# STUDIES ON THE REARRANGEMENT IN THE BIOSYNTHESIS OF SOME BISLACTONE ANTIBIOTICS.

A THESIS

PRESENTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

TO THE UNIVERSITY OF GLASGOW

BY

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**To my family**

**and in memory of my late father.**

## Acknowledgements

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February 1993.

## Summary :

Part I of this work is concerned with detailed studies on the biosynthesis of the bislactone antibiotics ethisolide **1** and avenaciolide **2** produced by the fungi *Penicillium decumbens* and *Aspergillus avenaceus* respectively. The alkyl-α-methyleneglutaric acid skeleton of these is thought to arise from an alkylcitric acid via an alkylitaconic acid, since the latter have previously been shown to act as efficient precursors of the antibiotics. Direct evidence that their skeletons are assembled by condensation of a preformed fatty acid with oxaloacetate has been provided by intact incorporation of deuterium labelled fatty acids. These results depended on their being clearly different from the pattern found upon feeding $d_3$-acetate, where enrichment was mainly in the fatty acid derived part of the molecule.

In other feedings with $d_4$-succinate and $d_2$-fumarate ethisolide **1** was obtained with enrichment mainly in the two terminal methylene positions. In several experiments with $d_2$-fumarate no enrichment of avenaciolide **2** was observed, possibly due to inhibition of growth since [$^{14}$C]-fumarate has previously shown good incorporation. However $d_4$-succinate gave avenaciolide again showing enrichment mainly in both terminal methylene positions. This is surprising since succinate and fumarate would be incorporated via [3S, $3d_1$]-oxaloacetate and hence via a [$d_1$]-alkylitaconic acid. In order to establish whether the loss of integrity of the deuterium label occurs during formation of the alkylitaconic acid or in the subsequent rearrangement, it was hoped to examine $d_4$-succinate incorporation into relevant alkylitaconic acid derivatives if these could be produced in sufficient quantity by the fungi under study. Extensive studies based on analytical HPLC led to the detection of n-butylitaconic acid in *P. decumbens* but the quantity produced did not allow analysis of its deuterium content when $d_4$-succinate had been fed. Feeding/trapping experiments also indicated that n-decylitaconic acid was not produced in detectable quantities by *A. avenaceus* when fed with $d_4$-succinate. A plausible mechanism for the rearrangement of the alkylitaconic acids to β-alkyl-α-methyleneglutaric acids involves a cyclopropylmethyl radical *e.g.* **79** with consequent loss of integrity of the two hydrogens of the methylene radical grouping.

Part II of this work deals with the synthesis of bromomethylcyclopropane dicarboxylic acid derivatives which could act as precursors to such radicals. One approach aimed at 1,3-dehydrohalogenation of the dibromo derivative **113** prepared from the dimethyl ester of α-methylene-β-propylglutaric acid. A number of nucleophilic bases simply debrominated the ester and treatment with DBU resulted in 1,2-dehydrohalogenation to give a vinyl bromide **114**. Treatment with NaOMe however afforded three products, the vinyl methyl ether **115** corresponding to the above vinyl bromide (52 %) together with an inseparable mixture (48%) containing roughly equal amounts of the vinyl bromide **114** and the desired bromomethylcyclopropane **107**.

In a different approach cyclopropane derivatives were prepared via addition of diazoalkanes to appropriate alkenes. Dimethyl bromomesaconate **118** itself appeared to be unsuitable affording complex mixtures, but the corresponding acetoxy compound **119** reacted smoothly with diazomethane or diazobutane each to give a single pyrazoline which lost $N_2$ upon photolysis to give acetoxymethylcyclopropanes *e.g.* **121** and **129**. It was aimed to convert these to bromomethyl compounds via the corresponding hydroxymethyl compounds. This involved selective acetate hydrolysis but facile lactonisation occurred involving a *cis* methoxycarbonyl grouping. With careful work up it was possible to obtain the hydroxymethyl derivative **141** from the corresponding diisopropyl ester **139** but attempted conversion of this to the corresponding bromomethylcyclopropane **143** again afforded a lactone **142**. An attempt to obtain the latter bromomethyl compound via its tosyloxymethyl analogue was unsuccessful since decomposition of the appropriate tosyloxymethylpyrazoline **146** gave a complex mixture. However the bromomethylcyclopropanes **158** and **159** were obtained in excellent yield by hydrobromination of the lactone **142**.

In relation to the stereochemistry of the proposed cyclopropylmethyl radical, the preparation of the isomeric cyclopropanes with the ester groups *cis* was also studied briefly using citraconic anhydride derivatives *e.g.* the tosyloxymethylpyrazoline **155**, but no useful results were obtained.

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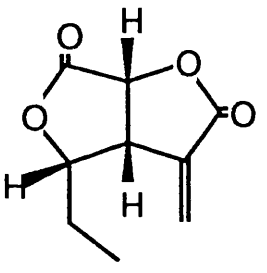
# CHAPTER 1 : Introduction.

## GENERAL INTRODUCTION .

Ethisolide **1**, avenaciolide **2** and their analogues isoavenaciolide **3**, canadensolide **4** form a part of a group of natural products which have a similar biogenetic origin and a number of which have interesting biological activity. These fungal metabolites, which contain an α-methylene lactone ring fused to a saturated lactone ring, have received considerable attention in recent years because of their novel structures and owing to their various kinds of biological activity *e.g.* inhibition of fungal germination, antibacterial or antitumor activity, or plant growth inhibition. [1-3]

The present work concerns four different stages of the biosynthesis of the bislactone antibiotics ethisolide **1** [4] and avenaciolide **2** [5] produced by *P. decumbens* and *A. avenaceus* respectively. Interpretation of the results obtained from feeding labelled precursors has led to the conclusion that a late stage in the biosynthesis of **1**, **2** and the related ethisic acid **5** involves a remarkable skeletal rearrangement of an alkylitaconic acid to an alkyl-α-methyleneglutaric acid (Scheme **4**). As discussed later in this introduction, a plausible mechanism for this rearrangement involves a cyclopropyl methyl radical [6] intermediate (Scheme **7**) and one feature of the present work also involves synthesis of relevant cyclopropane dicarboxylic acid derivatives as a prelude to model studies of this rearrangement. Another aspect which has been studied relates to the stereochemistry of this rearrangement.



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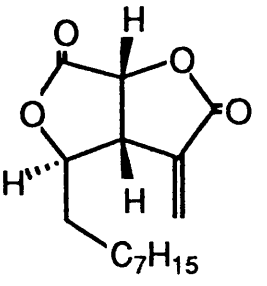

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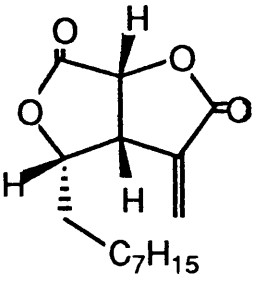

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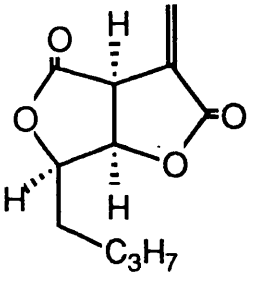

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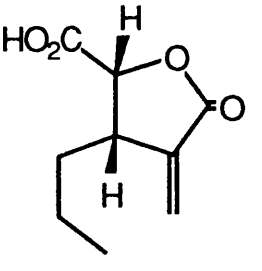


(5)

## Fungi and secondary metabolism .

Traditionally, fungi are classified as members of the thallophyta [7] a division of the plant kingdom. Today, many biologists have divided living organisms into four Kingdoms. The Animal kingdom, the kingdom of Green plants, the Fungal kingdom and the kingdom of bacteria and their allies. [8]

Most plants contain chlorophyll, a green substance which enables them to photosynthesise their organic food from $CO_2$ and water. Fungi differ from green plants in their lack of chlorophyll and so have to live off other plants and animals.

There are around 50.000 species of fungi, ranging in size and shape from tiny moulds to large bracket fungi. They contain fruit bodies which produce spores from which new fungi can grow. The main part of the fungus exists as a mass of tiny threads known as "MYCELIUM". This lives all year round buried in the plant or animal matter that it feeds on.

Some fungi obtain their basic materials such as sugar and starch from dead organic matter and are called saprophytes [8] like *Coriolus versicolor.* Others live either as parasitic or symbiotic fungi on living animals or plants, which they may harm or benefit. Many parasites damage and some even kill the plant or the animal they live on e.g. *Polyporus betulinus* known as *Birch polypore* which eventually kills the birch tree it grows on. [8]

Symbiotic fungi grow on living plants, but do not damage them. In this case, the fungus evolves a state of equilibrium with the host plant whereby both derive benefit. *Amanita muscaria* [9]

grows symbiotically with birch or pine trees as its mycelium grows around the tree roots. The tree provides the fungus with sugars which it needs and the fungus passes on the tree nutrients which it has taken from the leaf litter. This process helps birch trees to survive in poor soil.

Fungi have been of great importance in relation to human affairs for a very long time. Some saprophytic fungi are known to cause considerable damage to our food stores and buildings, like *Merulius lacrymans* (Dry rot) which lives on timber inside buildings. It makes the wood crack and eventually crumble away.

Much harm is done by parasitic fungi as they can destroy crops and other plants. Examples of fungal diseases are *Ceratocystis ulmi* (Dutch Elm) which has caused the death of Elm trees in Europe and North America. In man and other mammals, unlike plants, most diseases are caused by bacteria and viruses. However, there are some human afflictions in which fungal pathogens play a major role. The commonest are the dermatophytes, species of *Microporum* and *Trichophyton*, which are able to digest keratin and, of course, Athlete's Foot disease which is caused by a parasitic fungus that lives on the skin of the sole of people's feet. The skin becomes hard and cracks between the toes. [7,8]

However, not all fungi are harmful [8] and a few of them have been exploited industrially for making food and drink. Yeast has been used for thousands of years to make alcohol and bread as it breaks sugars in fruit juice to give alcohol or in bread dough to release carbon dioxide which makes the bread rise.

Some fungi like *Penicillium* which is a saprophytic mould growing on fruits, are exploited commercially to produce medicines *e.g.* penicillin, organic acids, enzymes and vitamins. They are used extensively in scientific research, giving crucial information about the biochemical pathways in living cells. [7, 8]

One of the vital and fundamental processes to living organisms is a complex web of enzyme-catalysed reactions which begins with $CO_2$ and photosynthesis and leads to diverse compounds called primary metabolites [10,11] *e.g.* amino acids, acetyl coenzyme A, sugars and nucleotides. These are involved in essential life processes and the biochemical reactions which lead to their production proceed in cycles (e.g. the citric acid cycle) and are referred to as primary metabolism.

Secondary metabolites are distinguished from the primary metabolites which form their main source, as having a restricted taxonomic distribution [11]. The question of the general role of secondary metabolites in the life of plants and micro-organisms and their metabolic function remain obscure and essentially unresolved [9] although they are important to the organism that produces them. [11]

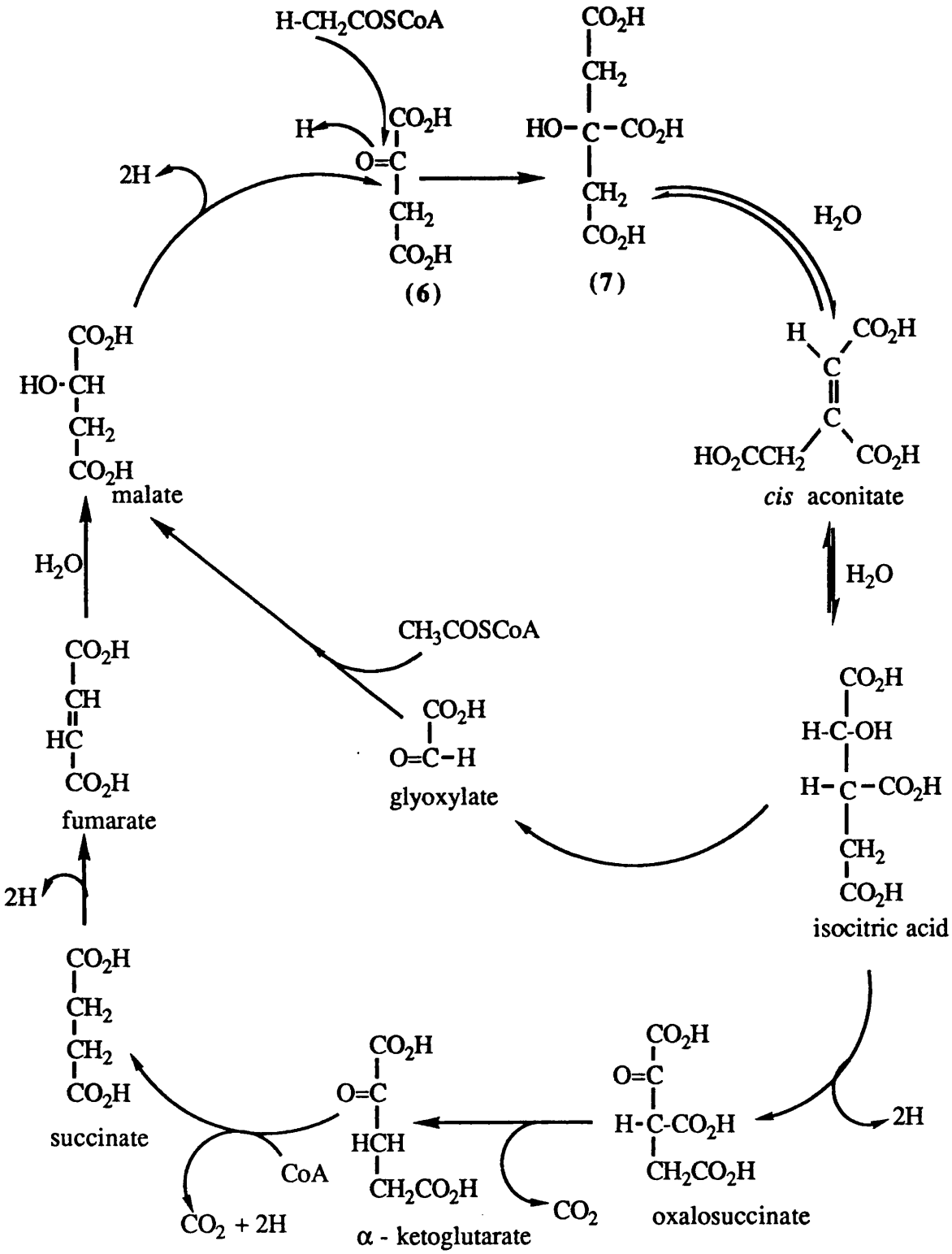
Unlike primary metabolism, secondary metabolism is not an invariable activity of organisms. [12] Several microbial antibiotics inhibit their own synthesis (*vide supra*) [13] and feedback and regulation may also occur by way of primary metabolites. Some pathways of antibiotic biosynthesis share the initial part of the pathway of biosynthesis with that of primary metabolite which controls, by feedback regulation, enzymes of its own synthesis. If these fall on the common pathway their inhibition may reduce

thereby the synthesis of the antibiotic. [10] Even in unicellular micro-organisms, secondary biosynthesis can usually be associated with a particular phase of life-cycle, in differentiated multicellular organisms it may occur only in certain parts and at certain times. Thus, the understanding of some mechanisms and their regulation, which lead to the formation of some natural products is even more preliminary. [12]

## Secondary metabolites derived from an acetate-derived chain and a TCA cycle intermediate .

Acetyl coenzyme A, a reactive thioester, is the main substrate entering the tricarboxylic acid cycle (TCA, Scheme **1**). It plays a key role not only in the generation of phosphorylating and reducing agents but it is also a source of important metabolic intermediates. [14] Two vital pathways of primary biosynthesis are derived from acetyl CoA itself, one via acetoacetyl CoA leading to isoprenoids and the other via malonyl-Co A to fatty acids. [11, 12]

One of the crucial primary metabolic intermediates is citric acid **7**. The effect of dilution rate on the specific activities of selected enzymes of the TCA cycle has been studied [15] and it was demonstrated that the activity of enzymes involved in the TCA cycle has an important influence on the progress of the citric acid fermentation. It has been reported that the effect of agitation on citric acid production is extremely important for the successful progress of fermentation. Citric acid is produced from the condensation of acetyl CoA with oxaloacetic acid (**6**, an intermediate in the TCA.) and the enzyme responsible for this reaction is citrate synthase. The results of these studies showed that the accumulation of citric acid is accompanied by an increase in the activity of citrate synthetase (CS) and a decrease in the activity of aconitate hydratase (ACH) and isocitrate dehydrogenase (ICDH) the enzymes responsible for the degradation of citric acid in the TCA cycle. [14] Other citrate synthases like methylcitrate and decylcitrate are responsible for the production

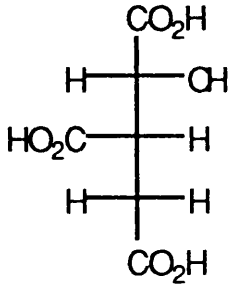



**Scheme 1 :** The tricarboxylic acid cycle (TCA).

of (-)-methylcitric acid **9** and (-)-decylcitric acid **10** respectively, have been isolated and investigated. it has been reported that (-)-methylcitric acid **9** is formed by condensation of propionyl-Co A with oxaloacetate. [16]

The formation of (-)-decylcitric acid **10** isolated from *Penicillium spiculisporum* is catalysed by decylcitrate synthase and the reported metabolite which was claimed to be (+) and (-) - decylcitric acid has been corrected. [17] This was shown to be a mixture of (-)-decylcitric acid **10** and (+)-isocitric acid **8**. The properties of 2-decylcitrate synthase described [18] as very specific for the C-4 diacid species, were non-specific with respect to the fatty acid chain moiety used and that a range of shorter chain substrates could be utilised.

The absolute configuration of (-)-decylcitric acid **10** from *P. spiculisporum*, of methylcitric acid **9** from *Candida lipolytica*, of norcaperatic acid **11** from *Cantharellus floccosus* and of agaricic acid **12** from *Polyporus officianalis* have been reported [19] as shown.



