THE INFLUENCE OF CONFIGURATION ON THE NIOtic
AND ESTERASE INHIBITORY POWERS OF CERTAIN
SYNTHETIC URETHANES.

by

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INTRODUCTION and DISCUSSION

The alkaloid physostigmine (eserine) has been shown by various workers to have the formula (I) first suggested for it by Stedman and Barger in 1925 (1.)

\[ \text{CH}_3\text{NHCOO} \]

\[ \text{CH}_3\text{O} \]

That it contains a urethane grouping has been shown by Polonovsky and Nitzberg (2.), who obtained the alkaloid by the action of methyl isocyanate on eseroline (II.) the phenolic body which results from the action of alkali upon physostigmine.

This alkaloid is well known on account of its action on the eye, as it produces when instilled into that organ a contraction of the iris along with reduction in the intracocular pressure. In view of the fact that eseroline has not these nor any of the other pharmacological characteristics of the alkaloid (which is a general parasympathetic stimulant), it was decided by Stedman to synthesise a number of simpler compounds containing the urethane grouping with a view to a comparison of their physiological action with that of physostigmine.
The first compounds synthesised by him (3.) were the methyl-, ethyl- and phenyl-urethanes from ortho-, meta- and para-dimethylaminophenols, from hordenine and from 8-hydroxyquinolone. Of these the last group were very feebly basic so that their salts were hydrolysed in solution with precipitation of the base, and they were not tested on the eye. Those of the first group also were weak bases, and their hydrochlorides became acid in solution. Some of their derivatives were also rather irritant. The urethane from hordenine was more satisfactory. It was, however, observed that the hydrochlorides of the bases of these two groups possessed in general the property of causing contraction of the iris in the case where the ester was one of methyl- or ethyl-carbamic acid, but this property was absent from the phenylurethanes. The methylurethanes were the most active. The methyliodides were active in some cases also.

The next series of compounds synthesised were those from the three isomeric hydroxybenzyldimethylamines (4.). These being stronger bases than the aminophenols it was thought that they would be more suitable for general pharmacological examination. Here the activity was confined to the methylurethanes. Urethanes from choline iodides and from tropine were found to be inactive, so it was concluded that the
hydroxyl group with which the methylecarbamic acid
was esterified must be phenolic.

Stedman and Stedman (5.) next carried out the
synthesis of the isomeric α-hydroxyphenylethyldimeth-
ylamine, and prepared their methylecarbamates. As
will be seen from the formula
\[ \text{CH}_3\text{NH} \cdot \text{CO} \cdot \text{C}_6\text{H}_4\cdot \text{CH} \cdot (\text{CH}_3) \cdot \text{N}(\text{CH}_3) \cdot 2 \]
these compounds contain an asymmetric carbon atom, and they were
synthesised with a view to their resolution in order
that the physiological activity of the enantiomorphs
might be compared; but at the time at which the
present research was undertaken, all attempts at
separation had failed. The miotic activity of the
racemic compounds were, however, tested. The tests
showed that one of them, the hydrochloride of the
methylurethane of α₂m₂-hydroxyphenylethyldimethyl-
amine, had a remarkable activity. It was estimated
in the preliminary tests (Stedman and Stedman, loc.
cit.,) as being of the same order as that of
physostigmine. This compound was given the name
miotine hydrochloride. A full pharmacological
investigation by White and Stedman (6.) later
confirmed this estimate, and indeed a remarkable
parallelism exists between the physiological action
of the hydrochlorides of miotine and physostigmine.
Two distinct objects were in view when the synthesis of the compounds described in the present paper was undertaken. The first of these was suggested by the great intensity of physiological action of miotine hydrochloride compared with that of the methylurethane of m-hydroxybenzylidimethylamine. The second object will be discussed later.

The substitution of a methyl group for a hydrogen atom as shown in the formulae

\[
\begin{align*}
\text{CH}_2\cdot\text{N}(\text{CH}_3)_2 & \quad \text{and} \quad \text{CH}(\text{CH}_3)\cdot\text{N}(\text{CH}_3)_2
\end{align*}
\]

may be regarded as having two separate effects on the nature of the molecule. In the first place it converts the compound from a derivative of toluene to one of ethylbenzene and in the second it converts it from a symmetrical to an asymmetrical form. The difference in the nature of the molecule due to the first of these effects does not seem to be very great, and it was suggested that asymmetry as such in this molecule has potent effects on its physiological properties.

This question has received considerable attention from Cushny (7, 8.) mainly in connection with tropeines. His arguments are briefly stated
below. As the tropine part of the atropine molecule is the same in all the compounds mentioned it is indicated by the symbol T.

Atropine (III.) has a very much greater physiological effect than homatropine (IV.), and this has a greater effect than benzoyltropine (V.), phenylacetyltropine (VI.) and hydratropyltropine (VII.). The ratios are given as approximately 300 : 10 : 1, the three last compounds having activities which are nearly the same. It is to be observed that III., IV., and VII. possess an asymmetric carbon atom, while III. and IV. possess in addition a hydroxyl group. Cusnny notes also that compounds analogous to III. and IV. but possessing a group such as a halogen atom or an acetyl radical in place of the hydroxyl group are less active. (These observations, quoted by him (7.) are by various workers.) From these circumstances he reasonably deduces that the presence of the hydroxyl group is the main cause of the great activity of III. and IV., but as the isomeric d. and l. homatropine also differ in physiological activity
he considers that asymmetry is also a factor. In his own words (7, p. 122):

"... it would appear that while the presence of an asymmetric carbon atom is of significance in intensifying the action of this group, as is shown by the comparison of d- and l-homatropine, its full effect is obtained only when it is accompanied by OH." (The group referred to is that of the substituted phenylacetyltropines under discussion.)

It might be commented that the difference between the physiological actions of d- and l-homatropine are in the ratio of only 1:2 (Cushny, 7.), and moreover that it is questionable whether this difference is in any case to be attributed to the fact of asymmetry per se rather than to the relative shapes of the two enantiomorphic molecules. In addition the attribution of increased activity to the homatropines on account of their asymmetry on the ground that the two isomers differ pharmacologically would only be acceptable if it were shown that in the case of the less active (non-hydroxy) compounds with asymmetric carbon no such difference existed. None of these compounds has been used, however, except in the racemic form.

The same argument is applied to compounds having the atropine skeleton. Here the substances
adduced have all the same asymmetry as atropine itself except two, atropyltropine (VIII.) and cinnamoyaltropine (IX.)

\[
\begin{align*}
\text{VIII} & \quad \text{C}(:\text{CH}_2)\cdot\text{CO} \cdot \text{CT} \\
\text{IX} & \quad \text{CH} \cdot \text{CH} \cdot \text{CO} \cdot \text{CT}
\end{align*}
\]

These have no activity, and only a little, respectively. The deduction is drawn that symmetry coupled with the lack of a hydroxyl group is responsible for this, but it may surely be pointed out that these are the only substances in Cushny's table (7, p.122) which possess double bonds in the side chain, and that such a structure is well known to exert a very great influence on the general properties of a molecule, especially when it forms part of a conjugated system.

Cushny states his general conclusions thus (7):-

"The typical atropine action...... is greatly intensified by the presence of hydroxyl and of asymmetric carbon in the side chain, and the highest degree of activity is reached only when tropine is combined with an acid of the benzene series, which contains a hydroxyl and an asymmetric carbon in the side chain, the whole molecule being laevorotatory."
Even the dextrorotatory isomer is 
considerably more active than the nearest 
homologues which do not possess the asymmetric 
carbon, and it might be supposed that the 
presence of the latter in itself has a certain 
intensifying action whatever the sign of the 
rotation. This cannot be definitely asserted,
however, because in the instances examined by 
me the asymmetric carbon was always accompanied 
by OH, and this appears to intensify the 
action in some degree."

The above was written before the activity of 
hydrotropyltropine (VII.) had been measured, but the 
argument was restated in a similar way later,
(Cushny, 8.) the smallness of the activity being 
explained by the statement that the asymmetric carbon 
atom has little effect in the absence of hydroxyl, 
the same argument as previously applied to compounds 
where the hydroxyl group of atropine had been 
replaced by CH₃COO, Cl etc. The object of the 
present discussion is to show that not only is it not 
permissible for the statement underlined in the 
above quotation to be "definitely asserted", but that 
there is no real reason for making it at all— that 
in fact all that can be stated is that the atropine 
action of compounds of this nature is greatly 
intensified by the presence of hydroxyl, and that if 
such a hydroxy-compound is optically active the
dextro form is found to be less active than the laevo.

In addition to the case of the tropane Cushny brings forward (loc. cit.) that of the adrenalin sympathetomimetics. Barger and Dale (9.) have prepared many compounds having constituents analagous to that of adrenalin (X.).

\[
\begin{align*}
\text{X} & : \text{CH(OH)} \cdot \text{CH}_2 \cdot \text{NH} \cdot \text{CH}_3 \\
\text{XI} & : \text{CH(OH)} \cdot \text{CH}_2 \cdot \text{NH}_2 \\
\text{XII} & : \text{CH}_2 \cdot \text{CH}_2 \cdot \text{NH} \cdot \text{CH}_3
\end{align*}
\]

They showed that the adrenalin-like action of these drugs on the whole increased as their constitution approximated to that of this substance. For comparison they used \text{d,l.} adrenalin. The most active compound reported by them was \text{d,l.} noradrenalin (XI.) and this was moreover the only catechol derivative besides adrenalin with either a hydroxyl group in the side chain or an asymmetric carbon atom.

\text{d,l.} adrenalin comes next in activity and then methyl-
amicinethylocatechol (XII.). The activities of these two are in the ratio of 5:7 and 1:10 respectively referred to noradrenalin, so that there is a greater falling off from \text{X.} to \text{XII.} than from \text{XI.} to \text{X.}

From these data Cushny concludes as follows (7.):

"I have shown that in .... adrenalin the \text{d,}
form is 12 to 15 times as powerful as the \text{l,}
so that the toxicity of the $\text{dl}$ form must be 6 to 7 times that of the $\text{d}$. The increase in activity found by Barger and Dale in passing from the methylaminooethylcatechol to the $\text{dl}$-adrenalin is wholly due to the presence of the laevo-rotatory component, and thus to the asymmetric carbon atom."

In making this comparison Cushny has chosen the $\text{d}$-adrenalin, which is certainly much more powerful (14 times from the above data) than methylaminooethylcatechol, but such a choice is quite arbitrary. Taking the average figure found by various observers (10, 11, 12.) we may say that natural adrenalin is 15 times as active as its dextro-rotatory isomeride, so that methylaminooethylcatechol is if anything more active than $\text{d}$-adrenalin. On this evidence, chosen no more arbitrarily than that used by Cushny, we might conclude equally well that the asymmetric carbon atom has if anything a derogatory effect on the physiological potency, the rise in activity in the case of the laevo compound being due to the presence of hydroxyl and that the inhibitory effect of the dextro configuration is less than that of the laevo.

The only logical conclusion that can be made from this part of the work of Barger and Dale appears to the author to be the simple one that the
constitution \[\text{HO} \quad \text{CH(OH)} \cdot \text{CH}_2 \cdot \text{NH} \cdot \text{R} \] is, in the type of compound studied, optimal for the sympathomimetic action, the laevo form being more active than the dextro.

It will be seen from what has been said that although it may be maintained by some that asymmetry is in itself a source of physiological potency the point has in no way been proved and indeed strong doubts may be entertained that there is any evidence at all in favour of this view. The series of urethanes represented by formulae XIII. to XVI. appeared to present, however, an admirable test of the point at issue.

\[
\begin{align*}
\text{XIII} & : \quad \text{O} \cdot \text{CO} \cdot \text{NH} \cdot \text{CH}_3 \\
\text{XIV} & : \quad \text{O} \cdot \text{CO} \cdot \text{NH} \cdot \text{CH}_3 \\
\text{XV} & : \quad \text{G} \cdot \text{CH}_3 \cdot \text{N} \cdot \text{CH}_3 \\
\text{XVI} & : \quad \text{CH} \cdot \text{C}_2 \cdot \text{H}_5 \cdot \text{N} \cdot \text{CH}_3
\end{align*}
\]

It will be seen that the question of the masking of the effect of asymmetry by the presence of the physiologically powerful hydroxyl group does not arise. If then asymmetry is in itself a matter of importance in this connection XIII. and XV. ought to
fall into one class and XIV. and XVI. into another when they are compared pharmacologically. That this was not so will be apparent from the list on p. 75. The activity of the hydrochloride of the isopropyl derivative XV. is much greater than that of its isomeride XVI., while it is certainly not less than that of miotine hydrochloride XIV. The compound XIII. is at the bottom of the list.

The methiodides of XIII., and XIV. were not included amongst the compounds tested in this series, but it is to be concluded from a table compiled by Stedman (4) that the activity in the case of the unsubstituted derivative (XIII.) is very small even in 2% solution, while that of the methiodide of miotine (XIV.) is stated (5.) to be "quite small" when tested in 1.5% solution. As the methiodides of the present series were tried in 1% solution it will be apparent from the observations that there is little likelihood that the methiodide of XV. is less active than those of XIII. and XIV. and that in fact it is not improbable that the activity rises to a maximum in the case of XV., giving the same order of activity as in the case of the hydrochlorides. There is evidently a definite falling away from the methiodide of XV. to that of the isomeride XVI. This point is not insisted upon, however, except in so far as there is no evidence that activity is attributable to
asymmetry in these cases, particularly in view of
the last statement of the preceding sentence.

It may be remarked here that Stedman (4.) gives
the hydrochloride of XVII. in the table already
referred to, and attributes to it an activity of
only ++ in 2% solution as against + for the
methiodide of the same base. In view of the results
of the present series of eye tests it was thought
better to verify the position of this hydrochloride
in Stedman's table. Accordingly the hydrochlorides
of its ortho and para isomerides, which are also
included in the table, were tried for miotic activity.
It was found (p. 6) that whereas the activity of the
ortho compound was found to be little if at all in
excess of that of the meta, that of the para
isomeride was not noticeable in 0.1% solution. The
order is thus,

\[ o > m > p \]

as against Stedman's

\[ o > p > m. \]

It would appear therefore that the meta compound has
been assigned too little activity, and should be
listed as ++++. The explanation is probably that
Stedman's observations were on different cats, for
it is noticeable that in some animals there appears
to be much more tendency for the drug to be lost via
the lachrymal duct than is the case with others. In
the present series the same cat was used throughout.
After the above results had been obtained in the
present work an observation was published (White and
Stedman, 6.) attributing greater activity to the
meta compound than to the para, in agreement with
the present observations; but the discrepancy
existing between this statement and that of (4.) was
not commented upon.

