REACTIONS OF SOME CARBOXYLIC ACID DERIVATIVES

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WILLIAM VINCENT RAFTERY

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<th>Abbreviation</th>
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<tr>
<td>Benzyloxybutyric acid</td>
<td>4-Benzyloxybutyric acid</td>
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<tr>
<td>Benzyloxyvaleric acid</td>
<td>5-Benzyloxyvaleric acid</td>
</tr>
<tr>
<td>DCC</td>
<td>Dicyclohexylcarbodiimide</td>
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<tr>
<td>RDS</td>
<td>Rate-determining step</td>
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<td>NMR</td>
<td>Nuclear magnetic resonance</td>
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<td>MS</td>
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<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
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<tr>
<td>UV</td>
<td>Ultraviolet</td>
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<tr>
<td>G.U.</td>
<td>Glasgow University</td>
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A B S T R A C T

Aromatic esters of 4-hydroxybutyric and 5-hydroxyvaleric acids have been synthesised for the first time and their hydrolysis studied in the presence of a variety of catalysts. Generally the rates were greatly increased over those for the analogous non-hydroxy-substituted esters. The hydrolysis of phenyl 4-hydroxybutyrate is catalysed by hydroxide ions at pH's greater than 3.6 and by hydronium ions at pH's less than 3.6, there being no observable spontaneous hydrolysis. Catalysis by phosphate, acetate and imidazole was also observed. The first two of these probably act as general base catalysts but imidazole is probably a nucleophilic catalyst. The hydrolysis in moderately concentrated perchloric acid yielded data from which Hammett and Bunnett plots were constructed. The factors influencing the rates of ring closure for 5 and 6-rings have been analysed. An alternative explanation has been suggested to explain the inversion of configuration that occurs in the rearrangement of 4 and 5-alkoxyacyl chlorides to the alkyl 4 and 5-chloro-esters, respectively. The first example of alkoxy-cleavage in the
rearrangement of a 4-alkoxyacyl chloride is reported.

A novel synthesis of 2-cyanobenzoic acid is reported. Evidence for the existence of an unsubstituted isoimide was shown using infrared spectroscopy. No intermediate was detected in the acid catalysed hydrolysis of 2-cyanobenzoic acid to phthalamic acid. The intermediacy of phthalimide in this reaction was eliminated.

Attempts to study a novel type of catalysis by boric acid were thwarted by the failure to prepare esters of myo-inositol-2-carboxylic acid. The preparation and hydrolysis of 4-hydroxybutyronitrile were attempted.
INTRODUCTION

For many years reactions have been known which proceed at rapid rates and follow stereospecific courses owing to the presence of a second functional group in the molecule. It was soon recognised that not only was the nature of this second functionality important but also its juxtaposition to the reacting group. The name "Neighbouring Group Participation" was given to this phenomenon which has been used to explain a diverse range of chemical reactions. Of particular interest were those compounds that hydrolysed at rates that were comparable to those induced by the presence of an appropriate enzyme. This lead to the suggestion that the enzymic and non-enzymic reactions proceed by analogous mechanisms. Although such a hypothesis is attractive and work has shown the importance of certain functional groups
in enzymes it is difficult to establish definitely due to the complexity of enzymes. It is this that has prompted the search for compounds that are similar to the proposed active site in enzymes. The work described herein is a continuation of these studies.
Proposed Mechanisms for Trypsin and Chymotrypsin

Various chemical functionalities have been suggested to explain the unusual mode of reaction of esters and amides with these enzymes. Although the exact mechanism of the reaction is still keenly debated, most contributors now agree on the following qualitative description. The ester (or amide) and enzyme are in equilibrium with a loose complex of the two. This can either revert very rapidly to the initial enzyme and ester or arrange itself such that it undergoes further irreversible reaction to produce a relatively stable acyl-enzyme and alcohol (or phenol). The acyl-enzyme is further hydrolysed to the acid and original enzyme which is then available for further reaction. That part of the enzyme involved in these changes is called the "active site".

Controversy has raged over the mechanisms of these reactions with the result that a variety of chemical groups present in the enzyme have at some time been implicated. Some of these are the hydroxy-group of serine-195, the imidazolyl group of histidine-57, the amino-group of isoleucine-16 and the carboxy-group of aspartic acid-102. In an attempt to
elucidate the mechanism a wide variety of substituted esters has been studied. The following is a short review of this work.

**Intramolecular Hydroxy-Participation in the Hydrolysis of Esters**

In principle it is possible for a hydroxy-group intramolecularly bonded to an ester group to interact in three distinct ways. The first is by nucleophilic catalysis which can further be subdivided into three classes. The hydroxy-group may be unionised (A), ionised (B) or partially ionised (C).
An alkoxide ion could interact by general base catalysis (D).

Thirdly, the hydrogen of the hydroxy-group could be partially bonded to either of the oxygen atoms in the ester (E and F). This would be described as general acid catalysis.

An example of nucleophilic catalysis is the base catalysed hydrolysis of methyl 1-benzyl-1-acetyl-cis 5,7-dihydroxy-cis-decahydroisoquinoline-8a-carboxylate. This hydrolysis is 7,700 times faster than the analogous compound having a trans 7-hydroxy-group. A 1,4-interaction of the methoxy carbonyl and hydroxy-group presumably occurs on formation of the boat conformation. Such interaction is sterically impossible for the trans isomer.

A mechanism similar to that above was proposed to explain the rapid hydrolysis of a 2-amino-aromatic ester in the presence of benzaldehyde. Thus in the reaction of
4-nitrophenyl leucine at pH 6.83 benzaldehyde was six times more effective as a catalyst than imidazole despite the latter being a stronger base by fourteen powers of ten.

\[
\text{Ph-CH} + \text{NH}_2 \text{C-CO}_2 \text{Ar} \rightarrow \text{Ph-CH} \text{NH}_2 \text{C-CO}_2 \text{Ar} \rightarrow \text{Ph-CH} \text{NHR} + \text{ArOH}
\]

A similar mechanism has been proposed for the carbon dioxide catalysed hydrolysis of 2-amino-esters. Jencks et al. have suggested that the greater catalytic activity of tris-(hydroxymethyl)-aminomethane over water may be due to intramolecular general base catalysis in much the same way as proposed for the active site in \( \alpha \)-chymotrypsin.
However, this was later shown not to be so, the increased activity being due solely to the lower pK$_a$ of the alcohol.

A phenolic hydroxy-group can similarly cause great rate increases. Thus, methyl-2-hydroxytriptoate hydrolyses on warming with sodium hydroxide in aqueous methanol whereas methyl triptoate required heating with potassium hydroxide in diethylene glycol at 130° for one hour. The authors proposed either nucleophilic attack by the oxygen anion or the kinetically equivalent general acid-specific base catalysis.
The enhanced reactivity of N-acetylserinamide with 4-nitrophenyl acetate over other alcohols was thought to be much greater than expected. This prompted the authors to suggest the following mechanism.

Later, other work showed that its activity is no more than would be expected from its pKa and confirmed this by showing that 2,2,2-trifluoroethanol which has a similar pKa to N-acetylserinamide but which cannot act as a general acid behaves in the same manner. The hydrolysis of 3-substituted phenyl acetates in the presence of cyclohexaambose or cycloheptaamylose causes rate increases of 250 (for t-butyl) over that in the aqueous solution. However, only small rate increases were observed for 4-substituted phenyl acetates.
The difference was attributed to the aromatic residue in the 3-substituted esters entering the cyclohexaamose torus from the side bound by the secondary hydroxy-groups. This situates the ester linkage opposite the acidic secondary hydroxy-groups. However, interaction between the two sites occurs only with meta-substituents in the aromatic residue, presumably caused by the substituents forcing the two sites into intimate contact. Para-substituted esters were believed to position themselves the opposite way in the torus such that "the ester function is located in close proximity to the primary hydroxy-groups," thus indicating that a prerequisite of interaction is the presence of an acidic hydroxy-group.

Facilitation of the alkaline hydrolysis of alicyclic axial acetates by a hydroxy-group bearing a 1,3-diaxial juxtaposition to the acetate is well established. Thus, cholestane-3\( \beta \), 4\( \beta \)-diol 3-monoacetate and cholestane-3\( \beta \), 4\( \beta \)-diol 4-monoacetate hydrolyse about eight times faster than cholestane-3\( \beta \)-\( \alpha \)l acetate and sixteen times faster than 3\( \gamma \)-methoxy-cholestane-4\( \beta \)-\( \alpha \)l acetate.\(^{12}\)
The stabilisation is believed to be caused by general acid catalysis by the alcoholic group to either the alkyl or acyl oxygen which in turn activates the ester group to attack by nucleophiles.

Rate enhancements up to 4000 have recently been reported in similar systems.¹³
One of the few quantitative studies on hydroxy-participation was on the alkaline hydrolysis of the following systems.\textsuperscript{14}

The rate differences between 1 - 5 were never greater than 33 and were impossible to correlate with hydrogen bonding in the ground state (in carbon tetrachloride solution). The authors criticise a mechanism previously suggested\textsuperscript{15} in which the rate enhancement is attributed to stabilisation of the leaving group since in a bimolecular reaction the rate-determining step is the formation of the tetrahedral
intermediate and any rate enhancement must be explained on the basis of an increase in this step. However, this criticism would not be valid if the mechanism was concerted or indeed if the RDS was breakdown of the tetrahedral intermediate, as has been suggested. These same authors criticise a mechanism in which the hydroxy-group is bonded to either the acyl or alkyl oxygen atoms in the transition state.

\[
\begin{align*}
\text{8} & \quad \text{9} \\
\text{H} & \quad \text{H} \\
\text{R}^2 & \quad \text{R}^2 \\
\text{C} & \quad \text{C} \\
\text{O} & \quad \text{O} \\
\text{O} & \quad \text{R}^1 \\
\text{R}^1 & \quad \text{R}^1
\end{align*}
\]

An ester involving carbonyl oxygen (8) as opposed to alkyl oxygen (9) hydrogen bonding should hydrolyse faster since there is more dipole structure in the former. However, their studies show that 3 which exhibits strong hydrogen bonding between the carbonyl and hydroxy-groups is hydrolysed slower than 4 which only shows weak hydrogen
bonding between these same two groups. This is not a valid criticism since when referring to earlier work they state that "though hydrogen bonding in the ground state may be determined via infrared analysis to be to the ether oxygen the kinetically important species could be that with hydrogen bonding to the carbonyl group." Similarly, although carbonyl-hydroxy-hydrogen bonding may occur in the ground state this may not be so in the transition state. This is particularly so in aqueous solution since all intramolecular hydrogen bonding may be negated by intermolecular bonding with water. The cyclopentyl system is a poor model on which to study hydrogen bonding or kinetic studies since many conformers can exist and thus any physical or chemical properties determined are a composite of all which makes detailed analysis very difficult.

Even for two different substituents four conformers exist considering just the envelope conformation.
Involvement of a neighbouring phenolic group has been invoked many times to explain rate increases over analogous compounds. N-carbobenzyloxy-Ω-benzylaspartyl-o-hydroxyanilide (10) hydrolyses $10^4$ times faster than its para-substituted compound.\textsuperscript{18}

\[
\begin{align*}
\text{PhCH}_2\text{O-}\text{CO-NH-CH-CONH} & \quad \text{PhCH}_2\text{O-}\text{CO-CH}_2 \quad 10 \\
\text{PhCH}_2\text{O-}\text{CO-NH-CH-CONH} & \quad \text{PhCH}_2\text{O-}\text{CO-CH}_2 \\
\text{PhCH}_2\text{O-}\text{CO-CH}_2 & \quad \text{PhCH}_2\text{O-}\text{CO-CH}_2 \\
\text{PhCH}_2\text{O-}\text{CO-CH}_2 & \quad \text{PhCH}_2\text{O-}\text{CO-CH}_2 \\
\text{PhCH}_2\text{O-}\text{CO-CH}_2 & \quad \text{PhCH}_2\text{O-}\text{CO-CH}_2 \\
\end{align*}
\]

The reaction is believed to proceed via a two-step mechanism, the second step being rate-determining. The possible mechanisms proposed are:

**step 1**

**step 2**
General base catalysis has been proposed for the hydrolysis of catechol monoacetate,\textsuperscript{19} catechol monochloroacetate,\textsuperscript{20} catechol monocinnamoate,\textsuperscript{18} salicylamide,\textsuperscript{21} phenyl salicylate,\textsuperscript{22} catechol monobenzoate\textsuperscript{22} and 4-nitrophenyl-5-nitrosalicylate.\textsuperscript{23} In all these examples it is kinetically impossible to distinguish between an intramolecularly general acid catalysed reaction of the unionised ester with hydroxide ion and an intramolecularly general base catalysed reaction of the ionised ester with water. However, the latter mechanism is usually preferred since the reaction of esters with nucleophiles other than water was not enhanced.\textsuperscript{23} A reaction related to the above was recently reported as the first example of intramolecular bifunctional general acid-base interaction (non-nucleophilic) by two hydroxy-groups in the hydrolysis of a simple carboxylic ester. On the basis of a bell-shaped pH-rate profile the authors proposed that the hydrolysis of methyl 2,6-dihydroxybenzoate proceeded by one of the following mechanisms.\textsuperscript{24}
The rate enhancement calculated for mechanisms 11 and 12 over that for methyl salicylate are 0.6 and 5.4 respectively. Such a small rate difference in changing from monofunctional catalysis to bifunctional catalysis is at first surprising but in view of the known stability of 2,6 di-substituted esters may nevertheless be so. However, it is futile to compare its rate of hydrolysis with that of methyl 2,6-dimethylbenzoate since the ester linkage in the latter is essentially encased in a hydrophobic atmosphere. An alternative explanation is that the ester linkage in the dianion of methyl 2,6-dihydroxybenzoate is greatly stabilised by extended conjugation with the aromatic ring and thus the free energy of activation is much greater than in a less conjugated system. In order to determine if the phenomenon reported is real it would have to be compared to the hydrolysis of methyl 2,4-dihydroxybenzoate.

Intramolecular Keto-Particiation in the Hydrolysis of Esters

The alkaline or base catalysed hydrolysis of methyl 2-formyl benzoate was found to be $10^5$ greater than that anticipated considering electronic and steric factors alone. By following the reaction at the isobestic point of the starting
material and eventual products an intermediate was observed in the morpholine catalysed reaction which prompted the authors to propose the following mechanism.

\[
\begin{align*}
\text{CHO} & + \text{R}_2\text{NH}^+ \rightarrow \text{C} & \text{O}_2\text{Me} \\
\text{MeO} & + \text{MeOH} \\
\text{CHO} & + \text{R}_2\text{NH}^+ \rightarrow \text{C} & \text{O}_2\text{Me} \\
\end{align*}
\]

The rate constant for the alkaline hydrolysis was of the order of magnitude of that expected for the rate constant for the hydration of the aldehyde by hydroxide ions so that the latter may be the RDS.

The alkaline hydrolysis of methyl 2-benzoyl-6-methyl benzoate is also believed to proceed via the same mechanism. The nuclear methyl group causes the ester group to be
approximately perpendicular to the plane of the ring so that the negatively charged oxygen atom approaches the ester group from a perpendicular direction. Further work on 6-substituted 2-keto-benzoates has recently appeared but whereas the former regard the RDS as cyclisation, the latter regard it as the bimolecular reaction between the ester and hydroxide anion. The same phenomenon has been reported in other systems. In at least one instance a rate increase has been observed in which the aldehydic group is situated on the phenolic as opposed to the acidic residue. Thus, cinnamoylsalicylaldehyde hydrolyses $10^4$ times faster than phenyl cinnamate in basic solution. The very rapid hydrolysis is attributed to the ready formation of a hydroxylic adduct or conjugate base thereof which then interacts with the neighbouring ester group.
Intramolecular Carboxylate Participation in the Hydrolysis of Esters

The hydrolysis of phenyl succinate and phenyl glutarate has been shown to follow the dissociation curve of the acid, the rate being proportional to the concentration of the anionic species. The reaction was compared to the intermolecularly catalysed hydrolysis of esters but in view of recent work...
this was not valid since the inter and intramolecularly
catalysed reactions proceed through different mechanisms.
The authors proposed that the intramolecular reaction
proceeded via nucleophilic attack by the carboxylate anion
at the ester linkage with concomitant fission of the aryloxy-
anion on the basis of a correlation between the rate of reaction
and the $pK_a$'s of the phenols, the difference in the latter
being attributed essentially to changes in the entropies of
activation. Thus, a tetrahedral intermediate is never formed.
An anhydride was identified as an intermediate in the hydrolysis
of 4-methoxyphenyl 7-oxabicyclo (2, 2, 1) hept-2-ene-5,
6-exo-dicarboxylate. Since the two carboxy-groups are
adjacent and rigidly fixed the rate of cyclisation to the
anhydride is much faster than in the alicyclic systems, thus
allowing spectrophotometric observation of the anhydride if
its hydrolysis is not too rapid. Hydrolysis of methyl and
ethyl phthalate has similarly been shown to involve large rate
increases but the rate is proportional to the concentration of
undissociated acid.\textsuperscript{37-39} In an attempt to characterise the
"cross-over" point between rates proportional to the
undissociated and dissociated acid various mono-esters of phthalic acid were hydrolysed having leaving groups of varying affinity. Thus, for poor leaving groups (e.g. methoxy) the rate was proportional to the undissociated acid while for good leaving groups (e.g. phenoxy) the rate was proportional to the dissociated anion. For propargyl phthalate the rate was independent of pH, the two modes of reaction being equally effective. The mechanism for phenyl phthalate is nucleophilic displacement since the anhydride was observed spectrophotometrically. For the acid catalysed reaction it is impossible to observe the anhydride because of its faster decomposition than formation. The possible mechanisms suggested are:

1. \[
\begin{align*}
\text{CO}_2\text{R} & \xrightleftharpoons{} \text{CO}_2\text{H} \\
\text{OH} & \text{OR} \\
\text{C} & \text{O} \\
\text{C} & \text{O} \\
+ \text{ROH} & 
\end{align*}
\]
An alternative mechanism for the latter reaction has been proposed.
Thus, the intermediate 13 is formed in a rapid and reversible step and breaks down only slowly with loss of the poor leaving group, methanol. Intermediate 13 has previously been suggested to be formed on dissolving phthalic anhydride in ethanol.\textsuperscript{41}
The hydrolysis of acetylsalicylic acid was shown to be eighty times greater than 4-acetoxybenzoic acid at pH 6.0 and on the basis of experiments using labelled water the authors proposed an intramolecular nucleophilic carboxylate attack for the former.

\[
\begin{align*}
\text{O} & \quad \text{C} & \quad \text{O} & \quad \text{C} & \quad \text{O} & \quad \text{Me} \\
\text{C} & \quad \text{O} & \quad \text{Me} \\
\end{align*}
\]

This work has recently been repeated but no incorporation of the label into salicylic acid was found. This prompted the authors to suggest that the reaction proceeded by a general base mechanism.
Substituents in the aromatic residue are very important in influencing the course of the reaction since 3,5-dinitroacetylsalicylate hydrolyses via an intramolecular-nucleophilic mechanism. Similarly a carboxylate group at the 6-position profoundly affects the reaction, the rate in this instance being 6000 times greater than for acetylsalicylic acid. The mechanism proposed is:
The rapid hydrolysis of 14 compared to acetylsalicylic acid and the bell-shaped pH rate profile caused Morawetz and Oreskes\textsuperscript{45} to suggest that the reaction proceeds via bifunctional catalysis.
or
However, the authors assumed that both compounds hydrolyse by a common mechanism and since this has been shown to be probably not so their comparison is invalid. A more plausible mechanism has been suggested.\textsuperscript{44}

\[ 
\text{Intramolecular Imidazole Catalysis in the Hydrolysis of Esters}
\]

The hydrolysis of phenyl esters of 4-(4-imidazoyl) butyric acid are about $10^6$ greater than the corresponding intermolecular reactions.\textsuperscript{46} The mechanism of the reactions could be nucleophilic attack by the imidazole residue, general base attack by the imidazole residue on water or general acid with concomitant attack by hydroxide anion (see below).
It is impossible to differentiate kinetically between these but in view of the known mechanism of reaction of imidazole with 4-nitrophenyl acetate\textsuperscript{47} the authors preferred a nucleophilic mechanism. An unusual feature of the system in that the apparent $pK_b$ of the imidazole is substituent dependent and different from that of the corresponding methyl ester. This dichotomy was explained by postulating a substituent dependent equilibrium before the RDS.

\[
\begin{align*}
\text{imidazole} & \rightleftharpoons \text{imidazole}^{+} \quad \text{ArOH} \\
15 & \\
\text{imidazole} & \rightarrow \text{imidazole}^{+} + \text{ArOH}
\end{align*}
\]
Surprisingly, whereas the methyl ester of 15 only showed specific base catalysis 16 showed very rapid hydrolysis especially about pH7 where the imidazole exists to a great extent as its free base.
This was the first reported example of imidazole catalysis of an alkyl ester.

Nucleophilic catalysis was originally proposed\textsuperscript{48} for the hydrolysis of 4-\(\text{[2-acetoxyphenyl]}\) imidazole but in light of further work is now believed to proceed via a general base mechanism.\textsuperscript{40}

**Intramolecular Amide Participation in the Hydrolysis of Esters**

The alkaline hydrolysis of 4-nitrophenyl hippurate proceeds very rapidly via attack by the oxygen atom.\textsuperscript{49} The RDS is the hydrolysis of L-phenyloxazolin-5-one, which was observed spectrophotometrically, to the acid. The reaction scheme outlined is:

\[
\begin{align*}
\text{R}^2\text{CONHCH}_2\text{CO}_2^- & \rightarrow \text{R}^2\text{CONHCH}_2\text{CO}_2^- \\
\text{N} & \rightarrow \text{R}^2\text{CONHCH}_2\text{CO}_2^- \\
\text{O} & \rightarrow \text{R}^2\text{CONHCH}_2\text{CO}_2^- \\
\text{H} & \rightarrow \text{R}^2\text{CONHCH}_2\text{CO}_2^- \\
\text{R}^1 & \rightarrow \text{R}^2\text{CONHCH}_2\text{CO}_2^- \\
\text{R}^2 & \rightarrow \text{R}^2\text{CONHCH}_2\text{CO}_2^- \\
\end{align*}
\]
The corresponding methyl ester does not hydrolyse by participation of the amido-group. A similar reaction but involving attack by the nitrogen atom is the alkaline hydrolysis of 17. This hydrolyses 80,000 times faster than the corresponding 4-substituted amide.

\chemical{\begin{align*}
{\text{amide}} & \rightarrow \text{ester} \\
{\text{amide}} & \rightarrow \text{amide} + \text{carboxylate} + \text{amide}
\end{align*}}
Similar examples have been shown in the hydrolysis of certain peptides and esters of substituted phthalamic acids.

**Intramolecular Amino-Catalysis in the Hydrolysis of Esters**

The hydrolysis of meta and para-substituted 4-(N,N-dimethyl amino) butyrates and 5-(N,N-dimethyl amino) valerates results in large rate enhancements over the corresponding bimolecular reaction and this has been attributed to nucleophilic attack by the amino-group.

![Chemical Reaction Diagram]

A similar explanation has been suggested for the hydroxide-ion catalysed lactamisation of methyl ω-amino α-(toluene-p-sulphonamido) butyrates and valerates and for the rapid hydrolysis of 8-acyloxyquinolines although a general base mechanism has recently been suggested for the latter.
Miscellaneous Intramolecular Electrophilic Catalysis in the Hydrolysis of Esters

Esters with a quaternary ammonium cation suitably disposed have been shown to cause appreciable rate increases over the simple base.\(^5\)\(^8\)

\[
\begin{align*}
\text{18} & \quad \text{COCH}_2\text{CH}_2\text{NEt}_3 \\
\text{19} & \quad \text{COCH}_2\text{CH}_2\text{NEt}_2 \\
\text{20} & \quad \text{COCH}_2\text{CH}_2\text{NHET}_2
\end{align*}
\]

Thus, \text{18} hydrolyses 20 times faster than \text{19} and 20 1000 times faster than \text{19}. The very rapid hydrolysis of 20 has been attributed to general acid catalysis.

The hydrolysis of phenyl salicylate in borate buffers was found to be about 100 times greater than that of phenyl 2-methoxybenzoate and phenyl benzoate.\(^2\)\(^2\) The authors
proposed prior complexation of the ester with boric acid which in turn activated the ester linkage to attack by water.\textsuperscript{22, 59}

\[
\text{PhCO}_2\text{Ph} \quad + \quad \text{B(OH)}_4^- \quad \rightarrow \quad \text{PhCO}_2\text{B(OH)}_4^- + \text{H}_2\text{O}
\]

However, neighbouring hydroxy-group participation has also been suggested as an explanation for this enhanced activity (ref. 60, p.32).

\[
\text{PhCO}_2\text{Ph} \quad + \quad \text{B(OH)}_3 \quad \rightarrow \quad \text{PhCO}_2\text{B(OH)}_3 + \text{PhOH}
\]
Hydrolysis of Nitriles involving Neighbouring Group Participation

An extensive study of the preparation and stability to hydrolysis of a wide variety of cyano-acids has already been recorded. The hydrolysis occurs for 3 and 4 (but not for 2, 5 and 6) cyano-acids and is particularly fast if the cyano-group is tertiary or aromatic bound. In such reactions an acid catalysed and a spontaneous hydrolysis occurs but the latter is invariably much smaller. The enhancement caused by the 3-phenyl groups in, for example, 3-phenyl-3-cyanopropionic acid over 3-cyanopropionic acid is not outlined but presumably is at least partly steric. Similarly the enhancement by the 2, 2-dimethyl groups in 2, 2-dimethyl-3-cyanobutyric acid is presumably a result of the "geminal effect" caused by a decrease in the unfavourable rotamer distribution. In conformity with the above the very rapid hydrolysis of ocba was noted and later the rate law in acidic solution was determined. The reaction was shown to be first order with respect to undissociated ocba and hydroxonium ions between pH's 1.5 and 3.5. However, at lower pH's the rate was less than linear and this was believed to be a result of a change in the rate-determining step.
That is, between pH 1.5 and 3.5 the rate-determining step is Step 2 whereas at pH's less than 1.5, Step 1 is the RDS.

An alternative mode of attack by water on the protonated isophthalimide cation 24 is at the carbonyl position.
It was hoped to differentiate between these two mechanisms by hydrolysing ocba in water enriched with oxygen-18 since in the former only the amide group would contain the label as opposed to the carboxy-group in the latter. A reaction related to the above is the second-order Beckmann rearrangement. When 2-oximino-ketones(27) possessing the anti-configuration are treated with strong acids they are cleaved to the corresponding nitrile and carboxylic acid.

\[
\begin{align*}
\text{27} & \quad \text{H}^+ \\
R^1\text{C}=\text{C}-\text{R}^2 & \quad \rightarrow \\
R^1\text{C}=\text{C}-\text{R}^2 + \text{H}_2\text{O} & \quad \rightarrow R^1\text{CO}_2\text{H}+R^2\text{CN}+\text{H}_2\text{O}
\end{align*}
\]

If, however, the carboxylic acid and nitrile are not separate entities but are bound to the same molecule then further reaction to the amic acid can readily occur as for ocba. Such a reaction is the preparation of camphoramic acid(28) from the corresponding oximino-ketone by treatment with concentrated hydrochloric acid.
The formation of unsubstituted isoimides has been proposed many times in the literature to explain both the very ready hydrolysis of cyano-acids to amic acids\textsuperscript{61, 65, 69} and the rearrangement of cyano-acids to imides or derivatives thereof.\textsuperscript{70-74} Thus in the Bucherer synthesis of hydantoins:

\[
\begin{align*}
\text{R}_2\text{C} & \text{NH}_2 + \text{CO}_2 \rightarrow \text{R}_2\text{C} \text{CONH}_2 \\
\text{R}_2\text{C} & \text{C} = \text{N} \\
\end{align*}
\]
In one instance a mechanism was proposed that failed to consider the possible interaction of cyano-acids, the authors preferring a mechanism involving preferential hydration of a nitrile to an amide. The reaction could readily be explained by the rearrangement of an isomimide to imide or by hydrolysis of an isomimide hydrochloride to the amic acid followed by ring closure to the imide.

A reaction of biosynthetic interest which may also be related to the ready hydrolysis of cyano-acids has recently been reported. The authors showed that 3-cyanoalanine in the form of 4-glutamyl-3-cyanoalanine is a major product of fixation of inorganic cyanide in VICIA SATIVA seedlings. Similarly the biosynthesis of asparagine and aspartic acid from cyanide in L-SYLVESTRIS has been shown to be the following:

\[
\begin{align*}
\text{CN}^- + \text{CONH}_2\text{CH}_2\text{CO}_2\text{H} \rightarrow \text{CN}^- + \text{CH}_2\text{CONH}_{\text{GLUTAMYL}}\text{CHNH}_2\text{CO}_2\text{H} \rightarrow \text{CN}^- + \text{CH}_2\text{CONH}_2\text{CH}_2\text{CO}_2\text{H}
\end{align*}
\]
The von Richter reaction involves a novel type of neighboring group participation which in many ways is closely analogous to the mechanism proposed for the hydrolysis of ocba.

\[
\begin{align*}
 & \text{The rearrangement of isoimides to imides is also very important in that it is related to the Chapmann rearrangement, the detailed mechanism of which has not been elucidated. Although much work has been done on the Chapmann rearrangement there are some reactions where such a rearrangement if possible would clearly explain the formation}
\end{align*}
\]
of anomalous products. For example, $2-N^{15}$ amino-pyridine(29) when treated with hydrochloric acid or aqueous ammonia undergoes exchange of the two nitrogen atoms. The author preferred a mechanism involving hydration causing disruption of the aromaticity of the aromatic system followed by recyclisation and elimination of water.
A simpler mechanism which has been suggested for analogous systems is:

\[
\begin{align*}
\text{Cl} & \text{NH} \\
H & \text{H}_2 \text{O} u
\end{align*}
\]

The very ready hydrolysis of nitriles to amides in the presence of salts of some transition elements\(^9\) or in the presence of manganese dioxide\(^1\) has been observed. The former has since been studied quantitatively in the nickel(II) catalysed hydrolysis of phenanthroline nitrile.\(^2\) Comparison of the rate constant for the nickel(II) catalysed reaction with that for basic hydrolysis shows the former to be \(10^7\) greater. This was attributed to the very low entropy of activation change (\(+14\ \text{e.u.}\)) as opposed to that in basic hydrolysis (-20 e.u.).
The mechanism outlined for the reaction is:

The hydrolysis of some nitriles is a very facile process and at least in two instances awaits an explanation. Thus, the preparation of 1-cyanoformamide from cyanogen defied synthesis in good yield due to further hydrolysis. A reaction which may be closely related to the above was the attempted preparation of 4-cyano-1 H-2, 3-benzoxazine(30) from the corresponding chloride. In all experiments only the amide
could be isolated.