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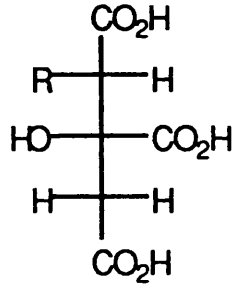


**(9)** R = Me

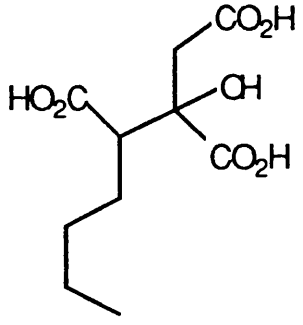
**(10)** R = $C_{10}H_{21}$

**(11)** R = $C_{14}H_{29}$

**(12)** R = $C_{16}H_{33}$

In connection with the present work, the isolation in these laboratories of another alkylcitric acid, namely n-butylcitric acid **13** from *P. decumbens* [20], is described later (Scheme **11**) and this further supports the intermediacy of an alkylcitric acid in the biosynthesis of the metabolites ethisolide **1** and avenaciolide **2**.

Ethisolide **1**, avenaciolide **2** and the *P. canadense* metabolites canadensolide **4**, an anti fungal antibiotic, dihydrocanadensolide **14**, an anti-ulcer compound, and canadensic acid **15**, appear to be the products of condensation of the keto group of oxaloacetic acid with an acetate derived fatty acid chain. [21] Those resulting from condensation at the α-position of the fatty acid like **4**, **14** and **15** may be referred to as "Type A" metabolites i.e. having an alkylitaconic acid skeleton. Only a few natural products possessing a $C_3$ unit linked to the β-position of a fatty acid are known. These include the antibiotics **1** and **2** and may be referred to as "Type B" metabolites i.e. having an α-methylene-β-alkylglutaric acid skeleton and these will be discussed later.

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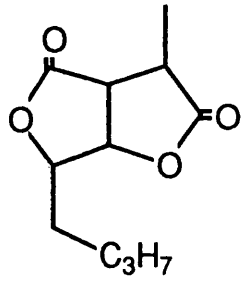


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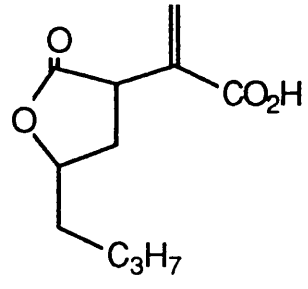
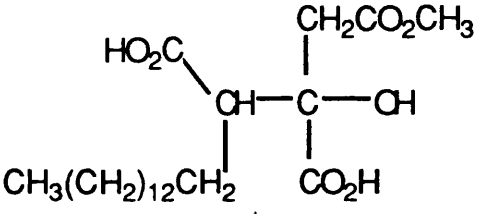



**(15)**

Type "A" compounds include caperatic acid a lichen product [22] whose structure has now been established as **16**. Spiculisporic acid **17** isolated from *P. funiculosum* [23] is evidently produced by condensation of α- ketoglutaric acid a TCA intermediate and lauric acid derived from acetate, followed by cyclisation, while (+)-norrangiformic acid **18** isolated from *Cladonia mitis* [24] probably results from dehydroxylation of caperatic acid **16**, or by an elimination-reduction as it occurs in the interconversion of malic and succinic acids in the TCA cycle.

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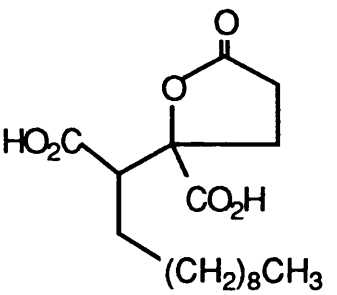


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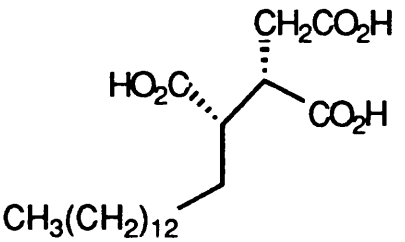


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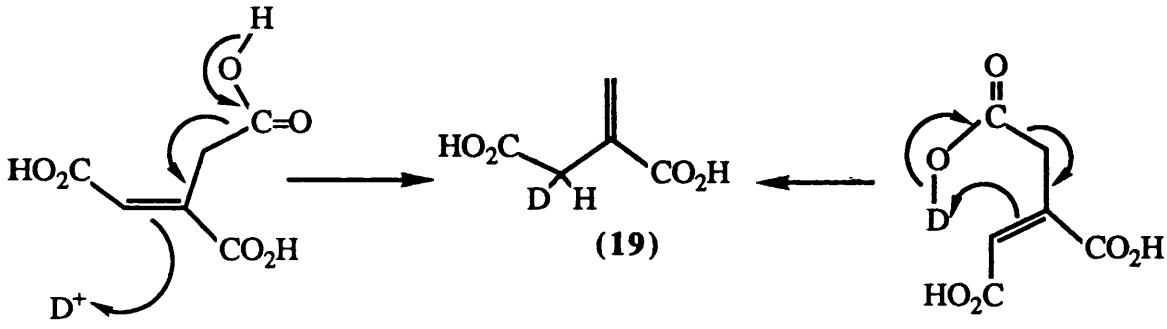
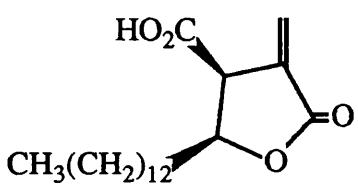



**Figure 1.**

The majority of natural products of this type are based upon an alkylitaconic acid skeleton which may be presumed to arise by a process similar to that established for the formation of itaconic acid **19** itself from citric acid using cell free extracts of *A.terreus* namely via dehydration [25, 26] followed by a concerted decarboxylation and double bond migration as in Figure **1**.

There are many examples of this type of compound produced by lichens, e.g. (-)-allo-protolichesterinic acid **20** [27, 28] and (+)-roccellaric acid **21** [28], in which hydroxylation at the β position of the fatty acid and lactonisation has also occurred. Two lichen acids acaranoic acid **22** and acarenoic acid **23** are unusual in having a δ-lactone structure. [29, 30]

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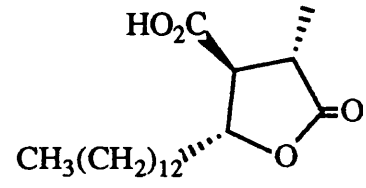


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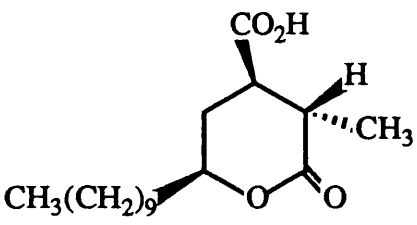

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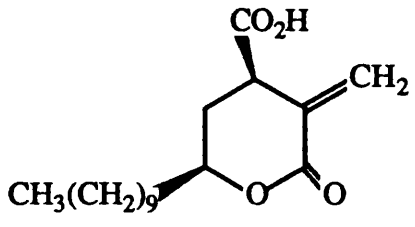
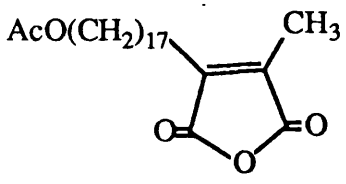


(23)

There are more examples of metabolites which may be listed under the class of carbonyl-methylene condensation products [31], such as 17-acetoxyheptadecylcitraconic anhydride **24** isolated from the fungus *A. wentii* [32], the compound **25** from the organism *Arthrinium sacchari* [33] and **26** by the bacterium *Stachybotrys atra.*



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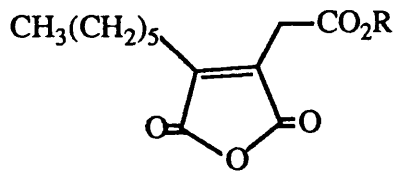
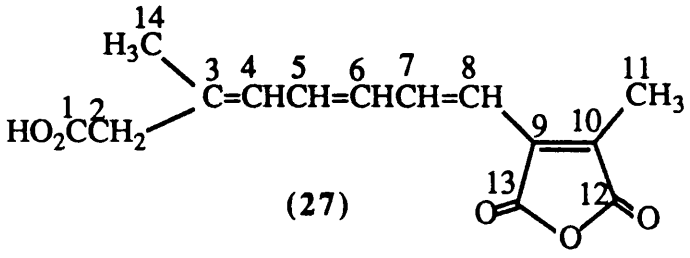


**(25) R = H**

**(26) R = CH3**

The biosynthesis of itaconitin **27** which at one time could be isolated from *Aspergillus gorakhpurensis* [34] showed that acetate and malonate were incorporated into C-1 to C-9 and also into C-13, indicating that this portion was derived from a fatty acid (Figure. 2).



(27)

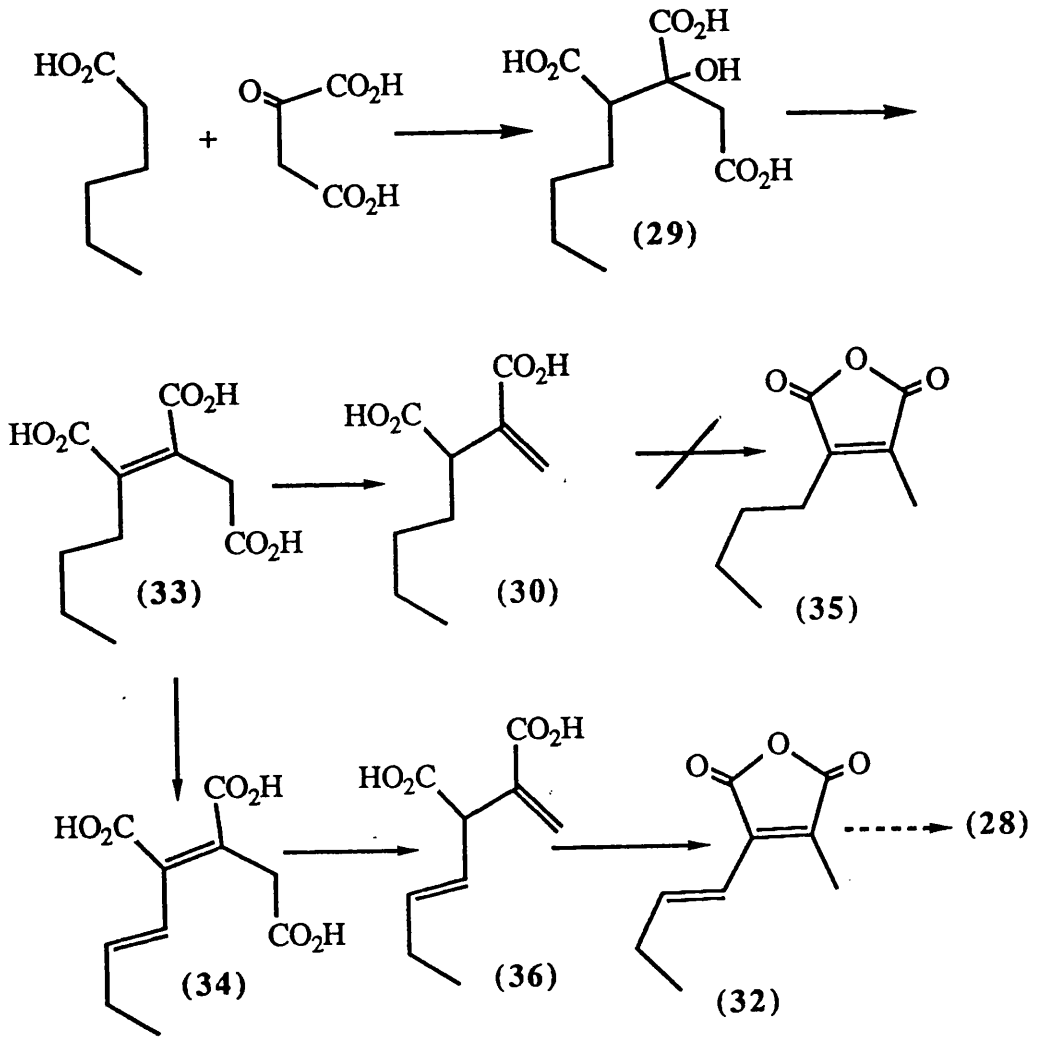
**Figure 2**

The nonadride group of fungal metabolites e.g. glauconic acid **28** [35] may arise by dimerisation of a $C_9$ unit, which could be derived from an alkylcitric acid *e.g.* **29** as outlined in Scheme **2**.

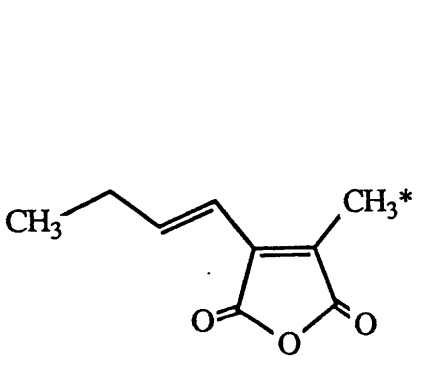
When [2,3-$^{13}C_2$]-succinate was fed to *Penicillium purpurogenum* glauconic acid **28** was obtained with label specifically incorporated into the $C_3$ residue. [36] Base-catalysed dimerisation of **32** gives an epimer of glaucanic acid **31** in 5% yield. It was also found that the [$^{14}$C]-labelled anhydride **32** incorporated into **28** [50.8%] and **31** [4.3 %] in *P. purpurogenum*. [35]

Earlier work has shown that while the doubly unsaturated anhydride **32** is an excellent precursor (incorporation 51.5%) the unsaturated anhydride **35** gave only a low incorporation (0.25%).[35]

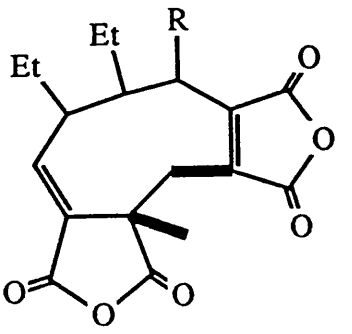
Recently, butylitaconic acid **30** labelled with deuterium in the terminal methylene positions was fed to cultures producing glauconic **28** and glaucanic **31** acids and no deuterium was detected in the metabolites. [37] These results suggest a pathway for these metabolites in which transformation from the *cis* aconitic acid **33** to the anhydride **32** does not proceed via alkylitaconic acid **30** but instead may involve desaturation of **33** followed by decarboxylation to give the diene **36** before isomerisation and cyclisation to give **32**. (Scheme **2**).




**Scheme 2**



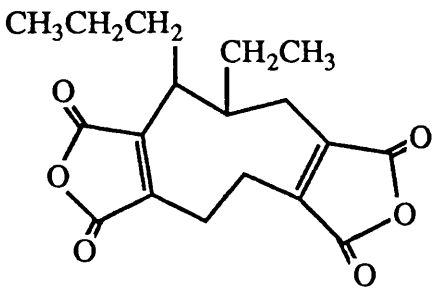

(32)



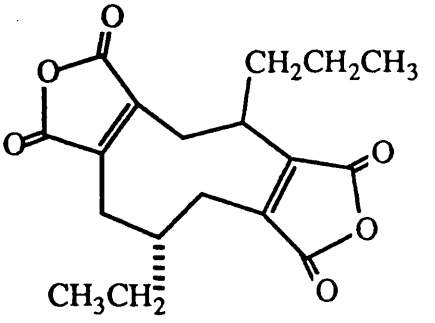

**(28)** R = OH

**(31)** R = H

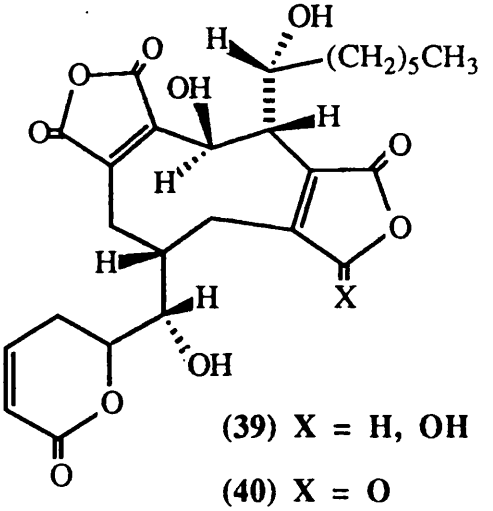
There are other compounds which belong to this group of natural products *e.g.* byssochlamic acid **37** which has been isolated from *Byssochlamys nivea* and *Paecilomyces varioti* [38], heaveadride **38** from *Helimenthosporum heveae* [39] and the rubratoxins A **39** and B **40**. [40, 41]



(38)



(37)



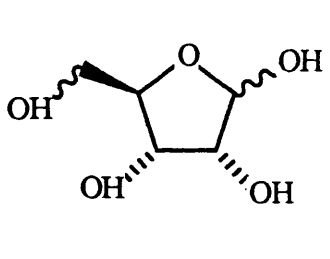
**(39) X = H, OH**

**(40) X = O**

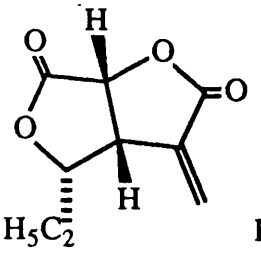
In the present work the most relevant "Type A" compounds are the lactone metabolites **4, 14** and **15** of *P.canadense*. The relative and absolute stereochemistry [42] of these has been fully established by various studies including synthesis of canadensolide **4** in optically active as well as racemic form. [43]

The Type "B" metabolites whose biosyntheses are investigated here include the antibiotics ethisolide **1** from *P. decumbens* and avenaciolide **2** from *A.avenaceus*. The interest of other workers has also been directed towards the synthesis of **1** and **2** because of their characteristic fused bislactone structure as well as their biological activity.