The conclusions reached above suggest at once
that in compounds of this nature the structure XVII.

\[
\begin{align*}
\text{XVII} & : \quad \text{OCO.NH.CH}_3 \\
\text{CR(CH}_3\text{).N(CH}_3\text{)}_2 & \\
\text{XVIII} & : \quad \text{OCO.NH.CH}_3 \\
\text{C(CH}_3\text{)(C}_2\text{H}_5\text{).N(CH}_3\text{)}_2
\end{align*}
\]

is optimal for miotic activity. It was decided
therefore to undertake the synthesis of the compound
XVIII. In order to see whether the nature of the radical $\mathbf{R}$ also presented an optimum. Unfortunately however, experimental difficulties in its preparation have so far precluded the investigation of this compound.

There seems little doubt, though perhaps the final proof will not be available until miotine has been tested in its optically active forms, that the mere presence of a methyl group in the side chain confers in this group of compounds peculiar physiological power and this appears to be distinct from its effect in causing asymmetry, as the presence of two methyl groups increases this effect so far as it is possible to judge, while an ethyl group causes a large drop in activity. That the methyl group possesses a chemical nature essentially different from that of an ethyl group is not in accordance with general observation, so that the hypothesis that it is the shape of the molecule which is optimal when we have a methyl group present seems most tenable. If we assume for the moment that $\mathbf{d}$ and $\mathbf{l}$ miotine will be found to have different physiological activities, as is probable from general experience, then one of these forms must possess the optimal configuration. Now the compound containing two methyl groups possesses in a way both configurations at once, so that every
molecule would perhaps be a closer approximation to the best possible than would half the molecules of dl. mixture. The effect of this might easily be that the potency of the symmetrical compound would exceed that of dl. mixture, but be about the same as, or rather less than that of whichever stereoisomeric side of the latter was most active. If it is permissible to draw an analogy it may be pointed out that a glove possessing four fingers of equal length and thumbs at both extremities could be worn on either hand, so that a fixed number of such gloves would cover a greater number of, say, right hands than would a similar number of one-thumbed gloves in pairs of opposites. (It is assumed, of course, that the back and front of a hand differ sufficiently to make it impossible for a glove to be worn reversed.) The theory really makes the distinction between configuration and asymmetry which has been insisted on in criticisms made of the papers of Cushny. Whether this distinction is valid remains to be seen, but if this argument is correct it should be found on resolution that d. and l. miotinine differ to a fair extent in activity, and moreover that the compound XVIII. is less active than its dimethyl analogue. Until this evidence is forthcoming the theory is still speculative. Also a more accurately quantitative test than that of
action on the iris seems necessary.

The second object which was held in view when the synthesis of these compounds was undertaken was an extension of the work of Stedman and Stedman (13.) on the inhibition of esterases by urethanes of the type under discussion.

The action of physostigmine on the heart has been attributed by Loewi and Navratil (14.) to its power of arresting the destruction of the acetylcholine which has been shown to be almost certainly produced in that organ on stimulation of the vagus. That the destructive agent is an esterase seems to be exceedingly probable. (15, 16.) The power of destroying acetylcholine has been shown by Plattner (17.) to be met with in a large variety of tissues, including the liver, and moreover such power was in all cases inhibited by physostigmine.

Now it has been shown (6) that miotine possesses all the pharmacological characteristics of physostigmine, as do also in less degree those other urethanes of the same class which were tested. Included in these properties is of course vagus sensitisation.

It has been shown by Matthes (16.) that the destruction of acetylcholine by the esterase of blood is inhibited in a similar way by miotine and by physostigmine.

On these grounds it was decided by Stedman and
Stedman (13.) to investigate the effect of their synthetic urethanes on the hydrolysis of simple esters by the enzymes present in animal tissues, as it seems probable that the same esterases would be operative in the two cases. They employed the enzyme from pig's liver. They found that the compounds had indeed a marked inhibitory action, and that this was specific to the urethanes, the hydroxycompounds and a variety of other substances proving inactive. The active urethanes tested were the hydrochlorides of the methyl urethanes of (1) the isomeric hydroxyphenyldimethylamines, (2) the isomeric hydroxybenzylidimethylamines and (3) the isomeric α-hydroxyphenylethylidimethylamines. They found that (a) the inhibitory action of the members of any one series differed amongst themselves but were of the same order; (b) the hydrochlorides of series (1) were most active; (c) the methiodides were in general less active than the corresponding hydrochlorides; (d) the inhibitory action of a given urethane is considerably smaller at pH 6.8 than in a slightly alkaline medium; (e) the effect of pH was greater with low concentrations of inhibitor, the action falling off with dilution more rapidly in acid solution; (f) the effect of dilution in diminishing the inhibition was greater in the case of the methiodides than in that of the hydrochlorides.
(g) the urethane grouping is not hydrolysed by liver esterase; (h) there is no correspondence between the relative inhibitory powers of the urethanes when using different substrates (methyl butyrate and tributyrin); (i) there is no relation between the order of inhibitory power of the urethanes and their miotic activities.

From these facts the authors make the suggestion that two factors are mainly concerned in determining the activity of a given urethane salt; firstly the adsorbability, which would depend on the pH and the strength of the base, the bases being only sparingly soluble; and secondly the exact chemical nature of the compound, which is presumed to enter into some form of union with the enzyme. The suggestion that the union is of the same nature as that of the enzyme and substrate is strongly supported by two pieces of evidence; that the inhibitors, though esters, are not hydrolysed by the esterase, and that for inhibition to take place the enzyme must be brought into contact with the inhibitor before addition of the substrate. One of their observations is admitted by Stedman and Stedman to be difficult of explanation on this theory, viz. that the different inhibitors act to different relative extents when the substrate is varied. It has, however, occurred to the writer, particularly in view of his own
experience, that the difference of pH (8.9 and 7.9) at which the experiments were done in the two cases might be sufficient in itself to account for this. That the different groups of three position isomerides used by Stedman and Stedman might be expected to differ largely within themselves in general chemical properties, such as basic strength, is in accordance with the general theories of induced polarities. It would therefore perhaps be too much to expect that their relative esterase inhibiting actions should be measures of miotic activity, even though the two actions were at bottom the same. This was the second reason for the synthesis of the compounds described here. These were the methyl urethanes of the following bases:

- $\alpha$-m-hydroxyphenyl-$\text{propyldimethylamine}$ (p)
- $\alpha$-m-hydroxyphenylisopropyldimethylamine (i)
- $\alpha$-m-hydroxyphenyl-$\text{butyldimethylamine}$ (bu)
- $\alpha$-m-hydroxyphenylbenzyl-$\text{dimethylamine}$ (3)

The hydrochlorides and the methiodides of these were employed, and in addition the corresponding derivatives of $\text{m-hydroxybenzyldimethylamine}$ (b) and of $\alpha$-m-hydroxyphenylethyl-$\text{dimethylamine}$ (mitine, e), for specimens of which the writer is indebted to Dr E. Stedman. The formulae of all these compounds are given below, and for convenience they will be referred to in the sequel by the letters shown.
Whether the hydrochlorides or methiodides are meant will be clear from the context.

It will be seen that with the exception of the compound $B$ they form members of a homologous series, so that their general properties might be expected to differ quantitatively but not to any extent qualitatively. The exception mentioned was included as an example of a slightly different class of compound as a point of general interest. It had originally been intended to prepare the methylurethanes of $\alpha$-m-hydroxyphenyl-$n$-amyldimethylamine but neither this nor any of its derivatives could be persuaded to crystallise, and so it had to be omitted from the series.

The first experiments were tried with pig's liver, and the methods employed were exactly as used by Stedman and Stedman, a brief account of which
will be found in the experimental portion (pp 78, 80).
The results, which are shown in Table I, show that
the order of descending activity of the hydrochlorides with methyl butyrate as substrate was
\[ e;B;b;(i,E);bu \]  
(pH, 8.9)
(All similar lists in the sequel begin with the most active substances. The letters in brackets indicate equal activity or a difference of activity within the limit of experimental error. In the latter case the order is, however, as found, actual equals being separated only by commas.)

When tributyrin was used as substrate the order was (Table III.):
\[ (e;i;b);(E,E);bu. \]  
(pH, 8.2)
It will be seen that two derivatives (i and E) are not similarly placed here, but that otherwise there is no discrepancy. If the abbreviated list obtained by omitting these
\[ e;E;bu;b \]
is compared with the list of miotic activities (p
e;E;bu;E)
it is seen that there is a certain amount of correspondence, but that the first member of the homologous series (b) is out of place. As such members are well known often to have rather different properties from the remainder, this is perhaps not altogether surprising. The list, then, when
whittled down to

agrees with the list on miotic activities.

In the case of the methiodides the differences were in both experiments rather small. The actual activities of these compounds are also less. The order for methyl butyrate (Table II.) is as follows:

\((E;E;Bu)\) (pH, 8.9)

and for tributyrin (Table IV.)

\((E;E;Bu)\) (pH, 8.2)

The position of miotine methiodide is the only important discrepancy here, but the differences between members of this series are small enough never to be very far from the limit of error, and it is doubtful whether it is allowable to draw any conclusions at all. The order of miotic activity for the four compounds (p 76) is

\(i:E:E:Bu\)

which, except in the case of the non-homologous compound B, at any rate does not contradict the order of inhibitory powers.

As has been seen the results above are very far from satisfactory in showing any similarity between miotic activity and inhibitory power, though what evidence there is may be summarised thus: that the order

\(E;E;Bu\)

is, within the limits of experimental error, the same
for all experiments, both on the enzyme and on the eye, but that the other three compounds exhibit a lack of any concurrency whatsoever.

As it was thought that the enzymes elaborated by the pig might differ from those of the cat sufficiently to mask the coincidence looked for it was decided to try the effect of the inhibitors on the esterase from the liver of this animal. It was found that extracts prepared in the same way as in the case of the pig had tributyrin activities of the same order, but that the methyl butyrate activity was very small - roughly one-tenth. The activities of the hydrochlorides on the cat esterase using tributyrin as substrate were as follows (Table IX):

$$E;:;\text{E;};(e;i);(\text{E;},\text{bu}) \quad \text{ (pH, 8.2)}$$

With the exception of the first two members of the series, which it will be noticed from the table had an altogether larger order of activity than the others, the order conforms so far as it goes to that of the miotic powers. The methiodides gave with the same substrate (Table X.) the order

$$E;:(e;\text{E;});(\text{i;},\text{bu}) \quad \text{ (pH 8.0)}$$

Here again there is nothing contrary to the opinion that the order is always the same for the three bases, e;E;bu, but very little to support it, owing, it may be surmised, to the crudeness of the methods of measurement employed.
The measurement of the inhibition of the cat's liver esterase when using methyl butyrate as substrate gave rise to considerable trouble owing to the fact that the activity of this enzyme preparation in hydrolysing methyl butyrate was too small to enable buffered solutions to be conveniently used. Efforts were therefore made to follow the hydrolysis by continuous titration of the butyric acid as liberated. This offered no difficulty in itself, but on the other hand it was found to be extremely difficult to maintain conditions of pH when the different inhibitors were mixed with the enzyme previous to the actual hydrolysies. It was found not only that the actual activities of the inhibitors changed with pH, but that they changed at different rates, so that the order of potency varied according to the alkalinity of the solution. (The pH during actual hydrolysis had apparently little effect within the narrow limits occurring in the experiments.) Owing to the colour of the esterase preparations it was found to be very difficult to judge the pH using indicators, and all that can be said is that the solutions were always more alkaline than pH 7.6 (the extremity of the range of brom-thymol-blue) but probably not more than pH 9 or thereabouts - possibly less owing to the buffering action of the proteins present.

One series of experiments (Table VII.), however,
gave in the case of the hydrochlorides, after correction (see p 86) the following list of activities. (The corrections have no effect on the order greater than is covered by experimental error.)

\((b;B);i;e;E;Bu\)

Here again the order

\(i;e;E;Bu\)

is in conformity with the miotic activities; the always exceptional compounds \(B\) and \(b\) falling out of place, though the compound \(i\) is this time correctly situated.

For the methiodides the same table gives:-

\(b;(E;p);i;(e,Bu)\)

or, selecting those methiodides tested on the eye:

\((E;p);i;Bu\)

This is not in accordance with the miotic action except in so far as the compounds \(p\) and \(Bu\) are concerned.

In another experiment on the hydrochlorides, where all the hydrolyses took place simultaneously the order (Table VIII.) was

\(i;b;(e;Bu;p);B\)

Eliminating the compounds \(b\) and \(B\) we get:

\(i;(e;Bu;p)\)

which at least does not markedly contradict the eye tests.

In consequence of these findings the suggestion
(which has already been made above in connection with the observations of Stedman and Stedman), that the difference in pH between the experiments on the two substrates might account for the different orders of activity of the inhibitors when using methyl butyrate and tributyrin, was tested by using the same enzyme-substrate mixture for both experiments, these being carried out simultaneously. The results of this experiment (Table XI.) which involved the urethane hydrochlorides, show a methyl butyrate order of

\[ i;e;(b,p) \]

for one group of urethanes, and a tributyrin order of

\[ (i;e,p);b \]

The second group of three hydrochlorides gave with methyl butyrate the order

\[ bu;p;B \]

and a tributyrin order of

\[ (bu,B;p) \]

That these lists convey much information cannot be argued. There is not enough evidence to show either marked correspondence or marked divergence in the two cases. The actual times are shown in the table for comparison, as true simultaneity was not achieved, the same stalagmometer being used for all experiments, to ensure the comparability of results in this respect. It is quite probable that this lack of time-correspondence accounts for the
discrepancies, as the pH of the enzyme extract had changed considerably during the time taken to perform the experiments in the first batch, if one judges by the difference in the methyl butyrate activities of the derivative P, which was common to each group. It is noticeable that the corresponding difference in the tributyrin series is small enough to be covered by the experimental error.

Owing to the difficulty of obtaining constancy of experimental conditions these experiments were not carried further.

The following general conclusions have been reached in the above discussion:

(1.) That there was some evidence of a general correspondence between miotic activity and esterase inhibiting activity in the case of the hydrochlorides of the urethanes of the homologous series beginning with miotine, including the dimethyl derivative in the case of liver esterase from the cat but not in the case of that of the pig when methyl butyrate was substrate.

(2.) The correspondence in the case of the methiodides was very incomplete, but this may be owing to experimental error masking the somewhat small differences between the activities of these compounds.
(3.) The urethanes from the unsubstituted hydroxybenzyldimethylamine and from that which had a substituted phenyl group showed no correspondence in the matter under discussion, being in general more active on the enzyme than on the eye when compared with the others.

