SYNTHETIC AND ENZYMATIC STUDIES RELATED TO BRANCHED-CHAIN AMINO ACID METABOLISM

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CONTENTS

ABBREVIATIONS

Me	Methyl
Et	Ethyl
Pr	Propyl
i-Pr	Iso-propyl
Bu	Butyl
t-Bu	Tertiary-butyl
Ph	Phenyl
R	Alkyl
Ar	Aryl
Tos	Toluene-4-sulphonyl
NMR	Nuclear magnetic resonance
s	Singlet
d	Doublet
t	Triplet
q	Quartet
m	Multiplet
b	Broad
J	Coupling constant
ppm	Parts per million
TMS	Tetramethylsilane
IR	Infra red
sh	shoulder
MS	Mass spectrum
CD	Circular dichroism

VCD	Vibrational circular dichroism
$[\alpha]$	Specific rotation
c	Concentration (g/100 ml)
ee	Enantiomeric excess
GLC	Gas-liquid chromatography
R_T	Retention time
HPLC	High pressure liquid chromatography
TLC	Thin-layer chromatography
mp	Melting point
bp	Boiling point
DME	1,2-Dimethoxyethane
THF	Tetrahydrofuran
ADC	Acetolactate decarboxylase
ALS II	Acetolactate synthase isozyme II
PLE	Pig liver esterase
FAD	Flavin adenine dinucleotide
TPP	Thiamine pyrophosphate
EEDQ	1-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
gly	Glycine

ACKNOWLEDGEMENTS

I would like to thank Professor D.H.G.Crout for the constant encouragement and advice which he has provided throughout the course of this work.

Thanks are due to Novo Industri A/S for the gift of acetolactate decarboxylase and for helpful discussions concerning the nature of the enzyme. I must also thank Dr J.V.Schloss of the Du Pont Company for provision of acetolactate synthase isozyme II.

Dr A.F.Drake is to be thanked for the measurement of vibrational circular dichroism spectra.

The financial assistance of the S.E.R.C. and especially of ICI Plant Protection Division is gratefully acknowledged.

LIST OF FIGURES

DECLARATION

The work described in this thesis is the original work of the author except where acknowledgement is made to work and ideas previously described. It was carried out in the Department of Chemistry, University of Warwick between October 1984 and September 1987 and has not been submitted previously for a degree at any institution.

PUBLICATIONS

Parts of the research described in this thesis have appeared in the scientific literature as follows.

1. Catalysis by Di-n-butyltin Oxide of a Tertiary Ketol Rearrangement: Synthesis of Intermediates and Analogues of Valine and Isoleucine Biosynthesis.
   Crout, D.H.G., and Rathbone, D.L., J. Chem. Soc. Chem. Commun., 1987, 290-291.

2. Biotransformations with Acetolactate Decarboxylase: Unusual Conversions of Both Substrate Enantiomers into Products of High Optical Purity.
   Crout, D.H.G., and Rathbone, D.L., J. Chem. Soc. Chem. Commun., 1987, in press.

3. Applications of Vibrational Infra Red Circular Dichroism to a Biological Problem: Stereochemistry of Proton Exchange in Acetoin (3-hydroxybutan-2-one) Catalysed by Acetolactate Decarboxylase.
   Drake, A.F., Siligard, G., Crout, D.H.G., and Rathbone, D.L., J. Chem. Soc. Chem. Commun., 1987, in press.

SUMMARY

Several sytheses of α-acetolactate analogues (2-hydroxy-3-oxo carboxylic esters) and related compounds were developed. The action of the enzyme acetolactate decarboxylase upon these compounds was studied.

A synthesis of 3-bromo-2-oxo carboxylic acids, esters and amides was developed. Inhibition studies using methyl 3-bromo-3-methyl-2-oxo butanoate with pig liver esterase indicated that the esters were not suitable mechanism-based inhibitors of the enzyme.

A synthesis of 3-hydroxy-2-oxo carboxylic esters was developed. These compounds were found to isomerise, *via* alkyl group migration, to the corresponding 2-hydroxy-3-oxo carboxylic esters upon treatment with catalytic quantities of dibutyl tin oxide. Cyclic substrates gave access to 6, 7 and 8-membered ring-expanded products.

A one-step synthesis of 2-hydroxy-3-oxo carboxylic esters, in high yield, from the corresponding αβ-unsaturated esters was developed. This involved the use of acidic manganate (VII) in aqueous acetone and was found to be superior to any synthesis of acyclic 2-hydroxy-3-oxo carboxylic esters published to date.

The enzyme acetolactate decarboxylase (ADC) was found to decarboxylate the (S)-isomers of α-acetolactate and its analogues to give the corresponding (R)-α-hydroxyketones in high optical purity. The (R)-substrates were decarboxylated to (R)-α-hydroxyketones *via* prior isomerisation to (S)-α-acetolactate analogues by a tertiary ketol rearrangement involving carboxylate group migration. As a result α-acetolactate analogues with non-identical substituents at the 2- and 3-positions gave structurally different α-hydroxyketone products upon decarboxylation with ADC. In the case of α-acetolactate, a substrate with identical substituents at the 2- and 3-positions, *both* enantiomers were converted into (R)-(-)-acetoin with an enantiomeric excess greater than 98%. This represents an unusual example of the enzymic conversion of both enantiomers of a racemic substrate into a single enantiomer of product. The behaviour of the enzyme towards a range of substrates gave some insight into the nature of the enzyme active site.

ADC was found to catalyse, stereospecifically, the incorporation of deuterium into the (R)-isomer of racemic acetoin at the methine position. The chirality of the deuterated and non-deuterated components of the partially deuterated mixture was analysed *in situ* by vibrational circular dichroism measurements.

Acetolactate synthase isozyme II (ALS II) was used to generate α-acetolactate and its homologues from simple 2-oxo carboxylic acids. These were decarboxylated *in situ* by ADC to give the corresponding α-hydroxyketones of high optical purity. In one example α-acetohydroxybutyrate was generated by the action of ALS II upon 2-oxobutanoate. The structure of the single α-hydroxyketone prepared by ADC-catalysed decarboxylation of the α-acetohydroxybutyrate indicated that the (S)-isomer of α-acetohydroxybutyrate had been generated.

Model studies were carried out on 2-hydroxycyclohexanone for the stereospecific reduction of the enzymatically produced α-hydroxyketones to 1,2-diols. No signifiant chiral induction was observed with sodium borohydride in a range of solvents or with lithium aluminium hydride.

INTRODUCTION

The work presented in this thesis falls into three main areas:

a) The synthesis of potential mechanism-based inhibitors of hydrolytic enzymes.

b) The synthesis of 2-alkyl-2-hydroxy-3-oxo carboxylic esters.

c) The action of the enzyme acetolactate decarboxylase upon 2-alkyl-2-hydroxy-3-oxo carboxylic esters.

Irreversible enzyme inhibitors

Many classes of molecules have been shown to cause specific inhibition of some target enzyme. The effects *in vivo* can be beneficial, as in the inhibition of microbial penicillinases, or the consequences can be toxic. An understanding of the mechanism of the inhibition process can help in the rational designing of drugs with optimum *in vivo* specificity.

Specific inhibitors of hydrolytic enzymes, peptidase inhibitors in particular, have a use in the purification of enzymes from culture media. For example, in the process of obtaining one particular enzyme from a cell paste, enzymes additional to the desired one may become concentrated in the purified fractions. If one of these extraneous enzymes has protease activity then it may destroy other proteins, and these may include the enzyme. It is therefore neccessary to inhibit protease action during enzyme isolation.

Irreversible enzyme inhibitors may be divided into two categories. The first comprises active site directed reagents. These molecules are structural analogues of normal substrates but have some

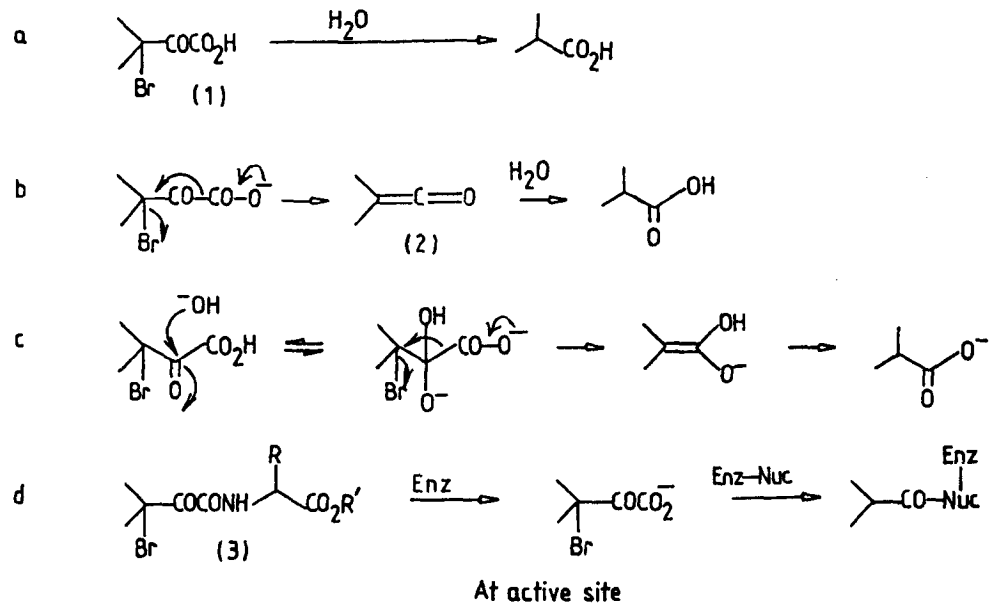
reactive functionality, such as a haloketone, epoxide, sulphonyl halide or phosphoryl halide. Having the required likeness to normal substrates they bind to the target enzyme in the usual manner of enzyme-substrate interaction. Their mode of action relies upon some nucleophilic amino acid side-chain of the target enzyme being close enough to the reactive group of the inhibitor molecule for there to be a displacement reaction between them leading to covalent modification of the active site.

The second category of irreversible enzyme inhibitor differs from the first in that the reactive functionality is latent in the inhibitor molecule. The reactivity is only uncovered during the enzymic process after binding of the enzyme and substrate. The specific action of the enzyme is neccessary to convert the original innocuous substrate into the reactive entity. The activation only occurs at the active site. If chemical reaction occurs between the activated substrate and the active site of the enzyme before the two can diffuse apart then only the target enzyme is modified and has its activity destroyed.

A review by Walsh[1] covers the different classes of mechanism-based inhibitors that have been studied.

A mechanism-based inhibitor of hydrolytic enzymes

The idea for a mechanism-based inhibitor of hydrolytic enzymes arose from the observation that 3-bromo-3-methyl-2-oxobutanoate (1) reacts with water to give isobutyric acid[2] (Scheme 1.a).



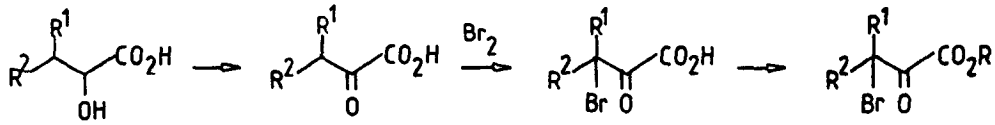
Scheme 1

This observation may be explained by either of two mechanisms. The first (Scheme 1.b) involves loss of carbon dioxide and bromide ion to generate a ketene (2) which is then hydrated. The second (Scheme 1.c) postulates a reversible nucleophilic attack at the carbonyl group followed by loss of carbon dioxide and bromide ion to generate the observed product.

If the acid (1) is masked as an ester or amide (3) then neither of the two postulated mechanisms can operate. If the protected species (3) is incubated with an enzyme capable of hydrolysing the peptide bond then the free acid will be generated at the active site of the enzyme. Any adjacent nucleophile at the active site might then be acylated by either of the two mechanisms postulated (Scheme 1.d).

Synthesis of potential mechanism-based inhibitors of hydrolytic enzymes

In order to synthesise 3-bromo-2-oxo esters and amides such as compound (3) it was decided to attempt bromination of 2-oxo acids followed by esterification or coupling to suitable protected amino acids. It was planned to use 2-hydroxy acids as precursors of the 2-oxo acids (Scheme 2).



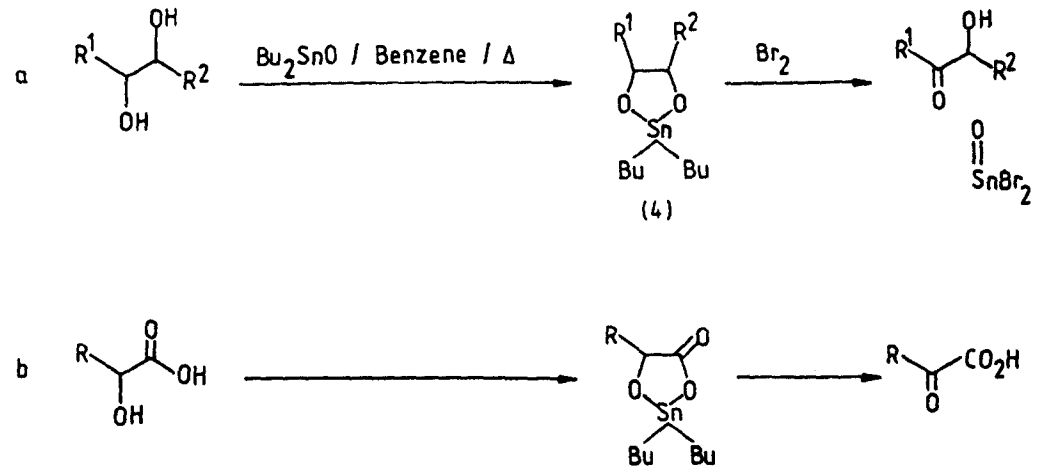
Scheme 2

Synthesis of 2-oxo carboxylic acids

Literature methods for the oxidation of 2-hydroxy acids to 2-oxo acids often involve more than one step, for example, formation of the t-butyl amide to protect the carboxyl function[3] prior to oxidation. Some methods employ vigorous conditions which might destroy sensitive functional groups present in the molecule, for example, the preparation of benzoylformic acid by alkaline potassium manganate (VII) oxidation of mandelic acid.[4]

It was decided to develop a mild procedure for the direct conversion of 2-hydroxy acids to 2-oxo acids.

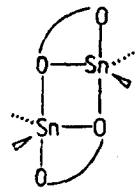
A mild method for the oxidation of 1,2-diols to hydroxyketones[5] has been reported. This involves the brominolysis of dioxostannolan derivatives of 1,2-diols (Scheme 3.a). It was decided to explore the potential of this method for the oxidation of 2-hydroxy acids (Scheme 3.b).



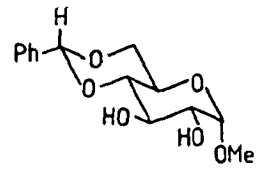
Scheme 3

Reaction of equimolar amounts of 1,2-diols and dibutyl tin oxide in refluxing benzene gave the five-membered cyclic dialkyl tin derivatives (4).[6] Linear structures have been ruled out since no OH stretch could be observed in the infrared spectrum. Molecular weight estimations[7] have shown these compounds to be monomeric in dilute or polar solvents and to exist as dimers at higher concentrations in non-polar solvents. There is also evidence for higher oligomers. Mossbauer parameters[8] are consistent with a dimer in the solid state, pentacoordinate at tin.

David and co-workers[9] performed ^{119}Sn NMR experiments on a range of dioxostannolans derived from chiral 1,2-diols. All ^{119}Sn resonances were observed 121-180 ppm upfield of the 4-coordinate Me_4Sn reference, a range characteristic of tin with coordination greater than four. Only one resonance for each compound was observed implying that the two tin atoms of the dimers present were in a symmetrical environment. C_2 symmetry is the only possible symmetry from the association of two chiral monomers.



(5)



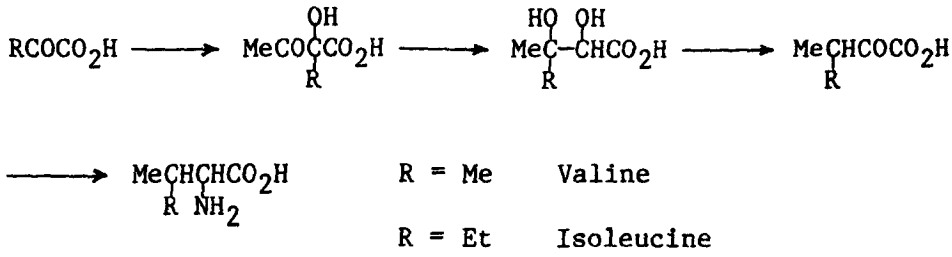
(6)

This suggests the structure (5). Within each monomeric unit of the dimer, tin binds equatorially to one oxygen atom of the parent diol, apically to the other and apically to the equatorial oxygen of the other monomeric subunit acting as a ligand to the first. X-ray data show the structure of dioxostannolan of (6) in the solid state to be of the above described geometry.[10]

David and co-workers[5] worked with a range of glycols derived from partially protected glucose and galactose. These were reacted with dibutyl tin oxide in benzene with azeotropic removal of water. Evaporation of the benzene gave the crude dioxostannolans which were used without purification. Brominolysis of the dioxostannolans was carried out by addition of bromine in dichloromethane to either the dioxostannolan in benzene in the presence of 4A molecular sieves or the dioxostannolan in dichloromethane in the presence of tributyl tin methoxide at room temperature. Yields were slightly higher by the latter procedure but the former was preferred since this made chromatographic separation of the product hydroxyketones less difficult. The reaction occured at the speed of titration with bromine. However, in the absence of hydrogen bromide scavengers the reaction often became slow after the addition of two thirds of the stoichiometric amount of bromine. It was thought that acid generated

in the medium by the reaction may have been attacking unreacted dioxostannolan causing Sn-O bond fission and generating unreactive material. Yields were found to be higher in the presence of a hydrogen bromide scavenger.

Inhibitors of the valine-isoleucine biosynthetic pathway

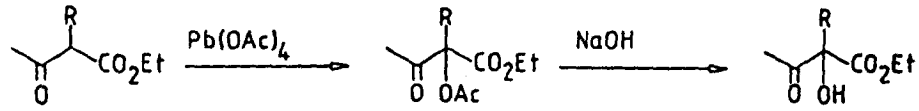


Scheme 4. The valine-isoleucine biosynthetic pathway

Two new classes of powerful herbicides have been developed which act by inhibiting the first common enzyme of the valine-isoleucine biosynthetic pathway, acetolactate synthase, (acetolactate pyruvate lyase (carboxylating), E.C.4.1.3.18)[10,11] (Scheme 4).

These comprise the sulphonylureas sulfometuron methyl and chlorosulfuron, marketed by Du Pont, and the imidazolinones 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)nicotinic acid and 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-3-quinoline carboxylic acid marketed by American Cyanamid. These herbicides are the most potent so far discovered. Consequently there is much commercial interest in the biosynthesis of valine and isoleucine. In particular, the natural products from the acetolactate synthase-catalysed step, α-acetolactate, α-acetohydroxybutyrate and their analogues are required for biological testing.

Synthesis of 2-hydroxy-3-oxo carboxylic esters



(7) R = Me

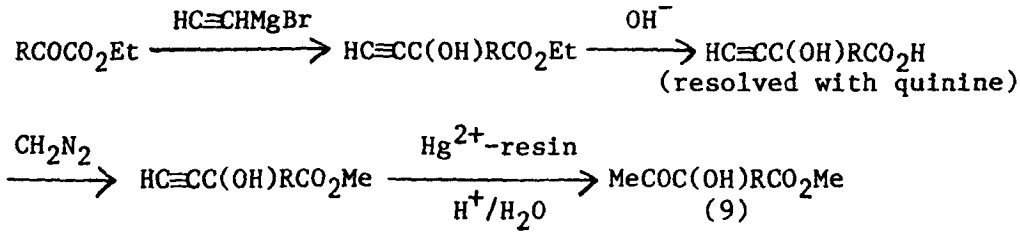
(8) R = Et

Scheme 5

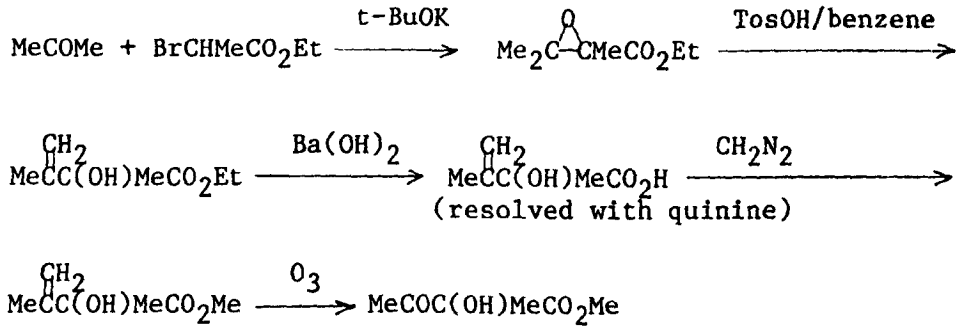
The standard syntheses of the natural substrates α-acetolactate (7) and α-acetohydroxybutyrate (8) are based on the acetoxylation of 2-methyl and 2-ethylacetoacetates respectively, as developed originally by Krampitz[12] (Scheme 5). These syntheses proceed in low and variable yield[2] particularly when carried out on a small scale.

Other, multi-step, syntheses have been developed with a view to the introduction of isotopic labels or the production of optically pure material.

The two published chiral syntheses[13,14] both involve the chemical resolution of a stable acid which was later transformed into the more labile β-keto acid. The synthesis developed by Hill and co-workers[13] (Scheme 6) involved attack of a Grignard reagent upon ethyl pyruvate in very low yield. Neglecting the resolution steps, the overall yield of methyl α-acetolactate (9) was only 10%.



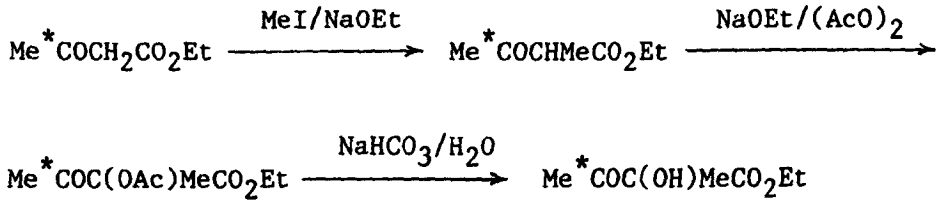
Scheme 6



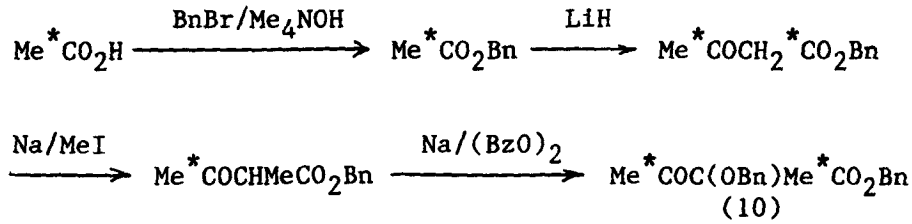
Scheme 7

The syntheses by Crout and co-workers[14] (Scheme 7) produced methyl -acetolactate in 34% yield, neglecting the resolution steps.

Syntheses of -acetolactate have been developed[2] which allow isotopic substitution by incorporation of labelled ethyl acetoacetate (Scheme 8) or labelled acetic acid (Scheme 9). The former gave ethyl -acetolactate in 42% yield. The latter gave the dibenzyl-protected -acetolactate (10) in 10% overall yield.



Scheme 8



Scheme 9

Bn = benzyl; Bz = benzoyl

Several recent syntheses of 2-hydroxy-3-oxo carboxylic acids proceed by epoxidation of the enol form of 3-oxo carboxylic esters, either as the equilibrium mixture with the 3-oxo carboxylic ester or trapped as the silyl enol ether.[15-20] During work up, rearrangement of the 2,3-epoxy-3-hydroxycarboxylic ester gives the 2-hydroxy-3-oxo carboxylic ester. Langlois and co-workers,[17] using this method, achieved a 76% yield of methyl 1-hydroxycyclopentan-2-one carboxylate (11) (Scheme 10).

(11)

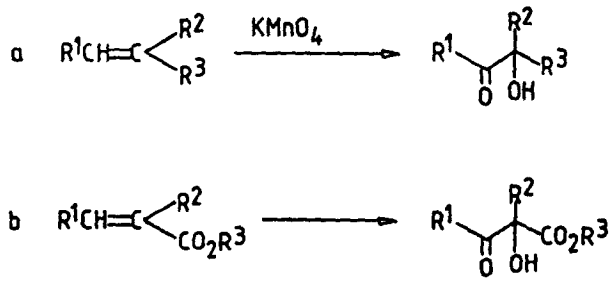
Scheme 10

However, this procedure failed completely with acyclic systems such as α-acetolactate. The other references cited above involve polycyclic systems where the 2-hydroxy-3-oxo carboxylic ester is part of a cyclohexane ring.

It was decided to develop a synthesis of 2-hydroxy-3-oxo carboxylic esters applicable to α-acetolactate and its analogues.

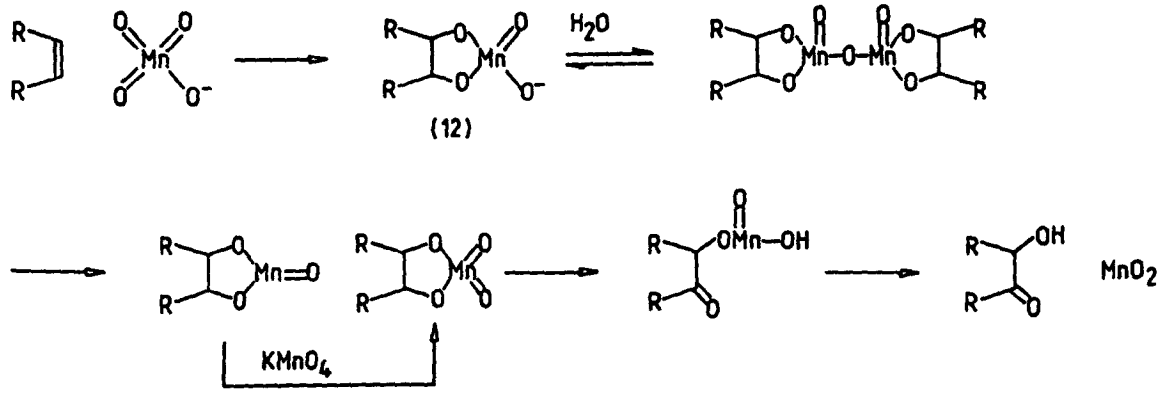
It is well known that at acidic pH potassium manganate (VII) will convert alkenes into hydroxyketones[21,22] (Scheme 11.a).

It was decided to attempt to extend this chemistry to 2-alkyl-substituted αβ-unsaturated esters to generate analogues of α-acetolactate (Scheme 11.b).



Scheme 11

For the reaction of potassium manganate (VII) with alkenes, the diol is the major product at pH >9 for cyclic systems or pH >12 for acyclic compounds.[23] The hydroxyketone is the major product at pH 4 to pH 6 and both products are observed at intermediate pH. The hydroxyketones are not formed via the diols since the latter are stable to potassium manganate (VII) under the hydroxyketone-forming conditions.[24,25] Formation of the diols, the reaction stereochemistry[26], ^{18}O labelling[27] and kinetic studies[28,29] support the existence of the hypomanganate intermediate (12) (Scheme 12).



Scheme 12

The kinetic results support the view that the diol and the hydroxyketone are formed from a common intermediate since the second-order rate constant is the same at pH 6.8 and pH 13.[28]

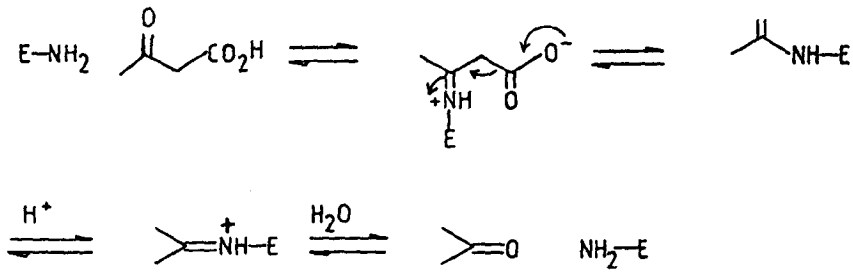
Deuterium labelling studies performed by Wolfe and Ingold[23] support the mechanism detailed in Scheme 12. There is oxidative decomposition of the cyclic hypomanganate ester (12) followed by hydrolysis of the resulting manganate (IV) ester, in that order, to give the hydroxyketone and manganese dioxide. The dimerisation of the cyclic hypomanganate ester (12) followed by disproportionation to the manganate (IV) and (VI) species is qualitatively similar to the well known oxidative and hydrolytic reactions of inorganic hypomanganate (MnO_4^{3-}).[23]

Acetolactate decarboxylase

2-Alkyl-2-hydroxy-3-oxo acids are analogues of the natural substrates for the enzyme acetolactate decarboxylase (2-hydroxy-2-methyl-3-oxobutanoate carboxy-lyase, EC 4.1.1.5). The natural substrates for this enzyme are α-acetolactate (2-hydroxy-2-methyl-3-oxobutanoate) (7) and α-acetohydroxybutyrate (2-ethyl-2-hydroxy-3-oxobutanoate) (8).

The mechanism of enzymic decarboxylation of β-keto acids

The mechanism of action of the enzyme acetoacetate decarboxylase (EC 4.1.1.4) in decarboxylating the normal substrate, acetoacetate, has been well established.[30] It is summarised in Scheme 13.



Scheme 13

The ε-amino group of a lysine residue at the active site forms a protonated Schiff's-base with the carbonyl group of the acetoacetate substrate. This "electron-sink" facilitates the withdrawl of electrons from the carboxylate group resulting in loss of carbon dioxide. Product is liberated by hydrolysis of the Schiff's base.

The above mechanism is consistent with the following observations. Formation and later hydrolysis of a Schiff's base must lead to exchange of the carbonyl group oxygen atom with oxygen from water. This has been observed.[31] Acetoacetate decarboxylase is not inhibited by sodium borohydride alone but is irreversibly inhibited by sodium borohydride in the presence of acetoacetate.[32] This is consistent with the formation of a Schiff's base between the enzyme and substrate which is then reduced by the sodium borohydride, thereby preventing release of product by hydrolysis.

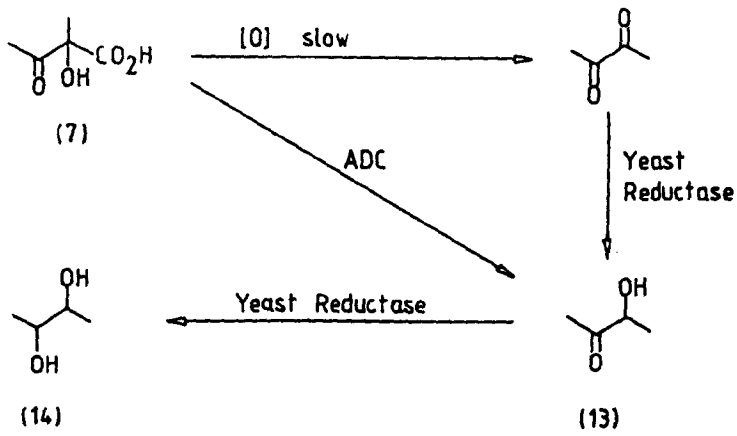
Sodium borohydride has been used to trap Schiff's base intermediates. Incubation of the the enzyme with sodium borohydride and 3-^{14}C-acetoacetate[32] resulted in incorporation of radioactivity into the protein which could not be removed by dialysis. Acid hydrolysis followed by chromatography yielded a single radioactive compound identified as ε-N-isopropyllysine.[33]

The uses of acetolactate decarboxylase

This enzyme is of great potential use to the brewing industry.[34] The fermentation of beer is divided into two major stages; a main fermentation and a maturation. During the main fermentation, over a period of about six days, the bulk of the fermentable sugars is metabolised. During maturation the beer becomes saturated with carbon dioxide, the colloidal stability is improved and the flavour of the beer is allowed to reach its final level. Except for the adjustment in flavour, which can take up to three weeks, the other properties can be achieved within a short process time using known technology. Flavour maturation is the rate limiting step in beer maturation.

The volatile diketone, butan-2,3-dione, plays a major role in determining the flavour of the beer. The precise adjustment of the concentration of this compound in beer is a very important objective in the maturation process. In small concentrations it contributes to the desired flavour but above 0.1 ppm it conveys an objectionable taste to the beer.

The source of butan-2,3-dione in the beer is the non-enzymatic oxidative decarboxylation of α-acetolactate (7), produced by the yeast in the main fermentation (Scheme 14). The butan-2,3-dione once produced is converted enzymatically to acetoin (13) and thence to 2,3-butandiol (14) by yeast cell reductases during maturation. The oxidative decarboxylation of the α-acetolactate is slow compared to the ensuing reductase-catalysed steps and is therefore the rate limiting step in the removal of butan-2,3-dione and its precursor, α-acetolactate, from beer.



Scheme 14

Addition of acetolactate decarboxylase to maturing beer by-passes the rate limiting production of butan-2,3-dione by channelling α-acetolactate directly to acetoin (Scheme 14). Godtfredsen and co-workers[34] have shown that the maturation process can be shortened to less than twenty four hours, simply by adding the enzyme to the beer, and still produce beer of the same quality as that matured conventionally. This represents a four-fold reduction in the overall brewing time and is potentially a great cost saving modification.

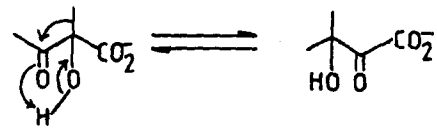
At the time of writing, acetolactate decarboxylase has not yet been used on a commercial scale in the brewing industry, because insufficient quantities of the purified enzyme are available.

The properties of α-acetolactate and acetoin

The absolute configurations of the enantiomers of methyl α-acetolactate have been found to be (S)-(+)- and (R)-(-)- by correlation with primary standards.[35] Similarly, acetoin has been found to have the absolute configurations (R)-(-) and (S)-(+).[36]

α-Acetolactate is unstable in aqueous solution and undergoes decarboxylation. At low pH this process is rapid. At high pH a degenerate rearrangement[13] occurs leading to racemisation of optically pure α-acetolactate. The racemisation occurs at a significant rate only above pH 12.9.[37] The mechanism of the rearrangement has been studied using isotopically labelled α-acetolactate[2] and has been shown to be an intramolecular tertiary ketol rearrangement with migration of the carboxylate group (Scheme 15.a) rather than migration of methyl group (Scheme 15.b).

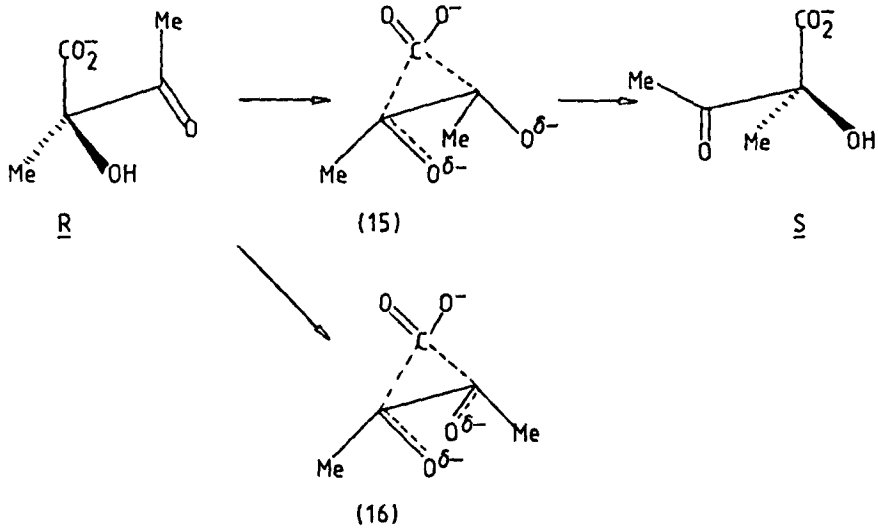
a 

b 

Scheme 15

Since the base-catalysed rearrangement of α-acetolactate results in racemisation, the transition state must be partly, if not wholly, represented by a meso-structure (15), in which a syn arrangement of the C-O bonds is found.

If the anti arrangement of the C-O bonds (16) were to pertain exclusively in the transition state then racemisation could not occur since the configuration at the new chiral centre after rearrangement would be the same as the configuration at the chiral centre in the starting material.



The stereochemistry of the acetolactate decarboxylase-catalysed decarboxylation of α-acetolactate

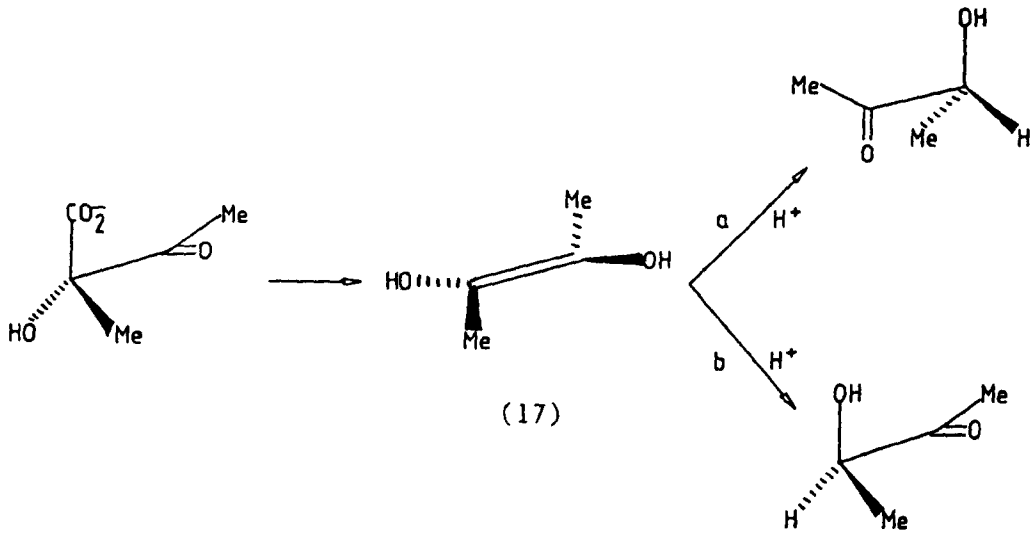
Much work has been done using acetolactate decarboxylase isolated from Aerobacter aerogenes. Loken and co-workers[38] have established the pH optimum for the decarboxylation to lie between pH 6.2 and 6.4 in phosphate buffers. The enzyme was shown not to be dependent on divalent metal cations and also not inhibited by ethanol. Its specificity for α-acetolactate and its analogues has been demonstrated by Juni.[39] It does not act upon acetoacetic acid or 2-oxobutan-1,4-dioic acids.

The (S)-isomers of α-acetolactate (7) and α-acetohydroxybutyrate (8), the biological precursors of valine and isoleucine respectively, are the prefered substrates[39] for the enzyme from Aerobacter aerogenes. The (R)-isomers are decarboxylated also but at 1/20 the rate of decarboxylation of the (S)-isomers.

The acetoin product from the decarboxylation of (S)-

α-acetolactate was found to have the (R)-(-)-configuration.[13]

It is presumed that the reaction procedes via an enediol intermediate (17) (Scheme 16).



(17)

Scheme 16

Protonation of the intermediate (17) might occur at the carbon atom that was originally the acetyl carbon atom of α-acetolactate (Scheme 16.a) or at the carbon atom that was originally the chiral centre (Scheme 16.b).

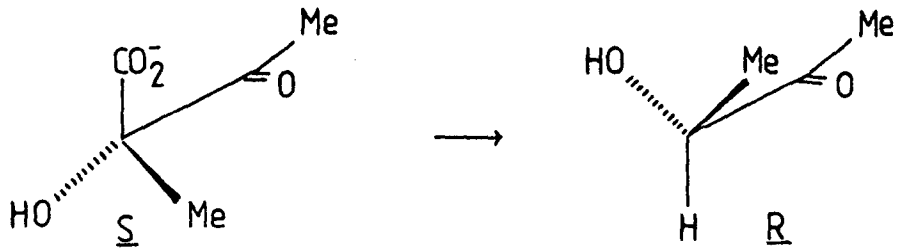
$$Me^{*}COC(OH)MeCOO^{-} \xrightarrow{ADC} Me^{*}COCH(OH)Me \xrightarrow{MeMgI} Me_2{}^{*}C(OH)CH(OH)Me$$
(18)

$$\xrightarrow{NaIO_4} Me_2{}^{*}CO + MeCHO$$

Scheme 17

The site of protonation has been established by Crout and co-workers.[37] [3-^{14}C]-α-Acetolactate was incubated with the acetolactate decarboxylase isolated from Klebsiella aerogenes. The acetoin produced

was alkylated with methyl magnesium iodide and the diol (18) was cleaved with sodium periodate. The cleavage products were isolated as the 2,4-dintrophenylhydrazones (Scheme 17). Most of the radioactivity (80%) was found to reside in the acetone component of the periodate cleavage and the remainder (20%) in the acetaldehyde portion. This showed that protonation of the postulated enediol intermediate in the enzymatic decarboxylation occured at the carbon atom to which the carboxylate group was originally attached. This result, together with the earlier observation that (R)-acetoin is the product obtained from (S)-α-acetolactate, shows that the carboxylate group was replaced by hydrogen with inversion of configuration (Scheme 18).