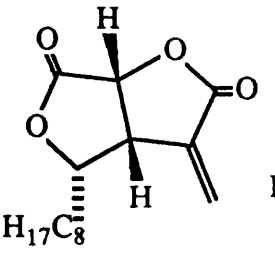
Isoavenaciolide **3** has been synthesised in racemic form [44] and as its natural enantiomer [42, 46-48] and ethisolide **1** has also been synthesised in racemic form [44b, 49] and in optically active form [47] from D-ribose **41**. Recently, Chida [50] has reported total synthesis of (-)-ethisolide **42** and (-)-isoavenaciolide **43** from L-quebrachitol **44**.



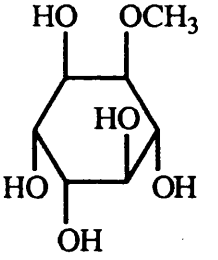

(41)




(42)




(43)




(44)

**Recent studies on the biosynthesis of alkylitaconic acid and α-methylene-β-alkylglutaric acid fungal metabolites.**

Previous incorporation studies [51] in these laboratories have shown that ethisolide **1**, avenaciolide **2** and the above *P.canadense* metabolites **4**, **14** and **15** to have similar biosynthetic origins.

The studies which gave the crucial information were the feedings of [1-$^{13}$C], [2-$^{13}$C] and [1,2-$^{13}C_2$] acetate and [2,3-$^{13}C_2$] succinate to cultures of *P.canadense* and *P.decumbens*. [51] The labelled acetate clearly showed C-1 to C-8 of the *P.canadense* metabolites **4**, **14** and **15** and C-1 to C-6 of ethisolide **1** (Figure. **3**) to be derived via the acetate - malonate pathway.

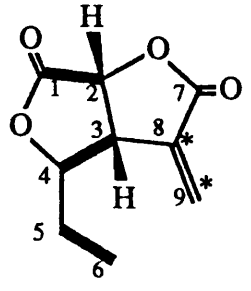
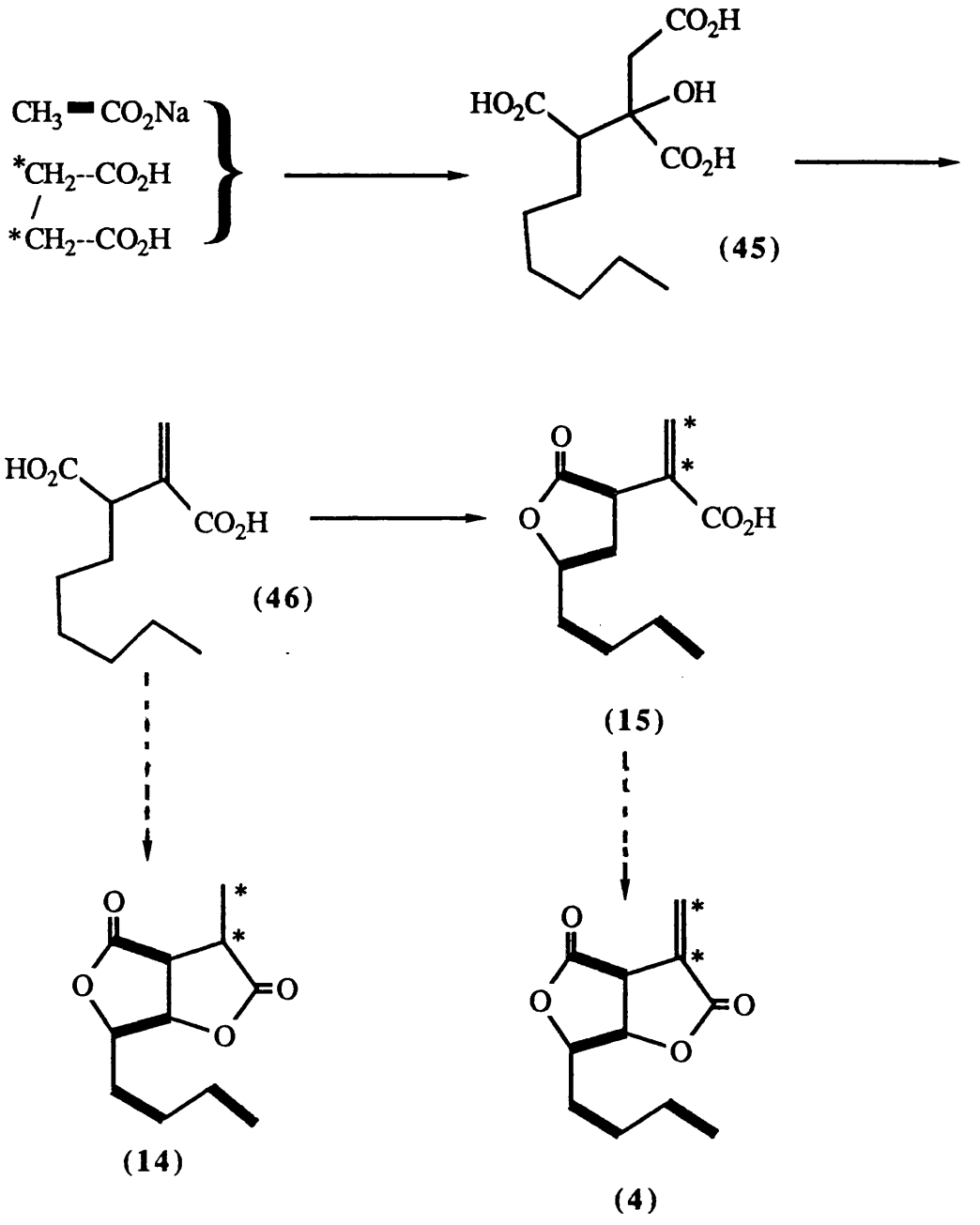



**Figure 3**

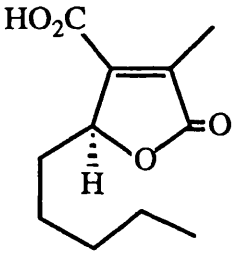
The intact incorporation of the doubly labelled succinate on the other hand, showed C-9 to C-11 of the metabolites **4**, **14** and **15** and C-7 to C-9 of ethisolide **1** to originate from a C-4 acid such as oxaloacetate. The results of these labelling studies as shown in (Scheme **3**) suggested the intermediacy of hexylcitric acid **45** and hexylitaconic acid **46** in the biosynthesis of the metabolites


CH3 CO2Na
*CH2--CO2H
*CH2--CO2H
HO2C
CO2H
OH
CO2H
(45)
HO2C
CO2H
(46)
O
*
*
O
CO2H
(15)
O
*
*
O
O
O
(14)
O
*
*
O
O
O
(4)

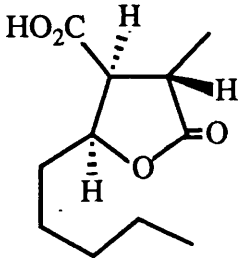
**Scheme 3**

**4**, **14** and **15**. This was proved by the efficient incorporation of $^{14}C$-labelled **46** into **4** (20.6%), **14** (11.9%) and **15** (33.6%) by *P.canadense*. [51]

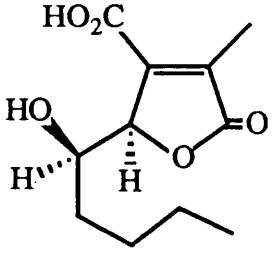
Apart from canadensic acid **15**, McCorkindale and coworkers [52] have isolated a number of other related metabolites from cultures of *P.canadense* These include isocanadensic acid **47**, dihydroisocanadensic acid **48** and hydroxyisocanadensic acid **49** which have the "Type A" lactone structure. [52]



**(47)**



**(48)**

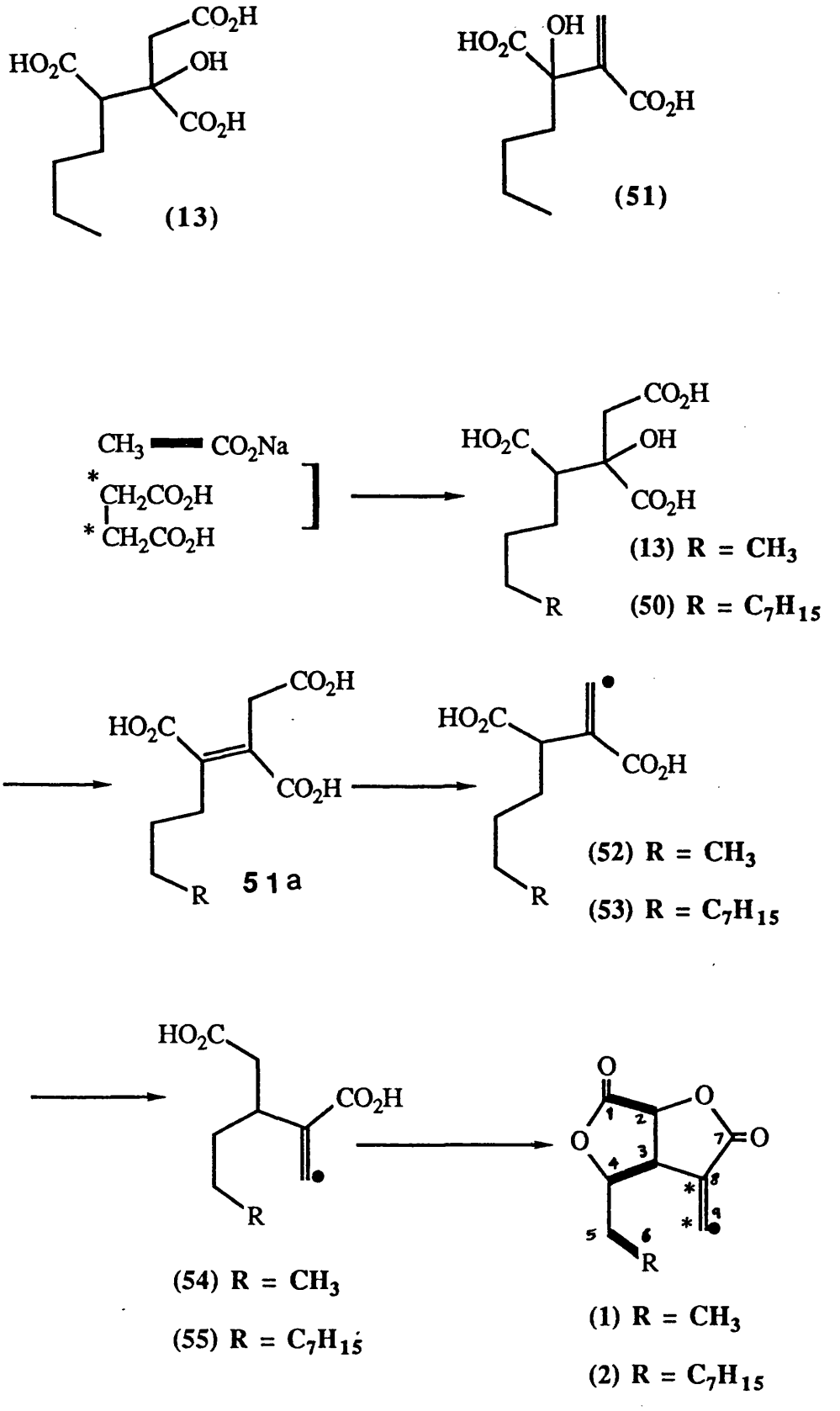


**(49)**

Avenaciolide **2** a "Type B" metabolite can be derived formally by condensation of a $C_3$ unit with the β-position of a fatty acid chain and Tanabe suggested that this type of compound may be formed by condensation of succinic acid with a β-keto acid. [53] This was supported by incorporation of [1-$^{13}C$] and [2-$^{13}C$] - acetate into avenaciolide **2** in *A. avenaceus* (Scheme **4**). Initially, The incorporation of variously $^{13}C$- labelled acetates and succinates into ethisolide **1** with the expected labelling pattern (Scheme. **4**) did lend support to the Tanabe theory and it was clear that a certain amount of acetate was incorporated into the $C_3$ unit via succinate derived by one turn of the tricarboxylic

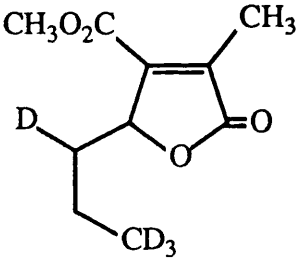
acid cycle (Scheme 1).

However, the isolation[20] of two "Type A" metabolites namely butylcitric acid **13** and α-butyl-α-hydroxyitaconic acid **51** (whose details are described in the discussion) from the same cultures (*P.decumbens*) strongly suggested a different pathway (Scheme 4) in which the early stages of the biosynthesis are similar to that of canadensolide **4** biosynthesis (Scheme 3) but rearrangement of the alkylitaconic acid intermediate occurs to give the α-methylene-β-alkylglutaric acid skeleton present in ethisolide **1**. This was shown to be correct by incorporation (10.4%) of [$CH_2$-$^{14}$C]-n-butylitaconic acid **52** into ethisolide **1** with activity specifically in the terminal methylene group as determined by ozonolysis.[51] Similarly , the corresponding [$CH_2$-$^{14}$C]-n-decylitaconic acid **53** was specifically incorporated (7.7%) into avenaciolide **2**. Furthermore, It has been found that a sample of α-[$CH_2$-$^{14}$C]-β,n-propylglutarate **54** was also incorporated (26%) by *P. decumbens* into ethisolide **1** with the label all in the terminal methylene group and β-nonyl-α-[$CH_2$-$^{14}$C]-glutarate **55** incorporated (11%) by *A. avenaceus*[55] into avenaciolide **2** again with the activity in the terminal methylene group (Scheme 4). This was found even a better precursor to ethisolide **1** than n-butylitaconic acid **52**.

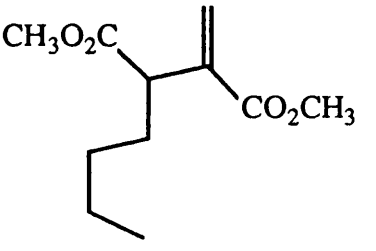

(13)
(51)
(13) R = CH3
(50) R = C7H15
51a
(52) R = CH3
(53) R = C7H15
(54) R = CH3
(55) R = C7H15
(1) R = CH3
(2) R = C7H15

Scheme 4

Recently a further "Type A" metabolite decumbic acid **77** was isolated in small quantities as its methyl ester **56** from cultures of *P.decumbens* and incorporation of deuterium from $CD_3CO_2Na$ into the fatty acid part of this metabolite was as expected. [56a] A method of selective esterification of the minor acidic broth metabolites (in the presence of large amounts of citric acid) was then discovered and this led to the isolation of butylitaconic acid itself as its methyl ester **57**. This further supports the intermediacy of this key intermediate in ethisolide **1** biosynthesis. [56a]



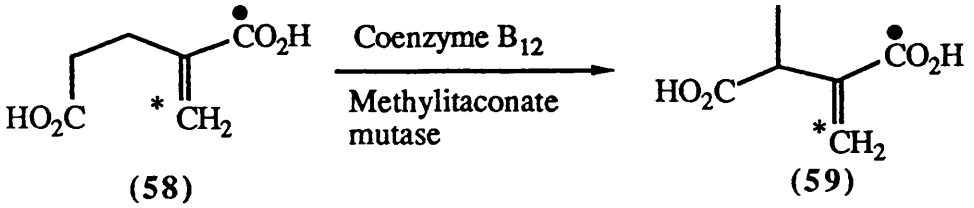

(56)




(57)

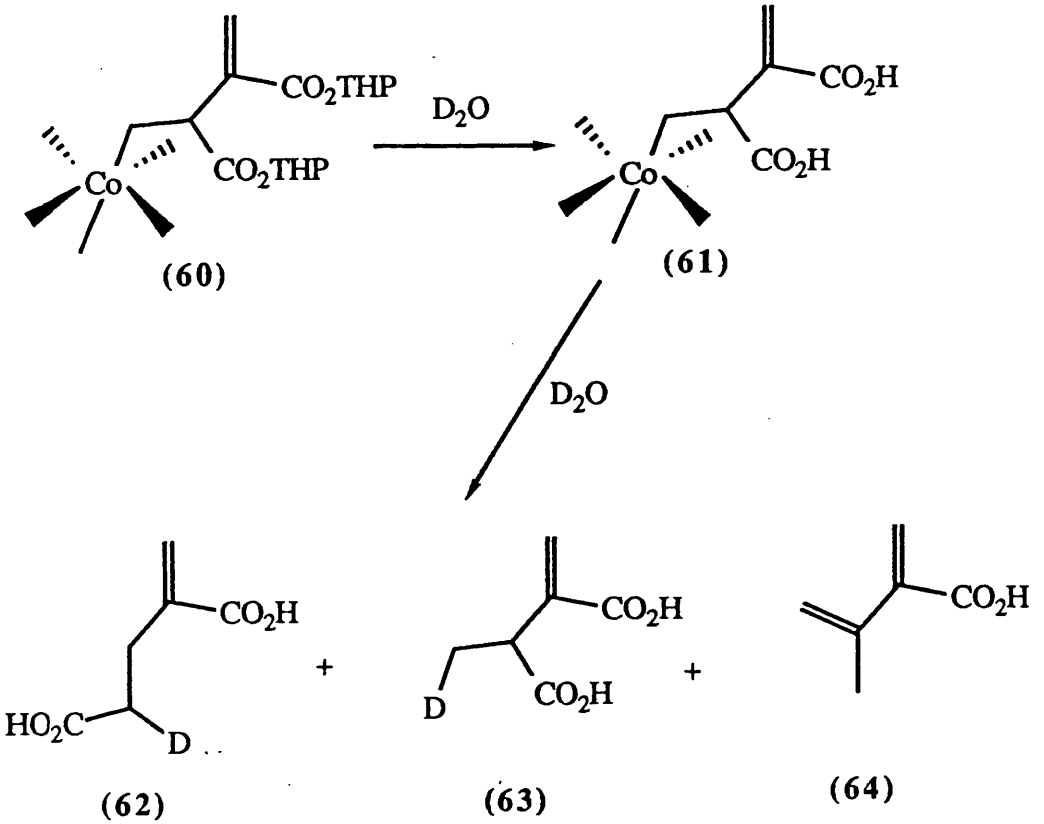
The rearrangement involved is analogous to the $B_{12}$ -coenzyme /α-methylene glutarate mutase interconversion of methylitaconic acid **58** and α-methyleneglutaric acid **59** (Figure **4**). [57] Dowd reported a model reaction [58, 59] which mimics the rearrangement reaction. In the model, bis(tetrahyropyranyl)methylitaconate **60** was attached through the 4-position to the cobalt atom of $B_{12}$ yielding the model intermediate **61**. When the model reaction was carried out in $D_2O$, carbon-skeleton rearrangement occurred spontaneously at ambient temperature, the products α-

methyleneglutaric acid **62** and methylitaconic acid **63** were labelled on the γ-carbon [60] (Scheme **5**). This result indicates that the position of deuterium was once occupied by cobalt and therefore establishing that the acrylate is the migrating group in the coenzyme $B_{12}$-dependent rearrangement step. [61] (Figure. **4**).