(4.) The order of the activities on the enzyme seems to be greatly influenced by the pH at which the two are in contact.

The suggestion (Stedman and Stedman, 13), which has already been mentioned, that the effect of pH depends on the strength of the urethanes as bases seems to provide a key to the situation. It is to be expected that the basic strengths of the members of the homologous series would differ progressively and only slightly, but that there would be the greatest change when the carbon atom attached to the basic nitrogen atom added an alkyl group where it possessed none before, or added an extra such group to that already bound, though the second change would be less effective than the first. The loss of basic strength on addition of the phenyl group would be very considerable. Applying this to the case of the urethane hydrochlorides the compound containing the phenyl group might be much more active than those without this group in alkaline
solution, even though it were less so at a lower pH. This is in accordance with experiment (p87); and in addition the pH of the body tissues is less than that of any of the solutions employed in the enzyme tests, so that relatively less miotic activity than enzyme activity is to be expected with this compound, a state of affairs which is actually observed to be so. (An alternative explanation of this last effect is that if the base is too weak it might be precipitated in the eye before reaching the receptor, an effect not paralleled in the experiments in vitro.) Applying this argument to the unsubstituted compound it is apparent from the fact that it has in general an effect on the esterase out of proportion to its miotic activity, that it is probably a weaker base than its higher homologues, and for a similar reason the appearance of the dimethyl compound too far down in the list (see p21) in one case where the pH was high (8.9) can be accounted for by supposing that the addition of a second alkyl group to the side-chain effects a further, though small, increase in the strength of the base. These conclusions cannot, however, be verified until the relative strengths of these substances as bases have been measured.

In the case of the methiodides the evidence is very scanty owing mainly to the differences between
the activities of the substances being too small to permit of accurate measurement by the methods employed. The quaternary bases will, of course, be much stronger than the tertiary, and it is possible that the differences are as small as they are because all these bases are of approximately equal strength.

The obvious substrate to have used for these experiments was, of course, acetylcholine in the form of one of its salts and the reason for not employing this substance was the instability of acetylcholine chloride coupled with the difficulty of testing its purity, as it is very deliquescent, does not recrystallise from solvents and has no sharp melting point - due perhaps to its deliquescence. In view of the fact, however, that discrepancies had always existed, as has been seen, between the order of the activities of the urethanes as inhibitors and as miotics, it was decided to reopen the question of the use of acetylcholine chloride.

A specimen of this substance was accordingly prepared and an experiment tried with the enzyme from cat's liver using the continuous titration method. It was found that hydrolysis took place only very slowly, about 1 c.c. of \( \frac{N}{50} \) alkali being
required to neutralise the acetic acid set free in 30 minutes by 10 c.c. of enzyme preparation at pH 7.6. Of this about 0.3 c.c. was shown by a control experiment to be due to non-enzymic hydrolysis, so that though the enzyme definitely does hydrolyse acetylcholine the rate is far too small for experiments with inhibitors to be practicable.
EXPERIMENTAL

I. Preparation of Urethanes.

Note: Unless otherwise stated analyses in this section have been carried out by the author.
"(Schoeller)" indicates that the analysis is by Dr A. Schoeller of Berlin; "(Roth)", by Dr Roth of Heidelberg.

m-Methoxybenzaldehyde

The m-methoxybenzaldehyde which was used for this and certain of the following preparations was obtained by the methylation of technical m-hydroxybenzaldehyde with sodium hydroxide and dimethyl sulphate according to the procedure described by Chakravarti, Haworth and Perkin (18). As will be seen below it was found necessary for the purpose of these syntheses to take special precautions at this stage to avoid any formation of m-hydroxybenzyl alcohol by excess of sodium hydroxide, and the solution was in fact never allowed to become alkaline during the addition of caustic soda, as it was by these authors. In addition the product was carefully distilled, only that portion being retained whose boiling point ranged over 1°.
m-Methoxyphenylethylcarbinol

12 g. of magnesium were dissolved in 250 c.c. of dry ether and 75 g. of ethyl iodide. To this was added 60 g. of m-methoxybenzaldehyde. The mixture was allowed to stand for a short time and then poured on ice. The precipitate was dissolved by the addition of 50 c.c. of concentrated hydrochloric acid and the water layer rejected. The ether layer was dried with potassium carbonate, the ether evaporated and the residue distilled in vacuo. The m-methoxyphenylethylcarbinol was obtained as a colourless oil b.p. 135 - 138°/11 mm. The yield was 62 g., i.e. 85% of theory. There was a little viscous residue. On analysis (Schoeller) 4.962 mg. gave 13.030 mg. CO₂ and 3.69 mg. H₂O. This corresponds to C, 71.6%; H, 8.3%. C₁₀H₁₄O₂ requires C, 72.3%; H, 8.5%.

α-m-Methoxyphenyl-n-propylidimethylamine

The above product (62 g.) was now dissolved in 200 - 300 c.c. of benzene and saturated with hydrogen bromide. A little calcium bromide was added to absorb the water set free. The dark-coloured solution was evaporated to small bulk in vacuo at a low temperature (35°C.) in order to avoid possible loss of hydrogen bromide with the formation of a double bond, a reaction commonly
met with in derivatives having a halogen atom in this position. The residual oil was dissolved in 150 c.c. of cooled benzene and added to a solution of 33 g. of anhydrous dimethylamine in 150 c.c. of ice-cold benzene. It was allowed to stand until no more dimethylamine hydrobromide appeared to be separating out, i.e. for a few days. The solid was removed by filtration and the filtrate shaken out with hydrochloric acid (50 c.c. of the concentrated acid diluted with water). The extract was washed with ether, made alkaline with sodium hydroxide and extracted with ether. The ether was removed by evaporation, after drying with potassium carbonate, and the residual oil distilled under diminished pressure. There was a very small lower-boiling fraction besides the main fraction consisting of α-methoxyphenyl-α-propyldimethylamine. The latter boiled at 119°/11 mm., and weighed 40 g., i.e. 53.5% of theory. On combustion (Schoeller) 5.101 mg. gave 13.900 mg. CO₂ and 4.53 mg. H₂O. This corresponds to C, 74.6%; H, 9.9%. C₁₂H₁₉ON requires C, 74.6%; H, 9.9%.

The hydrochloride was prepared by addition of insufficient alcoholic hydrochloric acid to a solution of the base in dry ether. The ether was poured off and the sticky salt washed several times with this solvent. It was then crystallised from
methylethyl ketone, when it formed clusters of small needles which deliquesced slowly in moist air. They melted at 141°C. Analysis gave the following results: 17.06 mg. substance gave 10.75 mg. AgCl, giving 15.59% Cl, whereas C₁₂H₁₉ON·HCl requires 15.45% Cl.

The methiodide was obtained by addition of methyl iodide to an acetone-ether solution of the base, when it crystallised slowly. Recrystallised from acetone it formed plates melting at 187°C. On analysis (Schoeller) 2.345 mg. methiodide gave 1.123 mg. I, giving 38.1% I. C₁₂H₁₉ON·CH₃I requires 37.9% I.

The picrate crystallised on standing when the base and an alcoholic solution of picric acid were mixed, after addition of some water. It recrystallised from alcohol or aqueous alcohol in yellow stoutish prisms m.p. 117°C.

(The lower boiling fraction mentioned above was in some earlier experiments found in larger quantity. It was identified by conversion to the hydrochloride, which formed flat prisms from acetone melting at 171°C. Analysis indicated that this was m-methoxybenzylidimethylamine hydrochloride. Direct comparison with authentic specimens by mixed melting point of this and also of the picrates prepared from it confirmed this view. The formation
of this product is due to the action of excess alkali in the preparation of the m-methoxybenzaldehyde, as explained above. The m-methoxybenzyl alcohol is of course regenerated unchanged after reaction with the magnesium ethyl iodide. Care greatly diminished the amount formed, though a small quantity was always present.

**α-m-Hydroxyphenylm- propyldimethylamine**

20 g. of the above base were boiled with 80 c.c. of constant-coiling hydrobromic acid for 5 - 6 hours under reflux. The solvent was removed in vacuo and the residual syrup dissolved in water. A saturated solution of sodium carbonate precipitated the oily base which was extracted with ether. The ether solution after drying over potassium carbonate and evaporation of the ether left a brownish oil which solidified on standing. It was purified by distillation in vacuo when it boiled at 149 - 150° at 8 mm. as a colourless oil which solidified slowly on cooling. The product weighed 14 g. (75% of theory). It was not yet pure as it melted slowly from 90°. After recrystallisation from ligroin it formed flat prisms melting at 103°. On analysis (Schoeller), 4.663 mg. gave 12.615 mg. CO₂ and 3.91 mg. H₂O, corresponding to 73.8% C and 9.4% H. C₁₁H₁₇O requires 73.7% C and 9.6% H.
The hydrochloride crystallised on standing an acetone solution of the base after the addition of a little alcoholic hydrochloric acid. It recrystallised from acetone in prisms melting at 163°. 20.05 mg. of this compound gave 13.23 mg. of AgCl on precipitation. This gives 16.32% Cl, as against 16.45% Cl required by C_{11}H_{17}ON.HCl.

The methiodide crystallised on standing the base and methyl iodide in acetone solution after addition of benzene. Recrystallised from acetone it formed prisms melting at 150°. An analysis showed that 18.14 mg. substance gave 13.18 mg. of AgI. This corresponds to 39.27% I. C_{11}H_{17}ON.CH I requires 39.56% I.

The picrate, prepared exactly as in the case of the methoxy derivatives, formed long yellow prisms from aqueous alcohol, melting at 141°.

This base does not appear to yield a crystalline phenyl urethane at all readily on treatment with phenyl isocyanate and so this was not prepared.

The impurity removed from the required base in the above preparation by recrystallisation from ligroin was obtained from the mother-liquors. Recrystallised from benzene it formed silky prisms m.p. 108°. Its hydrochloride formed rhombes from acetone, m.p. 173° and its methiodide, prisms from acetone, m.p. 170°. By mixed m.p. with an authentic
specimen of m-hydroxybenzyldimethylamine the base was shown to be identical with that substance, as is confirmed by the melting point of the hydrochloride.

A specimen of m-hydroxybenzyldimethylamine was shown to be identical with that substance, as is confirmed by the melting point of the hydrochloride.

**Methyurethane of α-m-hydroxyphenylm-1-propylidimethylamine**

The products obtained by mixing methyl isocyanate with α-m-hydroxyphenylm-1-propylidimethylamine in benzene solution and evaporating subsequently in vacuo were always syrups and refused to crystallise. Neither would they yield crystalline hydrochlorides or methiodides. One specimen, however, which had stood for some months in the benzene solution, gave a crystalline hydrochloride after the following treatment:-

The solvent was distilled off in vacuo in the cold as far as possible. The syrup remaining was rubbed up with dry ether. A certain amount did not dissolve and was separated off. Insufficient hydrochloric acid in alcohol was added to the ethereal solution, and the syrupy hydrochloride thus precipitated was freed from alcohol as far as possible by rubbing up with dry ether. On standing the solution in a little acetone of the product thus obtained it crystallised out. It recrystallised from acetone in triangular pyramids melting at 153°.
Analysis showed that 0.1690 mg. of hydrochloride gave 0.0888 mg. AgCl, corresponding to 13.00% Cl, the formula $C_{13}H_{20}O_2N_2\cdot HCl$ requiring 13.01%.

The methiodide was got by a similar procedure, substituting methyl iodide for the alcoholic hydrochloric acid. Recrystallised from alcohol-ether it gave stout prisms melting at 111° with effervescence. 15.42 mg. of this substance gave on analysis 9.10 mg. of AgI. This corresponds to 31.93% I, whereas $C_{13}H_{20}O_2N_2\cdot CH_3I$ requires 33.50% I. The substance was therefore again recrystallised from acetone after which 28.58 mg. of it gave 17.00 mg. of AgI, or 32.12% I. It was therefore supposed that the crystals might contain a molecule of water of crystallisation, as this requires 31.98% I, in good agreement with these results. 44.32 mg. of the salt were dried in vacuo at 70 - 80° to constant weight, when there was a loss of 2.02 mg. This corresponds to 4.51% of the whole, whereas the formula $C_{13}H_{20}O_2N_2\cdot CH_3I \cdot H_2O$ requires 4.54%. Moreover estimation of the iodine in the dried substance gave a result of 33.75%, 32.61 mg. giving 20.38 mg. of AgI.

From the foregoing it would appear that the reaction is very slow in benzene solution, and dry ether was therefore tried as solvent. After standing for two days with considerable excess of
methyl isocyanate the product was worked up for the hydrochloride as above. It was seeded in acetone solution, but only a small quantity of crystalline hydrochloride resulted, the rest remaining oily in spite of seeding. It appeared that the reaction in ether was too slow to be convenient.

The base (2g.) was therefore dissolved in excess (3 c.c.) of methyl isocyanate. Heat was given out, and so the mixture was cooled in ice. After standing overnight and removing excess methyl isocyanate in vacuo without heat the syrup was rubbed up with dry ether; when a considerable quantity remained undissolved. The ether solution was separated and converted to the hydrochloride as before, when a yield of 1.1 g. was got. A later preparation, using 4 g. of base and 3 c.c. of methyl isocyanate gave 3.5 g. (53% of theory) of urethane hydrochloride.

All attempts to crystallise the free urethane failed. The syrup remaining after rubbing up with ether in the above preparation was found to be very soluble in water. This solution gave an oil with sodium carbonate, which on shaking out with ether, drying with potassium carbonate and evaporation of the solvent did not crystallise. It gave a crystalline hydrochloride, however, on treatment as described before. This was shown by mixed melting point to be identical with the urethane hydrochloride.
just described. The syrup appears therefore to be a salt of the urethane, but all attempts to crystallise it failed. In this way, however, the yield stated above can be considerably increased, being in fact practically theoretical.

m-Methoxyphenylmethylcarbinol

This substance was prepared from m-methoxybenzaldehyde and magnesium methyl iodide as described by Stedman and Stedman (5), except that no acid was added after decomposition of the reaction mixture with ice. Instead, the ether layer was merely syphoned off, which it was found could be done with little loss. This method was used in this and later Grignard syntheses as it was found to have the advantage that very little sticky residue is left on distilling the product. This substance seems to be formed when the crude carbinol contains a little iodide formed by the action of hydriodic acid from the magnesium iodide and hydrochloric acid, as it was noticed that in preparations where much of it was left there was a little iodine vapour visible in the distilling apparatus even though the crude carbinol had been shaken out with alkali. Presumably the iodo-compound loses hydrogen iodide forming a styrene which polymerises. The freed hydriodic acid is then able to react with a further
molecule of carbinol and so on indefinitely.