Treatment of $\Delta^1,9$-2-octalone (31) with an alkaline solution of potassium cyanide resulted in the formation of only the amide as a result of keto-participation.
This type of phenomenon had also been observed in similar systems. Carbonyl-nitrile interaction was suggested to explain the formation of mesitamide when 1-cyanoisopropyl mesitoate was treated with alkali.

An alternative mechanism would be attack by base at the carbonyl position with concurrent cyclisation followed by a Chapman rearrangement.
Examples of hydroxy-participation with nitriles are the formation of unsubstituted cyclic imidates and their hydrochlorides. A recent example was reported whereby treatment of 2-((hydroxydiphenylmethyl)ferrocenylmethyl)-trimethylammonium iodide(33) with potassium cyanide in aqueous ethanol resulted in the formation of 34. The authors proposed the following mechanism:
However, in view of the respective basicities of the oxygen and nitrogen atoms the usual imidate structure (35) seems more plausible than 34.
Similar participation was suggested for the reaction of ammonia with substituted 2-cyanobutyrolactones (36).\textsuperscript{91}

Other examples of neighbouring group participation involving the nitrile group are listed.\textsuperscript{92–100}
EXPERIMENTAL

Melting points were measured on a Kofler-Reichert hot stage melting point apparatus and are uncorrected.

IR spectra were obtained using a Unicam SP 200 or Perkin-Elmer 237 spectrophotometer unless otherwise stated and were calibrated with a polystyrene film.

NMR spectra were determined as approximately 10% solutions (CDCl₃) on 60MHz using a Varian T-60 or A-60 and Perkin-Elmer R-10. Chemical shifts were measured downfield from internal tetramethyl silane and are quoted as tau values.

UV spectra for qualitative work were obtained using a Unicam SP 800.
Elemental analysis are quoted as percentages.

The preparation of esters of 4-hydroxybutyric acid has previously proven difficult. The most general procedure so far developed, the reaction of the appropriate ester and a secondary borane, suffered from the formation of a secondary alcohol due to incomplete selectivity of the borane. This method too is restricted to the relatively inactive esters and thus may not be used to prepare hydroxy-aryl esters. The only previous report of such an ester is that of 4-hydroxy-trichlorophenyl butyrate but no details of its preparation or physical properties were reported. Similarly only aliphatic esters of 5-hydroxyvaleric acid have been reported. Due to the expected instability of these esters an easily removed hydroxy-protecting group is necessary. An ideal choice is the benzyl group since under mild hydrogenation conditions it forms the alcohol and toluene and thus can be used in the presence of groups that under more forcing conditions would also be reduced (e.g. aromatic and
nitrilic compounds). The preparation of the benzyloxy-acids is modified slightly from that reported in the literature. 108

The Preparation of Benzyloxybutyric Acid

Toluene (300 ml, previously dried by refluxing over calcium hydride) in a round-bottomed flask (3 l) with an isomantle, dropping funnel, water condenser and motor stirrer was brought to reflux. Finely ground potassium hydroxide (140 g, 2.5 moles) was added to the vigorously stirred mixture. 4-Butyrolactone (Koch-Light "Puriss" grade, 86 g, 1 mole) was added over an hour. Benzyl chloride (380 g, 3 moles, previously dried by distilling under water pump vacuum) was added dropwise over an hour and then the whole refluxed for a further 40 hours. The toluene was removed on a rotary evaporator and the residue refluxed for 4-5 hours with potassium hydroxide solution (1 l; 2 N). The cooled solution was shaken with ether (200 ml) three times and the aqueous layer retained. The latter was acidified with concentrated hydrochloric acid which was then extracted by shaking with three aliquots of ether (200 ml) and then separating the ethereal layers. These were combined, washed four times
with water and finally dried over anhydrous sodium sulphate. Evaporation of the solvent on a rotary evaporator gave a yellow oil (150 ml) which was distilled. The fraction, b.p. 140-144°/1.0 mm Hg is benzyloxybutyric acid. Yield 107 g (55%).

The NMR spectrum showed a singlet at \( \gamma = 2.75 \) (5 H), a singlet at \( \gamma = 5.58 \) (2 H), a triplet centred at \( \gamma = 6.58 \) (2 H, splitting 6 cps), a triplet centred at \( \gamma = 7.60 \) (2 H, splitting 6 cps), an asymmetric quintet centred at \( \gamma = 8.13 \) (2 H, splitting 6 cps) and a singlet at \( \gamma = -1.45 \) (1 H).

The IR spectrum showed intense absorption in the ranges 1710 cm\(^{-1}\) and 3700-2400 cm\(^{-1}\) typical of carboxylic acids.

The Preparation of Benzyloxyvaleric Acid

The procedure was identical to that for benzyloxybutyric acid. The 5-valerolactone\(^{110}\) was prepared by Baeyer-Villiger oxidation of cyclopentanone (BDH)\(^{111}\) with pertrifluoracetic acid and immediately used for the above reaction. A 45% yield of benzyloxyvaleric acid, b.p. 171-175°/1.5 mm Hg was obtained.
The NMR spectrum showed a singlet at $\gamma = 2.75$ (5H), a singlet at $\gamma = 5.60$ (2H), a triplet centred at 5.60 (2H, splitting 4 cps), an asymmetric triplet centred at $\gamma = 7.70$ (2H, splitting 6 cps), a complex region centred about $\gamma = 8.4$ (4H) and a singlet at $\gamma = -1.7$ (1H).

The IR spectrum showed intense absorption centred at 3000 cm$^{-1}$, 1700 cm$^{-1}$ and 730 cm$^{-1}$ typical of a carboxylic acid containing an aromatic residue.

Benzyloxyvaleric acid had not previously been prepared and was characterised by its 2-naphthyl ester.

**Attempted Preparation of p-Tolyl Benzyloxybutyrate**

Dicyclohexylcarbodiimide$^{112a}$ (6.8 m moles) in pyridine (1 ml) was added slowly to a stirred ice-cold solution of benzyloxybutyric acid (6.2 m moles) and p-cresol in pyridine (2 ml). A white precipitate separated. The mixture was left at 0° for a further two hours and then overnight at room temperature. Filtration of the precipitate and evaporation of the solvent gave an oil from which a crystalline material separated. Although the infrared spectrum (absorption at 1750 cm$^{-1}$) indicated the product was formed it proved
impossible to separate it, by either physical or chemical means, from the impurities.

Attempted Preparation of p-Tolyl Benzyloxybutyrate via the Acid Chloride

Thionyl chloride (0.04 moles) was added to benzyloxybutyric acid (0.02 moles) at 40° over one hour. After the complete addition of the acid the mixture was kept at 40° for one hour and then the excess thionyl chloride removed by distillation under reduced pressure. A dark brown liquid was formed. This was added to p-cresol (0.02 moles) in pyridine (20 ml, previously dried over sodium hydroxide). The mixture immediately darkened. After refluxing for 30 minutes the cooled mixture was poured into hydrochloric acid (160 ml; 2 N) to yield a black tar. Since no oil or solid separated from the mixture the desired ester was obviously not formed.

Attempted Preparation of p-Tolyl Benzyloxybutyrate

Benzyloxybutyric acid (10 m moles) and p-cresol (10 m moles, ground to a fine powder) were placed in a flask fitted with a drying tube. Thionyl chloride (22 m moles) was added and the whole allowed to stand at 40° for a further 3 hours.
The excess thionyl chloride was distilled off under reduced pressure (water pump) and the resultant oil distilled at 1 mm Hg. The IR spectrum showed the distillate to be rich in 4-butyrolactone. A repeat of the experiment but in the absence of p-cresol gave the same result. Thus the ester is not formed due to the breakdown of the acid chloride to 4-butyrolactone and (presumably) benzyl chloride.

**Preparation of 2-Naphthyl Benzyloxybutyrate**

DCC (0.039 moles) in dried pyridine (6 ml) was added slowly to a stirred, ice-cold homogeneous solution of 0.034 moles each of benzyloxybutyric acid and 2-naphthol in pyridine (9 ml). The whole was stirred at 0° for a further 2 hours and then at room temperature overnight. The precipitate was filtered and the pyridine in the filtrate removed on a rotary evaporator. On cooling the resultant oil solidified. This brown solid was recrystallised three times with just a little absolute ethanol and then from a larger volume of ethanol and some charcoal. A 40% yield of the product, m.p. 68.5-69.0° was obtained.

The NMR spectrum shows a complex region between
\( \gamma = 2-3 \) (12 H), a singlet at \( \gamma = 5.55 \) (2 H), a triplet centred at \( \gamma = 6.48 \) (2 H, splitting 6 cps), an asymmetric triplet centred at \( \gamma = 7.32 \) (2 H, splitting 6 cps) and an asymmetric quintet centred at \( \gamma = 7.98 \) (2 H, splitting 6 cps).

The IR spectrum shows intense absorption at 1750 cm\(^{-1}\) typical of aromatic esters.

The mass spectrum shows no parent ion (320) but only fragments (234, 177, 144, 115, 91) as a result of breakdown.

\[
\text{PhCH}_2\text{O(CH}_2\text{)}_4\text{C}^0\text{O} \quad \rightarrow \quad \begin{array}{c}
\text{m/e 320} \\
\text{[PhCH}_2\text{O} \text{[C}_6\text{H}_4\text{]+[C}_6\text{H}_4\text{OH]++[C}_6\text{H}_4\text{]+[CH}_2\text{]+[H]+[OH]+]} \\
\text{m/e 234} \\
\text{m/e 144} \\
\text{m/e 115} \\
\text{m/e 91}
\end{array}
\]
The breakdown of $^{114}$naphthol to fragments $^{m/e}$ 115 is well documented in the literature but how it and fragment $^{m/e}$ 234 are formed in the first instance does not seem readily explicable.

Analysis (Rapid Elemental). Found: C, 78.91; H, 6.37 and C, 78.69; H, 6.45. $C_{21}H_{18}O_3$ requires C, 78.75; H, 6.25

**Preparation of 2-Naphthyl Benzylxyvalerate**

The procedure was essentially as above but methylene chloride was used as solvent. After filtering the precipitate and evaporating the solvent an oil was formed from which a crystalline material separated after addition of a mixture of petrol (60-80°) and ethyl acetate. After standing for a day the precipitate was filtered. The solvent was evaporated from the filtrate and the residue distilled at 0.5 mm Hg. The fraction distilling at about 200° was taken up in methylene chloride, cooled to 0° and shaken with ice-cold sodium hydroxide solution (0.5N). After separation of the aqueous layer the organic layer was washed with water. The solvent was removed from the organic layer after drying with anhydrous sodium sulphate and the oil then chromatographed. The pure
component was distilled, b.p. 170-180°/0.20 mm Hg.
Yield 59%.
Gas chromatographic analysis on a 0.5% APL column at 250°
showed only one component having a retention time of 3.94
minutes.
The NMR spectrum showed a complex region between
\( \gamma = 2\text{-}3 \) (12H), a singlet at \( \gamma = 5.50 \) (2H), a triplet centred
at \( \gamma = 6.50 \) (2H, splitting 6 cps), an asymmetric triplet
centred at \( \gamma = 7.4 \) (2H, splitting 6 cps) and a complex region
centred at about \( \gamma = 8.2 \) (4H).
The IR spectrum showed intense absorption at 1750 cm\(^{-1}\)
typical of an aromatic ester.
The mass spectrum showed no parent ion (334) but only fragments
(144, 115, 101 and 91) as a result of breakdown.
Analysis (G.U.). Found: C, 79.02; H, 6.61. \( \text{C}_{22}\text{H}_{22}\text{O}_{3} \)
requires C, 79.01; H, 6.63.

**Preparation of 2-Naphthyl Methoxybutyrate**

The procedure was essentially that for 2-naphthyl
benzyloxyvalerate\(^{115}\) but pyridine was used as solvent. The
product was recrystallised from aqueous ethanol and was
shown to yield only one spot by thin layer chromatography, m.p. 31.5-32.5°. Yield 21%.

Analysis (Rapid Elemental). Found: C, 73.71; H, 5.48 and C, 73.95; H, 5.60. \( \text{C}_{15} \text{H}_{16} \text{O}_3 \) requires C, 73.75; H, 6.60.

The mass spectrum showed a parent ion of \( m/e \ 244 \) and fragment ions of 186, 144, 127, 115, 101, 69 and 58 all of which can be accounted for by the well documented fragmentation of esters.\(^{116}\)

The NMR spectrum showed a complex splitting pattern between \( \gamma = 2-3 \ (7 \text{H}) \), a triplet centred at \( \gamma = 6.50 \ (2 \text{H}, \text{splitting 6 cps}) \), a singlet at \( \gamma = 6.65 \ (3 \text{H}) \), an asymmetric triplet centred at \( \gamma = 7.30 \ (2 \text{H}, \text{splitting 6 cps}) \) and an assymetrical quintet centred at \( \gamma = 7.95 \ (2 \text{H} \text{splitting 6 cps}) \).

The IR spectrum showed an intense band at 1750 cm\(^{-1}\) typical of an aromatic ester.

**Preparation of 2-Naphthyl Butyrate**

The procedure was essentially that in the literature.\(^{117}\)

The butyroyl chloride was prepared as in reference 113. The product was found to be a low melting solid. Recrystallisation
from ether twice by cooling to \(-15^\circ\) gave a 6% yield of product, m. p. 24.0-25.0\(^\circ\).\textsuperscript{118}

Analysis (Rapid Elemental). Found: C, 78.63; H, 6.38 and C, 78.79; H, 6.49. \(C_{14}H_{14}O_2\) requires C, 78.48; H, 6.59.

Preparation of meta-substituted Phenyl Esters of Benzyloxybutyric and Benzyloxyvaleric Acids

The procedure in all cases is essentially that in the literature.\textsuperscript{119} Thus, a stirred solution of the acid (0.03 moles) and phenol (0.03 moles) in dried pyridine (10 ml) was cooled to \(0^\circ\). Thionyl chloride (0.03 moles) was added over about one hour. The mixture was left for 12 hours at \(0^\circ\) and then poured into water (80 ml). The whole was shaken well, the oil separated and the procedure repeated. The oil was again separated, dissolved in ether and this solution shaken with a cadmium chloride solution. The precipitate formed was filtered and more cadmium chloride solution added and the procedure repeated until no more precipitate formed. The ether layer was dried, the solvent evaporated and the resultant oil heated gently at 0.001 mm Hg (oil diffusion pump) in order to distil the unreacted phenol. The oil was then chromatographed
on a silica gel column using 12% ether in petrol (60-80°).
The pure fractions were combined, dried, the solvent
evaporated and the oil distilled using a molecular still at
0.001 mm Hg. The maximum yield of pure compound was not
usually attempted since this would have required repeated
chromatographic separation. The yields were usually about
30%. The compiled results for all these preparations are
given below.

<table>
<thead>
<tr>
<th></th>
<th>n = 3</th>
<th></th>
<th>n = 4</th>
<th></th>
<th>Supplier of Corresponding Phenol</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Theoretical</td>
<td>Found</td>
<td>Theoretical</td>
<td>Found</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>H</td>
<td>C</td>
<td>H</td>
<td>C</td>
</tr>
<tr>
<td>Me</td>
<td>76.03</td>
<td>7.09</td>
<td>75.84</td>
<td>7.15</td>
<td>Me</td>
</tr>
<tr>
<td>I</td>
<td>51.53</td>
<td>4.33</td>
<td>51.59</td>
<td>4.33</td>
<td>I</td>
</tr>
<tr>
<td>Cl</td>
<td>66.99</td>
<td>5.62</td>
<td>67.33</td>
<td>5.58</td>
<td>Cl</td>
</tr>
<tr>
<td>F</td>
<td>70.82</td>
<td>5.95</td>
<td>70.27</td>
<td>6.46</td>
<td>F</td>
</tr>
<tr>
<td>H</td>
<td>75.53</td>
<td>6.71</td>
<td>75.59</td>
<td>6.81</td>
<td>H</td>
</tr>
</tbody>
</table>
With the exception where \( X = F \) all the above analyses were determined at G.U. The analysis for \( X = F \) was determined by Bernhardt. The IR spectra all showed absorption at 1750 cm\(^{-1}\) typical of an aryl ester.

The NMR for the benzyloxybutyrates for the substituents H, F, Cl and I all showed absorption in the range \( \gamma = 2.2-3.3 \), a singlet at \( \gamma = 5.5 \) (2 H), a triplet centred at \( \gamma = 6.4 \) (2 H, splitting 6 cps), a triplet centred at \( \gamma = 7.3 \) (2 H, splitting 6 cps) and a quintet centred at \( \gamma = 8.0 \) (2 H, splitting 6 cps). The NMR spectrum for the Me substituent showed absorption in the range \( \gamma = 2.5-3.3 \) (9 H), a singlet at \( \gamma = 5.5 \) (2 H), a triplet centred at \( \gamma = 6.5 \) (2 H, splitting 6 cps), a triplet centred at \( \gamma = 7.4 \) (2 H, splitting 6 cps), a singlet at \( \gamma = 7.7 \) (3 H) and an asymmetrical quintet centred at \( \gamma = 8.0 \) (2 H). The NMR spectra for the benzyloxyvalerates for the substituents H, Me and Cl all showed absorption in the range \( \gamma = 2.4-3.3 \), a singlet at \( \gamma = 5.5 \) (2 H), a triplet centred at \( \gamma = 6.5 \) (2 H, splitting 6 cps), a triplet centred at \( \gamma = 7.4 \) (2 H, splitting 6 cps) and a complex region in the range \( \gamma = 8.0-8.5 \) (4 H). In addition the Me substituent had
a singlet at $\delta = 7.7$ (3 H).

**Preparation of Phenyl Butyrate**

Phenol (0.10 mole, AR) in pyridine (20 ml) was added to a stirred, ice-cold solution of butyroyl chloride $^{113}$ (0.10 mole) in pyridine (20 ml) and the whole left at 0° overnight. The remainder of the procedure was as for the previous esters. The b.p. was 143° at water pump vacuum. $^{120}$ Yield 46%.

Analysis (G.U.). Found: C, 73.22; H, 7.23. $C_{10}H_{12}O_2$ requires C, 73.15; H, 7.37.

**Attempted Preparation of 5-endo-Benzylxynorbornane-2-carboxylic Acid**

The preparation was performed as for benzyloxibutyric acid. The lactone $^{121}$ was prepared by treating norborn-2-ene-5-endo-carboxylic acid with sulphuric acid. $^{122}$ The crude oil obtained was distilled at 2 mm Hg. A sublimate, formed over a wide range of temperatures, was shown to be a mixture of the starting lactone and norborn-2-ene 5-endo-carboxylic acid. A 14.5% yield of a pale green viscous liquid distilled between 170° and 200°. However, the NMR
spectrum indicated that it was not simply the desired product but also probably contained isomers as a result of isomerisation at the 2 and 6 carbon atoms of the norbornyl residue.

**Preparation of 2-Naphthyl 6-Keto-Norbornane-2-endo-Carboxylate**

6-Keto-norbornane-2-endo-carboxylic acid was prepared according to the literature. DCC (0.02 mole) in methylene chloride was slowly added to a stirred ice-cold solution of the acid (0.02 mole) and 2-naphthol (0.02 mole) in methylene chloride. After the addition of DCC was complete the reaction was stirred at 0° for a further two hours and then at room temperature overnight. The precipitate was filtered and the solvent evaporated from the filtrate. The oil formed soon solidified and was then recrystallised four times from ethanol. TLC in 50% ether in petrol showed only one component, m.p. 115.5-119.5°.

Analysis (Rapid Elemental). Found: C, 76.76; H, 5.76 and C, 76.51; H, 5.90. C\textsubscript{18}H\textsubscript{16}O\textsubscript{3} requires C, 77.12; H, 5.75.
Preparation of Phenyl 6-Keto-Norbornane-2-endo-Carboxylate

Condensation of phenol and 6-keto-norbornane-2-endo-carboxylic acid was achieved as in the above reaction. After evaporation of the solvent the oil was dissolved in ether. On standing a precipitate formed and was filtered. The solvent was evaporated from the filtrate and the solid formed recrystallised from ethanol. The product showed only one component on TLC, m.p. 85.0-86.0°. Yield 55%.

Analysis (G.U.). Found: C, 73.02; H, 6.10. \( \text{C}_{14} \text{H}_{14} \text{O}_{3} \) requires C, 73.03; H, 6.13.

Attempted Reduction of 2-Naphthyl 6-Keto-2-endo-Carboxylate

Water (60 ml), concentrated hydrochloric acid (0.5 ml) and platinum oxide (0.249 g, Johnson Matthey) were stirred over an atmosphere of hydrogen until the catalyst was reduced. Ethanol (150 ml), ethyl acetate (50 ml) and 2-naphthyl 6-keto-2-endo-carboxylate (1.069 g, 3.8m moles) was added and the whole stirred over an atmosphere of hydrogen for about 15
hours. After cessation of further uptake of hydrogen (0.0165 moles) the catalyst was filtered off and some of the solvent removed using a rotary evaporator such that the temperature was always below 30°. After cooling this solution white crystals separated. The TLC of these in petrol as eluant showed at least two components. Further examination of these crystals was abandoned as the NMR spectrum showed only protons at high fields and the IR spectrum exhibited no absorption in the carbonyl region. The filtrate obtained after separating the above crystals was treated with excess silver carbonate to neutralise the acid. After separation of the silver salts the solvent was removed, again using the rotary evaporator, at below 30°. TLC of this solution showed the presence of at least two components. The major component of this mixture was shown by comparison of NMR and IR spectra to be the lactone derived from 6-endo-hydroxy-norbornane-2-endo-carboxylic acid. No 2-naphthol was detected at any stage of the reaction. This is not surprising since the ratio of the number of moles of hydrogen absorbed to the number of moles of starting ester is approximately
4.3 and thus further reduction has occurred. It is noteworthy that at least some reduction of the ketonic group is occurring but that the product breaks down spontaneously because of the conditions employed and/or the favourable disposition of the two functional groups of the product.

**Attempted Reduction of 2-Naphthyl 6-Keto-Norbornane-2-endo-Carboxylate**

Ethyl acetate (5 ml, dried over potassium carbonate) was added to 2-naphthyl 6-keto-norbornane-2-endo-carboxylate (0.50 g, 1.8 moles) and 10% palladium on charcoal (0.15 g, Johnson Matthey). The whole was stirred over an atmosphere of hydrogen. A rapid uptake of hydrogen occurred (approximately 48 ml at 22°) followed by no further change. TLC in 50% ether in petrol showed three components which are probably just separable by column chromatography. The catalyst was filtered and the solvent evaporated. The NMR spectrum of the crude product showed the presence of aromatic protons. Some of the crude product (0.2 g) was separated using preparative plate chromatography (Kieselgel G) with 50% ether in petrol as solvent. Exposure by iodine showed complete
separation of that component with the lowest $R_f$ which preliminary experiments had shown to be the major component. Careful separation of this section of the plate followed by elution with ethyl acetate showed that it was contaminated with that component with the highest $R_f$. This second component is only present in trace amounts. Recrystallisation from ethanol gave crystals, m.p. 82.0-86.0° but still did not remove the impurity.

The IR spectrum showed intense absorption at 1750 cm$^{-1}$ (aromatic ester) but no absorption at 3500 cm$^{-1}$ (hydroxy-group).

The NMR spectrum shows complex splitting patterns between

- $\gamma = 2.70-3.40$ (3H), $\gamma = 6.50-6.85$ (1H), $\gamma = 6.85-7.10$ (2H), $\gamma = 7.10-7.45$ (4H), $\gamma = 7.70-8.10$ (4H) and $\gamma = 8.10-8.60$ (5H).

The mass spectrum showed fragments corresponding to a charge to mass ratio of 285, 284, 283, 149, 148, 147, 137, 136, 109 and 81. Their formation can be accounted for by the well documented fragmentation of esters.
The IR, NMR and MS spectra are consistent with the following structure:

Since 10% palladium is a very mild catalyst the above reduction will probably occur with all catalysts. In order to avoid this complexity the phenyl ester was prepared.

**Attempted Reduction of Phenyl 6-Keto-Norbornyl-2-endo-Carboxylate**

Phenyl 6-keto-norbornyl-2-endo-carboxylate (220 mg) and platinum oxide (100 mg, Johnson Matthey) were stirred in an atmosphere of hydrogen (previously dried by passing through a column of silica gel) for 15 hours. The catalyst was separated and the solvent removed from the filtrate using an oil pump. Part of the residue (80 mg) was purified using preparative plate chromatography (Kieselgel G) and 50% ether
in petrol as eluant. The band nearest the spotting point was carefully removed, eluted with ethyl acetate, the kieselgel filtered and the solvent evaporated using an oil pump. The product was recrystallised from a mixture of ethyl acetate and petrol, yielding white crystals, m.p. 102.0-103.7°.

The analysis was inconsistent with the formation of phenyl 6-hydroxy-norbornane-2-carboxylate but was consistent with 6-keto-norbornane-2-endo-carboxylic acid. Analysis (G.U.). Found: C, 62.51; H, 6.50. \( \text{C}_{14} \text{H}_{16} \text{O}_3 \) requires C, 72.59; H, 6.94 whereas \( \text{C}_8 \text{H}_{10} \text{O}_3 \) requires C, 62.33; H, 6.54. The melting point of 6-keto-norbornane-2-endo-carboxylic acid is 101.5-103.5° and 103-4°. NMR and IR spectra were superimposable on that of an authentic sample.

When the above reaction was repeated but using 10% palladium on charcoal as catalyst the starting material was recovered unchanged.

The above difficulties were hoped to be circumvented by the use of a secondary borane as reducing agent. Boranes would seem to be ideal despite their ability to react with both ketonic and ester groups since it has been shown that
their reaction with ketones is much faster than that with esters. Furthermore the reaction with norbornanone has been shown to yield almost 100% endo-2-norbornanol due to the steric inhibition associated with attack from below the norbornyl residue. This same effect would be expected to be even greater in norbornan-2-ones substituted in the 6-endo-position. It has also been shown that aromatic residues are unaffected by diborane. The synthetic problems seemed even further simplified when a new air-stable borane was prepared recently.

**Attempted Preparation of 9-Borabicyclo(3.3.1) Nonane**

The procedure was exactly as described in the above paper. On cooling, what should have been a solution of 9-borabicyclo (3.3.1) nonane in tetrahydrofuran, no product separated. Further cooling in an acetone/cardice mixture also caused no separation of the desired product. Partial evaporation of the solvent followed by cooling brought about no further change. All the solvent was then evaporated, yielding an oil which remained unchanged on standing. The IR spectrum of this oil did not seem consistent with that quoted
The experiment was repeated, but the amount of borane in the THF solution was estimated. Despite the use of an efficient bubbler the uptake of borane was only about one quarter of that expected. Thus borane did not appear to be as soluble in THF as is usually assumed. The procedure was accordingly adjusted by only adding one quarter of the theoretical amount of 1,5-cyclo-octadiene and evaporating three-quarters of the solvent after the reflux period. On cooling, a white solid separated which was filtered in an atmosphere of nitrogen and then dried in a desiccator. All the physical data for this product was at variance with that in the literature.

**Preparation of 2-Naphthyl 4-Hydroxybutyrate**

2-Naphthyl benzylxybutyrate (4 g, 0.0125 moles) and 10% palladium on charcoal (0.60 g) in ethyl acetate (40 ml) were stirred in an atmosphere of hydrogen for about 12 hours. The reaction was shown to be complete by testing for the starting ester by TLC. The desired product appears to decompose on a TLC plate to 2-naphthol and 4-butyrolactone. The uptake
of hydrogen was about 600 ml at room temperature (0.025 moles). The catalyst was separated by filtration after drying with anhydrous sodium sulphate. The solvent was removed using an oil pump yielding an off-white coloured product. This was dissolved in anhydrous ether (6 ml) which was then slowly cooled in a cardice/acetone mixture. The white precipitate was filtered and dried in a desiccator, m.p. 51.5 - 55.5°. After one further recrystallisation the melting point was 54.5 - 56.0°.

The IR spectrum showed absorption at 1730 cm⁻¹ (aromatic ester) and 3500 cm⁻¹ (hydroxy-group).

The NMR spectrum shows a complex region between γ = 2 - 3 (7 H), a triplet centred at γ = 6.23 (2 H, splitting 6 cps), an asymmetric triplet centred at γ = 7.28 (2 H, splitting 6 cps) and a quintet centred at γ = 8.00 (2 H, splitting 6 cps).

The mass spectrum showed no parent ion but only the breakdown products, 2-naphthol and 4-butyrolactone and fragments further derived from these (m/e 144, 115, 86, 58).

Analysis (Rapid Elemental). Found: C, 74.06; H, 6.34 and C, 74.00; H, 6.34 and C, 72.49; H, 5.35 and C, 72.50; H, 5.32. C₁₄H₁₄O₃ requires C, 73.05; H, 6.09.
Preparation of 2-Naphthyl 5-Hydroxyvalerate

2-Naphthyl 5-benzyloxyvalerate (0.77 g, 2.34 mmoles) and 10% palladium on charcoal (77 mg, Johnson Matthey) in ethyl acetate (10 ml) were hydrogenated over about 9.5 hours. TLC in 50% ether-benzene showed a trace of the starting material. Hydrogenation was complete after another hour. The total uptake of hydrogen was 78 ml at room temperature (3.2 mmoles). After separation of the catalyst the solvent was evaporated using an oil pump. The resultant solid was recrystallised from a mixture of petrol (40-60°) and anhydrous ether by cooling to 5°. The crystals were filtered and evacuated in a desiccator using an oil pump, mp. 45.0-46.0°. Yield 0.0612 g (11%).

The NMR spectrum shows two asymmetric triplets centred at \( \tau = 6.30 \) (2 H, splitting 6 cps) and 7.35 (2 H, splitting 6 cps), a complex pattern in the range \( \tau = 8.0-8.6 \) and a complex aromatic pattern in the range \( \tau = 2-3 \) (7 H). The presence of the hydroxy-group was not visible on the NMR spectrum but was on the IR spectrum (broad band centred about 3250 cm\(^{-1}\)).

Analysis (G.U.). Found: C, 73.52; H, 6.49. \( C_{15} H_{16} O_{3} \)
Preparation of Phenyl 4-Hydroxybutyrate

Phenyl 4-benzyloxybutyrate (0.20 g, 74.1 m moles) and 10% palladium on charcoal (0.02 g) in ethyl acetate (4 ml, previously dried over potassium carbonate) were stirred in an atmosphere of hydrogen saturated in ethyl acetate and dried by passing through silica gel. The presence of any starting material was detected by TLC. After two hours the catalyst was separated and the solvent immediately evaporated using an oil pump. The sample was evacuated for a further two hours to remove traces of ethyl acetate. The sample was immediately analysed.