(58) (59)

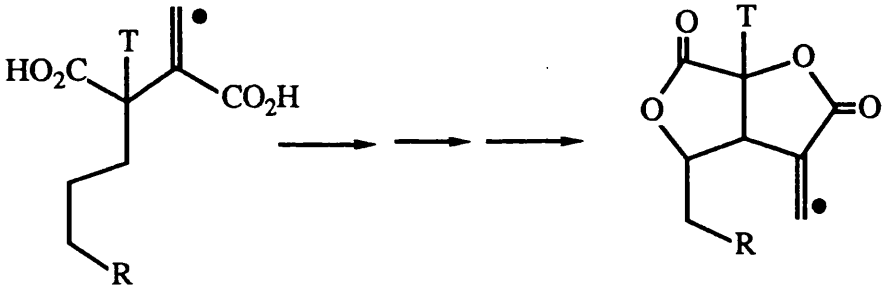
**Figure 4**




(60) (61) (62) (63) (64)

**Scheme 5**

It is not possible to study the labelling pattern of the presumed methyleneglutaric acid intermediates directly as these could not be detected in *P. decumbens* culture fluids. However, by studying the samples of **1** and **2** produced from feeding variously labelled acids **65** or **66**, conclusions could be drawn for most of stereochemical changes involved. McCorkindale et. al., firstly showed that n-butylitaconic acid **67** labelled with [$^3$H] at the α-position and with [$^{14}$C] at the terminal methylene carbon, afforded ethisolide 1 with the same tritium/carbon ratio as the administered **67**.[62] Similar results were obtained upon feeding the corresponding labelled n-decylitaconic acid **68** to *A. avenaceus*. This suggests retention of the α-hydrogen atom on **1** and **2** (Figure **5**). The presence of tritium at the 2' position in the ethisolide sample obtained from **67** was not proved since no degradative methods had been established.




**(67) R = $CH_3$** **(1) R = $CH_3$**

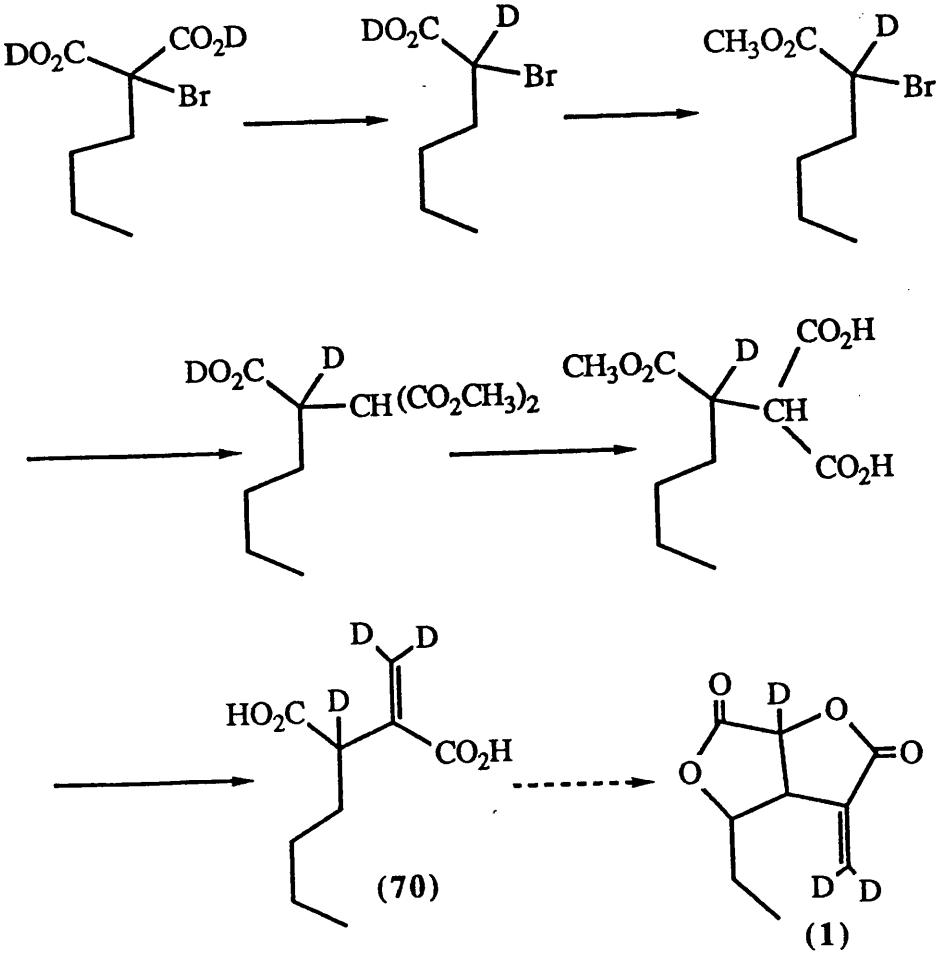
**(68) R = $C_7H_{15}$** **(2) R = $C_7H_{15}$**

**Figure 5**

The deuteriated acid **69** generated [62] from commercially available [$^2$H]-labelled paraformaldehyde was efficiently incorporated by *P. decumbens* into ethisolide **1** labelled only in

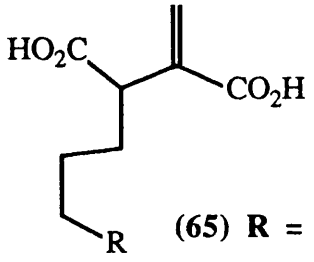
the terminal methylene positions as shown by $^2H$ nmr. Hence labelling of the terminal methylene group can be used as an internal reference check of deuterium retention from elsewhere in the molecule.

The required deuteriated **70**, labelled with $^2H$ at the α-position (60%) of that at either of the terminal methylene positions, was prepared as shown in (Scheme **6**). This was administered to cultures of *P. decumbens* to give ethisolide **1** with deuterium enrichment at C-2 again of ca. 60% of that at either of the terminal methylene positions. This result indicates complete retention of the $^2H$ atom at C-2 of ethisolide **1**, confirming the study with tritium labelled material.



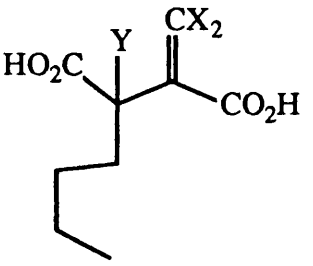

**Scheme 6**

The stereochemistry of the rearrangement proposed in ethisolide **1** was also studied by feeding various $^{2}H$-labelled alkylitaconic acids **72**, **73** and **74** and in each case, as internal standard, with $^{2}H$ in the terminal methylene group. Clear results already obtained for **70** as described above and now obtained for **75** gave ethisolide **1** showing retention of one $^{2}H$ atom from C-2 and one $^{2}H$ from C-3 respectively. On the other hand, 2/3 and 1/2 respectively of the $^{2}H$ label was retained from **73** and **74** respectively. This can be rationalised in terms of the rearrangement proceeding partly with retention of configuration and partly with inversion of configuration of these centers. [62]



**(65)** R = $CH_3$

**(66)** R = $C_7H_{15}$



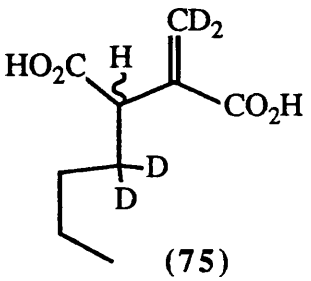
**(69)** Y = (2RS)-2-$^{1}H$, X = D

**(70)** Y = (2RS)-2-$^{2}H$, X = D

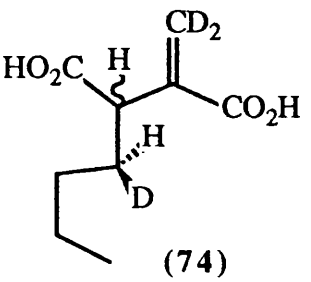
**(71)** Y = (2R)-2-$^{1}H$, X = H

**(72)** Y = (2R)-2-$^{2}H$, X= H

**(73)** Y = (2R)-2-$^{2}H$, X = D

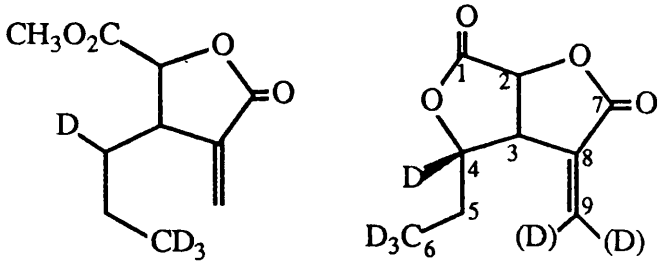


**(75)**



**(74)**

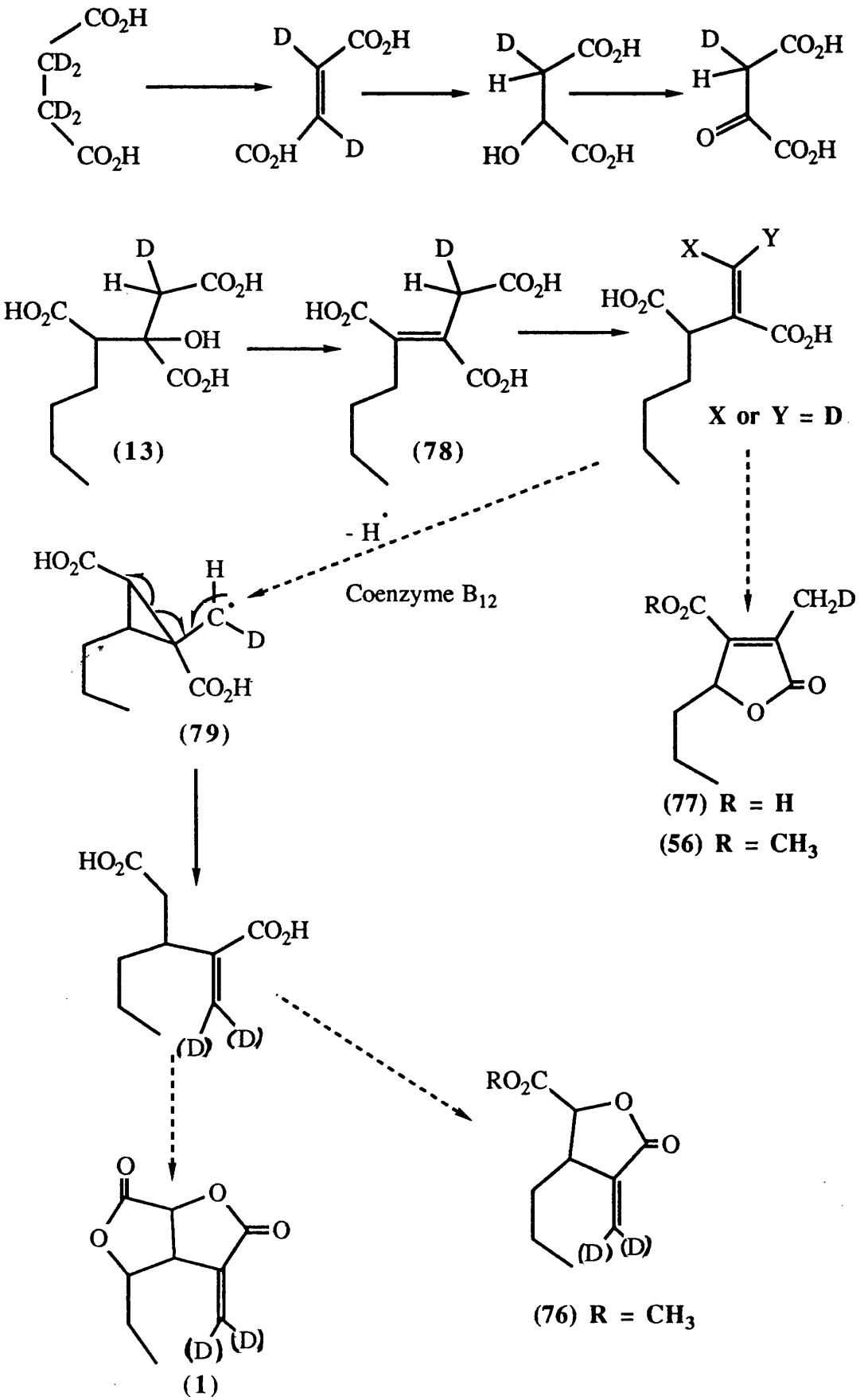
In the recent studies cultures of *P.decumbens* were also fed with $CD_3CO_2Na$. [56a] Incorporation of deuterium into both ethisolide **1** and the related minor metabolite ethisic acid **5** was mainly into the fatty acid portion of these metabolites, *i.e.* deuterium being located at positions C-4 and C-6 (the deuterium at C-2 is lost in the dehydration of the alkylcitric acid to give **51a**. (See Scheme **4** page 22). There was however a small amount of deuterium appearing in the $C_3$ unit, *i.e.* the terminal methylene positions (Figure **6**). This was in keeping with the known incorporation of acetate via succinate derived by one turn of the tricarboxylic acid cycle mentioned earlier and incubation of $d_4$-succinate and $d_2$-fumarate with cultures of *P.decumbens* gave ethisolide **1** and ethisic acid **5** with a substantial incorporation of deuterium into the $C_3$ unit (Scheme **7**).




**(76)** **(1)**

**FIGURE 6**

What was unexpected in these results was that both of the terminal methylene positions were labelled. It would be expected that succinate incorporation occurs via the TCA cycle, via fumarate, [3S-3$d_1$]-L-malic acid and hence [3S-3$d_1$]-oxaloacetate. [63] It might be expected that the subsequent steps involving formation of an alkylcitric acid **13** dehydration to an alkylaconitic acid **78** and decarboxylation to give an alkylitaconic acid **65** (Scheme **7**) would be stereospecific leading to only one of the terminal methylene hydrogens being labelled with deuterium.




**Scheme 7**

It would be desirable to know whether the apparent randomisation of deuterium is due to lack of specificity in the forementioned processes or whether randomisation occurs during the subsequent rearrangement of the itaconic acid **65**. Ideally, the incorporation of deuterium into the minor metabolite butylitaconic acid **65** or its α-hydroxy analogue **51** should be studied. However, it may be assumed that the readily available type A metabolites canadensolide **4** and canadensic acid **15** which have a hexylitaconic acid skeleton would be derived by a similar pathway and in canadensic acid **15**, deuterium was found to be incorporated into only one of the two terminal methylene positions, namely the hydrogen *cis* to the carboxyl group (Figure **7**). [56a]

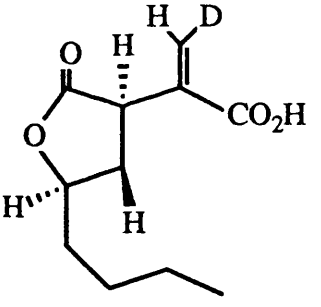
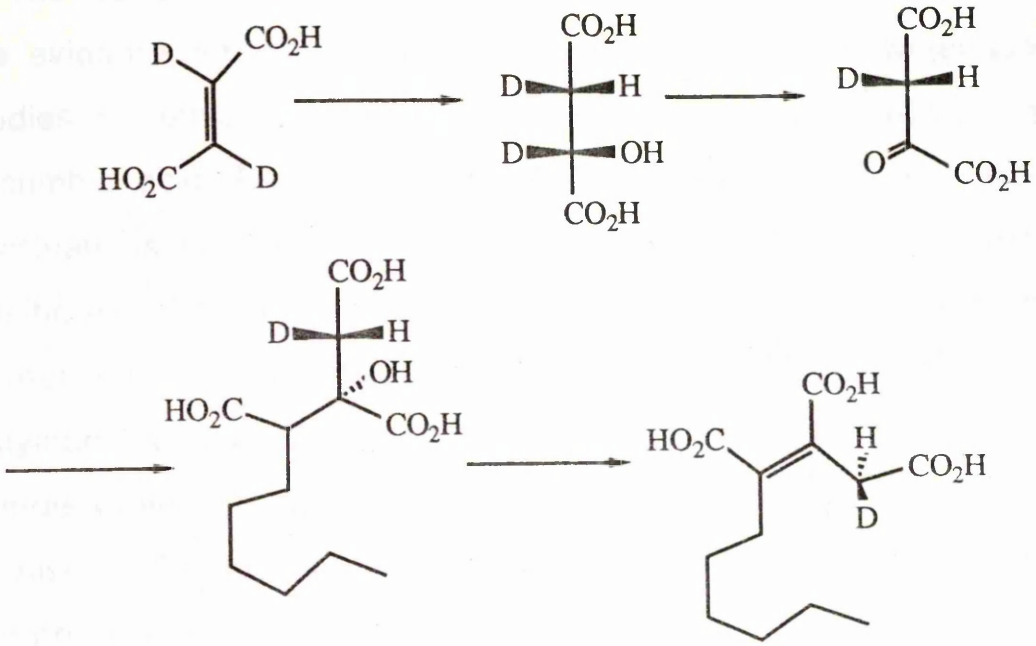


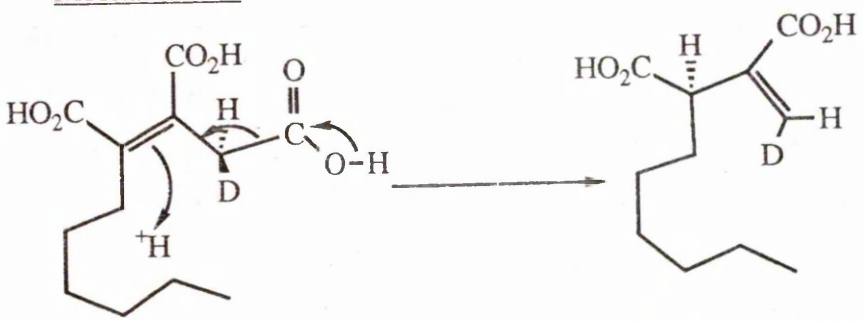

**Figure 7**

This result gives rise to a number of interesting conclusions regarding the stereochemistry involved in the biosynthesis, both of canadensic acid **15** and ethisolide **1**. The proven stereochemical character of fumarate hydratase leads directly to the assignment of malate, derived succinate-$d_4$ then from fumarate-$d_2$ as [3S,2S-2,3$d_2$]-malate. The specifically labelled

malate would be expected to continue along the pathway described in Scheme **8**, until it reaches the alkylitaconic acid. At this stage there are two concerted mechanisms are available for the decarboxylation step to give hexylitaconic acid. In mechanism (I), the $CO_2$ is lost from the opposite side of the molecule to which the proton is added, thereby resulting in the deuterium being *trans* to the $-CO_2H$ group in **15**. In mechanism (II), the $CO_2$ is lost from the same side of the molecule as the proton is added giving a *cis* relationship between the deuterium atom and the $CO_2$ group. As the enriched proton in this experiment appears *cis* to the $-CO_2H$ group, it may be presumed to be in the same configuration in the hexylitaconic acid intermediates thus leading support for mechanism (II).