**m-Methoxyacetophenone**

This was prepared after the method of Klages and Eppelsheim (20) by oxidation of the above carbinol by means of sulphuric acid and sodium dichromate. The ketone was obtained as a yellowish oil, b.p. 122 - 123°/13 mm.

**m-Methoxyphenylisopropenylbenzene**

This compound resulted from the first attempt to prepare m-methoxyphenyldimethylcarbinol. 31 g. of the m-methoxyacetophenone were run into a solution of 15 g. of magnesium in 500 c.c. of dry ether and 85 g. of methyl iodide. The Grignard compound was decomposed with ice and the ether layer separated without the use of acid according to the method already described. After drying with potassium carbonate, removing the ether and distilling in vacuo there was obtained 80 g. of a colourless oil boiling at 102 - 105°/13 mm. Further purification of this was not attempted. This substance had a characteristic odour unlike that of the carbinols previously prepared, and moreover decolorised cold potassium permanganate solution. It took up bromine at once. On these grounds and because of its low boiling point it was presumed to consist mainly of the styrene formed by the action of the excess of
Grignard reagent on the ketone. This is borne out by the work of Béhal and Tiffeneau (21) who found that this compound is produced when excess (three molecules) of magnesium methyl iodide are allowed to react with the ethyl ester of m-methoxybenzoic acid, though they indicate that this reaction is more sluggish than appears to be so in this case. They also state (loc. cit.) that the required carbinol can be prepared by the action of two molecules of magnesium methyl iodide on the same ester, and it was decided therefore to employ this method of preparation. As will be seen in the sequel, however, the unsaturated compound can be used equally as well as the carbinol in the present synthesis.

In addition to the preparation of m-methoxyphenylisopropenylbenzene from ethyl m-methoxybenzoate by Béhal and Tiffeneau, who did not distil under reduced pressure, the substance has been obtained by von Auwers (22) by methylation of the corresponding phenol. He gives its boiling point as 99°/12 mm., in good agreement with the observations of the author (a purer specimen, as will be seen below, boiled constantly at 102°/13 mm.)
m-Methoxybenzoic acid

This was prepared from the corresponding aldehyde by oxidation in the cold with a slight excess of aqueous potassium permanganate, to which it was added slowly with stirring. When all had been added excess of permanganate was destroyed by warming with a little methyl alcohol. The solution was filtered and from the filtrate the required acid was precipitated in good yield as a white crystalline solid by addition of hydrochloric acid. It was recrystallised from hot water after which it melted at 106.5°. (The melting point is given variously as 105° (23), 106 - 107° (24), 109° (21), 110° (25), 104.5° (26), and 107 - 108° (27). In most of these cases the acid was prepared by the methylation of m-hydroxybenzoic acid. The preparation by oxidation of m-methoxybenzaldehyde does not appear to have been previously described.)

Ethyl m-methoxybenzoate

The esterification of m-methoxybenzoic acid by means of alcohol and dry hydrogen chloride is described by Perkin (28). In the present synthesis the following method was used.

The acid (75 g.) obtained as above was boiled for three hours with one fifth of its weight of concentrated sulphuric acid and twice its weight of
absolute alcohol. The excess alcohol was removed under diminished pressure and the residue poured into water. The oil which separated out was extracted with ether and unchanged acid removed from the ether solution by shaking out with a solution of sodium carbonate. This gave, on addition of hydrochloric acid, 19 g. of m-methoxybenzoic acid recovered unchanged. The ether extract gave, after drying with potassium carbonate, removing ether and distilling, 40 g. of ester b.p. 250°. (Perkin (loc. cit.) gives this as 260 - 261° (corr.), and Behal and Tiffeneau (loc. cit.) as 250 - 252°).

m-Methoxyphenyldimethylcarbinol

40 g. of ethyl m-methoxybenzoate were dissolved in 100 c.c. of dry ether and to this solution was added with stirring a solution of magnesium methyl iodide obtained from 63 g. (2 molecules) of methyl iodide in 500 c.c. of dry ether. After refluxing for half an hour the mixture was treated with ice and the ether layer separated, no acid being added. It was dried with potassium carbonate, the ether evaporated and the residue distilled. 31 g. of the carbinol, i.e. 84% of theory, were obtained as a colourless oil b.p. 130°/13 mm. It had not the odour previously noted in the styrene, and did not react with cold potassium permanganate solution.
The boiling point of this compound under diminished pressure is not given by Béhal and Tiffeneau, who distilled at 242°/770 mm.

α-m-Methoxyphenylisopropyldimethylamine

This compound was obtained first by the successive action of hydrogen bromide and dimethylamine on the impure m-methoxyphenylisopropenylbenzene described before. The preparation was exactly as in the case of the corresponding normal propyl derivative as already described, except that no water separated during the saturation with hydrobromic acid. 70 g. of styrene and 40 g. of dimethylamine were used. The dimethylamine hydrobromide separated slowly, the reaction appearing to be complete in three days. The product was worked up as in the previous case and the base obtained in poor yield (about 10 g. or 11% of theory). It boiled at 125 - 127°/13 mm.

The hydrochloride crystallised on addition of a little alcoholic hydrochloric acid solution to a solution of the base in acetone. Recrystallised from this solvent it melted at 96°.

The methiodide formed large octagonal tablets when a solution of the base in acetone was allowed to stand with a little methyl iodide. It was
recrystallised from acetone. The crystals turned brown and decomposed at about 180°, but without melting. Analysis: 23.65 mg. of substance gave 16.52 mg. of AgI or 37.76%. C₁₂H₁₉ON, CH₃I requires 37.90%.

The picrate crystallised on standing a solution of the base in alcoholic picric acid. It recrystallised from alcohol in yellow hexagonal plates melting at 138°.

The carbinol prepared according to the method of Behal and Tiffeneau gave, on subjecting to the same treatment with hydrogen bromide and the dimethylamine as was given to the unsaturated compound, the same base and in about the same yield. (31 g. of carbinol and 22 g. of dimethylamine gave a yield of 5 g. of base, i.e. 14% of theory.) This boiled at the same temperature as the previous preparation and moreover the picrate, hydrochloride and methiodide were shown by the method of mixed melting point to be identical with those just described.

In order to discover if possible the reason for the smallness of the yields in the above preparations the benzene solution remaining from the latter of them after extraction of the base was dried over potassium carbonate and the benzene distilled off. The residue was distilled under reduced pressure, when practically all (17 g.) came over as a
colourless oil b.p. 102°/13 mm. By its boiling point, odour and action on cold potassium permanganate it appeared to be identical with the liquid prepared from m-methoxyacetophenone, though a purer specimen. It would therefore appear as though the dimethylamine abstracted hydrobromic acid from the bromo-derivative, leaving a double bond. To verify this conclusion the recovered styrene (17 g.) was put through the treatment with hydrogen bromide and dimethylamine as before. The dimethylamine hydrobromide was on this occasion weighed, and there was found to be 11 g., almost the theoretical quantity, but at the same time the yield of base was only 3 g. (not distilled). This represents 13% of theory which is of the same order as before obtained. The picrate and methiodide were used in order to establish the identity of this base with that already described.

As it appears from the foregoing that the bromide of this carbinol readily loses hydrogen bromide with formation of a double bond it was thought desirable to consider the possibility that by successive formation of the bromide from the carbinol, splitting off of hydrogen bromide to form a double bond and readdition in the opposite sense the isomeric compound \( \text{CH}_3\text{OC}_6\text{H}_4\text{CH} (\text{CH}_3) \cdot \text{CH}_2\text{Br} \)
might be formed. The corresponding base 
\( CH_3C_6H_4CH(CH_3)CH_2N(CH_3)_2 \), possesses, however, an 
asymmetric carbon atom.

An acid tartrate was therefore prepared by 
mixing in alcoholic solution equimolecular quantities 
of the base and of \( d \)-tartaric acid. The alcohol was 
evaporated and the residue, which was crystalline, 
recrystallised from acetone. It appeared to be 
homogeneous and was obtained in theoretical yield. 
It melted sharply at 76°. It seems therefore 
probable that the base obtained has no asymmetric 
carbon atom.

\( \alpha \)-m-Hydroxyphenylisopropylidimethylamine

The combined methoxy-amines from the above 
preparations were boiled for five hours with constant 
boiling hydrobromic acid. After extraction exactly 
as already described in the case of \( \alpha \)-m-hydroxy-
phenylm.-propylidimethylamine, there was obtained a 
good yield of hydroxy-amine as a crystalline solid. 
It was not distilled owing to the small quantity 
available. It was slightly brown, and so it was 
recrystallised from benzene, when it formed 
colourless flat prisms melting at 129°. Duplicate 
estimations of nitrogen by micro-Kjeldahl gave the 
following results:--

5.20 mg. of substance required 3.46 c.c. of 0.01 N. 
HCl for titration of liberated NH₃
8.55 mg. of substance required 4.67 c.c. of 0.01 N HCl for titration of liberated NH₃. These figures correspond to N, 7.82% and N, 7.65%.

C₁₇H₁₇ON requires N, 7.82%.

The hydrochloride crystallised at once on adding alcoholic hydrochloric acid to an acetone solution of the base. It recrystallised from alcohol in long needles, m.p. 213°. Analysis:— 21.82 mg. of this substance gave 14.66 mg. of AgCl, or Cl, 16.82% C₁₇H₁₇ON.HCl requires Cl, 16.45%.

The methiodide separated from a solution of the base in acetone on standing with a little methyl iodide. It was recrystallised from alcohol-ether, when it formed prisms melting at 151°. Analysis:— 20.98 mg. of the substance gave 15.41 mg. of AgI, or I, 39.68%. C₁₇H₁₇ON.CH₃I requires I, 39.56%.

This base did not readily yield a crystalline phenyl urethane with phenyl isocyanate.

Methyl urethane of α-m-hydroxyphenylisopropyldimethylamine.

1 g. of α-m-hydroxyphenylisopropyldimethylamine was treated with 1.5 c.c. of methyl isocyanate. The mixture became warm and the base slowly dissolved. On standing for a short time a mass of crystals separated. The mixture was left overnight, and then the methyl isocyanate was removed as far as possible in vacuo at room temperature. The crude urethane
was treated with ether, when the crystals dissolved, leaving a sticky substance. The ether solution was evaporated, when the urethane crystallised out. It still contained a little impurity, and was found to be best purified by dissolving it in hot light petroleum, cooling thoroughly as quickly as possible, filtering off a little oil which separated, and setting aside the filtrate. The pure urethane came out in hexagonal plates, melting at 96°. Duplicate analyses (Roth) gave the following results:

<table>
<thead>
<tr>
<th>Substance</th>
<th>CO₂ (mg)</th>
<th>H₂O (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.319 mg. substance</td>
<td>10.42</td>
<td>3.07</td>
</tr>
<tr>
<td>4.103 mg. &quot;</td>
<td>9.87</td>
<td>3.07</td>
</tr>
</tbody>
</table>

These figures correspond to C, 65.81%; H, 8.48%; and C, 65.60%; H, 8.37%; respectively.

In a micro-Kjeldahl determination 10.97 mg. of base required for the titration of the ammonia set free 9.05 c.c. of 0.01 N HCl. This gives N, 10.54%

C₁₃H₂₉NO₂ requires C, 66.10%; H, 8.54%; N, 10.87%.

The hydrochloride came out at once in crystalline form when an acetone solution of the base was treated with alcoholic hydrochloric acid. It recrystallised from methyl alcohol in prisms melting at 234° with effervescence. Analysis:

24.20 mg. of substance gave 12.79 mg. of AgCl. This corresponds to Cl, 13.07%. C₁₃H₂₀N₂HCl requires Cl, 13.01%.

The methiodide was prepared by allowing the
solution of the base in acetone to stand overnight with a little methyl iodide, when it had crystallised out. It formed clusters of needles on recrystallising from alcohol after addition of ether. On heating rapidly these melt at 160° and at once reset to a solid which decomposes slowly if heated further. If heating is carried out slowly the crystals soften somewhat below this temperature and do not melt until a much higher temperature is reached. At about 230° there is no solid left, but much gas has been given off at lower temperatures. Analysis:-
25.12 mg. of substance gave 15.64 mg. of AgI, corresponding to I, 33.65%. C₂₀H₃₀₂N₂₄H₃I requires 33.50% of I.

The sticky substance which separated when the crude urethane was originally treated with ether was found to crystallise from acetone. It was recrystallised from alcohol by addition of ether, forming hexagons melting at 143°. As in the case of compounds produced in analogous fashion in other preparations it was very soluble in water and gave the urethane (m.p. 96°) on treatment with sodium carbonate, and so appears to be a salt of this base. No acid could be recovered from the sodium carbonate extract by treatment with acid and subsequent extraction with ether, though at the same time it is to be remembered that the quantity of substance
available was small.

The usual test for sulphur by means of sodium fusion and sodium nitroprusside was positive. Analyses gave the following figures:

4.993 mg. of substance gave 8.810 mg. of CO₂ and 2.970 mg. of H₂O. (Schoeller) This corresponds to C, 48.11%; H, 6.66%.

14.81 mg. of substance requires in a micro-Kjeldahl estimation 11.56 c.c. of 0.01 N HCl.

11.14 mg. of substance requires in a micro-Kjeldahl estimation 8.76 c.c. of 0.01 N HCl. These correspond to N, 10.93% and N, 11.02% respectively.

15.40 mg. of substance gave 9.52 mg. of BaSO₄ in a micro-Carius estimation.

10.78 mg. of substance gave 6.49 mg. of BaSO₄ in a micro-Carius estimation. These give S, 8.49% and S, 8.27% respectively.

No formula was deduced from these figures and owing to the small quantity of substance available no further investigations were made. The sulphur present presumably comes from the methyl isocyanate as this was prepared by the action of dimethyl sulphate on potassium cyanate. A considerable quantity of quicklime was always mixed with these in order to slow down the reaction (it also seems to improve the yield of methyl isocyanate, as its
replacement by pure sand results in a reduction of this). It would therefore appear strange that any acid should distil off. Such an acid, moreover, must be of low boiling point as the methyl isocyanate was always redistilled before use.