Analysis (G.U.). Found: C, 66.32; H, 6.72. \( \text{C}_{10} \text{H}_{12} \text{O}_3 \) requires C, 66.65; H, 6.71.

The NMR spectrum showed two asymmetric triplets centred at \( \gamma = 6.30 \) (2 H, splitting 6 cps) and \( \gamma = 7.35 \) (2 H, splitting 6 cps), a quintet centred at \( \gamma = 8.04 \) (2 H, splitting 6 cps) and a complex pattern between \( \gamma = 2.4 - 3.3 \) (5 H).

The IR spectrum showed the presence of the aromatic ester (1750 cm\(^{-1}\)) and hydroxy-group (3400 cm\(^{-1}\)).
Preparation of m-Tolyl 4-Hydroxybutyrate

The procedure was as above.

The NMR spectrum showed a complex splitting pattern between

\[ \gamma = 2.50 - 3.50 (4H), \]  

a triplet centred at \( \gamma = 6.25 (2H, \) splitting 6 cps), an asymmetric triplet centred at \( \gamma = 7.35 \) (2H, splitting 6 cps), a singlet at \( \gamma = 7.67 (3H) \) and a quintet centred at \( \gamma = 8.01 (2H, \) splitting 6 cps).

The IR spectrum showed intense absorption at 3500 cm\(^{-1}\) (hydroxy) and 1750 cm\(^{-1}\) (aromatic ester).

Analysis (G.U.). Found: C, 68.22; H, 7.45. \( C_{11}H_{14}O_3 \) requires C, 68.02; H, 7.23.

Preparation of m-Chlorophenyl 4-Hydroxybutyrate

Attempts to prepare the above ester using the procedure for phenyl 4-hydroxybutyrate resulted in mainly the formation of m-chlorophenol and 4-butyrolactone. The same problem was also encountered in all the esters of 5-hydroxyvaleric acid. It has been previously noted that dioxan solutions (0.15 M) of the esters appeared to be more stable at \(-40^\circ\) than the neat liquid esters at the same temperature. Thus by reducing the
esters in a dioxan solution it was hoped to prevent lactonisation.

A solution of m-chlorophenyl benzyloxybutyrate (0.15 M, 60 mg of ester in 1.33 ml of dioxan, BDH 'special for spectroscopy') and 10% palladium on charcoal (12 mg) was reduced by stirring with hydrogen, previously dried by passing through silica gel and saturated with dioxan, for two hours. TLC showed the absence of any starting material. Any loss of dioxan was compensated for and the catalyst then removed by filtering through glass paper. The solution was stoppered and kept at -40° when not in use. A sample (10 μl) of this solution injected into water (3.0 ml) showed that little or no phenol was present since the UV spectrum of the ester is markedly different from that of m-chlorophenol. m-Fluorophenyl 4-hydroxybutyrate and phenyl, m-chlorophenyl and m-tolyl 5-hydroxyvalerates were also prepared using the same procedure.

**Attempted Preparation of m-Iodophenyl 4-Hydroxybutyrate**

Reduction of m-iodophenyl benzyloxybutyrate in both ethyl acetate and dioxan was unsuccessful. The starting material was recovered unchanged.
Preparation of Myo-inositol-2-Carboxylic Acid (39)

The preparation of the above compound was a six-stage reaction sequence. Myo-inositol (37) (Koch-Light "Puriss" grade) was converted to scyllo-inosose (38) by bacterial oxidation. The other five stages are as outlined in the literature.

Reaction Scheme 2
The nitrogen dioxide (Air Products) used in the oxidation to the acid was untreated. The product (which does not have a clearly defined melting point\textsuperscript{131}) was shown to be an acid by spotting a sample of an aqueous solution on to a silica TLC plate. On spraying with a weakly alkaline solution of bromo-cresol green the spot appeared yellow against a blue background.

\textbf{Preparation of Scyllitol-2-Carboxylic Acid (40)}

The first three steps of this six-stage reaction sequence are as above\textsuperscript{129}. The other reactions were as for references 130 and 131.

\begin{align*}
\text{Reaction Scheme 3}
\end{align*}
Attempted Preparation of Phenyl Myo-inositol-2-Carboxylate

1) DCC (20.6 mg, 0.1m moles) in pyridine (2 ml) was added to myo-inositol-2-carboxylate (22.4 mg, 0.1m moles) and phenol (9.4 mg, 0.1m moles) in pyridine (5 ml) at 0°, with stirring. After standing overnight the precipitate of dicyclohexyl urea was filtered and the pyridine evaporated using an oil pump. The residue was dissolved in a mixture of chloroform and water. The aqueous layer was separated and freeze-dried. A sample of the resultant white product when added to water liberated no phenol even on the addition of sodium hydroxide solution.

2) Since the myo-inositol-2-carboxylic acid is not very soluble in pyridine the above reaction was repeated dissolving the acid and phenol in a mixture of pyridine (4 ml) and water (1 ml). Work up as in 1) again yielded a white product. Again no phenol was liberated when some of this was added to either alkaline or neutral solution so presumably the ester was never formed or is very rapidly hydrolysed. Other solvents used were dimethyl formamide and dimethyl sulphoxide (at room temperature), but in neither case was there any evidence for the formation of a stable ester.
3) Myo-inositol-2-carboxylic acid (11 mg, 5 x 10^{-2} m moles) and phenol (4.7 mg, 5 x 10^{-2} m moles) were refluxed for 18 hours in dimethoxyethane (50 ml) using a soxhlet containing molecular sieves (BDH, Type 5A) to trap any water formed in the reaction. The solvent was removed on a rotary evaporator. The IR spectrum of the resultant oil showed absorption about 1720 cm\(^{-1}\). A sample of this oil when added to water in a UV cell showed the presence of phenol. Repeat scans in both neutral and alkaline solution showed no further liberation of phenol. After standing for a day the oil solidified and was recrystallised from methanol. The resultant crystals, m.p. 181.5-182.5\(^{0}\), showed absorption in the IR at 1710 cm\(^{-1}\) and in the range 700-860 cm\(^{-1}\) characteristic of an aromatic carboxylic acid. The compound was positively shown to be an acid (using bromo-cresol green) and its aromaticity proven by NMR spectroscopy. It would thus appear that simple dehydration of myo-inositol-2-carboxylic acid has occurred.

4) Since the acid was not very soluble in dimethoxyethane the experiment was repeated using a mixture of solvents in one of which the acid is soluble. The experiment was repeated using phenol (2.5 mg) and myo-inositol-2-carboxylic acid
(5 mg) in dioxan (30 ml, BDH "Spectroscopic" grade) and dimethyl sulphoxide (1 ml, BDH "Spectroscopic" grade). After refluxing for 20 hours the dioxan was removed using a rotary evaporator and a sample of the resultant dimethyl sulphoxide solution added to water in a UV cell. The presence of phenol was clearly visible but repeat scans in both neutral and alkaline solution did not show the result of any further formation of phenol.

Since the preceding experiments had shown that either the phenyl myo-inositol-2-carboxylate was either very reactive or simply not being formed an attempt was made to prepare the corresponding methyl ester.

**Attempted Preparation of Methyl Myo-Inositol-2-Carboxylate**

1) Myo-inositol-2-carboxylic acid (70 mg, 0.31m moles) was refluxed for 18 hours in a solution of hydrogen chloride in methanol (1% w/v; 100 ml). The starting acid dissolved only slowly, presumably as it reacts. On cooling excess silver carbonate (Johnson Matthey) was added to neutralise the acidic solution. After filtering the solvent was evaporated resulting in the formation of a discoloured oil. The IR spectrum
showed a band centred at 1715 cm\(^{-1}\) (which may be two overlapping bands) and the absence of the characteristic absorbance of a carboxylic acid in the 3300 cm\(^{-1}\) region. The fingerprint region is also appreciably different from the starting acid. Attempts to recrystallise the product from methanol (Analar) and ethanol (absolute) failed to form any crystals. On evaporation of the solvent the IR spectrum showed that decomposition to the starting acid has occurred.

2) The above experiment was repeated using the recovered acid (25 mg) from above but reflux was continued for two days. After neutralising the solution and filtering the precipitate, the solvent was evaporated. The IR spectrum of the off-white solid showed no absorption at 1715 cm\(^{-1}\) but showed an intense band at 1640 cm\(^{-1}\). This same band was shown to be present on re-examining the IR spectrum from the previous reaction, but its intensity is much weaker. It would appear that the ester was formed but that it then reacted further to another product.

3) A milder preparation of methyl esters is the reaction of diazomethane with the corresponding carboxylic acid. This also has the further advantage that it occurs even under
heterogenous conditions. A diazomethane solution was prepared as outlined by Vogel p. 971. This was standardised by allowing a known volume of the ethereal solution to react with an excess of benzoic acid (Analar) and then titrating with sodium hydroxide solution (0.10N) to determine how much of the benzoic acid was unreacted.

Thus, one equivalent of diazomethane in anhydrous ether was added to myo-inositol-2-carboxylic acid in dried methanol (30 ml). The colour of the diazomethane immediately disappeared. More diazomethane solution was added but after standing for a few minutes the solution again became colourless. It was suspected that the inositol was catalysing (at least partly) the decomposition of diazomethane. Finally a very large excess of diazomethane solution was added and left for two days. The solvent was removed on a rotary evaporator at about 30° yielding an oil. On addition of dried methanol a white compound (20 mg) separated which was filtered and the methanol removed by evacuation. The IR spectrum of this material showed the presence of a group absorbing at 1722 cm⁻¹ (the acid absorbs at 1740 cm⁻¹).
The fingerprint region in parts resembled that of the acid but in others was appreciably different. Attempts were made to show the presence of any acid present using TLC (polyamid). Both the product and myo-inositol-2-carboxylic acid have \( R_f \) values of one in methanol and of zero in ethanol, chloroform and isopropanol (plus a drop of acetic acid). All attempts at using a mixed solvent system caused streaking in which it was impossible to determine if there was any acid in the product or any other impurity.
Preparation of 4-Hydroxybutyronitrile and 5-Hydroxyvaleronitrile

Although there are reports in the literature of the preparation of 4-hydroxybutyronitrile and 5-hydroxyvaleronitrile the experiments below indicate that probably in no instance were the compounds pure. In reference 138 the product ratios from the reaction of 5-hydroxyvaleric acid and ammonia are given but no details of this determination are given.

4-Hydroxybutyronitrile

a) The procedure was outlined as in reference 143 which is a general method of aliphatic nitrile syntheses from the corresponding alkyl chloride or bromide.

3-Chloropropan-1-ol (B. Newton Maine Ltd., 5.95 g, 63.3 m moles) was added over 10-15 minutes to a rapidly stirred solution of sodium cyanide (3.33 g, 70 m moles) in dimethyl-sulphoxide (15.6 ml) at 80°. The temperature of the mixture rose to about 90°. After maintaining the mixture at this temperature for 10 minutes it was cooled and water (37 ml) and chloroform (12 ml) added. The chloroform layer was separated and the extraction repeated four times. The combined chloroform solutions were dried with anhydrous
magnesium sulphate and the chloroform removed on a rotary evaporator. The IR spectrum of the yellow oil indicated the presence of a nitrile in the product. The crude product was distilled at 1 mm Hg. A component, b.p. 32-40°, was shown by IR spectroscopy not to contain a nitrile group. The liquid residue (about 0.2 ml), however, did contain a nitrile. It thus appeared that the reaction proceeded in very low yield. One possible cause of this is the non-homogeneity of the sodium cyanide and the 3-chloropropan-1-ol.

b) The experiment above was repeated but the sodium cyanide was ground to a fine powder. After the addition of about 4g of 3-chloropropan-1-ol the temperature of the mixture suddenly rose to approximately 140°. After cooling to 80° with a water bath the remainder of the 3-chloropropan-1-ol was added causing the temperature to rise to 135°. On cooling, water (37 ml) was added and the whole extracted three times with chloroform (12 ml). The combined chloroform extracts were dried with anhydrous magnesium sulphate and the solvent evaporated. The brown liquid formed was distilled at 3.5 mm Hg. The fraction, b.p. 20-85°, was
shown by IR spectroscopy to contain no nitrile group whereas that fraction, b.p. 85-90°, did contain a nitrile (2280 cm⁻¹). The NMR spectrum of this sample showed a singlet at γ = 7.4 which is due to dimethyl sulphoxide. Since this appears to be the major component the experiment was abandoned and some of the literature preparations attempted.

c) The procedure of the following experiment is as in reference 132.

3-Chloropropan-1-ol (22.0 g, 0.23 moles) was refluxed in a mixture of ethanol (102 ml), water (29 ml) and potassium cyanide (19.2 g, 0.30 moles) for eight hours. The precipitate was filtered and the solvent removed from the filtrate using a rotary evaporator. The brown sludge was extracted three times with ether (100 ml). After drying the combined ethereal fractions with anhydrous magnesium sulphate the solvent was evaporated. The oil formed (6 ml) was distilled at 3 mm Hg. That fraction, b.p. 82-90°, was redistilled. The IR and NMR spectra indicated that 4-hydroxybutyronitrile was not the only compound formed.

Reflux of a sample (0.7 ml) of the fraction, b.p. 82-90°, in
benzene (10 ml, Analar, dried over sodium) for 2.5, 4.5 and 6.0 hours caused the 4-hydroxybutyronitrile (absorbance at 2280 and 3500 cm$^{-1}$) to partly cyclise to iminobutyrolactone (absorbance at 1695 cm$^{-1}$). The conversion, however, was not complete.

d) The above experiment was repeated but the reflux time was now only three hours. Work-up in the usual manner showed the presence of much 3-chloropropan-1-ol in addition to the other products.

e) The following preparation was as described in reference 133.

Potassium cyanide (5.2 g, 0.08 moles, BDH "Technical" grade) in water (8 ml) was added dropwise over 30 minutes to a stirred, refluxing solution of 3-bromopropan-1-ol (10.8 g, 77m moles, B. Newton Maine Ltd.) in absolute ethanol (40 ml) and the whole stirred under reflux for eight hours and then for eighteen hours at room temperature. The solvent was evaporated and the product extracted three times with ether (40 ml). The combined ethereal fractions were dried with anhydrous sodium sulphate and the solvent evaporated.
The oil formed was distilled at 2 mm Hg. The IR and NMR spectra of the fractions, however, showed the formation of a mixture of products. The total "yield" of products was about 15%.

f) The above experiment was repeated but using twice the molar ratio of potassium cyanide. The same products were obtained, again in poor yield (15%). The latter may be caused by poor extraction of the brown sludge with ether. Further extraction, however, resulted in only modest increases to the yield.

g) Potassium cyanide (20.8 g, 0.32 moles) in water (32 ml) was added over about 30 minutes to a stirred, refluxing solution of 3-bromopropan-1-ol (10.8 g, 80 moles) in absolute ethanol (40 ml) resulting in the immediate formation of a white precipitate. Towards the end of the addition of the potassium cyanide solution the reaction mixture became slightly discoloured and over the next half-hour darkened very much more. The solvent was evaporated and the residue refluxed with ether (250 ml) for four hours. On cooling the ether layer was decanted, dried with anhydrous sodium sulphate
and the solvent evaporated. The TLC of the yellow oil formed showed three products but no 3-bromopropan-1-ol. Distillation at 3 mm Hg gave three fractions, b.p. 86-90°, 90-93° and 93-100° of total yield about 40%. The IR and NMR spectra indicated a higher proportion of 4-hydroxybutyronitrile than had any previous preparation.

h) The above experiment was repeated but the potassium cyanide solution was added over only fifteen minutes. The solvent was evaporated on a rotary evaporator and the residue formed heated for 1.5 hours with ether. The ether layer was decanted, dried with anhydrous sodium sulphate and the solvent evaporated. A yellow liquid (4.6 ml) remained which was distilled at 3.5 mm Hg. The two fractions, b.p.'s 82-89° and 89-92°, showed a very high ratio of 4-hydroxybutyronitrile to iminobutyrolactone. Attempts to purify a sample by column chromatography using laboratory grade silica as a support failed.

i) A repeat of the above reaction but adding the potassium cyanide solution over only 5-10 minutes and refluxing the brown sludge with ether for only 30 minutes resulted in a 67%
yield of the product containing a high percentage of 4-hydroxybutyronitrile. When distilled the fraction, b.p. 74.5-90.0°C, showed only the presence of one component on TLC in ether. However, the IR spectrum of this sample, even after standing over molecular sieves (BDH Type 5A) for four days still showed a band centred at 1650 cm\(^{-1}\). This impurity is probably 4-hydroxybutyramide or 2-pyrrolidone (see Discussion). An attempt was made to remove this impurity by chromatographing a sample of the above liquid on silica (Koch-Light "200-300 mesh") using 80% ether in petrol as eluant. The first seven fractions collected showed the presence of two compounds on TLC and the IR spectrum showed the presence of a band at 1650 cm\(^{-1}\). The next seven fractions were shown by TLC, using ether as eluant, to be "pure". These were combined, dried with anhydrous sodium sulphate and the solvent evaporated. The impurity characterised by absorbance at 1650 cm\(^{-1}\) was still present but its intensity was much weaker than in the starting material.

The NMR spectrum of this sample showed two triplets at \(\tau = 6.3\) (–CH\(_2\)OH) and 7.5 (–CH\(_2\) CN), a quintet centred
at $\gamma = 8.2 \ (\text{CH}_2 \text{CH}_2 \text{CH}_2)$ all with a splitting constant of 6 cycles per second and integrating for two protons and a broad band centred at $\gamma = 6.8 \ (1 \ H)$ which disappeared on shaking with D$_2$O. The above data is thus consistent with slightly impure 4-hydroxybutyronitrile.

An attempt was made to purify the 4-hydroxybutyronitrile by forming an acetal with dihydropyran. The acetal was prepared by adding three drops of concentrated hydrochloric acid to a mixture of crude 4-hydroxybutyronitrile (5.0 g, 60m moles) and dihydropyran (5.0 g, 60m moles). A vigorous reaction occurred and after cooling in water the mixture was allowed to stand for 45 minutes. Sodium hydroxide solution (10 ml; 10% w/v) and ether (30 ml) were added and the whole shaken vigorously. The ethereal layer was separated, washed with water, dried with anhydrous sodium sulphate and the solvent evaporated. The oil formed was distilled, b.p. 126-130°/1 mm Hg. Treatment of this product with hydrochloric acid (2N), followed by extraction with ether, gave a mixture of products which TLC in 50% ether in benzene showed to contain three components of similar $R_f$ values. The experiment was, therefore, abandoned.

j) Since the benzyl group has served as an ideal hydroxy-
protecting group in the preparation of esters of 4-hydroxybutyric and 5-hydroxyvaleric acids it was hoped that it might be used similarly for the preparation of 4-hydroxybutyronitrile and 5-hydroxyvaleronitrile. Thus, 4-benzyloxybutyronitrile was prepared as outlined below.

**Preparation of 3-Benzylloxypropan-1-ol**

a) The synthetic procedure is as outlined in reference 144. The actual preparation was performed by Donald McLean to whom I am very grateful.

Small pieces of sodium (12.5 g, 0.54 moles) were added cautiously to 1,3-propanediol (120 g, 1.58 moles, Koch-Light) covered with dry xylene (100 ml) at 130°. Benzyl chloride (75 g, 0.60 moles, purified by distillation) was added in small portions and the reaction completed by boiling the whole for 15 minutes. On cooling benzene was added to increase the precipitation of sodium chloride. The latter was filtered, the filtrate washed with water and the solvent evaporated. The oil was distilled at reduced pressure. TLC in petrol shows the presence of a second component having an \( R_f \) of 0.5. The product was chromatographed using 10% ether in petrol as eluant. A white crystalline compound was quickly
eluted whose NMR spectrum indicated it to be bibenzyl. In reference 144 no details are given of the presence of this impurity. The other component to be eluted was shown by IR and NMR spectra and TLC to be 3-benzyloxypropan-1-ol. This was distilled, b.p. 118-119°/0.4 mm Hg. Yield 18 g (7%).

b) The preparation of 3-benzyloxypropan-1-ol was repeated using a different procedure. Dimethylformamide (350 ml, dried) was added to sodium hydrate (50 g, 1.0 mole, Alfa Inorganic Inc., 52%), previously washed four times with anhydrous ether. 1,3-Propanediol (76 g, 1.0 mole) was added to the stirred solution over about twenty minutes followed by benzyl chloride (112.5 g, 1.0 mole) added over about 30 minutes. On addition of the latter a strongly exothermic reaction occurred necessitating external cooling. After standing for three days at room temperature the product was extracted with ether, the ethereal solution dried and the solvent evaporated. This crude product was distilled, b.p. 127-150°/15 mm Hg. This product was further purified by column chromatography (silica), eluting with 25% ether in petrol. The pure fractions were combined, dried, the
ether evaporated and distilled, b.p. 138-9\(^{\circ}\)/15 mm Hg.\(^{145}\) Yield 45.2 g (27%).

In reference 144 the authors converted the above alcohol to the nitrile via the chloride. Since then, however, a one-step reaction for the conversion of an alcohol to a nitrile has been reported in the literature.\(^{146}\)

**Attempted Preparation of 4-Benzylxybutyronitrile**

a) 3-Benzylxypropan-1-ol (3.32 g, 20m moles), triphenylphosphine (5.25 g, 20m moles) and carbon tetrachloride (2.0 ml, 20m moles, Analar – previously dried over calcium hydride) were heated on a steam bath. After a few minutes a violent reaction occurred after which dimethyl sulphoxide (20 ml) was added and the whole heated at 100\(^{\circ}\) for five minutes. Sodium cyanide (0.98 g, 20m mole, BDH) was added to the reaction mixture immediately causing it to become discoloured. The whole was heated with stirring for another ten minutes. On cooling the product was poured into a dilute solution of sodium sulphate and the organic compounds extracted with chloroform. The chloroform layer was washed again with sodium sulphate solution followed by water. The organic layer was separated, dried with anhydrous sodium sulphate and the solvent evaporated.
The fraction, b.p. 145°/18 mm Hg was chromatographed (silica) using 10% ether in petrol and the pure components combined, the solvent evaporated and the resultant oil (1.5 ml) distilled, b.p. 140°/15 mm Hg.\textsuperscript{145}

The NMR spectrum showed a singlet at $\tau = 2.70$ (5H), a singlet at $\tau = 5.53$ (2H), two overlapping triplets centred at $\tau = 6.40$ (2H, splitting 6 cps) and $\tau = 6.44$ (2H, splitting 6 cps) and a quintet centred at $\tau = 8.00$ (2H, splitting 6 cps).

The IR spectrum showed the absence of absorption in the 2200-2300 cm\textsuperscript{-1} (nitrile).

Thus, the physical properties of the product indicate that 3-benzyloxypropyl chloride and not 4-benzyloxybutyronitrile has been formed.

b) The above experiment was repeated but after the addition of dimethyl sulphoxide and sodium cyanide the mixture was heated at 140° for ten minutes. The IR spectrum of the crude product showed it to contain some of the nitrile but the majority was 3-benzyloxypropyl chloride.

c) Since the above reaction is producing mainly impure 3-benzyloxypropyl chloride, the alcohol was converted to
the chloride as in reference 144. TLC in 5% ether in petrol showed the presence of two components in the distillate. These were separated by column chromatography. Yield 40 g (80%), b.p. 125°/15 mm Hg (Lit. 129°/16 mm Hg).

Pure 3-benzyloxypropyl chloride (37 g, 0.20 mole) was heated to 90° with dimethyl sulphoxide (400 ml). Sodium cyanide (19.6 g, 0.40 mole) was added, with stirring, causing the temperature to rise slowly. The whole was finally heated at 150° for 15 minutes. On cooling the reaction mixture was poured into water and the organic products extracted with ether. The ethereal layer was washed with hydrochloric acid (6N) and water. After drying with anhydrous sodium sulphate the solvent was evaporated, and the resultant oil chromatographed (silica) using 16% ethyl acetate in petrol as eluant. The colourless oil obtained, b.p. 160°/15 mm Hg is 4-benzyloxybutyronitrile (Lit. 144 157°/12 mm Hg). Yield 19.8 g (56%).

The NMR spectrum showed a singlet at \( \gamma = 2.70 \) (5 H), a singlet at \( \gamma = 5.5 \) (2 H), a triplet centred at \( \gamma = 6.48 \) (2 H, splitting 6 cps), an asymmetric triplet centred at \( \gamma = 7.57 \) (2 H, splitting 6 cps) and an asymmetric quintet centred at
\( \gamma = 1.90 \) (2H, splitting 6 cps).

The IR spectrum showed absorbance at 2270 cm\(^{-1}\) (medium).

Gas liquid chromatography on 1% SE 30 at 125\(^\circ\) shows the presence of only one compound, having a retention time of 7.716 minutes.

Analysis (G.U.). Found: C, 75.46; H, 7.47; N, 7.86.

C\(_{11}\)H\(_{13}\)NO requires C, 75.40; H, 7.48; N, 7.99.

**Preparation of 4-Hydroxybutyronitrile**

Hydrogen, dried by passing through silica gel, was passed over a stirred mixture of 4-benzyloxybutyronitrile (1.0 g, 5.7 m moles), palladium black (0.50 g, Johnson Matthey), 5 drops of an ethyl acetate solution containing dry hydrogen chloride and ethyl acetate (130 ml, previously dried over potassium carbonate). After 4 hours TLC in 16% ethyl acetate in petrol showed the absence of any starting material. The acid was neutralised by addition of a little sodium bicarbonate (Analar) after which the salts and catalyst were separated. The solvent was evaporated using an oil pump. Distillation of the resultant oil gave a colourless oil, b.p. 70\(^\circ\)/1 mm Hg, whose IR spectrum showed
no absorption at 1695 cm\(^{-1}\) (iminobutyrolactone) or at 1760 cm\(^{-1}\) (butyrolactone) but did absorb intensely at 3500 (broad), 2280 and 1080 cm\(^{-1}\). There was, however, slight absorption at 1650 cm\(^{-1}\). It is impossible to detect the presence of 4-butyrolactone and 4-hydroxybutyramide in the product by TLC since previous experiments had shown the method to be insensitive to low concentrations of 4-butyrolactone while 4-hydroxybutyramide has a similar \(R_f\) value to the nitrile.

The NMR spectrum indicated that the product was \(\gtrsim 95\%\) 4-hydroxybutyronitrile consisted of two asymmetric triplets centred at \(\gamma = 5.8\) (CH\(_2\)-OH) and 7.5 (CH\(_2\)-CN) and a quintet centred at \(\gamma = 8.1\), all having a splitting constant of 6 cycles per second and integrating for two protons. The hydroxy-group which also appeared at \(\gamma = 7.5\) (1 H) disappeared on adding D\(_2\)O.

Analysis (G.U.). Found: C, 51.0; H, 7.89; N, 14.19 and C, 49.80; H, 7.94; N, 15.18. \(C_4H_7NO\) requires C, 56.45; H, 8.29; N, 16.46.

GLC analysis using a QF1 column on a Perkin Elmer F11 showed only 1 peak which "tailed" in the characteristic manner of a compound(s) decomposing on the column. Attempts
to purify the 4-hydroxybutyronitrile by preparative plate chromatography only caused further complications due to the formation of 4-butyrolactone and 4-hydroxybutyramide. These samples had the same retention time as the starting compound thus indicating probable decomposition of 4-hydroxybutyronitrile on the column.

**Attempted Preparation of 5-Hydroxyvaleronitrile**

Since a sample of 4-hydroxybutyronitrile (purity greater than 95%) had been obtained by the reaction of a reactive alkyl halide and sodium cyanide the same method was attempted for 5-hydroxyvaleronitrile. The procedure was varied, however, in attempting to make the corresponding iodo-compound instead of the bromo-compound as outlined in reference 147 for the preparation of 3-iodopropan-1-ol.

Freshly distilled 4-chlorobutan-1-ol (10.8 g, 0.10 mole, B. Newton Maine Ltd.), sodium iodide (30 g, 0.20 mole, BP) and acetone (100 ml, Analar) were heated under reflux. The TLC in chloroform after 30 minutes and 3 hours showed two compounds in addition to the unreacted starting material. The pH of the mixture was about one indicating that the product had decomposed to hydrogen chloride and tetrahydrofuran.
b) 4-Chlorobutan-1-ol (21.6 g, 0.20 mole), acetone (100 ml) and finely ground potassium cyanide (19.6 g, 0.40 mole) were placed in a round-bottomed flask. On addition of finely ground sodium iodide (60 g, 0.40 mole) a transient yellow colour appeared. After the further addition of acetone (100 ml) the whole was refluxed for two hours. On cooling the white solid was filtered and solvent evaporated from the filtrate causing the formation of more precipitate. Chloroform was added to this mixture and the precipitate filtered. On evaporation of the solvent a discolourd liquid having a very weak band at 2200 cm\(^{-1}\) and an intense band at 1680 cm\(^{-1}\) in the IR spectrum was formed. The latter may be due to 5-iminovalerolactone which is reported to absorb at 1676 cm\(^{-1}\).\(^{142}\)

c) The experiment above was repeated but refluxing for 10 hours when no starting material remained. After filtration of the salts the crude product was distilled at 0.05 mm Hg. Chromatography using silica (Koch-Light "200-300 mesh") and eluting with 50% ethyl acetate in petrol gave a fraction which although not pure showed the presence of a nitrile in the IR spectrum. The only absorbance in the carbonyl region was a moderately intense band at 1650 cm\(^{-1}\).
d) Following a report\textsuperscript{148} for the ready conversion of an aldehyde to the corresponding nitrile an attempt was made to convert 5-hydroxypentanal to 5-hydroxybaleronitrile.

Dihydropyran (BDH) was converted to 5-hydroxypentanal as outlined in reference \textsuperscript{149}.

