lithium aluminium hydride to N-methyl-β-hydroxymethyl-pyrrolidine according to the method of Wu and Feldkamp (1961).

B.p. 99-100°/17 mm., n_D^{25} 1.4661, yield 47% (44 g.) [Wu and Feldkamp (1961), B.p. 94-6°/15 mm., n_D^{25} 1.4662]

1) N-ethyl-β-hydroxymethylpyrrolidine.

This compound was prepared by exactly the same method as described on page 93, except ethylamine was used this time instead of methylamine.

B.p. 104-6°/18 mm., n_D^{25} 1.4691, yield 49% (58 g.) [Wu and Feldkamp (1961), B.p. 110-11°/20 mm., n_D^{25} 1.4693]

II. Acylation of the aminoalcohols.

The acyl derivatives prepared were:

(i) Acetyl (when R = H and R' = H)

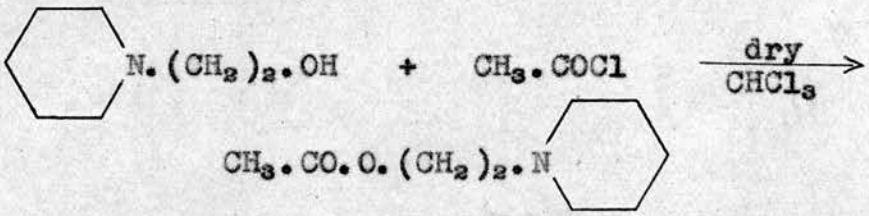
(ii) Diphenylacetyl (when R = Ph and R' = H)

(iii) Benziloyl (when R = Ph and R' = OH ; only for series [1] and [2]).

(i) Preparation of acetyl derivatives.

Acetyl derivatives were prepared by the action of acetyl chloride on the aminoalcohol dissolved in dry chloroform.

e.g. The preparation of N-(acetoxyethyl)-piperidine.



Acetyl chloride (78.5 g., 1 mole) was added dropwise, with continuous stirring, to an ice-cooled mixture of N-piperidinoethanol (64.5 g., 0.5 mole) dissolved in dry chloroform. The product was poured into cold anhydrous ether (1 litre). The melting point of the hydrochloride, after recrystallisation from ethyl methyl ketone, was 189°.

This was dissolved in the minimum quantity of water, made alkaline with sodium carbonate solution, extracted with ether, dried with anhydrous sodium sulphate and distilled. N-(acetoxyethyl)piperidine boiled at 95°/13 mm., yield 49% (42g.).

Yields and physical constants are shown in Table

TABLE ~~XX~~

Acetate of	Yield	m.p. of ester hydrochloride	Solvent	b.p. of ester	Ref.
N-piperidinoethanol	49	189°	ethyl methyl ketone	95°/13 mm.	1
N-pyrrolidinoethanol	60	124		91-3/16	2
N-methyl-β-hydroxy-piperidine	71	140		72/11	3
N-ethyl-β-hydroxy-piperidine	67	176			4
N-methyl-γ-hydroxy-piperidine	*				
N-ethyl-γ-hydroxy-piperidine	*				
N-methyl-β-hydroxy-methylpiperidine	*				
N-ethyl-β-hydroxy-ethylpiperidine				62-3/0.1	
N-methyl-α-hydroxy-methylpyrrolidine	59	72	ethyl methyl ketone	81/13	5
N-ethyl-α-hydroxy-methylpyrrolidine	*				
N-methyl-β-hydroxy-methylpyrrolidine	*				
N-ethyl-β-hydroxy-methylpyrrolidine	*				

1. Matkovics et al. (1961) give m.p. of hydrochloride 189°, ester b.p. 72°/2 mm.

2. Matkovics et al. (1961) give m.p. of hydrochloride 128°, ester b.p. 76°/5 mm.

3. Doyle et al. (1961) give ester b.p. 72°/11 mm.

4. Mikhlina et al. (1960) give m.p. of hydrochloride 179-81°.

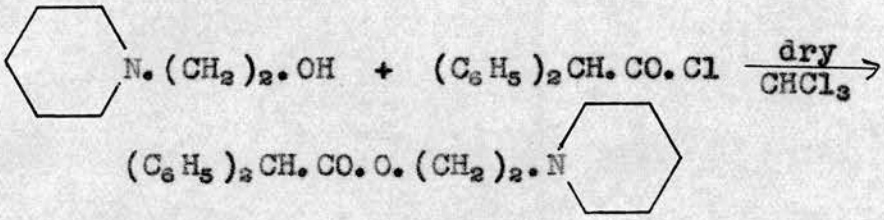
5. Renshaw and Cass (1939) give m.p. of hydrochloride 73-4°.