**m-Methoxyphenyln-...propylcarbinol**

This compound has been described by Klages (29), who prepared it from magnesium propyl iodide and m-methoxybenzaldehyde. In the present work magnesium propyl bromide was used in place of the iodide. The procedure was that used before for the preparation of m-methoxyphenylmethylcarbinol. Using 12 g. of magnesium, 250 c.c. of dry ether, 60 g. of propyl bromide and 60 g. of m-methoxybenzaldehyde there was obtained 66 g., or 83% of the theoretical yield of carbinol as a thick oil boiling at 145 - 147°/12 mm. Klages (loc. cit.) gives the boiling point as 151 - 152°/15 mm. The remainder consisted of a viscous residue.

**o-m-Methoxyphenyln-...butyldimethylamine**

The above carbinol (66 g.) was converted to this compound by successive treatment with hydrobromic acid in benzene and dimethylamine (31 g.) in benzene. Several days were required for the completion of the latter reaction. The base was isolated in the same manner as before and distilled. There was very
little low-boiling fraction. The required amine was a thick oil and boiled at 129 – 130°/11.5 mm. 36 g. or 47% of theory, were obtained. Analysis (Schoeller) gave the following results:–

4.991 mg. of substance gave 13.730 mg. of CO₂ and 4.53 mg. of H₂O. This corresponds to C, 75.0% and H, 10.2%. The formula C₁₃H₂₁ON requires C, 75.4%; H, 10.2%.

The hydrochloride was crystallised by the procedure described for the lower homologue (p.). It recrystallised from methylethyl ketone in fine white needles, but these deliquesced rapidly on exposure to the air, so that their melting point was not recorded.

The methiodide crystallised from a solution of the base in acetone on standing with a little methyl iodide. Recrystallised from the same solvent it gave triangular platelets melting at 119.5°.

Analysis:–

22.86 mg. of methiodide gave 15.36 mg. of AgI, corresponding to I, 36.32%. C₁₃H₂₁ON, CH₃I requires I, 36.38%.

The picrate crystallised out of an alcoholic solution of the base with picric acid. It recrystallised from alcohol in yellow needles, m.p. 99 – 100°.
α-m-Hydroxyphenyl-\-butyldimethylamine

30 g. of the methoxy-\-compound were boiled with 150 c.c. of constant-\-boiling hydrobromic acid for five hours, and the hydroxy-\-base isolated as in previous cases. The substance crystallised on standing after evaporating the ether extract, but was distilled to remove colour. All except a little tar came over as a colourless viscous syrup, which solidified very slowly. It boiled at 170°/12 mm. The yield was 26 g., i.e., 93% of the theoretical. It can be recrystallised from ether or ligroin and forms slender prisms melting at 107°.

Analysis (Schoeller):-

4.51 mg. of base gave 12.395 mg. of CO₂ and 4.01 mg of H₂O, corresponding to C, 74.9% and H, 9.9%.

C₁₅H₁₉O₂N requires C, 74.6% and H, 9.9%.

The hydrochloride was crystallised by careful addition of ether to a solution of the base in acetone to which had been added a little alcoholic hydrochloric acid. It recrystallised in tablets melting at 86° from methylethyl ketone. Analysis on two different specimens gave the following:-

8.42 mg. of substance gave 4.79 mg. of AgCl, corresponding to Cl, 14.37%.

16.78 mg. of substance gave 9.80 mg. of AgCl, corresponding to Cl, 14.46%.

As this indicated that the salt might contain water
of crystallisation, it was dried in vacuo. At temperatures not exceeding 60° it melted when treated in this way and the dried substance was therefore not analysed. 12.14 mg. of substance lost 0.88 mg. on drying, or 7.24%. \( \text{C}_{12}\text{H}_{19}\text{ON}, \text{HCl}, \text{H}_{2}\text{O} \) requires Cl, 14.34% and H₂O, 7.27%.

The methiodide crystallised on standing an acetone solution of the base with a little methyl iodide. It was recrystallised from acetone in thick colourless hexagonal tablets. These turned yellow at about 182° but by heating rapidly a fairly sharp melting point of 191 - 192° could be observed.

Analysis:-
13.83 mg. of substance gave 13.14 mg. of AgI. This corresponds with I, 37.71%. \( \text{C}_{12}\text{H}_{19}\text{ON}, \text{CH}_3\text{I} \) requires I, 37.90%.

The picrate crystallised from an alcoholic solution of the base with picric acid. It formed yellow rhombs m.p. 154° on recrystallisation from alcohol.

Phenylurethane of \( \alpha\)-m-hydroxyphenyl-\( \beta \)butyldimethylamine

This material was prepared by standing a solution of 4 g. of \( \alpha\)-m-hydroxyphenyl-\( \beta \)butyldimethylamine and 2.5 g. (a slight excess) of phenyl isocyanate for two days. A little crystalline deposit was filtered off (this is probably diphenyl urea)
and to the residue petroleum ether was added. The urethane crystallised on scratching. It was filtered off and washed with light petroleum. Yield 5.7 g., i.e. 88% of the theoretical. Recrystallised from ligroin after separation of a small quantity of insoluble substance it gave stout prisms melting at 96°. Analysis (Schoeller):—

4.811 mg. of substance gave 12.910 mg. of CO₂ and 3.29 mg. of H₂O. This corresponds with C, 73.2%; H, 7.7%. C₁₉H₂₄O₂N₂ requires C, 73.1%; H, 7.8%.

The hydrochloride crystallised at once on scratching when the base was dissolved in a little acetone and alcoholic hydrochloric acid added. It recrystallised from acetone containing a little alcohol in small rectangular tablets. These melted at 201° in a rapidly-heated bath. There was some effervescence. Analysis:

20.29 mg. of substance gave 8.39 mg. AgCl. This corresponds with Cl, 10.23%. C₁₉H₂₄O₂N₂HCl requires Cl, 10.18%.

The methiodide was crystallised as in the case of the phenol methiodide. It recrystallised from acetone-ether in prisms melting at 146°.

Methylurethane of α-m-hydroxyphenyl-n-butyldimethyl amine

Efforts to prepare this compound or its salts
in the crystalline form by carrying out the condensation with methyl isocyanate in solvents having failed, the method as described for the methylurethane of α-m-hydroxyphenylα-propyldimethylamine was tried with success. The hydrochloride and methiodide were obtained in exactly the same way. Here also it was found that there was a sticky uncrystallisable substance left on extraction of the urethane from the reaction mixture by means of ether, and moreover this substance when dissolved in water and treated with sodium carbonate gave the urethane.

In a later preparation 2 g. of the α-m-hydroxyphenylα-butyldimethylamine were dissolved in 1 to 2 c.c. of methyl isocyanate. The excess was removed in vacuo next day and the residue shaken with ether and sodium carbonate solution to decompose the salt-like compound. After drying with potassium carbonate the ethereal extract was divided into two equal portions, and these were converted into the hydrochloride (0.9 g.) and methiodide (1.25 g.) respectively by the procedure described. This represents a yield of 61% and 62% in the two cases.

The free base resisted all attempts at crystallisation.

The hydrochloride obtained as just mentioned was recrystallised from acetone. It formed prisms m.p. 143°.
Analysis:-

19.95 mg. of substance gave 10.04 mg. AgCl. This corresponds with Cl, 12.44% $\text{C}_4\text{H}_2\text{O}_2\text{N}_2\cdot\text{HCl}$ requires Cl, 12.34%.

The methiodide was recrystallised from acetone. It formed slender flat prisms. On heating to temperatures above about 163° it decomposed with effervescence. In a rapidly heated bath, however, it appeared to melt at 176° with much frothing.

Duplicate analyses resulted as follows:-

12.315 mg. of substance gave 7.43 mg. of AgI.
17.80 mg. of substance gave 10.61 mg. of AgI.

These correspond to I, 32.61% and I, 32.22% respectively. $\text{C}_4\text{H}_2\text{O}_2\text{N}_2\cdot\text{CH}_3\text{I}$ requires I, 32.40%.

**m-Methoxyphenylmethylethylcarbinol**

A solution of ethyl magnesium bromide from 54 g. of ethyl bromide in 500 c.c. of dry ether was run by means of a syphon into 73 g. of m-methoxyacetophenone (whose preparation has already been described) with stirring. This represents exactly the theoretical quantity. The above precautions were taken to avoid the formation of a styrene by the presence of excess of Grignard reagent, as happened when excess of magnesium methyl iodide was used (p 42).

The mixture was poured on ice and the ether layer separated without acid as described in previous cases. The ethereal solution was dried with
potassium carbonate, the ether evaporated off and the residue distilled in vacuo. The carbinol was obtained in a yield of 55 g., or 63% of theory, as a colourless oil b.p. 135°/13 mm. There was a considerable amount of syrupy residue.

Attempted preparation of α-m-methoxyphenylsec.-butyl-dimethylamine

The carbinol just described (55 g.) was treated with hydrobromic acid in benzene as usual. Water was liberated during the absorption. The excess of acid was removed under reduced pressure at 35°, and the residue treated with dimethylamine in benzene for 24 hours. Only 6 g. of dimethylamine hydrobromide had separated. Extraction as in previous similar preparations gave only 1.7 g. of oily base.

The benzene layer was dried over potassium carbonate and distilled under diminished pressure after removal of the benzene. 47 g. of a colourless oil, b.p. 120°/17 mm. was obtained, besides a little tarry residue. This oil is presumably either 3-methoxy 1'-ethylstyrene, CH₃OC₆H₄C(C₂H₅):CH₂, or the isomeric 3-methoxy 1':2'-dimethylstyrene, CH₃OC₆H₄C(CH₃):CH₂CH₃. It was not investigated further.

Owing to the very small yield of base obtained in the above experiment this synthesis was abandoned.
**m-Methoxyphenyl-n-butylcarbinol**

This material was obtained in a yield of 75 g. or 88% of the theoretical, by adding 60 g. of m-methoxybenzaldehyde to a solution of Grignard reagent from 66 g. of n-butyl bromide and 12 g. of magnesium in 250 c.c. of dry ether. The procedure was exactly as described under the preparation of m-methoxyphenylethylcarbinol. The substance was a colourless oil and boiled at 130 - 133°/3 mm.

**m-Methoxyphenyl-n-amylidimethylamine**

Treatment of the above carbinol (75 g.) with hydrobromic acid in benzene and then dimethylamine (35 g.) in the way described for previous similar preparations gave 43 g. (50% of theory) of m-methoxyphenyl-n-amylidimethylamine. This was a colourless oil and boiled at 146 - 148 /13 mm. There was a small lower-boiling fraction.

**Analysis (Schoeller):**

4.985 mg. of substance gave 13.820 mg. of CO₂ and 4.69 mg. of H₂O. This gives C, 75.6%; H, 10.5%.

C₁₄H₂₃O₃N requires C, 76.0%; H, 10.5%.

The hydrochloride was crystallised in the same way as that of the next lower homologue. Recrystallised from ethyl acetate or acetone it formed flat prisms m.p. 130.5°. **Analysis:**

13.34 mg. of substance gave 7.52 mg. of AgCl. This gives Cl, 13.94%. C₁₄H₂₃O₃N.HCl requires Cl, 13.77%.
The methiodide resisted all attempts at crystallisation.

The picrate crystallised from an alcoholic solution of the base and picric acid. It recrystallised from the same solvent as flat yellow prisms, m.p. 91°.

α-m-Hydroxyphenyl-n-amyldimethylamine

On boiling 22 g. of the base just described with constant-boiling hydrobromic acid for five hours and working up as in previous cases this material was obtained as a brown syrup which solidified on standing. It was distilled under diminished pressure when 18 g. (87% of theory) were obtained as a colourless exceedingly viscous syrup which very slowly crystallised. It boiled at 173°/10 mm. It was recrystallised from ligroin or ether when it formed pyramid-shaped crystals m.p. 91°. Analysis (Schoeller):

4.704 mg. of substance gave 12.990 mg. of CO₂ and 4.36 mg. of H₂O. This corresponds with C, 75.3%; H, 10.1%. C₁₃H₂₁ON requires C, 75.4%; H, 10.2%.

The hydrochloride could not be crystallised.

The methiodide was prepared by the action of methyl iodide on the base in acetone, and careful addition of ether. Recrystallised from acetone-ether it formed colourless hexagons, m.p. 141°.
Analysis:-
29.90 mg. of substance gave 20.21 mg. of AgI. This gives I, 36.54%. C_{13}H_{21}ON,CH_{3}I requires I, 36.38%.

The picrate was prepared as in the case of the methoxy-compound. It recrystallised from alcohol in yellow rhombs melting at 126°.

Phenylurethane of α-m-hydroxyphenyl-α-amyldimethylamine

This product was obtained in the manner already described for the next lower homologue. 4.3 g. of base and 2.5 g. of phenyl isocyanate in 20 c.c. of benzene gave 6.1 g. (90% of theory) of phenylurethane. Recrystallisation from ligroin gave a product melting at 103° as stout prisms. Analysis (Schoeller):-
4.620 mg. of substance gave 12.530 mg. of CO_{2} and 3.33 mg. of H_{2}O. This corresponds with C, 73.9%; H, 8.1%. C_{20}H_{26}O_{2}N_{2} requires C, 73.6%; H, 8.0%.

The hydrochloride was prepared as in the case of the lower homologue. It recrystallised in long prisms m.p. 166° from acetone containing a little alcohol. Analysis:-
23.35 mg. of substance gave 9.33 mg. of AgCl. This gives Cl, 9.88%. C_{20}H_{26}O_{2}N_{2},HCl requires Cl, 9.78%.

The methiodide was not obtained in the crystalline form.
The methylurethane of this compound, prepared by the action of methyl isocyanate upon it, was not obtained crystalline. Neither could its hydrochloride methiodide or picrate be persuaded to crystallise, though many procedures were tried, including that which had proved successful in other cases. A salt-like compound was present in the crude reaction mixture, as in all these preparations, but this did not crystallise.

The α-m-hydroxyphenyl-α-methylamylidimethylamine used was crystallised in some instances several times from various solvents, but without effect.

α-m-Methoxyphenylbenzyl alcohol

This material has been described by Stoermer (30), who prepared it by the action of phenyl magnesium iodide on m-methoxybenzaldehyde. In the present synthesis the same method was used, except that the bromide was substituted for the iodide. 60 g. of m-methoxybenzaldehyde were added to the solution obtained by the action of excess magnesium on 76 g. of bromobenzene in 500 c.c. of dry ether and the carbinol isolated as a colourless oil by the procedure used in previous cases. An attempt to distil the material failed, as it had too high a boiling point, even at 3 mm. Stoermer states that it can be crystallised, and melts at 41°, but it did not crystallise spontaneously, and as it was thought
that it would be pure enough for the present purposes no attempt was made to persuade it to do so. The yield appeared to be good.