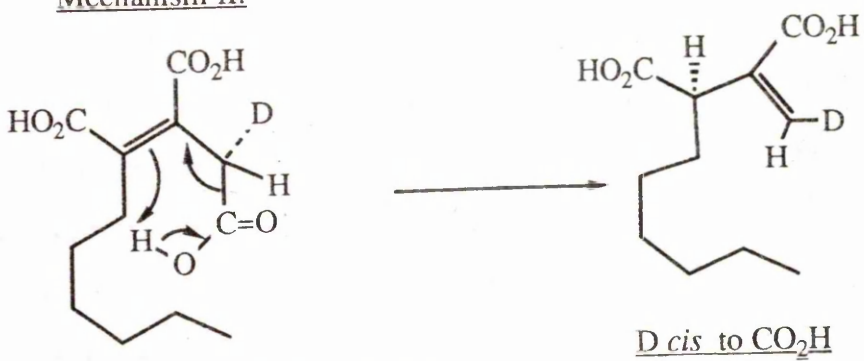


Mechanism I :



D *trans* to $CO_2H$

Mechanism II:



D *cis* to $CO_2H$

6 - membered ring transition state.

**Scheme 8**

The current work aimed amongst other things to substantiate the evidence already presented by the $^{13}C$ and $^{2}H$ incorporation studies on ethisolide **1** and its co-metabolites ethisic acid **5** and decumbic acid **77** and to examine whether deuterium label from succinate is incorporated in only one, or both terminal methylene positions of avenaciolide **2** and also hopefully to examine deuterium incorporation into the minor metabolites α,n-butylitaconic acid **65**, α,n-butyl-α-hydroxyitaconic acid **51** and α,n-decylitaconic acid **66**.

Also [$^{13}C$]- labelled n-butylitaconic acid **52** and α-methylene-β,n-propylglutaric acid **53** (Scheme **4**) have been shown to be potential intermediates in the biosynthesis of ethisolide **1**, and following an argument similar to that for canadensic acid **15** (Scheme **8**), it seems probable that the butylitaconic acid **65** derived from fumarate-$d_2$ would have deuterium in only one of the terminal methylene positions. This would imply that randomisation of the label may occur in the rearrangement of the three carbon piece into the β-position, as outlined in (Scheme **7**), and that a cyclopropyl methyl radical intermediate e.g. **79** may be involved.

This remarkable rearrangement merits further studies to explain the mechanisms involved. A contribution is made in this thesis by synthesis of alkylcyclopropane dicarboxylic acid derivatives related to the presumed intermediate **79** as discussed in detail in the discussion.

## CHAPTER 2 : Discussion (Part 1).

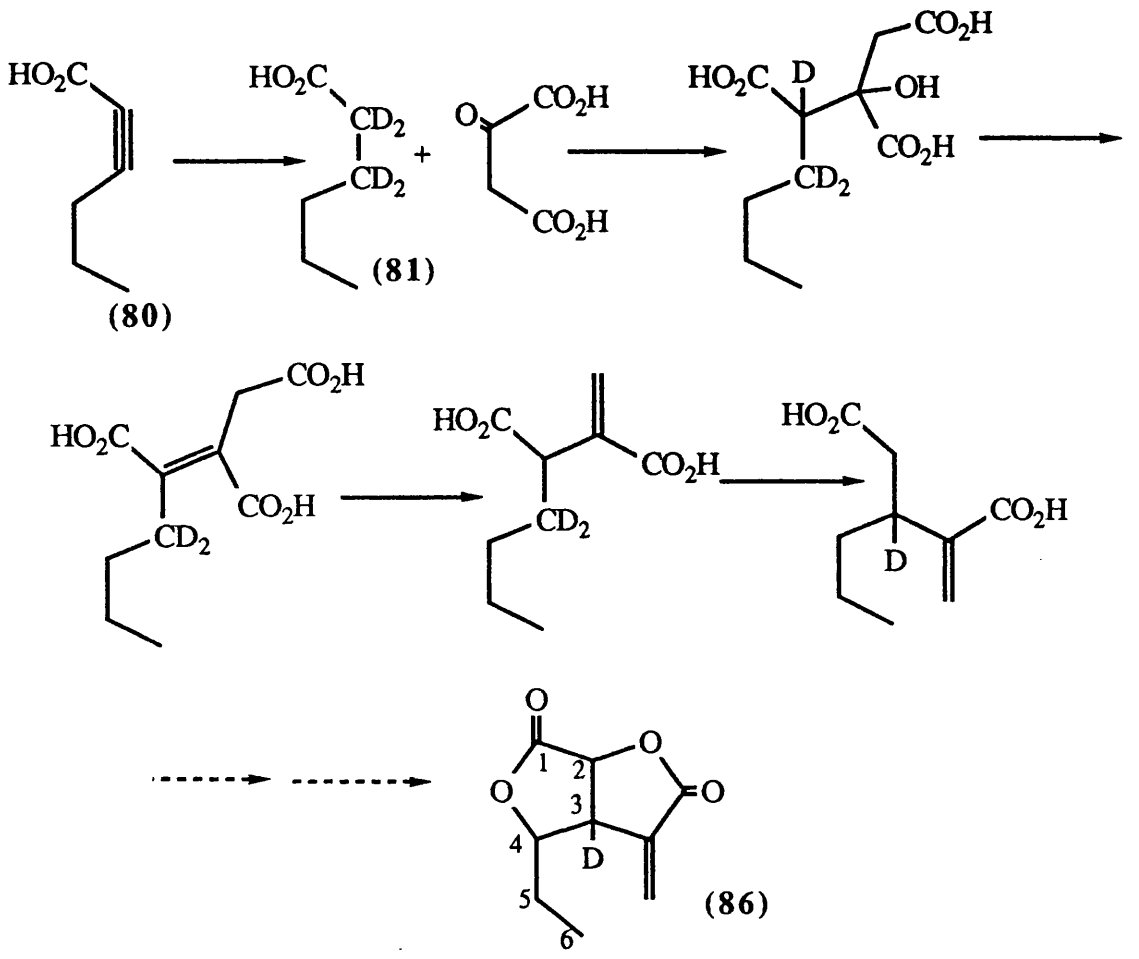
**Synthesis of potential biosynthetic intermediates and incorporation studies using *Penicillium implicatum* and *Aspergillus avenaceus*.**

The first part of this work is concerned with detailed biosynthetic studies of two fungal bislactone antibiotics ethisolide **1** and avenaciolide **2** which have been shown by previous [51] labelling studies to have considerable similarities in their biosynthetic origin. The labels $^{14}C$, $^{13}C$, $^{3}H$ and [$^{13}C$, $^{2}H_3$] from variously labelled acetate administered to cultures of *Penicillium decumbens* and *Aspergillus avenaceus* were found to be incorporated into **1** and **2** in a manner consistent with the operation of an alkylcitric-alkylitaconic acid pathway *i.e.* by condensation of an α-methylene group of a fatty acid (preformed from acetate/malonate) with the keto group of oxaloacetate, a tricarboxylic acid cycle (TCA cycle) metabolite.

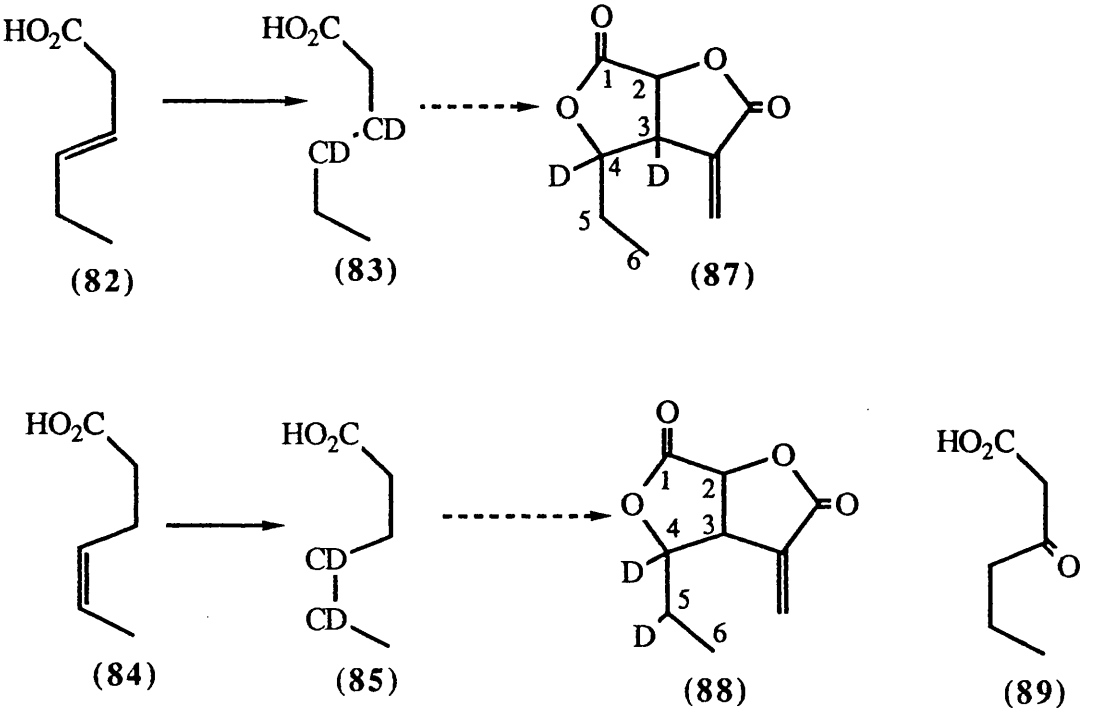
It was also found [56b] that the above labels from succinate were incorporated into the oxaloacetate derived part of the molecules **1** and **2**. The clearly observed incorporation of α-[$CH_2$-$^{14}C$]-n-butylitaconic acid **52** into ethisolide **1** [10.4%] and α-[$CH_2$-$^{14}C$]-n-decylitaconic acid **53** into avenaciolide **2** [7.7%] established the close similarity of their biosynthesis to that of the alkylcitric acid pathway (Scheme **4**). It was also found using deuteroacetate doubly labelled in addition with $^{13}C$ at either C-1 or C-2 that deuterium enrichment resulted at C-4 and C-6 of ethisolide **1** in a ratio 0.5 to 3 in keeping with a pronounced starter effect. On the other hand, the $^{13}C$ nmr spectrum confirmed the absence of deuterium at C-2 of the resulting ethisolide **1**, in keeping with its formation via an alkylaconitic acid **51a** (lacking hydrogen at C-2) as shown in Scheme **4** (p. 22).

In the present work the synthesis and intact incorporation of specifically labelled lauric acid **90** into avenaciolide **2** are described. A number of similar experiments on ethisolide **1** were previously carried out in these laboratories by Dr S. Miller. [64] These studies have been rounded off in the present work by collecting and writing up the data obtained for the first time and discussing their significance.

In order to establish the intact incorporation of hexanoic acid into ethisolide **1**, the [$^2$H ] labelled intermediates **81**, **83** and **85** were synthesised by catalytic deuteriation of hex-2-ynoic acid **80**, hex-3-enoic acid **82** and hex-4-enoic acid **84** respectively. [64] The intermediates 3,4-[$^2H_2$] and 4,5-[$^2H_2$]-hexanoic acids **83** and **85** were obtained with deuterium mainly on the expected positions (at δ1.6, 1.3 and at δ 1.26) as shown in the $^2$H nmr spectrum. These labelled precursors were fed to different surface cultures of *P.implicatum* [CMI 138002 ii] (formerly known as *P. decumbens*) and after incubation and the subsequent extraction with EtOAc substantial quantities of ethisolide **87** and **88** were obtained. The $^2$H nmr spectra of these samples showed prominent signals at δ 4.70, 4.00 for **87** and at δ 4.00, 1.70 for **88** indicating a high degree of deuterium enrichment at the C-3, C-4 and at the C-4, C-5 positions respectively. Breakdown of the labelled hexanoic acid to deuterium labelled acetate and incorporation of this into ethisolide does occur to a small extent as indicated by some enrichment at C-4 and C-6. Incorporation of deuterioacetate is known to result in enrichment only at these positions in the C-6 fatty acid chain of ethisolide. [56a, b] Thus incorporation of deuterium from the hexanoic acids **83** and **85** into C-3 and C-5 respectively of ethisolide can not be via acetate but indicates intact incorporation.




**Scheme 9**



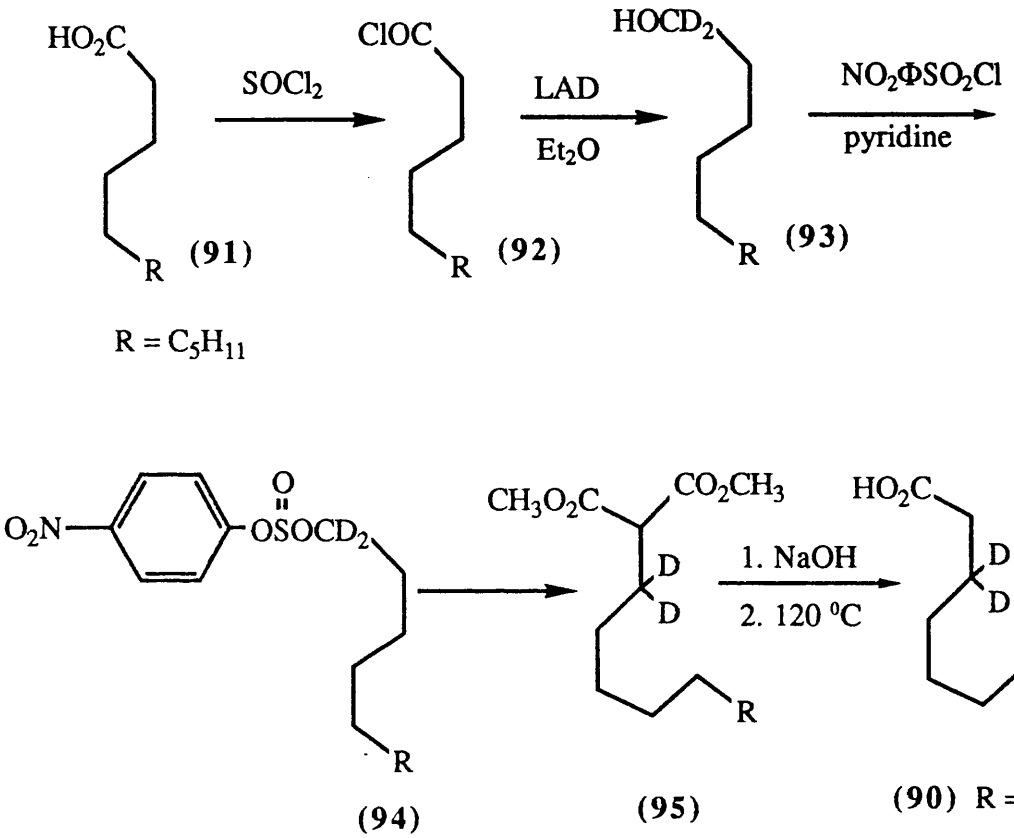
Another important result was obtained upon feeding 2,3-[$^2H_4$]-hexanoic acid **81** to *P.implicatum*.[64] The $^2H$ nmr spectrum of the former intermediate showed that deuterium to be mainly at the C-2 ( δ 2.27) and C-3 (δ 1.57 ) positions, in addition to some randomisation of small amounts of label to the adjacent positions probably due to the heterogeneous catalysis method used in the deuteriation of hex-2-ynoic acid **80**. The $^2H$ nmr spectrum of the corresponding ethisolide **86** obtained from this feeding experiment showed a strong signal at δ 4.07 indicating a substantial degree of deuterium incorporation at the C-3 position of ethisolide **86**. It was found that no incorporation of deuterium from the C-2 of hexanoic acid **81** was retained at C-2 of ethisolide **86**. It may be noted that the retention of deuterium in ethisolide from the 3-position of hexanoic acid **81** directly disproves the involvement of 3-oxohexanoic acid **89** suggested as an intermediate in the first biogenesis expounded by Turner *et.al.*.[65]