* Indicates that the ester hydrochlorides and the esters were unstable. In these circumstances the crude ester was used for quaternisation.

(ii) Preparation of diphenylacetyl derivatives.

The diphenylacetyl esters were prepared by the action of diphenylacetyl chloride on the aminoalcohol hydrochloride dissolved in dry chloroform.

e.g. The preparation of N-(diphenylacetoxyethyl)-piperidine



To an ice-cooled mixture of N-piperidinoethanol (12.9 g., 0.1 mole) in ether (500 ml.), an etherial solution of anhydrous hydrogen chloride was added dropwise, with continuous stirring, until the N-piperidinoethanol hydrochloride had been completely precipitated. This extremely hygroscopic hydrochloride was immediately transferred to a small wide-mouthed flask, containing dry chloroform (15 g.). Diphenylacetyl chloride (23.05 g., 0.1 mole) was added and the mixture heated under reflux for six hours.

A straw-coloured liquid was obtained. This was cooled, and poured into anhydrous ether (500 ml.). The hydrochloride of the diphenylacetyl derivative which precipitated was

filtered off and recrystallised from ethyl methyl ketone. It had a melting point of 131°.

The hydrochloride was added to a solution of sodium hydroxide (50%), extracted with ether, and the extract dried with sodium sulphate. The ether was distilled off but any attempt to distil the ester caused it to decompose. The undistilled material was found to be pure enough for conversion to the quaternary salt. In other preparations it was sometimes possible to distil the ester.

Yields and physical constants are shown in Table

TABLE XXI

Diphenylacetate of	Yield %	m.p. of ester hydrochloride	Solvent	b.p. of ester	Ref.
N-piperidinoethanol	76	131°	ethyl methyl ketone/ ethyl acetate		
N-pyrrolidinoethanol	82	127			
N-methyl-β-hydroxy-piperidine	63	192-3		155-60°/0.1mm.	1,2
N-ethyl-β-hydroxy-piperidine	46	190-1		194-6/0.15	3
N-methyl-γ-hydroxy-piperidine	48	117-19			4
N-ethyl-γ-hydroxy-piperidine				178-80/0.15	
N-methyl-β-hydroxy-methypiperidine	*				
N-ethyl-β-hydroxy-methylpiperidine	*				
N-methyl-α-hydroxy-methylpyrrolidine	68	133	ethyl methyl ketone/ ethyl acetate	164-5/0.3	5
N-ethyl-α-hydroxy-methylpyrrolidine	*				
N-methyl-β-hydroxy-methylpyrrolidine	*				
N-ethyl-β-hydroxy-methylpyrrolidine	*				