ω-m-Methoxyphenylbenzylidimethylamine

The crude carbinol just described was dissolved in benzene and saturated with hydrogen bromide in the usual manner. A little tar was separated and the excess acid removed as before. 35 g. of dimethylamine in benzene were added and the mixture stood for some days. On working up as usual and distilling there was obtained in addition to a little lower-boiling fraction, 65 g. of base as a thick colourless oil, b.p. 179°/12 mm. This represents a yield of 61% calculated on the m-methoxybenzaldehyde used. Analysis (Schoeller):- 5.080 mg. of substance gave 14.805 mg. of CO₂ and 3.68 mg. of H₂O. This gives C, 79.5%; H, 8.1%.

C₁₆H₁₉O₁N requires C, 79.7%; H, 7.9%.

The hydrochloride crystallised on standing an acetone solution of the base with a little alcoholic hydrochloric acid. It was recrystallised from acetone in rectangular prisms melting at 182°. Analysis:- 19.95 mg. of substance gave 10.35 mg. of AgCl. This corresponds with Cl, 12.82%. C₁₆H₁₉O₁N,HCl requires Cl, 12.77%.

The methiodide was not obtained in a crystalline form.
**ω-m-Hydroxyphenylbenzylidimethylamine**

43 g. of the methoxy-base were boiled under reflux with 150 c.c. of constant-boiling hydrobromic acid for five hours. On cooling, the hydrobromide of the required base separated out as a crystalline mass. It was filtered off. The mother liquors proved to contain practically none of the salt and so were rejected. The hydrobromide was dissolved in hot water (it is very little soluble in cold) and filtered from a little tar. (In some preparations the solution was further decolourised with charcoal, but this is unnecessary if the free base is to be distilled.) On addition of saturated sodium carbonate solution the amine separated as an oil. After cooling this set to a vitreous mass. This was dissolved in ether and the whole extracted with that solvent. After drying with potassium carbonate and driving off the ether the residue was distilled. Nearly the whole came over at 205 - 207°/10 mm. as a colourless viscous oil. The yield was 33 g., or 81% of the theoretical. On cooling it formed a hard vitreous mass which crystallised very slowly on standing. It recrystallised from ligroin as colourless tablets m.p. 94°.

Some of the hydrobromide which was prepared incidentally in the above process was recrystallised from hot water and then from acetone-ether, after which it melted at 228°.
Analysis:
21.87 mg. of substance gave 12.77 mg. of AgBr. This corresponds to Br, 24.84%. This indicated that the substance contained water of crystallisation and it was therefore dried in vacuo at 100°. 25.79 mg. of substance lost 1.42 mg. on drying, or 5.51% of the whole. C_{15}H_{17}ON, HBr, H_{2}O requires Br, 24.51%; E_{2}O, 5.52%

An analysis on the dried substance moreover gave the following:
24.37 mg. of substance gave 14.97 mg. of AgBr. This gives Br, 26.14%. C_{15}H_{17}ON, HBr requires Br, 25.95%.

The methiodide could not be crystallised.

The picrate crystallised on standing in alcoholic picric acid. It recrystallised from alcohol in yellow needles m.p. 129°.

**Phenylurethane of ω-m-hydroxyphenylbenzylidimethylamine**

47 g. of the ω-m-hydroxyphenylbenzylidimethylamine and 2.5 c.c. of phenyl isocyanate were dissolved in 20 c.c. of benzene and allowed to stand. Some of the urethane crystallised out. The benzene was removed in vacuo in the cold as far as possible and petroleum ether added to remove any remaining. The residue was crystallised from ligroin, a little insoluble non-basic matter being filtered off.

(This material is somewhat soluble in benzene - hence
the above procedure.) The urethane formed needles melting at 119°. The yield was 9.2 g, or 100% of theory. Analysis (Schoeller):-

4.850 mg. of substance gave 13.560 mg. of CO₂ and 2.78 mg. of H₂O. This gives C, 76.3%; H, 6.4%.

C₂₂H₂₂O₂N₂ requires C, 76.3%; H, 6.4%.

The hydrochloride was not obtained in a crystalline form.

The methiodide crystallised from acetone on addition of methyl iodide and standing. It was recrystallised from acetone in colourless pyramids. These turned brown at about 145°, but in a rapidly heated bath melted at about 186° with effervescence.

Methylurethane of ω-m-hydroxyphenylbenzyldimethylamine

In the first attempt at the preparation of this urethane 5 g. of ω-m-hydroxyphenylbenzyldimethylamine were dissolved in 30 c.c. of benzene and 5 c.c. (slight excess) of methyl isocyanate added. After some days crystals began to separate. When no more seemed to be being formed petroleum ether was added, which appeared to precipitate a little more. The clear liquid was decanted and the solid recrystallised from methyl alcohol by addition of ether. It weighed 40 g. and melted at 124° with frothing. It was thought at first that this might be the required urethane but analysis (see below) dismissed this idea.
The mother liquors were accordingly evaporated down in vacuo without heating. A syrup remained which would not crystallise by itself or on seeding with either the original phenol or the crystals just described. Attempts to prepare a crystalline hydrochloride or methiodide from this failed, but a picrate crystallised out on standing the solution of the substance in alcoholic picric acid. This recrystallised from acetone in small yellow double pyramids, m.p. 182°. Analysis:

0.2562 g. of substance gave 30.4 c.c. of N₂ at 17° and 737 mm. over 50% KOH. This gives N, 13.40%.

C₁₇H₂₀O₂N₂, C₆H₃O₇N₃ requires 13.66% N.

It seemed probable therefore that this was the picrate of the required urethane. The base was recovered from this by shaking up with ether and ammonia, but still did not crystallise, or yield any other crystalline derivatives. Recrystallisation of a little to the picrate showed that there was practically 100% yield, so that the base is stable to ammonia.

Better results were later got by using no solvent in preparing this urethane. 1 g. of the phenol was added to 1 c.c. of methyl isocyanate. It slowly dissolved with a little heat evolution. It was left overnight and the excess isocyanate removed in the cold in vacuo. The syrup was rubbed up with ether, when most of it dissolved, but a sticky
substance remained. The ether solution was evaporated and the residue crystallised from aqueous alcohol, or better from benzene and light petroleum. It formed square crystals, m.p. 80°. The base recovered from the picrate already referred to crystallised almost completely on seeding with this substance. Duplicate micro-Kjeldahl determinations resulted as follows:

6.69 mg. of substance required 4.66 c.c. of 0.01 N HCl for titration of liberated NH₃.
4.91 mg. of substance required 3.43 c.c. of 0.01 N HCl for titration of liberated NH₃.

These results give N, 9.75% and N, 9.78% respectively. C₁₇H₂₀O₂N₂ requires N, 9.86%.

The hydrochloride was crystallised by dissolving some of the crystalline base in ether, adding a slight excess of alcoholic hydrochloric acid and then a little more base until there was a slight excess of this. The ether was poured off from the flocculent non-crystalline hydrochloride. This was washed with ether and dissolved in a little acetone. After standing for two days crystals separated. It recrystallised from alcohol-ether in prisms melting at 204° with frothing. Analysis:

22.29 mg. of substance gave 10.08 mg. of AgCl. This gives Cl, 11.18%. C₁₇H₂₀O₂N₂HCl requires Cl, 11.06%.

The methiodide crystallised after some days on
standing a solution of the pure base in acetone and a little methyl iodide. It was recrystallised from alcohol-ether in rectangular prisms. These turned brown and decomposed at temperatures above about 140°, but did not melt. Analysis:-

23.11 mg. of substance gave 12.81 mg. of AgI. This gives I, 29.95%. C_{17}H_{20}O_{2}N_{2},CH_{3}I requires I, 29.80%.

The picrate has been described above.

The sticky substance remaining from the second preparation of the urethane was crystallised from alcohol-ether, and proved to be identical with the compound m.p. 124° obtained from the first preparation. It was soluble in water and gave the urethane on treatment with sodium carbonate and extraction with ether. It gave a positive sulphur test. Analyses were as follows:-

Combustion (Schoeller): 4.654 mg. of substance gave 9.345 mg. of CO₂ and 2.750 mg. of H₂O. This gives C, 54.74% and H, 6.61%.

Micro-Kjeldahl (duplicates): 10.19 mg. of substance required 6.30 c.c. of 0.01 N HCl for titration of the liberated NH₃.

10.82 mg. of substance required 6.68 c.c. of 0.01 N HCl for titration of the liberated NH₃. These correspond to N, 8.66% and N, 8.65% respectively.

Micro-Carius: 10.42 mg. of substance gave 5.16 mg. of BaSO₄. This gives S, 6.80%.
No formula could be deduced from these figures, and they do not seem to fit in any way the analyses carried out on the only other one of these salt-like compounds which crystallised, i.e. that from \( \alpha \)-m-hydroxyphenylisopropylidimethylamine. The matter was not investigated further, and the materials, which were available in only small quantities, were assumed to be probably rather impure.
II. Miotic Action of the Urethanes

In order to test the miotic action of the urethanes one drop of a solution of the hydrochloride or methiodide was introduced into the eye of a cat. The same cat was used for all the experiments, alternate eyes being treated. On no occasion was an experiment tried until both eyes were completely normal, usually 24 hours but sometimes 48 hours after the previous treatment. The same pipette was used throughout in order to ensure that the drops were of equal size. The solutions were made up in physiological saline.

In no case was there any indication that the substances induced irritation.

In the initial experiments the effect of a 1% solution of the hydrochlorides of the methyl urethanes of \( \alpha \)-m-hydroxyphenyl-\( \alpha \)-propyldimethylamine and of \( \alpha \)-m-hydroxyphenylisopropyldimethylamine were tried. It was, however, found that these produced a very great degree of miosis and that no distinction was observable between them. A solution of 0.1% was therefore tried and found to be very satisfactory.

In the cases of the methiodides, which were much less active, a 1% solution was used.

The author, who has not the necessary licence, is indebted to Dr E. Stedman for carrying out the tests described in this section.
The observations are given below.

1. \( \text{m-CH}_3\text{NH.CO.OC}_6\text{H}_4\text{.CH}_2\text{.N(CH}_3)_2\text{.HCl} \)
   Slight but distinct contraction after 20 - 25 minutes.

2. \( \text{m-CH}_3\text{.NH.CO.OC}_6\text{H}_4\text{.CH(CH}_3)_2\text{.N(CH}_3)_2\text{.HCl} \)
   Miosis noticed in 15 minutes increasing to a maximum in 25 minutes. Very marked.

3. \( \text{m-CH}_3\text{.NH.CO.OC}_6\text{H}_4\text{.C(CH}_3)_2\text{.N(CH}_3)_2\text{.HCl} \)
   Effect very similar to that in the preceding case - if anything greater.

4. \( \text{m-CH}_3\text{.NH.CO.OC}_6\text{H}_4\text{.CH(C}_2\text{H}_5)_2\text{.N(CH}_3)_2\text{.HCl} \)
   Rather little miosis after 20 - 25 minutes, but greater than in the case of 1.

5. \( \text{m-CH}_3\text{.NH.CO.OC}_6\text{H}_4\text{.CH(C}_3\text{H}_7)_2\text{.N(CH}_3)_2\text{.HCl} \)
   Contraction observable after 20 - 25 minutes; maximal in 35 - 40 minutes.
   Estimated as less than the preceding but greater than 1.

6. \( \text{m-CH}_3\text{.NH.CO.OC}_6\text{H}_4\text{.CH(C}_6\text{H}_5)_2\text{.N(CH}_3)_2\text{.HCl} \)
   A 0.1% solution had no effect. A 1% solution produced small but distinct miosis after 30 - 40 minutes and considerable salivation.

7. \( \text{o-CH}_3\text{.NH.CO.OC}_6\text{H}_4\text{.CH}_2\text{.N(CH}_3)_2\text{.HCl} \)
   Contraction noticed after 15 minutes.
and maximal in 20 - 25 minutes.

Equal to or possibly greater than that in the case of 1.

8. p-CH₃.NH.CO.OCH₃.H₂O.N(CH₃)₂, HCl

No effect in 0.1% solution.

9. m-CH₃.NH.CO.OCH₃.H₂O.C(CH₃)₂,N(CH₃)₂, CH₃I

One drop of 1% solution produced distinct miosis noticed after 15 - 20 minutes and maximal in 25 - 30 minutes.

10. m-CH₃.NH.CO.OCH₃.H₂O.CH(C₆H₅).N(CH₃)₂, CH₃I

Very small but perceptible contraction noticed after 30 minutes. Less than the preceding.

11. m-CH₃.NH.CO.OCH₃.H₂O.CH(C₃H₇).N(CH₃)₂, CH₃I

No effect was noticed with this compound in 1% solution.

12. m-CH₃.NH.CO.OCH₃.H₂O.CH(C₆H₅).N(CH₃)₂, CH₃I

Considerable salivation; after 30 - 40 minutes a small though clearly visible miotic action was observed.
III. Inhibition of Esterases by Urethanes

Substituents in the case of all urethanes used in this part of the work are in the meta position.

A. Experiments with pig's liver extracts.

The preparation of the liver powder was not in this case done by the author, who is indebted to Dr E. Stedman for this material. The method used by him was that of Willstätter and Memmen (31). The procedure is described in section B of this part.

The liver extracts were prepared from the liver powder according to the following procedure:

2 g. of the liver powder were left in contact for an hour or longer with 100 c.c. of 0.025 N ammonia solution. The whole was then centrifuged and the clear liquid treated with 0.5 N acetic acid until a precipitate began to appear. After standing for a short time this was centrifuged off. The solution was then dialysed for about two days through collodion membranes. Any further precipitate which had formed was removed by centrifuging and the solution was then ready for use. The same batch of extract was used for any one series of observations, but different series are not to be taken as being necessarily comparable with one another.

1. Methyl butyrate as substrate

The method used was that of Willstätter and
Memmen (loc. cit.) as adapted by Stedman and Stedman (13).