Preparation of the above labelled hexanoic acids suffered from the difficulties encountered preventing randomisation of label occurring during reduction of double bonds. In the present study to establish the analogous intact incorporation of dodecanoic acid **90** into avenaciolide **96** a route was chosen which would ensure complete specificity of the $^2H$ label at C-3 in dodecanoic acid **90**. (Scheme **10**)

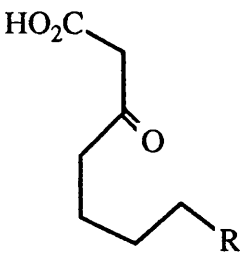
Decanoic acid **91** was converted into decanoyl chloride **92** by treatment with thionyl chloride followed by reduction with lithium aluminum deuteride (LAD) to give 1-[$^2H_2$]-decanol **93**. This exhibited in the IR spectrum a strong band at 3630 $cm^{-1}$ due to the free (OH) group. The latter was treated with *p*-nitrobenzensulfonyl chloride to afford the desired *p*-

nitrobenzenesulfonate derivative **94**, the structure ($C_{16}H_{23}D_2NO_5S$ ) was confirmed by mass measurement which gave a molecular ion at m/e 345.1576 and a strong characteristic peak at m/e 143 (11.6%) due to loss of the *p*-nitrobenzensulfonate group. The $^1H$ nmr spectrum showed doublets (*J* 12 Hz) at δ 8.40, 8.10 and as multiplet at δ 1.63 indicating the presence of the aryl protons and the methylene group ($-CD_2C\underline{H}_2-$) respectively, together with appropriate resonances for the alkyl group. The $^{13}C$ nmr also confirmed the structure by showing signals at δ 129.13 and δ 124.4 for the aromatic carbons, at δ 31.78 for the methylene carbon ($-\underline{C}H_2CD_2-$) together with signals as expected for the methyl and the methylene groups of the alkyl chain. The *p*-nitrobenzenesulfonate **94** gave after condensation with dimethyl sodiomalonate followed by distillation the desired dimethyl 1-$^2H_2$-decylmalonate **95** as colourless oil. The molecular formula $C_{15}H_{26}D_2O_4$ was established by high resolution mass spectroscopy which gave a molecular ion at m/e 274.2129 and a recognisable cracking pattern. The IR spectrum exhibited strong bands for the saturated dimethyl esters at 1755 and 1740 $cm^{-1}$. The $^1H$ nmr spectrum showed the expected signals for the carbomethoxyl methyl groups at δ 3.70. The methine proton which is α to the methyl esters appeared at δ 3.36 as a singlet showing no coupling as expected from the presence of two (2) deuterium atoms at the β position. The $^{13}C$ nmr showed signals of the appropriate multiplicity at δ 116.67 and 116.56 for non-conjugated (C=O) groups, at δ 51.49 for the methine carbon, together with appropriate resonances for the methoxyl and the alkyl groups.

Hydrolysis of **95** in 1M aqueous NaOH at room temperature gave the corresponding diacid in excellent yield. This was carefully heated at 120 $^0C$ for 2 hrs. to give after decarboxylation followed




**Scheme 10**

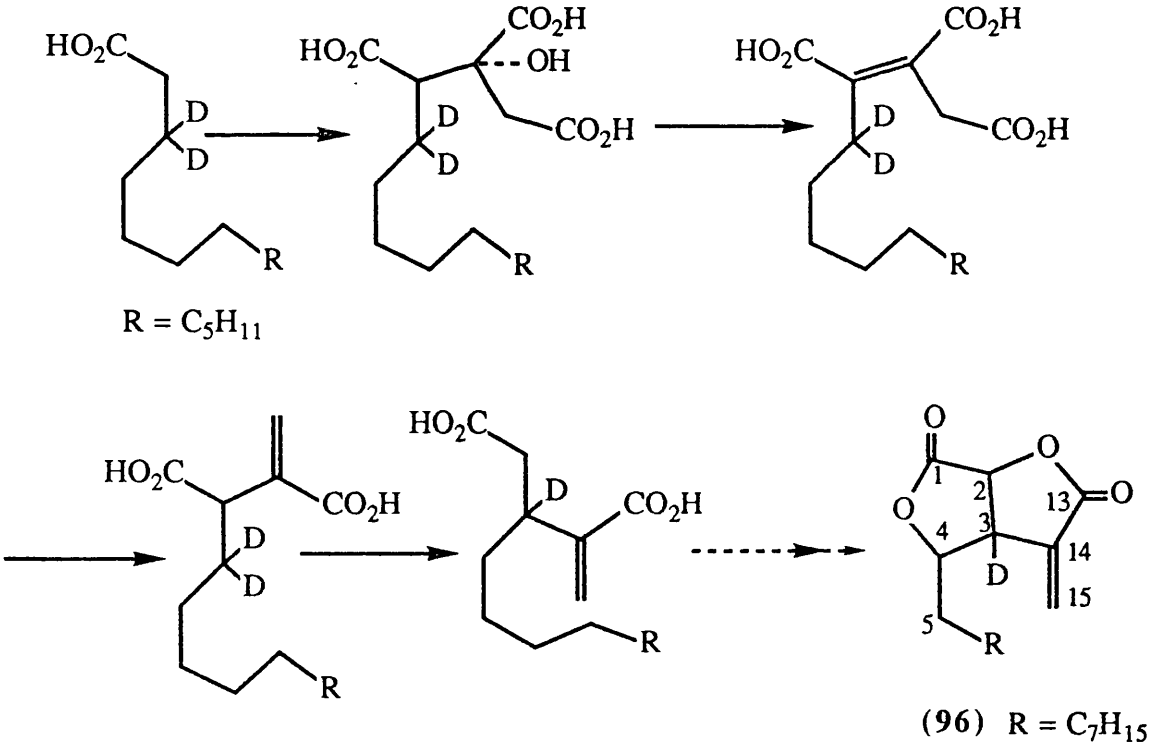



**(97)** R = $C_5H_{11}$

by TLC the desired deuterated dodecanoic acid **90** as white crystalline product m.p. 43-45 $^{0}C$ in 60% yield. This was characterised as $C_{12}H_{22}D_2O_2$ by mass spectroscopy which gave a molecular ion at m/e 202.1885 together with a recognisable cracking pattern. The $^{1}H$ nmr spectrum showed broad singlets at δ 11.5 for the hydroxyl group and at δ 2.32 corresponding to protons of the methylene group (-C$\underline{H}_2$C$D_2$-) at the α position. The $^{2}H$ nmr spectrum showed only one strong signal at δ 1.60 corresponding to the protons of the (-C$\underline{D}_2$-) group at the β-position indicating that the desired deuterated acid **90** was successfully prepared without randomisation of label elsewhere in the molecule. The IR spectrum showed a carbonyl (C=O) absorption at 1708 $cm^{-1}$ and it also exhibited a strong OH absorption at 3000 $cm^{-1}$. The $^{13}C$ nmr spectrum showed resonances of the expected chemical shift and multiplicities confirmed by a DEPT spectrum for this structure **90**. The assignments for lauric acid are known. [54a, b] Comparison of the labelled acid **90** with the unlabelled acid (Table **6**, page 116) shows the disappearance of the signal at 24.83 ppm for C-3 due to the presence of two deuterium atoms which greatly increase the relaxation time and result in loss of nuclear Overhauser enhancement. Also apparent are the typical upfield shifts of *ca.* 0.2 ppm for the adjacent (α) carbons C-2 and C-4 and of *ca.* 0.10 ppm for the (β) carbons C-1 and C-5 due to deuterium substitution at C-3. [54b]

The above labelled acid **90** was fed to surface cultures of *A.avenaceus* and after incubation and the subsequent extraction with ethyl acetate substantial quantities of avenaciolide **96** were obtained. The $^{2}H$ nmr spectrum showed a prominent signal at δ 3.59 indicating a moderate degree of deuterium enrichment at

the C-3 position of avenaciolide **96**, (Scheme **11**) but one which was consistent only with intact incorporation of the precursor **90**. This interesting result provides more evidence to the theory that the biosynthesis of avenaciolide **2** involves decylcitric acid formed by condensation of dodecanoic acid **90** and oxaloacetate (Scheme **11**). It may be noted that the retention of deuterium at the C-3 position of avenaciolide **2** and ethisolide **1** which corresponds to a position derived from the carbonyl group of acetate, rules out the involvement of oxo-dodecanoic acid **97** and/or oxo-hexanoic acid **89** and suggests that the incorporation of lauric acid **90** and hexanoic acids **81**, **83** and **85** into avenaciolide **2** and ethisolide **1** respectively must have been intact rather than via break down to acetate units.



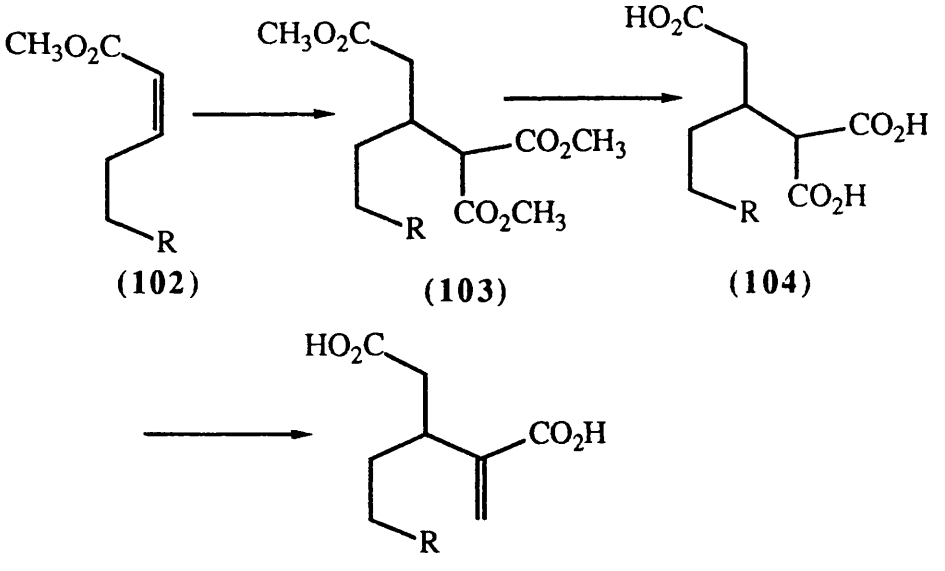

**Scheme 11**

## The biosynthesis of minor metabolites from *Penicillium decumbens* and *Aspergillus avenaceus*.

Evidence from incorporation studies on *P. decumbens* using labelled [2-$^{13}$C]-acetate showed that C-2, C-4 and C-6 of the dimethyl ester of α,n-butyl-α-hydroxyitaconic acid **99**, trimethyl butylcitrate **100** and methyl ethisate **76** to be derived via the acetate-malonate pathway (Scheme **12**). These results suggest that very closely related biosynthetic processes lead to the Type A and Type B compounds, *e.g.* **65** and **5** respectively, produced by *P.decumbens* and therefore ethisolide **1** and avenaciolide **2** are expected to be biosynthesised via Type A precursors. Thus, important information about the biosynthesis of ethisolide **1** and avenaciolide **2** could be derived by studying the biosynthesis of the minor metabolites n-butylitaconic acid **65** and decylitaconic acid **66** from the culture filtrates of the above fungi where these compounds are structurally related to the antibiotics **1** and **2**.

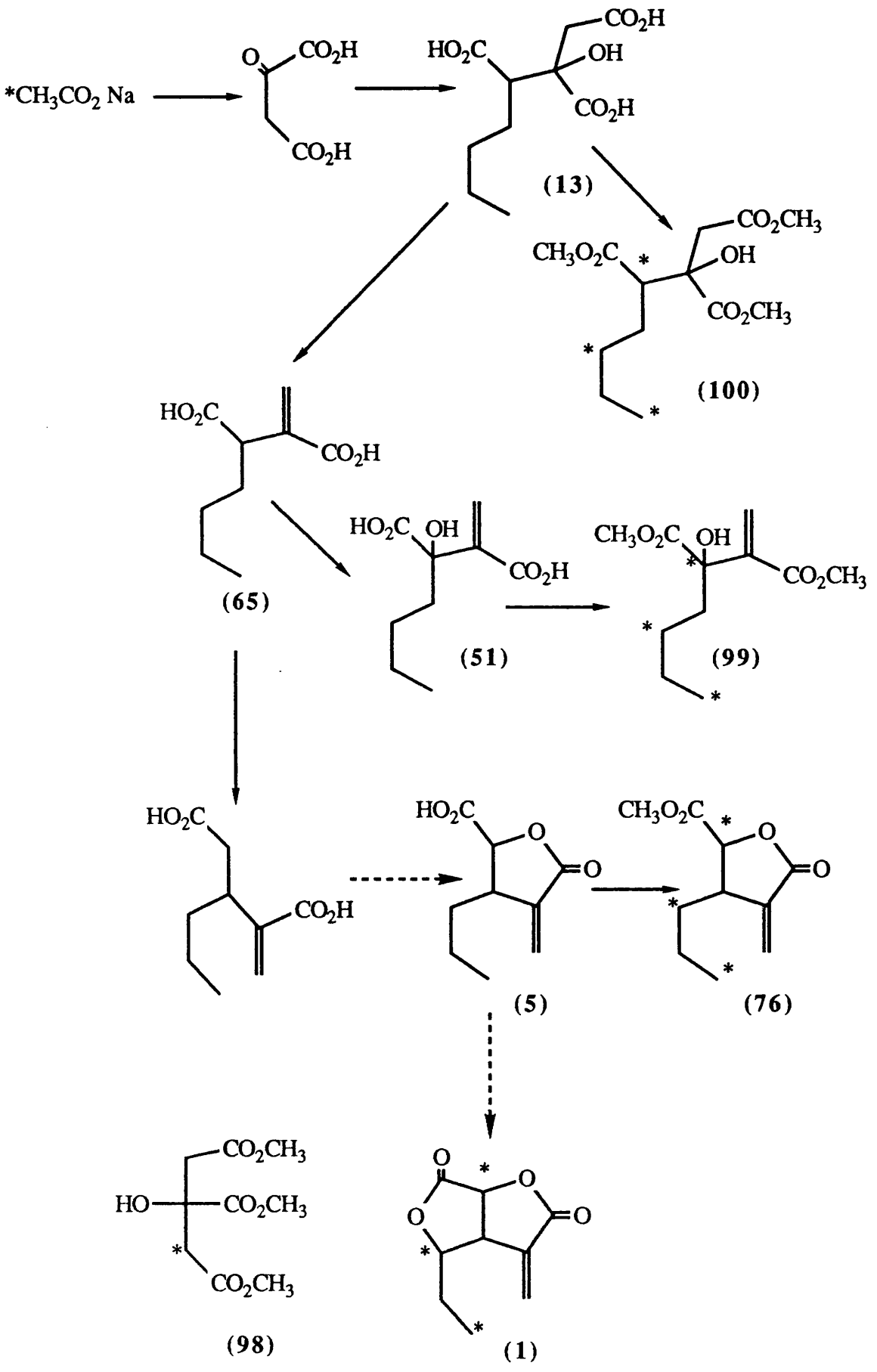
In order to follow the incorporation of deuterium into decylitaconic acid **66** a search for this minor metabolite in the aqueous broth of *A. avenaceus* was carried out. Unlike *P.decumbens,* the former fungus does not produce citric acid **7** or any other primary metabolites and this would not complicate the isolation of the desired acidic intermediates. In a previous experiment [62] in which radioactive acetate was fed to grown cultures of *A. avenaceus* in the presence of added inactive α-methylene-β,n-nonylglutaric acid **101**, the presence of decylitaconic acid was detected. It was thought that this perhaps accumulated owing to feed back inhibition [66] caused by the excess of α-methylene-β,n-nonylglutaric acid **101** present. It was hoped

that in a similar experiment in which deuterated succinate was fed it might be possible to isolate enough decylitaconic acid **66** to allow its labelling pattern to be determined. The required α-methylene-β,n-nonylglutaric acid **101** was synthesised as outlined in Scheme **13**. The appropriate α,β-unsaturated methyl ester **102** prepared by the Knoevenagel method and esterification underwent a Michael addition with dimethyl malonate, to give the trimethyl ester **103** which was in turn converted to the 2-carboxy-3,n-nonylglutaric acid **104** by treatment with aqueous NaOH. The conversion of the triacid **104** to the desired α-methylene-β,n-nonylglutaric acid **101** was affected by the methylenation procedure using aqueous formaldehyde and diethylamine.