Scheme 18

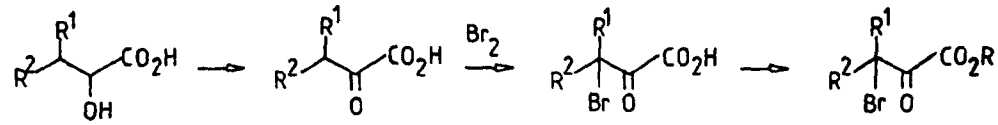
Relatively few decarboxylases are known to operate with overall inversion of configuration. These include 6-phosphonogluconate dehydrogenase,[40] sterol 4-carboxylic acid decarboxylase,[41] UDP glucuronate carboxylase (EC 4.1.1.35)[42] and aspartate β-decarboxylase.[43]

In contrast, retention of configuration has been observed in the enzymic α-decarboxylation of amino acids in all the cases studied so far.[44-50] Pyruvate kinase[51] and isocitrate dehydrogenase[52] are also known to operate with retention of configuration.

CHAPTER 1

The synthesis of 3-bromo-2-oxo carboxylic esters and amides: Potential mechanism-based inhibitors of hydrolytic enzymes

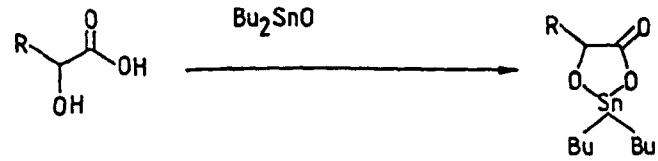
As described in the introduction, it was intended to follow the synthetic sequence below (Scheme 1.1).



Scheme 1.1

Synthesis of 2-oxo carboxylic acids using novel organo-tin chemistry

The dibutyl dioxostannolans (19) of a range of 2-hydroxy acids were prepared by reaction of equimolar quantities of the 2-hydroxy acid and dibutyl tin oxide in boiling benzene with azeotropic removal of water using a Dean-Stark apparatus (Table 1.1).



(19)

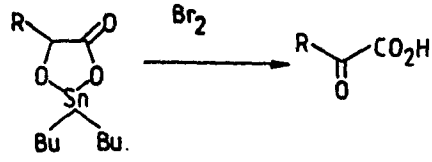
Table 1.1

Entry	R	dioxostannolan
19a	Ph	crystalline solid
19b	Me_2CH	glass
19c	Et	glass
19d	Me	glass

In only one case, (19a), was the dioxostannolan crystalline. The others were obtained as glasses and could not be crystallised.

David et al[5] found that the dioxostannolans from 1,2-diols decomposed on silica gel to regenerate the original 1,2-diols. Dioxostannolan (19a) behaved similarly, giving two spots by silica gel TLC, the less polar compound co-running with mandelic acid. This reactivity of dioxostannolans precluded the use of silica gel chromatography for their purification. The crude dioxostannolans were used as such.

Treatment of the dioxostannolan (19a) with one equivalent of bromine in dichloromethane gave the 2-oxo acid (20a) (Scheme 1.2). The reaction mixture was applied to a short silica gel column and washed with carbon tetrachloride to remove the organo-tin byproducts. The 2-hydroxy acid was eluted with ethyl acetate in nearly pure form (84% yield) and converted into its 2,4-dinitrophenylhydrazone.



(19) (20)

Scheme 1.2

Table 1.2

Entry	R	Yield/%
20a	Ph	84
20b	Me_2CH	43 *
20c	Et	0
20d	Me	0

*as the 2,4-dinitrophenylhydrazone

Similar treatment of (19b) gave the 2-oxo acid (20b) which was isolated as its 2,4-dinitrophenylhydrazone in 43% yield. Brominolysis of the dioxostannolans (19c) and (19d) simply resulted in reisolation of the 2-hydroxy acids.

This oxidation procedure worked well only for mandelic acid. This however, possesses no hydrogen at the 3-position and cannot be brominated to give the 3-bromo-2-oxo species required for inhibition studies with esterases. The 2-oxo acid (20b) was only obtained pure as its 2,4-dinitrophenylhydrazone. In order to use this compound for the synthesis of 3-bromo-3-methyl-2-oxobutanoic acid, the free 2-oxo acid would have to be generated, lowering an already poor yield. This oxidation method failed completely with the straight-chain examples (19c) and (19d) and offers no advantages over conventional literature procedures.

Oxidations using N-bromosuccinimide

A second approach to the generation of 3-bromo-2-oxo acids from 2-hydroxy acids was required. N-Bromosuccinimide is known to oxidise secondary alcohols to ketones[53] and 2-hydroxy esters to 2-oxo esters.[54]

Mild oxidations with N-bromosuccinimide have often been carried out in aqueous acetone or aqueous dioxane.[55,56] N-Bromosuccinimide becomes a more powerful oxidant in aqueous t-butyl alcohol or aqueous t-butyl alcohol in pyridine but with a concomitant decrease in selectivity. Light has been found not to be neccessary for the reaction. This, combined with the polar solvents in which the reaction occurs, implies a mechanism involving "positive" bromine rather than a

free radical pathway.

The by-products from the initial oxidation are succinimide and hydrogen bromide. If the latter has the opportunity to react with N-bromosuccinimide then bromine is generated which can brominate the ketone product from the initial oxidation. This has been used preparatively to generate α-bromoketones[57] and 3-bromo-2-oxo esters.[54] The presence of pyridine or calcium carbonate in the reaction mixture suppresses the bromination reaction by scavenging hydrogen bromide.[57]

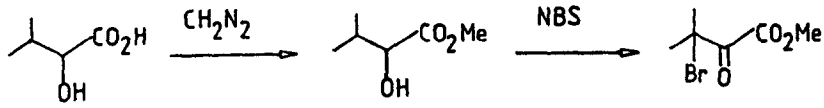
It was decided to prepare to methyl 3-bromo-3-methyl-2-oxobutanoate (21) (a suitable candidate for testing with pig liver esterase as a mechanism-based inhibitor) using the N-bromosuccinimide reaction, and similarly to prepare 3-bromo-3-methyl-2-oxobutanoic acid for coupling to amino acids as potential mechanism-based inhibitors of peptidases.

Very few 2-hydroxy acids are available commercially and which are suitable for transforming into the 3-bromo-2-oxo esters or amides for the enzyme inhibition studies envisaged here. By contrast, $\alpha\beta$-unsaturated esters are readily available commercially or readily synthesised. These may be di-hydroxylated and oxidised to 3-hydroxy-2-oxo esters with a range of alkyl substituents at the 3-position. It was decided to attempt to synthesise such compounds and to transform the remaining hydroxyl group into a leaving group by tosylation, mesylation or bromination.

Synthesis of 3-bromo-2-oxo carboxylic acids, esters and amides

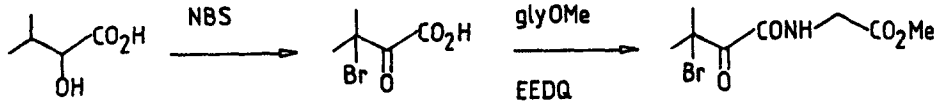
Commercially available 2-hydroxy-3-methylbutanoic acid was esterified with diazomethane in high yield. Treatment with two

equivalents of N-bromosuccinimide in boiling carbon tetrachloride gave methyl 3-bromo-3-methyl-2-oxobutanoate (21) in 83% yield (Scheme 1.3).



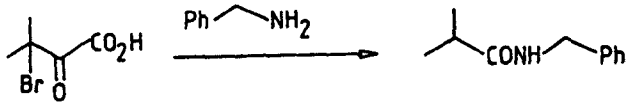
(21)

Scheme 1.3



(22) (23)

Scheme 1.4



(22) (24)

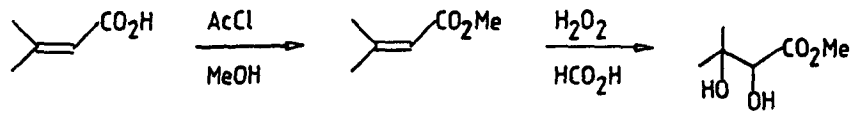
Scheme 1.5

Simliar treatment of 2-hydroxy-3-methylbutanoic acid with N-bromosuccinimide gave, essentially quantitatively, 3-bromo-3-methyl-2-oxobutanoic acid (22) (Scheme 1.4). Attempted coupling of the acid (22) to glycine methyl ester using standard peptide coupling procedures based on dicyclohexylcarbodiimide[58] failed. However, use of the coupling agent 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ)[59] gave the bromo keto amide (23) in 48% isolated yield. Reaction of the acid (22) with benzylamine in dry dichloromethane yielded the benzylamide of 2-methylpropanoic acid (24) (Scheme 1.5). Formation of this compound can be explained by either of the two

mechanisms dicussed in the main introduction for the interaction between 3-bromo-3-methyl-2-oxobutanoic acid and nucleophiles.

Synthesis of methyl 3-hydroxy-3-methyl-2-oxobutanoate

Commercially available 3,3-dimethylacrylic acid was esterified in 61% yield by the action of anhydrous methanol and hydrogen chloride. The latter was generated by addition of acetyl chloride to the methanol prior to reaction with the acid. A similar yield of the ester was obtained by the more conventional procedure of esterification with methanol and a catalytic amount of sulphuric acid.



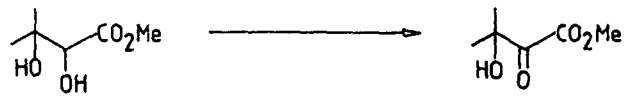
(25)

Scheme 1.6

Dihydroxylation with aqueous hydrogen peroxide and formic acid gave the 2,3-dihydroxy ester (25) in 61% yield after chromatography (Scheme 1.6).

Oxidation of the diol (25) using N-bromosuccinimide in boiling carbon tetrachloride in the presence of calcium carbonate gave only a 30% yield of methyl 3-hydroxy-3-methyl-2-oxobutanoate (26) after chromatography (Scheme 1.7).

Changing the solvent to aqueous dioxane[56] gave only a 42% yield of the ester (26). However, performing the reaction in carbon tetrachloride with illumination and heat from a tungsten lamp raised the isolated yield of (26) to 86%.



(25) (26)

Scheme 1.7

The increase in yield in the presence of light suggests that both ionic and free radical mechanisms occur under these conditions in the non-polar solvent, carbon tetrachloride.

In comparison with the above optimised oxidation procedure, the use of pyridinium chlorochromate[60] gave none of the desired product. Jones oxidation[61] gave a 34% yield of the ester (26). An attempt was also made to apply the procedure dicussed in the main introduction involving brominolysis of the dibutyl dioxostannolan derivatives of 1,2-diols to give hydroxyketones.[5] Reaction of the diol (25) with dibutyl tin oxide in boiling benzene gave the crude dioxostannolan derivative (27) (Scheme 1.8).

(25) (27) crude (7)

Scheme 1.8

Brominolysis of (27) gave the crude 3-hydroxy-2-oxo ester (26). However attempted purification by distillation gave rise to considerable amounts of methyl α-acetolactate (7). The implications of this are explored in chapter 4.

All attempts to manipulate the tertiary hydroxyl group of the 3-hydroxy-2-oxo ester (26) were unsuccessful. Details of the attempted

tosylation, mesylation and bromination are given in the experimental section, chapter 6.

CHAPTER 2

Inhibition of pig liver esterase

Pig liver esterase (EC 3.1.1.1) (PLE) has been shown to be composed of three different subunits.[62] Commercial preparations of PLE contain at least five isozymes, each comprising three subunits. Junge and co-workers[62] have isolated the isozymes by preparative isoelectric focussing. The two major components isolated, each trimeric in one subunit, were found to have markedly different kinetic properties.

One isozyme exhibited cholinesterase activity. It hydrolysed butyryl choline and other typical cholinesterase substrates (medium chain length mono-acyl esters) at a pH optimum between 8 and 9. This isozyme was inhibited by 0.01 M physostigmine and fluoride ion, both typical cholinesterase inhibitors.

The other major isozyme had a pH optimum of 6.5 and acted preferentially on short-chain aliphatic esters but not upon butyryl choline. It was inhibited by organophosphates and phenylmethyl sulphonyl fluoride but not by 0.01 M physostigmine or fluoride ion.

In conducting inhibition studies on PLE isozyme mixtures, the differing nature of the isozymes must be taken into account. It may be possible selectively to inhibit one of the major isozymes using one of the inhibitors mentioned above such that only one type of esterase activity is being examined.

Inhibition studies with methyl 3-bromo-3-methyl-2-oxobutanoate

Inhibition trials of PLE with methyl 3-bromo-3-methyl-2-oxobutanoate (21) were carried out at a constant pH of 7.2 by means of an autotitrator supplying aqueous sodium hydroxide. The ester (21) was incubated with PLE after which ethyl butyrate was added. The activity of the PLE was measured by the initial rate of hydrolysis of ethyl butyrate.

Methyl 3-bromo-3-methyl-2-oxobutanoate (21) was kept as a stock solution in dry methanol. Aliquots were mixed with water at pH 7.2. It was noticed that on dissolution there was a rapid uptake of alkali from the autotitrator, stoichiometric with the methyl 3-bromo-3-methyl-2-oxobutanoate added. In all the incubations, equilibration of the methyl 3-bromo-3-methyl-2-oxobutanoate with the water was allowed before addition of the PLE. Figure 2.1 shows the variation of residual esterase activity with incubation time.

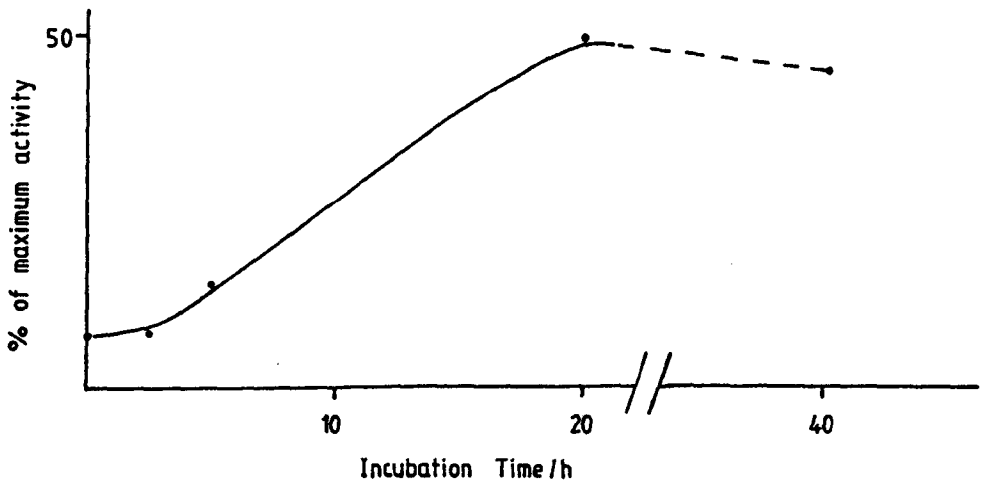
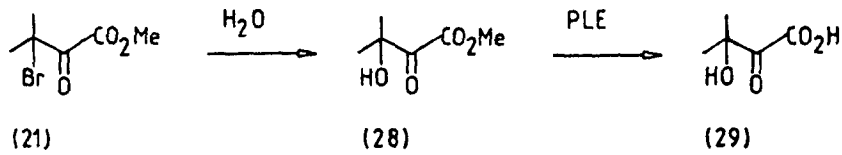


Figure 2.1

At the concentration of methyl 3-bromo-3-methyl-2-oxobutanoate used (0.76 mM) and an incubation time of 1 minute, 7% of the maxinmum

activity was observed. At longer incubation times the esterase activity recovered, reaching 50% of maximum activity after 20h. Incubation of PLE with 1.13 mM sodium bromide gave no measurable inhibition.

The interaction between the methyl ester (21) and water was investigated by proton NMR spectroscopy. Methyl 3-bromo-3-methyl-2-oxobutanoate dissolved in d_4-methanol was added to pH 7.2 phosphate buffer and the proton NMR spectrum was determined. The reaction was complete in less than the time it took to run the spectrum. The new product showed signals attributable to methyl 3-hydroxy-3-methyl-2-oxobutanoate (28) (Scheme 2.1).



(21) (28) (29)

Scheme 2.1

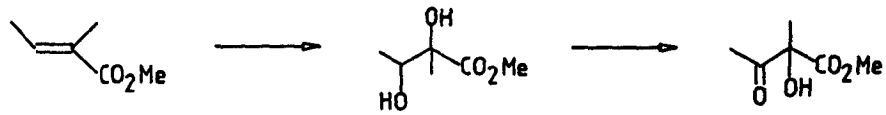
PLE was added to the solution. A slow hydrolysis of the methyl ester was observed by proton NMR.

From these observations the results from the inhibition studies may be rationalised as follows. Dissolution of the methanolic methyl 3-bromo-3-methyl-2-oxobutanoate in water led to loss of bromide ion to give methyl 3-hydroxy-3-methyl-2-oxobutanoate (28). This compound, not bromide ion, inhibited the PLE. The hydroxy compound (28) was itself hydrolysed to the acid (29) and methanol (Scheme 2.1) which together inhibited the PLE to a lesser extent, giving the observed recovery of activity.

Since the methyl 3-bromo-3-methyl-2-oxobutanoate (21) had been completely hydrolysed to methyl 3-hydroxy-3-methyl-2-oxobutanoate (28) before any PLE was added to the reaction, these results do not shed any light on the possible mechanism-based inactivation of PLE by bromo keto esters. There remains the possibility of attempting to synthesise and test chloro keto esters or other substrates in which the bromine has been replaced by a less labile leaving group.

CHAPTER 3

Synthesis of α-acetolactate analogues



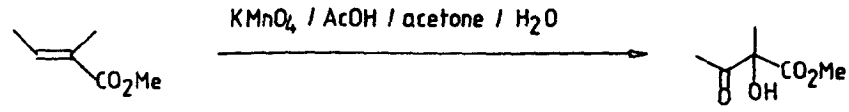
(30) (31) (9)

Scheme 3.1

Methyl α-acetolactate (9) was synthesised by oxidation of the dihydroxy ester (31) derived from methyl tiglate (30) by reaction with hydrogen peroxide-formic acid[63] (Scheme 3.1). Oxidation of the diol (31) with N-bromosuccinimide gave methyl α-acetolactate (9) in an isolated yield of 58%. A better yield of 70% was obtained following the procedure described in chapter 1 whereby the dioxostannolan derivative of the dihydroxy ester was subjected to brominolysis. The yield for the dihydroxylation step was 62%, giving α-acetolactate (9) from methyl tiglate (30) in an overall yield of 43%.

Manganate (VII) oxidation of αβ-unsaturated esters

A synthesis of 2-hydroxy-3-oxo carboxylic esters in one step from the corresponding αβ-unsaturated esters, and in high yield, was developed.



(30) (9)

Scheme 3.2

Following the method of Srinivasan and Lee[22] for the manganate (VII) oxidation of alkenes to α-hydroxyketones, methyl tiglate (30) was treated with potassium manganate (VII) in aqueous acetone in the presence of approximately 2% acetic acid at 25°C (Scheme 3.2). Methyl α-acetolactate (9) was isolated in 50% yield. Performing the reaction at lower temperatures resulted in higher yields (Table 3.1). The highest yield was obtained at -10°C.

Table 3.1

Temperature/°C	Yield/%
50	38
25	50
20	67
-10	80

A range of αβ-unsaturated esters was treated with potassium manganate (VII) and acetic acid in aqueous acetone at -10°C. High yields of the corresponding α-acetolactate ester analogues were obtained in all cases except for the cyclic derivative (33k) where a 62% yield was observed (Table 3.2).

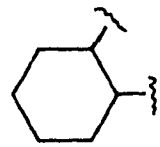
The products were purified by distillation except for the aryl analogues (33h,i,j). The p-nitrophenyl derivative (33i) was a crystalline solid easily purified by recrystallisation. Compounds (33h) and (33j), were viscous oils, which were purified by flash chromatography to avoid the risk of decomposition at the high temperatures required for distillation.

This procedure appears to have general applicability for the

synthesis of acyclic α-acetolactate analogues from αβ-unsaturated esters in high yield, and is far superior to any other method published in the literature to date.

(32) (33)

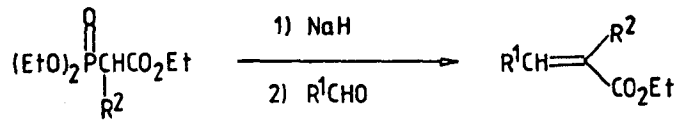
Table 3.2

Entry	R^1	R^2	R^3	Yield/%
33a[13]	Me	Me	Me	80
33b	Me	Et	Et	80
33c	Et	Et	Et	87
33d	Et	Me	Et	82
33e	Pr	Me	Et	70
33f	Bu	Me	Et	82
33g	t-Bu	Me	Et	80
33h	Ph	Me	Et	68 *
33i	p-NO_2Ph	Me	Et	83
33j	p-MeOPh	Me	Et	86
33k[15]			Me	62

* not optimised

Synthesis of $\alpha\beta$-unsaturated esters

Of the $\alpha\beta$-unsaturated esters used as substrates for the manganate (VII) oxidation described above, only methyl tiglate (30) and methyl 1-hydroxy-2-cyclopentanone carboxylate (32k) were available commercially. The others were synthesised by coupling aldehydes with the appropriate 2-alkyl-substituted phosphonate via the phosphonate modification[64] to the Wittig reaction (Scheme 3.3).



(34)

Scheme 3.3

Table 3.3

Entry	R^1	R^2	Yield/%	E-isomer/%
34a[65]	Me	Et	53	24
34b[66]	Et	Et	72	54
34c[67]	Et	Me	62	87
34d[65]	Pr	Me	68	86
34e[68]	Bu	Me	64	87
34f[69]	t-Bu	Me	71	54
34g	Ph	Me	81	100
34h	p-NO_2Ph	Me	38	100
34i	p-MeOPh	Me	67	100

It was found neccessary to use a slight excess of the phosphonate over the sodium hydride to ensure complete reaction of the latter. If sodium hydride was present during addition of the aldehyde to the

deprotonated phosphonate then the ensuing product mixtures contained appreciable amounts of $\beta\gamma$-unsaturated esters in addition to the desired $\alpha\beta$-unsaturated esters.

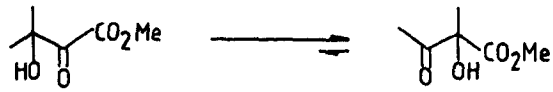
The aryl substituted esters (34g,h,i) gave exclusively the E-isomer. Isomer mixtures were obtained in the other cases. All of the esters were isolated as oils by distillation except for (34h) which is a crystalline solid. Appreciable amounts of the product (34h) co-crystallysed with unreacted p-nitrobenzaldehyde. The quoted yield of 38% refers to the isolated material only.

CHAPTER 4

The tertiary ketol rearrangement of 3-hydroxy-2-oxo esters

Scheme 4.1

The tertiary ketol rearrangement of α-hydroxyketones (Scheme 4.1) has been shown to be catalysed by heat,[70] hot metal surfaces,[71] light (via photoenolisation),[72] acids, bases and Lewis acids.



(26) (9)

Scheme 4.2

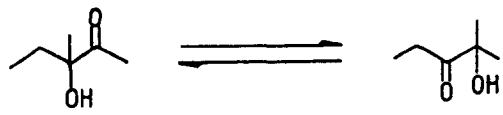
The tertiary ketol rearrangement of 3-hydroxy-2-oxo esters has not been described in the literature (except as a result of this work).[73] It was noted in chapter 1 that distillation of crude methyl 3-hydroxy-2-methyl-2-oxobutanoate (26) in the presence of organo-tin residues resulted in rearrangement to its isomer methyl α-acetolactate (9) (Scheme 4.2). The other methods described in chapter 1 to produce the hydroxy keto ester (26) involved distillation as the final purification step. In none of these was any rearrangement of (26) to methyl α-acetolactate (9) observed.

Compound (26) when treated with 5 mol% of dibutyl tin oxide in boiling toluene was observed, by GLC, to be converted quantitatively into methyl α-acetolactate (9) which was isolated in 81% yield.

Exposing methyl α-acetolactate to the same conditions resulted in no observable change and to re-isolation of (9).

Treatment of the ester (26) with a representative selection of Lewis acid catalysts under conditions known to effect the simple tertiary ketol rearrangement gave no rearrangement to methyl α-acetolactate. The use of aluminium trichloride-acetic anhydride-acetic acid,[74] aluminium t-butoxide[75] and alumina[75] failed to generate methyl α-acetolactate. Treatment of (26) with boron trifluoride etherate-acetic anhydride-acetic acid[76] led to acetylation of the tertiary hydroxyl group, to give the diester in 58% isolated yield, with no rearrangement. Methods involving aqueous acids and alkalis[77,78] are not applicable in this case since ester hydrolysis would occur with concomitant decarboxylation.

The simple ketol (35), when treated with dibutyl tin oxide in boiling toluene, was converted into a 61:39 mixture of the starting compound and its isomer (36) (Scheme 4.3).



(35) (36)

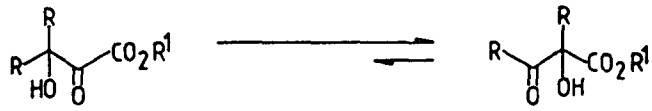
Scheme 4.3

This result shows that the carboxylic ester function is not essential to the rearrangement and that dibutyl tin oxide catalyses the simple tertiary ketol rearrangement, under essentially neutral conditions.

In order to explore the scope of this dibutyl tin oxide-catalysed rearrangement, a series of 3-hydroxy-2-oxo esters was prepared. Details of their synthesis is given at the end of this chapter.

Isomerisation of symmetrical substrates

Esters (37b) and (37c), higher homologues of the hydroxy keto ester (26), rearranged upon treatment with catalytic dibutyl tin oxide in boiling toluene quantitatively to give the α-acetolactate analogues in high isolated yield (Table 4.1). The diphenyl example (37d) gave an approximately 1:1 equilibrium mixture of starting material and its isomer (38d), from which the latter was isolated by chromatography in 38% yield.

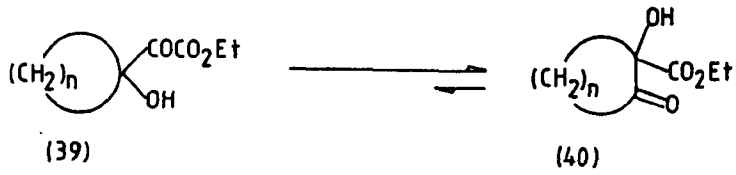


(37) (38)

Table 4.1

Entry	R	R^1	Yield/%
38a	Me	Me	81
38b	Pr	Et	80
38c	Bu	Et	88
38d	Ph	Et	38

Isomerisation of the cyclic substrates (39a,b,c) furnished the ring-expanded α-acetolactate analogues (40a,b,c) in high isolated yield (Table 4.2).



(39) (40)

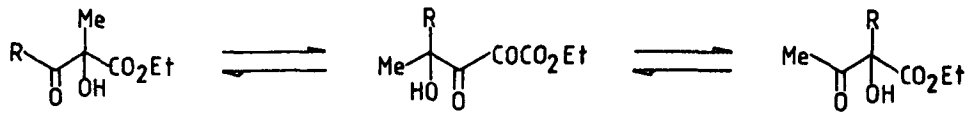
Table 4.2

Entry	n	Yield/%
40a	4	90
40b	5	79
40c	6	90

Formation of the six- and eight-membered ring products was quantitative. However, for the six- to seven-membered isomeration (39b) an equilibrium mixture of 97:3 was obtained in favour of the ring-expanded product.

Isomerisation of non-symmetrical substrates

The reversible nature of the rearrangement was demonstrated by exposing 2-hydroxy-3-oxo esters, with different alkyl substituents at the 2- and 3-positions, to the rearrangement conditions. In each case one of the alkyl substituents was methyl (Scheme 4.4).



(41) (42) (43)

Scheme 4.4

In all cases an equilibrium mixture of the two isomeric 2-hydroxy-3-oxo esters was obtained (Table 4.3). The proton NMR spectrum

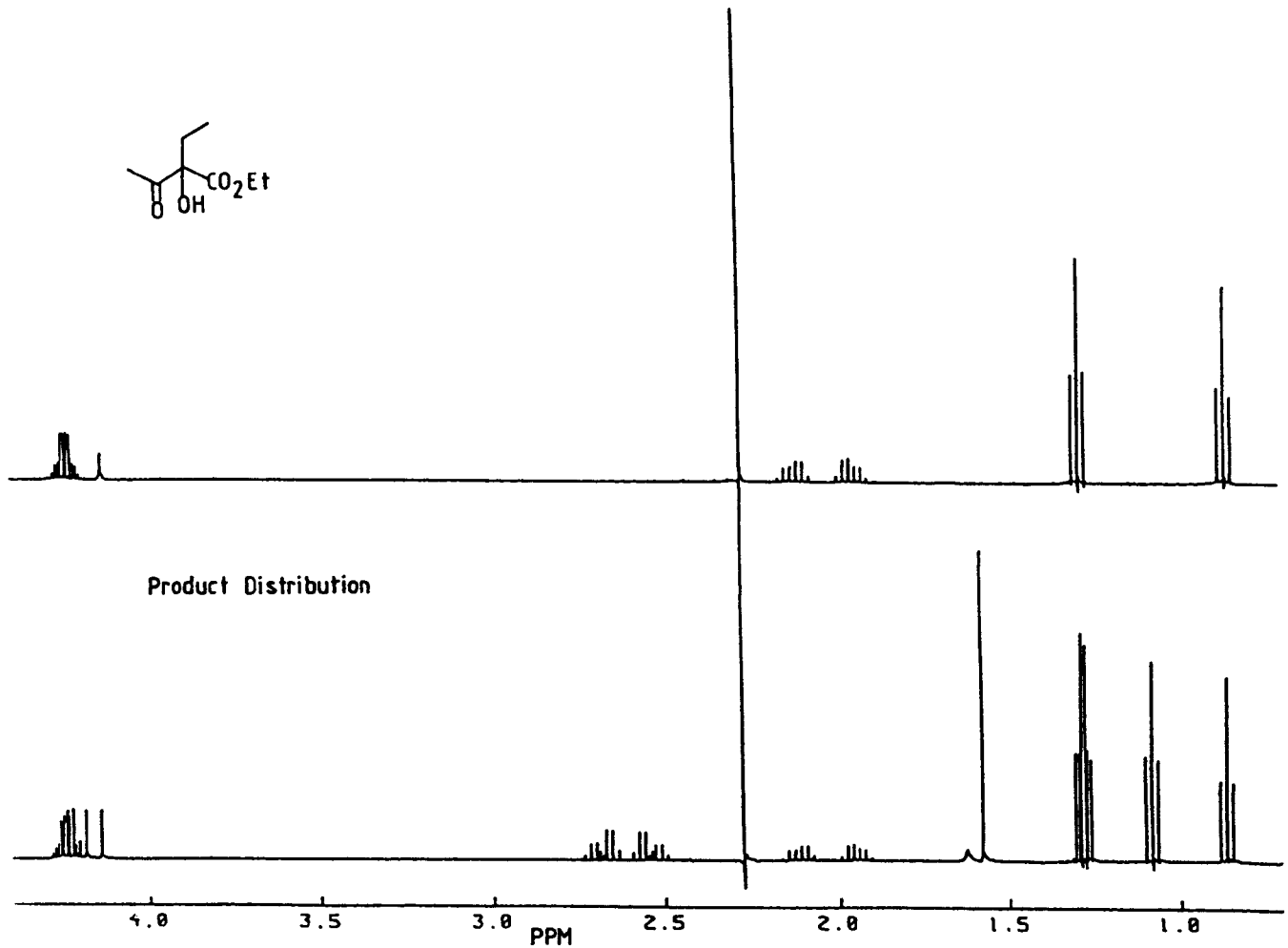


Figure 4.1

of the product mixture from the isomerisation of ethyl 2-ethyl-2-hydroxy-3-oxobutanoate (42a) (Figure 4.1) indicated that none of the 3-hydroxy-2-oxo ester was formed. The equilibrium mixtures for the isomeric pairs (41d, 43d) and (41g, 43g) were obtained by isomerisation of the corresponding 3-hydroxy-2-oxo esters.

Table 4.3

Entry	R	Ratio (41)/(43)
42a	Et	1.0
42b	Pr	2.0
42c	Bu	2.5
42d	i-Pr	0.9
42e	t-Bu	2.5
42f	p-NO_2Ph	1.2
42g	Ph	2.7
42h	p-MeOPh	6.6

On the basis of steric hindrance, the thermodynamic product distributions would be expected to favour the isomers with a substituent larger than methyl at the 3-position adjacent to the carbonyl group rather than at the more crowded 2-position. This is the general trend observed with the exception of the i-Pr example (42d).

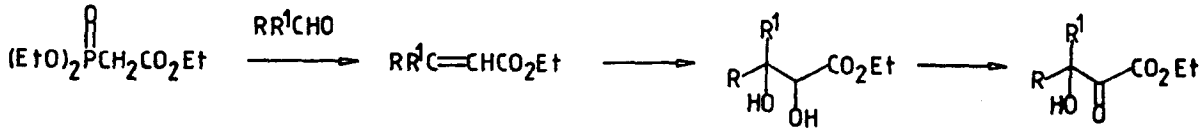
The observed product ratios correspond to small energy differences between the isomers, the most extreme case being only 350 $Jmol^{-1}$ at 298 K.

All the isomerisations were carried out in toluene, a solvent in which polar interactions should be minimal.

The components of the product mixtures could not be separated satisfactorily on TLC. However, resolution of the p-nitrophenyl example (42f) was achieved by HPLC on a C_{18} reverse phase column with methanol-water as eluant. In principle, each isomeric 2-hydroxy-3-oxo ester could be separated by preparative HPLC and the re-isolated starting material recycled. This gives access to the α-acetolactate analogues with bulky groups at the 2-position. To synthesise the αβ-unsaturated ester precursors required for the manganate (VII) reaction would be difficult. Preparation of the 2-substituted phosphonate would require harsh conditions and might prove to be difficult or impossible.

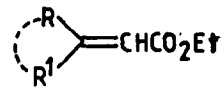
Thus for synthesis of α-acetolactate analogues with bulky groups at the 2-position, preparation of the less sterically hindered isomer, isomerisation to the product mixture and isolation of the rearranged product by preparative HPLC may be practical for the preparation of small quantities of such compounds.

Synthesis of 3-hydroxy-2-oxo carboxylic esters



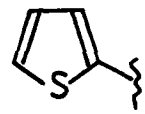
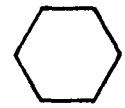
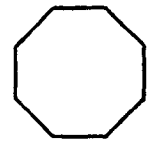
Scheme 4.5

Coupling of the appropriate ketones and ethyl bromoethanoate via the phosphonate modification to the Wittig reaction[64] gave the corresponding αβ-unsaturated ester series (Table 4.4).



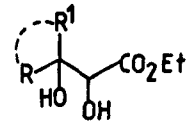
(45)

Table 4.4

Entry	R	R'	Yield/%	E-isomer/%
45a[107]	Pr	Pr	64	
45b	Bu	Bu	66	
45c[108]	Ph	Ph	70	
45d[109]	i-Pr	Me	70	85
45e[110]	Ph	Me	75	87
45f[111]		Me	51	85
45g[112]			70	
45h[81]			66	
45i[64]			76	
45j[81]			74	
45k[81]			74	

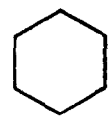
Di-hydroxylation using hydrogen peroxide-formic acid[63] (Table 4.5) and subsequent N-bromosuccinimide oxidation furnished the desired 3-hydroxy-2-oxo esters (47) (Table 4.6). Compounds (44a) and (44b) were prepared from the $\alpha\beta$-unsaturated esters without isolation of the dihydroxy ester (Table 4.7).

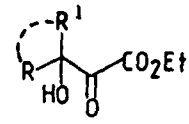
It was not found possible to carry the $\alpha\beta$-unsaturated esters (45f), (45g) and (45k) through the synthesis. All attempts to oxygenate the alkene double bond failed. Attempts using hydrogen peroxide-formic acid, osmium tetroxide[79,80] and *via* epoxidation with m-chloroperoxybenzoic acid were all unsuccessful.



(46)

Table 4.5

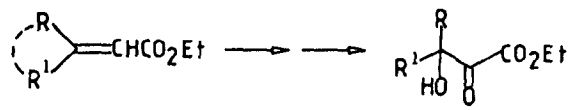
Entry	R	R'	Yield/%
46a	Pr	Pr	40
46b	Bu	Bu	30
46c	i-Pr	Me	30
46d	Ph	Me	46
46e			79
46f			39



(47)

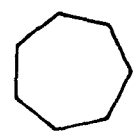
Table 4.6

Entry	R	R^1	Yield/%
47a	Pr	Pr	79
47b	Bu	Bu	93
47c	i-Pr	Me	19
47d	Ph	Me	78
47e			60
47f			51



(44)

Table 4.7

Entry	R	R^1	Yield/%
44a	Ph	Ph	30
44b			35

CHAPTER 5

Biotransformations with acetolactate decarboxylase

The properties of the enzyme used

The acetolactate decarboxylase (ADC) used in these studies was donated by Novo Industri A/S. It was used in crude form isolated from selected strains of Bacillus brevis.

Data for the purified enzyme have been obtained and information pertinent to this study is given below.

The temperature activity profile is shown in Figure 5.1. Under the assay conditions used the pure enzyme was stable up to 40°C. At high temperatures (>50°C) there is appreciable non-enzymatic decarboxylation of substrate.

The pH activity profile is shown in Figure 5.2. The most active range is between pH 5.5 and 6.5. However, 50% of maximum activity may be obtained at pH extremes of 4.3 and 7.2.

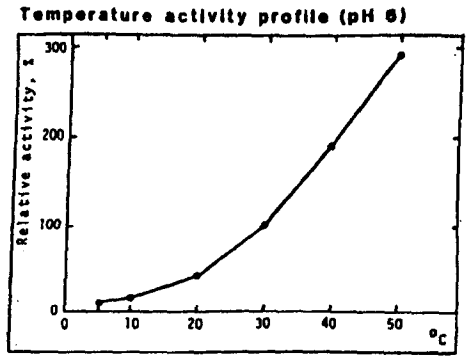


Figure 5.1

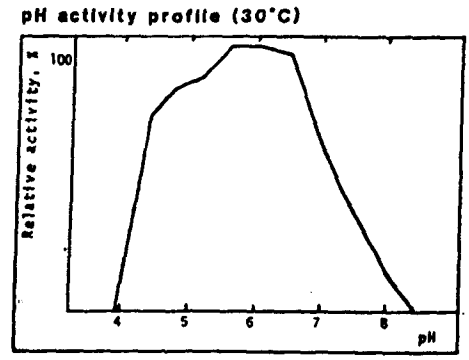
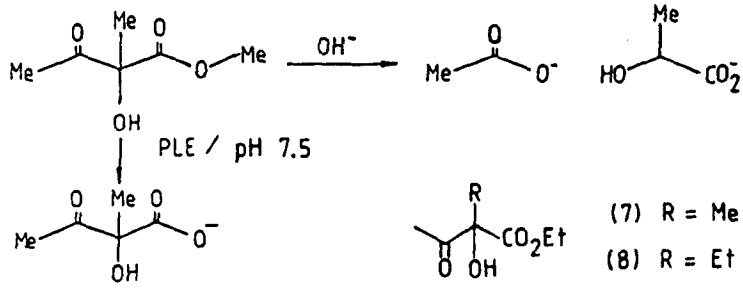


Figure 5.2

Acetolactate decarboxylase-catalysed decarboxylation of natural substrates

α-Acetohydroxybutyrate

α-Acetolactate (7) and α-acetohydroxybutyrate (8) were synthesised as their methyl and ethyl esters respectively by the method outlined in chapter 3. It was neccessary to generate the corresponding acids for incubation with the enzyme acetolactate decarboxylase (ADC). Hydrolysis with aqueous alkali carries with it the risk of β-keto ester cleavage (Scheme 5.1).



Scheme 5.1

However, it was found that hydrolysis of α-acetolactate analogues catalysed by pig liver esterase (PLE) was possible at pH 7.5 (the optimum pH for this enzyme[82]). Hydrolysis of an ester gives an alcohol and an acid with a consequent lowering of pH. Hence the progress of the reaction can be followed by titration with alkali, the amount of alkali added to maintain a constant given pH being proportional to the extent of reaction.

Ethyl α-acetohydroxybutyrate (33b) was hydrolysed at pH 7.5 using PLE. The progress of the reaction was monitored by the uptake of aqueous sodium hydroxide delivered by an autotitrator (Figure 5.3.a).