A stirred solution of 5-hydroxypentanal (5.0 g, 50m moles) and hydroxylamine hydrochloride (3.5 g, 50m moles, M and B) in ethanol (50 ml) was heated to reflux and ten drops of concentrated hydrochloric acid added. After refluxing for six hours and cooling to $0^\circ$ a white precipitate formed which was filtered. On evaporation of some of the filtrate more of the white precipitate formed and this too was separated. The IR spectrum of the filtrate showed a band at $2200 \text{ cm}^{-1}$. TLC in 50% ether in petrol showed the presence of three components whose separation would probably be difficult. Distillation of the filtrate showed the presence of a compound absorbing in the IR spectrum at $1730 \text{ cm}^{-1}$. The experiment was not pursued further.

e) An attempt to dehydrate the oxime of 5-hydroxypentanal using DCC in pyridine was attempted but was unsuccessful.
f) The preparation of 5-hydroxyvaleronitrile was attempted as detailed in reference 141. The preparation of diethyl cyanoethyl malonate was successful but its conversion to ethyl 4-cyanobutyrate failed. The reaction was repeated but using a method recently reported in the literature.\textsuperscript{150}

Diethyl cyanoethyl malonate\textsuperscript{141} (4.2 g, 20 m moles) and sodium cyanide (2.0 g, 40 m moles, M and B) in dimethyl sulphoxide were heated at 160° with stirring for four hours. The product was distilled at reduced pressure because of the tarry appearance of the reaction mixture. The distillate was poured into water and the organic fraction separated with petrol. The organic fractions were combined, dried and the solvent evaporated yielding a liquid (0.5 ml). The IR and NMR spectra indicated that this was probably the desired product.

g) Since it was suspected that the product was decomposing under the reaction conditions used the experiment was repeated but heating at 160° for only 75 minutes. The tarry reaction product was poured into water and the organic components extracted with petrol. IR spectroscopic analysis of the product indicated it to be mainly starting material. The
reaction was not further pursued for in addition to the above difficulties it was suspected that potassium borohydride reduction of ethyl 4-cyanobutyrate would yield other products in addition to 5-hydroxyvaleronitrile. The same difficulties have thus been encountered in the preparation of 5-hydroxyvaleronitrile as with 4-hydroxybutyronitrile. It was hoped to obviate these difficulties by protecting the hydroxy-group by the benzyl residue.

Preparation of 5-Benzylxoxyvaleronitrile

4-Benzylxoxybutyl chloride was prepared as described in reference 151. The product, however, was purified by column chromatography (silica) using 10% ether in petrol as eluant. Although the splitting pattern and chemical shifts of the various groups were as expected the integration of the aromatic and methylene protons attached to the aromatic ring are slightly greater than expected in comparison to the other protons.

5-Benzylxoxyvaleronitrile was prepared as for 4-benzylxoxybutyronitrile and purified using column chromatography (silica) eluting with 50% ether in petrol. The "pure" fractions were dried, the solvent evaporated and resultant oil distilled, b.p. 117-118°/0.5 mm Hg. (Lit. 161
The NMR spectrum showed a singlet at \( \gamma = 2.60 \) (5H), a singlet at \( \gamma = 5.45 \) (2H), a triplet centred at \( \gamma = 6.45 \) (2H, splitting 6 cps), a triplet centred at \( \gamma = 7.63 \) (2H, splitting 6 cps) and an asymmetric quintet centred at \( \gamma = 8.25 \) (4H, splitting 6 cps).

The IR spectrum showed absorbance at 2270 cm\(^{-1}\) (medium).

Although the NMR and IR spectra are as expected the analysis indicated the presence of an impurity.

Analysis (G.U.). Found: C, 74.40; H, 8.33; N, 7.86 and C, 74.22; H, 8.00; N, 6.57 and C, 74.39; H, 8.03; N, 6.90.

\( \text{C}_{12} \text{H}_{15} \text{NO} \) requires C, 76.16; H, 7.99; N, 7.40.

GLC analysis on 1% SE 30 column at 110° showed the presence of two compounds. A sample of pure 5-benzyloxyvaleronitrile was obtained using preparative GLC under the above conditions.

The retention time of the product is 14.17 minutes. Reduction to 5-hydroxyvaleronitrile was not attempted (see Discussion).
The corresponding \( \omega \)-hydroxyamides were prepared since they are possible products of hydrolysis of the \( \omega \)-hydroxynitriles.

**Preparation of 4-Hydroxybutyramide**

The preparation was as given in reference 152, m.p. 53-54.5\(^\circ\) (Lit. 53-54\(^\circ\), 152 46\(^\circ\), 153 52-3\(^\circ\), 154).

**Preparation of 5-Hydroxyvaleramide**

The preparative details are as given in reference 153. The product was recrystallised once from ethanol, m.p. 101.5-103.5\(^\circ\) and twice from ethyl acetate, m.p. 106.0-106.5\(^\circ\) and 106.0-107.0\(^\circ\) (Lit. 108\(^\circ\), 153 107.0-107.5\(^\circ\), 154).
Preparation of 2-Cyanobenzoic Acid

DCC (20.6 g, 0.101 mole) in dried chloroform (100 ml) was added over an hour with stirring to a slurry of phthalamic acid (16.5 g, 0.100 mole, Eastman Kodak) in dried methylene chloride (100 ml) at room temperature. After 16 hours sodium hydroxide solution (100 ml; N) was added, the whole vigorously shaken and the precipitate filtered. The aqueous layer was separated and twice treated with chloroform (25 ml) to extract any organic materials. Concentrated hydrochloric acid was added to the stirred ice-cold aqueous solution until the pH of the solution was three. The precipitate formed was filtered and dried in a desiccator. Recrystallisation from dried ethanol resulted in the formation of 6.0 g (40%) of 2-cyanobenzoic acid, m.p. 189-190° (Lit. 190° 61). The melting point, however, was characterised by undergoing a change at about 140° 155 (which may be only partial). Its further change to phthalimide was as recorded. 156 The product showed only one spot on TLC in ether and 50% ether in petrol (both containing a drop of acetic acid). Furthermore, the IR spectrum was superimposable on that of a sample prepared (by B. C. Ghosh), according to the method of
Sandmeyer. Although the yield is lower than that in reference 61 (40% as opposed to 57%) the procedure is much simpler than this and other methods so far reported for the preparation of 2-cyanobenzoic acid.

The only other reported synthesis of nitriles from amides using DCC are the formation of carbobenzoxy-β-cyano-L-alanine from carbobenzoxy-L-asparagine. The authors indicated that the reaction could be a general synthesis of cyano-acids from suitable amic acids using very mild conditions which unlike the usual reagents for the dehydration of amides would not react with hydroxy-groups. The reaction appears to be quite general and should be useful in the preparation of 2-cyano-acids of aromatic compounds. The previous difficulty in preparing 2-cyano-acids is exemplified by the absence of the 1,2; 2,1 or 2,3 derivatives of naphthalene in the literature.

The amic acids are usually readily available by treatment of the anhydride with ammonia although with asymmetric systems some difficulty might be experienced in separating the mixture of amic acids. 8-Cyano-naphth-1-oic acid, which has also defied synthesis, may probably now be
prepared since it is known that the corresponding amic acid is reasonably stable.\textsuperscript{162} Due to the known steric interaction of substituents in the 1,8 positions of naphthalene\textsuperscript{163} this "cyano-acid" may exist predominantly in the isoimide form (42).

\begin{center}
\begin{tikzpicture}
\begin{scope}[scale=0.6]
\draw[thick,black] (0,0) circle (0.5cm);
\draw[thick,black] (1,0) circle (0.5cm);
\draw[thick,black] (0,0.5) -- (1,0.5);
\draw[thick,black] (0,-0.5) -- (1,-0.5);
\draw[thick,black] (0,0) -- (1,-0.5);
\draw[thick,black] (0.5,0) -- (1,0.5);
\node at (0.5,0) {N};
\node at (0.5,0.5) {C};
\node at (0.5,-0.5) {C}
\node at (0.5,0.5) {O};
\node at (0.5,-0.5) {O};
\node at (1,0) {C};
\node at (1,0.5) {O};
\node at (1,-0.5) {OH};
\end{scope}
\end{tikzpicture}
\begin{tikzpicture}
\begin{scope}[scale=0.6]
\draw[thick,black] (0,0) circle (0.5cm);
\draw[thick,black] (1,0) circle (0.5cm);
\draw[thick,black] (0,0.5) -- (1,0.5);
\draw[thick,black] (0,-0.5) -- (1,-0.5);
\draw[thick,black] (0,0) -- (1,-0.5);
\draw[thick,black] (0.5,0) -- (1,0.5);
\node at (0.5,0) {N};
\node at (0.5,0.5) {C};
\node at (0.5,-0.5) {C};
\node at (0.5,0.5) {O};
\node at (0.5,-0.5) {O};
\node at (1,0) {C};
\node at (1,0.5) {O};
\node at (1,-0.5) {O};
\end{scope}
\end{tikzpicture}
\end{center}

Since N-substituted maleic isoimides have been prepared\textsuperscript{160} it seems probable that this reaction can be extended to the preparation of 1,2-substituted and unsubstituted maleic derivates.\textsuperscript{164} Similarly it may be of use in the preparation of 3-cyanobutyric acids. The use of the nitrile as an amide protecting group in suitable amic acids has already been indicated.\textsuperscript{165}
Evidence for the Intermediacy of Isophthalimide in the DCC Dehydration of Phthalamic Acid

DCC (2.06 g, 10m mole) in chloroform (10 ml) was added over about one hour to a stirred slurry of phthalamic acid in methylene chloride (10 ml). After 20 hours the white precipitate was filtered. The IR spectrum of the solution (using an identical solvent system in the reference beam) showed the presence of strong bands at 2200 (cyanide), 1860, 1790, 1780 and 1710 (broad) wave numbers. Those at 2220 and 1710 cm\(^{-1}\) are due to the formation of ocba. The other three bands are probably associated with the isophthalimide group. No information is available on the IR spectra of unsubstituted isothalamides but details for N-butylmaleisothalamide have been reported\(^{160}\) (\(\tilde{\nu} = 1821, 1805\) and 1695 cm\(^{-1}\)). On standing the filtrate from above precipitated a white solid whose IR spectrum showed the product to be ocba. After standing for a week at 0\(^{\circ}\) the white precipitate (again shown to be ocba) was filtered. The IR spectrum of the filtrate still showed the nitrile band at 2220 cm\(^{-1}\) but the bands associated with the isophthalimide had disappeared. Thus, although the isophthalimide is formed, it is converted slowly to ocba.
Preparation of Isophthalimide Hydrochloride

a) Dry hydrogen chloride was passed over a stirred solution of ocba (2.0g, 14m mole) in dried THF (20 ml). After about an hour a white precipitate appeared and this was immediately filtered and dried in a desiccator. The IR spectrum showed the presence of intense bands centred at 1870 and 1700 cm\(^{-1}\) and a moderately strong band at 3400 cm\(^{-1}\). The chloride equivalent, determined according to the method of Volhard,\(^{166}\) was 234 as opposed to the theoretical 183.

The mass spectrum (MS9) showed peaks at 183, 166, 165, 149, 148, 147, 122, 121, 105, 104, 91, 77, 76, 75 and 74. This indicated the presence of a parent ion of mass to charge ratio (\(^{m/e}\)) of 183, and a breakdown product of \(^{m/e}\) of 148. It is probably significant that the m-1 and m+1 ions associated with the latter are weaker in intensity. In view of the mode of preparation it is reasonable to propose that these ions are 43 and 44.

\[
\begin{align*}
\text{43} & \quad \text{Cl}^- \quad \text{Cl}^+ \quad \text{NH}_2 \\
\text{44} & \quad \text{NH}_2 \\
\end{align*}
\]
On standing the isophthalimide hydrochloride hydrolysed to phthalamic acid.

In an attempt to obtain a better chloride analysis the experiment above was repeated.

b) The experiment was repeated many times varying the reaction times. In no instance was any isophthalimide hydrochloride formed. Although a white precipitate sometimes formed this was due to "salting-out" of ocba. On standing the acidic solution precipitated phthalimide (IR spectrum superimposable on that of an authentic sample).

c) The preparation was essentially as described\(^{167}\) for camphoramic acid. The apparatus used was that described in reference 92.

Nitrogen, dried by passing through concentrated sulphuric acid and sodium hydroxide (solid), supported freshly distilled acetyl chloride (5.7 ml) and phthalamic acid (2.30g, 20m mole) at room temperature. As a result of evaporation more acetyl chloride had to be added over the two hour reaction time. The acetyl chloride was filtered and the white product washed twice with dried ether. Dried nitrogen was passed
through the product for 12 hours. The IR spectrum showed
the product to be phthalamic acid.

d) The experiment was repeated but heating phthalamic acid
(0.4g) in redistilled acetyl chloride (25 ml) at 50° for 18 hours.
The liquid was filtered and the precipitate washed twice with
dried ether. Dried nitrogen was passed over the precipitate
for two days. The IR spectrum only differed from that from
preparation a) in that the band at 3400 cm⁻¹ is now absent.
The latter is probably due to partial hydrolysis of
isophthalimide hydrochloride to phthalamic acid. The mass
spectrum showed peaks at 183, 148, 121, 120, 104, 77, 76,
75 and 74.

Infrared Spectra of 2-Cyanobenzoic Acid in a Mixture
of Dimethyl Sulphoxide, Deuterium Oxide and Deuterium Chloride
at Room Temperature

The spectra were run on a Unicam SP100 using calcium
fluoride cells of cell width 0.05 mm with an identical solvent
system in the reference beam unless otherwise stated.
Preliminary experiments had shown that a one molar solution
of ocba could be prepared by dissolving ocba in a mixture of
water and dimethyl sulfoxide in the ratio (volume) of seven to three. This solvent system was used throughout. The stock solution of deuterium chloride in deuterium oxide (Merck) was 6.46 N and was diluted as necessary using deuterium oxide (Koch-Light "Puriss" grade) and dimethyl sulfoxide (BDH "Spectroscopic" grade).

1) The IR spectrum of a 70:30 (by volume) mixture of deuterium oxide and DMSO against air showed some absorption at 2200 cm\(^{-1}\) and 1600 cm\(^{-1}\) but this was not sufficient to invalidate the subsequent experiments.

2) ocba (14.5 mg) in the solvent mixture (100 \(\mu\)l) showed bands centred at 2350 cm\(^{-1}\) and 1710 cm\(^{-1}\) characteristic of a nitrile and a carboxylic acid. Rescanning showed the appearance of a new peak at 1655 cm\(^{-1}\) due to phthalamic acid (see below).

3) Phthalamic acid (16.7 mg) in the solvent mixture (100 \(\mu\)l) showed intense bands at 1715 cm\(^{-1}\) and 1655 cm\(^{-1}\). The spectrum remained unchanged on standing.

4) The IR spectrum of a solution (0.1 molar in deuterium chloride) of deuterium oxide, deuterium chloride and DMSO
against air showed strong absorption in the 2200 cm\(^{-1}\) and 1600 cm\(^{-1}\) regions but this still was not sufficient to invalidate the experimental results.

5) On adding ocba to a solution of the above solvent the IR spectrum showed the characteristic bands at 2350 cm\(^{-1}\) and 1710 cm\(^{-1}\). On standing a new band appeared at 1655 cm\(^{-1}\) with a corresponding decrease in the intensity of the band at 2350 cm\(^{-1}\) again indicating the hydrolysis of ocba to phthalamic acid.

6) The IR spectrum of a solution (1.0 molar in deuterium chloride) of deuterium oxide, deuterium chloride and DMSO against air showed intense absorption in the region 2200 cm\(^{-1}\) and 1600 cm\(^{-1}\). Despite this (just) sufficient radiation was transmitted for the analysis below to be valid.

7) On addition of ocba to a solution of the above solvent (molar ratio of ocba to DCl \(\approx 1\)) the characteristic bands at 2350 cm\(^{-1}\) and 1710 cm\(^{-1}\) were again present. Repeat scans after 90, 150, 170 and 190 minutes showed increasing amounts of phthalamic acid.
8) On addition of ocba to a solution of the above solvent system (molar ratio of ocba to DCl ≈ 0.3) exactly the same phenomenon as in 7) was observed.

9) The procedure in 8) was repeated but using a molar ratio of ocba to DCl of 0.2 and 0.1 respectively. Similar results to 7) were again obtained.

The infinity spectrum of all the solutions in the above experiments were that of phthalamic acid. In no instance was a band characteristic of isophthalimide (1860, 1790 and 1780 cm\(^{-1}\)) or isophthalimide hydrochloride (1870 and 1700 cm\(^{-1}\)) observed even though the molar ratio of deuterium chloride to ocba was as high as ten.

**Test for Phthalimide as a Reaction Intermediate in the Hydrolysis of 2-Cyanobenzoic Acid**

The very ready hydrolysis of ocba in acidic media compared to 4-cyanobenzoic acid has been explained\(^{65}\) by the rapid formation of isophthalimide which then further reacts with water after protonation of the nitrogen atom. (Reaction Scheme No. 1, p. 38). An alternative explanation, which was not previously considered, is the acid catalysed
formation of isophthalimide, followed by rearrangement of phthalimide which is then hydrolysed to phthalic acid.

The rate-determining step would necessarily have to be the first, second or fifth step in order to be compatible with the known first order dependence on the concentration of (unionised) ocba and hydronium ion. An analogous mechanism has also been suggested for the isomerisation of N-phenyphalisoimide to N-phenylphthalimide. The above
suggestion, however, was shown to be untenable since phthalimide hydrolysed to phthalamic acid much slower than ocba under the same conditions. Only if the hydrolysis of phthalimide had been the same or faster than that of ocba would it have been consistent with the suggested mechanism.

Hydrolysis of Isophthalimide Hydrochloride

The UV spectrum of a solution of isophthalimide hydrochloride \((2 \times 10^{-4} \text{ M})\) in hydrochloric acid \((0.10 \text{ N})\) at room temperature was similar to phthalamic acid in the range 240–235 nm but different in the range 205–240 nm. The peak at 220 nm (absorbance 2.04, extrapolated) cannot be the result of a mixture of ocba and phthalamic acid since their respective absorbances at this wavelength are 1.22 and 1.59. A repeat scan after five minutes showed no change. Similarly, the UV spectrum of isophthalimide hydrochloride \((2 \times 10^{-4} \text{ M})\) was observed in aqueous acetate buffer, pH 4.19. Between 275 and 325 nm the spectrum was similar to that obtained in hydrochloric acid \((0.10 \text{ N})\) but the remainder of the spectrum was appreciably different. In neither instance could the spectra be interpreted in terms of phthalamic acid, ocba, phthalimide, phthalic acid, phthalic anhydride or simple mixtures of these.
Isophthalimide Hydrochloride

Absorbance

Wavelength (nm)

2.0

1.0

0.0

200 225 250 275 300 325

Isophthalimide Hydrochloride

Absorbance

Wavelength (nm)

2.0

1.0

0.0

200 225 250 275 300 325
Phthalic Anhydride

--- Hydrochloric acid (0.10 N)

---- Acetate buffer (pH 4.19)

Concentration $2 \times 10^{-4}$ M

Path length 10 mm

All spectra taken at room temperature
Determination of the Mode of Attack by Water on 2-Cyanobenzoic Acid

If the proposed mechanism for the hydrolysis of ocba is correct, then nucleophilic attack could occur at either the carbonyl or protonated imino-group. Thus, by hydrolysing ocba in labelled water it should be possible to determine the side of attack. Attack at the protonated imino-group should give phthalamic acid labelled in the carboxamide group while attack at the carbonyl group should give phthalamic acid labelled in the carboxy-group (see page 38). The mass spectrum of phthalamic acid (MS9) shows a distinct breakdown pattern which should lend itself in the determination of the mechanism.

\[
\begin{align*}
\text{OC} & \leftrightarrow \text{OC}^+ \\
\text{NH}_2 & \rightarrow \\
\text{OH}^+ & \leftarrow \\
\text{m/e} & 93 \\
\text{m/e} & 121 \\
\text{m/e} & 104
\end{align*}
\]
The peaks at m/e 148 and 122 indicated that there was more than one mode of fragmentation. However, the meta-stable ions at 55.54 (104 → 76), 71.48 (121 → 93) and 98.26 (149 → 121) indicated a very characteristic mode of breakdown which if occurred in the labelled phthalamic acid would pinpoint the exact site of the label.

The Isolation of Phthalamic Acid from Aqueous Solution

Since the hydrolysis of ocba occurs in aqueous acidic media separation of phthalamic acid by extraction with an organic solvent is impossible since it is very insoluble in all the common water immiscible solvents. Passing a solution of silver phthalamate through an acidic ion-exchange resin (IR 120) and freeze drying the resultant solution gave a compound whose IR spectrum was appreciably different from that of phthalamic acid. Evacuating a hydrochloric acid solution of phthalamic acid gave a sticky white solid whose IR spectrum was unlike that of authentic phthalamic acid. Attempts to neutralise the hydrochloric acid with an exact equivalent of sodium hydroxide and then preferably extracting the sodium chloride always resulted in appreciable
contamination. Sublimation even at reduced pressures is impossible due to decomposition to phthalic anhydride. The problem was overcome by neutralising the acidic solution with an exact equivalent of sodium hydroxide as follows (b).

a) Hydrochloric acid (0.10 M; 3.0 ml, BDH) and dioxan (0.5 ml, Merck "Spectroscopic") was heated to 45°. A dioxan solution of ocb (35 µl; 2 x 10^{-2} M) was injected and the UV spectrum scanned repeatedly on a Unicam SP800. After twenty-five minutes about 99% hydrolysis to phthalamic acid occurred.

b) 2-Cyanobenzoic acid (22 mg) in dioxan (0.5 ml) at 45° was added to hydrochloric acid (0.10 M; 3.0 ml, BDH) at 45° and the mixture heated for twenty-five minutes. The mixture was immediately cooled and the hydrochloric acid neutralised with sodium hydroxide solution (0.1 M; 3.0 ml, BDH).

The IR spectrum of the solid formed on freeze-drying this solution was identical to that of an authentic sample. Although some difficulties were experienced in obtaining the mass spectrum of this mixture, presumably due to the sodium chloride lattice trapping phthalamic acid, that eventually
obtained was essentially that of an authentic sample of phthalamic acid.

c) The experiment above was repeated on a much smaller scale. Hydrochloric acid (1.0 M; 10 μl, BDH), water (90 μl) and ocba (0.73 mg) in 16.6 μl of dioxan (0.3 M ocba in dioxan) were heated at 45° for 25 minutes. The sample was immediately cooled, the acid neutralised with sodium hydroxide solution (1.0 M; 10 μl, BDH) and the whole freeze-dried.

d) Oxygen-18 enriched water (90 μl, Miles Laboratories Inc., 18
62.57% H2O18), hydrochloric acid (10 μl; 1.0 M, BDH) and a dioxan solution of ocba (16.6 μl; 0.3 M) were heated at 45° in exactly the same manner as in c). The phthalamic acid (O18 enriched) was obtained by freeze-drying the neutralised solution. The mass spectrum of phthalamic acid (unlabelled) showed fragments m/e 166(w), 150 (w), 149(s), 148(s), 147(m), 130(w), 123(m), 122(s), 121(s), 106(m), 105(s), 104(s), 103(w). The mass spectrum of phthalamic acid (labelled) showed fragments m/e 168(w), 166(w), 151(m), 150(s), 149(m), 148(s), 147(m), 130(w), 124(m), 123(m), 122(s), 121(s), 107(m),
The mass spectra were measured on an AEI MS 12 at 115-120°.

**Dehydration of Labelled Phthalamic Acid**

Since the mass spectrum of labelled phthalamic acid defied detailed analysis it was converted to the corresponding oca by reaction with DCC. The MS of oca exhibits a simple breakdown pattern and since the mechanism of dehydration of amic acids by DCC is known it was hoped to be able to elucidate the site of the label.

A sample of labelled phthalamic acid (and sodium chloride) was prepared as previously described. An equivalent of DCC (1.03 mg) in chloroform (0.1 ml) and methylene chloride (0.1 ml) was added to the finely ground mixture and the whole left for twelve hours with occasional shaking. Sodium hydroxide solution (7 µl; N) was added, the white precipitate filtered and the organic layer separated. After further extraction of the aqueous layer with chloroform hydrochloric acid (7 µl; N) was added and the product freeze-dried. The MS of the resultant solid, however, did not show the anticipated simple spectrum of the unlabelled acid.
The complications in the MS of labelled phthalamic acid could be the result of contamination by phthalic acid as a result of further hydrolysis. The same complication in addition to the possible presence of some unreacted phthalamic acid could also account for the complicated spectrum of ocba. The mass spectrum of ocba (unlabelled) showed fragments \( m/e \ 148(w), 147(s) \ 131(w) \ 130(s) \ 106(w) \ 105(s) \ 104(m) \ 103(s) \ 102(s) \).

The mass spectrum of ocba (labelled) showed fragments \( m/e \ 150(m) \ 149(m) \ 148(m) \ 147(s) \ 106(s) \ 105(m) \ 104(s) \ 103(m) \).

The mass spectra were measured on an AEI MS 12 at 115–120°.

**Attempted Purification of Phthalamic Acid**

The TLC of phthalamic acid and phthalic acid on silica and "polyamid" plates in a variety of solvents showed extensive streaking and since this occurred in the same region of the plate separation by chromatography would be impossible. Some purification of phthalamic acid appeared to be possible since on "polyamid" plates (eluting with methanol) two distinctly separate components were observed. A sample
was duly purified using column chromatography and the product recrystallised from methanol (Analar), m.p. 140-4° (Lit. 147-8°). However, the product did not remelt at 238° (m.p. of phthalimide). The IR spectrum also showed the product not to be phthalamic acid.

Analysis (G.U.). Found: C, 52.68; H, 5.44; N, 15.64 whereas phthalamic acid \( (C_8 H_7 NO_3) \) requires C, 58.20; H, 4.27; N, 8.48. This is close to the analysis expected for ammonium phthalamate. \( C_8 H_{10} N_2 O_3 \) requires C, 52.74; H, 5.53; N, 15.38. This was confirmed by taking the IR spectrum of a sample of ammonium phthalamate prepared as in reference 171. Decomposition of phthalamic acid on recrystallisation from hot solvents has been noted before \(^{52} \) but the product was claimed to be phthalimide. This is somewhat surprising in view of the usual difficulty in hydrolysing amides.

Attempts to convert ammonium phthalamate to phthalamic acid \(^{171} \) gave a product which was less pure than the commercial product.
IR Spectroscopic Analysis of Oxygen-18 Enriched Phthalamic Acid

The failure of MS analysis to determine the form of the product prompted investigation by IR spectroscopic analysis. Although no details of oxygen-18 containing amides are known some work has been published on esters. Thus the frequencies of the carbonyl group (oxygen-18) in 4-bromo-phenyl benzoate \(^{169}\) is 1696 cm\(^{-1}\) as opposed to 1726 cm\(^{-1}\) in the corresponding oxygen-16 compound. Such a shift would similarly be expected with amides. The IR (potassium bromide disc) of a mixture of phthalamic acid and sodium chloride show bands between 1630-1670 cm\(^{-1}\) and 1680-1750 cm\(^{-1}\) due to the amide and carboxy-groups, respectively. The oxygen-18 enriched phthalamic acid only showed a broad band between 1630 and 1750 cm\(^{-1}\) in the carbonyl range. Thus neither mass spectral nor infrared techniques are useful in the interpretation of the site of the label.
Attempted Hydrolysis of 4-Hydroxybutyronitrile

The following spectra were observed in the range 200-300 nm on a Cary Model 14 spectrophotometer using an identical solvent system in the reference beam.

a) An aqueous solution of a solution of 4-hydroxybutyronitrile (1.41 x 10^-2 M) at 30^o showed a peak with a maximum at 230 nm (\( \varepsilon = 60 \)). Below 200 nm the sample absorbed intensely. Repeat scan over ten minutes showed no change in the spectrum. On addition of one drop of sodium hydroxide (N) the intensity of the peak at 230 nm decreased and progressively shifted to lower wave-numbers. Identification of the band at 230 nm was difficult since little UV spectroscopic data is available on non-aromatic imidates. However, butyronitrile (BDH), hexamethylene cyanohydrin (Eastman), 4-butyrolactone (Koch-Light "Puriss" grade), 4-hydroxybutyramid and pyrrolidone were all shown not to have a maxima in this range.

b) 4-Hydroxybutyronitrile was stable at 30^o in hydrochloric acid (0.05 N) at 30^o. The \( \varepsilon \) value of the band at 230 nm was unchanged after ten minutes.
c) The temperature of the above solution was raised to 50° but even after 2 hours the spectrum remained unchanged.

d) A solution of 4-hydroxybutyronitrile in hydrochloric acid (0.5 N) at 50° showed no change after one hour. The failure to observe any change in the UV spectrum (assuming the peak at 230 nm to be due to an impurity) may be due to rapid hydrolysis to the amide which is stable to the reaction conditions employed. This proposal was shown to be untenable since 4-hydroxybutyramide was readily hydrolysed under the same conditions (0.5 N HCl) in which the 4-hydroxybutyronitrile was stable.

e) The spectrum of 4-hydroxybutyronitrile in perchloric acid (2 M) at 50° was identical to that in aqueous solution. After 10 minutes the spectrum remained unchanged. However, after 12 hours there was a small increase in absorbance at all wavelengths. Most characteristic was a broad shoulder centred at 215 nm.

f) Exactly the same procedure as outlined in e) was carried out using perchloric acid (4 M). After 12 hours the spectrum showed slight increases at all wavelengths but especially so
between 200-225 nm. The intensity at 215 nm was too intense to be 4-butyrolactone.
4-Hydroxybutyronitrile

--- Aqueous solution

--- Weakly alkaline solution

Concentration $1.41 \times 10^{-2}$ M

Temperature $30^\circ$
Butyronitrile
Aqueous solution
Concentration $1.41 \times 10^{-2}$ M
Temperature $30^\circ$ C

Hexamethylene Cyanohydrin
Aqueous solution
Concentration $1.41 \times 10^{-2}$ M
Temperature $30^\circ$ C
**4-Butyrolactone**
Aqueous solution
Concentration $2 \times 10^{-3}$ M
Temperature $30^\circ$C

**4-Hydroxybutyramide**
Aqueous solution
Concentration $2 \times 10^{-3}$ M
Temperature $30^\circ$C
Pyrrolidone
Aqueous solution
Concentration $2 \times 10^{-3}$ M
Temperature 30°

4-Hydroxybutyronitrile
Hydrochloric acid (0.5 N)
Concentration $2 \times 10^{-3}$ M
Temperature 50°
4-Hydroxybutyronitrile
Perchloric acid (2 M)
Concentration $2 \times 10^{-3}$ M
Temperature 50°

--- Initial spectrum

--- Spectrum after 12 hours

4-Hydroxybutyronitrile
Perchloric acid (4 M)
Concentration $2 \times 10^{-3}$ M
Temperature 50°

--- Initial spectrum

--- Spectrum after 12 hours
Attempts\textsuperscript{137} to prepare 2-iminotetrahydrofuran from the corresponding imidate hydrochloride with anhydrous triethylamine resulted in the formation of tri-substituted sym-triazines.