**(101)** R = $C_7H_{15}$

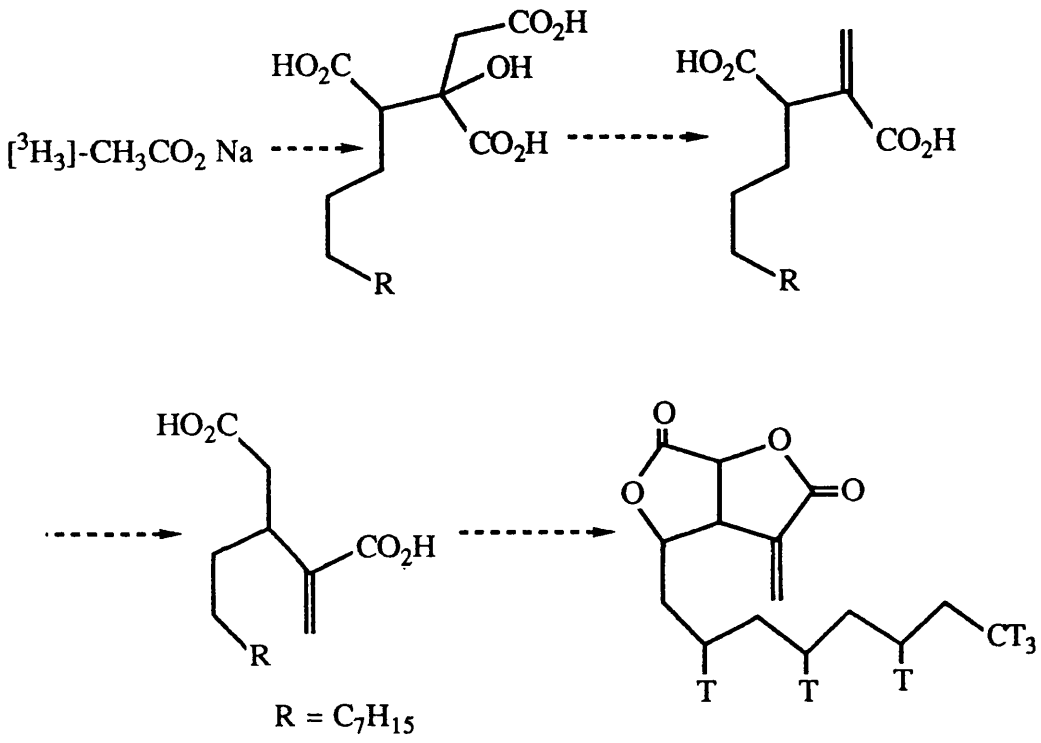
**Scheme 13**


*CH3CO2 Na
CO2H
O
CO2H
HO2C
OH
CO2H
CO2H
(13)
CO2CH3
CH3O2C
*
OH
CO2CH3
*
*
(100)
HO2C
CO2H
(65)
HO2C
OH
CO2H
(51)
CH3O2C
OH
*
CO2CH3
*
*
(99)
HO2C
CO2H
HO2C
O
O
(5)
CH3O2C
*
O
O
*
*
(76)
CO2CH3
HO
CO2CH3
*
CO2CH3
(98)
O
*
O
O
O
*
*
(1)

**Scheme 12**

[$^3$H]-Acetate, [$^2$H]-succinate, [$^2$H]-fumarate and a quantity of non-labelled α-methylene-β,n-nonylglutaric acid **101** were fed to surface cultures of *A. avenaceus*. After incubation and extraction of the culture fluid with ethyl acetate, the combined extracts were washed with aqueous $NaHCO_3$ and evaporation gave avenaciolide **105**. This contained 12.89% of the administered activity from [$^3$H]-acetate (Scheme **14**). The $^2$H nmr indicated that no incorporation of deuterium from [$^2$H]-succinate or fumarate has taken place into avenaciolide **2**. After treatment the $NaHCO_3$ layer with dilute acid and extraction with ethyl acetate a mixture of acids was obtained. Extensive preparative TLC separated the components into two main fractions, the more polar fraction contained as was expected substantial quantities of α-methylene-β,n-nonylglutaric acid **101** which contained 5.2% of the administered activity from [$^3$H]-acetate and the less polar fraction, contained an olefinic compound yet to be identified. It was hoped to isolate the decylitaconic acid **66** along with **101** from these acidic fractions but this could not be detected by either TLC or $^1$H nmr.

This experiment was repeated by feeding only [$^2$H]-succinate and a quantity of unlabelled **101**. After a similar work up, as discussed above, substantial quantities of avenaciolide **2** were obtained from the neutral fractions with no deuterium incorporation as was shown in the $^2$H nmr spectrum. The fed α-methylene-β,n-nonylglutaric acid **101** was recovered from the acidic fraction and again according to TLC and $^1$H nmr analysis, there was no sign of the desired decylitaconic acid **66**. As a result it has not been possible to follow the incorporation of deuterium from [$^2$H]-succinate into this key intermediate **66**.




R = $C_7H_{15}$

**(105)**

**Scheme 14**

It was hoped that n-butylitaconic acid **65** or its hydroxy analogue **51** might be isolated from the cultures of *P.decumbens* and therefore that it might be possible to examine whether the deuterium label is found in only one, or both terminal methylene positions of these molecules.

$^2H_4$-succinate was fed to 10 day old surface cultures of *P. decumbens* and after incubation, extraction of the culture fluid with ethyl acetate gave substantial quantities of ethisolide **1**. The $^2H$ nmr spectrum showed major signals at 6.68 and 5.95 ppm indicating that deuterium from $d_4$-Succinate is incorporated into both terminal positions of **1**. This is surprising since the terminal methylene hydrogens would be expected to be derived from the methylene group of a singly labelled [3S-3$d_1$]-oxaloacetate intermediate (Scheme **7** ). [see Introduction page 28].

After evaporation to dryness the mother liquor was treated with cold ether which separated more ethisolide **1** from a mixture of acids. Preparative TLC was therefore required to separate these, eluting with the solvent system $CHCl_3$:acetone:AcOH with the ratios (9:4:1) respectively. These gave two main fractions, the less polar fraction contained a further quantity of ethisolide **1** while the more polar fraction showed in the $^1H$ nmr spectrum, signals corresponding to one proton fine doublets at 6.31, 5.61 and at 5.04 ppm together with multiplets for a butyl and a hydroxyl group. It also showed a three proton doublet at 2.2 ppm (*J* 3 .00 Hz) and a one proton multiplet at 5.15 ppm corresponding to a vinyl methyl system. This was deduced to be a mixture of ethisic and decumbic acids **5** and **77** respectively, comparison being made with the data for authentic samples of the methyl esters of these acids.

It was hoped that n-butylitaconic acid **65** might be isolated along with the above metabolites but so far this could not be detected. The above experiment was repeated and after removal of ethisolide **1** by crystallisation, the mother liquor was again evaporated to dryness and extracted with cold ether to give a mixture of acids. The $^{1}H$ nmr spectra showed the presence of ethisic acid **5** and decumbic acid **77**, as discussed above, together with singlets at δ 6.56 and 5.87. These could correspond to the presence of a terminal methylene function, which is possibly part of the desired butylitaconic acid **65**. On the other hand, the $^{2}H$ nmr spectrum showed a strong signal at 2.67 ppm [probably due to natural abundance of $^{2}H$- succinate ] and no signals at δ 6.56 and 5.87 were present. The results of the above and previous experiments seemed to suggest that an early harvesting of the cultures could provide a better chance in the isolation of the minor metabolites in reasonable quantities. For this reason, $^{2}H_{4}$-succinate was fed to nine 09 day old surface cultures of *P. decumbens* and after incubation, extraction of the cultures fluid gave ethisolide **1** and a mixture of ethisic **5** and decumbic **77** acids. TLC and $^{1}H$ nmr analysis indicated that there was no sign of the key intermediate **65**.

It was thought that the feeding of the labelled precursors might have prevented the organism from producing this minor metabolite. It was therefore decided to carry out a similar search for this in unfed culture extracts. The aqueous broth of (09) day old surface cultures of *P. decumbens* was evaporated to dryness, extracted and the mother liquor was treated with aqueous $NaHCO_3$. After treatment with dilute acid, a silica gel column separated the mixture into two main fractions, the less polar, contained again decumbic acid **77** as a major component together with traces of ethisic acid **5**. The more polar fraction

was assayed by TLC and $^1H$ nmr and n-butylitaconic acid **65** could not be detected. It should be noted that no monolactonic metabolites (like ethisic acid **5** type) were detected in *A.avenaceus* cultures investigated earlier.

Since TLC was inefficient in separating the olefinic components of the aqueous broth of *P.decumbens* , HPLC has been investigated. C 18 and C 8 columns (5 υm) particles were tested on several standard compounds like the synthesised n-butylitaconic acid **65** and α-methylene-β,n-propylglutaric acid **106** and purified primary and secondary metabolites namely citric acid **7** and ethisolide **1** respectively.

Excellent results were obtained with the C 8 column and the chromatogram from elution with $H_2O$ (channel A) and $CH_3OH$ (channel B) showed narrow, sharp and symmetrical peaks and a fairly good distribution of the retention times for the above standard compounds either when injected separately or as a mixture.

The retention times (Rt) for ethisolide **1** and some acids particularly n-butylitaconic acid **65** [Rt=1.43 min.] using the final system mentioned under experimental are listed in Tables **1.1** and **1.2**.

Unlike ethisolide **1** which is a major product of *P.decumbens,* butylitaconic acid **65** is a minor metabolite. Since it is probably present in the cultures of the above fungus in only very small quantities, therefore it was not possible to be detected clearly by either TLC or $^1H$ nmr. HPLC was sought as an alternative means hoping that it would solve this difficulty.

Having established the excellent conditions for the above compounds, it was therefore decided to apply them to fungal extracts from different $^2H_4$- succinate feeding experiments. After removing ethisolide **1** by crystallisation, a sample from the

extracted aqueous broth was injected and the retention times were distributed from 0.31 to 1.48 min. The most interesting features from this chromatogram were the peaks at 1.11 and 1.48 min., which could be corresponding to n-butylitaconic acid **65** and α-methylene-β,n-propylglutaric acid **106** respectively. These results are quite compatible with those previously obtained for the above model studies for compounds **65** [Rt=1.48 min.] and **106** [ Rt=1.00 min. ] as listed in Table 1. Taking into account the slight variation in the different parameters involved in these experiments eg. pressure; room temperature ...etc.

Apparently, under these conditions the monolactonic compounds ethisic acid **5** and decumbic acid **77** gave a trace in which the only peak observed was at 0.31 min. This was concluded after a similar retention time was obtained for an injected reference sample of **5** and **77**.(see Table **1.1**). Hence either they have this retention time or are not detected at the wavelength used. In either case they would not interfere with the detection of n-butylitaconic acid **65** or α-methylene-β,n-propylglutaric acid **106**.

Although these results indicated that the detection and the separation of the components from the fungal extracts have been achieved, another system is required . This is to get a better and an even distribution of the retention times for all the primary and secondary metabolites and it would be also desirable to achieve a considerable shift in their retention time peaks from that of the solvent. An investigation to improve the present conditions was attempted by adding $H_3PO_4$ to the solvent system used earlier as discussed under experimental in Table **1.3**. Unfortunately, lack of time and access difficulty to the HPLC equipment prevented from carrying out quantitative studies using both systems in the isolation of the minor metabolites from *P.decumbens*.

It has been reported [56a] that butylitaconic acid and decumbic acid were isolated from unfed cultures of their corresponding fungus as their methyl esters **57** and **56** respectively. The mixture of the acids obtained from another 09 day old unfed cultures of the above fungus, was esterified by treatment with $Me_2SO_4$ and aqueous $NaHCO_3$ at 60 $^0$C for 03 days.

Extensive preparative TLC gave methyl decumbate **56** [the $^1$H nmr spectrum showed a characteristic methyl doublet at δ 2.2] and methyl ethisate **76** [characteristic signals for the terminal methylene protons as doublets at δ 6.51 and 5.52 in the $^1$H nmr ]. Dimethyl butylitaconate **57** could not be isolated or even detected as expected and the organism subsequently failed to yield this metabolite or its hydroxy analogue **51**, which has been previously isolated. [20]

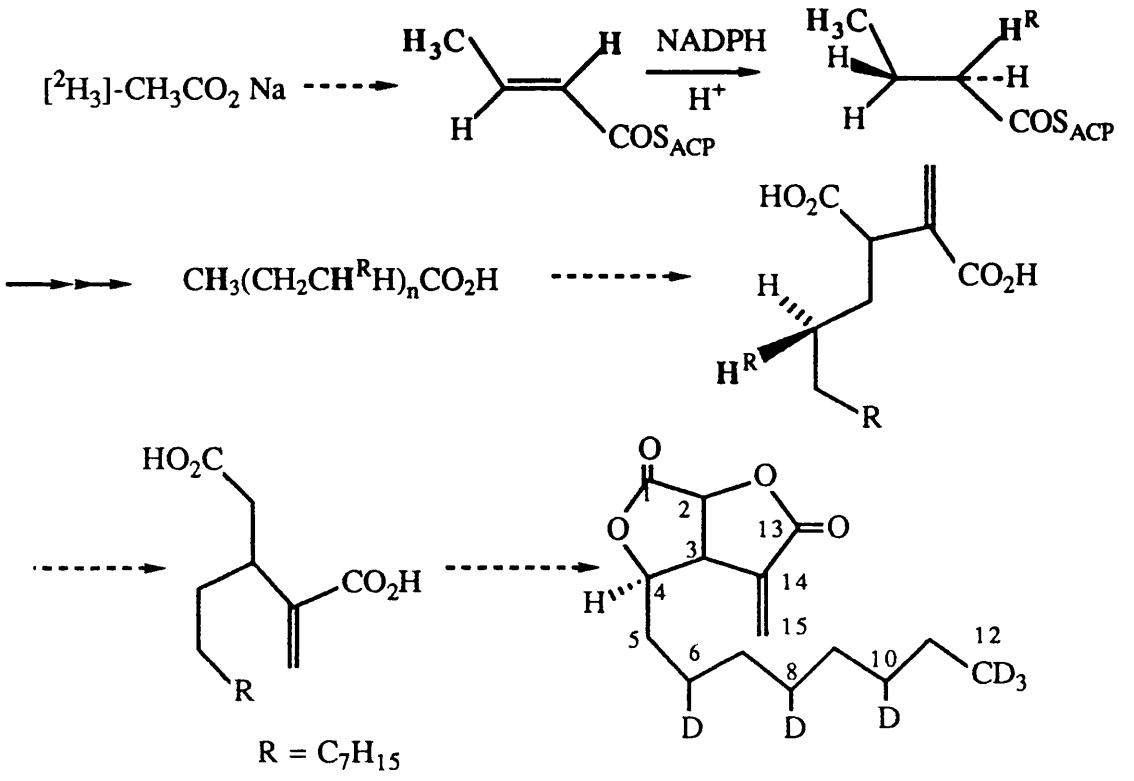
## Stereochemical aspects of the biosynthesis of the $C_3$ unit in ethisolide 1 and avenaciolide 2.

It was found in previous feedings that deuterium from $d_3$-acetate, was incorporated mainly into the fatty acid moiety of ethisolide **1** [56a] and of ethisic acid **5** (isolated [56a] as its methyl ester) with a small amount being located in the terminal methylene group. On the other hand deuterium from $d_4$-succinate or $d_2$-fumarate was incorporated mainly into the terminal methylene group of ethisolide **1** or of ethisic acid **5** and as with the acetate feed deuterium was located equally in both terminal methylene positions. It was hoped to confirm these unexpected results and examine the pattern of incorporation into avenaciolide **2** and the key intermediates butylitaconic acid **65** and decylitaconic acid **66**. Each of these acids has a terminal

methylene group as part of $C_3$ acrylic acid grouping, which has not undergone migration to the adjacent carbon atom.

$^2H_3$-Acetate was fed to surface cultures of *A. avenaceus* and after incubation, extraction of the culture fluid with ethyl acetate gave a substantial amount of avenaciolide **2**. The $^2H$ nmr showed major signals at $\delta$ 1.29 and 0.85 indicating that deuterium enrichment is mainly as expected at the C-12 methyl group and the methylene groups C-10, C-8 and C-6. There were two significant differences in the pattern of incorporation of deuterium from acetate into avenaciolide **2** as compared to ethisolide **1**. The first difference was that no enrichment was observed at C-4 in avenaciolide (Scheme **15**). This difference has previously been studied using $^{13}C$, $^2H$ doubly labelled precursors [56b] and is critically dependent on the stereochemistry at C-4. The fatty acid chain is evidently constructed with the same stereochemical consideration as in fatty acid biosynthesis [56c] *i.e.* the hydrogen atom at each even numbered position which is retained from acetate has the pro-R configuration (Scheme **15**). Hydroxylation of the chain as is normal evidently occurs with retention of configuration so that the pro-R hydrogen is lost or retained depending on the stereochemistry of hydroxylation. hence deuterium from acetate is retained at C-4 in ethisolide **1** (would be retained in isoavenaciolide **3**) but is lost at C-4 in canadensolide **4**, dihydrocanadensolide **14**, canadensic acid **15** and avenaciolide **2**. The present work using $^2H$ nmr is in keeping with these results.

The second difference observed in the pattern of incorporation of deuterium from $CD_3CO_2H$ into avenaciolide **2** as appeared to into ethisolide was that no deuterium incorporated via the Krebs cycle into the terminal methylene positions of avenaciolide **2**. Evidently the Krebs cycle is more active in *P. decumbens*.