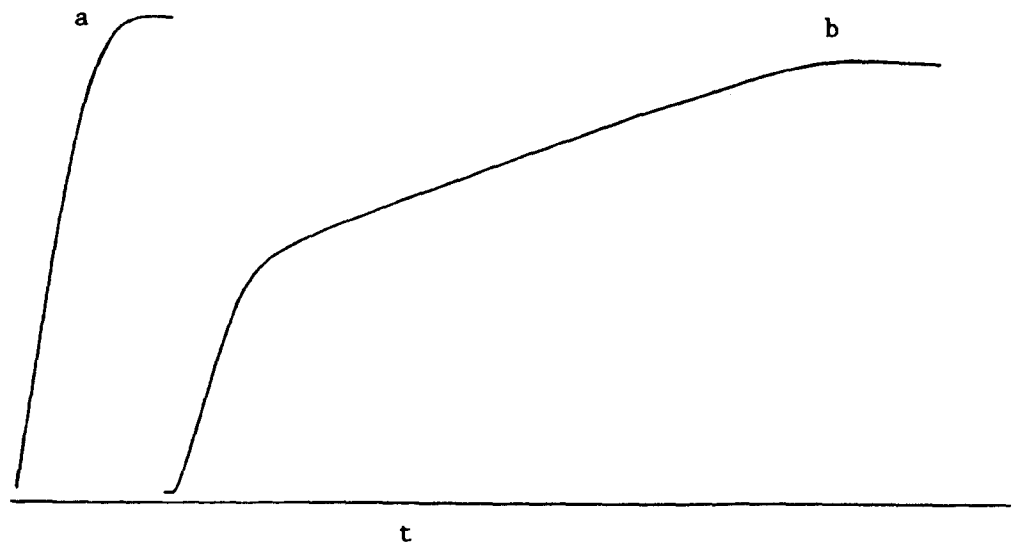


Figure 5.3

After complete hydrolysis the reaction mixture was acidified to pH 6.5. Decarboxylation of an acid gives rise to an increase in pH by loss of the weak acid, carbon dioxide, from the system. Progress of the decarboxylation can be monitored at constant pH by titration with acid.

ADC was added to the reaction mixture and the decarboxylation followed by uptake of hydrochloric acid delivered at pH 6.5 by an autotitrator (Figure 5.3.b).

The autotitrator trace for the decarboxylation reaction (Figure 5.3.b) showed two distinct rates; a faster reaction up to 50% completion and a slower reaction thereafter, suggesting that one enantiomer of the acid was being decarboxylated at a higher rate than the other.

The hydrolysis and decarboxylation was also performed in phosphate buffer at pH 7.2 and monitored by proton NMR spectroscopy on a continuous wave spectrometer at 220 MHz. The PLE-catalysed

hydrolysis step was observed to generate ethanol and small changes in chemical shift were observed between the ester and the acid.

The ADC-catalysed decarboxylation step gave a rapid development of signals attributable to 3-hydroxypentan-2-one (48), (Figure 5.4.b), up to 50% reaction, and a slower consecutive development of signals attributable to the isomeric hydroxy ketone, 2-hydroxypentan-3-one (49), (Figure 5.4.c,d). The ethanol generated in the hydrolysis step was used as an internal standard to evaluate the quantities of acid and decarboxylated products present during the decarboxylation reaction. These data are displayed in the progress curves of Figure 5.5.

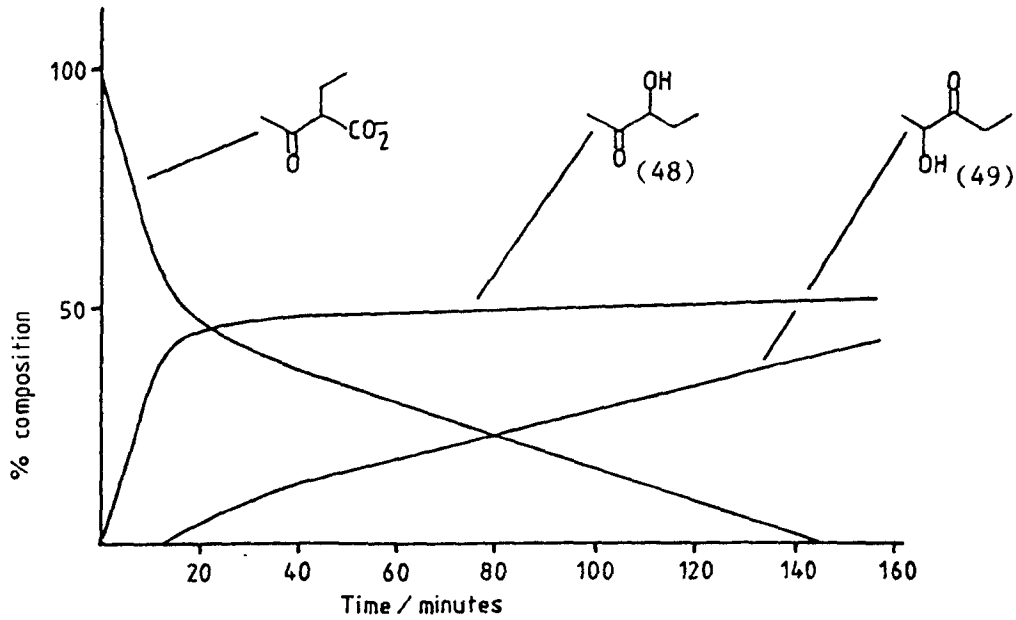
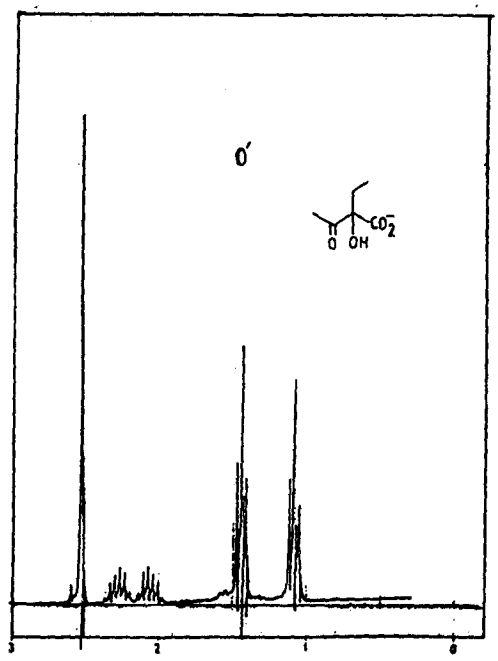


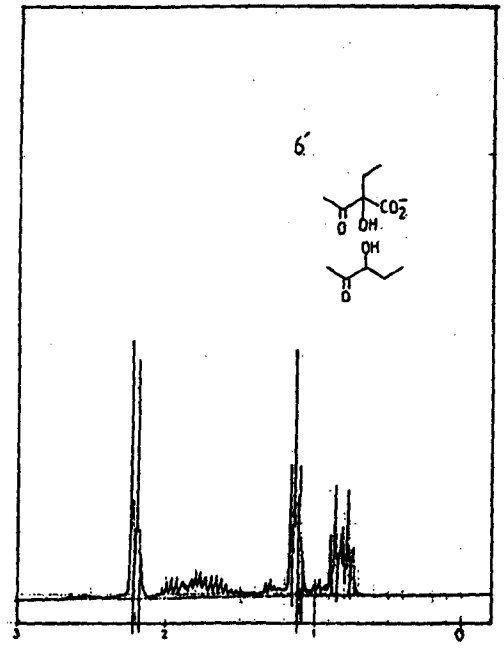
Figure 5.5

The notable feature of this experiment is that the generation of the two α-hydroxyketones was sequential. Production of ketol (48) had virtually ceased before any ketol (49) was observed.

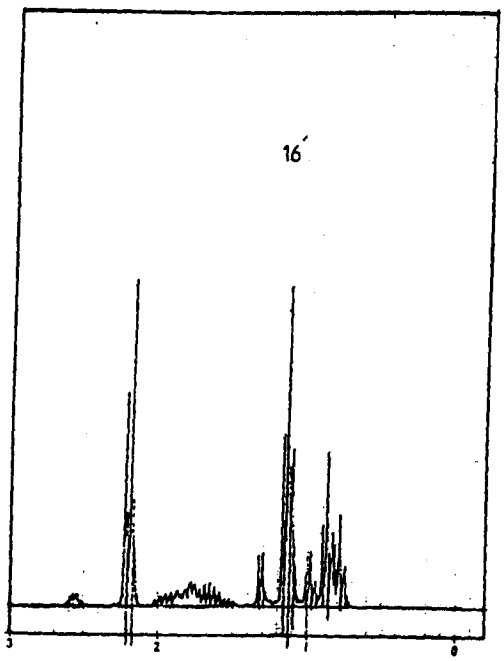
In a similar experiment, racemic ethyl α-acetohydroxybutyrate



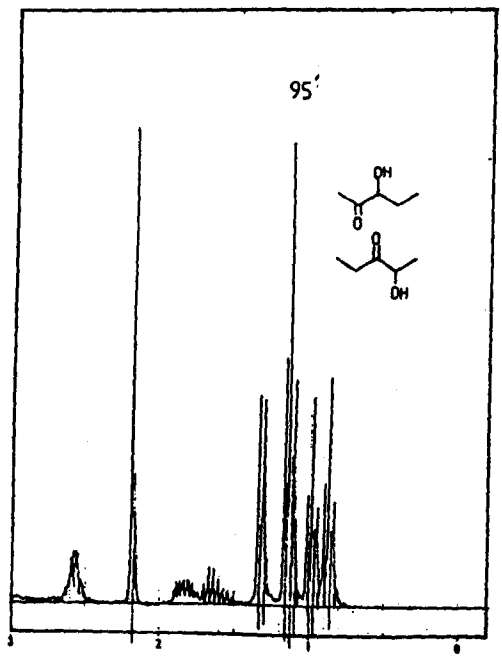
(a)



(b)



(c)



(d)

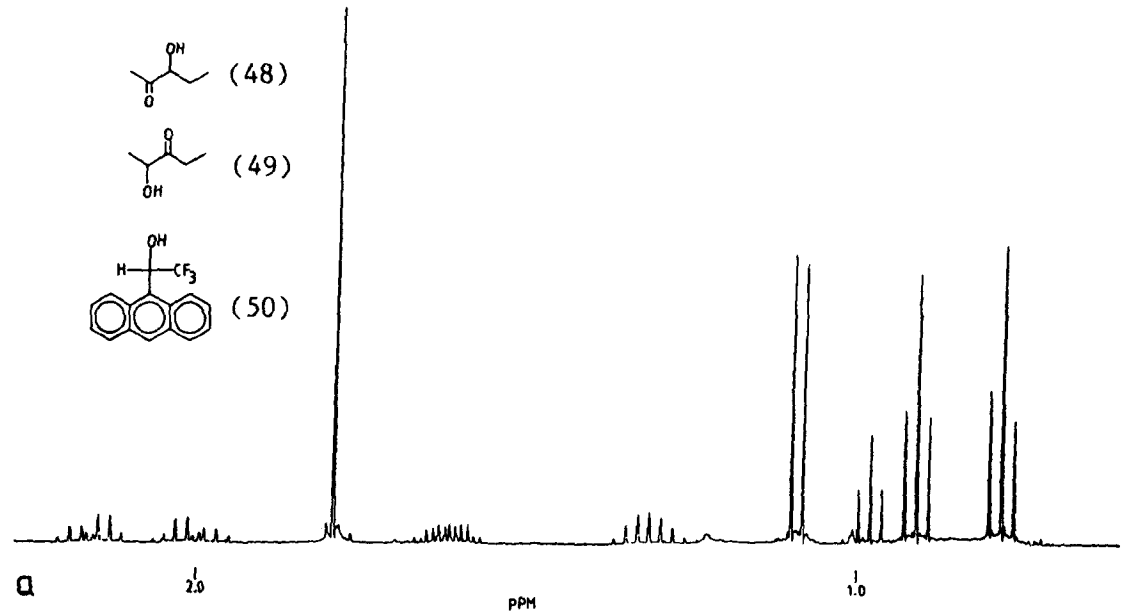
Figure 5.4

(33b) was treated with PLE and ADC consecutively at pH 7.2 in phosphate buffer. The hydroxyketone products were extracted into carbon tetrachloride and the proton NMR spectrum examined in the presence of a two-fold excess of the chiral solvating agent (S)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol (50)[83] (Figure 5.6.a). The spectrum was re-recorded in the presence of added racemic hydroxy ketones (Figure 5.6.b). The latter spectrum shows a splitting of the signals due to the enantiomer pairs of ketols (48) and (49) in rapid reversible equilibrium with the chiral solvating agent (50). The diagnostic peaks are the acetyl methyl of (48) at 0.8 ppm and the methyl doublet of (49) at 1.1 ppm. Comparison of the peak heights of the split signals revealed the optical purities of (48) and (49) to be 93% ee and >95% ee respectively.

Hill and co-workers[13] have suggested that the slow decarboxylations of the (R)-enantiomers of α-acetohydroxybutyrate (8) and α-acetolactate were caused by racemisation of the substrates to give the preferred (S)-isomers. However, the known, racemising, base-catalysed tertiary ketol rearrangement of α-acetohydroxy acids, which occurs by migration of the carboxylate group is unlikely here since it proceeds in vitro at a significant rate only above pH 12.9.[13,37]

That both α-hydroxyketones were generated in high optical purity implies that both the decarboxylation and the rearrangement were under enzymatic control. A rationalisation of the results is given in Scheme 5.2.

Here it is proposed that the (S)-isomer of α-acetohydroxybutyrate is decarboxylated with inversion of configuration to give (R)-3-hydroxypentan-2-one (48) rapidly. Furthermore, the (R)-isomer of



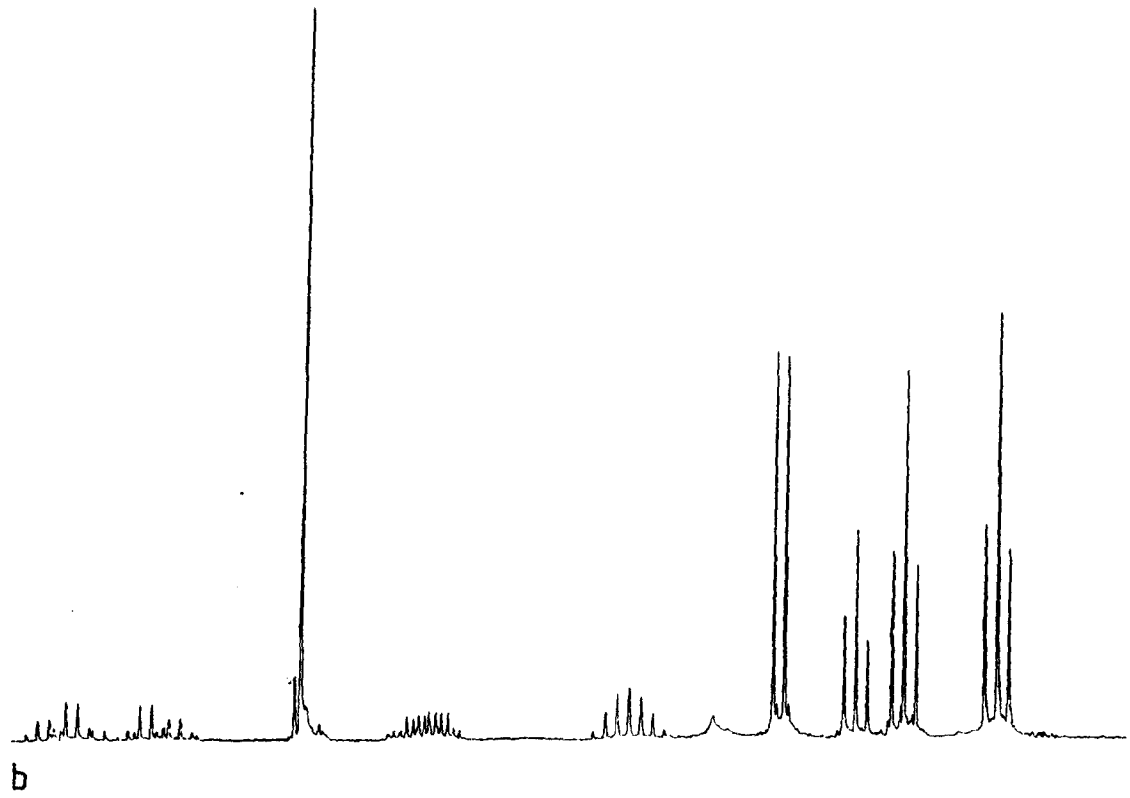
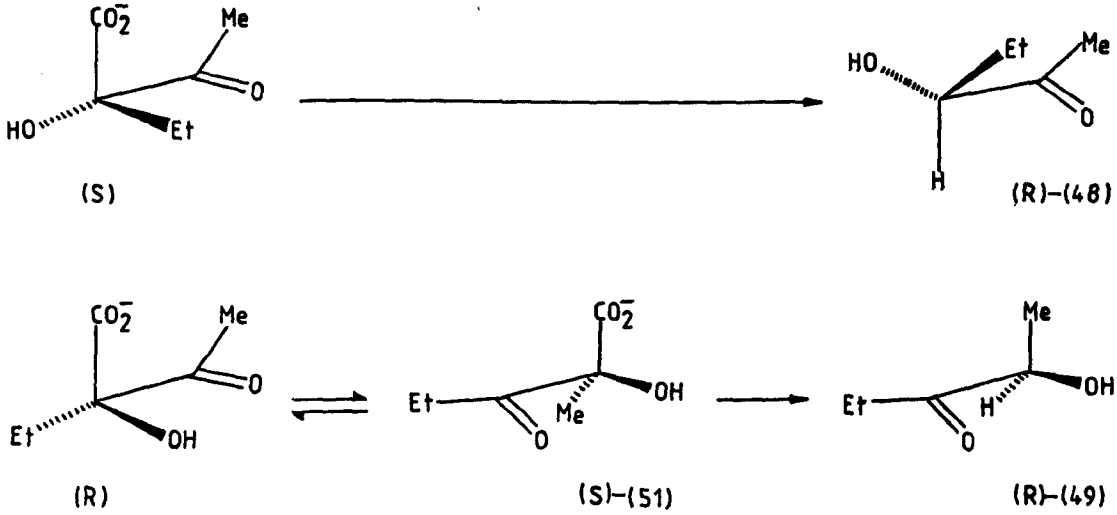


Figure 5.6

α-acetohydroxybutyrate is rearranged by the enzyme to (S)-2-hydroxy-2-methyl-3-oxopentanoate (51) and then decarboxylated with inversion of configuration to give (R)-2-hydroxypentan-3-one (49).



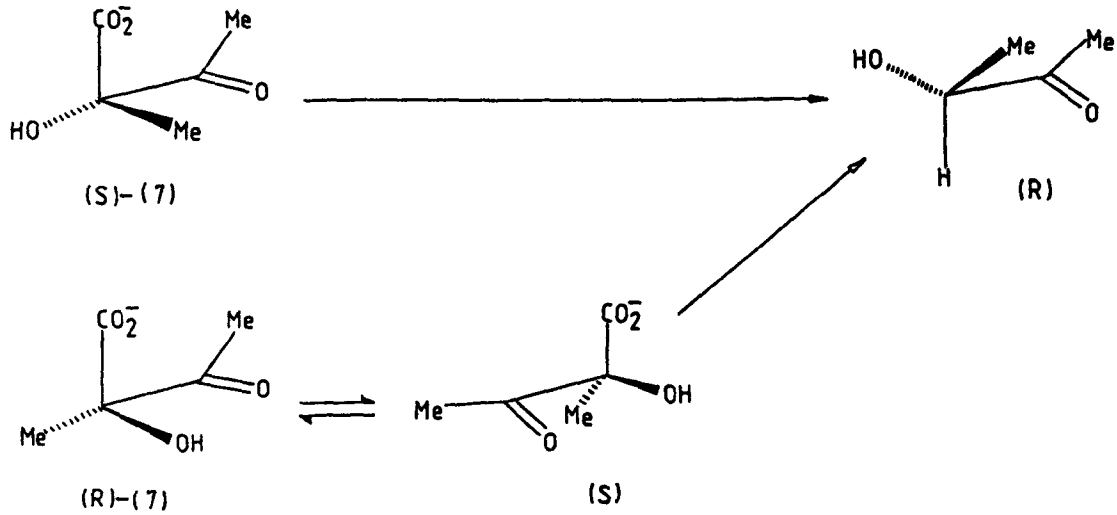
Scheme 5.2

To rearrange (R)-α-acetohydroxybutyrate (8) to (S)-2-hydroxy-2-methyl-3-oxopentanoate (51) with intramolecular migration of the carboxylate group requires a syn arrangement of the carbonyl oxygen atom and the hydroxyl oxygen atom. An anti arrangement would result in (R)-2-hydroxy-2-methyl-3-oxopentanoate.

The sequential production of the α-hydroxyketones (48) and (49) can be explained by making the assumption that (S)-α-acetohydroxy butyrate is much more tightly bound to the enzyme than (R)-α-aceto hydroxybutyrate and thus acts as a competitive inhibitor for the conversion of (R)-α-acetohydroxybutyrate to (R)-2-hydroxypentan-3-one.

α-Acetolactate

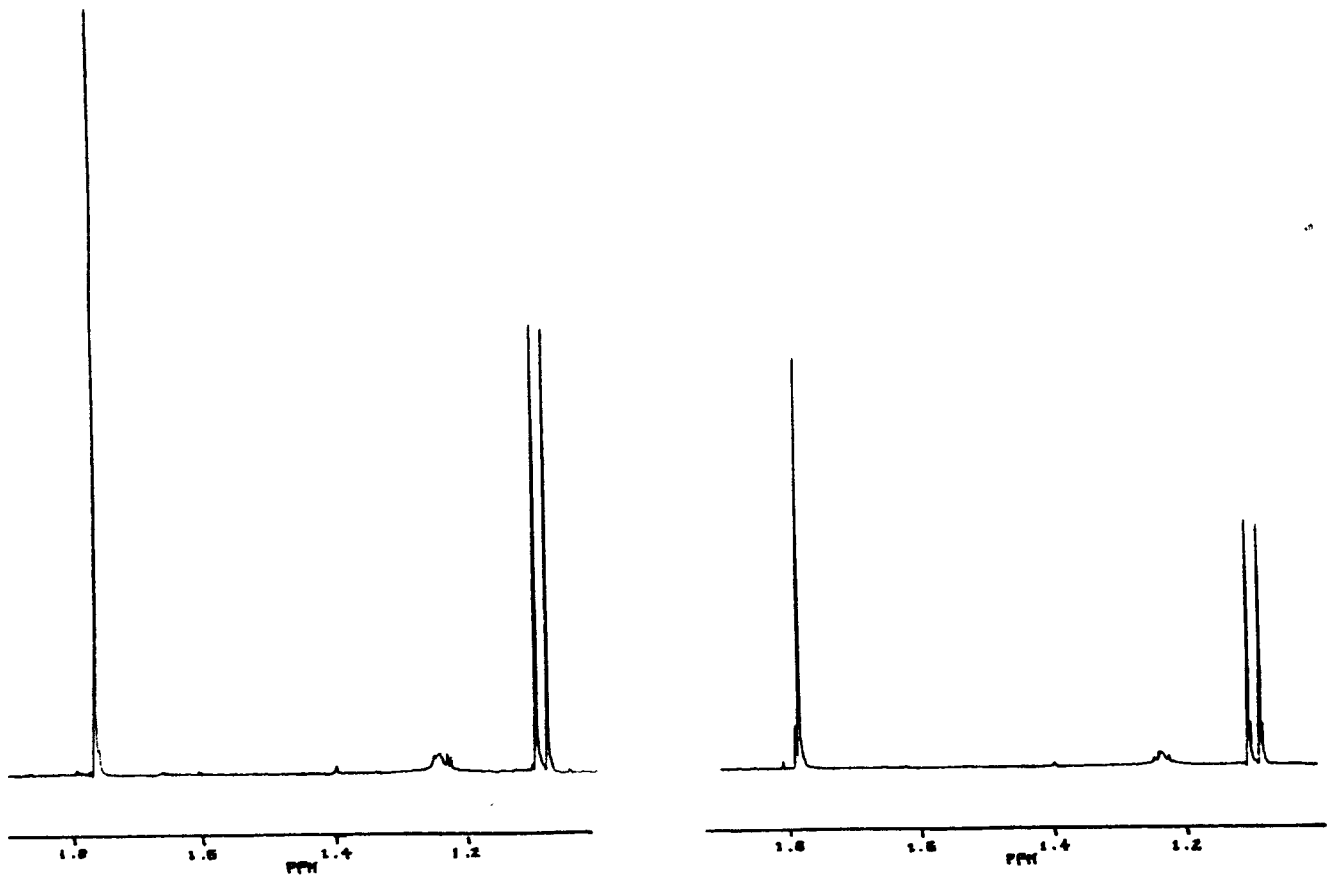
On the basis of the interpretation of the ADC-catalysed decarboxylation of α-acetohydroxybutyrate (8) it was predicted that complete decarboxylation of racemic α-acetolactate (7) should lead exclusively to (R)-(-)-acetoin, the (R)-isomer of α-acetolactate being converted into (R)-acetoin via prior rearrangement into (S)-α-acetolactate (Scheme 5.3).



Scheme 5.3

Sequential hydrolysis of racemic methyl α-acetolactate (7) with PLE and complete decarboxylation with ADC generated (R)-(-)-acetoin. NMR analysis as before indicated an ee of 92%.

The experiment was repeated but using the two enzymes simultaneously, not sequentially. Consequently, non-enzymatic decarboxylation of α-acetolactate was minimised. Only one enantiomer of the resulting acetoin could be detected by the NMR analysis indicating an ee of >98% (Figure 5.7)



a

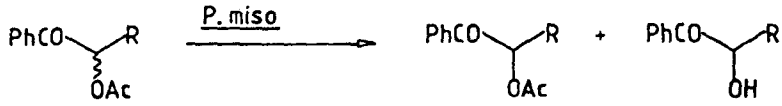
b (added racemate)

Figure 5.7

To confirm that (R)-(-)-acetoin was the product of the decarboxylation of α-acetolactate, the experiment was repeated inside a polarimeter cell. Comparison of the specific rotation obtained with that reported[84] for optically pure (R)-(-)-acetoin indicated that the product was indeed (R)-(-)-acetoin of 100% optical purity within experimental error.

The 100% conversion of racemic substrate into a single enantiomer of product by enzyme action alone is unknown in the literature except as a result of this work.[85] Sih[86] has published work in which a carefully chosen substrate was processed by a hydrolytic enzyme under pH conditions which caused racemisation of the starting material. That amounted to an in situ recycling of the "wrong" isomer to the prefered substrate in order for the enzyme to process the starting material completely.

Otha and co-workers[87] have investigated the production of optically pure α-hydroxyketones by enzymatic hydrolysis of the acetylated precursors (52) (Scheme 5.4).

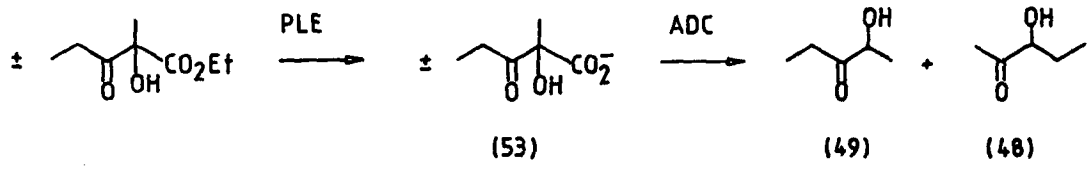


Scheme 5.4

Incubation of a range of α-acetoxyacylophenones (52) with the yeast Pichia miso IAM 4682 gave 50% de-acylation of starting material, both product and recovered starting material having high enantiomeric excesses. However, all of the examples quoted contained the PhCO fragment. No simple alkyl hydroxyketones were examined.

ADC-catalysed decarboxylation of unnatural substrates:

2-Hydroxy-2-methyl-3-oxopentanoate



Scheme 5.5

The PLE-catalysed hydrolysis of racemic ethyl 2-hydroxy-2-methyl-3-oxopentanoate showed apparent enantioselectivity, as indicated by the progress curve in Figure 5.8. After 50% hydrolysis there was a six-fold decrease in the rate of reaction.

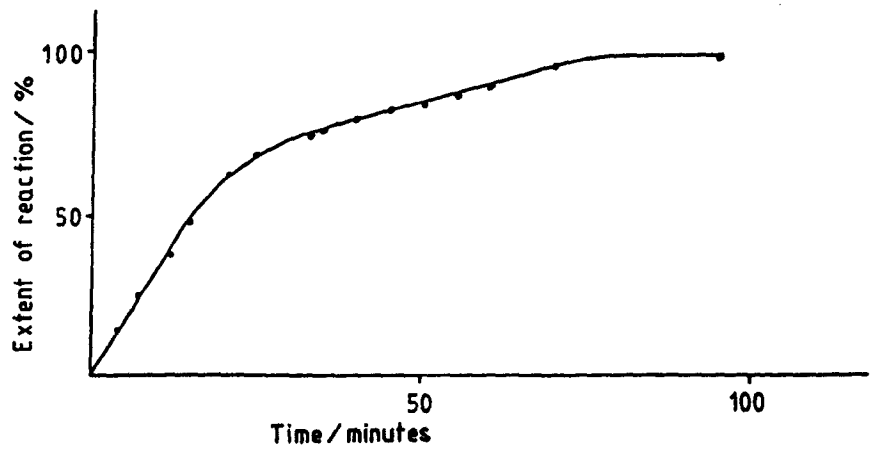


Figure 5.8

The completely hydrolysed substrate was treated with ADC in 1 M phosphate buffer at pH 7.2 and the decarboxylation followed by proton NMR. The production of signals corresponding to 2-hydroxypentan-3-one (49) was observed, followed by the slower growth of signals attributable to the isomeric α-hydroxyketone, 3-hydroxypentan-2-one (48) (Scheme 5.5). Overall, the same product distribution was obtained as that for the ADC-catalysed decarboxylation of the isomeric

substrate α-acetohydroxybutyrate (8).

The decarboxylation was also performed at pH 7.7 in 1 M phosphate buffer. The results were the same as above except that an unidentified compound was observed which disappeared during the course of the reaction as shown by the progress curves below (Figure 5.9).

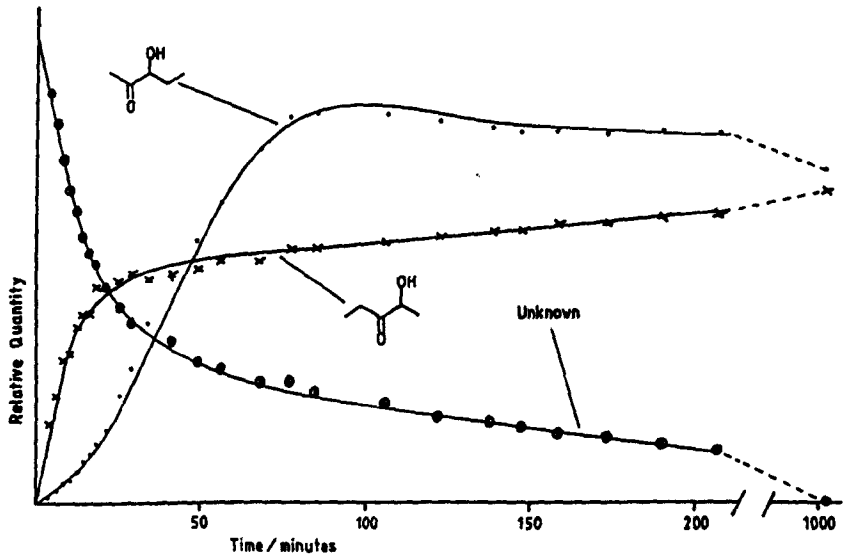
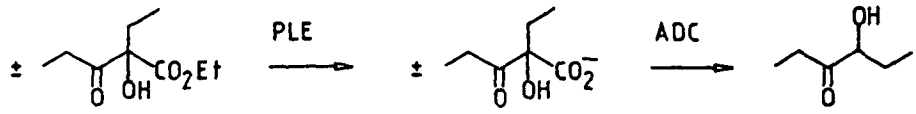


Figure 5.9

Its proton NMR spectrum was qualitatively similar to that of the acid (53) but shifted to lower field by about 0.1 ppm. Signals from both the acid (53) and the unidentified compound were observed together in the same spectra. This ruled out the possibility of rapidly reversible eqilibria such as protonated/non-protonated acid where a single, time-averaged set of signals would be seen. The sheer quantity of this unidentified compound also ruled ot the possibility of it being due to an enzyme-substrate complex. In relative molar terms the amount of ADC present with respect to substrate was vanishingly small.

2-Ethyl-2-hydroxy-3-oxopentanoate

Complete decarboxylation of racemic 2-ethyl-2-hydroxy-3-oxopentanoate with ADC gave 4-hydroxyhexan-3-one (54) (Scheme 5.6).



(55) (54)

Scheme 5.6

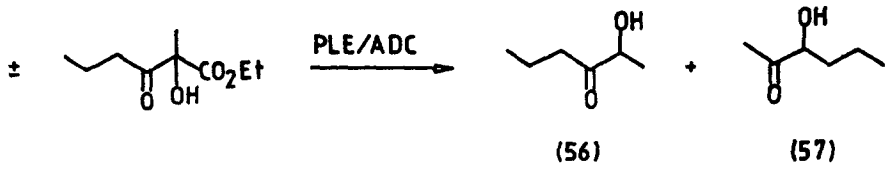
NMR analysis of the optical purity of the hydroxyketone (54) was not possible because of the complex splitting patterns of the signals in the proton NMR spectrum. It was attempted, by selective decoupling, to generate singlet resonances suitable for the observation of diastereomeric splitting on complexation with the chiral solvating agent (50). However, due to artifacts generated by the decoupling process, assignment of the signals from the minor isomer was not possible.

The decarboxylation of racemic 2-ethyl-2-hydroxy-3-oxopentanoate (55) was followed by polarimetry. A specific rotation of -76^{o} (c=0.48, 0.05 M phosphate buffer, pH 7.2, 289 nm, $26^{o}C$) was obtained for the α-hydroxyketone product (54). The specific rotation of optically pure 4-hydroxyhexan-3-one (54) is not known in the literature and so no estimate of optical purity could be made by comparison with such a value. However, the specific rotation obtained for the homologue, (R)-(-)-acetoin, by hydrolysis and decarboxylation of racemic methyl α-acetolactate conducted inside a polarimeter cell gave a value consistent with that obtained from chemically synthesised optically pure (R)-(-)-acetoin, within experimental error, as detailed on page 68. Thus the specific rotation obtained for 4-hydroxyhexan-3-one (54)

obtained by this method may be assumed to represent optically pure material.

2-Hydroxy-2-methyl-3-oxohexanoate

Incubation of racemic ethyl 2-hydroxy-2-methyl-3-oxohexanoate with PLE and ADC gave the two isomeric hydroxyketones (56) and (57). NMR analysis indicated ees of >90% and 92% respectively (Scheme 5.7).



(56) (57)

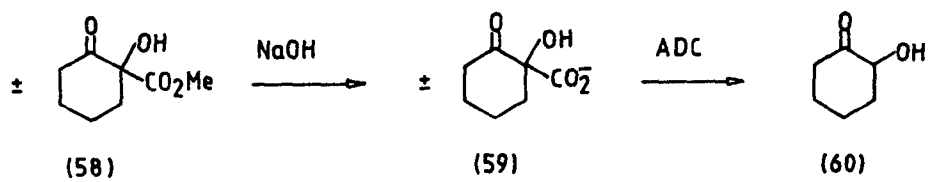
Scheme 5.7

2-Hydroxy-3-oxo-2,4,4-trimethylpentanoate

Ethyl 2-hydroxy-3-oxo-2,4,4-trimethylpentanoate proved not to be a substrate for PLE. Brief exposure to 3 M sodium hydroxide solution liberated the carboxylate ion. Treatment with ADC at pH 6.5 resulted in no change over a 2 h period. This tertiary-butyl α-acetolactate analogue was not a substrate for ADC.

1-Hydroxycyclopentan-2-one carboxylate

Methyl 1-hydroxycyclopentan-2-one carboxylate (58) was not a substrate for PLE. Hydrolysis to the acid (59) was achieved by brief exposure to 3 M potassium hydroxide solution (Scheme 5.8).



(58) (59) (60)

Scheme 5.8

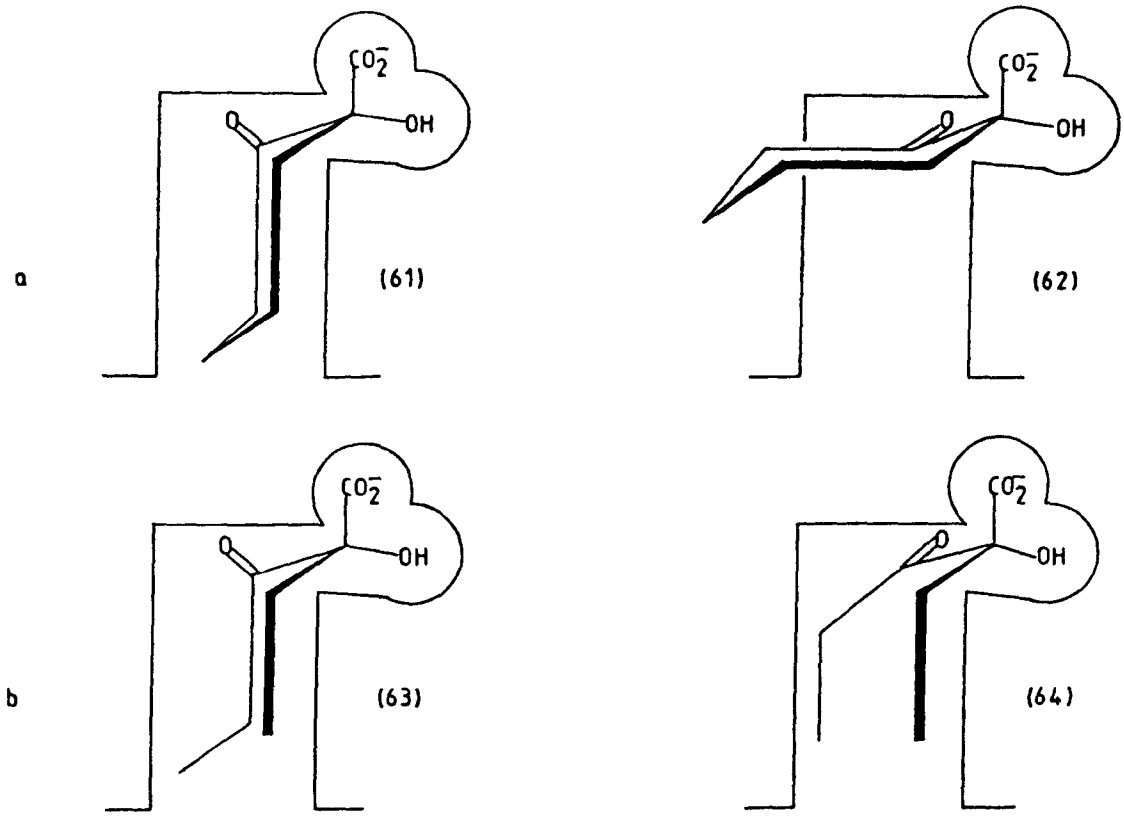
Treatment with ADC at pH 6.5 led to 43% reaction of the acid, as

judged by uptake of hydrochloric acid from an autotitrator. 2-Hydroxycyclohexanone (60) was isolated in 37% yield. Analysis of the optical purity of the hydroxyketone (60) by proton NMR was not possible owing to the gross overlapping of signals. Polarimetry also was of no use since the optical rotation of enantiomerically pure 2-hydroxycyclohexanone is not known in the literature.

The failure of ADC to decarboxylate the cyclic α-acetolactate analogue (59) completely may be rationalised as follows. The (S)-isomer was decarboxylated preferentially, as observed for α-acetolactate (7) and α-acetohydroxybutyrate (8). However, the enzyme, unable to decarboxylate the (R)-isomer of the cyclic substrate (59), was also unable to catalyse carboxylate migration to give the preferred (S)-isomer of (59).

For carboxylate migration, the carbon-carboxylate bond must be perpendicular to the plane of the trigonal carbonyl group. In the (R)-isomer of the cyclic substrate (59) this is most readily achieved when the carboxylate group is axial to the ring. However, if the (R)-isomer of (59) is bound at the carboxylate group and hydroxyl group with the ring in the chair form and the carboxylate group equatorial to the ring (61), then in order to bring the carboxylate group axial to the ring, and allow carboxylate group migration, the alternative chair conformation (62) must be adopted (Scheme 5.9.a).

If the shape of the active site is that envisaged in structure (61) then conformational change in the substrate to the alternative chair form (62) would not be possible. Hence, the substrate would not be able to adopt the conformation necessary for carboxylate group migration.



Scheme 5.9

In the case of the corresponding acyclic example, 2-ethyl-2-hydroxy-3-oxopentanoate (55), in which both enantiomers were found to be decarboxylated by ADC, the conformational restraints are much less severe. Assuming the same model for the active site, it can be seen using molecular models that the complete freedom of rotation about each sp^3 bond should allow the (R)-isomer of (55) to adopt either conformation, (63) or (64), (Scheme 5.9.b), within the constraints of the active site, thus allowing carboxylate group migration giving the preferred (S)-isomer.