However, the $\lambda_{\text{max}}$ and $\varepsilon$ values for these compounds are different from those of the 4-hydroxybutyronitrile ($\lambda_{\text{max}} = 259$ nm, $\log \varepsilon = 2.85$). Since the $\varepsilon$ value was independent of pH it is unlikely that the band(s) at 230 nm is due to either 2-iminotetrahydrofuran or its hydrochloride.
KINETIC EXPERIMENTAL DETAILS AND RESULTS

Solutions and Buffers

All chemicals used for the preparation of buffered and other solutions were of 'Analar' grade. For buffer catalysis a stock solution of buffer at a particular ionic strength was diluted with a stock solution of sodium or potassium chloride of the same ionic strength. Perchloric acid solutions were prepared by dilution of perchloric acid (72%, Analar). Weighed samples of this were titrated against standard sodium hydroxide, and thence the corresponding Ho value taken from reference 172. All solutions contained EDTA (10^{-4} M) which was shown to have no effect upon the observed rate constant. 'Spectrograde' dioxan (Merck) was used for the preparation of aqueous dioxan buffers and also for the stock solutions of the substrates.

pH Measurements

The pH of all buffer solutions was measured at the temperature of the kinetic experiment with a Radiometer TTT1 titrator and expansion scale or a Radiometer Model 26 pH meter, with an external temperature compensator. A
Radiometer type G 202 C glass electrode was used together with a type K 401 calomel electrode. The pH meter was standardised against commercial standard buffers complying to BS 1647, 1961.

**Spectrophotometric Rate Determinations**

A Hilger Gifford spectrophotometer was used to determine the observed rate constants in Tables 1 and 2. A Cary Model 14 or a Zeiss PMQ II was used to determine the remainder. The Cary Model 14 was fitted with a five cell compartment and thermostating arrangements. Constant temperature was achieved by using a Lauda electronic thermostating bath. The temperature in the cell was measured with a National Physical Laboratory calibrated thermometer. The temperature was measured before and after each run and was constant to ± 0.03°.

Spectrosil quartz UV cells (10 mm) were used. At least 30 minutes was allowed for temperature equilibration of the buffers in the cells. After injecting the solution of substrate into the buffer, the cell was removed, vigorously shaken and immediately returned to the cell holder.
The output from the spectrophotometers was fed into a Solartron Compact Data Logger which digitised the absorbance reading. The latter was transferred on to 5-channel paper tape, via a Creed punch, at convenient time intervals. Usually 100-700 values were taken. However, only about 30 absorbance readings were taken when the values were read off the chart recorder.

The first-order rate constants were determined using a generalised least-squares program, written by Dr. B. Capon following the procedure of Wentworth and Deming. Evaluation was performed on an English Electric KDF 9 computer.

The stock solutions of 2-naphthyl, phenyl, m-tolyl 4-hydroxybutyrates and 2-naphthyl 5-hydroxyvalerate were prepared by dissolving the ester in an appropriate amount of dioxan. The stock solutions of m-chlorophenyl and m-fluorophenyl 4-hydroxybutyrates and phenyl, m-chlorophenyl and m-tolyl 5-hydroxyvalerates were prepared in dioxan as detailed on p. 79. Preparation of the hydroxy-esters by the latter procedure inevitably results in
the presence of toluene in the stock solution. The $\lambda_{\text{max}}$ for toluene in water is 268 nm ($\varepsilon = 200$)$^{175}$ and as the calculation below shows this absorbance is small compared to that of the phenol which at the same concentration is of the order of 0.7.

$$A = \varepsilon \, c \, l$$
$$= 200 \times 2.5 \times 10^{-4}$$
$$= 0.05$$
TABLE 1: THE RATES OF THE IMIDAZOLE CATALYSED
HYDROLYSIS OF SOME 2-NAPHTHYL ESTERS AT
30.0°C IN A BUFFERED SOLUTION OF DIOXAN
IN WATER (20% v/v)

<table>
<thead>
<tr>
<th>Acid Residue</th>
<th>Buffer</th>
<th>I</th>
<th>$10^5k_{obs}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyric acid</td>
<td>Imidazole hydrochloride</td>
<td>0.10</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>13.4</td>
</tr>
<tr>
<td>4-Methoxybutyric acid</td>
<td>&quot;</td>
<td>&quot;</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>17.2</td>
</tr>
<tr>
<td>4-Hydroxybutyric acid</td>
<td>&quot;</td>
<td>&quot;</td>
<td>54.4</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>54.1</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>Sodium chloride</td>
<td>0.05</td>
<td>5.18</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>4.79</td>
</tr>
<tr>
<td>4-Methoxybutyric acid</td>
<td>&quot;</td>
<td>&quot;</td>
<td>7.65</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>7.60</td>
</tr>
<tr>
<td>4-Hydroxybutyric acid</td>
<td>&quot;</td>
<td>&quot;</td>
<td>35.5</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>34.7</td>
</tr>
</tbody>
</table>

The reactions were studied at 327 nm. The pH of the buffer solutions, pH (app), read from a pH meter using calomel-glass electrodes (calibrated for aqueous solution) was 7.02. The true pH was determined by first measuring the pH on a meter of a series of buffers in the above solvent system whose true pH
was already known. A plot of pH as read on the pH meter versus the true pH is linear. The pH of the above buffer solutions was determined from this graph (p. 181) and found to be 6.82. A concentrated solution of the ester in dioxan (25 µl, 4 x 10^{-2} M solution) was injected into the buffer solution (2.5 ml). The concentration of the ester in the buffer solution was 2.5 x 10^{-4} M.

The imidazole catalytic rate constants, \( k_{IM} \), for the 2-naphthyl esters of butyric acid, 4-methoxybutyric, and 4-hydroxybutyric are 1.70, 1.92, and 3.82 x 10^{-3} M^{-1} s^{-1}, respectively.
**TABLE 2**: THE RATES OF THE HYDROXIDE CATALYSED HYDROLYSIS OF 2-NAPHTHYL 4-HYDROXY-BUTYRATE AT 30.0° IN A BUFFERED SOLUTION OF DIOXAN IN WATER (20% v/v)

<table>
<thead>
<tr>
<th>pH (app)</th>
<th>Buffer</th>
<th>I</th>
<th>$10^5k_{obs}$(s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.38</td>
<td>Phosphate</td>
<td>0.05</td>
<td>5.55</td>
</tr>
<tr>
<td>6.38</td>
<td>&quot;</td>
<td>&quot;</td>
<td>5.52</td>
</tr>
<tr>
<td>6.96</td>
<td>&quot;</td>
<td>&quot;</td>
<td>18.2</td>
</tr>
<tr>
<td>6.96</td>
<td>&quot;</td>
<td>&quot;</td>
<td>18.2</td>
</tr>
<tr>
<td>7.03</td>
<td>&quot;</td>
<td>&quot;</td>
<td>21.9</td>
</tr>
<tr>
<td>7.51</td>
<td>&quot;</td>
<td>&quot;</td>
<td>68.8</td>
</tr>
<tr>
<td>7.51</td>
<td>&quot;</td>
<td>&quot;</td>
<td>69.4</td>
</tr>
<tr>
<td>7.94</td>
<td>&quot;</td>
<td>&quot;</td>
<td>167.0</td>
</tr>
<tr>
<td>7.94</td>
<td>&quot;</td>
<td>&quot;</td>
<td>165.0</td>
</tr>
<tr>
<td>8.05</td>
<td>&quot;</td>
<td>&quot;</td>
<td>220.0</td>
</tr>
<tr>
<td>8.05</td>
<td>&quot;</td>
<td>&quot;</td>
<td>211.0</td>
</tr>
<tr>
<td>8.37</td>
<td>&quot;</td>
<td>&quot;</td>
<td>502.0</td>
</tr>
<tr>
<td>8.37</td>
<td>&quot;</td>
<td>&quot;</td>
<td>507.0</td>
</tr>
<tr>
<td>8.37</td>
<td>&quot;</td>
<td>&quot;</td>
<td>503.0</td>
</tr>
<tr>
<td>8.37</td>
<td>&quot;</td>
<td>&quot;</td>
<td>506.0</td>
</tr>
<tr>
<td>8.32</td>
<td>Phosphate</td>
<td>0.025</td>
<td>409.0</td>
</tr>
<tr>
<td>8.32</td>
<td>Sodium chloride</td>
<td>0.025</td>
<td>400.0</td>
</tr>
</tbody>
</table>

The reaction was studied at 327 nm. A concentrated solution of the ester in dioxan (10 μl; 0.15 M solution) was injected into the buffer solution (3.0 ml). The concentration of the ester in the buffer solution was $5 \times 10^{-4}$ M.
TABLE 3: THE RATES OF THE HYDOXIDE CATALYSED HYDROLYSIS OF 2-NAPHTHYL 5-HYDROXY-VALERATE AT 30.0° IN A BUFFERED SOLUTION OF DIOXAN IN WATER (20% v/v)

<table>
<thead>
<tr>
<th>Buffer</th>
<th>pH (app)</th>
<th>I</th>
<th>$10^5 k_{obs}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate</td>
<td>7.40</td>
<td>0.05</td>
<td>3.95</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td></td>
<td>3.88</td>
</tr>
<tr>
<td>&quot;</td>
<td>7.94</td>
<td>0.05</td>
<td>13.1</td>
</tr>
<tr>
<td>Phosphate</td>
<td>8.32</td>
<td>0.025</td>
<td>24.4</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>&quot;</td>
<td>0.025</td>
<td>26.7</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td></td>
<td>25.9</td>
</tr>
<tr>
<td>Phosphate</td>
<td>8.37</td>
<td>0.05</td>
<td>30.3</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td></td>
<td>30.3</td>
</tr>
</tbody>
</table>

All other variables were as outlined in Table 2.
<table>
<thead>
<tr>
<th>Buffer</th>
<th>pH (app)</th>
<th>I</th>
<th>$10^5 k_{\text{obs}}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate 6.93</td>
<td>0.05</td>
<td>17.3</td>
<td></td>
</tr>
<tr>
<td>Phosphate 7.47</td>
<td>&quot;</td>
<td>51.3</td>
<td></td>
</tr>
<tr>
<td>Phosphate 7.96</td>
<td>&quot;</td>
<td>166.0</td>
<td></td>
</tr>
<tr>
<td>Phosphate 8.41</td>
<td>&quot;</td>
<td>502.0</td>
<td></td>
</tr>
</tbody>
</table>

The wavelength at which the reactions were studied was 270 nm. The other variables were as outlined in Table 2.
TABLE 5: THE RATES OF THE HYDROXIDE CATALYSED HYDROLYSIS OF m-TOLYL 4-HYDROXYBUTYRATE AT 30.0° IN A BUFFERED SOLUTION OF DIOXAN IN WATER (20% v/v)

<table>
<thead>
<tr>
<th>Buffer</th>
<th>pH (app)</th>
<th>I</th>
<th>$10^5k_{obs}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate</td>
<td>6.96</td>
<td>0.050</td>
<td>11.7</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>12.1</td>
</tr>
<tr>
<td>Phosphate</td>
<td>7.40</td>
<td>0.050</td>
<td>35.7</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>36.0</td>
</tr>
<tr>
<td>Phosphate</td>
<td>7.94</td>
<td>0.050</td>
<td>115.0</td>
</tr>
<tr>
<td>Phosphate</td>
<td>8.37</td>
<td>0.050</td>
<td>319.0</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>307.0</td>
</tr>
<tr>
<td>Phosphate Sodium chloride</td>
<td>8.32</td>
<td>0.025</td>
<td>269.0</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>274.0</td>
</tr>
</tbody>
</table>

The reactions were studied at 273 nm. The other variables were as in Table 2.
The reactions were studied at 270 nm. A concentrated solution of the ester (10 μl; 0.15 M) was injected into the buffer solution (3.0 ml).

\[ k_{\text{OH}} = 5.42 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}. \]

The graph of log \( k_{\text{obs}} \) versus pH is shown on p. 154.
### Table 7: The Rates of the Acid and Hydroxide Catalysed Hydrolysis of Phenyl 4-Hydroxybutyrate at 50.0°C in Buffered Aqueous Solution

<table>
<thead>
<tr>
<th>Buffer</th>
<th>pH</th>
<th>I</th>
<th>$10^5k_{obs}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrochloric acid (0.10 N)</td>
<td>1.10</td>
<td>0.100</td>
<td>56.8</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>56.8</td>
</tr>
<tr>
<td>Chloracetate</td>
<td>2.85</td>
<td>0.050</td>
<td>1.36</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1.38</td>
</tr>
<tr>
<td>Chloracetate</td>
<td>3.50</td>
<td>0.050</td>
<td>0.76</td>
</tr>
<tr>
<td>Acetate</td>
<td>3.96</td>
<td>0.050</td>
<td>2.01</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2.01</td>
</tr>
<tr>
<td>Acetate</td>
<td>5.21</td>
<td>0.050</td>
<td>9.25</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>9.69</td>
</tr>
<tr>
<td>Acetate</td>
<td>5.68</td>
<td>0.050</td>
<td>27.1</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>27.3</td>
</tr>
<tr>
<td>Phosphate</td>
<td>6.02</td>
<td>0.050</td>
<td>62.1</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>61.1</td>
</tr>
<tr>
<td>Chloracetate</td>
<td>2.06</td>
<td>0.050</td>
<td>6.55</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>6.52</td>
</tr>
<tr>
<td>Chloracetate</td>
<td>2.08</td>
<td>0.040</td>
<td>6.16</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>&quot;</td>
<td>&quot;</td>
<td>6.19</td>
</tr>
<tr>
<td>Chloracetate</td>
<td>2.13</td>
<td>0.025</td>
<td>5.52</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>&quot;</td>
<td>&quot;</td>
<td>5.48</td>
</tr>
</tbody>
</table>

All other variables were as outlined in Table 6.

$$k_{H^+} = 7.10 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$$

$$k_{OH^-} = 1.04 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$$

The graph of $\log k_{obs}$ versus pH is shown on p. 156.
TABLE 8: THE RATES OF THE PERCHLORIC ACID CATALYSED HYDROLYSIS OF PHENYL 4-HYDROXYBUTYRATE AT 30.0° IN AQUEOUS SOLUTION

<table>
<thead>
<tr>
<th>Molarity</th>
<th>(10^5k_{obs} \text{ (s}^{-1})</th>
<th>-log (k_{obs})</th>
<th>-H(_o)</th>
<th>(a_{H_2O})</th>
<th>-log (a_{H_2O})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.000</td>
<td>122</td>
<td>2.9136</td>
<td>0.30</td>
<td>0.9950</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>122</td>
<td>2.9136</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>119</td>
<td>2.9245</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.000</td>
<td>319</td>
<td>2.4962</td>
<td>0.85</td>
<td>0.9057</td>
<td>0.043</td>
</tr>
<tr>
<td></td>
<td>307</td>
<td>2.5129</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.995</td>
<td>622</td>
<td>2.2062</td>
<td>1.33</td>
<td>0.8260</td>
<td>0.0830</td>
</tr>
<tr>
<td></td>
<td>624</td>
<td>2.2048</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.947</td>
<td>1140</td>
<td>1.9431</td>
<td>1.76</td>
<td>0.7387</td>
<td>0.1315</td>
</tr>
<tr>
<td></td>
<td>1160</td>
<td>1.9355</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.021</td>
<td>2160</td>
<td>1.6655</td>
<td>2.32</td>
<td>0.6067</td>
<td>0.2170</td>
</tr>
<tr>
<td></td>
<td>2140</td>
<td>1.6696</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All other variables were as outlined in Table 6.
### TABLE 9: THE RATES OF THE PERCHLORIC ACID CATALYSED HYDROLYSIS OF PHENYL BUTYRATE AT 30.0°C IN AQUEOUS SOLUTION

<table>
<thead>
<tr>
<th>Molarity</th>
<th>$10^5 k_{obs} (s^{-1})$</th>
<th>$-\log k_{obs}$</th>
<th>$-\log a_{H^+}$</th>
<th>$a_{H_2O}$</th>
<th>$-\log a_{H_2O}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.000</td>
<td>6.71</td>
<td>4.1733</td>
<td>0.30</td>
<td>0.9950</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>6.88</td>
<td>4.1624</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.000</td>
<td>12.4</td>
<td>3.9066</td>
<td>0.85</td>
<td>0.9057</td>
<td>0.043</td>
</tr>
<tr>
<td></td>
<td>12.8</td>
<td>3.8928</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.995</td>
<td>19.4</td>
<td>3.7122</td>
<td>1.33</td>
<td>0.8260</td>
<td>0.0830</td>
</tr>
<tr>
<td></td>
<td>19.3</td>
<td>2.7144</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.947</td>
<td>27.2</td>
<td>3.5654</td>
<td>1.76</td>
<td>0.7387</td>
<td>0.1315</td>
</tr>
<tr>
<td></td>
<td>26.6</td>
<td>3.5751</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.021</td>
<td>35.8</td>
<td>3.4461</td>
<td>2.32</td>
<td>0.6067</td>
<td>0.2170</td>
</tr>
<tr>
<td></td>
<td>35.7</td>
<td>3.4473</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All other variables were as outlined in Table 6.
\[
\text{LHS: } \text{HO(}\text{CH}_2\text{)}_3\text{CO}_2\text{Ph} \\
\text{RHS: } \text{H(}\text{CH}_2\text{)}_3\text{CO}_2\text{Ph}
\]
\[ \text{log molarity} \]

\[ -\log k_{\text{obs}} \]

- **H(CH_2)_3CO_2Ph**  RHS
- **HO(CH_2)_3CO_2Ph**  LHS
\[ \log k_{\text{obs}} - \log a_{\text{H}_2\text{O}} \]

Graph showing log-log plot with points for two compounds:

- \( \times \) \( \text{CH}_3\text{CH}_2\text{CH}_2\text{CO}_2\text{Ph} \) on the RHS
- \( \circ \) \( \text{HO(CH}_2\text{)}_3\text{CO}_2\text{Ph} \) with \( w^* = -2.46 \) on the LHS

The graph illustrates a linear relationship in the log-log scale.
\[ \text{CH}_3\text{CH}_2\text{CH}_2\text{CO}_2\text{Ph} \quad \phi = 0.98 \quad \text{RHS} \]
\[ \text{HO}(\text{CH}_2)_3\text{CO}_2\text{Ph} \quad \phi = 0.60 \quad \text{LHS} \]

\[ \text{log } k_{\text{obs}} + H_0 \]

\[ H_0 + \log \text{molarity} \]

\[ -39 \]

\[ -37 \]

\[ -35 \]

\[ -33 \]

\[ -15 \]

\[ -10 \]

\[ -55 \]

\[ -5.0 \]

\[ -4.5 \]

\[ 163. \]
TABLE 10: THE RATES OF THE HYDROLYSIS OF PHENYL 4-HYDROXYBUTYRATE IN AQUEOUS PERCHLORIC ACID (1.000 M)

<table>
<thead>
<tr>
<th>$10^5 k_{obs}$ (s$^{-1}$)</th>
<th>$-\log k_{obs}$</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>47.1</td>
<td>3.3270</td>
<td>20.10$^o$</td>
</tr>
<tr>
<td>46.9</td>
<td>3.3288</td>
<td>&quot;</td>
</tr>
<tr>
<td>122</td>
<td>2.9136</td>
<td>29.97$^o$</td>
</tr>
<tr>
<td>122</td>
<td>2.9136</td>
<td>&quot;</td>
</tr>
<tr>
<td>295</td>
<td>2.5302</td>
<td>40.04$^o$</td>
</tr>
<tr>
<td>289</td>
<td>2.5391</td>
<td>&quot;</td>
</tr>
<tr>
<td>659</td>
<td>2.1811</td>
<td>49.97$^o$</td>
</tr>
<tr>
<td>680</td>
<td>2.1675</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

All other variables were as outlined in Table 6.

Linear least squares treatment of data yields

$\Delta H^\# = 16.8 \pm 0.1 \text{ k cal mol}^{-1}$

$\Delta S^\# = -18.4 \pm 0.3 \text{ eu}$
### TABLE 11: The Rates of the Hydrolysis of Phenyl Butyrate in Aqueous perchloric Acid (1.000 M)

| $10^5 k_{obs} (s^{-1})$ | $-\log k_{obs}$ | Temperature  
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>6.71</td>
<td>4.1733</td>
<td>30.18°</td>
</tr>
<tr>
<td>6.88</td>
<td>4.1624</td>
<td></td>
</tr>
<tr>
<td>17.2</td>
<td>3.7645</td>
<td>40.07°</td>
</tr>
<tr>
<td>16.6</td>
<td>3.7799</td>
<td></td>
</tr>
<tr>
<td>41.2</td>
<td>3.3851</td>
<td>49.97°</td>
</tr>
<tr>
<td>38.5</td>
<td>3.4145</td>
<td></td>
</tr>
<tr>
<td>40.3</td>
<td>3.3947</td>
<td></td>
</tr>
</tbody>
</table>

All other variables were as outlined in Table 6.

Linear least squares treatment of data yields

\[ \Delta H^\ddagger = 17.2 \pm 0.2 \text{ kcal mol}^{-1} \]
\[ \Delta S^\ddagger = -22.9 \pm 0.6 \text{ e u} \]
TABLE 12: THE RATES OF HYDROLYSIS OF PHENYL 4-HYDROXYBUTYRATE AT 50.0° IN DIFFERENT AQUEOUS ACETATE BUFFERS OF TOTAL IONIC STRENGTH 0.20

<table>
<thead>
<tr>
<th>I(acetate)</th>
<th>I(potassium chloride)</th>
<th>pH</th>
<th>(10^5 k_{\text{obs}}(s^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.20</td>
<td>0</td>
<td>5.17</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12.0</td>
</tr>
<tr>
<td>0.16</td>
<td>0.04</td>
<td>5.16</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11.9</td>
</tr>
<tr>
<td>0.12</td>
<td>0.08</td>
<td>5.14</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10.8</td>
</tr>
<tr>
<td>0.08</td>
<td>0.12</td>
<td>5.14</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.87</td>
</tr>
</tbody>
</table>

All other variables were as outlined in Table 6. The graph of \(k_{\text{obs}}\) versus acetate concentration is shown on p. 167.

\[ k_{\text{OAc}} = 1.83 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}. \]
### Table 13: The Rates of Hydrolysis of Phenyl 4-Hydroxybutyrate at 30.0° in Different Aqueous Phosphate Buffers of Total Ionic Strength 0.20

<table>
<thead>
<tr>
<th>I(phosphate)</th>
<th>I(potassium chloride)</th>
<th>pH</th>
<th>$10^5 k_{obs}$ (s$^{-1}$)</th>
<th>$10^2 \times$ Molarity of Na$_2$HPO$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.20</td>
<td>0</td>
<td>7.30</td>
<td>193</td>
<td>6.05</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>192</td>
<td>&quot;</td>
</tr>
<tr>
<td>0.16</td>
<td>0.04</td>
<td>7.29</td>
<td>186</td>
<td>4.84</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>185</td>
<td>&quot;</td>
</tr>
<tr>
<td>0.12</td>
<td>0.08</td>
<td>7.27</td>
<td>176</td>
<td>3.63</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>178</td>
<td>&quot;</td>
</tr>
<tr>
<td>0.08</td>
<td>0.12</td>
<td>7.26</td>
<td>168</td>
<td>2.42</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>168</td>
<td>&quot;</td>
</tr>
<tr>
<td>0.04</td>
<td>0.16</td>
<td>7.25</td>
<td>156</td>
<td>1.21</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>158</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

All other variables were as outlined in Table 6. The graph of $k_{obs}$ versus phosphate concentration is shown on p. 169.

$k_{PHOS} = 6.78 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$. 
### TABLE 14: THE RATES OF HYDROLYSIS OF PHENYL 4-HYDROXYBUTYRATE AT 30.0° IN DIFFERENT AQUEOUS IMIDAZOLE BUFFERS OF TOTAL IONIC STRENGTH 0.20

<table>
<thead>
<tr>
<th>I(imidazole hydrochloride)</th>
<th>I(potassium chloride)</th>
<th>pH</th>
<th>$10^5 k_{obs} (s^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.20</td>
<td>0</td>
<td>7.14</td>
<td>292</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>296</td>
</tr>
<tr>
<td>0.16</td>
<td>0.04</td>
<td>7.15</td>
<td>254</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>257</td>
</tr>
<tr>
<td>0.12</td>
<td>0.08</td>
<td>7.16</td>
<td>218</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>215</td>
</tr>
<tr>
<td>0.08</td>
<td>0.12</td>
<td>7.17</td>
<td>175</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>176</td>
</tr>
<tr>
<td>0.04</td>
<td>0.16</td>
<td>7.17</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>135</td>
</tr>
</tbody>
</table>

All other variables were as outlined in Table 6.

- $k_{OH^-} = 8.19 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$
- $k_{obs}$ (extrapolated) = $9.7 \times 10^{-4} \text{ s}^{-1}$ at zero imidazole buffer concentration
- $k_{IM} = 9.90 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$
- $k_{OH^-}/k_{IM} = 8.29 \times 10^5$
$10^3 k_{obs}$ vs. (imidazole)
<table>
<thead>
<tr>
<th>n</th>
<th>X</th>
<th>$10^5k_{obs} (s^{-1})$</th>
<th>$-log k_{obs}$</th>
<th>$\sigma_{177}$</th>
<th>$\lambda (nm)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>H</td>
<td>54.4</td>
<td>3.2644</td>
<td>0</td>
<td>270</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>56.8</td>
<td>3.2457</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>56.8</td>
<td>3.2457</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>Me</td>
<td>54.5</td>
<td>3.2636</td>
<td>-0.07</td>
<td>273</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>54.2</td>
<td>3.2660</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>Cl</td>
<td>50.2</td>
<td>3.2993</td>
<td>0.37</td>
<td>274</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>50.7</td>
<td>3.2950</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>F</td>
<td>48.6</td>
<td>3.3134</td>
<td>0.35</td>
<td>267</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>49.3</td>
<td>3.3072</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>95.9</td>
<td>3.0182</td>
<td>0</td>
<td>270</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>94.5</td>
<td>3.0246</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>Me</td>
<td>100</td>
<td>3.0000</td>
<td>-0.07</td>
<td>273</td>
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<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>99.3</td>
<td>3.0031</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>Cl</td>
<td>85.5</td>
<td>3.0680</td>
<td>0.37</td>
<td>274</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>87.7</td>
<td>3.0570</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

The buffer was contained in water. A concentrated solution of the ester (10 μl, 0.15 M) was injected into the buffer solution (3 ml). The graphs of $log k_{obs}$ versus $\sigma$ is shown on p. 173.

For n = 3 and 4, $\rho = -0.13$ (approx.).
x n=4 RHS
⊙ n=3 LHS

log k_{obs}
TABLE 16: THE RATES OF HYDROLYSIS OF
\[\text{HO}-(\text{CH}_2)_n\text{CO}_2\text{m}-\text{X}\cdot\text{Ar}\]
IN AQUEOUS PHOSPHATE BUFFER
(pH = 6.02; I = 0.05) AT 50.0°

<table>
<thead>
<tr>
<th>n</th>
<th>X</th>
<th>(10^5 k_{obs} \text{ (s}^{-1})</th>
<th>-log (k_{obs})</th>
<th>(\delta_m)</th>
<th>(\lambda (\text{nm}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>H</td>
<td>62.1</td>
<td>3.2069</td>
<td>0</td>
<td>270</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>61.1</td>
<td>3.2140</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>Me</td>
<td>45.8</td>
<td>3.3391</td>
<td>-0.07</td>
<td>273</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>44.7</td>
<td>3.3497</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>Cl</td>
<td>144</td>
<td>2.8416</td>
<td>0.37</td>
<td>274</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>147</td>
<td>2.8327</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>143</td>
<td>2.8447</td>
<td>0.35</td>
<td>267</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>144</td>
<td>2.8416</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>6.06</td>
<td>4.2175</td>
<td>0</td>
<td>270</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>6.13</td>
<td>4.2125</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>Me</td>
<td>4.64</td>
<td>4.3335</td>
<td>-0.07</td>
<td>273</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>4.60</td>
<td>4.3372</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

All other variables were as outlined in Table 15. The graph of log \(k_{obs}\) versus \(\delta_m\) for \(n = 3\) is shown on p. 175.
\(\epsilon = 1.08\) (approx.).
### TABLE 17: THE RATES OF HYDROLYSIS OF

\[ \text{HO}-(\text{CH}_2)_n-\text{CO}_2\text{m}-\text{X} \cdot \text{Ar} \]

IN PERCHLORIC ACID (1.000 M) AT 30.0°

<table>
<thead>
<tr>
<th>n, X</th>
<th>(10^5 k_{\text{obs}} \text(s}^{-1})</th>
<th>(-\log k_{\text{obs}})</th>
<th>(\sigma_{m}^{177})</th>
<th>(\lambda \text{(nm)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>3, H</td>
<td>122</td>
<td>2.9136</td>
<td>0</td>
<td>270</td>
</tr>
<tr>
<td>&quot;</td>
<td>122</td>
<td>2.9136</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>119</td>
<td>2.9245</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>Me 121</td>
<td>2.9172</td>
<td>-0.07</td>
<td>273</td>
</tr>
<tr>
<td>&quot;</td>
<td>120</td>
<td>2.9208</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>Cl 110</td>
<td>2.9586</td>
<td>0.37</td>
<td>274</td>
</tr>
<tr>
<td>&quot;</td>
<td>110</td>
<td>2.9586</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>F 109</td>
<td>2.9626</td>
<td>0.35</td>
<td>267</td>
</tr>
<tr>
<td>&quot;</td>
<td>108</td>
<td>2.9666</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>4, H</td>
<td>252</td>
<td>2.5986</td>
<td>0</td>
<td>270</td>
</tr>
<tr>
<td>&quot;</td>
<td>264</td>
<td>2.5784</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>Me 273</td>
<td>2.5638</td>
<td>-0.07</td>
<td>273</td>
</tr>
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</tr>
<tr>
<td>&quot;</td>
<td>276</td>
<td>2.5591</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>Cl 230</td>
<td>2.6383</td>
<td>0.37</td>
<td>274</td>
</tr>
<tr>
<td>&quot;</td>
<td>231</td>
<td>2.6364</td>
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<td>&quot;</td>
</tr>
</tbody>
</table>

All other variables were as outlined in Table 15. The graphs of \(\log k_{\text{obs}}\) versus \(\sigma_{m}\) are shown on p. 177.

For \(n = 3\), \(\varepsilon = -0.11\) (approx.).