As there were in each case six urethane salts besides one control experiment the series was done in two runs, one being started immediately after the other was finished. The procedure was as follows:-

A weighed quantity of the salt of each urethane was dissolved up in water and diluted to a suitable extent, this having been determined by previous trial. 2 c.c. of this solution was added in each case to a mixture of 5 c.c. of the enzyme preparation and 5 c.c. of a diluted buffer solution (the buffer described below diluted 10 or 20 times). In the case of the control 2 c.c. of water were substituted for the solution of the urethane derivative. These were well mixed and allowed to stand at room temperature for one hour, after which equilibrium may be supposed to have been reached (Cf. Stedman and Stedman, ibid, 1153). Meanwhile the requisite number (3 or 4) of 100 c.c. graduated flasks containing 65 c.c. of water and 20 c.c. of buffer solution were placed in a thermostat at 30°. This buffer solution was composed of 1 volume of 2.5 N ammonium hydroxide to 2 volumes of 2.5 N ammonium chloride, and had a pH of 8.9. 1 c.c. of methyl butyrate was pipetted into each flask and dissolved
up. 10 c.c. of the enzyme-urethane solution were added to the flasks in turn. After each addition the volume was made up to 100 c.c., the flask well shaken, and 20 c.c. of its contents abstracted as rapidly as possible. The pipette was emptied into 25 c.c. of 0.2 N hydrochloric acid in 40 c.c. of water previously measured out. On each occasion the time was noted and the withdrawal of 20 c.c. repeated in the same manner every 20 minutes, four times in all for each substance. The excess hydrochloric acid was titrated with 0.2 N sodium hydroxide solution using bromocresol purple as indicator. A buffered solution (pH 7.3) of the indicator was used for comparison as the end-point became rather bad towards the end of the experiments when much butyric acid was present.

(a). Hydrochlorides of Urethanes

0.05 of a millimole of the salt was dissolved in 5 c.c. of water and 1 c.c. of this diluted to 100 c.c. 2 c.c. of this solution was employed as described. The final concentration is thus $2 \times 10^{-6}$ molar.

Results are shown in Table I.
(b) Methiodides of Urethanes

0.05 millimole of the methiodide was dissolved in 10 c.c. of water and 2 c.c. of this solution used. The final concentration is therefore $10^{-4}$ molar. The results are shown in Table II.

In the first instance the last two substances were inadvertently mixed with 5 c.c. of a buffer of double the usual concentration during contact with the enzyme previous to the hydrolysis. These observations are shown in brackets in the table. This produced a rise in inhibition. The experiment was therefore repeated in the case of these compounds and one other. The same liver extract was used, but appeared to have become more active in the interval of two days between the experiments. The inhibitions are also greater and in fact it has been observed on other occasions that such a parallelism commonly occurs. The inhibition shown in the last column of this part of the table is calculated proportionally to the value of the first member as shown in the first part, a procedure which seems justified by the similar relationship of the figures for the last two substances in both experiments.

2. Tributyrin as substrate

The hydrolysis of this substrate was followed by the stalagmometric method of Rona and Michaelis (32). The procedure used was that described by
Stedman and Stedman (13) for use in similar cases. Briefly it is as follows:

Up to 5 c.c. (depending on its activity) of the enzyme extract were mixed with 2 c.c. of \( \frac{M}{3} \) phosphate buffer of pH 8.2, and made up to 10 c.c. 1 c.c. of this was mixed with 1 c.c. of a solution of a suitable concentration (determined beforehand by trial) of the salt of each urethane. The mixtures were left for 1 hour before use.

In the case of the control water was used instead of the urethane solution.

One c.c. of the mixture was then pipetted into a mixture of 50 c.c. of a saturated filtered solution of tributylin in water and 5 c.c. of the phosphate buffer. This mixture had been previously placed in a thermostat at 20°, where it remained during the hydrolysis. The drop number was taken at once by means of a stalagmometer. The observation was repeated twice at intervals of 20 minutes. As nearly one hour is taken for each experiment the interval elapsing between the first and last member of a series of seven (including the control) was considerable. A second control was therefore done at the end of the series. No difference was, however, noticed between these.

(a) Hydrochlorides of Urethanes

0.05 millimole of these were dissolved in each
case in 25 c.c. of water and of this 2 c.c. were
diluted to 100 c.c. 1 c.c. of these solutions was
used. This gives a final concentration of about
$4 \times 10^{-7}$ molar. The results are shown in Table III.
It will be noticed that the second difference in the
case of the controls and one of the others is less
than the first. This is due to the velocity of the
reaction ceasing to be linear owing to disappearance
of too much substrate. The figures (in brackets) in
the next column are therefore got by doubling the
first reading.

(a) Methiodides of Urethanes

0.1 of a millimole of the methiodide was
dissolved in 5 c.c. of water and 1 c.c. diluted to
10 c.c. 1 c.c. of this solution was used, a final
concentration of approximately $2 \times 10^{-5}$ molar. The
remarks made in section (a) above regarding the
method of arriving at the total differences in the
case of the controls apply here also. The results
are shown in Table IV.

8. Experiments with cat's liver extract

The fresh liver (83 g.) of a fully-grown cat
was minced finely after removal of the gall-bladder
and as much fibrous tissue as was easily practicable.
It was ground up with 320 c.c. of acetone and stood
for one hour in a stoppered bottle. The extract was
filtered off and rejected and the solid residue
extracted similarly once again with acetone, once with 50% acetone-ether, and twice with pure ether. (The ether used had been recently recovered from previous liver extractions, by distillation, and so should be free of peroxide.) The residue was air-dried overnight and finely sieved. 9 g. of powder and 6 g. of fibrous residue were obtained. Two separate livers were used on different occasions. The second was that of a younger animal and gave a better yield of powder. The esterase content was about the same in the extracts from both powders.

2 g. of the powder were extracted as described in section A of this part, and the extract used in the following experiments.

1. Methyl butyrate as substrate.

A preliminary experiment with 5 c.c. of the ammoniacal extract before addition of acetic acid showed a very small degree of activity. The procedure was exactly as described in Section A(1) of this part, the pH being 8.9.

After precipitation of protein by acetic acid, dialysis and final centrifuging the activity was again tested by the same method, but no increase was noticed. A third test was carried out at a pH of 6.8 the method being the same as that used before except that 70 c.c. of \( \frac{8}{3} \) phosphate buffer and 15 c.c. of water were substituted for the 20 c.c. of ammonia
buffer and 65 c.c. of water, and that 5 c.c. of \( \frac{1}{3} \) phosphate buffer were substituted for the 5 c.c. of diluted ammonia buffer. Thymolphthalein was used as indicator. There was no marked rise in activity. The figures are quoted in Table V.

As the activity was too small to admit the employment of buffered solutions a continuous titration method was tried.

100 c.c. of water were warmed to 30° in a thermostat and a measured quantity of methyl butyrate dissolved in it. 10 drops of the chosen indicator were added, and then sufficient sodium hydroxide solution to bring the mixture to the required pH. This was always chosen as the most alkaline permitted by the indicator, the colour being matched with a standard. In the preliminary experiments the alkali was one-fifth normal. 5 c.c. of the enzyme extract were added at a definite time, the colour maintained by alkali addition and a reading taken every five minutes for twenty minutes. Tests were carried out with brom-cresol purple at a pH of about 6.8 and with brom-thymol blue at a pH of about 7.6, using 1 c.c. of methyl butyrate. A third test carried out with brom-thymol blue and ½ c.c. of methyl butyrate was done as it was thought that the large excess of substrate might possibly inhibit the enzyme. The results (Table VI) show that this is not so, and
1 c.c. of methyl butyrate was therefore used in all subsequent cases. It will be seen also that the activity is the same at pH 6.8 and 7.6. The latter was chosen on account of the better colour change of brom-thymol blue. Comparison of this table with Table V. shows moreover that there is no appreciable inhibition by the indicator or the alcohol in which it is dissolved, it being of course remembered that only one-fifth of the whole is titrated each time in buffered solution.

In view of the above results the alkali used in all the following experiments was \( \frac{N}{50} \) sodium hydroxide.

The method first employed in measuring the inhibitions was to add to 5 c.c. of the enzyme preparation 1 c.c. of the salt of the urethane derivative, to add to this 1 drop of brom-thymol blue and then make just alkaline with \( \frac{N}{50} \) alkali. (It was found to be necessary that this mixture should be rather alkaline as otherwise the inhibition rose during the hydrolysis, where the medium was of higher pH.) After standing for 1 hour 5 c.c. of the solution were used for the hydrolysis which was followed in the manner already described. The results got by this method were, however, widely discrepant, a circumstance correctly attributed to variations in the pH at which the enzyme-inhibitor
mixture was stood previous to hydrolysis. In order to avoid this difficulty the experiments were repeated with this variation; that the whole of the enzyme extract was made slightly alkaline before mixing with the different urethane solutions. The results of this were more satisfactory. They are shown in Table VII. The series, as will be seen, included both the methiodides and the hydrochlorides. The final concentration of the inhibitors was in all cases $2 \times 10^{-5}$ molar. As the observations were conducted two at a time rather a long interval (4 hours) elapsed between the first and the last, and the first one was therefore repeated. It will be seen that the inhibition has fallen off. This is due to a change in pH which always occurred when the esterase preparations were allowed to stand, as had indeed been observed previously. (This change is not due to bacterial action, as it occurs when the solution is saturated with chloroform. It is probably due to an enzyme, e.g. an oxidase.) As the change is small a correction has been applied to each inhibition proportional to the time which elapsed between it and the first pair. The corrected figures are in the last column. It is doubtful whether such a procedure is in general justifiable, but the differences here are small and the corrections are not significant.
In order to get over this difficulty of changing pH it was decided to repeat the experiment in exactly the same manner except that in this case a whole series of six titrations were carried out at once. Readings were taken every ten minutes. The results are shown in Table VIII. It will be noticed at once that the relative inhibiting powers of the different hydrochlorides shown bear no relation to those in Table VII. It was suggested that this was probably due to an accidental difference in the pH at which the enzyme and inhibitor were in contact. That this idea was well grounded was shown by an experiment in which two inhibitors, the hydrochlorides of the methyl urethanes of α-m-hydroxyphenylethylidimethylamine and ω-m-hydroxyphenylbenzylidimethylamine, were each used in three different concentrations so as to cover a good range of inhibitions. The experiment was done at two different values of pH, and it was found that whereas mitotine hydrochloride was the better inhibitor in each case in the less alkaline solution, at the higher pH the position was always reversed. The latter case gave, as expected, greater inhibitions.

2. Tributyrin as substrate

The power of hydrolysing tributyrin shown by the cat's liver extracts was about the same as that
of those from pig's liver, and the same method was used for measuring the inhibitions. The results in the case of the urethane hydrochlorides, using a phosphate buffer of pH 8.2 are shown in Table IX. The final concentration of the inhibiting salt was $4 \times 10^{-6}$ molar.

A similar experiment in the case of the methiodides gave the results in Table X. The pH in this case was 8.0 and the final concentration of the inhibitor $5 \times 10^{-5}$ molar.

An experiment was also conducted where methyl butyrate and tributyrin were hydrolysed by portions of the same enzyme-inhibitor mixture. The procedure for methyl butyrate was exactly as outlined above, four substances being employed in the first run and three in the second, one being common to both in order to show any variation which might have arisen through change in pH of the enzyme extract. This is seen from the results (Table XI.) to have occurred. The tributyrin experiments were carried out with 0.5 c.c. of the mixture of enzyme and urethane. The final concentration of the latter was $4 \times 10^{-6}$ molar in the case of methyl butyrate and $8 \times 10^{-7}$ molar in the case of tributyrin.
In the following tables of esterase inhibition experiments the urethanes used were of the general formula:

\[
\text{CH}_3\text{.NH.CO.O}\quad CR(R')\text{.N(CH}_3\text{)}_2
\]

The particular base whose hydrochloride or methiodide is referred to is therefore indicated by quoting the substituents \( R, R' \) only.
TABLE I.  (p 79)

Enzyme, pig's liver.
Substrate, methyl butyrate.
Inhibitors, hydrochlorides.
Ammonia buffer, pH, 8.9.

<table>
<thead>
<tr>
<th>Inhibitor (R, R')</th>
<th>Titrations</th>
<th>Differences</th>
<th>Total Difference</th>
<th>Percentage Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.5 11.6 13.9 15.95</td>
<td>2.1 2.3 2.05</td>
<td>6.45</td>
<td>-</td>
</tr>
<tr>
<td>H, H</td>
<td>9.4 10.3 11.4 12.6</td>
<td>0.9 1.1 1.2</td>
<td>3.2</td>
<td>50</td>
</tr>
<tr>
<td>H, CH₃</td>
<td>9.35 9.85 10.5 11.15</td>
<td>0.5 0.65 0.65</td>
<td>1.8</td>
<td>72</td>
</tr>
<tr>
<td>CH₃, CH₃</td>
<td>9.3 10.55 11.8 12.9</td>
<td>1.25 1.25 1.1</td>
<td>3.6</td>
<td>45</td>
</tr>
<tr>
<td>H, C₂H₅</td>
<td>9.2 10.35 11.5 12.8</td>
<td>1.15 1.15 1.3</td>
<td>3.6</td>
<td>45</td>
</tr>
<tr>
<td>H, C₃H₇</td>
<td>9.35 10.75 12.2 13.65</td>
<td>1.4 1.45 1.45</td>
<td>4.3</td>
<td>33</td>
</tr>
<tr>
<td>H, C₆H₅</td>
<td>9.25 10.3 11.25 12.2</td>
<td>1.05 0.95 0.95</td>
<td>2.95</td>
<td>54</td>
</tr>
</tbody>
</table>

TABLE II.  (p 80)

Enzyme, pig's liver.
Substrate, methyl butyrate.
Inhibitors, methiodides.
Ammonia buffer, pH, 8.9.

<table>
<thead>
<tr>
<th>Inhibitor (R, R')</th>
<th>Titrations</th>
<th>Differences</th>
<th>Total Difference</th>
<th>Percentage Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.6 11.65 13.85 15.9</td>
<td>2.05 2.2 2.05</td>
<td>6.3</td>
<td>-</td>
</tr>
<tr>
<td>H, H</td>
<td>9.55 10.7 11.7 12.85</td>
<td>1.15 1.0 1.15</td>
<td>3.3</td>
<td>48</td>
</tr>
<tr>
<td>H, CH₃</td>
<td>9.5 10.8 11.95 13.1</td>
<td>1.3 1.15 1.15</td>
<td>3.6</td>
<td>43</td>
</tr>
<tr>
<td>CH₃, CH₃</td>
<td>9.55 10.8 11.9 13.1</td>
<td>1.25 1.1 1.2</td>
<td>3.55</td>
<td>44</td>
</tr>
<tr>
<td>H, C₂H₅</td>
<td>9.55 10.75 11.95 13.15</td>
<td>1.2 1.2 1.2</td>
<td>3.6</td>
<td>43</td>
</tr>
<tr>
<td>(H, C₃H₇)</td>
<td>(9.8 10.7 11.7 12.6)</td>
<td>(0.9 1.0 0.9)</td>
<td>(2.8)</td>
<td>(56)</td>
</tr>
<tr>
<td>(H, C₆H₅)</td>
<td>(9.8 10.5 11.45 12.25)</td>
<td>(0.7 0.95 0.8)</td>
<td>(2.45)</td>
<td>(61)</td>
</tr>
<tr>
<td>Control</td>
<td>9.9 12.05 14.1 16.2</td>
<td>2.15 2.05 2.1</td>
<td>7.3</td>
<td>-</td>
</tr>
<tr>
<td>H, C₂H₅</td>
<td>9.8 10.65 11.55 12.55</td>
<td>0.85 0.9 1.0</td>
<td>2.75</td>
<td>62 (43)</td>
</tr>
<tr>
<td>H, C₃H₇</td>
<td>9.75 10.7 11.7 12.6</td>
<td>0.95 1.0 0.9</td>
<td>2.85</td>
<td>61 (42)</td>
</tr>
<tr>
<td>H, C₆H₅</td>
<td>9.85 10.6 11.35 12.2</td>
<td>0.75 0.75 0.85</td>
<td>2.35</td>
<td>68 (47)</td>
</tr>
</tbody>
</table>
TABLE III. (p 81)

Enzyme, pig's liver.
Substrate, tributyrin.
Inhibitors, hydrochlorides.
Phosphate buffer, pH, 8.2.