The same conclusions may be reached by similar arguments if it is assumed that the carbonyl group and the adjacent alkyl substituents are responsible for binding instead of the carboxylate group and the hydroxyl function.

If the ADC-catalysed decarboxylations proceed via an enediol intermediate, then the very fact that the cyclic substrate (59) was

decarboxylated means that the proposed enediol intermediate must be of the cis-geometry (65), (Scheme 5.10), since in this case the trans-geometry is obviously not possible.

(S)-(59) (65)

Scheme 5.10

Cis-enediol intermediates have precedent in the literature. Rose and co-workers[88,89] have published evidence for a cis-enediol intermediate in the manganese-dependent aldose-isomerase-catalysed reactions (Scheme 5.11).

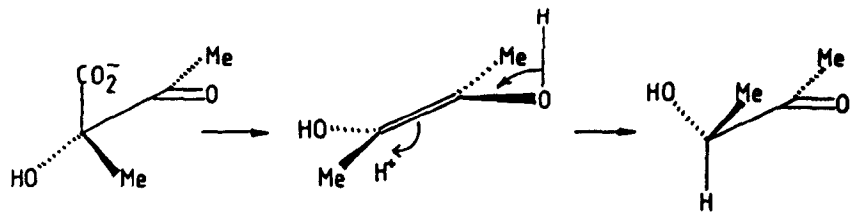
$$RCH(OH)CHO \rightleftharpoons RCOCH_2OH$$

Scheme 5.11

Deuterium labeling studies showed that the hydrogen transfer was intramolecular and suprafacial. This implied that the same group at the enzyme active site was involved in both proton abstraction and donation. In each of the eight cases examined, the stereochemical outcome could only be rationalised on the basis of protonation at C-1 or C-2 of a cis-enediol.

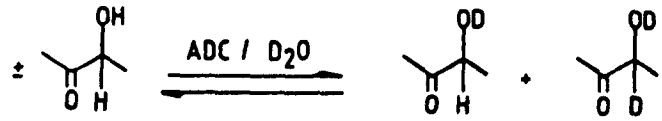
ADC-catalysed deuterium incorporation into acetoin

It is assumed that the ADC-catalysed decarboxylation of α-acetolactate proceeds via an enediol intermediate which is protonated to give the acetoin product (Scheme 5.12).



Scheme 5.12

The reversibility of the last step was demonstrated by incubation of racemic acetoin and ADC in D_2O. The reaction was followed by proton NMR spectroscopy. The quartet arising from the methine proton was seen to fall to half of its original integrated value with respect to the other acetoin signals. Deuterium NMR showed that deuterium had been incorporated at the carbon atom bearing the hydroxyl group. This observation suggested that the exchange was attributable to the reversible operation of the last step in the ADC-catalysed decarboxylation reaction and that exchange was stereospecific and probably limited to the (R)-(-)-isomer, the normal product of the decarboxylation (Scheme 5.13).



Scheme 5.13

Since the C-H and C-D stretching regions are widely separated, the use of vibrational circular dichroism (VCD) offered the opportunity to determine the absolute configurations of the deuterated and non-deuterated components of the mixture without prior chemical separation.

The measurement of VCD is still in its infancy compared to the more established electronic CD in the UV-visible region. Recent

advances in instrumentation have extended the range of VCD to 600 cm^{-1}. However, few such instruments exist and few specra have been published.

The vibrational infra red circular dichroism and normal infra red spectra of the partially deuterated acetoin mixture and (R)-(-)-acetoin of normal isotopic composition were determined separately in carbon tetrachloride. IR and CD measurements made in the C-D stretching region (2250cm^{-1} to 2050cm^{-1}) are shown in Figure 5.10. The stereoselective nature of the proton-deuteron exchange was demonstrated clearly by the negative CD bands found for the partially deuterated sample.

Figure 5.11 shows the IR and CD curves in the C-H stretching region. The non-deuterated component of the partially deuterated mixture gave rise to a CD spectrum positive in sign, whereas (R)-(-)-acetoin of normal isotopic composition exhibited a corresponding curve negative in sign. This indicated that the non-deuterated component from the enzyme-catalysed reaction was enriched in (S)-(+)-acetoin and that the deuterated component had the same configuration as (R)-(-)-acetoin. The intensity of the optical activity of the partially deuterated mixture was approximately half that of the normal (R)-(-)-isomer of acetoin. This is consistent with the inference, based on proton NMR observations made during the enzymatic experiment, that only one enantiomer of acetoin had undergone exchange.

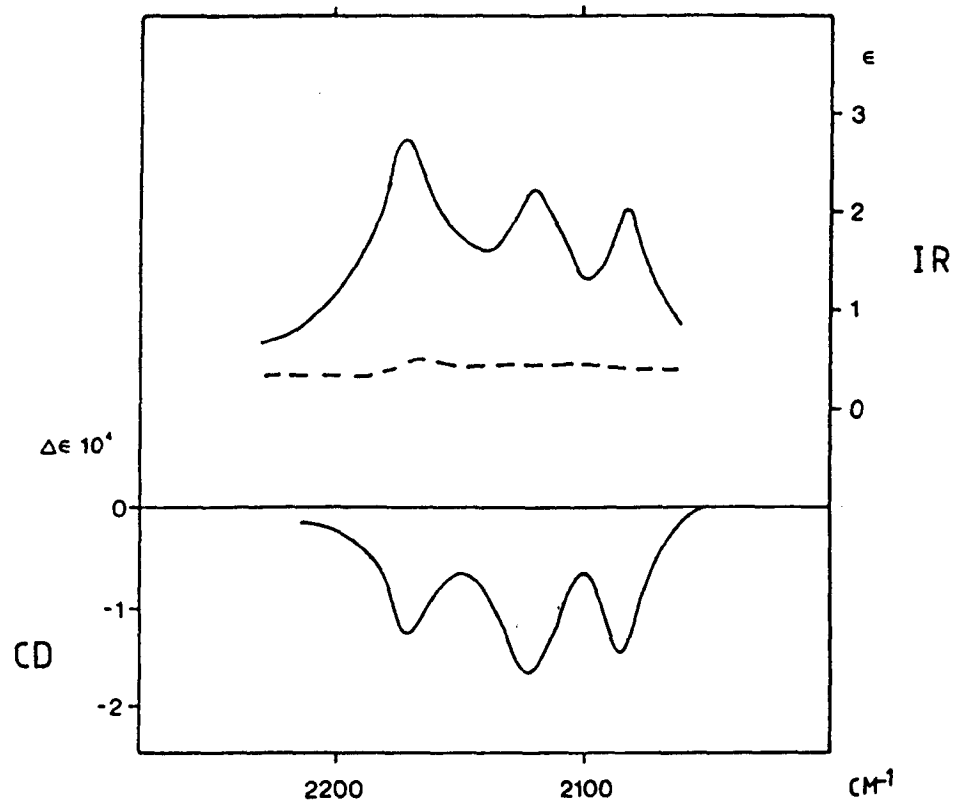


Figure 5.10

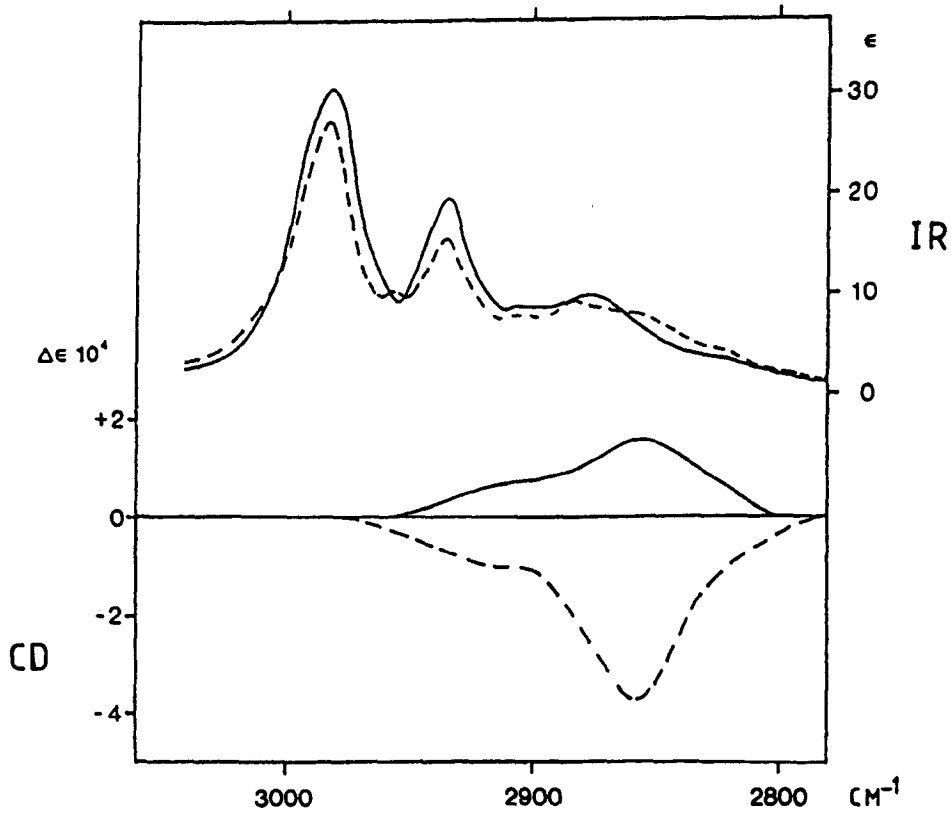


Figure 5.11

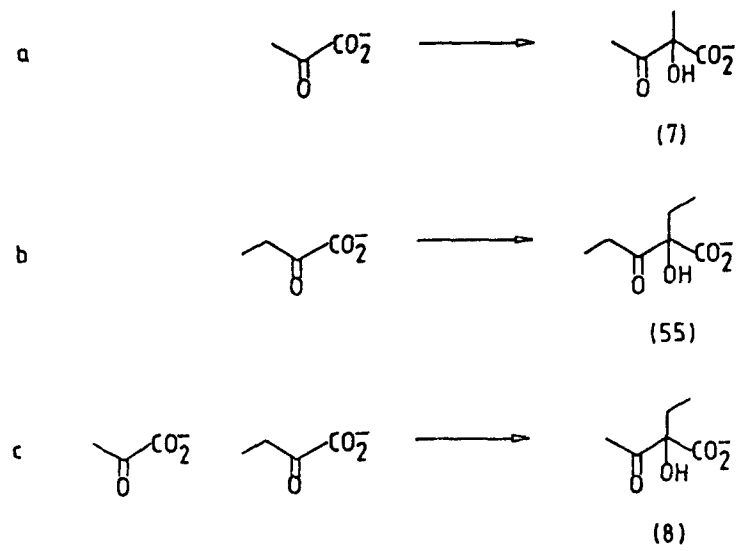
(———) Partially deuterated acetoin

(- - - -) (R)-(-)-acetoin of normal isotopic composition

Biotransformations with acetolactate synthase isozyme II and acetolactate decarboxylase

Acetolactate synthase isozyme II

Acetolactate synthase, the first common enzyme in the biosynthesis of valine and isoleucine, is found in bacteria, yeast and higher plants. In enteric bacteria there are several isozymes of acetolactate synthase with varying properties. Acetolactate synthase isozyme II (ALS II) from Salmonella typhimurium is distinguished from the other isozymes in that it is not inhibited by valine, leucine or isoleucine at concentrations up to 10 mM.[90]



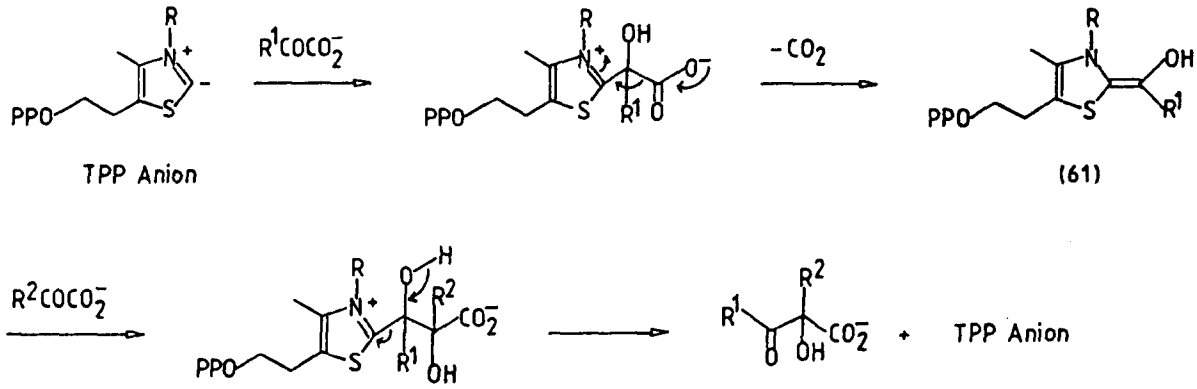
Scheme 5.14

ALS II is known to catalyse the homologous self-condensation reactions of pyruvate and 2-oxobutanoate to give α-acetolactate (7) and its higher homologue (55) (Scheme 5.14.a,b).[91]

However, in the presence of both substrates, the heterologous reaction dominates to produce α-acetohydroxybutyrate (8) (Scheme

5.12.c).[91]

The enzyme has two substrate binding positions.[91] The first utilises thiamine pyrophosphate (TPP) to bind keto acid and bring about decarboxylation, an essentially irreversible reaction. The "active aldehyde" (66) is condensed with keto acid bound at the second site to give the observed α-acetolactate analogues and regeneration of TPP (Scheme 5.15).



Scheme 5.15

Schloss and co-workers[92] have isolated hydroxyethyl TPP, the decarboxylated adduct of ALS II and pyruvate, using chemical quench experiments to trap the reaction intermediates in the enzymic reaction. They also incubated ALS II with an equimolar mixture of pyruvate and 2-oxobutanoate in which the carboxylate group of the pyruvate was enriched in ^{13}C. Measurement of the isotope rato of the evolved carbon dioxide by mass spectrometry indicated a 19-fold preference of pyruvate for the first site (carboxylate loss) over 2-oxobutanoate. The latter was found to be the preferred substrate for the second site.

It has been reported[93] that (S)-α-acetolactate, but not (R)-α-acetolactate, is a substrate for the reductoisomerise enzyme of

Salmonella typhimurium. From this it has been concluded[13] that the α-acetolactate generated, via the acetolactate synthase, is of the (S)-configuration.

Surprisingly, nothing has been reported concerning the configuration of the α-acetohydroxybutyrate (8) or higher homologues of α-acetolactate formed by condensations of keto acids catalysed by ALS.

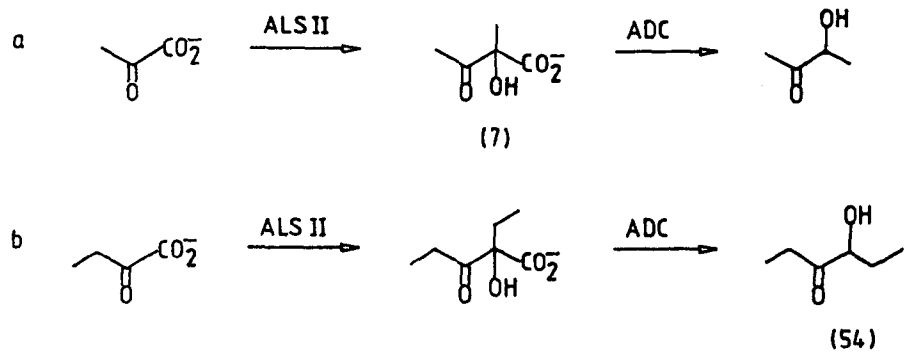
Schloss and co-workers[90] have developed a procedure for the large-scale production and purification of ALS II. Plasmid pDU9, which encodes the large and small subunits of Salmonella typhimurium ALS II and valine transaminase, was transformed into E.coli HB101. The enzyme was expressed in large quantities in the harvested E.coli. The enzyme-FAD complex is stable aerobically in the dark or anaerobically in light. Although the reactions catalysed by ALS II involve no net oxidation or reduction, the enzyme has a requirement for FAD, TPP and divalent metal cations. The metal plays no role in catalysis other than to assist in binding TPP to the enzyme.

A sample of purified ALS II was kindly donated by Dr J.V.Schloss. The studies reported here were conducted using this sample.

The combined use of ALS II and ADC

Homologous condensations

Sodium pyruvate was incubated with ADC and ALS II with its required cofactors, FAD and TPP (stoichiometric with the enzyme) and magnesium chloride. Under these conditions the enzyme has a half-life of approximately 3 h.[90] The reaction was followed by proton NMR spectroscopy. A rapid production of acetoin was observed (Scheme 5.16.a).



Scheme 5.16

No α-acetolactate was observed since sufficient ADC had been employed to process the acid (7) as soon as it was formed.

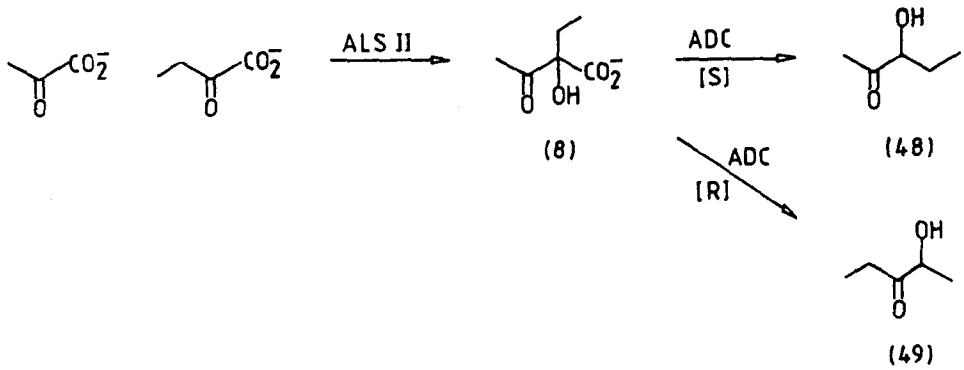
Similar incubation of sodium 2-oxobutanoate with the two enzymes gave the homologous α-hydroxyketone (54) (Scheme 5.14,b), at a rate about an order of magnitude slower than the pyruvate condensation. The condensation and decarboxylation was also followed by polarimetry. On the basis of complete conversion of starting material to 4-hydroxy hexan-3-one (54) the specific rotation of the product, so far unreported, was found to be -81^{o} at $26^{o}C$. This is very similar to the rotation of the lower homologue, (R)-(-)-acetoin with a rotation of -84^{o}.[84]

With these homologous condensations the product from the ADC-catalysed decarboxylation does not depend upon the configuration of the acetohydroxy acid precursor. In both cases the (R)-hydroxyketones were produced.

Treatment of 2-oxo-2-phenylethanoic acid with ADC and ALS II as before led to no observable reaction over a 10 h period. The acid was not a substrate for ALS II.

Heterologous condensations

An equimolar mixture of pyruvate and 2-oxobutanoate was incubated with ADC and ALS II as before. The reaction was followed by proton NMR spectroscopy.



Scheme 5.17

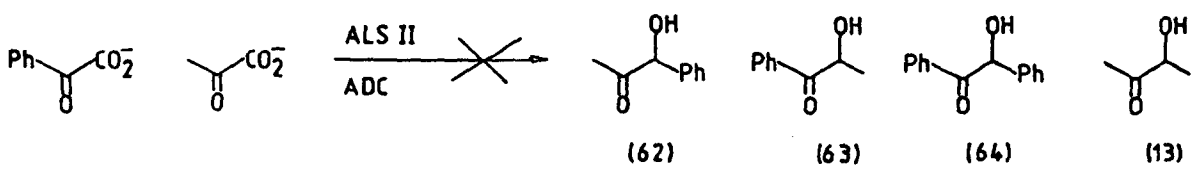
Observations up to 90% reaction indicated the exclusive generation of 3-hydroxypentan-2-one (48) (Scheme 5.17). This hydroxyketone arises from ADC-catlysed decarboxylation of (S)-α-acetohydroxybutyrate (8). None of the isomeric α-hydroxyketone, 2-hydroxypentan-3-one (49) (which would have been generated from the (R)-acid (8) by carboxylate migration prior to decarboxylation) was observed. This indicated that the ALS II-catalysed condensation of pyruvate and 2-oxobutanoate generated stereospecifically

(S)-α-acetohydroxybutyrate (8).

The remaining phase of the reaction saw the growth of diverse small signals which may be attributable to the homologous condensations of pyruvate or 2-oxobutanoate respectively followed by decarboxylation to give the corresponding hydroxyketones. The condensation and decarboxylation was also followed by polarimetry. On the basis of complete conversion of starting material to 3-hydroxy pentan-2-one (48) the specific rotation of the product, unknown in the literature, was found to be -94^{o} at $26^{o}C$. This value is similar to the rotations observed for acetoin and 4-hydroxyhexan-3-one obtained by the same method.

Here it has been possible to determine the stereochemical outcome of an enzymatic reaction, in a rather novel way, not by measurement of optical rotations or synthesis of diastereoisomers with a chiral auxilliary, but simply by observing which product was formed in a consecutive enzyme-catalysed reaction which forms different products depending upon the configuration of its substrate.

Treatment of an equimolar mixture of sodium pyruvate and 2-oxo-2-phenylethanoic acid with ADC and ALS II as before gave no reaction observable by proton NMR spectroscopy over a 10 h period. None of the possible products (67), (68), (69), or (13) arising from heterologous or homologous condensations of the keto acids and subsequent decarboxylation was observed (Scheme 5.18).



Scheme 5.18

These results indicate that 2-oxo-2-phenyl ethanoic acid is an inhibitor of the ALS II-catalysed condensation of pyruvate to α-acetolactate. This is not surprising in view of the results obtained by Stormer[94] with the pH 6 acetolactate-forming enzyme from Aerobacter aerogenes. 1 mM Phenylpyruvate and glyoxylate caused approximately 80% inhibition of the ALS-catalysed condensation of pyruvate to α-acetolactate. Competitive inhibition was observed implying that these two pyruvate analogues were not bound to the first site and decarboxylated, but perhaps competed with pyruvate for the second site.

The use of proton NMR spectroscopy to follow the ALS II-catalysed reactions represents a considerable improvement in assay technique over those in the recent literature.[92] Products are observed and identified directly as they form using a non-invasive technique. Subsequent reactions of the products may also be observed.

The advantages of combining ALS II and ADC

The combined use of ALS II and ADC to generate optically pure α-hydroxyketones from keto acids represents an advance in the application of enzymes to organic synthesis. The overall effect is not simply to degrade, in an enantioselective manner, a molecule generated by chemical synthesis, as is the case with hydrolytic enzymes and decarboxylases when used alone. Combining ALS II and ADC allows carbon-carbon bond formation in the condensation of simple achiral compounds to give optically pure chiral products of higher molecular mass and greater complexity than the starting materials.

The use of ALS II to transform commercially available 2-oxo acids

into α-acetolactate analogues for use with ADC obviates the need to synthesise them chemically. At the time of writing, the substrate acceptability range for ALS II is not known. Chemical synthesis, using the preferred methods as described in chapter 3, will still be neccessary to obtain α-acetolactate analogues inaccessible by ALS II-catalysed condensations of keto acids.

Chiral synthons from ADC-catalysed decarboxylations

Introduction

The optically pure α-hydroxyketones generated by ADC-catalysed decarboxylation of α-acetolactate analogues have a potential use as chiral building blocks. An extension to their usefulness would be their conversion to the corresponding 1,2-diols by stereoselective chemical reduction.

There are two possible products from a reduction of α-hydroxyketones; one from syn-selectivity (70) and the other from anti-selectivity (71) (Scheme 5.19).

(70) (71)

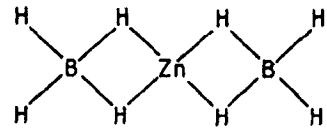
Scheme 5.19

Chemical methods have been developed for the stereoselective reduction of α-hydroxyketones.

Reductions giving anti-selectivity

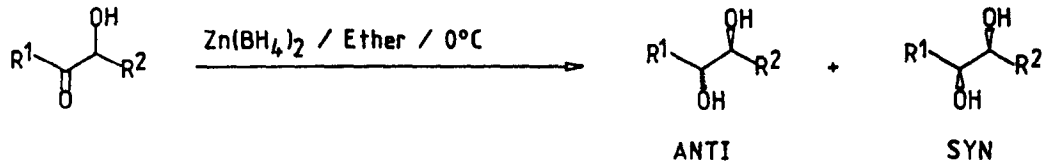
If the reaction conditions encourage chelation of the reducing agent to the substrate then the predominant diastereoisomer of the diol produced is generally the anti-isomer in accordance with Cram's cyclic model.[95]

Zinc borohydride



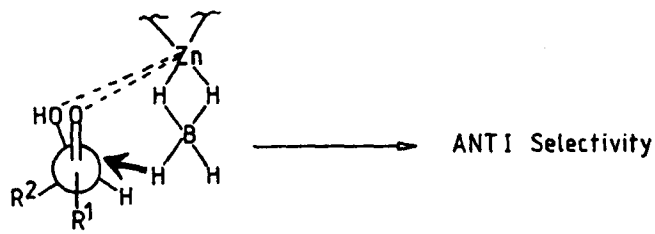
(67)

In solution in ether, zinc borohydride exists as a contact ion pair (72).[96]



Scheme 5.20

In reaction with α-hydroxyketones (Scheme 5.20) the zinc coordinates to the two oxygen atoms of the substrate. Hydride delivery to the carbonyl group is related to the relative stabilities of the two possible zinc-mediated transition states. The larger the substituents attached to the α-hydroxyketone the more the transition state leading to anti-selectivity is favoured (Scheme 5.21).

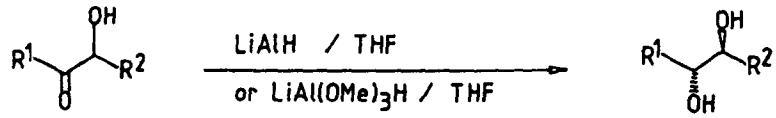


Scheme 5.21

Nakata and co-workers[97] subjected a series of α-hydroxyketones to reduction with zinc borohydride. They obtained anti-selectivity with diastereomeric excesses in the range of 54-98%.

The disadvantage of zinc borohydride is its low solubility in ether; concentrations greater than 0.1 M cannot be achieved. Other solvents such as 1,2-dimethoxyethane, in which zinc borohydride is much more soluble, compete with the α-hydroxyketone substrate for coordination to the zinc resulting in little selectivity in the reduction.[98]

Aluminium hydrides



Scheme 5.22

Katzenellenbogen and co-workers[99,100] have investigated the scope of lithium aluminium hydride and lithium trimethoxyaluminium hydride for the stereoselective reduction of α-hydroxyketones (Scheme 5.22). Their results showed that enlarging the bulk of R^1, the substituent attached to the carbonyl carbon atom, resulted in greater anti-selectivity. However, varying the size of R^2, the substituent adjacent to the hydroxyl group, had no regular or marked effect on the selectivity of the reduction (Table 5.1).

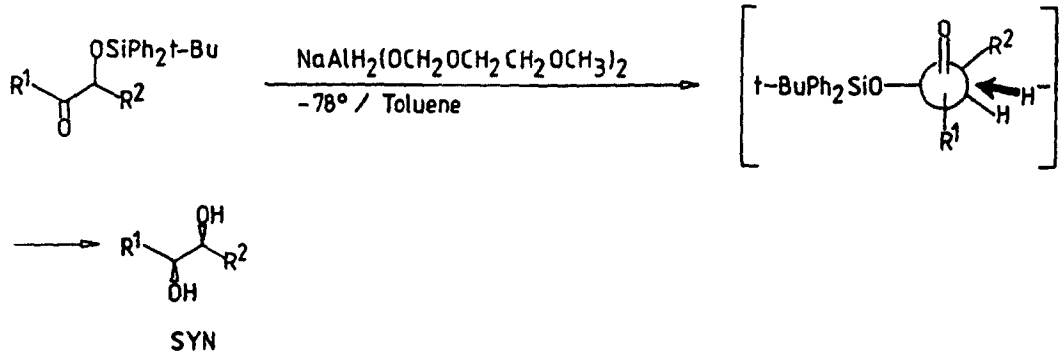
Table 5.1

R^2	R^2	% Anti-selectivity	
		$LiAlH_4$/THF	$LiAl(OMe)_3H$/THF
pentyl	Me	64	73
i-Pr	Me	58	46
t-Bu	Me	87	85
Ph	Me	87	85
Me	pentyl	70	78
Me	i-Pr	73	66
Me	t-Bu	75	77

Reductions giving syn-selectivity

Syn-selectivity in the reduction of α-hydroxyketones is favoured under conditions where the reducing agent does not complex with the substrate. The reaction can be considered to proceed via the open-chain model as proposed by Felkin.[101]

Sodium bis(2-methoxyethoxy)aliminium hydride ("Vitride")



Scheme 5.23

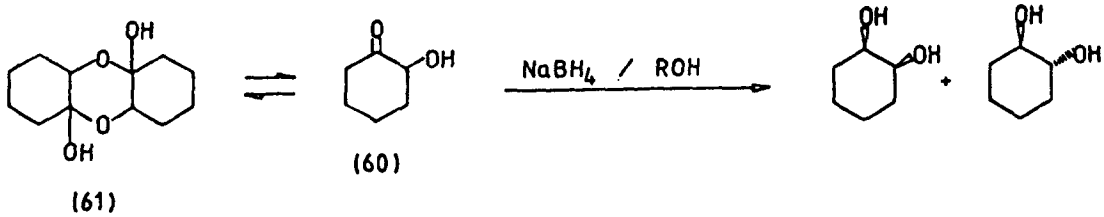
Nakata and co-workers[97] achieved the above conditions by blocking the alcohol with a very bulky protecting group and using a metal hydride of low coordinating ability (Scheme 5.23). They found that

syn-selectivity was good except where the substituent adjacent to the protected alcohol function, R^2, was methyl (Table 5.2).

Table 5.2

R^1	R^2	% Syn-selectivity
Me	pentyl	98
Me	i-Pr	96
Me	Ph	76
Et	Bu	93
Pr	Pr	86
Bu	Et	86
pentyl	Me	61
i-Pr	Me	46
Ph	Me	91

Model reactions on racemic 2-hydroxycyclohexanone



(61) (60)

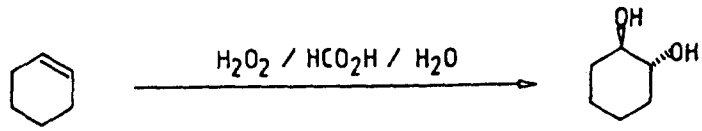
Scheme 5.24

α-Hydroxyketones such as acetoin and 2-hydroxycyclohexanone (adipoin) form stable crystalline dimers thought to be of the structure (61). The equilibrium favouring the monomer is established in water or polar protic solvents such as methanol or ethanol. If structure (61) is an accurate representation of the dimer then it is

necessary to regenerate the monomer before rection with a reducing agent can take place (Scheme 5.24).

The reduction of 2-hydroxycyclohexanone dimer with sodium borohydride at room temperature was performed in water and a range of alcohols. The product mixtures were analysed by ^{13}C NMR.

The ^{13}C NMR signals of cis and trans cyclohexane-1,2-diol are widely separated. It was possible to assign the two sets of signals by comparison with the ^{13}C NMR spectrum of authentic trans-cyclohexane-1,2-diol. This was synthesised by reaction of cyclohexene with aqueous hydrogen peroxide (Scheme 5.25).[63]



Scheme 5.25

Of the reductions attempted the only selectivity observed was when isopropanol was used as solvent (Table 5.3). Even in this case the selectivity was poor, only 60% of the trans isomer being formed.

Table 5.3

Solvent	% trans isomer
water	50
methanol	50
ethanol	50
isopropanol	60

The use of zinc borohydride or the aluminium hydrides requires the use of anhydrous aprotic solvents, such as ether or tetrahydrofuran, not conducive to the de-dimerisation of

α-hydroxyketone dimers. Solutions of monomeric acyclic α-hydroxyketones in ether can be obtained by continuous ether extraction of aqueous solutions in which the monomer-dimer equilibrium has been established. This cannot be done with tetrahydrofuran.

The 2-hydroxycyclohexanone monomer-dimer equilibrium in ether proved to be heavily in favour of the insoluble dimer. It was not possible to generate the monomer in solution in ether except at a very low concentration.

The dimer, suspended in ether, was treated with lithium aluminium hydride at $-78^{o}C$. A mixture of the cis and trans diols was obtained. There was a small excess of the cis isomer (60% of the mixture).

Of the reductions attempted above, none was of sufficient stereospecificity to be useful synthetically.

INTRODUCTION TO EXPERIMENTAL CHAPTERS

References are given to known compounds as they appear. Where possible data are compared.

The experimental data are presented in the format approved by the journal Synthesis. Elemental analyses are quoted in the style used by the Chemical Society.

Nuclear magnetic resonance spectra were recorded using the instruments listed below operating at the frequencies given in the table.

Spectrometer	Frequency / MHz		
	^{1}H	^{2}H	^{13}C
Bruker WH 400	400.13	61.41	100.62
Bruker AC 250			62.90
Perkin-Elmer R34	220		
Bruker WH 180			45.28
Bruker WH 90			22.63

Chemical shifts are quoted in ppm from tetramethylsilane as internal reference unless otherwise stated.

Mass spectra were recorded using a Kratos MS 80 spectrometer.

Infra red spectra were recorded using a Perkin-Elmer 580-B spectrophotometer.

Optical rotations were recorded using an Optical Activity LTD AA-1000 polarimeter. Rotations were measured at 589 nm in a 2 dm path length cell.

Vibrational infra red circular dichroism spectra were recorded by Dr A.F. Drake, Birkbeck College, London, using an own-built

spectrometer (Drake, A.F., J. Phys. E. Sci. Instrum., 1986, 19, 170). Measurements were performed for solutions in carbon tetrachloride.

Melting points were determined using a Gallenkamp apparatus and are quoted uncorrected.

Gas-liquid chromatographic analysis was performed using a Pye 204 gas chromatograph. The columns used were 6 feet in length. Nitrogen was used as carrier gas at a flow rate of 30 ml/minute.

High pressure liquid chromatography was performed using a Gilson system comprising the following components; a model 302 piston pump, a model 802C manometric module and a H M holochrome UV/Vis detector.

pH-Stat experiments were performed using a Radiometer Copenhagen RTS882 recording titration system.

Thin-layer chromatography was performed on Merck Kieselgel F_{254} 0.2 mm precoated plates. Spot detection was by UV fluorescence quenching, exposure to iodine vapour, potassium manganate spray or phosphomolybdic acid/ethanol spray.

Flash chromatography was performed on Merck Kieselgel 60 silica gel (230-400 mesh). Approximately 100 g of silica gel was used per g of compound. The flow of solvent through the column was assisted by compressed air such that the solvent front moved at approximately 4 cm/minute.

All solvents were distilled.

Ether refers to diethyl ether.

Petrol refers to the petroleum fraction boiling in the range 40-60^{o}C.

CHAPTER 6

Prepartion of the dioxostannolan of dl-mandelic acid

dl-Mandelic acid (6.573 g, 43 mmol) and dibutyl tin oxide (10.785 g, 43.3 mmol) were heated together in boiling toluene (200 ml) for 3.5 h with azeotropic removal of water using a Dean-Stark apparatus. The solvent was evaporated under reduced pressure and the residue was crystallized from chloroform to give the dioxostannolan as a white crystalline solid (11.916 g, 72%), mp 185-187°C. This was recrystallised from chloroform-benzene to a constant melting point of 194-196°C.

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ = 0.4-2.0 (complex, 18 H, butyl); 5.06 (d, 1 H, J=7 Hz, PhCH); 7.3 ppm (complex, 5 H, Ph).

IR (nujol mull): ν = 1640(sh), 1615 cm^{-1}.

MS (EI): m/z= 383$(M)^{+\cdot}$, 177$(SnBu)^+$, 105$(PhCO)^+$, 91$(PhCH_2)^+$.

Oxidation of dl-mandelic acid

dl-Mandelic acid (1.972 g, 12.97 mmol) and dibutyl tin oxide (3.315 g, 13.3 mmol) were heated together in boiling benzene (170 ml) for 40 h with azeotropic removal of water using a Dean-Stark apparatus. The solvent was evaporated under reduced pressure/40°C to give the crude dioxostannolan as a white crystalline solid. This was suspended in dry dichloromethane (30 ml) and the flask flushed with nitrogen. Bromine (0.7 ml, 12.0 mmol) was added dropwise with stirring over 3 minutes. A deep red homogeneous solution formed which was set aside, in the dark, at room temperature. Decolourisation was complete after 3 days. The solvent was evaporated under reduced pressure/30°C

and the residue was applied to a short silica column (60-120 mesh, 10 g) and eluted successively with carbon tetrachloride (450 ml) and ethyl acetate (400 ml). The ethyl acetate was evaporated under reduced pressure/40°C to give the crude keto acid as an oil (1.645 g, 11.0 mmol, 84%). This was dissolved in 2.5 M sulphuric acid (100 ml) and added to a solution of 2,4-dinitrophenylhydrazine (3 g in 2.5 M sulphuric acid (250 ml)). The 2,4-dinitrophenylhydrazone was filtered off and recrystallised to a constant melting point of 197-197.5°C from ethanol. Lit.[102] mp 196-197°C.

2-hydroxy-2-phenylethanoic acid:

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ= 7.55 (m, 2 H, ortho CH); 7.73 (m, 1 H, para CH); 8.35 (m, 2 H, meta CH); 9.0 ppm (bs, 1 H, CO_2H).

2,4-dinitrophenylhydrazone:

^{1}H NMR, 220 MHz (d_6DMSO/TMS): δ= 7.46 (complex, 3 H, meta+para Ph); 7.79 (m, 2 H, ortho Ph); 8.16 (d, 1 H, J=9.8 Hz, NHCCHCH); 8.43 (dd, 1 H, J=9.8,2.7 Hz, NHCCHCH); 8.97 (d, 1 H, J=2.7 Hz, $NO_2CCHCNO_2$); 14.00 ppm (s, 1 H, COOH).

IR (film): ν = 3200, 3100, 1680, 1615, 1595, 1505, 1340 cm^{-1}.

MS (EI): m/z= 330$(M)^{+\cdot}$, 286$(M-CO_2)^{+\cdot}$, 103$(PhCN)^+$.

High resolution MS: $C_{14}H_{10}N_4O_6$, calc. 330.0601, found 330.0592.

Elemental analysis: Found: C, 50.91; H, 3.03; N, 16.97. $C_{14}H_{10}N_4O_6$ requires C, 50.37; H, 2.93; N, 16.55.

Oxidation of racemic 2-hydroxy-3-methylbutanoic acid

Racemic 2-hydroxy-3-methylbutanoic acid (1.006 g, 8.5 mmol) and dibutyl tin oxide (2.143 g, 8.6 mmol) were heated together in boiling benzene (100 ml) for 5.5 h with azeotropic removal of water using a

Dean-Stark apparatus. The benzene was evaporated under reduced pressure/40°C to give a glass. This was suspended in dichloromethane (30 ml) under nitrogen. Bromine (0.45 ml, 8.8 mmol) was added in one portion and the mixture was stirred in the dark for 20 h. The colourless solution was evaporated under reduced pressure/40°C to give the impure keto acid as a yellow oil. This was suspended in 3 M sulphuric acid (100 ml) and filtered into a solution of 2,4-dinitrophenylhydrazine (1.8 g) in 3 M sulphuric acid (100 ml). The precipitate was filtered off to give the 2,4-dinitrophenylhydrazone as a yellow solid (1.07 g, 3.6 mmol, 43%). This was recrystallised from ethanol-petrol to give a yellow crystalline solid mp 145-146°C.
Lit.[103] mp 191-192°C.

^{1}H NMR, 220 MHz (d_6-DMSO/TMS): δ= 1.24 (d, 6 H, J=6.7 Hz, $CH(\underline{CH_3})_2$); 3.14 (m, 1 H, $\underline{CH}(CH_3)_2$); 5.60 (bs, 1 H, NH); 8.15 (m, 1 H, NHC$\underline{CH}$); 8.46 (m, 1 H, NHCC$\underline{H}$CH); 9.0 (m, 1 H, $NO_2CCHCNO_2$); 13.55 ppm (s, 1 H, CO_2H).

IR (film): ν = 1705, 1625, 1595 cm^{-1}.

MS (EI): m/z= 296$(M)^{+\bullet}$, 183$(C_6H_5N_3O_4)^+$.

High resolution MS: $C_{11}H_{12}N_4O_6$, calc. 296.0757, found 296.0753.

Attempted oxidation of 2-hydroxybutanoic acid and 2-hydroxypropanoic acid.

Treatment of these two hydroxy acids with dibutyl tin oxide and bromine as before led to recovery of starting material and no isolation of keto acids.