For \(n = 4\), \(\varepsilon = -0.16\) (approx.).
## TABLE 18: THE RATES OF HYDROLYSIS OF 
\( \text{HO-(CH}_2)_n\text{-CO}_2\text{m-X}\cdot\text{Ar} \)
IN AQUEOUS PHOSPHATE BUFFER 
\((\text{pH} = 7.47; \ I = 0.05) \text{ AT 30.0}^\circ \)

<table>
<thead>
<tr>
<th>n</th>
<th>X</th>
<th>(10^5k_{\text{obs}})</th>
<th>(-\log{k_{\text{obs}}})</th>
<th>(\sigma_\text{m}^{177})</th>
<th>(\lambda) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>H</td>
<td>239</td>
<td>2.6216</td>
<td>0</td>
<td>270</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>239</td>
<td>2.6216</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>Me</td>
<td>174</td>
<td>2.7595</td>
<td>-0.07</td>
<td>273</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>174</td>
<td>2.7595</td>
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<td>&quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>Cl</td>
<td>575</td>
<td>2.2403</td>
<td>0.37</td>
<td>274</td>
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<tr>
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<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>F</td>
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<td>2.2403</td>
<td>0.35</td>
<td>267</td>
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<td>3.8861</td>
<td>&quot;</td>
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</tr>
<tr>
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<td>3.9747</td>
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<tr>
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<td>3.9830</td>
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</tr>
<tr>
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<td>&quot;</td>
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<td>3.9788</td>
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<tr>
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<td>31.1</td>
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</tbody>
</table>

All other variables were as outlined in Table 15. The graphs of \(\log{k_{\text{obs}}}\) versus \(\sigma_\text{m}\) are shown on p. 179. \(\rho = 1.05\) (approx.).
TABLE 19: THE pH OF BUFFERED SOLUTIONS OF DIOXAN IN WATER (20% v/v) AT 30.0°

<table>
<thead>
<tr>
<th>Buffer</th>
<th>pH\textsuperscript{176}</th>
<th>pH (app)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>9.907</td>
<td>9.59</td>
</tr>
<tr>
<td>Acetate</td>
<td>5.292</td>
<td>5.15</td>
</tr>
<tr>
<td>Formate</td>
<td>4.180</td>
<td>4.09</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.629</td>
<td>2.62</td>
</tr>
</tbody>
</table>

pH (app) = 6.81 corresponds to pH = 7.02

The graph of pH versus pH (app) is shown on p. 181.
DISCUSSION

Much evidence has been accumulated that indicates that the hydroxy-group of serine-195 and the imidazolyl group of histidine-57 are implicated in the active site of both trypsin and chymotrypsin. The two mechanisms most consistent with this evidence are shown below.
Mechanism G involves nucleophilic (imidazoyle) and general acid (serine-195 hydroxy-group) catalysis while mechanism H depicts general base catalysis by the imidazolyl of nucleophilic attack by the serine hydroxy-group. Ideally to test this hypothesis a model system is required in which a primary hydroxy-group, an ester and an imidazolyl residue
are situated in exactly the same position with respect to each other as in the active site in the enzyme. The preparation of such systems is plagued by difficulties so that studies are commonly made on intramolecular reactions in the hope that they will shed some light on enzymatic reactions. Such results obtained, however, may have no bearing at all on enzymatic reactions. For example, in the intermolecular reaction the molecules can take up an infinite number of arrangements with respect to each other and by virtue of this alone may be able to react in a variety of ways. However, in enzymatic reactions the molecules are at least semi-rigidly fixed which restricts the number of ways in which it can act. A model system that has many similarities to the active site in trypsin and chymotrypsin are esters of 4-hydroxybutyric acid and 5-hydroxyvaleric acid. Thus, addition of an ester to the enzyme results in the rapid formation of a loosely bonded species which then breaks down with the resultant formation of a phenol (or alcohol) and an acyl-enzyme complex. The acyl-enzyme then undergoes a slower hydrolysis to form the acid with resultant
regeneration of the enzyme. Although the expulsion of the phenol is very much faster than the hydrolysis of the acyl-enzyme both reactions are very much faster than either the acid or base catalysed reactions of simple phenyl esters at the same pH. The hydroxy-group in the model systems is ideally situated for interacting with the ester linkage and thus closely resembles the loose complex formed between the ester and enzyme.

\[
\begin{align*}
\text{CO}_2\text{Ar} & \quad \text{CO}_2\text{Ar} \\
\text{OH} & \quad \text{OH}
\end{align*}
\]

One could anticipate that under the right conditions hydroxy-participation would occur with formation of a phenol and either 4-butyrolactone or 5-valerolactone which in turn would be hydrolysed to the corresponding hydroxy-acid. Bruice has suggested\(^{179}\) that since O-acetyl serine is more susceptible to hydrolysis than an ordinary ester the O-acyl-enzyme must be of a highly reactive form. He suggested that the O-acyl-enzyme took up a \textit{cis} conformation as opposed
to the usual trans which is more stable, and accordingly hydrolysed much quicker than "ordinary esters." As a model for the above he compared the hydrolysis of ethyl acetate and 4-nitrophenyl acetate with those of 4-butyrolactone and 5-valerolactone which by virtue of their ring size must exist in the cis form. The relative rate constants for the hydroxide catalysed reaction at 78° are 1 : 3640 : 461 : 3050, respectively. Presumably the greater instability of the cis esters is caused by lone-pair interaction between the oxygen atoms. In order to be directly comparable to the enzyme system an imidazolyl residue should be suitably disposed to the hydroxy-group to act as a general base. Such a situation would be very similar to the proposed active site in chymotrypsin.
The above mechanism very closely resembles mechanism \( H \) previously outlined for chymotrypsin (p. 183). A similar mechanism can be outlined corresponding to mechanism \( G \). However, the system in fact is complicated by a number of factors. Hydrolysis of 4-butyrolactone and 5-valerolactone has been shown to be catalysed by both imidazole and hydroxide ion and their relative magnitudes are dependent on the temperature and the concentration of the free base form of imidazole.\(^{180}\) Although the authors do not identify the mechanism of the imidazole catalysed reaction it probably is a general base by analogy with other systems.\(^{239}\)

\[
k_{\text{obs}} = k_{\text{IM}} \left( \frac{K}{K + a_{H^+}} \right) C_T + k_{\text{OH}^-} \left( \frac{K}{a_{H^+}} \right)
\]

where \( K \) is the dissociation constant for the imidozolinium cation and \( C_T \) is the total concentration of imidazole species. Thus the relative contributions to the hydrolysis in basic solution is:
where $K = 10^{-7} \text{M}$, $K_{\text{OH}} = 1.2 \times 10^{3} \text{M}^{-1} \text{min}^{-1}$, $k_{\text{IM}} = 1.2 \times 10^{-3} \text{M}^{-1} \text{min}^{-1}$ and $C_T = 1.0 \text{M}$. At pHs 5, 6, 7, 8 and 9 the above ratios are $10^{-3}$, $10^{-2}$, 0.05, 1.1, 10, respectively, so that in the pH range of interest (7 - 8) the two reactions are of comparable magnitude. At lower temperatures the hydroxide ion catalysed reaction dwarfs even further the imidazole catalysed reaction. Another complication is that imidazole is known to catalyse the hydrolysis of esters in at least two different ways. For esters with good leaving groups nucleophilic mechanism prevails while those with poor leaving groups hydrolyse via a general base mechanism. At first sight the general base mechanism appears to be less favourable than the nucleophilic reaction since it involves a very specific arrangement of three molecules. However, if a nucleophilic mechanism is denied to a particular ester because the $pK_a$ of
the conjugate acid of the leaving group is high then general base mechanism becomes significant. For the phenyl esters of 4-hydroxybutyric acid and 5-hydroxyvaleric acid a nucleophilic mechanism for hydrolysis is possible as for simple phenyl esters but now there is also the possibility of a general base catalysed reaction that is a bimolecular reaction and thus might be expected to be more favourable. In an attempt to distinguish between the two, the hydrolysis of the 2-naphthyl esters of 4-hydroxybutyric acid, 4-methoxybutyric acid and butyric acid were compared. The rates of hydrolysis at pH 6.82 in imidazole buffer of ionic strength 0.1 and 30.0°C are 4.0 : 1.3 : 1 (Table 1, p. 147). Since the esters of butyric acid and methoxybutyric acid hydrolyse by a nucleophilic mechanism this data indicates that either 2-naphthyl 4-hydroxybutyrate also hydrolyses by this mechanism or that the free energy of activation for the nucleophilic and general base mechanisms are similar and that the reaction proceeds by both mechanisms. In an attempt to resolve these difficulties phenyl 4-hydroxybutyrate was studied in imidazole, phosphate and acetate aqueous buffers since the hydrolysis of phenyl acetate has been
studied in a variety of buffers. Ordinarily the determination of the catalytic ratio for basic hydrolysis ($k_{\text{OH}^-}$) to that for imidazole ($k_{\text{IM}}$) and the catalytic ratio for phosphate ($k_{\text{PHOS}}$) to imidazole gives a positive indication to the mechanism of the imidazole catalysed reaction. Thus if imidazole acts as a nucleophile $k_{\text{IM}}/k_{\text{PHOS}}$ will be very large ($\sim 10^4$) because the phosphate dianion is such a weak nucleophile although of the same basicity. In contrast if imidazole acts as a general base the catalytic ratio will be about four. Similarly the hydroxide anion is a powerful nucleophile and experiments show that for imidazole acting as a nucleophile $k_{\text{IM}}/k_{\text{OH}^-}$ is $10^3 - 10^5$ whereas for general base reactions the ratio is $10^5 - 10^6$. All these calculations are based on general base reactions being termolecular but in the system studied if a general base mechanism is occurring it is probably a predominantly bimolecular reaction. Thus $k_{\text{OH}^-}/k_{\text{IM}} = 8.29 \times 10^5$ for phenyl 4-hydroxybutyrate (p.170) but this ratio is inflated due to $k_{\text{OH}^-}$ not being solely the result of attack of hydroxide ion at the carbonyl centre but in addition that due to any specific base catalysis at the alcohol residue. Similarly $k_{\text{IM}}/k_{\text{PHOS}} = 1.46$ (p.168) but
this could be due to the imidazole catalysed reaction proceeding by a predominantly nucleophilic mechanism (as reflected in its similar catalytic constant to that of phenyl acetate \( k_{\text{IM}} = 9.90 \times 10^{-3} \, \text{M}^{-1} \, \text{s}^{-1} \) at 30\(^\circ\)C and \( I = 0.20 \) (p. 170) as opposed to \( k_{\text{IM}} \) (phenyl acetate\(^{181}\)) = 8.90 \times 10^{-3} \, \text{M}^{-1} \, \text{s}^{-1} \) at 25\(^\circ\)C and \( I = 1.0 \) while that for phosphate is increased due to the possibility of an intramolecular reaction.

Comparison of the hydroxide catalytic constants shows that \( k_{\text{OH}} \) for phenyl 4-hydroxybutyrate is about 4000 times greater than that for phenyl acetate \( (8.19 \times 10^{3} \, \text{M}^{-1} \, \text{s}^{-1} \) at 30\(^\circ\)C and \( I = 0.20 \) (p. 170) as opposed to \( 1.27 \, \text{M}^{-1} \, \text{s}^{-1} \) at 25\(^\circ\)C and \( I = 1.0^{181}\)). Similarly the catalytic constant for the acetate catalysed reaction is approximately 200 times greater than that for phenyl acetate \( (1.83 \times 10^{-4} \, \text{M}^{-1} \, \text{s}^{-1} \) at 30.0\(^\circ\)C \( I = 0.20 \) and pH 5.15 (p. 166) as opposed to \( 3.84 \times 10^{-7} \, \text{M}^{-1} \, \text{s}^{-1} \) at 25\(^\circ\), \( I = 0.50 \) and pH 5.0.\(^{36}\) There is no data in the literature for the catalytic constant for phosphate dianion in the hydrolysis of phenyl acetate. However, p-nitrophenyl acetate has been studied\(^5\) and \( k_{\text{PHOS}} \) found to be \( 1.23 \times 10^{-4} \, \text{M}^{-1} \, \text{s}^{-1} \) at 25.0\(^\circ\), \( I = 0.50 \) and pH 5.9. Despite the activating p-nitro group this is still approximately 40 times
less than that for phenyl 4-hydroxybutyrate ($k_{PHOS} = 6.78 \times 10^{-3} \text{ M}^{-1} \text{s}^{-1}$ at 30.0°, $I = 0.20$ and pH 7.27 (p. 168)). A variety of mechanisms may be suggested for these reactions (p. 4 and 5). The kinetic data shows the rate of reaction to be first order in concentration of base and ester. Thus these species are involved in the formation of the rate-determining transition state but it is impossible to differentiate between the above mechanisms on the basis of the kinetic law since they are kinetically indistinguishable. For hydroxide, acetate and phosphate catalysis the hydroxy-group must be involved since their catalytic rate constants are so much greater than in the analogous non-hydroxy-substituted compounds. In contrast the catalytic rate constant for the imidazole catalysed reaction is similar to that for the unsubstituted ester so that the hydroxy-group may or may not be involved.

Mechanisms E and F are similar to those proposed to explain the enhanced reactivity of the monoacetates of 1, 3-diëxial alcohols. In view of recent work on a comparable simple model system in which a six-membered
ring transition state (as opposed to seven and eight in the aryl esters of 4-hydroxybutyric and 5-hydroxyvaleric acids, respectively) is potentially possible the above seems unlikely since no rate increases were found.  

Even with a semi-rigid system in which hydrogen bonding effects would be maximised little stabilisation of the transition state occurs since the cis and trans isomers hydrolyse at the same rate. Further scrutiny of mechanism F (as exemplified above) shows it to be unlikely since any stabilisation occurs in the breakdown of the tetrahedral intermediate whereas the RDS for phenyl esters is thought to be attack of the base at the ester group.  

Mechanism B involves a rapid pre-equilibrium of the alcohol followed by a rate-determining nucleophilic attack at the ester group. Alternatively, one could invoke general
base catalysis by the alkoxide ion but an identical kinetic equation would be obtained (Mechanism D).

\[
\begin{align*}
&\text{CO}_2\text{Ph} \quad \text{CO}_2\text{Ph} \\
&\quad \quad \leftrightarrow \quad \text{C}=\text{O} \\
&\quad \quad + \text{PhO}^- \quad \text{OH}
\end{align*}
\]

Now the rate is proportional to the concentration of alkoxide anion which is related to dissociation constant of the alcohol as shown below.

\[
\text{Rate} = k_2 (\text{RO}^-) \\
\text{and } K_a = \frac{(\text{RO}^-)(\text{H}^+)}{\text{(ROH)}} \\
\therefore \frac{k_2 K_a (\text{ROH})}{(\text{H}^+)} = k_{\text{obs}} (\text{ROH}) \\
\therefore k_2 = \frac{k_{\text{obs}} (\text{H}^+)}{K_a}
\]

At 30.0° and pH 7.16, \(k_{\text{obs}}\) for the hydroxide catalysed reaction is \(9.7 \times 10^{-4} \text{ s}^{-1}\) (p. 170).

\[
\therefore k_2 = \frac{9.7 \times 10^{-4} \times 10^{-7.16}}{10^{-16}} \approx 10^6 \text{ s}^{-1}
\]

where \(K_a \approx 10^{16}\)
This rate is well within the diffusion controlled rate\(^{186}\) 
\(\cong 10^{11} \text{ M}^{-1} \text{ s}^{-1}\) and so cannot be eliminated.

Mechanism C involves general base attack at the hydroxy-group.

The rate equations for the catalysed hydrolysis at a given pH are

\[
\text{Rate} = \frac{d(\text{Phenolate})}{dt} = k_2 (\text{Tet}) - k_{-2} (\text{Lac}) (\text{PhO}^-)
\]

\[
\frac{d(\text{Tet})}{dt} = k_1 (\text{B}) (\text{Est}) - k_{-1} (\text{BH}) (\text{Tet}) - k_2 (\text{Tet}) + k_{-2} (\text{Lac}) (\text{PhO}^-)
\]

and if the steady state approximation\(^{186}\) holds then

\[
(Tet) = \frac{k_1 (B) (Est) + k_{-2} (Lac) (PhO^-)}{k_{-1} (BH) + k_2}
\]

Since \(k_{-2}\) would be expected to be much smaller than \(k_2\), the rate equation simplifies to

\[
\frac{d(\text{Phenolate})}{dt} = \frac{k_1 k_2 (B) (Est)}{k_{-1} (BH) + k_2} = k_{\text{obs}} (\text{Est})
\]
At low buffer concentrations $k_2 \gg k_1(BH)$ and $k_{obs} = k_1(B)$

At higher buffer concentrations $k_1(BH)$ cannot be ignored and at sufficiently high buffer concentrations $k_1(BH) \gg k_2$.

Then, $k_{obs} = \frac{k_1 k_2(B)}{k_1(BH)} = \frac{k_1 k_2 K_{BH}}{k_1(H)} = \frac{k_1 k_2 K_{BH}(OH^-)}{k_1 K_w}$

Thus, at sufficiently high buffer concentrations the rate of reaction is independent of buffer concentration. Inspection of the graphs for $k_{obs}$ versus concentration of buffer at a given pH does indeed show a levelling off of the observed rates at high buffer concentrations for both acetate and phosphate buffers (p. 167 and 169). However, an alternative explanation could be complexation of the buffer. Also inspection of the rate equation for Mechanism B (Equation 1) shows

$$k_{obs} = \frac{k_2 K_a}{(H^+)}$$

and this is void of any dependence on the buffer concentration at a constant pH. It is proposed that at least for phosphate and acetate catalysis the mechanism operative is general
base. In contrast the graphs of $k_{obs}$ versus concentration of imidazole at a constant pH is linear (p. 171). This could simply arise by not studying the reaction at sufficiently high imidazole concentrations but more likely in this instance to occur by a change from an asymmetric to symmetric mechanism. Consider the symmetric reaction outlined below.

$$R^1C=O + N \xrightarrow{k_1(B)} R^2C-O \xrightarrow{k_2(BH)} R^1CO_2H + R^2OH$$

In such a sequence of reactions the rate constant can be shown, assuming the concentration of the tetrahedral intermediate is small, to be

$$\frac{k_1k_2(B)}{k_1 + k_2} - \frac{k_2k_1(B)}{k_1 + k_2}$$

and thus the observed rate is linearly dependent on the concentration of buffer. Another explanation of the phenomenon is that the reaction is concerted and irreversible so that the first step is rate-determining. The description of the intermediate (if any) will be discussed later (p. 223).
It is interesting to compare the hydrolysis of phenyl 2-hydroxyphenyl acetate and phenyl 4-hydroxyphenyl acetate. Undoubtedly the 2-hydroxy-group is the cause of the very rapid hydrolysis since it hydrolyses approximately $10^6$ faster than the corresponding 4-substituted compound. Since resonance effects are common to both systems and there is no obvious release of steric strain in the transition state it is reasonable to propose that the hydroxy-group acts in a more direct manner. Mechanisms E and F proposed for the hydrolysis of esters of 4-hydroxybutyric acid and 5-hydroxyvaleric acid are unlikely for the reasons previously proposed. However, mechanisms B and C are both probable on first considerations.

$$\begin{align*}
\text{OH} & \text{CO}_2\text{Ph} \\
\text{CH}_2 & \text{CO}_2\text{Ph}
\end{align*}$$

\[ \text{B} \quad \begin{align*}
\text{CH}_2 & \quad k_1 \\ 
\text{CO}_2\text{Ph} & \quad k_{-1} \quad k_2 \\
\text{CO}_2\text{Ph} & \quad k_{-2}
\end{align*} \]

\[ \text{CH}_2 + \text{PhO}^- \]
Assuming that $k_{-2}$ is small compared to $k_2$ then,

$$\text{Rate} = k_2 (\text{ArO}^-)$$

and since

$$K = \frac{(\text{ArO}^-) (H^+)}{(\text{ArOH})}$$

$$\text{Rate} = \frac{k_2 K (\text{ArOH})}{(H^+)}$$

$$\therefore k_{\text{obs}} = \frac{k_2 K}{(H^+)}$$

Now at pH 4.15, $k_{\text{obs}} = 1.05 \times 10^{-2} \text{ s}^{-1}$ at $25^\circ$.

$$\therefore k_2 = \frac{k_{\text{obs}} (H^+)}{K} = \frac{1.05 \times 10^{-2} \times 10^{-4.15}}{10^{-10}} \approx 10^4 \text{ s}^{-1}$$

This again is well within the diffusion controlled rate and thus differentiation between mechanism B and C is again impossible from the kinetic data alone. Although mechanism C cannot be eliminated the following considerations show that if it occurs $k_1$ cannot be the RDS. If $k_1$ was the RDS, then
\[
\frac{k_1}{a_{OH}^-} = \frac{k_{obs} \times a_H^+}{K_w}
\]

At pH 4.15 and 25\(^\circ\), \(k_{obs} = 1.05 \times 10^{-2} \text{ s}^{-1}\)

\[
\therefore k_1 = \frac{1.05 \times 10^{-2} \times 10^{-4.15}}{1.47 \times 10^{-14}} = 5.06 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}
\]

However, it has been shown\(^\text{188}\) that the rate of proton transfer from phenol to hydroxide ion (to form the phenoxide ion and water) is approximately \(1.4 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}\). Thus, if mechanism \(\text{C}\) is operative breakdown of the tetrahedral intermediate must be the RDS. The above calculation similarly eliminates a general base mechanism with concerted elimination of the phenoxide group. It is noteworthy that \(k_2\) is about 100 times greater for phenyl 4-hydroxybutyrate than phenyl 2-hydroxyphenyl acetate (assuming they both hydrolyse via mechanism \(\text{B}\)) despite the latter being a more rigid system. This is undoubtedly due to the alkoxide ion being a much more powerful nucleophile and poorer leaving group than the aryloxide ion.

Another mechanism kinetically equivalent to that for \(\text{B}\) and analogous to that proposed for similar systems is general base attack by the phenoxide anion. This mechanism was
eliminated, however, (at least at low pH's) by the spectrophotometric observation of dihydrocoumarin which then further hydrolysed to the phenolic acid. The absence of any appreciable acetate catalysis indicates that at least for acetate no mechanism analogous to mechanism B occurs although this does not eliminate this possibility for other bases, particularly strong bases like hydroxide. The exceptionally high value of the catalytic constant, $k_{\text{OH}^-} = 10^8 \text{M}^{-1} \text{s}^{-1}$, for hydroxide ion is about $10^4$ and $10^8$ faster than that for phenyl 4-hydroxybutyrate (p. 170) and phenyl acetate, respectively.

An attempt was made to prepare phenyl 6-endo-hydroxy (2, 2, 1) bicylo-2-carboxylate(46) in order to compare a rigid hydroxy-(alcohol) ester with a hydroxy-(phenol) ester.

This compound also has other features of interest. As previously mentioned the catalysis of chymotrypsin is believed to proceed via formation of a complex which places
the ester adjacent to the serine-195 hydroxy-group. Although non-rigid systems like esters of 4-hydroxybutyric acid bear some resemblance to this the above ester is even more like the proposed enzyme active site. Mention has already been made of the difficulty in differentiating between an exclusively nucleophilic and combined nucleophilic and general base mechanisms in the imidazole catalysed hydrolysis of phenyl 4-hydroxybutyrate. In 46 if a nucleophilic mechanism occurred the rate should be similar or slightly less (due to steric hindrance to attack) than that for phenyl 4-hydroxybutyrate. However, if a general base mechanism occurred to any extent in phenyl 4-hydroxybutyrate it would be many times magnified in the above system and presumably be reflected in a large rate enhancement. Unfortunately efforts to make the above compound failed. A system which has even more similarities to the active site in chymotrypsin in that the imidazole group is suitably fixed is shown below (47).
This system has the further advantage that the hydroxy-group can be protected by the benzyl group and thus readily removed under mild conditions. Similarly, the imidazolyl residue can be deactivated by forming its salt.

**Spontaneous Hydrolysis of Phenyl 4-Hydroxybutyrate**

Since the pH rate profile exhibited no plateau the acid and/or base catalysed reactions are greater than or of equal magnitude to the uncatalysed reaction. An upper limit on its magnitude can be determined from the cross-over point on the pH rate profile. At $50^\circ$ and $I = 0.05$ the uncatalysed rate for formation of phenol is less than or equal to $6.9 \times 10^{-6} \text{ s}^{-1}$ (p. 156).

**Involvement of Hydroxy-groups in Ester Hydrolysis**

In the introduction (p. 4) the possible ways in which an intramolecularly bonded hydroxy-group could theoretically interact in the hydrolysis of an ester were outlined. While there are many claims of general base and general acid catalysis, examples of nucleophilic catalysis are much less common. There are, however, two detailed mechanistic studies of bimolecular reactions involving the
hydroxy-group of an alcohol and an ester group. Mention was made in the introduction (p. 6) of the enhanced hydrolysis of certain esters in the presence of tris-(hydroxymethyl)-aminomethane and N-acetylsersinamide which was later shown to be due to the abnormally low pKₐ's of the alcohols. Similarly the enhanced rate of hydrolysis of m-substituted phenyl acetate in the presence of cycloamylose has been attributed to nucleophilic attack by one of the hydroxy-groups on the cycloamylose. The latter reaction is not strictly a bimolecular reaction since an intermolecular complex is formed with the substrate and since this is productively bound it is similar to an intramolecular reaction. The authors proved that the same acylated cycloamylose ester was formed as an intermediate and this prompted them to suggest that one of the hydroxy-groups interacted with the m-substituted phenyl acetate by a nucleophilic mechanism. Identification of the particular hydroxy-group was not definitive but blocking experiments indicated that it was probably bonded to C-2 but not C-6. This was further substantiated from the pH rate profile which
implicated a group having a $pK_a$ of 12.1. While this figure is low for the more common alcohols it is of similar
magnitude to that of the ribose moiety of adenosine (12.35)\(^{189}\) which also has two adjacent secondary groups. In both examples the combined inductive effects of the oxygen atoms and the stabilisation by intramolecular hydrogen bonding of the resultant alkoxide ion have been preferred as explanations. Finally, models showed that inclusion of the $m$-substituent in the torus from that side of the torus containing the secondary hydroxy-groups situated the ester in close proximity to the secondary hydroxy-groups. Furthermore, if the acetyl portion is inserted into the cavity from the secondary side until it protrudes from the primary hydroxy-side (which cannot be done for the $meta$-isomer), then the aromatic ring and most of the substituent are included in the cycloamylose cavity. "In this model the ester function is located in close proximity to the primary hydroxyl groups."\(^{11}\) This suggests that it is not steric restrictions that prevent nucleophilic attack by the primary hydroxy-groups but the very low concentration of the alkoxide ion (due to its high
PK\textsubscript{a}). The very rapid hydrolysis of the aryl 4 and 5-hydroxy-esters must cast some doubts on this.

The explanation for the low PK\textsubscript{a} of the alcohol is attractive and consistent with current ideas on activation and stabilisation energies. The relatively low PK\textsubscript{a} of 3, 6-anhydro-\(\alpha\)-D-glycopyranoside\(12.2\)\textsuperscript{190} could similarly be explained although here the two hydroxy-groups are 1, 3 and not 1, 2-bonded. However, the PK\textsubscript{a} of 1, 6-anhydro-\(\varepsilon\)-D-glucose\textsuperscript{190} is 13.53 despite the two hydroxy-groups having the same relationship to each other. This may suggest that the interpretation of PK\textsubscript{a}'s is not as simple as usually believed and that caution should be exercised in identifying hydroxy-groups when more than one is present in the molecule.

The benzaldehyde\textsuperscript{3, 4} and carbon dioxide\textsuperscript{4} catalysed reactions of 4-nitrophenyl esters of various 2-amino-acids may also involve participation of a neighbouring hydroxy-group. The authors of these papers differ in what they regard as the rate-determining step, one tentatively suggesting it is the formation of the carbinolamine intermediate\textsuperscript{3} while the others consider it as breakdown of
this intermediate. The two papers are discussed in detail since clarification of this point and others may be afforded by the research herein.

The reaction scheme outlined for the benzaldehyde catalysed reaction is as follows:

\[
\text{H} + \text{H}_2\text{N}\cdot\text{CHR}\cdot\text{CO}_2\text{Ar} \rightarrow \text{products}
\]

The above scheme is very much simplified since under the conditions in which the reaction occurred various equilibria are of importance. A more detailed mechanism is shown on p. 208.
Let $k_{\text{obs}}$ be the observed first-order rate constant for the formation of 4-nitrophenol and $E_T$, $EH$ and $E$ the concentrations of total ester, protonated ester and ester as free base, respectively.

\[
K_1 = \frac{(E) (H)}{(EH)} \quad K_2 = \frac{(C) (H)}{(CH)} \quad K_3 = \frac{(\text{cat}) (E)}{(C)} = \frac{k_{-3}}{k_3}
\]

and $K_W = (H) (OH)$

where $(H)$ and $(OH)$ are the concentrations of hydrogen ions and hydroxide ions, respectively. All other terms are defined as shown in the diagram. Under the reactions conditions
outlined the spontaneous and acid-catalysed hydrolysis of E are likely to be small in comparison with the aldehyde catalysed reaction. Furthermore STEPS 3 and 4 would be expected to be either hydroxide catalysed reactions or uncatalysed reactions. If the former the rate equation for hydrolysis is -

\[ k_{obs} (E_T) = k_1 (E)(OH) + k_2 (EH)(OH) + k_1' (C)(OH) + k_2' (CH)(OH) \]

\[ = k_1 (E)(OH) + k_2 (EH)(OH) + \frac{k_1' (cat) (E)(OH)}{K_3} + \frac{k_2' (C)(H)(OH)}{K_2} \]

\[ = k_1 (E)(OH) + k_2 (E)(H)(OH) + \frac{k_1' (cat) (E)(OH)}{K_3} + \frac{k_2' (cat) (E)(H)(OH)}{K_2 K_3} \]

Since the concentration of C and CH\textsuperscript{3} are very small,

\[ E_T = E + E_H \]

\[ \therefore \frac{E}{E_T} = \frac{K_1}{K_1 + (H)} \]

\[ \therefore k_{obs} = \frac{K_1}{K_1 + (H)} \left( \frac{k_1 (OH)}{K_1} + \frac{k_2 K_w}{K_1 K_3} + \frac{k_1' (cat)(OH)}{K_3} + \frac{k_2' K_w (cat)}{K_2 K_3} \right) \]
For a given pH a graph of $k_{obs}$ vs. (cat) should have

$$\text{gradient} = \frac{K_1}{K_1 + (H)} \frac{k_1'(OH)}{K_3} + \frac{k_2'K_w}{K_2 K_3}$$

$$\therefore \text{gradient} (K_1 + (H)) = \frac{K_1}{K_3} \frac{k_1'(OH)}{k_2'} + \frac{k_2'}{K_2}$$

A graph of gradient $(K_1 + (H))$ vs. $(OH)$ should be linear.

If STEPS 3 and 4 were uncatalysed, the equivalent rate equation for the reaction is

$$k_{obs} = \frac{K_1}{K_1 + (H)} \frac{k_1(OH)}{k_2K_w} + \frac{k_1'(cat)}{K_3} + \frac{k_2'(cat)(H)}{K_2 K_3}$$

For a given pH a graph of $k_{obs}$ vs. (cat) should have

$$\text{gradient} = \frac{K_1}{K_1 + (H)} \frac{k_1'(cat)}{K_3} + \frac{k_2'(H)}{K_2 K_3}$$

$$\therefore (K_1 + (H)) \text{ gradient} = \frac{K_1}{K_3} k_1' + \frac{k_2'}{K_2}$$

The estimated $pK_a$ for 4-nitrophenyl L-leucinate is 7.2.