1. Biel (1962) give m.p. of hydrochloride 193-4°; ester b.p. 160-3°/0.06 mm.

2. Doyle et al. (1961) give ester b.p. 155-62°/0.1 mm.

3. Biel et al. (1952) give m.p. of hydrochloride 195-6°; ester b.p. 191-2°/0.18 mm.

4. McElvain and Rorig (1948) give m.p. of hydrochloride 115-20°.

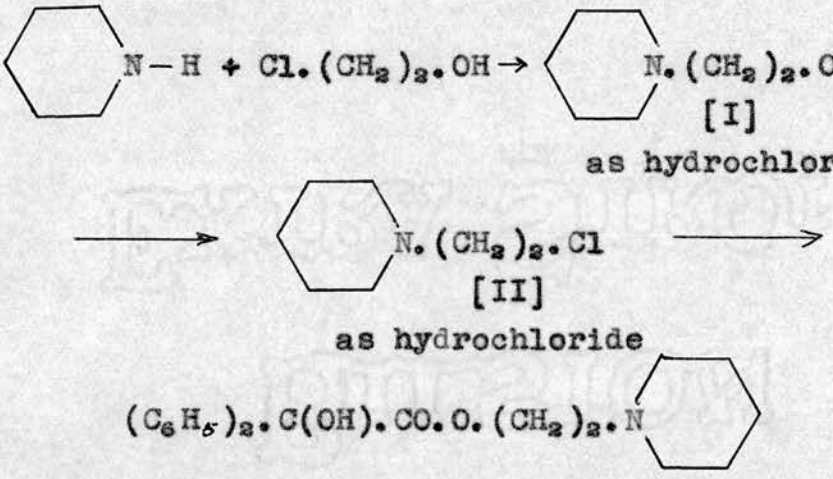
5. Blicke and Lu (1955) give m.p. of hydrochloride 134-5°.

* See page

(iii) Preparation of the benziloyl derivatives.

The benziloyl esters were prepared from the aminoethyl chloride by the method described by Horenstein and Pahlicke (1938) as modified by Burtner and Cusic (1943).

e.g. Preparation of N-(benziloyloxyethyl)-piperidine.



Piperidine (85 g., 1 mole) dissolved in anhydrous toluene (80 g.) was refluxed with ethylene chlorohydrin (80.5 g., 1 mole) for three hours. This was left to cool and N-piperidinoethanol hydrochloride (I) crystallised out and was filtered off.

This solid was then dissolved in dry chloroform (50 g.), treated dropwise with thionyl chloride (119 g., 1 mole), and refluxed for two hours. The mixture was left to cool and N-(β-chloroethyl)piperidine hydrochloride (II) crystallised out. This was filtered off and was

washed with small amounts of dry chloroform. The melting point of (II) was 228°. [Stach and Winter (1962) give 229-30°].

This was dissolved in the minimum quantity of water, made alkaline with sodium hydroxide solution (40%), extracted with ether, dried and distilled. The N-(β-chloroethyl)-piperidine boiled at 70°/14 mm. [Stach and Winter (1962) record 75°/16 mm.].

This product was dissolved in iso-propyl alcohol (200 g.), benzilic acid (22.83 g., 0.1 mole) was added, and the mixture was heated under reflux for four hours.

Anhydrous ether was added to the cooled solution until it became turbid, the mixture was then left and the hydrochloride was found to crystallise out slowly.

The product was filtered off, but, as it was extremely hygroscopic, it was not purified but converted immediately into the base by adding alkali and extracting with ether. The extract was dried with anhydrous sodium sulphate and distilled. The benziloyl ester of N-piperidinoethanol boiled at 196-8°/15 mm., yield 28% (94 g.). [Matkovics et al. (1961) give 141°/2 mm.].

The benziloyl ester of N-pyrrolidinoethanol, as its hydrochloride, was

obtained by the same method in 46% yield. The melting point was 93-4° (recrystallised from ethyl methyl ketone).

III. Quaternisation of the aminoesters.

The method employed was the same for all the compounds.

The aminoester (0.01 mole), dissolved in ethyl methyl ketone, was heated under reflux with methyl or ethyl halide (0.015 mole) for two hours. The quaternary ammonium halide crystallised slowly from the cold solution (sometimes it was necessary to add anhydrous ether to encourage crystallisation) and was filtered off then recrystallised.

The melting points, yields and analytical results are recorded in the following Tables.