Methylation of 2-hydroxy-3-methylbutanoic acid

To a solution of 2-hydroxy-3-methylbutanoic acid (4.027 g, 34 mmol) in ether (100 ml) was added a solution of diazomethane (generated from "Diazald") in ether dropwise until a yellow colouration persisted. A few drops of glacial ethanoic acid were added to discharge the colour. The solution was dried ($MgSO_4$) and evaporated under reduced pressure/20°C to give the volatile methyl ester as a pale yellow oil (4.339 g, 97%). (Lit.[104] bp 62-63°C/15 mmHg).

^1H NMR, 220 MHz ($CDCl_3$/TMS): δ= 0.87 (d, 3 H, J=6.7 Hz, CCH_3); 1.03 (d, 3 H, J=6.7 Hz, CCH_3); 2.08 (m, 1 H, CH); 2.80 (bs, 1 H, OH); 3.81 (s, 3 H, OCH_3); 4.07 ppm (d, 1 H, J=4 Hz, $\underline{C}HOH$).

IR (film): ν = 3480, 1730 cm^{-1}.

MS (+ve CI, ammonia): m/z= 150$(M+18)^+$, 133$(M+1)^+$.

High resolution MS: $C_6H_{13}O_3$, (M+H), calc. 133.0864, found 133.0859.

Methyl 3-bromo-3-methyl-2-oxobutanoate

N-Bromosuccinimide (10.6 g, 59.6 mmol) and methyl 2-hydroxy-3-methylbutanoate (3.855 g, 29.2 mmol) were heated together in boiling carbon tetrachloride (70 ml) under nitrogen for 7 h. The mixture was cooled (ice bath), filtered and evaporated under reduced pressure/35°C. The residue was distilled in a Kugelrohr apparatus (100°C/15 mmHg) to give a pale yellow oil (5.062 g, 24.2 mmol, 83%).

^1H NMR, 220 MHz ($CDCl_3$/TMS): δ= 2.02 (s, 6 H, $C(CH_3)_2$); 3.93 ppm (s, 3 H, OCH_3).

^{13}C NMR, 22.64 MHz ($CDCl_3$; $CDCl_3$=δ77.0 ppm): δ= 29.4 ($C(\underline{C}H_3)_2$); 52.7 (CBr); 60.7 (OCH_3); 162.5 ($\underline{C}O_2CH_3$); 190.0 ppm (CO).

IR (film): ν = 1745, 1720 cm^{-1}.

MS (EI): m/z= 208,210$(M)^{+\bullet}$.

High resolution MS: $C_6H_9BrO_3$, calc. 207.9711, found 207.9738.

GLC (10% SE30, 120°C); R_T= 370s (100%).

Elemental analysis: Found: C, 34.42; H, 4.40; Br, 38.11. $C_6H_9BrO_3$ requires C, 34.45; H, 4.31; Br, 38.28%.

3-Bromo-3-methyl-2-oxobutanoic acid

N-Bromosuccinimide (4.540 g, 25,5 mmol) and 2-hydroxy-3-methylbutanoic acid (1.496 g, 12.7 mmol) were heated together in boiling carbon tetrachloride (40 ml) under nitrogen for 2.25 h. The mixture was filtered and evaporated under reduced pressure/35°C to give a pale yellow oil (2.564 g, crude yield 103%).

^1H NMR, 220 MHz ($CDCl_3$/TMS): δ= 2.06 (s, 6 H, $C(CH_3)_2$); 9.40 ppm (bs, 1 H, CO_2H). Succinimide impurity δ= 2.83 ppm (s). Lit.[2] ($CDCl_3$/TMS) δ= 2.08 (s), 9.91 ppm (s).

IR (film): ν = 1715(b) cm^{-1}.

MS (EI): m/z= 197, 195$(M+1)^{+\bullet}$, 151, 149$(M-OEt)^+$

High resolution MS: $C_5H_7BrO_3$, calc. 193.9578, found 193.9589.

Estimated purity by ^1H NMR, 90%. Corrected yield, 93%.

Attempted coupling of 3-bromo-3-methyl-2-oxobutanoic acid and glycine methyl ester using dicyclohexyl carbodiimide

To a stirred suspension of 3-bromo-3-methyl-2-oxobutanoic acid (350 mg, 1.5 mmol) and glycine methyl ester hydrogen chloride (432 mg, 3.43 mmol) in dichloromethane (10 ml) was added triethylamine (0.47 ml, 3.4 mmol). To the resulting homogeneous solution was added, in one portion, dicyclohexyl carbodiimide (370 mg, 1.8 mmol). A white

precipitate formed after 5 minutes. After 7 h the mixture was filtered and the solid was washed with dichloromethane (20 ml). The dichloromethane was washed with 2M hydrochloric acid (50 ml). The aqueous layer was back-extracted with dichloromethane (4x50 ml). The combined dichloromethane portions were dried ($MgSO_4$) and evaporated under reduced pressure to give a pale yellow gum (669 mg). Analysis by ^1H NMR indicated none of the desired amide.

Coupling of 3-bromo-3-methyl-2-oxobutanoic acid and glycine methyl ester using EEDQ

A flask was charged with a magnetic follower, glycine methyl ester hydrogen chloride (128 mg, 1.01 mmol) and ethyl 1,2-dihydro-2-ethoxy-1-quinolinecarboxylate (EEDQ) (216 mg, 1.06 mmol), fitted with a suba seal stopper and flushed with nitrogen. To this was added, via syringe, dichloromethane (10 ml) and triethylamine (0.145 ml, 1.04 mmol). After 2 minutes 3-bromo-3-methyl-2-oxobutanoic acid (90%, 259 mg, 1.1 mmol) in dichloromethane (10 ml) was added to the homogeneous solution with stirring. A white precipitate formed and redissolved after a further 1 h at room temperature. After 20 h the solvent was evaporated under reduced pressure/35°C. The residue was suspended in ethyl acetate (30 ml), filtered and washed with saturated aqueous sodium hydrogen carbonate (10 ml). The aqueous phase was back-extracted with ethyl acetate (2x20 ml). The combined organic portions were washed with 1 M hydrochloric acid (20 ml) and the aqueous phase back-extracted with ethyl acetate (2x10 ml). The combined organic portions were dried ($MgSO_4$) and evaporated under reduced pressure/40°C and the residue was co-evaporated under reduced pressure with carbon

tetrachloride (20 ml) to give a white crystalline solid (231 mg, 86%). This was subjected to flash chromatography eluting with ethyl acetate-petrol 3:7. The amide, R_F 0.22 was isolated as a white crystalline solid (129 mg, 48%), mp 79-81°C.

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ= 2.09 (s, 6 H, $C(CH_3)_2$); 3.82 (s, 3 H, OCH_3); 4.13 (d, 2 H, J=5.8 Hz, $NHCH_2$); 7.44 ppm (bs, 1 H, NH).

IR (nujol mull): ν= 3330, 1750, 1715, 1695, 1675, 1540 cm^{-1}.

MS (+ve CI, ammonia): m/z= 283, 285$(M+18)^+$, 266, 268$(M+1)^+$.

High resolution MS: $C_8H_{13}BrNO_4$, (M+H), calc. 268.0007, found 268.0012.

Elemental analysis: Found C, 36.29; H, 4.45; N, 4.80; Br, 28.47.

$C_8H_{12}BrNO_4$ requires C, 36.09; H, 4.51; N, 5.26; Br, 30.07%.

Reaction of 3-bromo-3-methyl-2-oxobutanoic acid with benzylamine

To a stirred solution of benzylamine (338 mg, 3.16 mmol) in dichloromethane (2 ml) under nitrogen was added 3-bromo-3-methyl-2-oxobutanoic acid (250 mg, 90%, 1.08 mmol) dissolved in dichloromethane (3 ml). A white precipitate formed rapidly. After 2 h the reaction mixture was diluted with dichloromethane (10 ml) and washed with 1 M hydrochloric acid (30 ml) and saturated sodium hydrogen carbonate solution (30 ml). The aqueous layers were separately back-extracted with dichloromethane (3x15 ml). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure/40°C to give a white gum (70 mg). This was subjected to flash chromatography eluting with ethyl acetate-petrol 1:1. The benzylamide, R_F 0.3 was isolated as a white crystalline solid (49 mg, 0.28 mmol, 26%). mp 91-92°C. The mp was unchanged on recrystallisation from water.

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ= 1.16 (d, 6 H, J=6.7 Hz, $C(CH_3)_2$); 2.39

(septuplet, 1 H, J=6.7 Hz, CH); 4.42 (d, 2 H, J=5.3 Hz, $PhCH_2$); 6.05 (bs, 1 H, NH); 7.30 ppm (complex, 5H, Ph).

IR (nujol mull): ν = 3290, 3070, 1640, 1545 cm^{-1}.

MS (EI): m/z= 177$(M)^{+\cdot}$.

High resolution MS: $C_{11}H_{15}NO$, calc. 177.1154, found 177.1155.

Elemental analysis: Found: C, 74.60; H, 8.86; N, 8.01. $C_{11}H_{15}NO$ requires C, 74.58; H, 8.47; N, 7.91%.

Methylation of 3,3-dimethyl acrylic acid (a)

Acetyl chloride (60 ml, 0.844 mol) was added to dry methanol (150 ml) at 0°C. To this was added 3,3-dimethyl acrylic acid (80.6 g, 0.805 mol). The mixture was stirred at 40-50°C for 3.5 h to give a homogeneous solution. This was set aside at room temperature for 12 h. Fractions boiling up to 65°C were removed by distillation. The remaining solution formed two layers. The upper layer was removed and distilled at 135-138°C at atmospheric pressure (Lit.[105] bp 136°C) to give the methyl ester as a colourless liquid (55.973 g, 0.49 mol, 61%).

^1H NMR, 220 MHz ($CDCl_3$/TMS): δ = 1.90 (s, 3 H, CCH_3); 2.18 (s, 3 H, CCH_3); 3.69 (s, 3 H, OCH_3); 5.70 ppm (m, 1 H, CH).

IR (film): ν = 1725, 1665 cm^{-1}.

MS (EI): m/z= 114 $(M)^{+\cdot}$.

Methylation of 3,3-dimethyl acrylic acid (b)

3,3-Dimethyl acrylic acid (32.419 g, 0.324 mol) was dissolved in dry methanol (400 ml). Concentrated sulphuric acid (1 ml) was added and the solution was boiled for 19 h. The bulk of the methanol was

distilled off at atmospheric pressure. The residue was dissolved in dichloromethane (50 ml). The solution was washed with 5% sodium hydrogen carbonate solution (2x50 ml) and distilled at atmospheric pressure. The ester was collected at 130-134°C as a colorless oil (21.393 g, 0.188 mol, 58%).

Dihydroxylation of methyl 3,3-dimethylacrylate

To a solution of methyl 3,3-dimethylacrylate (3.670 g, 32.2 mmol) in 98% formic acid (20 ml) was added a 30% solution of hydrogen peroxide in water (5 ml, 48 mmol). The solution was maintained at 40°C for 20 h. The solvents were evaporated under reduced pressure, the residue treated with toluene (30 ml) and again evaporated under reduced pressure. The crude diol was subjected to flash chromatography eluting with ethyl acetate-petrol 1:1. The diol ester,[106] R_F 0.2, was isolated as a colourless oil (2.907 g, 19.6 mmol, 61%).

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ = 1.22 (s, 3 H, CCH_3); 1.30 (s, 3 H, CCH_3); 2.58 (bs, 1 H, OH); 3.19 (bs, 1 H, OH); 3.86 (s, 3 H, OCH_3); 4.01 ppm (d, 1H, J=8 Hz, CH).

IR (film): ν = 3460, 1735 cm^{-1}.

MS (+ve CI, ammonia): m/z= 166$(M+18)^+$, 149$(M+1)^+$.

GLC (3% SE30, 90°C); R_T= 231s, (100%).

Oxidation of methyl 2,3-dihydroxy-3-methylbutanoate using N-bromosuccinimide

A stirred suspension of N-bromosuccinimide (2.973 g, 16.7 mmol), calcium carbonate (1.68 g, 16.8 mmol) and methyl 2,3-dihydroxy-3-methylbutanoate (1.900 g, 12.8 mmol) in carbon tetrachloride (26 ml),

under nitrogen, was heated and illuminated by means of a 100W tungsten lamp. The temperature of the reaction mixture stabilised at 50°C. After 5 minutes a deep red colouration developed which disappeared over a 5 h period. The mixture was cooled (ice bath), flitered and the residue was washed with carbon tetrachloride (50 ml). The combined carbon tetrachloride portions were evaporated under reduced pressure. The residue was distilled in a Kugelrohr apparatus (120°C/10 mmHg) to give methyl 3-hydroxy-3-methyl-2-oxobutanoate as a pale yellow oil (1.604 g, 10.99 mmol, 86%).

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ = 1.53 (s, 6 H, $C(CH_3)_2$); 2.65 (bs, 1 H, OH); 3.92 ppm (s, 3 H, OCH_3).

IR (film): ν = 3500, 1740(sh), 1730 cm^{-1}.

MS (+ve CI, ammonia): m/z= 164$(M+18)^+$.

GLC (10% SE30, 110°C); R_T= 262s.

Attempted oxidation of methyl 2,3-dihydroxy-3-methylbutanoate using N-bromosuccinimide (a)

A stirred suspension of N-bromosuccinimide (7.870 g, 44.2 mmol), calcium carbonate (4.417 g, 44.1 mmol) and methyl 2,3-dihydroxy-3-methylbutanoate (6.520 g, 44.0 mmol) in carbon tetrachloride (150 ml) was heated at 60-70°C for 40 minutes under nitrogen. The mixture was cooled (ice bath), filtered and the residue was washed with cold dichloromethane (5x10 ml). The combined solutions were evaporated under reduced pressure. The residue was subjected to flash chromatography eluting with ethyl acetate-petrol 2:5. Methyl 3-hydroxy-3-methyl-2-oxobutanoate, R_F 0.53, was isolated as a colourless oil (1.925 g, 30%).

Attempted oxidation of methyl 2,3-dihydroxy-3-methylbutanoate using N-bromosuccinimide (b)

To a cooled (ice bath) solution of methyl 2,3-dihydroxy-3-methylbutanoate (1.062 g, 7.2 mmol) in dioxane (16 ml) and water (1.6 ml) was added N-bromosuccinimide (1.311 g, 7.36 mmol). The mixture was allowed to warm to room temperature. The resulting orange homogeneous solution was set aside at room temperature for 24 h and heated for 2 h at 40-60°C. 2 M Hydrochloric acid (10 ml) was added and the solution was extracted with ethyl acetate (4x30 ml). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was subjected to flash chromatography eluting with dichloromethane to give methyl 3-hydroxy-3-methyl-2-oxobutanoate as a pale yellow oil (446 mg, 42%). This was distilled using a Kugelrohr apparatus (150°C/15 mmHg) to give a pale yellow oil (277 mg, 26%).

Attempted oxidation of methyl 2,3-dihydroxy-3-methylbutanoate using pyridinium chorochromate[60]

A solution of methyl 2,3-dihydroxy-3-methylbutanoate (1.427 g, 9.6 mmol) in dichloromethane (10 ml) was added to a stirred suspension of pyridinium chlorochromate (3.235 g, 15.0 mmol) in dichloromethane (10 ml). A black precipitate formed. After 1.5 h dry ether (25 ml) was added and the supernatant liquor was decanted. The black solid residue was washed with ether (3x15 ml) and the combined ether-dichloromethane portions were filtered through florisil. Evaporation of the solvent under reduced pressure gave a colourless oil (159 mg). Proton NMR spectroscopy indicated a very complex mixture.

Attempted oxidation of methyl 2,3-dihydroxy-3-methylbutanoate using Jones reagent[61]

Concentrated sulphuric acid (23 ml) was added to a slurry of chromium trioxide (26.77 g, 0.268 mol) in water (20 ml) and diluted to a total volume of 100 ml with water.

Methyl 2,3-dihydroxy-3-methylbutanoate (973 mg, 6.57 mmol) was dissolved in acetone (20 ml). The chromic acid solution (2.1 ml, 5.63 mmol) was added dropwise. A dark green precipitate formed. Water (10 ml) was added, the supernatant liquor decanted and extracted with ethyl acetate (4x40 ml). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give a green oil. This was dissolved in ether and eluted through florisil with ether. The solution was evaporated under reduced pressure to give methyl 3-hydroxy-3-methyl-2-oxobutanoate as a pale yellow oil (324 mg, 2.2 mmol, 34%)

Attempted oxidation of methyl 2,3-dihydroxy-3-methylbutanoate via brominolysis of the dioxostannolan deivative

Methyl 2,3-dihydroxy-3-methylbutanoate (1.205 g, 8.14 mmol) and dibutyl tin oxide (2.075 g, 8.34 mmol) were heated together in boiling benzene for 8 h using a Dean-Stark apparatus for the azeotropic removal of water. The benzene was evaporated under reduced pressure and the solid residue was suspended in dichloromethane (10 ml). Bromine (0.45 ml, 8.77 mmol) was added dropwise to the stirred mixture. The deep red solution was set aside at room temperature for 12 h. The solvent was evaporated under reduced pressure, the oily residue was dissolved in carbon tetrachloride (2 ml) and applied to a

silica gel column (10 g, 60-120 mesh). The column was eluted sequentially with carbon tetrachloride (700 ml) and ethyl acetate (250 ml). The ethyl acetate portion was evaporated under reduced pressure to give a colourless oil (608 mg). Proton NMR indicated the presence of starting material, methyl 3-hydroxy-3-methyl-2-oxobutanoate and methyl 2-hydroxy-2-methyl-3-oxobutanoate in approximately equal amounts. The spectral data for these compounds are given pages 104 and 113 respectively.

Attempted tosylation of methyl 3-hydroxy-3-methyl-2-oxobutanoate (a).

Toluene-4-sulphonyl chloride (1.486 g, 7.8 mmol) was added to a cooled (ice bath) solution of methyl 3-hydroxy-3-methyl-2-oxobutanoate (1.035 g, 7.1 mmol) in dry pyridine (10 ml). The homogeneous solution was set aside at room temperature for 48 h. The pyridine was evaporated at high vaccuum/15^{o}C. The residue was dissolved in chloroform (30 ml). The solution was washed with 1 M hydrochloric acid (8x25 ml), dried ($MgSO_4$) and evaporated under reduced pressure to give toluene-4-sulphonyl chloride (968 mg) as identified by proton NMR.

Attempted tosylation of methyl 3-hydroxy-3-methyl-2-oxobutanoate (b).

To a stirred suspension of sodium hydride (80% dispersion in oil, 140 mg, 4.7 mmol) in 1,2-dimethoxyethane (DME) (5 ml) under nitrogen was added dropwise, over 5 minutes, a solution of methyl 3-hydroxy-3-methyl-2-oxobutanoate (592 mg, 4.05 mmol) and toluene-4-sulphonyl chloride (798 mg, 4.18 mmol) in DME (10 ml). The mixture was stirred vigorously for 20 minutes. Water (20 ml) was added and the solution was extracted with dichloromethane (5x30 ml). The combined extracts

were dried ($MgSO_4$) and evaporated under reduced pressure/30°C to give a yellow oil. Proton NMR indicated the presence of a very complex mixture.

Attempted mesylation of methyl 3-hydroxy-3-methyl-2-oxobutanoate

To a cooled solution (ice bath) of methyl 3-hydroxy-3-methyl-2-oxobutanoate (1.024 g, 7.0 mmol) and triethylamine (1 ml, 7.2 mmol) in dry dichloromethane (20 ml) was added methane sulphonyl chloride (0.6 ml, 7.5 mmol) with stirring. The solution was maintained at 0°C for 30 minutes and allowed to attain room temperature over 2 h. The solution was washed with 1 M hydrochloric acid (2x30 ml). The washings were back-extracted with dichloromethane (2x20 ml). The combined dichloromethane portions were dried ($MgSO_4$) and evaporated under reduced pressure to give a brown oil. Proton NMR indicated the presence of a very complex mixture.

Attempted bromination of methyl 3-hydroxy-3-methyl-2-oxobutanoate

To a solution of methyl 3-hydroxy-3-methyl-2-oxobutanoate (1.190 g, 8.15 mmol) in dry tetrahydrofuran (THF) (10 ml) was added carbon tetrabromide (5.440 g, 16.4 mmol) and triphenylphosphine (4.278 g, 16.3 mmol) with cooling from an ice bath. THF (20 ml) was added, the mixture stirred overnight at room temperature, flitered and evaporated under reduced pressure. The residue comprised a very complex mixture of compounds, as judged by proton NMR.

CHAPTER 7

Incubation of methyl 3-bromo-3-methyl-2-oxobutanoate with pig liver esterase

To water at 32°C was added a methanolic solution of methyl 3-bromo-3-methyl-2-oxobutanoate (100 µl, 79 mg/5 ml, 7.6 µmol). The pH was maintained at 7.2 by delivery of sodium hydroxide (107 mM) from an autotitrator. A rapid uptake of alkali (0.07 ml, 7.5 µmol) was observed. After 1 minute PLE (12 µl, 5 mg/ml, 8 units) was added and the pH was maintained at 7.2 as before. After a period of incubation aqueous ethyl butyrate (10 ml, 12.1 mM, 0.121 mmol) was added and the rate of hydrolysis was recorded by the uptake of alkali from the autotitrator at pH 7.2. The initial rate of hydrolysis of the ethyl butyrate was taken as a measure of the activity of the PLE.

Incubation time (minutes)	Observed activity (µmol EB/minute)	% of maximum activity
1	1.0	7
5	1.2	9
14	1.1	8
152	1.3	9
294	2.1	15
1200	7.1	50
1530	6.3	45
2430	6.4	45

EB = ethyl butyrate

Two control experiments were performed exactly as above:

a) pure methanol used in place of the methanolic methyl 3-bromo-3-methyl-2-oxobutanoate.

b) as (a) but using 1.13 mM sodium bromide solution in place of the water.

The same initial rate of hydrolysis of ethyl butyrate, 14.1 μmol/minute, was observed for both control experiments (a) and (b).

Hydrolysis of methyl 3-bromo-3-methyl-2-oxobutanoate followed by proton NMR spectroscopy

To 1 M pH 7.2 phosphate buffer (0.5 ml) in an NMR tube was added a solution of methyl 3-bromo-3-methyl-2-oxobutanoate (14 mg, 0.07 mmol) in d_4-methanol (50 μl). The proton NMR spectrum was determined immediately. Signals attributable to methyl 3-hydroxy-3-methyl-2-oxobutanoate alone were observed. After 10 minutes PLE (20 μl, 5 mg/ml, 13 units) was added and the solution incubated at 32°C. The proton NMR spectrum was determined periodically. Hydrolysis of the methyl ester was complete after 2 days.

Methyl 3-hydroxy-3-methyl-2-oxobutanoate:

^{1}H NMR, 220 MHz (H_2O/CH_3OH = δ 3.26 ppm): δ = 1.16 (s, 6 H, $C(CH_3)_2$); 3.75 ppm (s, 3 H, OCH_3).

3-Hydroxy-3-methyl-2-oxobutanoate:

^{1}H NMR, 220 MHz (H_2O/CH_3OH = δ 3.26 ppm): δ = 1.36 ppm (s, 6 H, $C(CH_3)_2$).

CHAPTER 8

Methyl 2,3-dihydroxy-2-methylbutanoate

To a solution of methyl tiglate (3.013 g, 27.2 mmol) dissolved in 98% formic acid (10 ml) was added an aqueous solution of hydrogen peroxide (30%, 4.5 ml, 44 mmol). The solution was maintained at 50°C for 16 h. The solvents were evaporated under reduced pressure/35°C and the residue co-evaporated with toluene (2x30 ml) under reduced pressure/35°C. The residue was subjected to flash chromatography eluting with ether-petrol 9:1. The diol ester was obtained as a pale yellow oil R_F (ether) 0.25 (2.682 g, 18.1 mmol, 67%). This was distilled in a Kugelrohr apparatus (140°C/10 mmHg) to give a colourless oil (2.497 g, 16.9 mmol, 62%).

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.16 (d, 3 H, J=7 Hz, $CHC\underline{H}_3$); 1.45 (s, 3 H, CCH_3); 2.24 (d, 1 H, J=8.5 Hz, $C\underline{H}(OH)$); 3.43 (s, 1 H, C(OH)); 3.8 ppm (complex, 4 H, $CHC\underline{H}_3$, $OC\underline{H}_3$).

IR (film): ν= 3475, 1735 cm^{-1}.

MS (+ve CI, ammonia): m/z= 166$(M+18)^+$, 149$(M+1)^+$.

GLC (10% SE30, 100°C); R_T= 490 s (100%).

Methyl 2-hydroxy-2-methyl-3-oxobutanoate (a)

Methyl 2,3-dihydroxy-2-methylbutanoate (515 mg, 3.5 mmol), calcium carbonate (370 mg, 3.7 mmol) and N-bromosuccinimide (640 mg, 3.6 mmol) were stirred together in dry carbon tetrachloride (10 ml) under nitrogen for 2 h with illumination and heat from a 100 W tungsten lamp. The mixture was filtered and the solid washed with carbon tetrachloride (20 ml). The combined carbon tetrachloride

portions were evaporated under reduced pressure to give the crude methyl 2-hydroxy-2-methyl-3-oxobutanoate (404 mg, 2.8 mmol 79%). This was distilled in a Kugelrohr apparatus (130°C/10 mmHg) to give a very pale yellow oil (295 mg, 2.02 mmol, 58%). Its spectral data were consistent with its formulation as methyl 2-hydroxy-2-methyl-3-oxobutanoate (see next page).

Methyl 2-hydroxy-2-methyl-3-oxobutanoate (b)

Methyl 2,3-dihydroxy-2-methylbutanoate (661 mg, 4.5 mmol) and dibutyl tin oxide (1.128 mg, 4.5 mmol) were heated together in boiling benzene (100 ml) for 8.5 h with azeotropic removal of water using a Dean-Stark apparatus. The benzene was evaporated under reduced pressure/40°C and the residue was subjected to high vacuum for 20 h. The resulting brown oil was suspended in dichloromethane (10 ml). Bromine (0.24 ml, 4.7 mmol) was added dropwise with stirring. A deep red homogeneous solution formed which became decolourised after 15 h. The solution was evaporated under reduced pressure/30°C and applied to a silica gel column (60-120 mesh, 10 g). This was eluted successively with carbon tetrachloride (500 ml) and ethyl acetate (200 ml). The ethyl acetate was dried ($MgSO_4$) and evaporated under reduced pressure/35°C to give the crude methyl 2-hydroxy-2-methyl-3-oxobutanoate as a pale yellow oil (600 mg, 4.1 mmol, 92%). This was distilled in a Kugelrohr apparatus (130°C/10 mmHg) to give a colourless oil (455 mg, 3.1 mmol, 70%). Its spectral data were consistent with its formulation as methyl 2-hydroxy-2-methyl-3-oxobutanoate (see next page).

Oxidation of methyl tiglate with potassium manganate (VII)

To a stirred solution of methyl tiglate (2.148 g, 18.8 mmol) and acetic acid (2.5 ml) in acetone (150 ml) and water at -10°C was added dropwise a solution of potassium manganate (VII) (6.5 g, 41 mmol) in acetone (130 ml) and water (50 ml). After stirring for 1 h at -10°C, the mixture was filtered through celite and the acetone evaporated under reduced pressure/35°C. The aqueous solution was extracted with dichloromethane (3x60 ml). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure/30°C. The residue was co-evaporated with carbon tetrachloride (2x30 ml) under reduced pressure/30°C to give a colourless oil (2.314 g, 15.8 mmol, 84%). This was distilled in a Kugelrohr apparatus (120°C/12 mmHg) to give methyl 2-hydroxy-2-methyl-3-oxobutanoate (2.195 g, 15.0 mmol, 80%).[14]

The reaction was repeated on the same scale at a range of temperatures.

Temperature/°C	Yield/%
50	38
25	50
20	67

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ = 1.63 (s, 3 H, C(OH)CH_3); 2.31 (s, 3 H, $COCH_3$); 3.83 (s, 3 H, OCH_3); 4.25 ppm (s, 1 H, OH). Lit.[14] 1.58 (s); 1.23 (s); 3.78 (s); 4.18 ppm (s).

^{13}C NMR, 22.63 MHz ($CDCl_3$/$CDCl_3$ = δ 77.0 ppm): δ = 21.4 (C(OH)CH_3); 23.8 (OCH_3); 52.9 ($COCH_3$); 80.8 (C(OH)); 171.6 (CO_2CH_3); 204.7 ppm ($COCH_3$).

IR (film): ν = 3470, 1750(sh), 1730 cm^{-1}.

MS (+ve CI, ammonia): m/z= 164$(M+18)^+$, 147$(M+1)^+$.

High resolution MS: $C_6H_{11}O_4$, (M+H), calc. 147.0657, found 147.0656.

GLC (3% SE30, 80°C); R_T= 270 s.

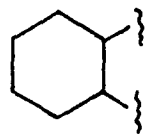
Elemental analysis: Found: C, 49.25; H, 7.13. $C_6H_{10}O_4$ requires C, 49.31; H, 6.85%.

Synthesis of 2-hydroxy-3-oxocarboxylic esters: general procedure;

To a stirred soloution of the $\alpha\beta$-unsaturated ester (20 mmol) and acetic acid (7 ml) dissolved in water (90 ml) and acetone (300 ml), maintained at -10°C using an ice-salt bath, was added potassium manganate (VII) (5.5 g, 34.8 mmol) in portions such that the reaction temperature remained below -10°C. The mixture was stirred for 1 h at -10°C. The black precipitate of manganese dioxide was filtered off through celite. The solid was washed with acetone (100 ml). The combined solutions were evaporated under reduced pressure/20°C to remove the acetone component. The aqueous solution was extracted with dichloromethane (4x100 ml). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure to furnish the crude ester which was purified by distillation in a Kugelrohr apparatus.

The following 2-hydroxy-3-oxocarboxylic esters were synthesised as above.

(32) (33)

Entry	R^1	R^2	R^3	Temp. /°C	bp /°C/mmHg	Yield /%
33a[14]	Me	Me	Me	-10	74-76/15	80
33b	Me	Et	Et	-10	120/15	80
33c	Et	Et	Et	-12	100/0.5	87
33d	Et	Me	Et	-10	110/2	82
33e	Pr	Me	Et	-10	80/0.25	70
33f	Bu	Me	Et	-12	110/0.2	82
33g	t-Bu	Me	Et	-12	90/0.5	80
33h	Ph	Me	Et	-10		68
33i	p-NO_2Ph	Me	Et	-10	mp 84-85°C	83
33j	p-MeOPh	Me	Et	-10		86
33k[15]			Me	-12	120/0.2	62

Ethyl 2-ethyl-2-hydroxy-3-oxobutanoate (33b)

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ = 0.87 (t, 3 H, J=7.3 Hz, C(OH)$CH_2\underline{CH_3}$); 1.30 (t, 3 H, $OCH_2\underline{CH_3}$); 2.05 (m, 2 H, C(OH)$\underline{CH_2}CH_3$); 2.27 (s, 3 H, $COCH_3$); 4.15 (s, 1 H, OH); 4.28 ppm (q, 2 H, J=7.1 Hz, $OCH_2\underline{CH_3}$).

^{13}C NMR, 22.63 MHz ($CDCl_3$/$CDCl_3$ = δ 77.0 ppm): δ = 7.1 (C(OH)$CH_2\underline{CH_3}$); 13.8 ($OCH_2\underline{CH_3}$); 24.4 (C(OH)$\underline{CH_2}$); 28.1 ($CO\underline{CH_3}$); 62.2 (OCH_2); 84.3 (C(OH)); 170.8 ($\underline{C}O_2CH_2$); 204.7 ppm ($\underline{C}OCH_3$).

IR (film): ν = 3480, 1750(sh), 1720 cm^{-1}.

MS (+ve CI, ammonia): m/z= 192$(M+18)^+$, 175$(M+1)^+$.

High resolution MS: $C_8H_{15}O_4$, (M+H), calc. 175.0970, found 175.0975

GLC (10% SE30, 120°C); R_T= 430 s.

Elemental analysis: Found: C, 54.89; H, 8.36. $C_8H_{14}O_4$ requires C, 55.16; H, 8.10%.

Ethyl 2-ethyl-2-hydroxy-3-oxopentanoate (33c)

^1H NMR, 220MHz ($CDCl_3$/TMS): δ= 0.86 (t, 3 H, J=7.6 Hz, $C(OH)CH_2\underline{CH_3}$); 1.08 (t, 3 H, J=7.5 Hz, $COCH_2\underline{CH_3}$); 1.30 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 2.05 (m, 2 H, $C(OH)\underline{CH_2}$); 2.65 (m, 2 H, $CO\underline{CH_2}$), 4.25 ppm (complex, 3 H, OH, OCH_2).

^{13}C NMR, 22.63 MHz ($CDCl_3/CDCl_3$ = δ 77.0 ppm): δ= 7.2 ($C(OH)CH_2\underline{CH_3}$, $COCH_2\underline{CH_3}$); 14.0 ($OCH_2\underline{CH_3}$); 28.3 ($C(OH)\underline{CH_2}$); 30.1 ($\underline{CH_2}CO$); 62.3 (OCH_2); 84.2 (C(OH)); 171.1 ($\underline{CO_2}CH_2$); 207.7 ppm ($\underline{CO}CH_2$).

IR (film): ν = 3480, 1745, 1720 cm^{-1}.

MS (EI): m/z= 188$(M)^{+\cdot}$.

High resolution MS: $C_9H_{16}O_4$, calc. 188.1049, found 188.1049.

GLC (3% SE30, 110°C); R_T= 310 s.

Elemental analysis: Found: C,57.65; H, 8.81. $C_9H_{16}O_4$ requires C, 57.45; H, 8.51%.

Ethyl 2-hydroxy-2-methyl-3-oxopentanoate (33d)

^1H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.09 (t, 3 H, J=7.5 Hz, $COCH_2\underline{CH_3}$); 1.29 (t, 3 H, $OCH_2\underline{CH_3}$); 1.59 (s, 3 H, $C(OH)\underline{CH_3}$); 2.65 (m, 2 H, $COCH_2$); 4.25 ppm (complex, 3 H, OH, OCH_2).

^{13}C NMR, 22.63 MHz ($CDCl_3/CDCl_3$ = δ 77.0 ppm): δ= 7.4 ($COCH_2\underline{CH_3}$); 13.6 ($OCH_2\underline{CH_3}$); 21.5 ($C(OH)\underline{CH_3}$); 29.5 ($\underline{CH_2}CO$); 62.1 (OCH_2); 80.5 (C(OH)); 171.4 ($\underline{CO_2}CH_2$); 207.4 ppm ($\underline{CO}CH_2$).

IR (film): ν = 3475, 1740(sh), 1725 cm^{-1}.

MS (EI): m/z= 174$(M)^{+\cdot}$.

High resolution MS: $C_8H_{14}O_4$, calc. 174.0892, found 174.0877.

GLC (10% SE30, 130°C); R_T= 340 s.

Elemental analysis: Found: C, 55.33; H, 8.18. $C_8H_{14}O_4$ requires C, 55.17; H, 8.05%.

Ethyl 2-hydroxy-2-methyl-3-oxohexanoate (33e)

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ= 0.92 (t, 3 H, J=7.6 Hz, $CH_2CH_2\underline{CH_3}$); 1.28 (t, 3 H, J=7.1 Hz, $COCH_2\underline{CH_3}$); 1.60 (complex, 5 H, $C(OH)\underline{CH_3}$, $CH_2\underline{CH_2}CH_3$); 2.57 (m, 2 H, $\underline{CH_2}CH_2CH_3$); 4.25 ppm (complex, 3 H, OCH_2, OH).

^{13}C NMR, 22.63 MHz ($CDCl_3/CDCl_3$ = δ77.0 ppm): δ= 13.2 ($CH_2CH_2\underline{CH_3}$); 13.8 ($OCH_2\underline{CH_3}$); 16.7 ($CH_2\underline{CH_2}CH_3$); 21.5 ($C(OH)\underline{CH_3}$); 38.0 ($\underline{CH_2}CH_2CH_3$); 62.2 (OCH_2); 80.7 (C(OH)); 171.4 ($\underline{CO_2}CH_2$); 206.8 ppm ($\underline{CO}CH_2$).

IR (film): ν = 3470, 1740(sh), 1720 cm^{-1}.

MS (EI): m/z= 188$(M)^{+\cdot}$.

High resolution MS: $C_9H_{16}O_4$, calc. 188.1048, found 188.1034.

GLC (10% SE30, 140°C); R_T= 390 s.

Elemental analysis: Found: C, 57.26; H, 8.63. $C_9H_{16}O_4$ requires C, 57.45; H, 8.51%.

Ethyl 2-hydroxy-2-methyl-3-oxoheptanoate (33f)

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ= 0.90 (t, 3 H, J=7.5 Hz, $CH_2CH_2CH_2\underline{CH_3}$); 1.28 (complex, 5 H, $OCH_2\underline{CH_3}$, $CH_2CH_2\underline{CH_2}CH_3$); 1.58 (complex, 5 H, $C(OH)\underline{CH_3}$, $CH_2\underline{CH_2}CH_2CH_3$); 2.60 (m, 2 H, $\underline{CH_2}CH_2CH_2CH_3$); 4.25 ppm (complex, 3 H, OCH_2, OH)

^{13}C NMR, 22.63 MHz ($CDCl_3/CDCl_3$ = δ77.0 ppm): δ= 13.7 ($OCH_2\underline{CH_3}$, $CH_2CH_2CH_2\underline{CH_3}$); 21.6 ($CH_2CH_2\underline{CH_2}CH_3$); 21.9 ($CH_2\underline{CH_2}CH_2CH_3$); 25.4 ($C(OH)\underline{CH_3}$); 35.9 ($CO\underline{CH_2}$); 62.2 ($OCH_2$); 80.7 (C(OH)); 171.3 ($\underline{CO_2}CH_2$); 206.9 ppm ($\underline{CO}CH_2$).

IR (film): ν = 3470, 1740(sh), 1725 cm^{-1}.

MS (EI): m/z= 202$(M)^{+\cdot}$.

High resolution MS: $C_{10}H_{18}O_4$, calc. 202.1205, found 202.1196.

GLC (3% SE30, 130°C); R_T= 260 s.

Elemental analysis: Found: C, 50.10; H, 9.19. $C_{10}H_{18}O_4$ requires C, 59.40; H, 8.91%.

Ethyl 2-hydroxy-3-oxo-2,4,4-trimethylpentanoate (33g)

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.24 (s, 9 H, $C(CH_3)_3$); 1.29 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 1.57 (s, 3 H, $C(OH)\underline{CH_3}$); 4.13 (s, 1 H, OH); 4.26 ppm (q, 2 H, J=7.1 Hz, OCH_2).

^{13}C NMR, 22.63 MHz ($CDCl_3/CDCl_3$ = δ 77.0 ppm): δ= 13.8 ($COCH_2\underline{CH_3}$); 23.9 ($C(OH)\underline{CH_3}$); 27.4 ($C(\underline{CH_3})_3$); 44.6 ($\underline{C}(CH_3)_3$); 62.3 (OCH_2); 80.7 (C(OH)); 172.3 ($\underline{CO_2}CH_2$); 210.5 ppm ($\underline{C}OC(OH)$).

IR (film): ν = 3500, 1740, 1710 cm^{-1}.

MS (+ve CI, ammonia): m/z= 220$(M+18)^+$, 203$(M+1)^+$.

High resolution MS: $C_{10}H_{18}O_4$, calc. 202.1205, found 202.1172.

GLC (3% SE30, 120°C); R_T= 700 s.

Elemental analysis: Found: C, 59.60; H, 9.27. $C_{10}H_{18}O_4$ requires C, 59.40; H, 8.91%.

Ethyl 2-hydroxy-2-methyl-3-oxo-3-phenylpropanoate (33h)

Pale yellow oil isolated by flash chromatography eluting with ethyl acetate-petrol 1:4 R_F 0.37.

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.15 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 1.74 (s, 3 H, $C(OH)\underline{CH_3}$); 4.24 (q, 2 H, J=7.1 Hz, OCH_2); 4.56 (bs, 1 H, OH); 7.48 (m, 2 H, Ph); 7.61 (m, 1 H, Ph); 8.03 ppm (m, 2 H, Ph).

^{13}C NMR, 22.63 MHz ($CDCl_3/CDCl_3$ = δ 77.0 ppm): δ= 13.6 ($OCH_2\underline{CH_3}$); 23.3 ($C(OH)\underline{CH_3}$); 62.2 (OCH_2); 79.5 (C(OH)); 128.4, 129.3 ($\underline{CH}\underline{CH}C(CO)\underline{CH}\underline{CH}$); 133.4 ($\underline{C}HCHCHCCO$); 160.4 ($COC\underline{C}H$); 172.3 ($\underline{CO_2}CH_2$); 195.9 ppm (CO).

IR (film): ν = 3475, 1740, 1695, 1600 cm^{-1}.

MS (+ve CI, ammonia): m/z= 223$(M+1)^+$.

High resolution MS: $C_{12}H_{15}O_4$ (M+H), calc. 223.0971, found 223.0981.

GLC (3% SE30, 150°C); R_T= 456 s (100%).

Elemental analysis: Found: C, 64.68; H, 6.40. $C_{12}H_{14}O_4$ requires C, 64.86; H, 6.31%.