Therefore, $pK_1 = 7.2$ and $K_1 = 6.31 \times 10^8$ M. The data in the literature has been re-analysed and tabulated on p. 211 $(K_1 + (H))$ in M, gradient in M$^{-1}$ s$^{-1}$ and gradient $(K_1 + (H))$ in s$^{-1}$.

Let $X = 10^8 (K_1 + (H)) \times \text{ gradient}$
<table>
<thead>
<tr>
<th></th>
<th>4-nitrobenzaldehyde</th>
<th>4-methoxybenzaldehyde</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.60</td>
<td>2.51</td>
<td>39.81</td>
</tr>
<tr>
<td>7.40</td>
<td>3.98</td>
<td>25.13</td>
</tr>
<tr>
<td>7.20</td>
<td>6.31</td>
<td>15.85</td>
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<tr>
<td>6.80</td>
<td>15.85</td>
<td>6.31</td>
</tr>
<tr>
<td>6.40</td>
<td>39.81</td>
<td>2.51</td>
</tr>
<tr>
<td>4.80</td>
<td>15.85</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>$10^8$ (H)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.82</td>
<td>10.29</td>
<td>12.62</td>
</tr>
<tr>
<td>9.1</td>
<td>114.8</td>
<td>92</td>
</tr>
<tr>
<td>5.4</td>
<td>119.7</td>
<td>46</td>
</tr>
<tr>
<td>2.4</td>
<td>110.7</td>
<td>26.7</td>
</tr>
<tr>
<td>0.074</td>
<td>117.7</td>
<td></td>
</tr>
<tr>
<td><strong>$10^8$ (K+&lt;H&gt;)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.27</td>
<td>2.98</td>
<td>1.98</td>
</tr>
<tr>
<td>1.88</td>
<td>0.89</td>
<td>0.89</td>
</tr>
<tr>
<td>1.08</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>20.02</td>
<td>20.38</td>
<td>23.74</td>
</tr>
<tr>
<td>19.72</td>
<td>19.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>961.5</td>
<td></td>
</tr>
<tr>
<td><strong>Gradient</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.27</td>
<td>2.98</td>
<td>1.98</td>
</tr>
<tr>
<td>1.88</td>
<td>0.89</td>
<td>0.89</td>
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<tr>
<td>1.08</td>
<td>0.40</td>
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<tr>
<td>20.02</td>
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<tr>
<td>19.72</td>
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<td></td>
</tr>
<tr>
<td>961.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Neither for the benzaldehyde nor for the substituted benzaldehydes is \((K_1 + (H)) \times \text{gradient}\) directly proportional to the concentration of hydrogen ions or hydroxide ions. Indeed the term \((K_1 + (H)) \times \text{gradient}\) is reasonably constant for the pH range studied for all three benzaldehydes. This would be consistent with \(k_1' = 0\) in the hydroxide catalysed reaction and \(k_2' = 0\) in the uncatalysed reaction. For the hydroxide catalysed breakdown of Ç there, however, is no very obvious reason why only \(k_1' = 0\). Similarly there is no obvious reason why only \(k_2' = 0\) for the uncatalysed reaction for the mechanism previously outlined. However, if the uncatalysed reaction did not involve formation of a cyclic compound but migration of a hydroxide ion (which then acts as a nucleophile) \(k_2'\) would be equal to zero.
Capon and Capon \(^3\) made no definite conclusion as to the rate-determining step in the above reactions but suggested that for the reaction with pyridine-2-aldehyde the bimolecular reaction was probably rate-determining since the latter compound was not a more efficient catalyst than pyridine-4-aldehyde. On the basis of the catalytic constants for the benzaldehyde catalysed hydrolyses of the 4-nitrophenyl esters of leucine, glycine and phenylalanine (11.4, 3.1 and \(5.3 \text{ M}^{-1} \text{s}^{-1}\), respectively) Hay and Main suggested that breakdown of the carbinolamine was rate-determining. While this is possible the author should exercise restraint in deductions of the mechanism of hydrolysis of such different esters on a difference of catalytic constant of less than four. The same authors suggested that the failure of pyridine-2-aldehyde to be a more efficient catalyst than pyridine-4-aldehyde was due to either intramolecular bonding between the hydroxy-group of the carbinolamine and the nitrogen of the pyridine residue or the steric hindrance involved in nucleophilic attack by the nitrogen of the pyridine residue. The former explanation is consistent with the observed greater catalytic constant for pyridine-2-aldehyde over that
for benzaldehyde. It does not explain why the catalytic constant for pyridine-4-aldehyde is the same as that for the 2-substituted isomer where such intramolecular bonding is impossible.

Comparison of the Inter and Intramolecularly Catalysed Reactions

Conversion of a reaction from an intermolecular to intramolecular reaction may from first considerations not be expected to effect the mechanism of reactions since both reacting groups are essentially the same. While this is sometimes true it is by no means general. The method often used to identify a change in mechanism is to compare the $\rho$ values for two series of reactions, for example inter and intramolecular reactions. While this is useful it has to be used with much reservation since even within one series of compounds the mechanism of reaction can change. For example, the acetate catalysed hydrolysis of a series of substituted phenyl acetates has recently been studied and the reaction shown to proceed via exclusive nucleophilic attack for esters with good leaving groups such as 2, 4-dinitrophenoxide and exclusive general base mechanism for esters with poor leaving groups like
Further, esters with leaving groups of intermediate stability hydrolyse by a dual mechanism. Previously it had been thought that at the mechanistic crossover point there would be a discontinuity in the Brønsted plot of the logarithm of the rate constant for the acetate catalysed reaction versus the $pK_a$ of the conjugate acid of the leaving group and that this could be used as a diagnostic tool to identify a change in mechanism. However, further work showed that at least for this reaction detection of this crossover point from the Brønsted plot is impossible for despite the fact that the reactions proceed by two different mechanisms all points can be fitted on the one plot. Since the slope of the Brønsted plot, $\beta$, is linearly related to the reaction constant $\rho$ it follows that a Hammett plot would similarly be linear and thus also fail to detect a change in mechanism. One can conclude that a change in either $\beta$ or $\rho$ probably reflects a change in mechanism but that little or no change in these parameters does not necessarily reflect no change in mechanism. For example, the intra and intermolecularly catalysed hydrolysis of aromatic esters by imidazole, tertiary amino and carboxylate show $\rho$ values of 1.35, 1.7, $^46$ 2.5,
2.2;\textsuperscript{54} and 2.5, 1.1,\textsuperscript{35} respectively. The similarity for the imidazole and tertiary amino-groups caused the authors to suggest that the inter and intramolecularly catalysed reactions proceed by the same mechanism. The \( \rho \) values for the acetate catalysed reactions were so different that the authors proposed that the intramolecular reactions were concerted reactions such that no tetrahedral intermediate was formed while the intermolecular reactions proceeded with formation of a tetrahedral intermediate following nucleophilic attack by acetate.\textsuperscript{35} In view of recent work\textsuperscript{36} on the acetate catalysed hydrolysis of esters it seems more likely that the intermolecular reaction proceeds via a general base mechanism at least for phenyl acetate. Hydrolysis of aryl esters of 4-hydroxybutyric acid and 5-hydroxyvaleric acid show \( \rho \) values of 1.08 (four values) and 1.71 (two values) in aqueous phosphate buffer, pH 6.02, \( I = 0.05 \), temperature = 50.0\textsuperscript{0} (p. 174), 1.13 (four values) and 1.05 (three values) in aqueous phosphate buffer, pH 7.47, \( I = 0.05 \), temperature = 30.0\textsuperscript{0} (p. 178), -0.13 (four values) and -0.13 (three values) in aqueous hydrochloric acid (0.10 N) at 50.0\textsuperscript{0} (p. 172) and -0.11 (four values) and -0.16 (three values)
in perchloric acid (1.00 M) at 30.0° (p. 176). This agrees closely with the values obtained in 60% acetone-water at 25° in acid (\(\phi = -0.22\)) and basic (\(\phi = 1.0\)) solution\(^{181}\) and with \(\phi = 1.0\) for that at 25.0°, \(I = 1.0\) in aqueous basic solution.\(^{176}\)

Since the \(\phi\) values in both acidic and basic media are similar to those of simple esters it can be concluded that the inter and intramolecular reactions are not necessarily dissimilar. It is worth considering at this juncture the factor(s) which cause a reaction to change its mechanism from nucleophilic to general base since if similarity of \(\phi\) values is insufficient criterion the phenomenon may be commoner than usually presumed. Consideration of the two groups alone would not expect to cause any change since they are essentially the same. The most obvious difference is that the collision frequency of the two groups is much increased in the intramolecular reaction but consideration of the tetrahedral intermediates formed would not in itself expect to lead to a change in mechanism. The only other obvious difference is in the other product formed. Thus hydrolysis of esters of 4-hydroxybutyric acid probably gives 4-butyrolactone
(a cis ester) as an initial product while the corresponding bimolecular reaction forms a trans ester. Similarly intramolecular cyclisation of the variously substituted phenyl succinates and glutarates to the corresponding succinic and glutaric anhydrides (cis anhydrides) is believed to occur in their hydrolysis while the intermolecular reaction forms the trans anhydride. Consider the reactions between a carboxylate anion and a phenyl ester in an intramolecular and intermolecular reaction. Assume that in both a tetrahedral intermediate is formed. Since acetate does not catalyse the hydrolysis of phenyl acetate to any appreciable extent by a nucleophilic mechanism the dissociation of the tetrahedral intermediate in the intermolecular reaction is almost completely to starting materials. In contrast since carboxylate catalyses the intramolecular reaction the tetrahedral intermediate must dissociate to an appreciable extent to products. The tetrahedral intermediate formed in both instances are shown below.
Inspection of the Newman projection formulae for these intermediates shows that 48 can exist in only one eclipsed conformation while 49 exists in three staggered conformations.
The intermediate 48 formed in the intramolecular reaction exists in an eclipsed conformation and thus will be a high energy intermediate. Breakdown of 50 results in the formation of succinic anhydride 51 which has a staggered conformation. The RDS is probably the formation of 50.

The expected free energy diagram for this reaction is shown on the next page (-----).
In contrast formation of \( 52 - 54 \) in the intermolecular reaction results in the formation of an intermediate with an eclipsed conformation. Although this too is a high energy intermediate it will not be as great as 50. Breakdown of \( 52 - 54 \) to acetic anhydride and phenoxide necessarily results in the formation of an eclipsed molecule (acetic anhydride 55) so that the free energy of activation of this step will be higher than the analogous step in the intramolecular reaction. Furthermore, since the proposed intermediates \( 52 - 54 \) have never been observed their concentrations must be very low. Thus the free energy of activation for the reverse reaction, \( \Delta G_2 \), must be very small. However, the free energy of activation for formation of 55 must be high since acetate does not catalyse the hydrolysis of phenyl acetate by a nucleophilic mechanism to any appreciable extent. The expected free energy diagram for this reaction is shown on the next page.
In the intermolecular reaction breakdown of the tetrahedral intermediate to \( 55 \) is the RDS. Since the nucleophilic mechanism becomes more important for better leaving groups, \( \Delta G_3 \) must be lower for these groups.

Comparison of the free energy diagrams for these intra and intermolecular reactions may not completely explain the large rate difference for this system. In the free energy diagrams succinic anhydride and acetic anhydride were shown to have the same free energy. However, this is not so since succinic anhydride is 7.4 k cal mol\(^{-1}\) more stable than acetic anhydride relative to their respective acids.\(^{192}\) If some of this stabilisation was reflected in the stability of the tetrahedral intermediate then the free energy of activation for the intramolecular reaction would be correspondingly lower (---). A further feature of interest in the intermolecular reaction is which of the conformers of \( 52 - 54 \) most readily forms the hydrolysis products. NMR studies have recently shown that acyclic
anhydrides\textsuperscript{193} (and esters) exist almost completely in the \textit{anti} form which must be much more stable than the \textit{syn} form. If some of this stabilisation energy is reflected in the transition state and intermediate that conformer of the tetrahedral intermediate most suited to forming the \textit{anti} form of acetic anhydride (55) will preferentially decompose to hydrolysis products. As shown below, \textit{52} is that conformer.

\begin{center}
\includegraphics[width=0.8\textwidth]{diagram.png}
\end{center}

\textbf{Description of the Tetrahedral Intermediate}

Both the carboxylate and alkoxide (or hydroxide) catalysed reactions have been described in terms of a tetrahedral intermediate which can partition either to starting material or to products. The results may be equally well described in terms of an asymmetric energy barrier such that no addition intermediate is formed. If the formation of this transition state is rate-determining there is little bond formation in the transition state whereas if decomposition is rate-determining there is much bond formation between the
ester and nucleophile. Differentiation between these two is obviously difficult but at least in some instances evidence for a tetrahedral intermediate has been obtained. One such example is the trapping of the tetrahedral intermediate formed on the aminolysis of a lactone.\textsuperscript{194} It is, however, possible that the tetrahedral intermediate is not being truly trapped but that the polarisation of the carbon-bromine bond is diverting the reaction to a course which it would not otherwise take. Probably the most convincing evidence is the incorporation of labelled oxygen into the starting material when some carboxylic acid derivatives are treated with labelled water in alkali.\textsuperscript{195}
However, not all acid derivatives show such incorporation. In such instances it is possible to suggest that $k_4$ is smaller than usual (which is very unlikely), that $k_2$ is very much greater than $k_{-1}$ or that the reaction does not proceed via a tetrahedral intermediate. Such experiments on phenyl benzoate in 50% dioxan-aqueous alkali at 25° showed that the rate of collapse of the tetrahedral intermediate to product to that to reactant is greater than 10^5. At least for phenyl benzoate $k_2 \gg k_{-1}$. For a given alkoxy or aryloxy-function esters formed from aromatic acids normally hydrolyse slower than those formed from aliphatic acids. Hence, if $k_1$ is the RDS for phenyl benzoates it is probably also for phenyl esters of aliphatic acids, assuming the absence of any unusual conjugative effects. Certainly $k_2$ is much greater than $k_{-1}$.

The free energy diagram for the intramolecular carboxylate catalysed reaction was discussed earlier (p.220). If the hydroxide catalysed hydrolysis of aryl 4-hydroxybutyrate proceeds via mechanism B (p. 4) a similar free energy diagram would be expected. A point of interest is the magnitude of $\Delta G_3$ since this determines if the tetrahedral intermediates has any real existence, in which case a minimum occurs on
the free energy diagram, or if it is a transition state when no such minimum will be evident. The lower limit of $k_2$ has been shown to be $10^9 \text{s}^{-1}$. The theoretical limits correspond at the lower limit to the translational energy of the molecule (0.6 k cal) when the departing group is vibrationally unexcited and at the upper limit to the energy associated with a molecular vibration when the departing group is fully stretched. An eclipsed structure is formed in the transition state (or tetrahedral intermediate) in the intramolecular reaction. Consideration of the various conformers of the starting material shows that while the semi-rigid structure is still held no release of conformational restrictions can occur.

\[
\begin{align*}
\text{OAr} & \quad +H^+ \\
\text{OAr} & \quad \text{or} \\
\text{OAr} & \quad \text{or}
\end{align*}
\]

There will thus be an appreciable energy barrier to reversion to the constrained form of the hydroxy-ester. In contrast decomposition to products was thought to be a facile reaction so that the cyclisation of the hydroxy-esters (and monophenyl esters of glutaric and succinic acids) is probably essentially
a concerted reaction. In contrast the tetrahedral intermediate formed by the aryl 5-hydroxyvalerates is staggered so that it may be relatively stable.

**Comparison of Rate of Ring Closure for Five and Six Membered Rings**

The rate of ring closure of a wide range of compounds has been shown to bear no simple relation to the size of the ring formed as the product. All experiments show that the relative rates depend on many factors. Indeed consideration of the relative rates of formation of say aliphatic cyclic ethers, anhydrides and esters (lactones) would not be expected to follow any simple correlation since the products contain 0, 2 and 1 sp² centres, respectively, and this will necessarily be reflected in their transition states. Similarly, the number of gauche interactions will be a function of the size of the ring and the hybridization within the ring. It is thus only meaningful to compare relative rates of ring formation when only one factor is different.

The cyclisation of 4-chlorobutyric acid and 5-chlorovaleric was studied in sodium hydroxide solution. The authors
proposed nucleophilic carboxylate attack to form the corresponding lactones which under the conditions rapidly solvolysed to the hydroxy-acid. The relative rate of formation of 4-butyrolactone to 5-valerolactone is 1:1. The acid catalysed lactonisation of 4-hydroxybutyric acid and 5-hydroxyvaleric acid in aqueous 1, 2-dimethoxyethane at \(25^\circ\) has been studied and the relative rate found to be 1:6.4. (In aqueous solution the ratio is 1:17.0). Similarly the acid catalysed hydrolysis of 4-hydroxybutyramide and 5-hydroxyvaleramide at \(20^\circ\) shows a rate ratio of 1.0:2.6. However, even consideration of cyclisation of different compounds to a common intermediate is complicated by the rate-determining step being formation of the ring (as is probable with aryl esters of 4-hydroxybutyric acid and 5-hydroxyvaleric acid), breakdown of the resultant tetrahedral intermediate, or a function of both when the two rates are similar. Consider for example the hydrolysis of 4-hydroxybutyramide. The activation free energy associated with ring closure would be expected to be similar to that for phenyl 4-hydroxybutyrate since the electrophilicity of the carboxyl group is not greatly dependent on the substituent. Yet its hydrolysis in acidic
solution is only 20 times greater than that for butyramide whereas that for phenyl 4-hydroxybutyrate is about 200 times greater than that for phenyl acetate (p.251). This indicates that decomposition to the product is favourable for the ester but unfavourable for the amide. This is not surprising since the high $pK_a$ of ammonia shows the amide to be a very poor leaving group in comparison with phenoxide ($pK_a$ of phenol is 10) so that release of conformational strain in cyclisation is offset by the activation energy required to rupture the carbon-nitrogen bond. The authors showed that the reaction was catalysed by the presence of borate and phosphate buffers and in view of what was previously mentioned this would reflect an increase in the rate of decomposition of the tetrahedral intermediate. A series of papers on the hydrolysis of 4-hydroxybutyranilide and derivatives thereof showed the same phenomenon which the authors attributed to a general base-general acid mechanism. Thus this reaction produces aniline (or ammonia) rather than the amide or anilide anion and since this is thermodynamically much more favourable an appreciable rate increase results. In contrast the leaving group in the base catalysed hydrolysis of aryl
esters is the aryloxy-anion. However, this is probably not true of all esters for at least for the carboxylate catalysed intramolecular hydrolysis of esters the reaction has been shown to be proportional to the concentration of the anionic species for good leaving groups whereas for poor leaving groups the rate is proportional to the concentration of the undissociated acid (or a kinetic equivalent thereof). The latter is usually interpreted in terms of protonation of the leaving group either prior to or concerted with cyclisation to the anhydride thus making it a better electrophile.

The specific base catalysed reaction for the hydrolysis of the aryl esters of 4-hydroxybutyric acid hydrolysed between 10 and 20 times faster than those of 5-hydroxyvaleric acid (p. 174 and 178). This could be accounted for if the hydroxy-group in the 4-hydroxy-esters was more acidic than that in the 5-hydroxy-esters although this is unlikely. Another explanation could be the increased entropy change in freezing an additional methylene unit in the transition state for the 5-hydroxy-esters. A feature of these reactions which has previously received little attention is the relative ease of formation of exo double bonds in 5 and 6 rings (assuming the
reaction proceeds via the lactone). Thus, Brown et al. have shown that exo double bonds in 5-rings are relatively stable while in 6-rings they are relatively unstable. They postulated that reactions will proceed in such a manner as to favour the formation or retention of an exo double bond in the 5-ring and to avoid the formation or retention of the exo double bond in the 6-ring systems. Consideration of the aryl-hydroxy-esters shows that $\text{57}$ should be formed faster than $\text{56}$.

\[ \text{56} \quad \text{57} \]

$\text{56}$ and $\text{57}$ can revert to starting material or to products (lactone + aryloxy-anion) and for both these reactions $\text{56}$ should be faster than $\text{57}$.

The rearrangement of 4 and 5-alkoxyacyl chlorides to the alkyl 4 and 5-chloro-esters has many similarities to the above reactions. The proposed mechanism is shown below.
4-Ethoxybutyroyl chloride rearranges forty times faster than 5-ethoxyvaleroyl chloride. However, like the hydroxy-esters interpretation of relative rates is complicated by there being three possible steps. However, if one can consider the reaction proceeding via the intermediate 59 rather than 58 similar considerations as for the hydroxy-esters can be made.

\[
\begin{array}{c}
\text{R} \\
\text{Cl} \\
\text{O}^+ \\
\text{O} \\
\text{(CH}_2\text{)}_{n-2} \\
\text{CH}_2
\end{array}
\]

\(59\)

- \(k_1\) for \(n = 4\) will be smaller than for \(n = 5\).
- \(k_{-1}\) for \(n = 4\) will be greater than for \(n = 5\).
- \(k_2\) for \(n = 4\) will be greater than for \(n = 5\).

The relative \(k_2\)'s is probably greater than forty since the standing concentration of intermediate 59 when \(n = 4\) is probably much smaller than for \(n = 5\).

Other features of interest in this reaction are that it is intramolecular and that inversion occurs at the terminal carbon atom. The latter has previously been assumed to be
due to steric hindrance preventing the alternative mode of attack. However, since the reaction is intramolecular it is reasonable to consider it as a concerted reaction.\textsuperscript{209} Consider the particular intermediate for $n = 4$ (60).

To obtain the observed products the C(4)-O bond must break. Energetically it is more favourable if backside displacement of the leaving group occurs (as shown). Such a displacement situates the incipient chloride ion close to the incipient carbonium ion in the transition state. Reaction of these two sites inevitably results in inversion at C(4). If a concerted mechanism does occur this has similar ramifications for the corresponding valerates.

Previously no example of alkoxy-cleavage to the lactone had been reported.\textsuperscript{198} In an attempt to prepare aryl esters of the benzyloxy-acids the corresponding acyl chloride was prepared. Addition of thionyl chloride to benzyloxybutyric acid (p. 57) resulted in formation of 4-butyrolactone and presumably
benzyl chloride. The change in the usual course of reaction is probably due to the greater stability of the benzyl cation over primary alkyl cations. If this is reflected in a weakening of the benzyl-oxygen bond the benzyl group will be more electrophilic in character than the primary alkyl groups. Furthermore, since intermediates of type 58 are unlikely to have any real existence under the conditions in which the reactions are carried out the benzyl and chloro-groups are probably cis to each other in the transition state.

\[
\text{\begin{tikzpicture}
\node[draw] (a) {$\text{Cl}$};
\node[draw] (b) [above right of=a] {$\text{O}$};
\node[draw] (c) [below left of=a] {$\text{CH}_2\text{Ph}$};
\end{tikzpicture}}
\]

It would be of interest to know if inversion occurred in this reaction too.

**General Base Catalysis and the Mechanism of Chymotrypsin**

Consider a proton being transferred from one basic species (A) to which it is fully bonded in the ground state to a second basic species (B).
If A and B are identical the transition state occurs when the proton is exactly midway between A and B. However, if B is more basic than A the proton will be nearer to A than B in the transition state.

\[ B - HA - H \]

Say the basicity of B is much greater than A. Before the transition state is reached the proton will have to be very close to A.

\[ B - H \quad A - H \]

From the above diagram the activated complex is obviously of very high energy and as such will be very reactive. Stabilisation can occur by reversion to starting material or by further reaction with another species if this is possible. On this argument the greater the difference in basicity of the two relevant sites the
more reactive is the more basic site once the proton has been removed. There is obviously some limiting factor and this is the high activation energy required to bring about such a transition.

Examples of reactions in which general base catalysis by phenoxide anion has been proposed have already been listed. Of particular interest is the hydrolysis of phenyl salicylate which is hydrolysed 50 times faster than phenyl-methoxybenzoate. This, however, is very small compared to $10^8$ for phenyl 2-hydroxyphenyl acetate over phenyl acetate (p•201) which indicates that at least for esters with good leaving groups the rate of intramolecular nucleophilic attack by phenoxide is much greater than that by general base mechanism assuming that the ring size is not critical. Consider now the acetate catalysed hydrolysis of phenyl acetate. In effect an acetate anion partially abstracts a proton from water which then reacts with the ester.

$$\text{MeC}=O\text{H-O} \xrightarrow{\text{MeCO}_2\text{H} + \text{PhO}^-} 2\text{MeCO}_2\text{H} + \text{PhO}^-$$
By virtue of being a termolecular reaction this reaction is unfavourable to a nucleophilic mechanism which is bimolecular but if the latter is impossible for some reason then the general base mechanism may be the predominant or exclusive mechanism. The reaction must be concerted since very little charge accumulation must occur on the oxygen of water as otherwise the reaction would be almost completely reversible. General base mechanism thus permits the hydrolysis of esters which are sometimes stable to a nucleophilic mechanism. In effect this mechanism permits the hydrolysis of esters and amides by a weakly basic species even when the difference between the $pK_a$ of the conjugate acid of the leaving group and catalyst is of the order of 23 $pK$ units. This contrasts with a nucleophilic mechanism where the difference is less than 3 $pK$ units. As already mentioned, in non-rigid systems the rates of general base mechanism are relatively slow but it is feasible that if the three relevant groups were held in the right juxtaposition this mechanism could be much favoured and result in very large rate increases. It is just this that is proposed for the enzymes trypsin and chymotrypsin where the basic species
is an imidazolyl unit of a histidine residue. However, even this assumes that the ratio of catalytic rate constants is of the same order for water as for primary alcohols. Recent work has shown that there may be no justification for this assumption. In aqueous solution 2-nitrophenyl acetate is known to be hydrolysed by nucleophilic and general base mechanisms. In methanol, however, the reaction (methanolysis) proceeds wholly by a nucleophilic mechanism to form acetic anhydride.

Recently a paper has appeared in which the authors propose that the general base–general acid mechanism at least for amides is unlikely in enzymes. The authors studied N-n-butyl-8-hydroxy-1-naphthoamide in sodium hydroxide (0.10 N) at 25°C. The authors argued that as the aryloxide anion is $10^3$ times more basic than imidazole the abstraction of a proton from water should be $10^3$ times faster than in the enzyme. Conversely elimination of the free amine by proton donation by the conjugate acid should be $10^3$ faster in the enzyme than the model system since the imidazolinium ion is a stronger acid than the naphthol. At the pH of the experiment approximately the same ratio of free base to
conjugate base is present as in chymotrypsin so that one would expect a similar rate of hydrolysis for the model system as for an amide catalysed by chymotrypsin. However, no appreciable hydrolysis occurred and the rate was at least $10^5$ times slower than that of the enzymatic system. While the result is of interest the limitations of the comparison to the enzyme should be noted. The authors assume that the rate increase caused by the enhanced basicity of the system over the enzyme in the first step is nullified by the diminished acidity of the conjugate acid in the second step. However, the observed rate is that of the slowest reaction in the sequence so that in the enzyme system the greater acidity of the imidazolinium ion is irrelevant if its formation is rate-determining. As indicated the substituted naphthoxide anion has an $sp^2$ orbital on its oxygen atom that is ideally situated for either a nucleophilic or general base mechanism. That a nucleophilic mechanism does not occur is not surprising since the $pK$ difference of naphthol and butylamine is at least 23. However, in view of the known general base catalysis of salicylamide it is surprising on first consideration that this mechanism does not prevail. Although a general base
mechanism is frequently postulated in reactions rarely are steric and conformational restrictions emphasised. If such a mechanism was to prevail in this model system a water molecule would have to be accommodated such that one hydrogen and oxygen atom would be in the plane of the ring, with the amide group perpendicular to the plane of the ring.

Since the peri-positions of the naphthalene ring are only 2.44A apart[^213] this would probably necessitate much steric crowding. Indeed since the spatial volume occupied by the oxygen-hydrogen bond is greater than that of the lone pairs on the oxygen it is probable that even if a water molecule is situated between the peri-positions it is ill-disposed to be involved in a general base mechanism.
This raises the further question, assuming that the general base mechanism of chymotrypsin is correct, as to what other restrictions may be involved in such a model system. Thus proton abstraction could occur either with or without inversion of the hydroxy-oxygen atom of serine-195. It is obviously impossible to test if such considerations are important from intermolecular reaction studies and as yet no experiments have been devised which would discriminate between these.

The hydrolysis of phenyl acetate in the presence of imidazolyl substitute \( \beta \)-cyclodextrins has already been reported.\textsuperscript{214} In this system it is possible that an imidazolyl general base catalysed reaction could occur but only small rate increases were reported.

**Boric Acid Catalysis of Esters**

The understanding of enzymatic systems has stimulated the development of many model systems in which an activating group, such as hydroxy, imidazolyl, amino or carboxy-group, are bonded intramolecularly to the reacting functionality. While these studies have been illuminating in terms of simple chemical interaction between various functionalities they have
not usually been of much value in interpreting phenomena in enzymes. Consider, for example, the reaction between an ester and chymotrypsin. The ester and enzyme are believed to be involved in a very rapid equilibrium with a loose complex whose exact bonding is not fully understood but known to be weak.¹⁷₈ This Michaelis complex, in turn, interacts with the serine-195 hydroxy-group resulting in the formation of an acyl-enzyme complex which hydrolyses to the enzyme and acid. Most model systems are designed to imitate that part of the enzyme sequence corresponding to the Michaelis complex but since forces of the type operative in the enzyme are difficult to engineer in model systems the two reacting groups are usually bound intramolecularly and so unlike the enzyme the model cannot dissociate reversibly. Such a system could possibly be developed using boric acid since the latter is known to reversibly form covalent adducts with hydroxylic compounds at a rate which is fast relative to the rates of many other reactions. As has already been mentioned phenyl salicylate hydrolyses 100 times as fast as phenyl 2-methoxybenzoate²² which prompted the authors to propose electrophilic catalysis by boron while others suggested the possibility of
reversible complexation with boric acid followed by irreversible hydroxy-participation of the resultant adduct. 59

Similarly, formaldehyde reacts with phenol in the presence of boric acid to form 2-hydroxymethylphenol whereas in the absence of boric acid no such product is formed. 215 Again a rapid and reversibly formed complex of boric acid was proposed. The hydrolysis of chloroethanol to ethylene glycol is catalysed by a substituted boric acid. Again a rapid reversible formation of a boron adduct was proposed. 216

It is obvious from these examples that boric acid is indeed an ideal complexing agent. In imitating the enzyme system one requires an ester which when complexed is relatively reactive to that when uncomplexed. If the system was to be a model for chymotrypsin one would prefer the activating group to be a hydroxy-group and, at that, preferably not one bonded to boron. Such a system could be generated by complexing boric acid and esters of myo-inositol-2-carboxylic acid.
This has the further similarity that the alcoholic groups are more acidic than aliphatic alcohols and probably of the order of that of serine-195.\(^1\) Again the acyl-intermediate is of the cis configuration and thus expected to be rapidly hydrolysed to the acid. As a standard it was hoped to use esters of scyllitol-carboxylic acid which could not react by the same type of participation.
Such complexes are well known in the literature and the equilibrium constants have been calculated for the unsubstituted inositols. That for cisinositol is $1.1 \times 10^7$ at $22^\circ$ in contrast to 25 for myoinositol. The difference is a reflection of the number of groups in the complexed inositol that have an axial configuration. With cisinositol there are only equatorial substituents so that $K$ is very large. In contrast myoinositol has two groups axial while scyllitol which would have three axial hydroxy-groups does not form a complex in solution. The preparation of the respective acids caused no difficulties but conversion of these to either an aliphatic or aromatic ester proved impossible. The difficulty experienced was no doubt partly due to the insolubility of the acid in the usual solvents used in preparing esters.