SERIES [1]

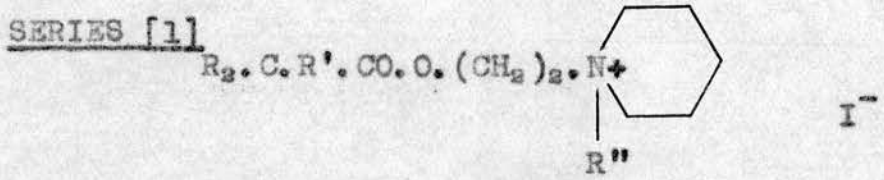


TABLE XXII

R	R'	R''	Yield %	m.p. °C	Solvent
H	H	Me	64	109	ethyl methyl ketone/ethyl acetate
H	H	Et	58	85	
Ph	H	Me	61	153	iso-propyl alcohol + ether
Ph	H	Et	75	141	
Ph	OH	Me	53	110	
Ph	OH	Et	69	135-7	

TABLE XXIII

R	R'	R''	M.W.	Percentage Found			Percentage Required		
				C	H	I^-	C	H	I^-
H	H	Me	313.2	38.62	6.14	41.45	38.35	6.44	40.52
H	H	Et	327.2	40.51	6.50	38.69	40.37	6.78	38.78
Ph	H	Me	465.4	56.72	5.78	27.27	56.77	6.06	27.26
Ph	H	Et	479.4	57.81	6.31	26.64	57.61	6.31	26.47
Ph	OH	Me	481.4	55.48	6.10	26.22	54.88	5.85	26.36
Ph	OH	Et	494.4	56.02	6.02	25.91	55.86	6.11	25.66

SERIES [2]

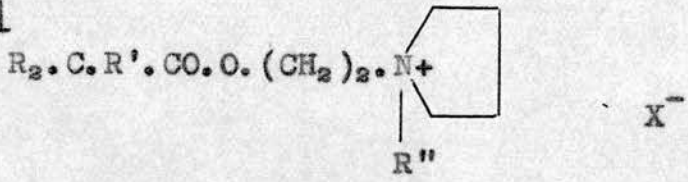


TABLE XXIV

R	R'	R''	X^-	Yield %	m.p. °C	Solvent
H	H	Me	I	61	63-4	ethyl methyl ketone/ethyl acetate
H	H	Et	I	70	58	
Ph	H	Me	I	68	181-2	iso-propyl alcohol + ether
Ph	H	Et	I	59	150	
Ph	OH	Me	Br	55	211	
Ph	OH	Et	Br	74	200-2	

TABLE XXV

R	R'	R''	X^-	M.W.	Percentage Found			Percentage Required		
					C	H	Halide	C	H	Halide
H	H	Me	I	299.2	36.04	6.19	42.73	36.13	6.06	42.41
H	H	Et	I	313.2	38.24	6.36	40.29	38.35	6.44	40.52
Ph	H	Me	I	450.3	55.54	5.97	28.04	56.01	5.82	28.18
Ph	H	Et	I	465.4	57.00	5.90	27.42	56.77	6.06	27.27
Ph	OH	Me	Br	420.3	60.10	5.91	19.18	60.00	6.23	19.01
Ph	OH	Et	Br	434.4	60.70	6.51	18.57	60.82	6.49	18.39

SERIES [3]

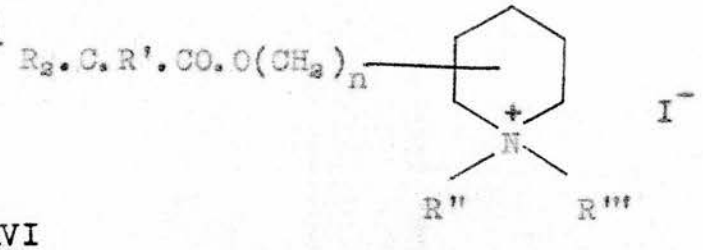


TABLE XXVI

R	R'	R''	R'''	n	Point of attachment to ring	Yield %	m.p. °C	Solvent
H	H	Me	Me	0	β	73	155	ethyl methyl ketone/ethyl acetate
H	H	Me	Et	0	β	52	117-8	
Ph	H	Me	Me	0	β	64	186	iso-propyl alcohol
Ph	H	Me	Et	0	β	58	68-70[2] d	
H	H	Me	Me	0	γ	83	169	ethyl methyl ketone/ethyl acetate
H	H	Me	Et	0	γ	81	242	
Ph	H	Me	Me	0	γ	72	214	iso-propyl alcohol + ether
Ph	H	Me	Et	0	γ	48	185	
Ph	H	Et	Et	0	γ	23	162	
H	H	Me	Me	1	β	85	130-1[2]	ethyl methyl ketone/ethyl alcohol
Ph	H	Me	Me	1	β	76	216	