Ethyl 2-hydroxy-2-methyl-3-oxo-3-(4-nitrophenyl)-propanoate (33i)

Pale yellow solid recrystallised to constant melting point (84-85°C) from ethyl acetate-petrol.

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.19 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 1.75 (s, 3 H, CCH_3); 4.27 (q, 2 H, J=7.1 Hz, OCH_2); 8.26 ppm (complex, 4 H, Ar).

^{13}C NMR, 100.62 MHz ($CDCl_3$/$CDCl_3$ = δ77.0 ppm): δ= 13.9 ($OCH_2\underline{CH_3}$); 23.4 ($C(OH)\underline{CH_3}$); 63.1 (OCH_2); 80.0 (C(OH)); 123.6 ($\underline{C}HC(NO_2)\underline{C}H$); 130.5 ($\underline{C}HC(CO)\underline{C}H$); 138.7 ($CH\underline{C}(CO)CH$); 150.2 ($NO_2C$); 172.5 ($\underline{C}O_2CH_2$); 194.1 ppm (CO).

IR (film): ν = 3480, 3105, 1745, 1725, 1695, 1595 cm^{-1}.

MS (+ve CI, ammonia): m/z= 285$(M+18)^+$, 268$(M+1)^+$.

High resolution MS: $C_{12}H_{17}N_2O_6$, $(M+NH_4)$, calc. 285.1087, found 285.1050.

Elemental analysis: Found: C, 53.84; H, 4.89; N, 5.32. $C_{12}H_{13}NO_6$ requires C, 53.93; H, 4.87; N, 5.24%.

Ethyl 2-hydroxy-2-methyl-3-(4 methoxyphenyl)-3-oxopropanoate (33j)

Pale yellow viscous oil isolated by flash chromatography eluting with ethyl acetate-petrol 1:1 R_F 0.46.

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.16 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 1.74 (s, 3 H, $C(OH)\underline{CH_3}$); 3.89 (s, 3 H, $PhOCH_3$); 4.23 (q, 2 H, J=7.1 Hz, OCH_2); 4.69 (bs, 1 H, OH); 6.96 (m, 2 H, Ar); 8.04 ppm (m, 2 H, Ar).

^{13}C NMR, 22.63 MHz ($CDCl_3/CDCl_3$ = δ77.0 ppm): δ= 13.7 ($OCH_2\underline{C}H_3$); 23.6 ($C(OH)\underline{C}H_3$); 55.4 (OCH_3); 62.1 (OCH_2); 79.2 (C(OH)); 113.7 ($\underline{C}HC(OCH_3)\underline{C}H$); 125.7 ($CO\underline{C}CH_3$); 131.9 ($\underline{C}HC(CO)\underline{C}H$); 163.8 ($CH_3O\underline{C}$); 172.2 ($\underline{C}O_2CH_2$); 194.2 ppm (CO).

IR (film): ν= 3470, 1740, 1685, 1605, 1515 cm^{-1}.

MS (EI): m/z= 253$(M+1)^{\cdot+}$.

High resolution MS: $C_{13}H_{17}O_5$, (M+H), calc. 253.1076, found 253.1054.

GLC (3% SE30, 180°C); R_T= 474 s (97%).

Elemental analysis: Found: C, 61.96; H, 6.52. $C_{13}H_{16}O_5$ requires C, 61.90; H, 6.35%.

Methyl 1-hydroxycyclohexan-2-one carboxylate (33k)[15]

^1H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.8 (complex, 6 H, $C(OH)\underline{CH_2}\underline{CH_2}\underline{CH_2}$); 2.15 (m, 2 H, $COCH_2$); 3.82 (s, 3 H, OCH_3); 4.39 ppm (s, 1 H, C(OH)).

^{13}C NMR, 22.63 MHz ($CDCl_3/CDCl_3$ = δ 77.0 ppm): δ= 21.7 ($C(OH)CH_2\underline{CH_2}$); 26.8 ($COCH_2\underline{CH_2}$); 37.5 ($C(OH)\underline{CH_2}$); 38.6 ($COCH_2$); 52.8 ($OCH_3$); 80.6 (C(OH)); 170.5 ($\underline{C}O_2CH_3$); 207.1 ppm ($\underline{C}OCH_2$).

IR (film): ν= 3460, 1745(sh), 1720 cm^{-1}.

MS (EI): m/z= 172$(M)^{\cdot+}$.

High resolution MS: $C_8H_{12}O_4$, calc. 172.0736, found 172.0727.

GLC (10% SE30, 130°C); R_T= 640 s.

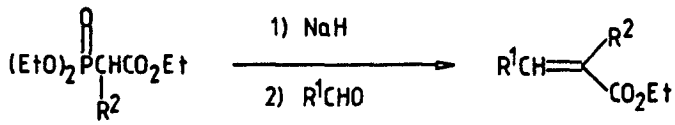
Elemental analysis: Found: C, 56.14; H, 7.16. $C_8H_{12}O_4$ requires C, 55.81; H, 6.98%.

Synthesis of $\alpha\beta$-unsaturated esters: general procedure;

To a stirred suspension of sodium hydride (2.28g, 95 mmol) in 1,2-dimethoxyethane (DME) (30 ml) under nitrogen was added a solution of the phosphonate (100 mmol) in DME (30ml), with cooling from an ice

bath, at a rate such the reaction temperature remained below 35°C. After stirring at room temperature for a further 15 minutes a solution of the aldehyde (110 mmol) in DME (30 ml) was added, in the same manner. Upon completion of the reaction a jelly-like precipitate was deposited. The mixture was poured onto water (150ml) and extracted with dichloromethane (4 100ml). The combined dichloromethane extracts were dried ($MgSO_4$) and evaporated under reduced pressure to furnish the crude $\alpha\beta$-unsaturated ester as a mixture of Z- and E-isomers. The mixture was purified by distillation.

The following $\alpha\beta$-unsaturated esters were synthesised by the above procedure.



(34)

Entry	R^1	R^2	Time /h	Yield /%	E-isomer /%	bp /°C/mmHg
34a[65]	Me	Et	2	53	24	61-63/15
34b[66]	Et	Et	6	72	54	63-69/15
34c[67]	Et	Me	20	62	87	60-62/15
34d[65]	Pr	Me	5	68	86	110/15
34e[68]	Bu	Me	15	64	87	140/15
34f[69]	t-Bu	Me	48	71	54	120/15
34g	Ph	Me	15	81	100	84-85/0.25
34h	p-NO_2Ph	Me	24	38	100	mp 78-79°C
34i	p-MeOPh	Me	30	67	100	130/0.5

Ethyl 2-ethylbut-2-eneoate (34a)

Lit.[65] bp 97-102°C/65 mmHg.

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.01 (m, 3 H, $CCH_2\underline{CH_3}$); 1.30 (m, 3 H, $OCH_2\underline{CH_3}$); 1.81 (d, 2 H, J=7.6 Hz, E $CH\underline{CH_3}$); 1.96 (d, 1 H, J=7.1 Hz, Z $CH\underline{CH_3}$); 2.33 (m, 2 H, CCH_2); 4.21 (overlapping q, 2 H, J=7.1 Hz, OCH_2); 5.97 (q, 0.3 H, J=7.1 Hz, Z CH); 6.84 ppm (q, 0.7 H, J=7.6 Hz, E CH).

IR (film):ν= 1715, 1655 cm^{-1}.

MS (EI): m/z= 142$(M)^{\ddagger}$.

High resolution MS: $C_8H_{14}O_2$, calc. 142.0993, found 142.0992.

GLC (10% SE30, 110°C); R_T= 305(E), 358(Z) s.

Ethyl 2-ethylpent-2-eneoate (34b)

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.05 (complex, 6 H, $CCH_2\underline{CH_3}$, $CHCH_2\underline{CH_3}$); 1.31 (m, 3 H, $OCH_2\underline{CH_3}$); 2.25 (complex, 4 H, CCH_2, $CH\underline{CH_2}$); 4.20 (complex, 2 H, OCH_2), 5.86 (t, 0.4 H, J=7.6 Hz, Z CH); 6.75 ppm (t, 0.6 H, J=7.6 Hz, E CH).

IR (film):ν= 1715, 1645 cm^{-1}.

MS (EI): m/z= 156$(M)^{\ddagger}$.

High resolution MS: $C_9H_{16}O_2$, calc. 156.1150, found 156.1159.

GLC (10% SE30, 120°C); R_T= 323(Z), 399(E) s.

Ethyl 2-methylpent-2-eneoate (34c)

Lit.[67] bp 60-80°C/12 mmHg.

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.05 (t, 3 H, J=7.5 Hz, $CHCH_2\underline{CH_3}$); 1.30 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 1.83 (s, 2.5 H, E CH_3); 1.89 (s, 0.5 H, Z CH_3); 2.19 (m, 1.7 H, E $CH\underline{CH_2}$); 2.45 (m, 0.3 H, Z $CH\underline{CH_2}$); 4.21 (q, 2 H, J=7.1 Hz; OCH_2); 5.94 (m, 0.15 H, Z CH); 6.77 ppm (m, 0.85 H, E CH).

IR (film): ν = 1710, 1650 cm^{-1}.

MS (EI): m/z= 142$(M)^{+\cdot}$.

GLC (10% SE30, 110°C); R_T= 330(E), 440(Z) s.

Ethyl 2-methylhex-2-eneoate (34d)

Lit.[65] bp 107-112°C/53 mmHg.

^1H NMR, 220MHz ($CDCl_3$/TMS): δ = 0.94 (t, 3 H, J=8 Hz, $CH_2CH_2\underline{CH_3}$); 1.29 (t, 3 H, J=7 Hz, $OCH_2\underline{CH_3}$); 1.46 (m, 2 H, $CH_2\underline{CH_2}CH_3$); 1.83 (s, 2.6 H, E CH_3); 1.89 (s, 0.3 H, Z CH_3); 2.15 (m, 1.75 H, E $\underline{CH_2}CH_2CH_2$); 2.41 (m, 0.25 H, Z $\underline{CH_2}CH_2CH_3$); 4.20 (q, 2 H, J=7 Hz, OCH_2); 5.93 (m, 0.15 H, Z CH_3); 6.77 ppm (m, 0.85 H, E CH).

IR (film): ν = 1715, 1655 cm^{-1}.

MS (EI): m/z= 156$(M)^{+\cdot}$.

High resolution MS: $C_9H_{16}O_2$, calc. 156.1150, found 156.1136.

GLC (10% SE30, 120°C); R_T= 377(Z), 498(E) s.

Ethyl 2-methylhept-2-eneoate (34e)

Lit.[68] bp 69-71°C/3 mmHg.

^1H NMR, 220MHz ($CDCl_3$/TMS): δ = 0.93 (t, 3 H, J=7.1 Hz, $CH_2CH_2CH_2\underline{CH_3}$); 1.4 (complex, 7 H, $CH_2CH_2\underline{CH_2}CH_3$, $OCH_2\underline{CH_3}$); 1.84 (s, 2.5 H, E CH_3); 1.89 (s, 0.5 H, Z CH_3); 2.18 (m, 1.6 H, E $\underline{CH_2}CH_2CH_2CH_3$); 2.45 (m, 0.4 H, Z $\underline{CH_2}CH_2CH_2CH_3$); 4.20 (q, 2 H, J=7.1 Hz, OCH_2); 5.95 (m, 0.2 H, Z CH); 6.77 ppm (m, 0.8 H, E CH).

IR (film): ν = 1715, 1655 cm^{-1}.

MS (EI): m/z= 170$(M)^{+\cdot}$.

High resolution MS: $C_{10}H_{18}O_2$, calc. 170.1307, found 170.1338.

GLC (3% OV17, 120°C); R_T= 208(Z), 299(E) s.

Ethyl 2,4,4-trimethylpent-2-eneoate (34f)

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.09 (s, 4.3 H, Z $C(CH_3)_3$); 1.18 (s, 4.7 H, E $C(CH_3)_3$); 1.30 (overlapping t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 1.90 (d, 1.4 H, J=1.6 Hz, Z CCH_3); 1.96 (d, 1.6 H, J=1.6 Hz, E CCH_3); 4.20 (overlapping q, 2 H, J=7.6 Hz, OCH_2); 5.47 (s, 0.5 H, Z CH); 6.83 ppm (s, 0.5 H, E CH).

IR (film): ν = 1725, 1710, 1645 cm^{-1}.

MS (EI): m/z= 170$(M)^{+\cdot}$.

High resolution MS: $C_{10}H_{18}O_2$, calc. 170.1307, found 170.1306.

GLC (10% SE30, 130°C); R_T= 240(E), 370(Z) s.

Ethyl 2-methyl-3-phenylprop-2-eneoate (34g)

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.35 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 2.13 (d, 3 H, J=1.6 Hz, CCH_3); 4.29 (q, 2 H, J=7.1 Hz, OCH_2); 7.41 (complex, 5 H, Ph); 7.73 (d, 1 H, J=1.6 Hz, CH).

IR (film): ν = 3060, 1710, 1640 cm^{-1}.

MS (EI): m/z= 190$(M)^{+\cdot}$.

High resolution MS: $C_{12}H_{14}O_2$, calc, 190.0994, found 190.0996.

GLC (3% SE30, 150°C); R_T= 397 s (95%).

Ethyl 2-methyl-3-(4-nitrophenyl)-prop-2-eneoate (34h)

The aldehyde component, 4-nitrobenzaldehyde, was added as a solid, in one portion, to the lithiated phosphonate. The reaction was exothermic and required external cooling with an ice-bath. After work up as above the ester was obtained as a yellow crystalline solid which was recrystallised to constant melting point (78-79°C) from ethyl acetate-petrol. A substantial amount of the ester co-crystallised with unreacted 4-nitrobenzaldehyde.

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.37 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 2.13

(d, 3 H, J=1.7 Hz, CCH_3); 4.32 (q, 2 H, J=7.1 Hz, OCH_2); 7.56 (m, 2 H, Ar); 7.73 (bs, 1 H, CH, 8.28 ppm (m, 2 H, Ar).

IR (film):ν = 3120, 1700, 1630, 1600 cm^{-1}.

MS (EI): m/z= 235$(M)^{+\cdot}$.

High resolution MS: $C_{12}H_{13}NO_4$, calc. 235.0844, found, 235.0841.

Elemental analysis: Found: C, 61.29; H, 5.56; N, 5.79. $C_{12}H_{13}NO_4$ requires C, 61.28; H, 5.53; N, 5.96%.

Ethyl 2-methyl-3-(4-methoxyphenyl)prop-2-eneoate (34i)

^1H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.36 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 2.16 (d, 3 H, J=1.6 Hz, CCH_3); 3.87 (s, 3 H, OCH_3); 4.30 (q, 2 H, J=7.1 Hz, OCH_2); 6.98 (m, 2 H, Ar); 7.46 (m, 2 H, Ar); 7.73 ppm (bs, 1 H, CH).

IR (film):ν= 1705, 1605, 1510 cm^{-1}.

MS (EI): m/z= 220$(M)^+$, 191$(M-Et)^+$, 175$(M-OEt)^+$, 146$(M-CO_2Et)^+$.

High resolution MS: $C_{13}H_{16}O_3$, calc. 220.1100, found 220.1109.

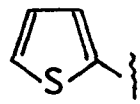
GLC (3% SE30, 180°C); R_T= 408 s (98%).

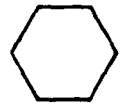
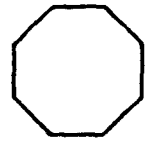
CHAPTER 9

Synthesis of αβ-unsaturated carboxylic esters

The following αβ-unsaturated esters were synthesised from triethyl phosphonoethanoate and the appropriate ketone via the phosphonate modification to the Wittig reaction as detailed in chapter 8.

(45)

Entry	R	R^1	Scale /mmol	Reaction time/h	Yield /%
45a[107]	Pr	Pr	93	190	64
45b	Bu	Bu	87	72	66
45c[108]	Ph	Ph	9	48	70
45d[109]	i-Pr	Me	76	6.5	70
45e[110]	Ph	Me	127	5	75
45f[111]		Me	86	72	51
45g[112]			68	20	70
45h[81]			177	15	66

Entry	R R^1	Scale /mmol	Reaction time/h	Yield /%
45i[64]		77	17	76
45j[81]		46	20	74
45k[81]		149	168	74

Ethyl 3-propylhex-2-eneoate (45a)

Colourless oil. bp 92-94/15 mmHg. Lit.[107] bp 166.5°C/28 mmHg.

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ = 1.94 (complex, 6 H, butyl CH_3); 1.28 (t, 3 H, J=7.6 Hz, $OCH_2\underline{CH_3}$); 1.50 (complex, 4 H, butyl CH_2); 2.13 (m, 2 H, butyl CH_2); 2.57 (m, 2 H, butyl CH_2); 4.15 (q, 2 H, J=8.0 Hz, OCH_2); 5.64 ppm (bs, 1 H, CH).

IR (film): ν = 1715, 1645 cm^{-1}

MS (EI): m/z=184$(M)^+_\bullet$, 156$(M-Et)^+$, 139$(M-OEt)^+$.

High resolution MS: $C_{11}H_{20}O_2$, calc. 184.1463, found 184.1459.

GLC (3% SE30, 120°C); R_T = 356 s (97%).

Ethyl 3-butylhept-2-enoate (45b)

Colourless oil. bp 116-118/15 mmHg

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ = 0.93 (complex, 6 H, butyl CH_3); 1.2-1.65 (complex, 11 H, butyl CH_2, $OCH_2\underline{CH_3}$); 2.15, 2.63 (m, 2 H, CCH_2); 4.17 (q, 2 H, J=7 Hz, $O\underline{CH_2}CH_3$); 5.67 ppm (bs, 1 H, CH).

IR (film): ν = 1720,1645 cm^{-1}.

MS (EI): m/z=212$(M)^+_\bullet$, 183$(M-Et)^+$, 167$(M-OEt)^+$, 155$(M-Bu)^+$.

High resolution MS: $C_{10}H_{18}O_2$, calc. 170.1307, found 170.1338.

GLC (3% SE30, 130°C); R_T= 573 s (100%).

Ethyl 3,3-diphenylprop-2-enoate (45c)

Colourless oil. Kugelrohr distilled; 220°C/1 mmHg. Lit.[108] bp 152-156°C/0.1 mmHg.

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ= 1.11 (t, 3 H, J=7.5 Hz, $OCH_2\underline{CH_3}$); 4.07 (q, 2 H, J=7.5 Hz, OCH_2); 6.38 (s, 1 H, CH); 7.3 ppm (complex, 10 H, Ph).

IR (film): ν = 3045, 1720, 1660, 1615, 1595 cm^{-1}.

MS (EI): m/z=252(M)$^+\cdot$, 223(M-Et)$^+$, 207(M-OEt)$^+$, 178(M-COOEt)$^+$.

High resolution MS: $C_{17}H_{16}O_2$, calc. 252.1151, found 252.1151.

GLC (3% SE30, 210°C); R_T= 254 s (94%).

Ethyl 3,4-dimethylpent-2-enoate (45d)

Reaction mixture refluxed for 6.5h. Product Kugelrohr distilled; colourless oil bp 110°C/15 mmHg. Lit.[109] bp 46-48°C/0.7 mmHg.

Data for E isomer.

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ= 1.07 (d, 6 H, J=6.9 Hz, $CH(\underline{CH_3})_2$); 1.28 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 2.14 (s, 3 H, $CHC\underline{CH_3}$); 2.35 (m, 1 H, $\underline{CH}(CH_3)_2$); 4.16 (q, 2 H, J=7.1 Hz, $OC\underline{H_2}CH_3$); 5.70 ppm (bs, 1 H, CH).

IR (film): ν= 1715, 1645 cm^{-1}.

MS (EI): m/z= 156(M)$^+\cdot$, 127(M-Et)$^+$, 111(M-OEt)$^+$, 83(M-COOEt)$^+$.

High resolution MS: $C_9H_{16}O_2$, calc. 156.1150, found 156.1148.

GLC (3% SE30, 120°C); R_T= 387 s (E, 85%), 444 (Z, 15%). (Purity 98%)

Ethyl 3-phenylbut-2-enoate (45e)

Colourless oil. bp 92°C/0.3 mmHg. Lit.[110] bp 127°C/10 mmHg.

^{1}H NMR, 220 MHz($CDCl_3$/TMS): δ= 1.3 (complex, 3 H, $OCH_2\underline{CH_3}$); 2.58 (s, 3 H, $CHC\underline{CH_3}$); 4.20 (m, 2 H, $OC\underline{H_2}CH_3$); 6.15 (bs, 1 H, CH); 7.40 ppm (complex, 5 H, Ph).

IR (film): ν = 1740, 1715, 1630 cm^{-1}

MS (EI): m/z= 190$(M)^{+\cdot}$, 161$(M-Et)^{+}$, 145$(M-OEt)^{+}$.

High resolution MS: $C_{12}H_{14}O_2$, calc. 190.0993, found 190.0986.

GLC (3% SE30, 150°C); R_T= 190 s (Z, 13%), 320 s (E, 87%)

Ethyl 3-methyl-3-(2-thienyl)but-2-enoate (45f)

Isolation by flash chromatography eluting with 20% ether in petrol. E isomer, R_F=0.6, Z isomer, R_F=0.5.

Data for E isomer.

^1H NMR, 220 MHz ($CDCl_3$/TMS): δ = 1.32 (t, 3 H, J=7.1, $OCH_2\underline{CH_3}$); 2.63 (s, 3 H, $CHCCH_3$); 4.22 (q, 2 H, J=7.1 Hz, OCH_2CH_3); 6.28 (s, 1 H, CH); 7.07 (dd, 1 H, J=4.9, 4.9 Hz, SCHCHCH); 7.3 ppm (complex, 2 H, SCHCHCH).

IR (film): ν = 3105, 1710, 1615, 1520 cm^{-1}.

MS (EI): m/z= 196$(M)^{+\cdot}$, 124$(M-COOEt)^{+}$.

High resolution MS: $C_{10}H_{12}SO_2$, calc. 196.0558, found 196.0575.

GLC (3% SE30, 150°C); R_T= 300 s (Z, 15%), 400 s(E,85%).

Ethyl cyclobuylidene ethanoate (45g)

Kugelrohr distilled; 120°C/10 mmHg.

^1H NMR, 220 MHz ($CDCl_3$/TMS): δ = 1.27 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 2.10 (complex, 2 H, $CCH_2\underline{CH_2}$); 2.86, 3.16 (m, 2 H, CCH_2); 4.17 (q, 2 H, J=7.7 Hz, OCH_2); 5.63 ppm (complex, 1 H, CH).

IR (film): ν = 1715,1675 cm^{-1}.

MS (EI): m/z=140$(M)^{+\cdot}$, 112$(M-Et)^{+}$, 95$(M-OEt)^{+}$, 67$(M-COOEt)^{+}$.

High resolution MS: $C_8H_{12}O_2$, calc. 140.0838, found 140.0838.

GLC (3% SE30, 100°C); R_T= 324 s (100%).

Ethyl cyclopentylidene ethanoate (45h)

Colourless oil. bp 69-72°C/0.6 mmHg. Lit.[81] bp 71-72.5°C/1 mmHg.

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ = 1.28 (t, 3 H, J=7.5 Hz, $OCH_2\underline{CH_3}$); 1.7 (complex, 4 H, $CCH_2\underline{CH_2}\underline{CH_2}$); 2.45, 2.78 (m, 2 H, CCH_2); 4.26 (q, 2 H, J=7.5 Hz, OCH_2); 5.83 ppm (m, 1 H, CH).

IR (film): ν = 1715, 1655 cm^{-1}.

MS (EI): m/z=154$(M)^+$, 126$(M-Et)^+$, 109$(M-OEt)^+$, 81$(M-COOEt)^+$.

High resolution MS: $C_9H_{14}O_2$, calc. 154.0994, found 154.0993.

GLC (3% SE30, 110°C); R_T= 370 s (98%).

Ethyl cyclohexylidene ethanoate (45i)

Colourless oil. bp 120°C/0.3 mmHg. Lit.[64] bp 88-90°C/10 mmHg.

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ = 1.28 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 1.63 (complex, 6 H, CH_2); 2.19 (m, 2 H, CH_2); 2.85 (m, 2 H, CH_2); 4.16 (q, 2 H, J=7.1 Hz, OCH_2); 5.62 ppm (s, 1 H, CH).

IR (film): ν = 1720, 1655, 1455 cm^{-1}.

MS (EI): m/z= 168$(M)^+$, 140$(M-Et)^+$, 123$(M-OEt)^+$, 95$(M-COOEt)^+$.

High resolution MS: $C_{10}H_{16}O_2$, calc. 168.1150, found 168.1143

GLC (3% SE30, 130°C); R_T= 267 s (100%).

Ethyl cycloheptylidene ethanoate (45j)

Colourless oil. bp 100°C/0.07 mmHg. Lit.[81] 94-95°C/1 mmHg.

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ = 1.29 (t, 3 H, J=7.3 Hz, $OCH_2\underline{CH_3}$); 1.5-1.8 (complex, 8 H, ring CH_2); 2.38 (m, 2 H, ring CH_2); 2.88 (m, 2 H, ring CH_2); 4.16 (m, 2 H, OCH_2); 6.73 ppm (bs, 1 H, CH).

IR (film): ν = 1715, 1635 cm^{-1}.

MS (EI): m/z= 182$(M)^+$, 154$(M-Et)^+$, 137$(M-OEt)^+$, 109$(M-COOEt)^+$.

High resolution MS: $C_{11}H_{18}O_2$, calc. 182.1307, found 182.1298.

GLC (3% SE30, 150°C); R_T= 230 s (98%).

Ethyl cyclooctylidene ethanoate (45k)

Colourless oil. bp 74-75°C/0.25 mmHg. Lit.[81] bp 81-83°C/0.58 mmHg.

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ= 1.2-2.0 (complex, 12 H, ring CH_2, $OCH_2\underline{CH_3}$); 2.33 (m, 2 H, CH_2); 2.44 (m, 1 H, CH_2); 2.77 (m, 2 H, CH_2); 4.26 (q, 2 H, J=7.1 Hz, OCH_2); 5.76 ppm (bs, 1 H, CH).

IR (film): ν= 1710, 1630 cm^{-1}.

MS (EI): m/z= 196$(M)^+_{\cdot}$, 168$(M-Et)^+$, 151$(M-OEt)^+$.

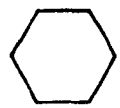
High resolution MS: $C_{12}H_{20}O_2$, calc. 196.1464, found 196.1462.

GLC (3% SE30, 150°C); R_T= 344 s (94%)

Dihydroxylation of $\alpha\beta$-unsaturated esters

The following $\alpha\beta$-unsaturated esters were dihydroxylated by the method described below for ethyl cyclopentylidene ethanoate.

(45) (46)

Entry	R	R^1	Scale /mmol	Temp. /°C	Time /h	Yield /%
46a	Pr	Pr	29	50-60	17	40
46b	Bu	Bu	20	50-60	20	30
46c	i-Pr	Me	20	40-50	15	30
46d	Ph	Me	41	40	20	46
46e			45	40-50	16	79
46f			25	40-50	20	35

Ethyl 3-(1-hydroxycyclopentyl)-2-hydroxyethanoate (46e)

To a stirred solution of ethyl cyclopentylidene ethanoate (8.515 g, 45.3 mmol) in 98% formic acid (30 ml) was added a 30% solution of hydrogen peroxide (8.5 ml, 82 mmol). The mixture was stirred at 40-50°C for 16 h, evaporated under reduced pressure/35°C, and co-evaporated with toluene (2x40 ml) under reduced pressure/35°C. The residue was distilled in a Kugelrohr apparatus (150°C/0.15 mmHg) to give the 2,3-dihydroxy ester as a pale yellow oil (6.722 g, 35.8 mmol, 79% yield).

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ= 1.33 (t, 3 H, J=7.6 Hz, $OCH_2\underline{CH_3}$); 1.7 (complex, 8 H, ring CH_2); 2.23 (s, 1 H, $CH_2C(O\underline{H})CH_2$); 3.30 (d, 1 H, J=6.7 Hz, CH(O<u>H</u>)); 4.06 (d, 1 H, J=6.7 Hz, CH); 4.28 ppm (m, 2 H, OCH_2).

IR (film): ν= 3475, 1735 cm^{-1}.

MS (+ve CI, ammonia): m/z= 206$(M+18)^+$, 188$(M)^+$, 171$(M-OH)^+$.

High resolution MS: $C_9H_{17}O_4$ (M+H), calc. 189.1126, found 189.1136.

GLC (3% SE30, 130°C); R_T= 320 s (90%).

Ethyl 2,3-dihydroxy-3-propylhexanoate (46a)

Isolated by flash chromatography, eluting with ethyl acetate-petrol 1:1. Colourless oil, R_F 0.56.

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ= 0.94 (complex, 6 H, propyl CH_3); 1.4 (complex, 11 H, propyl CH_2, $OCH_2\underline{CH_3}$); 2.41 (bs, 1 H, CH(OH)); 3.15 (bd, 1H , J=7.1 Hz, PrCOH); 4.11 (d, 1 H, J=7.1 Hz, C<u>H</u>(OH)); 4.32 ppm (dq, 2 H, J=7.1, 1.3 Hz, OCH_2).

IR (film): ν= 3470, 1730 cm^{-1}.

MS (+ve CI, ammonia): m/z= 291$(M+1)^+$.

High resolution MS: $C_{11}H_{23}O_4$ (M+H), calc. 219.1596, found 219.1592.

GLC (3% SE30, 150°C); R_T= 267 s (96%).

Ethyl 3-butyl-2,3-dihydroxyheptanoate (46b)

Isolated by flash chromatography, eluting with ethyl acetate-petrol 1:4. Colourless oil, R_F 0.43.

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ= 0.92 (complex, 6 H, butyl CH_2); 1.2-1.75 (complex, 15 H, butyl CH_2, $OCH_2\underline{CH_3}$); 2.38 (bs, 1 H, C(OH)); 3.13 (bd, 1 H, J=7.3 Hz, CH(O$\underline{H}$)); 4.13 (d, 1 H, J=7.13 Hz, C$\underline{H}$(OH)); 4.33 ppm (q, 2 H, J=7.1 Hz, OCH_2).

IR (film): ν= 3490, 1735 cm^{-1}.

MS (+ve CI, ammonia): m/z= 264$(M+18)^+$, 246$(M)^+$, 229$(M-OH)^+$, 188$(M-Bu)^+$.

High resolution MS: $C_{13}H_{30}NO_4$ ($M+NH_4$), calc. 264.2174, found 264.2154.

Ethyl 2,3-dihydroxy-3,4-dimethylpentanoate (46c)

Isolated by flash chromatography, eluting with ethyl acetate-petrol 1:3. Colourless oil R_F 0.2, (mixture of diastereoisomers).

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ= 0.96 (complex, 6 H, $CH(\underline{CH_3})_2$); 1.09, 1.14 (s, 3 H, C(OH)$\underline{CH_3}$); 1.34 (complex, 3 H, $OCH_2\underline{CH_3}$); 1.83, 2.05 (m, 1 H, C$\underline{H}(CH_3)_2$); 2.46, 2.54 (s, 1 H, C(O$\underline{H}$)), 3.29, 3.37 (d, 1 H, J=7 Hz, CH(O$\underline{H}$)); 4.15 (complex, 1 H, C$\underline{H}$(OH)); 4.31 ppm (complex, 2 H, OCH_2).

IR (film): ν= 3480, 1730 cm^{-1}.

MS (EI): m/z= 191$(M+1)^+$, 173$(M-OH)^+$, 147$(M-Pr)^+$, 117$(M-COOEt)^+$.

High resolution MS: $C_9H_{19}O_4$ (M+H), calc. 191.1283, found 191.1291.

Ethyl 2,3-dihydroxy-3-phenylbutanoate (46d)

Isolated by flash chromatography, eluting with ethyl acetate-petrol 1:4. Pale yellow oil R_F 0.2, (mixture of diastereoisomers).

^{1}H NMR, 220 MHz ($CDCl_3$/TMS) (major diastereoisomer): δ= 1.17 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 1.63 (s, 3 H, $C(OH)\underline{CH_3}$); 3.17 (d, 1 H, J=6.4 Hz, $CH(\underline{OH})$); 3.35 (s, 1 H, PhCOH); 4.18 (q, 2 H, J=7.1 Hz, OCH_2); 4.35 (d, 1 H, J=6.4 Hz, CH); 7.4 ppm (complex, 5 H, Ph).

IR (film): ν= 3480, 1730, 1495 cm^{-1}.

MS (+ve CI, ammonia): m/z= 242$(M+18)^+$, 224$(M)^+$, 207$(M-OH)^+$.

High resolution MS: $C_{12}H_{20}NO_4$ ($M+NH_4$), calc. 242.1392, found 242.1389.

GLC (3% SE30, 170°C); R_T= 294 s.

Ethyl 2-(1-hydroxycyclohexyl)-2-hydroxyethanoate (46f)

Isolated by flash chromatography, eluting with ethyl acetate-petrol 1:4. Colourless oil R_F 0.13.

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ= 1.15-1.95 (complex, 13 H, ring CH_2, $OCH_2\underline{CH_3}$); 2.67 (s, 1 H, C(OH)); 3.56 (d, 1 H, J=7.6 Hz, $CH(\underline{OH})$); 4.02 (d, 1 H, J=8.0 Hz, $\underline{CH}(OH)$); 4.33 ppm (q, 2 H, J=7.1 Hz, OCH_2).

IR (film): ν= 3470, 1730 cm^{-1}.

MS (EI): m/z= 203$(M+1)^+$, 185$(M-OH)^+$, 81(cyclohexyl-H)$^+$.

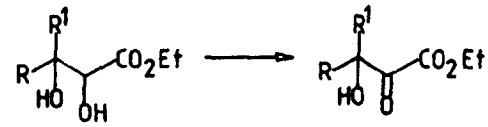
High resolution MS: $C_{10}H_{19}O_4$ (M+H), calc. 203.1284, found 203.1265.

GLC (3% SE30, 150°C); R_T= 291 s (98%).

Elemental analysis: Found: C, 59.15; H, 8.94. $C_{10}H_{18}O_4$ requires C, 59.41; H, 8.91%.

Oxidation of 2,3-dihydroxy esters with N-bromosuccinimide

The following 2,3-dihydroxy esters were oxidised by the method described below for ethyl 2,3-dihydroxy-3-propylhexanoate.



(47)

Entry	R	R^1	Scale /mmol	Time /h	Yield /%
47a	Pr	Pr	10.2	3	79
47b	Bu	Bu	4.5	15	93
47c	i-Pr	Me	5.6	5	19
47d	Ph	Me	9.4	14	78
47e			10.8	17	60
47f			6.8	6	51

Ethyl 3-hydroxy-2-oxo-3-propylhexanoate (47a)

Ethyl 2,3-dihydroxy-3-propylhexanoate (2.229 g, 10.2 mmol), N-bromosuccinimide (1.966 g, 11.0 mmol) and calcium carbonate (1.060 g, 10.6 mmol) were stirred together in dry carbon tetrachloride (40 ml) under nitrogen with illumination and heat from a 100 W tungsten lamp. After 15 minutes a deep red colouration developed which dissipated over a 3 h period to give a yellow solution and a yellow solid. The mixture was cooled (ice bath), filtered and the solid washed with carbon tetrachloride (2x10 ml). The combined carbon tetrachloride portions were evaporated under reduced pressure to give a yellow oil. This was subjected to flash chromatography éluting with ethyl acetate-petrol 1:7. Ethyl 3-hydroxy-2-oxo-3-propylhexanoate was obtained as a yellow oil R_F 0.34 (1.733 g, 8.02 mmol, 79%).

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ = 0.89 (t, 6 H, J=7.6 Hz, $CH_2CH_2\underline{CH_3}$); 1.15 (m, 2 H, $C(OH)CH_2\underline{CH_2}$); 1.37 (complex, 5 H, $OCH_2\underline{CH_3}$, $C(OH)CH_2\underline{CH_2}$); 1.80 (m, 4 H, $C(OH)\underline{CH_2}$); 3.28 (s, 1 H, OH); 4.37 ppm (q, 2 H, J=7.1

Hz, OCH_2).

IR (film): ν = 3520, 1740, 1720 cm^{-1}.

MS (+ve CI, ammonia): m/z= 217$(M+1)^+$, 143$(M-CO_2Et)^+$, 115$(M-COCO_2)^+$.

High resolution MS: $C_{11}H_{21}O_4$ (M+H), calc. 217.1439, found 217.1409.

GLC (PEG 20M, 180°C); R_T= 597 s (100%)

Elemental analysis: Found: C, 61.28; H, 9.39. $C_{11}H_{20}O_4$ requires C, 61.11; H, 9.26%.

Ethyl 3-butyl-3-hydroxy-2-oxoheptanoate (47b)

Yellow oil isolated by flash chromatography eluting with ethyl acetate-petrol 3:17 R_F 0.52.

^1H NMR, 220MHz ($CDCl_3$/TMS): δ= 0.88 (t, 6 H, J=7.1 Hz, $CH_2CH_2CH_2\underline{CH_3}$); 0.97-1.50 (complex, 11 H, butyl CH_2, $OCH_2\underline{CH_3}$); 1.85 (m, 4 H, $C(OH)\underline{CH_2}$); 3.29 (bs, 1 H, OH); 4.36 ppm (q, 2 H, J=8.0 Hz, OCH_2).

IR (film): ν = 3515, 1740, 1725 cm^{-1}.

MS (EI): m/z= 245$(M+1)^+$, 171$(M-CO_2Et)^+$.

High resolution MS: $C_{13}H_{25}O_4$ (M+H), calc. 245.1752, found 245.1754.

GLC (3% SE30, 200°C); R_T= 545 s (99%).

Elemental analysis: Found: C, 63.57; H, 9.91. $C_{13}H_{24}O_4$ requires C, 63.93; H, 9.84%.

Ethyl 3,4-dimethyl-3-hydroxy-2-oxopentanoate (47c)

Colourless oil isolated by flash chromatography eluting with ethyl acetate-petrol 1:3 R_F 0.43. There was accidental loss of product during work up.

^1H NMR, 220MHz ($CDCl_3$/TMS): δ= 0.87, 0.99 (d, 3 H, J=7.1 Hz, $CH\underline{CH_3}$); 1.38 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 1.44 (s, 3 H, $C(OH)\underline{CH_3}$); 2.26 (m, 1 H, CH); 3.12 (s, 1 H, OH); 4.37 ppm (q, 2 H, J=7.1 Hz, OCH_2).

IR (film): ν= 3520, 1740(sh), 1725 cm^{-1}.

MS (EI): m/z= 189$(M+1)^{+}$, 115$(M-CO_2Et)^{+}$, 87$(M-COCO_2Et)^{+}$.

High resolution MS: $C_9H_{17}O_4$ (M+H), calc. 189.1127, found 189.1130.

GLC (3% SE30, 100°C); R_T= 409 s (100%).

Ethyl 3-hydroxy-2-oxo-3-phenylbutanoate (47d)

Pale yellow oil isolated by flash chromatography eluting with ether-petrol 1:4 R_F (ether-petrol 2:3) 0.3.

^1H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.23 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 1.82 (s, 3 H, $C(OH)\underline{CH_3}$); 4.05 (bs, 1 H, OH); 4.23 (q, 2 H, J=7.1 Hz, OCH_2); 7.4 ppm (complex, 5 H, Ph).

IR (film): ν= 3500, 3060, 1740(sh), 1725, 1600, 1495 cm^{-1}.

MS (+ve CI, ammonia): m/z= 240$(M+18)^{+}$, 223$(M+1)^{+}$, 177$(M-OEt)^{+}$, 121$(M-COCO_2Et)^{+}$, 105$(PhCO)^{+}$.

High resolution MS: $C_{12}H_{15}O_4$ (M+H), calc. 223.0971, found 223.0998.

Elemental analysis: Found: C, 64.66; H, 6.50. $C_{12}H_{14}O_4$ requires C, 64.86; H, 6.31%.

Ethyl 2-(1-hydroxycyclopentyl)-2-oxoethanoate (47e)

Pale yellow oil isolated by flash chromatography eluting with ether-petrol 1:1 R_F 0.25.

^1H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.38 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 1.85 (complex, 6 H, ring CH_2); 2.13 (complex, 2 H, ring CH_2); 3.06 (bs, 1 H, OH); 4.37 ppm (q, 2 H, J=7.1 Hz, OCH_2).

^{13}C NMR, 22.63 MHz ($CDCl_3$/$CDCl_3$ = δ 77.0 ppm): δ= 13.8 (CH_3); 24.9 ($C(OH)CH_2\underline{CH_2}\underline{CH_2}$); 38.9 ($\underline{CH_2}C(OH)\underline{CH_2}$); 62.2 ($OCH_2$); 86.3 (C(OH)); 162.9 ($\underline{CO_2}CH_2$); 198.2 ppm (CO).

IR (film): ν = 3505, 1725(b) cm^{-1}.

MS (EI): m/z= 186$(M)^{+}$, 168$(M-H_2O)^{+}$, 113$(M-CO_2Et)$; 85$(M-COCO_2Et)^{+}$.

High resolution MS: $C_9H_{14}O_4$, calc. 186.0892, found 186.0890.