<table>
<thead>
<tr>
<th>Inhibitor (R, R')</th>
<th>Drop Numbers</th>
<th>Differences</th>
<th>Total Difference</th>
<th>Percentage Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>120 105 92</td>
<td>15</td>
<td>(30)</td>
<td></td>
</tr>
<tr>
<td>H, H</td>
<td>120 113 105</td>
<td>7</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>H, CH₃</td>
<td>121 115 107</td>
<td>6</td>
<td>14</td>
<td>53</td>
</tr>
<tr>
<td>CH₃, CH₃</td>
<td>120 114 106</td>
<td>6</td>
<td>14</td>
<td>53</td>
</tr>
<tr>
<td>H, C₆H₅</td>
<td>120 111 102</td>
<td>9</td>
<td>18</td>
<td>40</td>
</tr>
<tr>
<td>H, C₆H₇</td>
<td>120 107 97</td>
<td>13</td>
<td>(36)</td>
<td>13</td>
</tr>
<tr>
<td>H, C₆H₆</td>
<td>120 111 102</td>
<td>9</td>
<td>18</td>
<td>40</td>
</tr>
<tr>
<td>Control</td>
<td>120 105 92</td>
<td>15</td>
<td>(30)</td>
<td></td>
</tr>
</tbody>
</table>

TABLE IV. (p 82)

Enzyme, pig's liver.
Substrate, tributyrin.
Inhibitors, methiodides.
Phosphate buffer, pH, 8.2.

<table>
<thead>
<tr>
<th>Inhibitor (R, R')</th>
<th>Drop Numbers</th>
<th>Differences</th>
<th>Total Difference</th>
<th>Percentage Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>120 103 90</td>
<td>17</td>
<td>(34)</td>
<td></td>
</tr>
<tr>
<td>H, H</td>
<td>121 114 107</td>
<td>7</td>
<td>14</td>
<td>59</td>
</tr>
<tr>
<td>H, CH₃</td>
<td>121 115 109</td>
<td>6</td>
<td>12</td>
<td>65</td>
</tr>
<tr>
<td>CH₃, CH₃</td>
<td>121 113 105</td>
<td>8</td>
<td>16</td>
<td>53</td>
</tr>
<tr>
<td>H, C₆H₅</td>
<td>121 113 106</td>
<td>8</td>
<td>16</td>
<td>53</td>
</tr>
<tr>
<td>H, C₆H₇</td>
<td>121 113 105</td>
<td>6</td>
<td>13</td>
<td>62</td>
</tr>
<tr>
<td>H, C₆H₆</td>
<td>121 115 108</td>
<td>6</td>
<td>13</td>
<td>62</td>
</tr>
<tr>
<td>Control</td>
<td>121 104 90</td>
<td>17</td>
<td>(34)</td>
<td></td>
</tr>
</tbody>
</table>

TABLE V. (p 84)

Enzyme, cat's liver.
Substrate, methyl butyrate.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Buffer, pH</th>
<th>Titrations</th>
<th>Differences</th>
<th>Total Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammoniacal</td>
<td>Ammonia, 8.2</td>
<td>9.75 10.6 10.3</td>
<td>.25 .3</td>
<td>.55</td>
</tr>
<tr>
<td>Dialysed</td>
<td>Ammonia, 8.2</td>
<td>9.75 10.1 10.35</td>
<td>.3 .15</td>
<td>.55</td>
</tr>
<tr>
<td>Dialysed</td>
<td>Phosphate, 6.2</td>
<td>9.2 10.1 10.4</td>
<td>.3 .3</td>
<td>.6</td>
</tr>
</tbody>
</table>
TABLE VI. (p 84)
Enzyme, cat's liver.
Substrate, methyl butyrate.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>pH</th>
<th>cc. of substrate</th>
<th>Readings</th>
<th>Differences</th>
<th>Total Diff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brom-cresol purple</td>
<td>6.8</td>
<td>1.0</td>
<td>0.05</td>
<td>-0.4</td>
<td>0.75 1.0 1.3</td>
</tr>
<tr>
<td>Brom-thymol blue</td>
<td>7.6</td>
<td>1.0</td>
<td>1.45</td>
<td>1.75</td>
<td>2.1 2.4 2.75</td>
</tr>
<tr>
<td>Brom-thymol blue</td>
<td>7.6</td>
<td>0.5</td>
<td>2.75</td>
<td>3.05 3.3 3.55 3.8</td>
<td>.5  .25 .25 .25 .25 .25 .25 .25</td>
</tr>
</tbody>
</table>

TABLE VII. (p 86)
Enzyme, cat's liver.
Substrate, methyl butyrate.
Inhibitors, hydrochlorides and methiodides.
Indicator, brom-thymol blue.

<table>
<thead>
<tr>
<th>Time P.M.</th>
<th>Inhibitor (R, R')</th>
<th>Readings</th>
<th>Percentage Inhibition</th>
<th>Correction</th>
<th>% inhibition (corrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Control</td>
<td>0.0 2.3 4.5 6.7 8.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.55</td>
<td>H, H HCl</td>
<td>0.0 0.6 1.3 2.0 2.7</td>
<td>70</td>
<td>0</td>
<td>70</td>
</tr>
<tr>
<td>3.57</td>
<td>H, CH₃ HCl</td>
<td>0.0 1.0 1.9 2.9 3.8</td>
<td>57</td>
<td>0</td>
<td>57</td>
</tr>
<tr>
<td>4.36</td>
<td>CH₃, CH₃ HCl</td>
<td>0.0 0.8 1.6 2.4 3.2</td>
<td>64</td>
<td>2</td>
<td>66</td>
</tr>
<tr>
<td>4.38</td>
<td>H, C₂H₅ HCl</td>
<td>0.0 1.0 2.1 3.1 4.1</td>
<td>54</td>
<td>2</td>
<td>56</td>
</tr>
<tr>
<td>5.25</td>
<td>H, C₃H₇ HCl</td>
<td>0.0 1.3 2.6 3.9 5.2</td>
<td>42</td>
<td>4</td>
<td>46</td>
</tr>
<tr>
<td>5.22</td>
<td>H, C₆H₅ HCl</td>
<td>0.0 0.7 1.4 2.0 2.6</td>
<td>71</td>
<td>4</td>
<td>75</td>
</tr>
<tr>
<td>6.06</td>
<td>H, CH₃ CH₃I</td>
<td>0.0 1.4 2.7 4.0 5.3</td>
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<td>46</td>
</tr>
<tr>
<td>7.58</td>
<td>H, CH₃ CH₃I</td>
<td>0.0 1.8 3.6 5.3 7.0</td>
<td>21</td>
<td>12</td>
<td>33</td>
</tr>
<tr>
<td>6.58</td>
<td>CH₃, CH₃ CH₃I</td>
<td>0.0 1.6 3.3 5.1 6.8</td>
<td>24</td>
<td>9</td>
<td>33</td>
</tr>
<tr>
<td>7.00</td>
<td>H, C₂H₅ CH₃I</td>
<td>0.0 1.6 3.2 4.8 6.3</td>
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<td>9</td>
<td>32</td>
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<td>7.28</td>
<td>H, C₃H₇ CH₃I</td>
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<td>30</td>
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<tr>
<td>7.30</td>
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<td>33</td>
<td>10</td>
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<tr>
<td>7.36</td>
<td>H, H HCl</td>
<td>0.0 0.9 1.9 2.7 3.7</td>
<td>58</td>
<td>(12)</td>
<td>(70)</td>
</tr>
</tbody>
</table>
TABLE VIII.

Enzyme, cat's liver.
Substrate, methyl butyrate.
Inhibitors, hydrochlorides.
Indicator, brom-thymol blue.

<table>
<thead>
<tr>
<th>Inhibitor (R, R')</th>
<th>Readings</th>
<th>Differences</th>
<th>Total Difference</th>
<th>Percentage Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.9 21.1 25.3 29.4 33.5</td>
<td>4.2 4.2 4.1 4.1 16.6</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>H, H</td>
<td>16.6 17.2 19.0 20.1 21.3</td>
<td>1.2 1.2 1.1 1.2 4.7</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>H, CH₃</td>
<td>19.1 20.3 21.6 22.8 24.1</td>
<td>1.2 1.2 1.1 1.2 5.0</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>CH₃, CH₃</td>
<td>18.5 19.5 20.4 21.4 22.4</td>
<td>1.0 1.0 1.0 1.0 3.9</td>
<td>71</td>
<td></td>
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<tr>
<td>H, C₂H₅</td>
<td>19.0 20.3 22.2 23.6 24.9</td>
<td>1.3 1.3 1.4 1.3 5.3</td>
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<tr>
<td>H, C₃H₇</td>
<td>18.4 19.5 20.6 22.2 23.5</td>
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<td>59</td>
<td></td>
</tr>
<tr>
<td>H, C₆H₅</td>
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<td>1.7 1.5 1.8 1.6 6.6</td>
<td>60</td>
<td></td>
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</tbody>
</table>
TABLE IX.  (p 88)

Enzyme, cat's liver.
Substrate, tributyrin.
Inhibitors, hydrochlorides.
Phosphate buffer, pH, 8.2.

<table>
<thead>
<tr>
<th>Inhibitor (R, R')</th>
<th>Drop Numbers</th>
<th>Differences</th>
<th>Total Difference</th>
<th>Percentage Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>121 108 97</td>
<td>13 11</td>
<td>24</td>
<td>75</td>
</tr>
<tr>
<td>H, H</td>
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<td>75</td>
</tr>
<tr>
<td>H, CH₃</td>
<td>121 112 103</td>
<td>9 9</td>
<td>18</td>
<td>75</td>
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<tr>
<td>CH₃, CH₃</td>
<td>121 112 102</td>
<td>9 10</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>H, C₆H₅</td>
<td>121 111 100</td>
<td>10 11</td>
<td>21</td>
<td>13</td>
</tr>
<tr>
<td>H, C₃H₇</td>
<td>121 111 100</td>
<td>10 11</td>
<td>21</td>
<td>13</td>
</tr>
<tr>
<td>H, C₃H₅</td>
<td>121 120 118</td>
<td>1 2</td>
<td>3</td>
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</table>

TABLE X.  (p 88)

Enzyme, cat's liver.
Substrate, tributyrin.
Inhibitors, methiodides.
Phosphate buffer, pH, 8.0.

<table>
<thead>
<tr>
<th>Inhibitor (R, R')</th>
<th>Drop Numbers</th>
<th>Differences</th>
<th>Total Difference</th>
<th>Percentage Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>122 107 95</td>
<td>15 12</td>
<td>(30)</td>
<td>-</td>
</tr>
<tr>
<td>H, H</td>
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<td>6 6</td>
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<td>60</td>
</tr>
<tr>
<td>H, CH₃</td>
<td>123 117 111</td>
<td>6 6</td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td>CH₃, CH₃</td>
<td>122 115 107</td>
<td>7 8</td>
<td>15</td>
<td>50</td>
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<tr>
<td>H, C₆H₅</td>
<td>123 117 111</td>
<td>6 6</td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td>H, C₃H₇</td>
<td>123 114 106</td>
<td>9 8</td>
<td>17</td>
<td>43</td>
</tr>
<tr>
<td>H, C₃H₅</td>
<td>123 129 115</td>
<td>4 4</td>
<td>8</td>
<td>73</td>
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</tbody>
</table>
**TABLE XI.** (p 88)

Enzyme, cat's liver.
Substrates, methyl butyrate and tributyrin.
Inhibitors, hydrochlorides.
Phosphate buffer, pH, 8.0. (Tributyrin)
Indicator, brom-thymol blue. (Methyl butyrate)

<table>
<thead>
<tr>
<th>Time (P.M.)</th>
<th>Inhibitor (R, R')</th>
<th>Readings</th>
<th>Differences</th>
<th>Total Difference</th>
<th>Percentage Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>H, H</td>
<td>10.8 15.4 20.4 25.2 29.9</td>
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<td>19.1</td>
<td>-</td>
<td>74</td>
</tr>
<tr>
<td>H, CH₃</td>
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<td>1.1 1.3 1.3 1.3</td>
<td>5.0</td>
<td>77</td>
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</tr>
<tr>
<td>CH₃, CH₃</td>
<td>12.3 13.3 14.5 15.6 16.7</td>
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<td>0.9 0.9 1.1 1.0</td>
<td>3.8</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>H, C₂H₅</td>
<td>13.2 14.4 15.6 16.9 18.2</td>
<td>1.2 1.2 1.3 1.3</td>
<td>5.0</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>H, C₂H₅</td>
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<td>1.9 2.0 1.9 2.0</td>
<td>7.8</td>
<td>59</td>
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</tr>
<tr>
<td>H, C₃H₇</td>
<td>17.0 18.8 20.7 22.5 24.4</td>
<td>1.8 1.9 1.8 1.9</td>
<td>7.4</td>
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<tr>
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<tr>
<td>H, H</td>
<td>122 116 108</td>
<td>6 8</td>
<td>14</td>
<td>77</td>
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<tr>
<td>H, CH₃</td>
<td>122 119 115</td>
<td>3 4</td>
<td>7</td>
<td>80</td>
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<tr>
<td>CH₃, CH₃</td>
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<td>122 120 114</td>
<td>2 6</td>
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<td>3 4</td>
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<td>H, C₆H₅</td>
<td>124 121 117</td>
<td>3 4</td>
<td>7</td>
<td>77</td>
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</table>
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