Despite this failure it is worth considering the expected kinetic rate equations for the reaction. Assuming the reaction is irreversible and that $k_2$ is the RDS,

$$k_{\text{obs}} = k_2 \text{ (Borate Complex)} \text{ (OH)}$$

Now let $K_B = \frac{1}{K_A} = \frac{\text{(Borate Complex)}}{\text{(B(OH)}_3 \text{)}} \text{ (Ester)}$
As the boric acid concentration is increased more and more of the ester exists as the complex

\[ \text{Rate} = k_2 \times \frac{c_B}{K_A + c_B} \text{(Ester)} (\text{OH}^-) \]

where \( c_B \) is the concentration of boric acid. At low boric acid concentrations \( K_A \gg c_B \) and

\[ \text{Rate} = k_2 \frac{c_B}{K_A} \text{(Ester)} (\text{OH}^-) = k_2 c_B K_B \text{(Ester)} (\text{OH}^-) \]

At a given pH the rate is expected to be linear at low concentrations of boric acid but non-linear at high concentrations as shown below.

In addition to acting as a tridentate ligand boric acid is also known to act as a monodentate ligand and as previously mentioned reactions have been suggested in which it acts as a monodentate ligand.\(^{215-6}\) Even if rate increases had been observed it would not necessarily have been due to the formation of a tridentate ligand. Bidentate complexes are readily formed with
cis alcohols but the only one possible in the above system is unlikely to cause any marked effect since the two hydroxy-groups are disposed away from the ester linkage.

However, if monodentate complexes are feasible then at least two forms of interaction are possible.
Such complexation will give rise to similar rate equations as already derived for boric acid as a tridentate ligand.

Evidence for such a mechanism could be the relatively rapid hydrolysis of phenyl 3-hydroxybutyrate in boric acid buffers compared to that in glycine buffers at the same pH.\textsuperscript{184}

**The Acid Hydrolysis of Esters**

The acid catalysed hydrolysis of esters can occur with either acyl or alkyl oxygen fission.\textsuperscript{218} The latter, however, only occurs if the alkyl group can form a stable carbonium ion so that for esters of primary aliphatic alcohols and phenols the mechanism is exclusively acyl fission. Further work on the detailed mechanism of ethyl acetate has recently been reported.\textsuperscript{219} The authors assumed rapid protonation of the ester followed by rate-determining attack of this conjugate acid by \( n \) molecules of water. From the derived rate equation \( n \) was shown to be two prompting the authors to suggest either

\[ \begin{align*}
\text{I} & \quad \text{or} \quad \text{J} \\
\end{align*} \]
With esters of 4-hydroxybutyric and 5-hydroxyvaleric acid other mechanisms, in addition to those already discussed are possible.

\[ K \]
\[ \text{CO}_2H \]
\[ + \text{ArOH} \]

\[ L \]
\[ \text{CO}_2H \]
\[ + \text{ArOH} \]

\[ M \]
\[ + \text{ArOH} + \text{H}_2\text{O} \]
Mechanism K involves general acid catalysis of the ester group (probably via the carboxyl oxygen atom) with simultaneous attack by water as proposed for the hydrolysis of N, N-diethylammonium ethyl benzoate. It might be expected, since alcohols are much weaker bases than amines, that this mechanism could be eliminated but as the following calculation shows this is not so.

\[ k_{\text{obs}}(E_T) = k'(H_2O) \text{(conjugate acid of ester)} \]

\[ = k'(H_2O) \frac{(H^+) \text{} (E_T)}{K + (H^+)} \]

where \( E_T \) is the total concentration of ester and \( K \) the acid association constant for the protonated alcohol group. Although the latter is not known it would be expected to be of the order of that for protonated methanol which is known to be 100. At 50.0° the observed rate in hydrochloric acid (0.10 N) for the hydrolysis of phenyl 4-hydroxybutyrate is 5.60 \( \times \) 10^{-4} s^{-1} (p. 172).

\[ \therefore k_{\text{obs}} = k' \left( \frac{55}{0.1} \right) = \frac{5.60 \times 10^{-4}}{100} \text{ s}^{-1} \]

\[ \therefore k' = \frac{5.60 \times 10^{-4} \times 10^2}{5.5} \approx 10^{-2} \text{ M}^{-1} \text{ s}^{-1} \]

which is far less than that of a diffusion controlled reaction.
This mechanism differs from L and M (but not \( I \) and \( J \)) in that the hydroxy-acid as opposed to the lactone is initially formed as a reaction product. However, differentiation on this basis is impossible due to the difficulty in isolating the hydroxy-acid without cyclisation. Mechanisms L and M are analogous to I and J but involve the hydroxy-group of the alcohol instead of a second molecule of water.

The rate of the hydrolysis of phenyl 4-hydroxybutyrate in the range pH 0 \( \rightarrow \) 3.0 is first order in the concentrations of hydroxonium ions and the hydroxy-ester (p. 155). The acid catalytic constant is \( 7.10 \times 10^{-3} \text{ M}^{-1} \text{s}^{-1} \) at 30.0\(^\circ\)C. This is approximately 200 times faster than that for phenyl acetate at 25\(^\circ\)C in 60% acetone \( 221 \) (\( k = 2.7 \times 10^{-5} \text{ M}^{-1} \text{s}^{-1} \)). Presumably this enhancement is a result of intramolecular participation by the hydroxy-group by mechanism K, L or M.

The rates of hydrolysis of substrates in moderately concentrated aqueous acids was originally studied by Zucker and Hammett. \( 222 \) For some substrates they found that log \( k_{\text{obs}} \)
was linearly related to $-H_o$ while for others $\log k_{obs}$ was linearly related to the logarithm of the acid concentration. They proposed that the transition state for the former did not involve reaction with water in the transition state while the transition state for the latter did involve water. Most substrates do not yield a predicted gradient of 1.00 and also some substrates do not give linear relationships for either plot. Later, Bunnett suggested improvements to the original Hammett-Zucker hypothesis. For some substrates a plot of $\log k_{obs} + H_o$ vs. $\log a_{H_2O}$ was linear. If the gradient, $w$, of this plot was between -2.5 and 0 water was not involved in the transition state. If $w$ was between +1.2 and +3.3 water acts as a nucleophile and if $w$ was greater than 3.3 water acts as a proton transfer agent. However, if the above plot was not linear $\log k_{obs} - \log (\text{acid concentration})$ vs. $\log a_{H_2O}$ was plotted. If the gradient, $w^*$, was less than -2 water is probably acting as a nucleophile in the transition state. If $w^*$ was greater than -2 water probably acts as a proton transfer agent in the transition state.
The plot of log $k_{\text{obs}}$ vs. $H_0$ was linear for four of the five points for phenyl 4-hydroxybutyrate yielding a gradient of -0.55 (p. 155). The same plot for phenyl butyrate was curved having an approximate gradient of -0.4 (p. 155). The plot of log $k_{\text{obs}}$ vs. acid concentration is curved for phenyl 4-hydroxybutyrate and phenyl butyrate (p. 160). The plots of log $k_{\text{obs}} + H_0$ vs. log $a_{H_2O}$ for phenyl 4-hydroxybutyrate was approximately linear having a $w$ value of -0.35 (p. 161) which would indicate that water was not involved in the transition state. The analogous plot for phenyl butyrate was curved (p. 161). The plot of log $k_{\text{obs}} - \log$ (acid concentration) vs. log $a_{H_2O}$ for 4-hydroxyphenyl butyrate was approximately linear having a $w^*$ value of -2.46 (p. 162). This would seem to indicate that water was acting as a nucleophile and is thus in contradiction to the result afforded by the previous plot. The analogous plot for phenyl butyrate was curved (p. 162).

A linear relationship also exists between plots of log $k_{\text{obs}} + H_0$ versus $H_0 + \log$ (acid concentration). Indeed these plots are usually more linear than those previously outlined. The plot for phenyl 4-hydroxybutyrate had a gradient, $\phi$, of
The analogous plot for phenyl butyrate had \( \phi = 0.98 \) (p. 163). It is noteworthy that in both instances all points fitted on the line (within experimental error). The smaller \( \phi \) value for the intramolecular reaction is in accord with the change in hydration on going to the transition state being smaller. The value of \( \phi (0.60) \) for phenyl 4-hydroxybutyrate is close to that for water being involved as a nucleophile (0.22 - 0.56) and that for water involved as a proton transfer agent (\( > 0.58 \)).

Although the mode of the form of the interaction of the hydroxy-group in the intramolecular reaction is not definitely established that it does interact is beyond reasonable doubt. Thus the rates of hydrolysis of phenyl 4-hydroxybutyrate is 18 and 60 times greater than that of phenyl butyrate in 5.021 M and 1.000 M perchloric acid, respectively (p. 157-8). While the enthalpies of activation are similar the entropy of activation for phenyl 4-hydroxybutyrate is about 4.5 e.u. less than that for phenyl butyrate (p. 164-5). This indicates that the transition state for the intramolecular reaction requires less arrangement of the reacting species than the intermolecular
reaction which again is indicative of participation by the intramolecularly bonded hydroxy-group.
PREPARATION OF 2-CYANOBENZOIC ACID

A simple one-step preparation of 2-cyanobenzoic acid has been devised starting from the readily available phthalamic acid. Although other ways of preparing this acid are known \(^{67, 158, 225}\), none are as convenient or use such mild conditions. Dehydration using DCC of amic acids to the corresponding cyano-acids has previously been reported in the literature \(^{75, 160}\), but the preparation of 2-cyanobenzoic acid appears to be the first example of an aromatic cyano-acid being prepared by this means. The reaction appears to be general for all amides with a suitably disposed carboxy-group. The preparation of the analogous cyano-acids has previously suffered from isomerisation to the corresponding imide and/or hydrolysis to the amic acid. Since this reaction obviates these difficulties it should allow the preparation of a wide range of cyano-acids which have not previously been accessible.

Infra-red Spectrum of Isophthalimide

The dehydration of phthalamic acid in a mixture of chloroform and methylene chloride using DCC was studied by IR spectroscopy. Previous work \(^{75, 160}\) has shown that suitable secondary
amides form isoimides and the IR spectrum of both N-alkyl and N-aryl derivatives have been recorded.\textsuperscript{160, 226} Although unsubstituted amic acids have been dehydrated by the same method no reported attempt has been made to either isolate the unsubstituted isoimide or observe its IR spectrum.

Isolation of isophthalimide proved impossible. However, the IR solution spectrum showed intense bands in the carbonyl region at 1780, 1790 and 1860 cm\textsuperscript{-1} that were not present in the spectra of phthalamic acid, DCC, ocba or dicyclohexyl urea (p. 113). They are, presumably, due to the isoimide group. Also present were peaks at 2200 and 1710 cm\textsuperscript{-1} due to ocba. On standing some ocba precipitated. This was accompanied by a decrease in the intensity of the bands due to isophthalimide but not at first with those due to ocba. It thus appears that ocba slowly precipitates from solution causing the isophthalimide in solution to undergo isomerisation to ocba.

Unsubstituted isoimides have been suggested as intermediates to explain the rapid hydrolysis\textsuperscript{61, 65, 69} and isomerisation of certain cyano-acids.\textsuperscript{70-74} Isophthalimide was first suggested\textsuperscript{227} to explain the formation of ocba when
phthaloyl chloride was treated with ammonia. However, the above IR data is the only positive evidence for their existence.

**Preparation of Isophthalimide Hydrochloride**

The initial attempt at cyclising ocba using hydrogen chloride was shown to have been successful when later the same compound was prepared as indicated in the literature.\(^{227}\) The only marked difference in their IR spectra was a band at 3400 cm\(^{-1}\) which was probably due to phthalamic acid resulting from the ready hydrolysis of isophthalimide hydrochloride. Subsequent attempts to repeat the experiment, however, resulted in the formation of phthalimide. Aqueous acid hydrolysis of isomides is known\(^{225}\) to result in the formation of the corresponding amic acid. This could arise by either nucleophilic attack by water at the protonated imine or carbonyl groups but which occurs is not yet known. Since isophthalimide hydrochloride was once isolated it would seem that it is an intermediate. Knowing this, a plausible interpretation of the result is that the protonated isophthalimide cation, \(^{24}\), combines with the chloride ion forming \(^{61}\) but that this reaction is reversible. Chloride ions could also attack the carbonyl group forming the acid chloride,
62, which then eliminates hydrogen chloride resulting in the formation of phthalimide, 63.

\[
\begin{array}{c}
\text{Cl} & \text{NH}_2 \\
\text{C} & \text{O} & \text{C} & \text{O} \\
\text{H}_2 \text{c} & \text{H}_2 \text{c}
\end{array} \xrightarrow{\text{Cl}^-} \xrightarrow{+\text{Cl}^-} 
\begin{array}{c}
\text{NH}_2 \\
\text{C} & \text{O} & \text{C} & \text{O} \\
\text{H}_2 \text{c} & \text{H}_2 \text{c}
\end{array}
\]

\[61 \rightarrow 24\]

The only stabilisation of 61 from 24 is as a result of the gain in bonding energy from forming a carbon-chlorine bond. In contrast the formation of 62 results in a gain in bonding energy as a result of the formation of a carbon-chlorine bond but also that gained from the conjugation of the amide group with the aromatic residue. Similarly formation of phthalimide results in even greater stabilisation. The free energy diagram of these changes can be illustrated as shown on the next page.
The formation of phthalimide is obviously the thermodynamically more favourable reaction. Furthermore, the activation free energy for $63 \rightarrow 62$ and $62 \rightarrow 24$ are probably so large that the formation of phthalimide is irreversible.

Some evidence that $61$ exists is gleaned from the mass spectrum of the so-called isophthalimide hydrochloride (see p. 116). That is, no difficulty was experienced obtaining the spectrum unlike ionic salts which are very involatile.

Attempted Observation of Intermediates in the Hydrolysis of 2-Cyanobenzoic Acid

The previous kinetic data on the hydrolysis of ocba was conducted in aqueous solution at concentration of $2 \times 10^{-4} \text{ M}$.
A number of modifications had to be made to these conditions if any intermediate was to be observed using IR spectroscopy. In order to obtain a spectrum the concentration of ocba had to be about one molar. However, ocba is only slightly soluble in aqueous solution thus necessitating the use of a mixture of solvents. Since an isoimide and protonated isoimide were thought to be intermediates and these are known to absorb in the carbonyl range (p. 113-4) it was necessary to use a solvent system that does not absorb intensely in this region. Solutions of ordinary water absorb intensely in the carbonyl region but deuterium oxide and deuterium chloride in deuterium oxide do not. The solvent system that had none of these shortcomings and which was used for these experiments was a mixture of deuterium oxide and dimethyl sulphoxide. It must be emphasised that conclusions deduced from these experiments are true only for this solvent (and at this particular concentration of ocba), and are not necessarily true for aqueous solution.

Previous work had shown that between pH's 1.5 and 3.5 the rate of hydrolysis of ocba was first-order in ocba and the
concentration of hydroxonium ions. At lower pH's the above kinetic equation no longer fitted the observed kinetic results, the observed rate levelling off until it eventually becomes independent of pH. An explanation for the behaviour at pH's less than 1.5 is that there is a change in the RDS from STEP 2 to STEP 1 (Reaction Scheme No. 1, p. 38) or at least that STEP 2 becomes of similar magnitude to STEP 1. Such an occurrence would of course alter the whole form of the kinetic equation and indeed if such a change did occur the observed rate would be independent of pH in accordance with the observed results. Similarly, if the isomerisation of ocba to isophthalimide became rate-determining the same kinetic result would be obtained. An alternative explanation is that at pH's less than 1.5 the protonated isophthalimide cation, 24, is stable. If this were true then as the acidity is increased the anion derived from ocba, ocba and isophthalimide should all be converted to 24 which should be observable in the IR spectrum. However, no such species was ever observed even when the molar ratio of deuterium chloride to ocba was as high as ten. In all instances hydrolysis of ocba to
phthalamic acid was observed. Under these conditions at least the concentration of both isophthalimide and protonated isophthalimide must be very small. The failure to observe 24 suggests that the change in the kinetic form of the rate equation below pH 1.5 is caused by a change in the RDS from STEP 2 to STEP 1 (p. 38) or the isomerisation of ocba to isophthalimide.

**Elimination of Phthalimide as an Intermediate**

As suggested (p. 119) phthalamic acid could possibly be formed from ocba by the intermediate formation of phthalimide from isophthalimide. The thermal rearrangement of ocba to phthalimide is well documented.70-74 If, however, it could be shown that any step in this proposed reaction path was slower than the observed rate of ocba under identical conditions that mechanism would be untenable. The catalytic constant for the acid catalysed hydrolysis of phthalimide124 at 100° is 9.83 x 10^-5 M^-1 s^-1. That for ocba65 in the pH range 1.5 - 3.5 at 60° is 0.47 M^-1 s^-1. The possibility of the intermediacy of phthalimide in the acid catalysed hydrolysis of ocba is thus eliminated.
Determination of the Mode of Attack by Water
in the Acid Catalysed Hydrolysis of 2-Cyanobenoic Acid

The acid catalysed hydrolysis of 2-cyanobenoic acid is thought to proceed via the protonated isophthalimide cation, 24 (p. 38). Nucleophilic attack by oxygen labelled water at the protonated imine and carbonyl groups would result in 64 and 65 forms of phthalamic acid, respectively.

![Chemical Structures]

By comparing the intensities of the \((C_8H_6NO_2)^+\) ions it was hoped to deduce the mode of attack since if 64 was exclusively formed there would be no loss of the label whereas if 65 was exclusively formed half the label would be lost.

The labelled water contained 62.57% oxygen-18. A sample of this (90 μl) was mixed with hydrochloric acid (10 μl). Thus, the percentage of oxygen-18 labelled water is

\[
\frac{90}{100} \times 62.57\% = 56.7\% \text{ and, therefore, 43.3\% is unlabelled water.}
\]
If attack occurred exclusively at the protonated imine group the following species would be formed.

\[
\begin{align*}
\text{NH}_2 & \quad \rightarrow \quad [\text{NH}_2]^{+} \\
\text{CO}_2\text{H} & \quad \rightarrow \quad [\text{CO}]^{+} \\
\text{m/e} \ 167(56.7\%) & \quad \text{m/e} \ 150(56.7\%) \\
\text{NH}_2 & \quad \rightarrow \quad [\text{NH}_2]^{+} \\
\text{CO}_2\text{H} & \quad \rightarrow \quad [\text{CO}]^{+} \\
\text{m/e} \ 165(43.3\%) & \quad \text{m/e} \ 148(43.3\%)
\end{align*}
\]

The ratio of the intensities of the ions of \text{m/e} 148 and 150 would thus be 1.0 : 1.3, respectively, if this mechanism occurred. If attack occurred exclusively at the carbonyl group the following species would be formed.
The ratio of the intensities of the ions of $m/e$ 148 and 150 would now be 2.5 : 1. It was hoped that by comparing these two intensities the mechanism would be elucidated. However, it was later realised that the ion of $m/e$ 150 could be oxygen-18 enriched phthalic anhydride which could be formed by either of the two mechanisms shown below.
Until the mode of formation of phthalic anhydride from phthalamic acid is known comparison of the intensities of the ions of $m/e$ 148 and 150 is useless.

Comparison of the intensities of the ions of $m/e$ 147 and 149 (the phthalimides) could have overcome this problem. However, the MS of unlabelled phthalamic acid showed a relatively intense peak at $m/e$ 149 which would complicate any detailed analysis.

Analysis of the Kinetic Data

The failure of the previously derived kinetic equation to explain the levelling off of the observed rate for the hydrolysis of ocba at pH's less than 1.5 prompted closer inspection of the assumptions therein.

In the pH range 1.5 - 3.5 the rate of hydrolysis of ocba was found to be proportional to the concentration of hydroxonium ions. It was assumed that there was a rapid reversible equilibrium between ocba and isophthalimide, that STEP 2 was the RDS (Reaction Scheme No. 1, p. 38) and that the formation of 25 was irreversible. However, no evidence was obtained to support these assumptions. It is interesting in view of this
to see what other kinetic equations are also consistent with the kinetic data.

If STEP 1 is acid catalysed and is also the RDS the rate equation takes the form

\[ \text{Rate} = k_2 (AH)(H) \]

which is what was found experimentally.

If \( k_5 \) was rate-determining the rate equation would take the form

\[ \text{Rate} = k_5 \]

Using the steady state approximation, \(^{(24)}(HgD)\)

\[ \frac{d}{dt} (25) = k_4 (24) (H_2O) - k_5 (25) = 0 \]

\[ \therefore (25) = \frac{k_4}{k_5} (24) (H_2O) \]

\[ \therefore \text{Rate} = k_4 (24) (H_2O) = \frac{k_4}{K_3} (23) (H) (H_2O) \]

\[ = \frac{K_2}{K_3} \frac{(23)}{(22)} (H) (H_2O) \]

where \( K_2 = \frac{(23)}{(22)} \) and \( K_3 = \frac{(23) (H)}{(24)} \)

Thus this too is consistent with the observed kinetics as well as that with STEP 2 as the RDS. However, all these kinetic equations fail to explain the levelling off of the rate at pH's
less than 1.5. Ghosh proposed that the RDS might then be
STEP 1 and not STEP 2 or that 24 was stable under these
conditions. However, if, for some reason, STEP 2 was a
rapid reversible reaction at pH's less than 1.5 then

\[ K_4 = \frac{(24) (H_2O)}{(25) (H)} \]

\[ \text{Rate} = k_5 \frac{(24) (H_2O)}{K_4} = k_5 \frac{(23) (H_2O)}{K_3 K_4} \]

\[ = k_5 \frac{K_2}{K_3 K_4} (22) (H_2O) \]

The rate is now independent of pH as observed at pH's less
than 1.5. While this offers an explanation for the levelling
off of the rate it in no way explains why STEP 2 is irreversible
at pH's greater than 1.5 but reversible at lower pH's.

Finally, in an attempt to explain the levelling off of the
rate the steady state approximation was used. If in Reaction
Scheme No. 1 (p.38) the RDS is STEP 2 the rate of formation
of phthalamic acid is given by the equation

\[ \text{Rate} = k_4 (24) (H_2O) \] (2)

Now \[ \frac{d(23) + (24)}{dt} = k_2 (22) - k_{-2} (23) - k_4 (24) (H_2O) = 0 \]
270.

\[ k_2 (22) - \frac{k_{-2} K_3 (24)}{(H)} - k_4 (24) (H_2O) = 0 \]

\[ (24) = \frac{k_2 (22)}{k_{-2} K_3 + k_4 (H_2O)} \]

Substituting in (2) gives

\[ \text{Rate} = \frac{k_2 k_4 (22)}{k_{-2} K_3 + k_4 (H_2O)} \]  

(3)

The rate of hydrolysis is also given by the equation

\[ \text{Rate} = k_{\text{obs}} \left( (21) + (22) \right) \]

Since

\[ K_1 = \frac{(21) (H)}{(22)} \]

\[ \text{Rate} = k_{\text{obs}} \left( \frac{K_1}{(H)} + 1 \right) (22) \]  

(4)

Equations (3) and (4) are rate equations for the same equation.

Equating these gives, after rearrangement

\[ k_{\text{obs}} = \frac{k_2 k_4}{\left( k_{-2} K_3 + k_4 (H_2O) \right) \left( 1 + \frac{K_1}{(H)} \right)} \]

\[ = \frac{k_2}{\left( \frac{k_{-2} K_3 + (H_2O)}{k_4 (H)} \right) \left( 1 + \frac{K_1}{(H)} \right)} \]
Further rearrangement and substitution for \( \frac{k_2}{k_2} \) gives

\[
\frac{1}{k_{\text{obs}}} = \frac{K_1 K_3}{k_4 K_2 (H)^2} + \frac{1}{(H)} \left( \frac{K_3}{k_4 K_2} + \frac{1}{k_2} (H_2O) \right) + \frac{(H_2O)}{k_2}
\]

At low acid concentrations \((H)\) is small so that \( \frac{1}{(H)^2} \) is very large. Thus a plot of \( \frac{1}{k_{\text{obs}}} \) versus \( \frac{1}{(H)^2} \) should have a gradient of \( \frac{K_1 K_3}{k_4 K_2} \) and an intercept (extrapolated) of \( \frac{(H_2O)}{k_2} \).

<table>
<thead>
<tr>
<th>pH</th>
<th>(10^3 k_{\text{obs}} \text{ (s}^{-1})</th>
<th>(1/k_{\text{obs}} \text{ (s)})</th>
<th>((H)(M))</th>
<th>(1/(H)(M^{-1}))</th>
<th>(1/(H)^2 \text{ (M}^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.20(H_2O)</td>
<td>14.7</td>
<td>68.03</td>
<td>1.585</td>
<td>0.6308</td>
<td>0.03979</td>
</tr>
<tr>
<td>0.20(H_2O)</td>
<td>14.5</td>
<td>68.97</td>
<td>6.310 \times 10^{-1}</td>
<td>1.585</td>
<td>2.513</td>
</tr>
<tr>
<td>1.06</td>
<td>12.6</td>
<td>79.37</td>
<td>8.710 \times 10^{-2}</td>
<td>11.48</td>
<td>1.318 \times 10^{2}</td>
</tr>
<tr>
<td>1.51</td>
<td>9.98</td>
<td>100.2</td>
<td>3.090 \times 10^{-2}</td>
<td>32.36</td>
<td>1.047 \times 10^{3}</td>
</tr>
<tr>
<td>1.87</td>
<td>6.45</td>
<td>155.0</td>
<td>1.349 \times 10^{-2}</td>
<td>74.07</td>
<td>5.486 \times 10^{3}</td>
</tr>
<tr>
<td>2.07</td>
<td>4.13</td>
<td>242.1</td>
<td>8.511 \times 10^{-3}</td>
<td>117.5</td>
<td>1.380 \times 10^{4}</td>
</tr>
<tr>
<td>2.47</td>
<td>1.92</td>
<td>520.8</td>
<td>3.388 \times 10^{-3}</td>
<td>295.2</td>
<td>8.715 \times 10^{4}</td>
</tr>
<tr>
<td>3.04</td>
<td>0.997</td>
<td>1003</td>
<td>9.120 \times 10^{-4}</td>
<td>1096.0</td>
<td>1.202 \times 10^{6}</td>
</tr>
<tr>
<td>3.51</td>
<td>0.533</td>
<td>1876</td>
<td>3.090 \times 10^{-4}</td>
<td>3236.0</td>
<td>1.047 \times 10^{7}</td>
</tr>
</tbody>
</table>

Inspection of the above data shows that at least in the pH range studied there is no linear relationship between \( 1/ k_{\text{obs}} \) and \( 1/(H)^2 \).

While no further information can be obtained from this data this treatment may nevertheless be valid at higher pH's.
THE HYDROLYSIS OF 4-HYDROXYBUTYRONITRILE

Reduction of 4-benzyloxybutyronitrile with hydrogen gave a product whose NMR and IR spectra were consistent with that of 4-hydroxybutyronitrile (p. 102). The analysis, however, showed that it was not pure. Also the UV spectrum in aqueous solution had a band at $\lambda_{\text{max}} = 230$ nm which is absent in the spectrum of butyronitrile. Since the impurity could be the result of spontaneous hydrolysis the UV spectra of 4-hydroxybutyramide, pyrrolidone and 4-butyrolactone were compared with it. However, they were all appreciably different (p. 136-141). Only a qualitative study of the hydrolysis 4-hydroxybutyronitrile was attempted in view of its impure state. However, no very rapid hydrolysis occurred in either acidic (hydrochloric and perchloric) or basic (sodium hydroxide) solution.
REFERENCES


39. M. L. Bender, F. Chloupek, M. C. Neveu, *J. Amer. Chem. Soc.*, 1958, 5384 reported that the hydrolysis of methyl phthalate was proportional to the concentration of the anion but more recent work contradicts this. 37, 38


b) E. Sondheimer, R. W. Holley, *ibid.*, 1954, 2467.

c) E. Sondheimer, R. W. Holley, *ibid.*, 1957, 3767.


67. M. P. Cava, R. P. Stein, J. Org. Chem., 1966, 1866 proposed a second-order Beckmann rearrangement to explain the formation of ethyl 2-cyanobenzoate when benzocyclobutadienequinone was treated with hydroxylamine hydrochloride in ethanol.


    b) K. Watanabe, *ibid.*, 1964, 1325.


c) A. Salamon, Muegyetemi Közlemények, 1949, 72 and Chemical Abstracts, 1951, 552.


120. "Dictionary of Organic Compounds" gives b.p. 227 - 8°/760 mm Hg and 110 - 111°/17 mm Hg.


145. Reference 144 gives the boiling points of 3-benzyloxy-propyl chloride, 4-benzyloxybutyronitrile and 3-benzyloxypropan-1-ol as 129°/16 mm Hg, 157°/12 mm Hg and 155°/23 mm Hg, respectively.


158. J. Scheiber, Chem. Ber., 1912, 2401 has summarised the preparations of ocba.


161. See reference 10 in reference 159 above.


164. There are very few substituted cis 3-cyano-acrylic acids reported in the literature. Neither the cis nor the trans form of the parent compound are known.


184. M. I. Page, personal communication.


   
   


216. a) R. L. Letsinger, S. Dadidegaonker, W. J. Vullo, 


b) M. L. Bender, F. J. Kézdy, *J. Amer. Chem. Soc.*, 1964, 
3704.


Vol. 1, Interscience Publishers Inc., New York, 