GLC (3% SE30, 140°C); R_T= 580 s (100%).

Ethyl 2-(1-hydroxycyclohexyl)-2-oxoethanoate (47f)

Pale yellow oil isolated by flash chromatography eluting with ethyl acetate-petrol 1:4 R_F 0.63.

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.15-1.45 (complex, 4 H, ring CH_2, $OCH_2\underline{CH_3}$); 1.50-1.95 (complex, 9 H, ring CH_2); 2.85 (s, 1 H, OH); 4.35 ppm (q, 2 H, J=7.1 Hz, OCH_2).

^{13}C NMR, 62.896 MHz ($CDCl_3$/$CDCl_3$ = δ 77.0 ppm): δ= 13.9 (CH_3); 20.6 ($\underline{CH_2}CH_2C(OH)CH_2\underline{CH_2}$); 24.9 ($C(OH)CH_2CH_2\underline{CH_2}$); 33.3 ($\underline{CH_2}C(OH)\underline{CH_2}$); 62.2 ($OCH_2$); 77.8 (C(OH)); 163.3 ($\underline{CO_2}CH_2$); 199.1 ppm (CO).

IR (film): ν = 3520, 1740(sh), 1720 cm^{-1}.

MS (EI): m/z= 201$(M+1)^+$, 183$(M-OH)^+$, 99$(M-COCO_2Et)^+$.

High resolution MS: $C_{10}H_{17}O_4$ (M+H), calc. 201.1126, found 201.1130.

GLC (3% SE30, 130°C); R_T= 380 s (98).

Elemental analysis: Found: C, 59.55; H, 8.48. $C_{10}H_{16}O_4$ requires C, 60.00; H, 8.00%.

Oxidation of ethyl 3,3-diphenylprop-2-eneoate to ethyl 3,3-diphenyl-3-hydroxy-2-oxopropanoate (44a)

To a solution of ethyl 3,3-diphenylprop-2-eneoate (4.369 g, 17.3 mmol)in 98% formic acid (12 ml) was added a 30% solution of hydrogen peroxide in water (5 ml, 48 mmol). The mixture was stirred et 65°C for 12 h and evaporated under reduced pressure/40°C to give an pale brown solid. This was suspended in carbon tetrachloride (50 ml). Calcium carbonate (1.703 g, 17 mmol) and N-bromosuccinimide (3.00 g, 16.8 mmol) were added and the mixture stirred under nitrogen with illumination and heat from a 100 W tungsten lamp for 20 h. The mixture

was filtered and evaporated under reduced pressure/35°C to give a yellow oil. The product was isolated by flash chromatography eluting with ethyl acetate-petrol 1:4 R_F 0.25 as a yellow oil (1.477 g, 30%).

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ = 1.25 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 4.05 (s, 1 H, OH); 4.26 (q, 2 H, J=7.1 Hz, OCH_2); 7.40 ppm (complex, 10 H, Ph).

IR (film): ν = 3500, 3060, 1740(sh), 1725, 1600, 1495 cm^{-1}.

MS (+ve CI, ammonia): m/z= 302$(M+18)^+$, 285$(M+1)^+$, 239$(M-OEt)^+$, 183$(M-COCO_2Et)^+$.

High resolution MS: $C_{17}H_{17}O_4$ (M+H), calc. 285.1127, found 285.1148.

Elemental analysis: Found: C, 72.07; H, 5.76. $C_{17}H_{16}O_4$ requires C, 71.83; H, 5.63%.

Oxidation of ethyl cycloheptylidene ethanoate to ethyl 2-(1-hydroxycycloheptyl)-2-oxoethanoate (44b)

To a solution of ethyl cycloheptylidene ethanoate (3.177 g, 17.4 mmol) dissolved in 98% formic acid (10 ml) was added a 30% solution of hydrogen peroxide in water (5 ml, 48 mmol). The mixture was strirred at 60-80°C for 17 h, evaporated under reduced pressure/40°C and co-evaporated with toluene (50 ml) under reduced pressure/40°C. High vacuum was applied for 2 h to remove traces of toluene. The residue was dissolved in carbon tetrachloride (50 ml). N-bromosuccinimide (3.028 g, 17.0 mmol) and calcium carbonate (1.741 g, 17.4 mmol) were added and the mixture was stirred under nitrogen for 5 h with illumination and heat from a 100 W tungsten lamp. A deep red colouration formed which gave way to a yellow solution and an orange solid. The mixture was filtered and evaporated under reduced

pressure/35°C to give a pale yellow oil. This was subjected to flash chromatography eluting with ethyl acetate-petrol 1:4. Ethyl 2-(1-hydroxycycloheptyl)-2-oxoethanoate, R_F 0.1, was isolated as a yellow oil (1.291 g, 6.03 mmol, 35%).

^1H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.38 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 0.95-1.90 (complex, 10 H, ring CH_2); 2.12 (m, 2 H, ring CH_2); 2.93 (s, 1 H, OH); 4.37 ppm (q, 2 H, J=7.1 Hz, OCH_2).

^{13}C NMR, 45.28 MHz ($CDCl_3/CDCl_3$ = δ77.0 ppm): δ= 13.7 ($OCH_2\underline{CH_3}$); 22.0 ($\underline{CH_2}C(OH)\underline{CH_2}$); 29.1 ($\underline{CH_2}CH_2C(OH)CH_2\underline{CH_2}$); 37.2 ($C(OH)CH_2CH_2\underline{CH_2}\underline{CH_2}$); 62.0 ($OCH_2$); 80.6 (C(OH)); 163.5 ($\underline{CO_2}CH_2$); 199.5 ppm (CO).

IR (film): ν= 3500, 1735(sh), 1720 cm^{-1}.

MS (EI): m/z= 215$(M+1)^+$, 197$(M-OH)^+$, 113$(M-COCO_2Et)^+$.

High resolution MS: $C_{11}H_{19}O_4$ (M+H), calc. 215.1283, found 215.1265.

GLC (3% SE30, 150°C); R_T= 410 s (100%).

Elemental analysis: Found: C, 60.70; H, 8.39. $C_{11}H_{18}O_4$ requires C, 61.68; H, 8.41%.

Attempted isomerisation of methyl 3-hydroxy-3-methyl-2-oxobutanoate using aluminium trichloride and acetic anhydride

Methyl 3-hydroxy-3-methyl-2-oxobutanoate and aluminium trichloride (619 mg, 4.6 mmol) were suspended in glacial acetic acid (20 ml) and acetic anhydride (5 ml) and set aside at room temperature for 20 h. Water (60 ml) was added and the solution brought to pH 7 with solid sodium hydrogen carbonate. The mixture was extracted with dichloromethane (100 ml). The dichloromethane was dried ($MgSO_4$) and evaporated under reduced pressure/40°C to give a yellow oil (62 mg).

Proton NMR spectroscopy indicated a complex mixture containing neither starting material nor methyl 2-hydroxy-2-methyl-3-oxobutanoate.

Attempted isomerisation of methyl 3-hydroxy-3-methyl-2-oxobutanoate using aluminium trichloride

Methyl 3-hydroxy-3-methyl-2-oxobutanoate (76 mg, 0.5 mmol) and aluminium trichloride (37 mg, 0.3 mmol) were heated together in boiling toluene (2 ml) under nitrogen for 16 h. Water (5 ml) was added and the mixture was taken to pH 8 with saturated sodium hydrogen carbonate solution, filtered and extracted with dichloromethane (6x10 ml). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure/40°C to give a yellow oil (17 mg). Proton NMR spectroscopy indicated a complex mixture containing neither starting material nor methyl 2-hydroxy-2-methyl-3-oxobutanoate.

Attempted isomerisation of methyl 3-hydroxy-3-methyl-2-oxobutanoate on neutral alumina

Methyl 3-hydroxy-3-methyl-2-oxobutanoate (111 mg, 0.76 mmol) was stirred with neutral alumina (1.6 g, grade I) for 5 days. Benzene (10 ml) was added and the slurry stirred for 20 h. The benzene was evaporated under reduced pressure/40°C and the alumina extracted with dichloromethane in a soxhlet apparatus. The dichloromethane was filtered through celite and evaporated under reduced pressure/40°C to give a white gum (16 mg). Proton NMR spectroscopy indicated a complex mixture containing some starting material but no methyl 2-hydroxy-2-methyl-3-oxobutanoate.

Atempted isomerisation of methyl 3-hydroxy-3-methyl-2-oxobutanoate using aluminium t-butoxide

Methyl 3-hydroxy-3-methyl-2-oxobutanoate (115 mg, 0.79 mg) and aluminium t-butoxide (907 mg, 3.7 mmol) were stirred together in boiling toluene (10 ml) under nitrogen for 24 h. The mixture was acidified with 4 M hydrochloric acid and the toluene evaporated under reduced pressure/40°C. Water (2 ml) was added and the mixture extracted with dichloromethane (5x10 ml). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give a pale brown solid (30 mg). Proton NMR spectroscopy indicated a complex mixture containing neither starting material nor methyl 2-hydroxy-2-methyl-3-oxobutanoate.

Attempted isomerisation of methyl 3-hydroxy-3-methyl-2-oxobutanoate using boron trifluoride etherate

To a solution of methyl 3-hydroxy-3-methyl-2-oxobutanoate (240 mg, 1.6 mmol) in glacial acetic acid (10 ml) and acetic anhydride (1.6 ml) was added boron trifluoride etherate (1.6 ml). The homogeneous solution was set aside at room temperature for 17 h. Water (50 ml) was added and the solution extracted with dichloromethane (3x50 ml). The combined extracts were washed with saturated sodium hydrogen carbonate solution (50 ml). The aqueous phase was back-extracted with dichloromethane (3x50 ml). The combined dichloromethane portions were dried ($MgSO_4$) and evaporated under reduced pressure/35°C to give a yellow oil, spectral data consistent with its formulation as methyl 3-acetoxy-3-methyl-2-oxobutanoate (262 mg, 1.39 mmol, 87%). This was distilled in a Kugelrohr apparatus (90°C/0.5 mmHg) to give a

colourless oil (173 mg, 0.92 mmol, 58%).

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.62 (s, 6 H, $C(CH_3)_2$); 2.08 (s, 3 H, $COCH_3$); 3.86 ppm (s, 3 H, OCH_3).

IR (film): ν= 1735 cm^{-1}.

MS (EI): m/z= 189$(M)^+$, 129$(M-OAc)^+$.

High resolution MS: $C_8H_{13}O_5$ (M+H), calc. 189.0763, found 189.0770.

GLC (3% SE30, 110°C); R_T= 239 s (100%)

Elemental analysis: Found: C, 51.51; H, 6.46. $C_8H_{12}O_5$ requires C, 51.06; H, 6.38%.

Isomerisation of 3-hydroxy-3-methylpentan-2-one

3-Hydroxy-3-methylpentan-2-one (256 mg, 2.2 mmol) and dibutyl tin oxide (27 mg, 0.11 mmo, 5 mol%) were heated together in boiling toluene (5 ml) under nitrogen for 14 days. The reaction was followed by GLC (PEG 20M, 100°C, R_T (starting material) 700 s, (new product) 770 s.) The reaction mixture was extracted with deuterium oxide (2 ml) and the proton NMR spectrum determined. The signals observed were coincident with those of authentic 3-hydroxy-3-methylpentan-2-one and 2-hydroxy-2-methylpentan-3-one.

3-Hydroxy-3-methylpentan-2-one:

^{1}H NMR, 220MHz (D_2O/HOD = δ5.00 ppm): δ= 0.96 (t, 3 H, $CH_2\underline{CH_3}$), 1.52 (s, 3 H, $C(OH)\underline{CH_3}$); 1.92 (m, 2 H, $\underline{CH_2}CH_3$); 2.42 ppm (s, 3 H, $COCH_3$).

2-Hydroxy-2-methylpentan-3-one:

1H NMR, 220MHz (D_2O/HOD = δ5.00 ppm): δ= 1.16 (t, 3 H, $CH_2\underline{CH_3}$), 1.53 (s, 6 H, $C(CH_3)_2$); 2.87 ppm (q, 2 H, $CO\underline{CH_2}CH_3$).

Integration of the proton NMR spectrum gave a ratio of 3-hydroxy-3-methylpentan-2-one to 2-hydroxy-2-methylpentan-3-one of 61:39.

Attempted isomerisation of methyl 2-hydroxy-2-methyl-3-oxobutanoate

Methyl 2-hydroxy-2-methyl-3-oxobutanoate (456 mg, 3.1 mmol) and dibutyl tin oxide (42 mg, 0.16 mmol) were heated together in boiling toluene (10 ml) under nitrogen for 18 h. No change was observed by GLC (PEG 20M, 70°C, R_T= 500 s). Evaporation of the solvent gave the starting materials as judged by proton NMR spectroscopy.

Dibutyl tin oxide-catalysed isomerisation of methyl 3-hydroxy-3-methyl-2-oxobutanoate

Methyl 3-hydroxy-3-methyl-2-oxobutanoate (727 mg, 4.98 mmol) and dibutyl tin oxide were heated together in boiling toluene (3 ml) under nitrogen for 22 h. The reaction was monitored by GLC (10 % SE30, 100°C, R_T (starting material) = 320 s, R_T (product) = 380 s.) The solvent was evaporated under reduced pressure/40°C. The residue was distilled in a Kugelrohr apparatus (140°C/10 mmHg) to give methyl 2-hydroxy-2-methyl-3-oxobutanoate as a colourless oil (587 mg, 4.02 mmol, 81%).

^{1}H NMR, 220MHz ($CDCl_3$/TMS): δ = 1.60 (s, 3 H, C(OH)$\underline{CH_3}$); 2.30 (s, 3 H, $COCH_3$); 3.83 (s, 3 H, OCH_3); 4.23 ppm (s, 1 H, OH).

^{13}C NMR, 22.63 MHz ($CDCl_3$/$CDCl_3$ = δ 77.0 ppm): δ = 21.4 (C(OH)$\underline{CH_3}$); 23.8 (OCH_3); 52.9 ($CO\underline{CH_3}$); 80.8 (C(OH)); 171.6 ($\underline{CO_2}CH_3$); 204.7 ppm ($\underline{CO}CH_3$).

IR (film): ν = 3505, 1735(b) cm^{-1}.

MS (+ve CI, ammonia): m/z= 164$(M+18)^+$, 147$(M+1)^+$.

High resolution MS: $C_6H_{11}O_4$, (M+H), calc. 147.0657, found 147.0656.

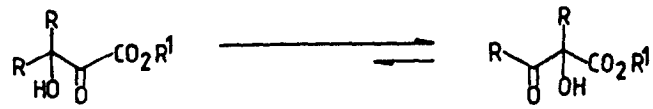
GLC (3% SE30, 80°C); R_T= 270 s.

Elemental analysis: Found: C, 49.25; H, 7.13. $C_6H_{10}O_4$ requires C, 49.31; H, 6.85%.

Dibutyl tin oxide-catalysed isomerisation of 3,3-dialkyl-3-hydroxy-2-oxocarboxylic esters

A range of 3,3-dialkyl-3-hydroxy-2-oxocarboxylic esters was subjected to the isomerisation conditions as described above for methyl 3-hydroxy-3-methyl-2-oxobutanoate.

a) Acyclic substrates having two identical alkyl substituents at the 3-position:



(37) (38)

Entry	R	R^1	Scale /mmol	Catalyst /mol%	Time /days	Yield /%
38a	Me	Me	5.0	3.0	1	81
38b	Pr	Et	4.7	5.0	4	80
38c	Bu	Et	3.6	4.4	7	88
38d	Ph	Et	6.3	4.4	6	38

Ethyl 2-hydroxy-3-oxo-2-propylhexanoate (38b)

Colourless oil isolated by flash chromatography eluting with ethyl acetate-petrol 1:9, R_F 0.28.

^{1}H NMR, 400MHz ($CDCl_3$/TMS): δ = 0.89 (t, 3 H, J=7.4 Hz, $C(OH)CH_2CH_2\underline{CH_3}$); 0.91 (t, 3 H, J=7.3 Hz, $COCH_2CH_2\underline{CH_3}$); 1.27 (complex, 5 H, $OCH_2\underline{CH_3}$, $C(OH)CH_2\underline{CH_2}$); 1.60 (m, 2 H, $C(OH)\underline{CH_2}CH_2$); 1.77, 2.06 (m, 2 H, $C(OH)\underline{CH_2}$); 2.48, 2.66 (dt, 1 H, J=18.0, 7.1 Hz, $COCH_2$); 4.17 (bs, 1 H, OH); 4.23 ppm (dq, 2 H, J=7.2, 2.2 Hz, $O\underline{CH_2}CH_3$).

IR (film): ν = 3500, 1745(sh), 1720 cm^{-1}.

MS (+ve CI, ammonia): m/z= 234$(M+18)^+$, 217$(M+1)^+$.

High resolution MS: $C_{11}H_{21}O_4$ (M+H), calc. 217.1440, found 217.1458.

GLC (PEG 20M, 180°C); R_T= 595 s.

Elemental analysis: Found: C, 61.64; H, 9.40. $C_{11}H_{20}O_4$ requires C, 61.11; H, 9.26%.

Ethyl 2-butyl-2-hydroxy-3-oxoheptanoate (38c)

Colourless oil isolated by flash chromatography eluting with ethyl acetate-petrol 1:4, R_F 0.5.

^1H NMR, 220MHz ($CDCl_3$/TMS): δ= 0.89 (m, 6 H, butyl CH_3); 1.15-1.65 (complex, 11 H, butyl CH_2, $OCH_2\underline{CH_3}$); 2.00 (m, 2 H, $C(OH)\underline{CH_2}$); 2.62 (m, 2 H, $COCH_2$); 4.20 (s, 1 H, OH); 4.26 ppm (q, 2 H, J=7.1 Hz, OCH_2).

IR (film): ν= 3500, 1745(sh), 1725 cm^{-1}.

MS (EI): m/z= 245$(M+1)^+$, 227$(M-OH)^+$, 171$(M-CO_2Et)^+$.

High resolution MS: $C_{13}H_{24}O_4$, calc. 244.1674, found 244.1652.

GLC (3% SE30, 160°C); R_T= 314 s (97%).

Elemental analysis: Found: C, 63.63; H, 9.73. $C_{13}H_{24}O_4$ requires C, 63.93; H, 9.84%.

Ethyl 2-hydroxy-3-oxo-2,3-diphenylpropanoate (38d)

Yellow oil isolated by flash chromatography eluting with ethyl acetate-petrol 1:4, R_F 0.3.

^1H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.24 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 4.25 (m, 2 H, OCH_2); 6.17 (s, 1 H, OH); 7.42-7.70 (complex, 8 H, Ph); 8.17 ppm (m, 2 H, Ph).

IR (film): ν= 3400, 3070, 1755, 1730, 1605, 1500 cm^{-1}.

MS (EI): m/z= 284$(M)^+$, 211$(M-CO_2Et)^+$, 105$(PhCO)^+$.

High resolution MS: $C_{17}H_{16}O_4$, calc. 284.1048, found 284.1054.

GLC (3% SE30, 200°C); R_T= 533 s (98%).

Elemental analysis: Found: C, 71.65; H, 5.64. $C_{17}H_{16}O_4$ requires C, 71.83; H, 5.63%.

b) Cyclic substrates giving ring-expanded products:

(39) (40)

Entry	n	Scale /mmol	Catalyst /mol%	Time /h	Yield /%
40a	4	6.3	2.0	14	90
40b	5	2.0	2.0	48	79
40c	6	4.9	2.6	16	90

Ethyl 1-hydroxycyclohexan-2-onecarboxylate (40a)

Colourless oil isolated by distillation in a Kugelrohr apparatus (100°C/0.8 mmHg).

^{1}H NMR, 220 MHz ($CDCl_3$/TMS): δ= 1.30 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 1.63-2.10 (complex, 5 H, ring CH_2); 2.63 (complex, 3 H, ring CH_2); 4.26 (q, 2 H, J=7.1 Hz, OCH_2); 4.39 ppm (bs, 1 H, OH).

^{13}C NMR, 45.28 MHz ($CDCl_3/CDCl_3$ =δ77.0 ppm): δ= 14.0 ($OCH_2\underline{CH_3}$); 21.9, 27.0, 37.6, 38.8 (ring CH_2); 62.2 (OCH_2); 80.62 (C(OH)); 170.0 ($\underline{CO_2}CH_2$); 207.2 ppm (CO).

IR (film): ν= 3460, 1745(sh), 1720 cm^{-1}.

MS (EI): m/z= 186$(M)^+_\bullet$, 142$(M-CO_2)^+_\bullet$, 113$(M-CO_2Et)^+$.

High resolution MS: $C_9H_{14}O_4$, calc. 186.0892, found 186.0890.

GLC (3% SE30, 120°C); R_T= 405 s (100%).

Elemental analysis: Found: C, 57.47; H, 7.54. $C_9H_{14}O_4$ requires C, 58.06; H, 7.53%.

Ethyl 1-hydroxycycloheptan-2-onecarboxylate (40b)

The reaction was followed by GLC (3% SE30, 130°C). R_T (starting material) = 415 s, R_T (product) = 489 s. The product mixture (starting material and product in the ratio 3:97) was isolated as a colourless oil by distillation in a Kugelrohr apparatus (120°C/0.8 mmHg).

^1H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.28 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 1.3-2.35 (complex, 8 H, ring CH_2); 2.60 (m, 1 H, ring CH_2); 2.97 (m, 1 H, ring CH_2); 4.23 (q, 2 H, J=7.1 Hz, OCH_2); 4.29 ppm (bs, 1 H, OH).

^{13}C NMR, 22.63 MHz ($CDCl_3/CDCl_3$ =δ77.0 ppm): δ= 13.8 ($OCH_2\underline{CH_3}$); 23.5, 26.8, 29.9, 34.3, 39.9 (ring CH_2); 61.9 (OCH_2); 83.2 (C(OH)); 170.5 ($\underline{CO_2}CH_2$); 209.4 ppm (CO).

IR (film): ν= 3480, 1750, 1715 cm^{-1}.

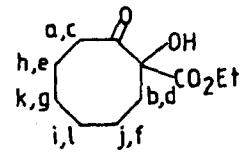
MS (EI): m/z= 200$(M)^{+\cdot}$, 127$(M-CO_2Et)^+$.

High resolution MS: $C_{10}H_{16}O_4$, calc. 200.1049, found 200.1047.

Elemental analysis: Found: C, 60.58; H, 8.49. $C_{10}H_{16}O_4$ requires C, 60.00; H, 8.00%.

Ethyl 1-hydroxycyclooctan-2-onecarboxylate (40c)

Isolated as a yellow oil by flash chromatography, eluting with ethyl acetate-petrol 3:17, R_F 0.28.



^1H NMR, 400 MHz ($CDCl_3$/TMS): δ= 0.90 (m, 1 H, c); 1.27 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 1.40 (m, 2 H, j, k); 1.75 (m, 4 H, f, g, h, i); 1.95 (m,

1 H, e); 2.15 (dt, 1 H, J=15.2, 4.0 Hz, d); 2.39 (dt, 1 H, J=12.2, 4.6 Hz, b); 2.73 (m, 1 H, b); 3.06 (td, 1 H, J=12.4, 3.7 Hz, a); 4.20 (q, 2 H, J=7.1 Hz, OCH_2); 4.42 ppm (bs, 1 H, OH).

The COSY-45 spectrum (400 MHz, $CDCl_3$/TMS) is given as a contour plot in Figure 9.1. Table 9.1 gives the nature of the coupling between the multiplets, as deduced from the COSY-45 spectrum. "2" indicates a 2-bond coupling and "3" indicates a 3-bond coupling.

Table 9.1

	a	b	c	d	e	f	g	h	i	j	k	l
l						3	3		2	3	3	
k					3		2	3	3			3
j		3		3		2			3			3
i						3	3			3	3	2
h	3		3		2		3				3	
g					3			3	3		2	3
f		3		3					3	2		3
e	3		3				3	2			3	
d		2				3				3		
c	2				3			3				
b				2		3				3		
a			2		3			3				

^{13}C NMR, 45.28 MHz ($CDCl_3/CDCl_3$ =δ77.0 ppm): δ= 13.7 ($OCH_2\underline{C}H_3$); 22.0, 23.9, 25.2, 29.9, 30.2, 36.7 (ring CH_2); 61.8 (OCH_2); 82.9 (C(OH)); 170.2 ($\underline{C}O_2CH_2$); 208.0 ppm (CO).

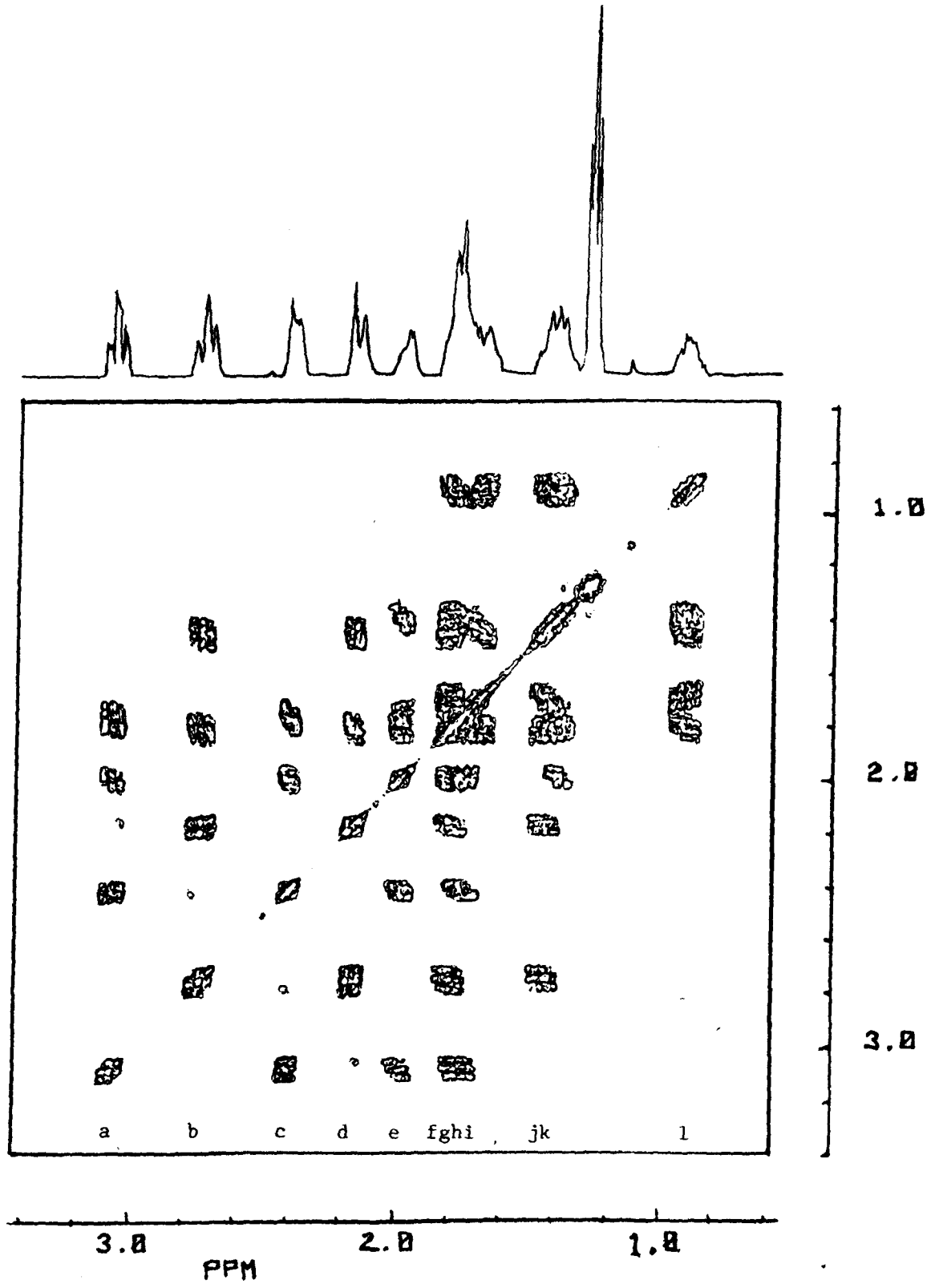


Figure 9.1

IR (film): ν = 3470, 1745, 1710 cm^{-1}.

MS (+ve CI, ammonia): m/z= 214$(M)^+$, 141$(M-CO_2Et)^+$.

High resolution MS: $C_{11}H_{18}O_4$, calc. 214.1205, found 214.1187.

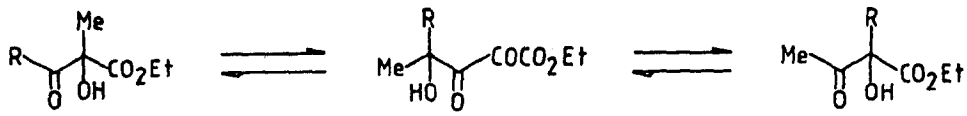
GLC (3% SE30, 150°C); R_T= 444 s.

Elemental analysis: Found: C, 61.81; H, 8.72. $C_{11}H_{18}O_4$ requires C, 61.68; H, 8.41%.

c) Acyclic substrates bearing non-identical substituents at the 3-position giving rise to product distributions:

General procedure:

The hydroxy keto ester (2 mmol) and dibutyl tin oxide (25 mg, 0.1 mmol, 5 mol%) were heated together in boiling toluene (5 ml) under nitrogen. The solvent was evaporated under reduced pressure. The residue was suspended in ether (10 ml), filtered and evaporated under reduced pressure to give the crude product mixture. Product distributions were analysed by integration of the proton NMR spectrum.



(41) (42) (43)

Starting material	R	Scale /mmol	Catalyst /mol%	Time /days	Recovery /%	Ratio (41)/(43)
41a[33d]	Et	2.3	6.7	0.9	71	1.0
41b[33e]	Pr	2.5	5.1	2.5	94	2.0
41c[33f]	Bu	3.1	5.2	5.2	92	2.5
42d[47c]	i-Pr	1.0	4.6	0.9	64	0.9
41e[33g]	t-Bu	1.5	7.2	3.0	82	2.5
41f[33i]	p-NO_2Ph	0.5	9.0	4.0	100	1.2
42g[47d]	Ph	1.0	4.0	0.9	48	2.7
41h[33j]	p-MeOPh	1.4	5.4	4.0	92	6.6

Proton NMR data for the starting materials may be found in chapter 8. The data given here are for the new products generated by the isomerisation procedure.

Ethyl 2-hydroxy-3-oxo-3-propylbutanoate (43b)

^{1}H NMR, 220MHz ($CDCl_3$/TMS): signals distinguishable δ= 0.94 (t, 3 H, J=7.1 Hz, propyl CH_3); 1.30 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 2.30 (s, 3 H, CH_3CO); 4.27 ppm (q, 2 H, J=7.1 Hz, OCH_2).

Ethyl 2-butyl-2-hydroxy-3-oxobutanoate (43c)

^{1}H NMR, 220MHz ($CDCl_3$/TMS): signals distinguishable δ= 2.29 ppm (s, CH_3CO).

Ethyl 2-hydroxy-2-(1-methylethyl)-3-oxobutanoate (43d)

^{1}H NMR, 220MHz ($CDCl_3$/TMS): signals distinguishable δ= 0.81 (d, 3 H, J=7.1 Hz, $CH\underline{CH_3}$), 0.95 (d, 3 H, J=7.1 Hz, $CH\underline{CH_3}$); 1.31 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 2.45 (s, 3 H, CH_3CO); 2.74 (m, 1 H, $\underline{CH}(CH_3)_2$); 4.17 ppm (bs, 1 H, OH).

Ethyl 2,4-dimethyl-2-hydroxy-3-oxopentanoate (41d)

^{1}H NMR, 220MHz ($CDCl_3$/TMS): signals distinguishable δ= 1.11 (dd, 6 H, J=6.7, 1.3 Hz, $CH(\underline{CH_3})_2$); 1.33 (t, 3 H, J=7.1 Hz, $OCH_2\underline{CH_3}$); 1.61 (s, 3 H, $C(OH)\underline{CH_3}$); 3.15 (m, 1 H, $\underline{CH}(CH_3)_2$); 4.17 ppm (bs, 1 H, OH).

Ethyl 2-(1,1-dimethylethyl)-2-hydroxy-3-oxobutanoate (43e)

^{1}H NMR, 220MHz ($CDCl_3$/TMS): signals distinguishable δ= 1.08 (s, 9 H, $C(CH_3)_3$); 2.32 ppm (s, 3 H, $C(OH)\underline{CH_3}$).

Ethyl 2-hydroxy-3-oxo-2-(4-nitrophenyl)butanoate (43f)

^{1}H NMR, 220MHz ($CDCl_3$/TMS): signals distinguishable δ= 2.25 (s, 3 H, CH_3CO); 7.74 ppm (m, 2 H, Ar).

HPLC (C_{18} reverse phase, methanol-water 3:1, 0.75 ml/minute, monitoring at 263 nm): R_T (41i)= 7.7 minutes; R_T (43i)= 8.8 minutes.

Ethyl 2-hydroxy-2-methyl-3-oxo-3-phenylpropanoate (41g)

^{1}H NMR, 220MHz ($CDCl_3$/TMS): signals distinguishable δ= 1.15 (t, 3 H, $OCH_2\underline{CH_3}$); 1.75 (s, 3 H, $C(OH)\underline{CH_3}$); 4.64 ppm (bs, 1 H, OH).

Ethyl 2-hydroxy-3-oxo-2-phenylbutanoate (43g)

^{1}H NMR, 220MHz ($CDCl_3$/TMS): signals distinguishable δ= 1.31 (t, 3 H, $OCH_2\underline{CH_3}$); 2.23 (s, 3 H, CH_3CO); 4.85 ppm (bs, 1 H, OH).

Ethyl 2-hydroxy-2-(4-methoxyphenyl)-3-oxobutanoate (43h)

^{1}H NMR, 220MHz ($CDCl_3$/TMS): signals distinguishable δ= 2.20 (s, 3 H, CH_3CO); 3.86 ppm (s, 3 H, CH_3OPh).

CHAPTER 10

PLE-catalysed hydrolysis and ADC-catalysed decarboxylation of racemic ethyl 2-ethyl-2-hydroxy-3-oxobutanoate followed by autotitration

Ethyl 2-ethyl-2-hydroxy-3-oxobutanoate (112 mg, 0.64 mmol) dissolved in water (5 ml) was treated with PLE (100 µl, 5 mg/ml, 65 units) at 32°C. The hydrolysis was followed at pH 7.5 by the uptake of 1.104 M aqueous sodium hydroxide from an autotitrator. Reaction was complete after 1h. The solution was acidified to pH 6.5 with hydrochloric acid. ADC (1.1 mg, Novo batch 2) was added. The decarboxylation at pH 6.5 and 32°C was followed by the uptake of 1.115 M hydrochloric acid from an autotitrator.

Uptake of alkali: 0.475 ml, 0.52 mmol

Uptake of acid: 0.457 ml, 0.51 mmol

Consequtive PLE-catalysed hydrolysis and ADC-catalysed decarboxylation of racemic ethyl 2-ethyl-2-hydroxy-3-oxobutanoate followed by proton NMR spectroscopy

To a solution of ethyl 2-ethyl-2-hydroxy-3-oxobutanoate (20 mg, 0.11 mmol) in 1 M pH 7.4 phosphate buffer (0.6 ml) was added PLE (40 µl, 10 mg/ml, 52 units). The hydrolysis was followed by proton NMR at 220 MHz on a continuous wave instrument. Reaction was complete after 50 minutes. ADC (0.4 mg, Novo batch 1) was added and the decarboxylation followed by proton NMR as before. Over a period of time there was a development of signals attributable to 3-hydroxypentan-2-one. Thereafter a slower development of signals attributable to 2-hydroxy pentan-3-one was observed. The relative

amounts of starting material and products were calculated from the ratio of the peak heights to the peak height of the triplet from the ethanol generated in the hydrolysis step.

Acid; $CH_3COC(OH)(Et)CO_2H$

Product 1; $CH_3COCH(OH)Et$

Product 2; $EtCOCH(OH)CH_3$

Time /minutes	Peak heights relative to ethanol triplet		
	Acid	Product 1	Product 2 (x2)
8	1.36	0.52	0.00
13	1.00	0.69	0.00
18	0.92	0.85	0.10
21	0.89	0.87	0.10
25	0.84	0.90	0.13
44	0.69	0.94	0.26
48	0.66	0.94	0.28
53	0.61	0.94	0.31
111	0.23	0.96	0.59
153	0.00	0.97	0.80

3-Hydroxypentan-2-one:

^1H NMR, 220 MHz, ($H_2O/CH_3CH_2OH = \delta$ 1.14 ppm): δ = 0.86 (t, 3 H, CH_2CH_3); 1.70 (m, 2 H, CH_2); 2.18 ppm (s, 3 H, CH_3CO).

2-Hydroxypentan-3-one:

^1H NMR, 220 MHz, ($H_2O/CH_3CH_2OH = \delta$ 1.14 ppm): δ = 0.98 (t, 3 H, CH_2CH_3); 1.33 (d, 3 H, $CHCH_3$); 2.56 ppm (m, 2 H, CH_2CH_3).

Consecutive PLE-catalysed hydrolysis and ADC-catalysed decarboxylation of racemic ethyl 2-ethyl-2-hydroxy-3-oxobutanoate and analysis of the optical purity of the products

Generation of racemic ketol mixture: Ethyl 2-ethyl-2-hydroxy-3-oxobutanoate (50 mg, 0.28 mmol) and ethyl 2-methyl-2-hydroxy-3-oxopentanoate (50 mg, 0.28 mmol) were treated with 3 M aqueous potassium hydroxide (2 ml). After 2 minutes the solution was acidified to pH 1 with concentrated hydrochloric acid and saturated with sodium chloride. Carbon tetrachloride (0.5 ml) was added, the water adsorbed onto crushed 4A molecular sieves, and the mixture filtered through a glass wool plug.

Enzymatic generation of ketol mixture: Ethyl 2-ethyl-2-hydroxy-3-oxobutanoate (38 mg, 0.22 mmol) was suspended in 1 M pH 7.2 phosphate buffer (0.8 ml). PLE (100 µl, 5 mg/ml, 65 units) was added and the hydrolysis followed by proton NMR at 30°C. After 3 h ADC (0.9 mg, Novo batch 1) was added and the decarboxylation followed by proton NMR at 30°C. The decarboxylation was complete after 1.5 h. The solution was saturated with sodium chloride. Magnesium sulphate and carbon tetrachloride (0.8 ml) were added and the emulsion filtered through a glass wool plug. d_6-Benzene (0.1 ml) was added and the proton NMR spectrum at 400 MHz determined in the presence of 2 equivalents of (S)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol. The spectrum was re-run in the presence of added racemic ketol mixture.

3-Hydroxypentan-2-one:

^{1}H NMR, 400 MHz (CCl_4, d_6-benzene/benzene= δ 7.20 ppm): δ = 0.78 (t, 3 H, J=7.37 Hz, $CH_2\underline{CH_3}$); 1.31 (m, 1 H, CH_2); 1.63 (m, 1 H, CH_2); 1.78 ppm (s, 3 H, CH_3CO).

Peak heights: major peak 1.78 ppm 162 mm

minor peak 1.81 ppm 6 mm

Enantiomeric excess = 93%

2-Hydroxypentan-3-one:

^{1}H NMR, 400 MHz (CCl_4, d_6-benzene/benzene=δ 7.20 ppm): δ= 0.91 (t, 3 H, J=7.31 Hz, $CH_2\underline{CH_3}$); 1.09 (d, 3 H, J=7.06 Hz, $CH\underline{CH_3}$); 2.09 ppm (m, 2 H, CH_2).

Splitting of the doublet at 1.09 ppm was only observed in the presence of added racemic compound. This gave an estimated enantiomeric excess of enzymatically generated 2-hydroxypentan-3-one of >95%.

Sequential PLE-catalysed hydrolysis and ADC-catalysed decarboxylation of racemic methyl 2-hydroxy-2-methyl-3-oxobutanoate

To a solution of racemic methyl 2-hydroxy-2-methyl-3-oxobutanoate (34.5 mg, 0.24 mmol) dissolved in 1 M pH 7.2 phosphate buffer (0.7 ml) was added PLE (100 μl, 5 mg/ml, 65 units) at 30°C. The hydrolysis of the methyl ester (followed by proton NMR spectroscopy) was complete after 1.5 h. ADC (0.8 mg, Novo batch 1) was added and the reaction was monitored as before at 30°C. The decarboxylation to give 3-hydroxy butan-2-one was complete after 1.5 h. Carbon tetrachloride (0.9 ml) was added, the water adsorbed onto crushed 4A molecular sieves, and the mixture filtered through a glass wool plug. d_6-Benzene (0.2 ml) was added and the proton NMR spectrum determined at 400 MHz in the presence of 2 equivalents of (S)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol. The spectrum was re-determined in the presence of added racemic 3-hydroxybutan-2-one.

3-Hydroxybutan-2-one:

^{1}H NMR, 400 MHz (CCl_4, d_6-benzene/benzene = δ 7.2 ppm): δ = 1.06 (d, 3 H, J=7.1 Hz, $CHC\underline{H}_3$); 1.73 ppm (s, 3 H, $COCH_3$).