1. Biel (1962) gives 70° decomposes.
2. Doyle et al. (1962) give 130-1°C.

TABLE XXVII

R	R'	R''	R'''	n	Point of attachment to ring	M.W.	Percentage Found			Percentage Required		
							C	H	I^-	C	H	I^-
H	H	Me	Me	0	β	299.2	36.03	6.06	42.79	36.13	6.06	42.41
H	H	Me	Et	0	β	313.2	38.52	6.07	40.68	38.35	6.44	40.52
Ph	H	Me	Me	0	β	450.3	55.88	5.77	28.41	56.01	5.82	28.18
Ph	H	Me	Et	0	β	465.4	56.70	6.33	27.03	56.77	6.06	27.26
H	H	Me	Me	0	γ	299.2	36.32	6.05	42.57	36.13	6.06	42.41
Ph	H	Me	Me	0	γ	450.3	55.91	5.75	28.54	56.01	5.82	28.18
Ph	H	Me	Et	0	γ	465.4	57.03	6.02	27.73	56.77	6.06	27.26
Ph	H	Et	Et	0	γ	479.4	57.53	6.38	26.22	57.61	6.31	26.47
H	H	Me	Me	1	β	313.2	38.04	6.32	40.19	38.35	6.44	40.52
Ph	H	Me	Me	1	β	465.4	56.62	6.09	27.56	56.77	6.06	27.26

SERIES [4]

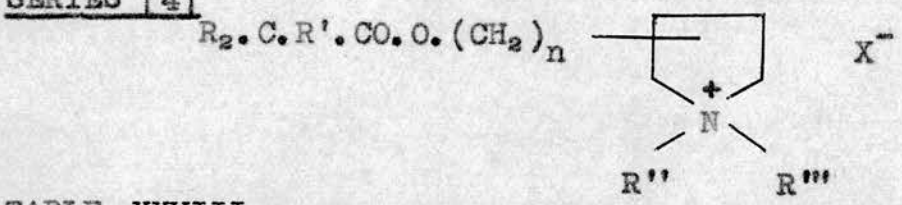


TABLE XXVIII

R	R'	R''	R'''	n	Point of attachment to ring	X^-	Yield %	m.p. °C	Solvent
H	H	Me	Me	1	α	I	74	125[1]	ethyl methyl ketone/ethyl acetate
H	H	Me	Et	1	α	I	58	165	
Ph	H	Me	Me	1	α	I	92	179	iso-propyl alcohol
Ph	H	Me	Et	1	α	I	56	154	
H	H	Me	Me	1	β	I	23	130	ethyl methyl ketone/ethyl acetate
H	H	Me	Et	1	β	I	31	151	
Ph	H	Me	Me	1	β	Br	76	148	iso-propyl alcohol + ether
Ph	H	Me	Et	1	β	Br	61	92s.	

1. Renshaw and Cass (1939) give 127-8°.

s. Sealed tube.

TABLE XXIX

R	R'	R''	R'''	n	Point of attachment to ring	X^-	M.W.	Percentage Found			Percentage Re		
								C	H	Halide	C	H	
H	H	Me	Me	1	α	I	299.2	36.10	5.83	42.35	36.13	6.06	
H	H	Me	Et	1	α	I	313.2	38.32	6.32	40.20	38.35	6.44	
Ph	H	Me	Me	1	α	I	450.3	55.94	5.77	28.04	56.01	5.82	
Ph	H	Me	Et	1	α	I	465.4	56.49	5.66	27.06	56.77	6.06	
H	H	Me	Me	1	β	I	299.2	36.22	5.85	42.29	36.13	6.06	
H	H	Me	Et	1	β	I	313.2	38.41	6.28	40.46	38.13	6.44	
Ph	H	Me	Me	1	β	Br	404.4	62.53	6.65	20.16	62.36	6.48	
Ph	H	Me	Et	1	β	Br	418.4	62.91	6.52	19.38	63.14	6.74	

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