Peak heights:	major peak	1.73 ppm	134 mm
	minor peak	1.73 ppm	5 mm

Enantiomeric excess = 93%

Treatment of racemic methyl 2-hydroxy-2-methyl-3-oxobutanoate with PLE and ADC

To a solution of racemic methyl 2-hydroxy-2-methyl-3-oxobutanoate (23 mg, 0.16 mmol) and ADC (1.0 mg, Novo batch 1) in 1 M pH 7.2 phosphate buffer (0.6 ml) was added PLE (50 μl, 5 mg/ml, 32 units). The reaction was followed at room temperature by proton NMR spectroscopy. Conversion of substrate into 3-hydroxybutan-2-one was complete after 3 h. Carbon tetrachloride (0.5 ml) was added, the water adsorbed onto crushed 4A molecular sieves, and the mixture was filtered through a glass wool plug. d_6-Benzene (0.1 ml) was added and the proton NMR spectrum determined at 400 MHz in the presence of 2 equivalents of (S)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol. The spectrum was re-determined in the presence of added racemic 3-hydroxybutan-2-one.

3-Hydroxybutan-2-one:

^{1}H NMR, 400 MHz (CCl_4, d_6-benzene/benzene = δ 7.2 ppm): δ = 1.08 (d, 3 H, J=7.1 Hz, $CHC\underline{H}_3$); 1.76 ppm (s, 3 H, $COC\underline{H}_3$).

No splitting was observed. Both enantiomers were observed on addition of racemic 3-hydroxybutan-2-one. Enantiomeric excess >99%.

PLE-catalysed hydrolysis and ADC-catalysed decarboxylation of racemic methyl 2-hydroxy-2-methyl-3-oxobutanoate followed by polarimetry

A mixture of racemic methyl 2-hydroxy-2-methyl-3-oxobutanoate (33.4 mg, 0.23 mmol), ADC (2.9 mg, Novo batch 2) and PLE (50 μl, 5 mg/ml, 32 units) was made up to a volume of 5 ml in a volumetric flask with 1 M pH 7.2 phosphate buffer. An aliquot was transfered to a 2 dm path length cell. Its optical rotation at 298 nm was monitored at room temperature.

Final rotation = -0.711^{o} at $23^{o}C$.

Assuming total conversion of substrate to 3-hydroxybutan-2-one, then c=0.403 g/100 ml. Calculated specific rotation $[\alpha]_D = -88^{o}$. Lit.[84] $[\alpha]_D$ -84 (c=0.6, H_2O).

Sequential treatment of racemic ethyl 2-hydroxy-2-methyl-3-oxopentanoate with PLE and ADC at pH 7.2

To a solution of racemic ethyl 2-hydroxy-2-methyl-3-oxopentanoate (25 mg, 0.14 mmol) in 1 M pH 7.2 phosphate buffer (0.6 ml) was added PLE (100 μl, 5 mg/ml, 65 units) and the mixture incubated at $34^{o}C$. Hydrolysis of the ester was complete after 2 h as judged by proton NMR spectroscopy. ADC (0.8 mg, Novo batch 2) was added and the decarboxylation was followed by proton NMR spectroscopy. The relative amounts of 2-hydroxy-2-methyl-3-oxopentanoic acid, 2-hydroxypentan-3-one and 3-hydroxypentan-2-one were calculated by comparison of the relevant peak heights from the compounds with that of the methyl triplet of the ethanol generated in the PLE-catalysed hydrolysis step. The proton NMR data for the α-hydroxyketones are given on page 157.

Time /minutes	Peak heights as % of ethanol triplet	
	$EtCOCH(OH)CH_3$ (x2)	$CH_3COCH(OH)Et$
3	31	10
6	42	14
9	56	16
11	71	21
14	73	24
17	80	33
19	83	42
38	87	93

Sequential treatment of racemic ethyl 2-hydroxy-2-methyl-3-oxopentanoate with PLE and ADC at pH 7.7

To a solution of racemic ethyl 2-hydroxy-2-methyl-3-oxopentanoate (39 mg, 0.22 mmol) in 1 M pH 7.7 phosphate buffer (0.6 ml) was added PLE (20 μl, 10 mg/ml, 26 units). The hydrolysis at 29°C was followed by proton NMR spectroscopy. The extent of reaction was calcuated by the peak height ratio of the methyl singlet in the ester and the acid.

ADC (0.6 mg, Novo batch 1) was added and the decarboxylation was followed by proton NMR spectroscopy. The final product distribution comprised equimolar 2-hydroxypentan-3-one and 3-hydroxypentan-2-one. An unidentified compound was observed which disappeared during the course of the reaction. The relative amounts of starting material, hydroxyketones and the unidentified transient compound were calculated by comparison of representative peak heights with the ethanol generated in the PLE-catalysed step.

Transient compound:

^1H NMR, 220 MHz, ($H_2O/CH_3CH_2OH = \delta$ 1.04 ppm): δ = 1.13 (t); 1.85 (s); 2.60 ppm (m).

^1H NMR data for the α-hydroxyketones are given on page 157.

PLE-catalysed hydrolysis:

Time/minutes	% reaction	Time/minutes	% reaction
4	14	40	79
7	25	45	82
12	38	50	84
15	48	55	87
21	62	60	89
25	68	70	95
33	74	95	97
35	76		

ADC-catalysed decarboxylation:

Time	Peak heights as % of ethanol triplet		
/minutes	$EtCOCH(OH)CH_3$ (x2)	$CH_3COCH(OH)Et$	singlet 1.85 ppm
4	24	2	123
6	32	4	113
8	42	6	103
10	44	7	94
12	52	10	87
14	56	13	79
16	56	15	74
18	64	18	71

21	64	22	63
25	66	32	58
29	68	40	53
34	66	53	51
41	68	66	48
49	70	78	42
56	72	90	40
68	72	106	36
77	76	116	36
85	76	117	33
106	78	117	30
122	80	115	26
138	82	113	24
147	82	112	23
158	84	112	21
173	84	111	20
190	86	112	18
207	86	111	16
227	88	110	15
1083	94	100	0

The action of PLE and ADC on racemic ethyl 2-ethyl-2-hydroxy-3-oxopentanoate

To a solution of racemic ethyl 2-ethyl-2-hydroxy-3-oxopentanoate (25.6 mg, 0.14 mmol) and ADC (4.1 mg, Novo batch 2) in 1 M pH 7.2 phosphate buffer (0.6 ml) was added PLE (100 µl, 5 mg/ml, 65 units). The reaction was followed by proton NMR spectroscopy at 30°C. Total

conversion of starting material to 4-hydroxyhexan-3-one was observed after 2.5 h. Carbon tetrachloride (0.6 ml) was added, the water was adsorbed onto crushed 3A molecular sieves, and the mixture was filtered through a glass wool plug. d_6-Benzene (0.1 ml) was added and the proton NMR spectrum was determined at 400 MHz in the presence of 6 equivalents of (S)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol. The spectrum was re-determined in the presence of added racemic 4-hydroxyhexan-3-one. In neither spectrum was splitting of the signals observed. Selective decoupling of the relevant multiplets to generate singlets also showed no conclusive splittings.

4-Hydroxyhexan-3-one:

^{1}H NMR, 400 MHz (CCl_4, d_6-benzene/benzene = δ 7.2 ppm): δ = 0.77 (t, 3 H, J=7.5 Hz, $CHCH_2\underline{CH_3}$); 0.92 (t, 3 H, J=7.5 Hz, $COCH_2\underline{CH_3}$); 1.3 (m, 1 H, $CHCH_2$); 1.62 (m, 1 H, $CHCH_2$); 2.1 ppm (m, 2 H, $COCH_2$).

<u>The action of PLE and ADC on racemic ethyl 2-ethyl-2-hydroxy-3-oxopentanoate followed by polarimetry</u>

A mixture of racemic ethyl 2-ethyl-2-hydroxy-3-oxopentanoate (38.6 mg, 0.205 mmol), ADC (3.3 mg, Novo batch 2) and PLE (50 μl, 5 mg/ml, 32 units) was made up to a volume of 5 ml with 1 M pH 7.2 phosphate buffer in a volumetric flask. An aliquot was transferred to a 2 dm path length cell. Its optical rotation at 289 nm was followed at room temperature.

Final observed rotation = -0.727° at 27°C. Assuming total conversion of starting material to 4-hydroxyhexan-3-one, then c= 0.476 g/100 ml. Calculated specific rotation $[\alpha]_D$ = -76°.

The action of PLE and ADC on racemic ethyl 2-hydroxy-2-methyl-3-oxohexanoate

To a mixture of racemic ethyl 2-hydroxy-2-methyl-3-oxohexanoate (37 mg, 0.2 mmol) and ADC (7.3 mg, Novo batch 2) in 1 M pH 7.2 phosphate buffer (0.6 ml) was added PLE (100 μl, 5 mg/ml, 65 units). The mixture was agitated at 33°C and the reaction monitored by TLC (ethyl acetate-petrol 3:7); Starting material R_F 0.41, new products R_F 0.27. After 6 h no starting material remained. Carbon tetrachloride (0.8 ml) was added, the water was adsorbed onto crushed 3A molecular sieves, and the mixture was filtered through a glass wool plug. d_6-Benzene (0.1 ml) was added and the proton NMR spectrum was determined at 400 MHz in the presence of 2 equivalents of (S)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol. The spectrum was re-run in the presence of added racemic 2-hydroxyhexan-3-one and 3-hydroxyhexan-2-one.

3-Hydroxyhexan-2-one:

^1H NMR, 400 MHz (CCl_4, d_6-benzene/benzene = δ 7.2 ppm): δ = 0.75 (t, 3 H, J=7.4 Hz, $CH_2\underline{CH_3}$); 1.18-1.50 (complex, 6 H, propyl CH_2 from both hydroxyketones); 1.74 ppm (s, 3 H, $COCH_3$).

Peak heights:	major peak 1.74 ppm	147 mm
	minor peak 1.75 ppm	6 mm

Enantiomeric excess = 92%

2-Hydroxyhexan-3-one:

^1H NMR, 400 MHz (CCl_4, d_6-benzene/benzene = δ 7.2 ppm): δ = 0.81 (t, 3 H, J=7.2 Hz, $CH_2\underline{CH_3}$); 1.06 (d, 3 H, J=7.1 Hz, $CH\underline{CH_3}$); 2.01 ppm (m, 2 H, $COCH_2$).

Splittings were only observed on addition of racemic 2-hydroxyhexan-3-one. Estimated enantiomeric excess >95%.

The action of PLE on racemic ethyl 2-hydroxy-3-oxo-2,3,3-trimethylpentanoate

Racemic ethyl 2-hydroxy-3-oxo-2,3,3-trimethylpentanoate (41.9 mg, 0.21 mmol) suspended in water (5 ml) at 32°C was treated with PLE (20 µl, 5 mg/ml, 13 units) and maintained at pH 7.5 by titration with 0.11 M aqueous sodium hydroxide from an autotitrator. After 2 h there had been no uptake of alkali from the autotitrator.

The action of PLE on racemic methyl 1-hydroxy-2-oxocyclohexane carboxylate

Racemic methyl 1-hydroxy-2-oxocyclohexanecarboxylate was treated with PLE as above. No reaction was observed.

The action of ADC on racemic 2-hydroxy-3-oxo-2,3,3-trimethylpentanoic acid

Racemic ethyl 2-hydroxy-3-oxo-2,3,3-trimethylpentanoate (26 mg, 0.13 mmol) was treated with 3 M aqueous sodium hydroxide (0.2 ml, 0.6 mmol). After 2 minutes the solution was acidified to pH 7 with 4 M hydrochloric acid. 1 M pH 7.2 Phosphate buffer (0.6 ml) and ADC (2.2 mg, Novo batch 2) were added. The mixture was incubated at 30°C and its proton NMR spectrum recorded periodically. No change was observed over a 4 h period.

^{1}H NMR, 220 MHz (H_2O/CH_3CH_2OH = δ 3.55 ppm): δ = 1.10 (s, 9 H, $C(CH_3)_3$); 1.31 ppm (s, 3 H, $C(OH)CH_3$).

The action of ADC on racemic 1-hydroxycyclohexan-2-one carboxylate

Racemic methyl 1-hydroxycyclohexan-2-one carboxylate (286 mg, 1.66 mmol) was treated with 1 M aqueous potassium hydroxide (2 ml). After 1.5 minutes the solution was acidified to pH 7 with 5 M hydrochloric acid. ADC (1.8 mg, Novo batch 2) was added and the uptake of 1.01 M hydrochloric acid from an autotitrator at pH 6.5 and 32°C was monitored. There was no further reaction after 3 h. The solution was extracted with dichloromethane (4x20 ml). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure/30°C to give a yellow oil (69 mg, 0.6 mmol, 37%).

Uptake of acid = 0.711 ml (0.718 mmol, 43%).

^1H NMR, 220MHz ($CDCl_3$/TMS): δ= 1.1-2.8 (complex, 8 H, CH_2); 3.70 (bs, 1 H, OH); 4.15 ppm (dd, 1 H, J=7, 12 Hz, CH(OH)).

IR (film): ν= 3400, 1710 cm^{-1}.

MS (EI): m/z= 144$(M)^+$, 70$(M-CO_2)^+$.

High resolution MS: $C_6H_{10}O_2$, calc. 114.0680, found 114.0680.

ADC-catalysed deuterium incorporation into racemic acetoin

Racemic acetoin (31 mg, 0.35 mmol) and ADC (0.7 mg, Novo batch 1) were suspended in deuterium oxide (0.6 ml) and incubated at 43°C. The proton NMR spectrum was determined periodically. After 5 days the reaction mixture was diluted to a volume of 15 ml with water. Racemic acetoin (110 mg, 1.25 mmol) was added and the solution was extracted with ether continuously for 10 h. Water (1.5 ml) was added to the ether phase. The ether was evaporated under reduced pressure/20°C. The deuterium NMR spectrum of the remaining aqueous phase was determined.

^1H NMR, 220 MHz (D_2O/HOD = δ4.83 ppm): δ= 1.37 (complex, 3 H,

$CH(D)\underline{C}H_3$); 2.23 (s, 3 H, $COCH_3$); 4.43 ppm (q, 0.5 H, J=7.2 Hz, CH). ^2H NMR, 61.4 MHz (H_2O/H_2O = δ 4.76 ppm): δ = 4.33 ppm (s, CH_3CD).

ADC-catalysed deuterium encorporation into racemic acetoin and analysis by vibrational circular dichroism

Racemic acetoin (147 mg, 1.67 mmol) and ADC (3.1 mg, Novo batch 2) were suspended in deuterium oxide (2 ml) and incubated at 34°C. The proton NMR spectrum was determined periodically. After 4 days the reaction mixture was diluted with water (10 ml) and extracted with ether continuously for 10 h. Carbon tetrachloride (10 ml) was added and the ether was distilled off at atmospheric pressure. The vibrational circular dichroism spectrum of the carbon tetrachloride solution and that of a carbon tetrachloride solution of (R)-(-)-acetoin of normal isotopic content was determined by Dr A.F.Drake at the Department of Chemistry, Birkbeck College, London. These spectra are shown on page 78.

The action of ALS II and ADC on sodium pyruvate

Sodium pyruvate (49.4 mg, 0.45 mmol), ADC (3.4 mg, Novo batch 2), TPP (3.6 mg, 8.8 μmol), FAD (1.1 mg, 1.4 μmol) and magnesium chloride (12.2 mg, 0.13 mmol) were dissolved in 1 M pH 7.2 phosphate buffer (0.8 ml). ALS II (5 μl, 149 mg/ml, 11 units) was added and the solution was incubated in the dark at 37°C. The proton NMR spectrum was determined periodically. Acetoin was the sole observed product.

Time/h	% conversion	Time/h	% conversion
0.25	59	1.00	98
0.28	66		

The action of ALS II and ADC on sodium 2-oxobutanoate

Sodium 2-oxobutanoate (55 mg, 0.44 mmol), ADC (5.2 mg, Novo batch 2), TPP (3.8 mg, 9.3 mol), FAD (0.8 mg, 1.0 mol) and magnesium chloride (14 mg, 0.15 mmol) were dissolved in 1 M pH 7.2 phosphate buffer (0.8 ml). ALS II (5 1, 149 mg/ml, 11 units) was added and the solution was incubated in the dark at 37°C. The proton NMR spectrum was determined periodically. The sole observed product was 4-hydroxyhexan-3-one.

^{1}H NMR, 220 MHz, (H_2O/TSS): δ = 0.89 (t, 3 H, J=7.6 Hz, $CHCH_2\underline{CH_3}$); 1.03 (t, 3 H, J=7.6 Hz, $COCH_2\underline{CH_3}$); 1.76 (m, 2 H, $CH\underline{CH_2}$); 2.60 ppm (dq, 2 H, J=7.6,2.7 Hz, $COCH_2$).

Time/h	% conversion	Time/h	% conversion
0.7	15	7.7	86
2.9	63	10.5	91
3.7	70	23.8	97
5.3	79	33.4	97

The action of ALS II and ADC on sodium 2-oxobutanoate followed by polarimetry

Sodium 2-oxobutanoate (44.0 mg, 0.355 mmol) and ADC (3.0 mg, Novo batch 2) were dissolved in 0.1 M pH 7.2 phosphate buffer containing magnesium chloride (0.12 M), FAD (14 μM) and TPP (2.4 mM). ALS II (10 μl, 149 mg/ml, 22.3 units) was added and the solution was made up to 5 ml in a volumetric flask with more buffer solution. The solution was incubated at 37°C in the dark. Aliquots (1.03 ml) were removed periodically, diluted to a volume of 5 ml with water and the optical rotation was measured at 289 nm. The polarimeter had previously been

zeroed using the buffer solution diluted similarly.

On the basis of complete conversion of starting material to product, then the concentration of 4-hydroxyhexan-3-one in the polarimeter cell = 0.0848 g/100 ml.

Time /h	Rotation /°	temperature /°C	[α] /°
3.4	-0.122	26	-72
4.9	-0.133	27	-78
8.3	-0.137	26	-81

The action of ALS II and ADC on 2-oxo-2-phenylethanoic acid

2-Oxo-2-phenylethanoic acid (79.4 mg, 0.53 mmol), ADC (2.4 mg, Novo batch 2), TPP (3.5 mg, 8.6 μmol), FAD (1.1 mg, 1.4 μmol) and magnesium chloride (10.2 mg, 0.11 mmol) were dissolved in 1 M pH 7.2 phosphate buffer (0.8 ml). ALS II (5 μl, 149 mg/ml, 11 units) was added and the solution was incubated in the dark at 37°C. The proton NMR spectrum was determined periodically. There was no observable change over a 10 h period.

The action of ALS II and ADC on equimolar sodium pyruvate and sodium 2-oxobutanoate

Sodium pyruvate (56.5 mg, 0.514 mmol), sodium 2-oxobutanoate (63.8 mg, 0.515 mmol), ADC (5.5 mg, Novo batch 2), TPP (1.4 mg, 3.4 μmol), FAD (2.9 mg, 3.7 μmol) and magnesium chloride (10.5 mg, 0.11 mmol) were dissolved in 1 M pH 7.2 phosphate buffer (0.8 ml). ALS II (5 μl, 149 mg/ml, 11 units) was added and the solution was incubated in the dark at 37°C. The proton NMR spectrum was determined

periodically. The generation of signals attributable to 3-hydroxypentan-2-one was observed.

^{1}H NMR, 220 MHz, (H_2O/TSS): δ = 0.90 (t, 3 H, J=7 Hz, $CH_2\underline{CH_3}$); 1.75 (m, 2 H, CH_2); 2.21 ppm (s, 3 H, $COCH_3$).

Time/h	% conversion	Time/h	% conversion
0.15	15	4.93	95
1.05	87	7.72	95
2.62	92	21.00	96

The action of ALS II and ADC on equimolar sodium pyruvate and sodium 2-oxobutanoate followed by ploarimetry

Sodium pyruvate (34.0 mg, .309 mmol), sodium 2-oxobutanoate (37.7 mg, 0.304 mmol) and ADC (2.0 mg, Novo batch 2) were dissolved in 0.1 M pH 7.2 phosphate buffer containing magnesium chloride (0.12 M), FAD (14 μM) and TPP (2.4 mM). ALS II (10 μl, 149 mg/ml, 22.3 units) was added and the was solution made up to 5 ml in a volumetric flask with more buffer solution. The solution was incubated at 37°C in the dark. Aliquots (1.03 ml) were removed periodically, diluted to a volume of 5 ml with water and the optical rotation was measured at 289 nm. The polarimeter had previously been zeroed using the buffer solution diluted similarly.

On the basis of complete conversion of starting material to product, then the concentration of 3-hydroxypentan-2-one in the polarimeter cell = 0.128 g/100 ml.

Time /h	Rotation /°	temperature /°C	[α] /°
2.9	-0.225	26	-88
4.5	-0.238	27	-93
7.9	-0.240	26	-94

The action of ALS II and ADC on equimolar sodium pyruvate and 2-oxo-2-phenylethanoic acid

2-Oxo-2-phenylethanoic acid (78 mg, 0.52 mmol), sodium pyruvate (56 mg, 0.51 mmol), ADC (3.4 mg, Novo batch 2), TPP (4.9 mg, 12.0 μmol), FAD (2.0 mg, 2.6 mol) and magnesium chloride (14.3 mg, 0.15 mmol) were dissolved in 1 M pH 7.2 phosphate buffer (0.8 ml). ALS II (5 μl, 149 mg/ml, 11 units) was added and the solution was incubated in the dark at 37°C. The proton NMR spectrum was determined periodically. There was no observable change over a 10 h period.

Preparation of trans-cyclohexane-1,2-diol

To a solution of cyclohexene (5 ml, 49.3 mmol) in 98% formic acid (50 ml) was added a 30% solution of hydrogen peroxide in water (17 ml, 165 mmol). The stirred mixture was heated at 60-70°C for 2 h to give a homogeneous solution. The solvent was evaporated under reduced pressure/50°C and the residue was heated with sodium hydroxide (7 g, 175 mmol) in boiling water (50 ml) for 1 h. The solution was evaporated to dryness under reduced pressure/50°C. The residue was suspended in dichloromethane (50 ml) and filtered. The solid was washed with dichloromethane (3x40 ml). The combined dichloromethane portions were dried ($MgSO_4$) and evaporated under reduced pressure/40°C

to give the diol as a white crystalline solid (4.960 g, 40.4 mmol, 82%). This was recrystallised from acetone to give a white crystalline solid mp 102-103°C (2.42 g, 42%). Lit.[63] mp 102-103°C.

^{1}H NMR, 220 MHz, ($CDCl_3$/TMS): δ = 1.26 (complex, 4 H, CH_2); 1.70 (complex, 2 H, CH_2); 1.96 (complex, 2 H, CH_2); 3.36 (complex, 2 H, CH(OH)CH(OH)); 3.51 ppm (bs, 2 H, OH).

^{13}C NMR, 22.63 MHz ($CDCl_3$/$CDCl_3$ = δ77.0 ppm): δ = 24.2 ($C(OH)CH_2\underline{C}H_2\underline{C}H_2$); 32.8 ($\underline{C}H_2CH(OH)CH(OH)\underline{C}H_2$)); 75.6 ppm ($\underline{C}H(OH)\underline{C}H(OH)$).

IR (film): ν = 3300 cm^{-1}.

MS (EI): m/z= 116$(M)^{+\cdot}$, 98$(M-OH)^+$, 70$(C_5H_{10})^+$.

High resolution MS: $C_6H_{12}O_2$, calc. 166.0837, found 166.0837.

Reduction of 2-hydroxycyclohexanone with sodium borohydride in water

A suspension of 2-hydroxycyclohexanone dimer (199 mg, 1.7 mmol with respect to monomer) in water (10 ml) was stirred for 1.5 days to give a homogeneous solution. Sodium borohydride (52 mg, 1.4 mmol) was added and the mixture was stirred for 24 h. The solution was acidified to pH 1 with concentrated hydrochloric acid and extracted with dichloromethane (4x30 ml). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure/40°C to give the diol mixture as a white crystalline solid (64 mg, 32%).

Data for the trans-diol is given above.

Isomer mixture: Data for the cis-diol;

^{13}C NMR, 22.63 MHz ($CDCl_3$/$CDCl_3$ = δ77.0 ppm): δ = 21.4 ($C(OH)CH_2\underline{C}H_2\underline{C}H_2$); 29.9 ($\underline{C}H_2CH(OH)CH(OH)\underline{C}H_2$); 70.6 ppm ($\underline{C}H(OH)\underline{C}H(OH)$).

Relative peak heights cis-diol:trans-diol 1:1

Reduction of 2-hydroxycyclohexanone with sodium borohydride in methanol

To a mixture of 2-hydroxycyclohexanone dimer (475 mg, 4.2 mmol with respect to monomer) and sodium borohydride (154 mg, 4.0 mmol) under nitrogen was added dry methanol (15 ml). The mixture became hot and formed a homogeneous solution after 3 minutes. After 10 minutes no starting material was observed by TLC (ethyl acetate). After 30 minutes the solvent was evaporated under reduced pressure/40°C, the residue was dissolved in water (10 ml), acidified to pH 1 with dilute hydrochloric acid and extracted with dichloromethane (4x15 ml). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure/40°C to give the diol mixture as a white crystalline solid (133 mg, 27%).

Ratio of cis-diol:trans-diol as judged by ^{13}C NMR; 1:1.

Reduction of 2-hydroxycyclohexanone with sodium borohydride in ethanol

To a mixture of 2-hydroxycyclohexanone dimer (442 mg, 3.9 mmol with respect to monomer) and sodium borohydride (143 mg, 3.8 mmol) under nitrogen was added dry ethanol (15 ml). After 30 minutes no starting material was observed by TLC (ethyl acetate). After 50 minutes the solvent was evaporated under reduced pressure/40°C, the residue dissolved in water (10 ml), acidified to pH 1 with dilute hydrochloric acid and extracted with dichloromethane (4x15 ml). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure/40°C to give the diol mixture as a white crystalline solid (177 mg, 39%).

Ratio of cis-diol:trans-diol as judged by ^{13}C NMR; 1:1.

Reduction of 2-hydroxycyclohexanone with sodium borohydride in propan-2-ol

To a mixture of 2-hydroxycyclohexanone dimer (502 mg, 4.4 mmol with respect to monomer) and sodium borohydride (110 mg, 2.9 mmol) under nitrogen was added dry propan-2-ol (20 ml). The mixture was stirred vigorously at 0°C for 2 h and for 24 h at room temperature.The solvent was evaporated under reduced pressure/40°C, the residue dissolved in water (10 ml), acidified to pH 1 with dilute hydrochloric acid and extracted with dichloromethane (4x15 ml). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure/40°C to give the diol mixture as a white crystalline solid (145 mg, 28%). Ratio of cis-diol:trans-diol as judged by ^{13}C NMR; 1:1.45.

Reduction of 2-hydroxycyclohexanone with lithium aluminium hydride

To a stirred suspension of 2-hydroxycyclohexanone dimer (328 mg, 2.9 mmol with respect to monomer) in dry ether (50 ml) at -75°C under nitrogen was added a suspension of lithium aluminium hydride (500 mg, 13 mmol) in ether (40 ml) over a period of 20 minutes. The mixture was stirred at -75°C for 4 h and at room temperature for 10 h, poured onto water (30 ml) and acidified to pH 1 with 4 M hydrochloric acid. The aqueous layer was extracted with dichloromethane (4x50 ml). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure/35°C to give the diol mixture as a white crystalline solid (121 mg, 36%). mp 67-68°C

Ratio of cis-diol:trans-diol as judged by ^{13}C NMR; 1.5:1.

REFERENCES

1. Walsh, C., Tetrahedron, 1982, 38, 871-909.
2. Crout, D.H.G., and Hedgecock, J.R., J. Chem. Soc. Perkin Trans. 1, 1979, 1982-1989.
3. Anatol,J., and Medete, A., Bull. Soc. Chim. France, 1972, 189-192.
4. Organic Synthesis, 2nd Edition, Coll. vol. 1, 241-245
5. David, S., and Thieffry, A., J. Chem. Soc. Perkin Trans. 1, 1979, 1568-1573.
6. Considine, W.J., J. Organomet. Chem., 1966, 5, 263-266.
7. Mehrotra, R.C., and Gupta, V.D., J. Organomet. Chem., 1965, 4, 145-150.
8. Smith, P.J., White, R.F.M., and Smith, L., J. Organomet. Chem., 1972, 40, 341-353.
9. David, S., and Thieffry, A., Tetrahedron Lett., 1981, 22, 2647-2650.
10. Schloss, J.V., Van Dyk, D.E., Vasta, J.F., and Kutny, R.M., Biochemistry, 1985, 24, 4952-4959.
11. LaRossa, R.A., and Schloss, J.V., J. Biol. Chem., 1984, 259, 8753-8757.
12. Krampitz, L.O., Methods in Enzymology, 1957, 3, 277-283.
13. Hill, R.K., and Sawada, S., Bioorg. Chem., 1979, 8, 175-189.
14. Armstrong, F.B., Lipscomb, E.L., Crout, D.H.G., Mitchell, M.B., and Prakash, S.R., J. Chem. Soc. Perkin Trans. 1, 1983, 1197-1201.
15. Andriamialisoa, R.Z., Langlois, N., and Langlois, Y.,

Tetrahedron Lett., 1985, 3563-3566.

16. Heathcock, C.H., Mahaim, C., Schlecht, M.F., and Utawanit, T., J. Org. Chem., 1984, 49, 3264-3274.

17. Kutney, J.P., Bunzli-Trepp, U., Chan, K.K., de Souza, J.P., Fujise, Y., Honda, T., Katsube, J., Klein, F.K., Leutwiler, A., Morehead, S., Rohr, M., and Worth, B.R., J. Am. Chem. Soc., 1978, 100, 4220-4244.

18. Ando, M., Büchi, G., and Ohnuma, T., J. Am. Chem. Soc., 1975, 97, 6880-6991.

19. Büchi, G., Matsumoto, K.E., and Nishimura, H., J. Am. Chem. Soc., 1971, 93, 3329-3331.

20. Hubert, A.J., and Starcher, P.S., J. Chem. Soc. (C), 1968, 2500-2502.

21. Coleman, J.E., Ricciuti, C., and Swern, D., J. Am. Chem. Soc., 1956, 78, 5342-5345.

22. Srinivasan, N.S., and Lee, D.G., Synthesis, 1979, 520-521.

23. Wolfe, S., Ingold, C.F., and Lemieux, R.U., J. Am. Chem. Soc., 1981, 103, 938-939.

24. King, G., J. Chem. Soc. (C), 1936, 1788-1792.

25. Freeman, F., Fuselier, C.O., Tetrahedron Lett., 1975, 2133-2136.

26 Lemieux, R.U., Kullnig, R.K., and Moir, R.Y., J. Am. Chem. Soc., 1958, 80, 2237-2242.

27. Wilberg, K.B., and Saegebarth, K.A., J. Am. Chem. Soc., 1957, 79, 2822-2824.

28. Wilberg, K.B., and Geer, R.D., J. Am. Chem. Soc., 1966, 88, 5827-5832.

29. Lee, D.G., and Brownridge, J.R., J. Am. Chem. Soc., 1974, 96,

5517-5523.

30. Fridovich, I., in "The Enzymes," ed. Boyer, P.D., Academic Press, New York and London, 3rd edition, 1977, 6, 255-270.

31. Hamilton, G.A., and Westheimer, F.H., J. Am. Chem. Soc., 1959, 81, 6332-6333.

32. Fridovich, I., and Westheimer, F.H., J. Am. Chem. Soc., 1962, 84, 3208-3209.

33. Warren, S., Zerner, B., and Westheimer, F.H., Biochemistry, 1966, 5, 817-823.

34. Godtfredsen, S.E., and Ottesen, M., Carlesberg Res. Commun., 1982, 47, 92-102.

35. Robins, D.J., and Crout, D.H.G., J. Chem. Soc. (C), 1970, 1334-1336.

36. Blom, R.H., J. Am. Chem. Soc., 1945, 67, 494.

37. Crout, D.H.G., Littlechild, J., Mitchell, B., and Morrey, S.M., J. Chem. Soc. Perkin Trans. 1, 1984, 2271-2276.

38. Loken, J.P., and Stormer, F.C., Eur. J. Biochem., 1970, 14, 133-137.

39. Juni, E., J. Biol. Chem., 1952, 195, 715-726.

40. Lienhard, G.E., and Rose, I.A., Biochemistry, 1964, 3, 190-195.

41. Knapp, F.F., Goad, L.J., and Goodwin, T.W., Phytochemistry, 1977, 16, 1677.

42. Schutzbach, J.S., and Feingold, D.S., J. Biol. Chem., 1970, 245, 2476-2482.

43. Chang, C-C., Laghai, A., O'Leary, M.H., and Floss, H.G., J. Biol. Chem., 1982, 257, 3564-3569.

44. Palekar, A.G., Tate, S.S., and Meister, A., Biochemistry, 1971,

10, 2180-2182.

45. Battersby, A.R., Chrystal, E.J.T., and Staunton, J., J. Chem. Soc. Perkin Trans. 1, 1980, 31-42.

46. Leistner, E., and Spencer, I.D., J. Chem. Soc. Chem. Commun., 1975, 378-379.

47. Battersby.A.R., Nicoletti, M., Staunton, J., and Vleggaar, M., J. Chem. Soc. Perkin Trans. 1, 1980, 43-51.

48. Battersby, A.R., Staunton, J., and Tippet, J., J. Chem. Soc. Perkin Trans. 1, 1982, 455-459.

49. Orr, G.R., and Gould, S.J., Tetrahedron Lett., 1982, 3139-3142.

50. Allen, R.R., and Klinman, J.P., J. Biol. Chem., 1981, 256, 3233-3239.

51. Rose, I.A., J. Biol. Chem., 1970, 245, 6051-6056.

52. Rose, Z.B., J. Biol. Chem., 1966, 241, 2311-2313.

53. Filler, R., Chem. Rev., 1963, 63, 21-43.

54. Kruse, P.F., Geurkink, N., and Grist, K.L., J. Am. Chem. Soc., 1954, 76, 5796-5797.

55. Fieser, L.F., and Rajagopalan, S., J. Am. Chem. Soc., 1949, 71, 3935-3938.

56. Fieser, L.F., and Rajagopalan, S., J. Am. Chem. Soc., 1949, 71, 3938-3941.

57. Stuckwisch, C.G., Hammer, G.G., and Blau, N.F., J. Org. Chem., 1957, 22, 1678-1680.

58. Sheehan, J.C., and Hess, G.P., J. Am. Chem. Soc., 1955, 77, 1067-1068.

59. Belleau, B., and Malek, G., J. Am. Chem. Soc., 1968, 90, 1650-1651.

60. Piancatelli, G., Scettri, A., and D'Auria, M., Synthesis, 1982 245-257.

61. Bowers, A.J., Halsall, T.G., Jones, E.R.H., and Lemin, A.J., J. Chem. Soc., 1953, 2548-2560.

62. Junge, W., and Heymann, E., Eur. J. Biochem., 1979, 95, 519-525.

63. Swern, D., Org. React., 1953, 7, 378-433.

64. Wadsworth, W.S., and Emmons, W.D., J. Am. Chem. Soc., 1961, 83 1733-1737.

65. Tanikaga, R., Miyashita, K., Ono, N., and Kaji, A., Synthesis, 1982, 131-132.

66. Larock, R.C., J. Org. Chem., 1975, 40, 3237-3242.

67. Vieregge, H., Schmidt, H.M., Renema, J., Bos, H.J.T., and Arens, J.F., Rec. Trav. Chim., 1966, 85, 929-951.

68. White, J.D., Takabe, K., and Prisbylla, M.P., J. Org. Chem., 1985, 50, 5233-5244.

69. Kinstle, T.H., and Mandanas, B.Y., J. Chem. Soc. Chem. Commun., 1968, 1699-1700.

70. Aplin, R.T., and Frearson, M.J., Chem. Ind. (London), 1969, 1663-1664.

71. Stevens, C.L., Glenn, F.E., and Pillai, P.M., J. Am. Chem. Soc., 1973, 95, 6301-6308.

72. van der Westhuizen, J.H., Ferreira, D., and Roux, D.G., J. Chem. Soc. Perkin Trans. 1, 1980, 2856-2865.

73. Crout, D.H.G., and Rathbone, D.L., J. Chem. Soc. Chem. Commun., 1987, 290-291.

74. Turner, R.B., J. Am. Chem. Soc., 1953, 75, 3484-3488.

75. Wendler, N.L., Taube, D., and Walker, R.W., Tetrahedron, 1960,

11, 163-170.

76. Oliveto, E.P., Gerold, C., Rausser, R., and Hershberg, E.B., J. Am. Chem. Soc., 1957, 79, 3594-3597.

77. Fishman, J., J. Am. Chem. Soc., 1960, 82, 6143-6147.

78. Stevens, C.L., Treat, T.A., and Pillai, P.M., J. Org. Chem., 1972, 37, 2091-2097.

79. Weisner, K., Chan, K.K., and Demerson, L., Tetrahedron Lett., 1965, 2893-2897.

80. Van Rheenen, V., Kelly, R.C., and Cha, D.Y., Tetrahedron Lett., 1976, 1973-1976.

81. Wolinsky, J., and Erickson, K.L., J. Org. Chem., 1965, 30, 2208-2211.

82. Stoops, K.J., Horgan, D.J., Runnegar, M.T.G., and de Jersey, J., Biochemistry, 1969, 8, 2026-2033.

83. Pirkle, K.J., and Hoover, D.J., Top. Stereochem., 1982, 13, 263-331.

84. Crout, D.H.G., and Morrey, M., J. Chem. Soc. Perkin Trans. 1, 1983, 2435-2449.

85. Crout, D.H.G., and Rathbone, D.L., J. Chem. Soc. Chem. Commun., 1987, in press.

86. Fulling, G., and Sih, C.J., J. Am. Chem. Soc., 1987, 109, 2845-2846.

87. Otha, H., Ikemoto, Y., Chem. Lett., 1986, 1169-1172.

88. Rose, I.A., O'Connell, E.L., and Mortlock, R.P., Biochim. Biophys. Acta , 1969, 178, 376-379.

89. Hanson, K.R., and Rose, I.A., Accounts of Chemical Research, 1975, 8, 1-10.

90. Schloss, J.V., and Van Dyk, D.E., Methods in Enzymology, 1987, in press.

91. Abell, L.M., O'Leary, M.H., and Schloss, J.V., Biochemistry, 1985, 29, 3357.

92. Ciskanik, L.M., and Schloss, J.V., Biochemistry, 1985, 29, 3357.

93. Armstrong, F.B., Hedgecock, C.J.R., Reary, J.B., Whitehouse, D., and Crout, D.H.G., J. Chem. Soc. Chem. Commun., 1974, 351-352.

94. Stormer, F.C., J. Biol. Chem., 1968, 243, 3735-3739.

95. Cram, D.J., and Kopecky, K.R., J. Am. Chem. Soc., 1959, 81, 2748-2755.

96. Porai-Koshits, M.A., Antsyshkina, A.S., Pasynskii, A.A., Sadikov, G.G., Skripkin, Y.V., and Ostrikova, V.N., Inorg. Chim. Acta., 1979, 34, L285-L287.

97. Nakata, T., Tanaka, T., and Oishi, T., Tetrahedron Lett., 1983, 2653-2656.

98. Patel, D.V., Van Middlesworth, F., Donaubauer, J., Gannett, P., and Sih, C.J., J. Am. Chem. Soc., 1986, 108, 4603-4614.

99. Katzenellenbogen, J.A., and Bowlus, S.B., J. Org. Chem., 1973, 38, 627-632.

100. Katzenellenbogen, J.A., and Bowlus, S.B., J. Org. Chem., 1974, 39, 3309-3314.

101. Cherest, M., and Felkin, H., Tetrahedron Lett., 1968, 2199-2204.

102. Morgan, L.R., Schunior, R.J., and Boyer, J.H., J. Org. Chem., 1963, 28, 260-261.

103. Iwakura, Y., Toda, F, and Torii, Y., J. Org. Chem., 1966, 31, 2875-2878.

104. Mori, M., Bull. Chem. Soc. Jpn., 1961, 34, 453-454.

105. Gakhokidze A.M., J. Gen. Chem. (USSR), 1947, 17, 1327-1331.

106. Aberhart, D.J., J. Org. Chem., 45, 5218-5220.

107. Jones, W.A., Jacobson, J., and Martin, D.F., Science, 1966, 152, 1516-1517.

108. Banda, F.M., and Brettle, R., J. Chem. Soc. Perkin Trans. 1, 1977, 1773-1776.

109. Edwards, J.A., Mills, J.S., Sundeen, J., and Fried, J.H., J. Am. Chem. Soc., 1969, 91, 1248-1249.

110. Texier-Boullet, F., Villemin, D., Ricard, M., Moison, H., and Foucaud, A., Tetrahedron, 1985, 41, 1259-1266.

111. Miller, R.E., and Nord, F.F., J. Org. Chem., 1950, 15, 89-95.

112. Gajewski, J.J., and Burka, L.T., J. Am. Chem. Soc., 1972, 94 2554-2556.