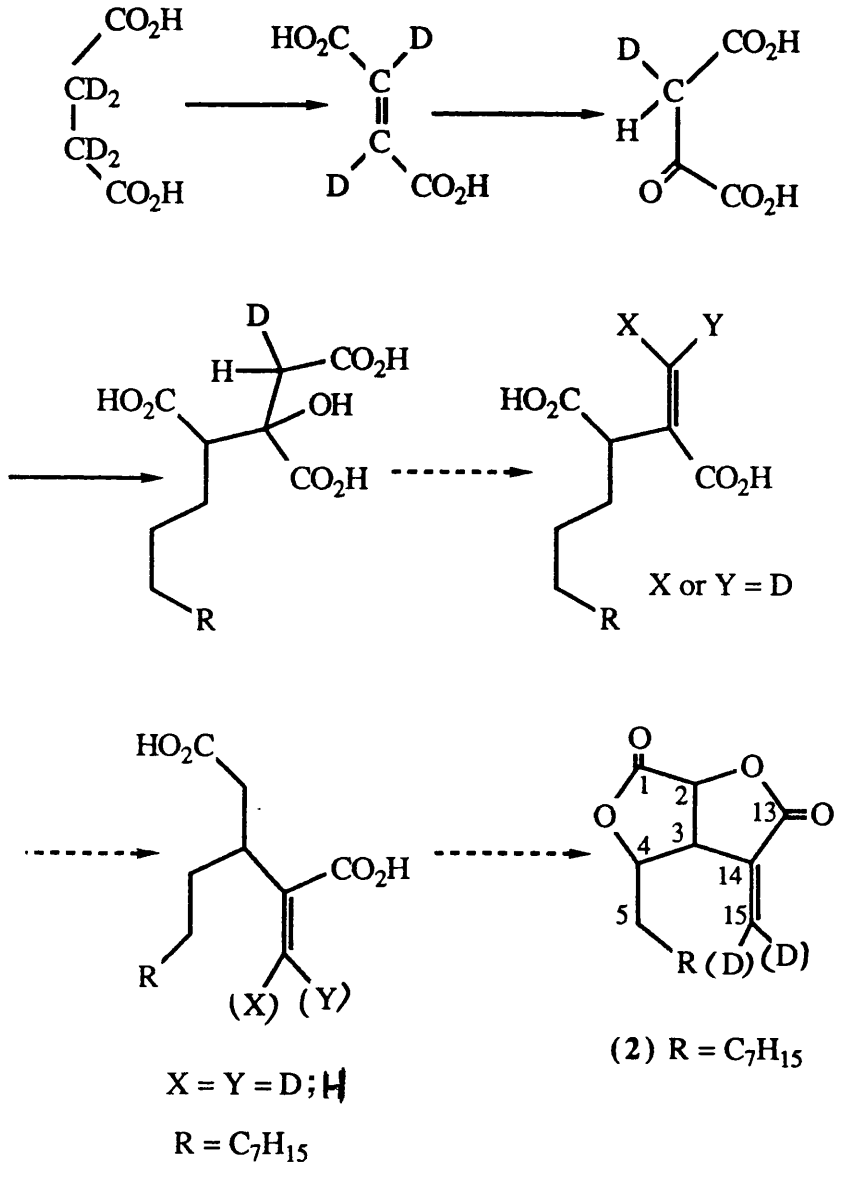



(2)

**Scheme 15**

$^2H_2$-fumarate was also fed to surface cultures of *A. avenaceus* and after incubation and extraction substantial quantities of avenaciolide **2** were obtained. The $^2H$ nmr spectrum showed prominent signals at δ 1.27 and 0.9 indicating that deuterium enrichment is mainly at the C-12 methyl group and the methylene groups C-10, C-8 and C-6 of **2**. On the other hand, there was no incorporation of deuterium into the terminal methylene positions. This is probably due to inhibition of the biosynthesis at the feeding level required of a [$^2H$] -precursor. This contrasts with ethisolide **1** biosynthesis by *P. decumbens* discussed earlier since feeding $d_2$-fumarate affords ethisolide showing deuterium enrichment mainly in both terminal methylene positions.

However, the $^2H$ nmr spectrum of avenaciolide **2** isolated upon feeding $^2H_4$-succinate showed signals at δ 6.46 and 5.82 indicating the incorporation of deuterium into both terminal methylene positions of avenaciolide **2** (Scheme **16**). This result is in complete accord with that previously reported for ethisolide **1** and its co-metabolite methyl ethisate **76** and this further supports the similarity of their biosynthetic pathways. The incorporation of [$^2H$] from succinate into both terminal positions of avenaciolide **2**, is evidently via the Krebs cycle. Thus, as discussed for ethisolide **1** earlier [see pages 28 and 47] incorporation of deuterium from succinate is presumably via a monodeuterio oxaloacetate and presumably the same factors result in the apparent scrambling of label into both terminal methylene positions of the antibiotic **2**. As postulated for ethisolide **1** rearrangement of an alkylitaconate intermediate to an alkyl-α-methyleneglutarate intermediate via a cyclopropylmethyl radical could account for the observed result. The following studies on cyclopropylmethyl derivatives aimed to clarify this point.


CO2H
CD2
CD2
CO2H
HO2C
D
C
C
D
CO2H
D
CO2H
C
H
O
CO2H
D
H
CO2H
HO2C
OH
CO2H
R
X
Y
HO2C
CO2H
R
X or Y = D
HO2C
CO2H
R
(X)
(Y)
X = Y = D; H
R = C7H15
O
O
1
2
O
13
O
4
3
14
15
5
R
(D)
(D)
(2) R = C7H15

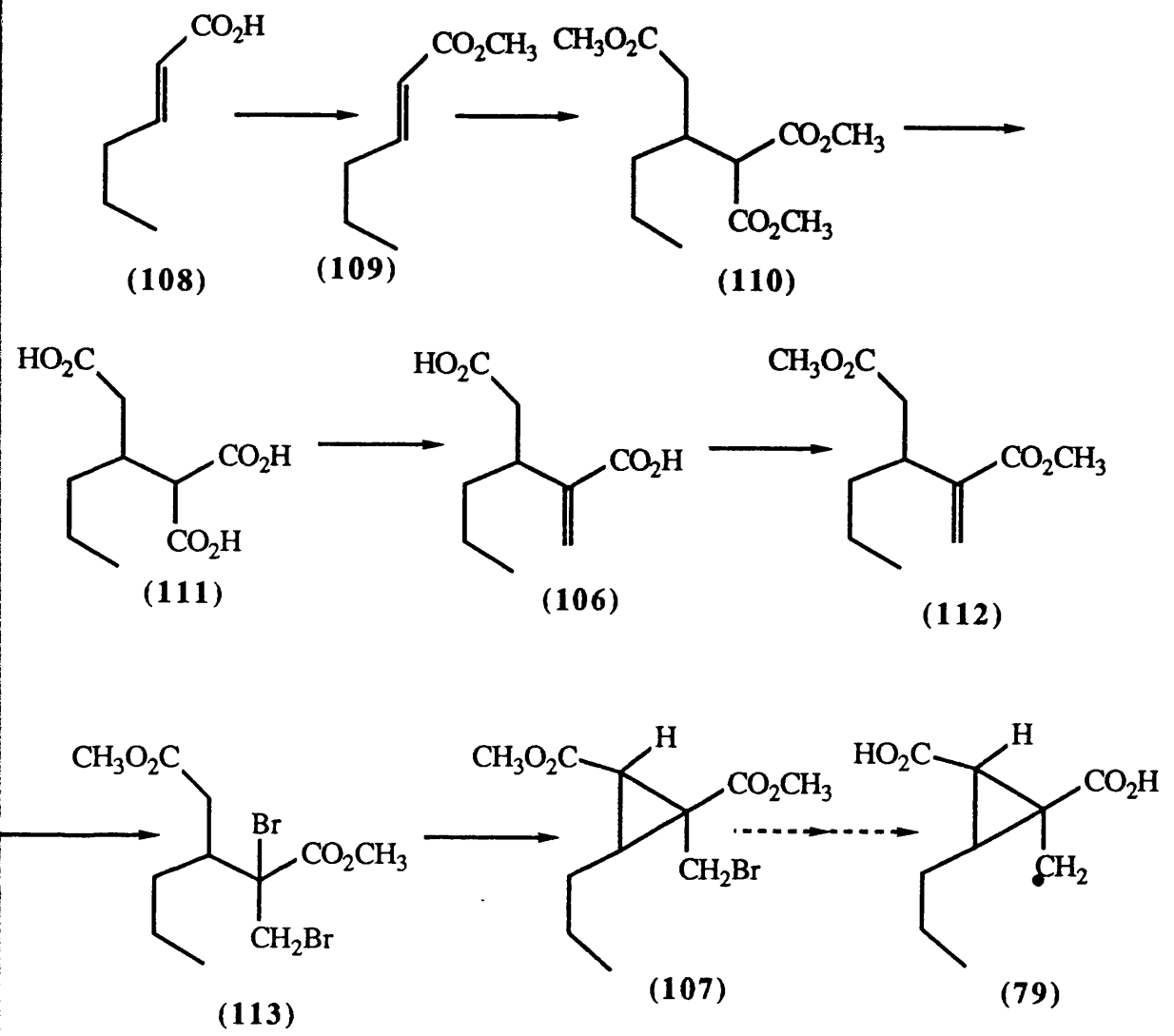
**Scheme 16**

## CHAPTER 3 : Discussion (Part 2).

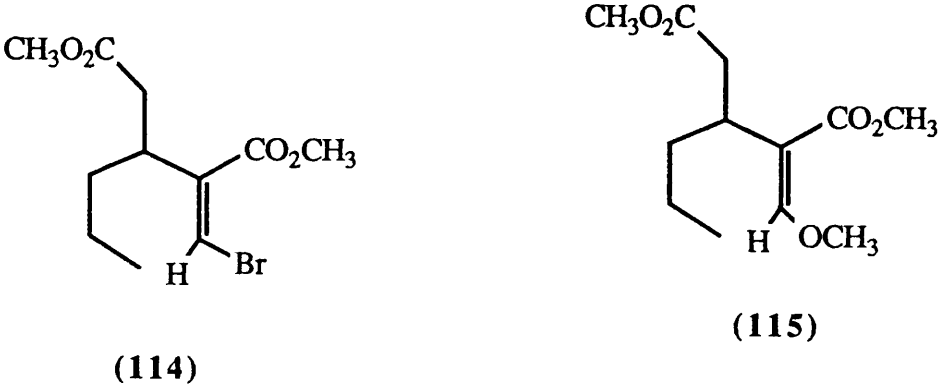
## 1. Synthesis of cyclopropanes via 1,3-elimination reactions.

As discussed earlier the biosynthesis of the bislactone antibiotics ethisolide **1**, avenaciolide **2** and isoavenaciolide **3** and the metabolite ethisic acid **5** is of the alkylcitric acid type and appears to subsequently involve rearrangement of an α-alkylitaconic acid eg. **65** to an α–methylene-β,n-propylglutaric acid e.g. **106**. This could by analogy with the conversion of methylitaconic acid into α–methyleneglutaric acid be considered to be a coenzyme $B_{12}$-dependent enzymatic rearrangement. A plausible mechanism for this rearrangement involves the cyclopropane intermediate (**79**, R=$CH_3$ ). The cyclopropane ring is a common unit in a large number of natural products and compounds of pharmaceutical interest. [67, 68] The present work is directed towards generating the proposed intermediate **79** in the biosynthesis of ethisolide **1** and the minor metabolite ethisic acid **5** via the bromomethylcyclopropane **107**. A possible route to prepare the bromomethylcyclopropane **107** via α–methylene-β-n-propylglutaric acid **106** is outlined in Scheme **17**.

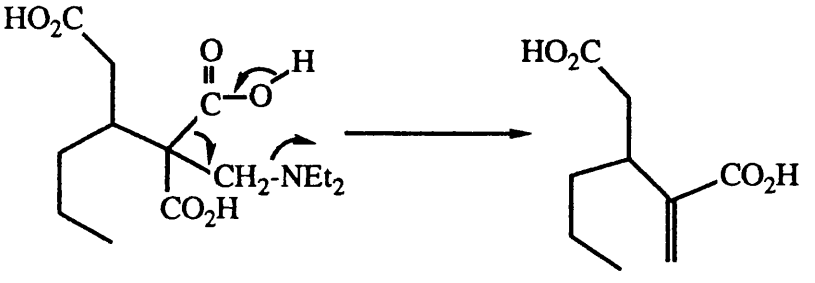
The starting material was commercially available *trans* 2-hexenoic acid **108** which was converted to its methyl ester **109** by refluxing with methanolic HCl prepared by adding $CH_3COCl$ to anhydrous methanol. This ester underwent Michael addition of dimethyl malonate to give the trimethyl ester **110**. Careful hydrolysis of this with aqueous NaOH produced 2-carboxy-3-n-propylglutaric acid **111**. Decarboxylation occurs as an undesired side reaction if conditions are too vigorous but the NMR of the


CO2H
CO2CH3
CH3O2C
CO2CH3
CO2CH3
(108)
(109)
(110)
HO2C
CO2H
CO2H
(111)
HO2C
CO2H
(106)
CH3O2C
CO2CH3
(112)
CH3O2C
Br
CO2CH3
CH2Br
(113)
CH3O2C
H
CO2CH3
CH2Br
(107)
HO2C
H
CO2H
CH2
(79)

**Scheme 17**


CH3O2C
CO2CH3
H
Br
(114)
CH3O2C
CO2CH3
H
OCH3
(115)

product showed the absence of the decarboxylation product. The conversion of the triacid **111** to the desired α-methylene-β-n-propylglutaric acid **106** was affected by methylenation with aqueous formaldehyde and diethylamine. This is thought to involve a Mannich type of reaction followed by aminodecarboxylation as shown in Scheme **18** :




**(101)**

**Scheme 18**

The various intermediates in this route and the final product **106** were identified by comparison with the spectra and samples of authentic material. [69]

Esterification of **106** with methanolic HCl gave the dimethyl ester **112** as colourless oil in 58% yield. This was characterised as $C_{11}H_{18}O_4$ by microanalysis and mass spectroscopy. The $^1H$ nmr spectrum showed singlets at δ 3.71 and 3.59 indicating the presence of the two methoxyl groups and it also exhibited in the IR spectrum a C=O absorption at 1730 and 1720 $cm^{-1}$ and conjugated ($C=CH_2$) absorption at 1620 $cm^{-1}$. The $^{13}C$ nmr showed signals of the appropriate multiplicity at δ 172.78. and 167.10 for non-conjugated and conjugated C=O groups respectively, at δ 142.37 and 125.19 for the terminal methylene

carbons and at δ 37.84 for the methine carbon together with appropriate resonances for the methoxyl and the propyl groups.

Bromination of the dimethyl ester **112** in $CCl_4$ gave the required dibromo compound **113** as *threo* and *erythro* mixture. This was purified by flash chromatography in EtOAc/petrol with the ratios (20:80) to ensure the absence of unreacted alkene **112** and again **113** was obtained as an inseparable (1:1) mixture of *threo & erythro* isomers (one spot on TLC). The mass spectrum showed no ion corresponding to the molecular ion and the highest ions at m/z 295 and 293 can be readily explained as a clear loss of $^{\bullet}Br$ and also showed abundant ions at m/z 263, 261 due to loss of HBr and $-OCH_3$ from both parent ions 376 and 372 respectively, ions at m/z 235, (233) and 214 are due to loss of $Br/CO_2CH_3$ and $Br_2$ respectively together with ions at m/z 171 and 155 due loss of the propyl and the carbomethoxyl groupings from the ion m/z 214.

In the $^1H$ nmr the absence of the olefinic hydrogens was again evident and bromomethyl groupings appeared as two AB quartets at δ 4.10, 3.88 ($J_{AB}$ 10.50 Hz) and at δ 3.98, 3.85 ($J_{AB}$ 11.00 Hz) respectively. The methylene protons α to the methyl ester and the (-CH-) group appeared as multiplets at δ 2.5-2.8 and at δ 1.75 respectively, together with appropriate resonances for the methoxyl and the propyl groups. The $^{13}C$ nmr spectrum showed a high number of signals which could be accounted for when doubling of signals was considered owing to the presence of diastereoisomers. In a DEPT spectrum, the $-CH_2Br$ and -CBr carbons for the two isomers appeared at δ 38.82, 38.28 and δ 71.98, 69.37 respectively and one of the ester carbonyl groups

was downfield of the other by ca. 1.8 ppm, probably due to the halogen substituent on the α-carbon.

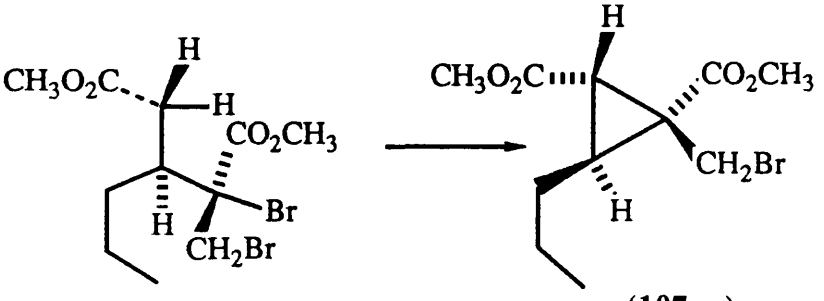
It was hoped that the bromomethylcyclopropane **107** could be prepared by dehydrohalogenation of **113** with base. [70, 71] (Scheme **17**). The first attempts were made using pyridine, $Al_2O_3$ or $Ag_2O$ in DMF and t-butyl lithium/THF which according to the $^1H$ nmr spectra gave unchanged starting material **113**. NaH/THF, KOAc/EtOH and $K_2CO_3$/MeOH gave a mixture of unchanged starting material **113** along with traces of the debrominated ester **112**.

When the reaction was carried out under milder conditions using DBU/THF at -70 $^oC$, the vinyl bromide **114** was obtained. This compound exhibited in the IR a conjugated -C=CHBr absorption at 1630 $cm^{-1}$, and was assigned structure **114** on the basis of its mass spectrum and its $^{13}C$ nmr (DEPT) which showed a signal corresponding to the vinyl carbon (-CH-) at δ 124.31 whose proton resonated in the $^1H$ spectrum at δ 7.65 as a singlet. The methylene protons (H-2) and the methine proton (H-3) appeared as the AB and X parts of an ABX system at δ 2.6 and δ 3.5 respectively, in addition to appropriate resonances for the methoxyl and propyl groups.

Treatment of the dibromo compound **113** with sodium methoxide in refluxing methanol gave three compounds of interest. The product was obtained as a mixture which TLC in EtOAc/petrol (1:2) separated into two fractions. The less polar fraction contained an inseparable mixture of two compounds thought to be again the above vinyl bromide **114**, which showed in

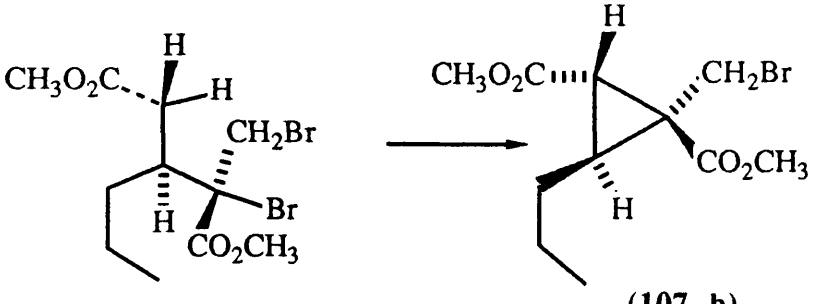
the $^1H$ nmr a characteristic singlet at δ 7.65 for the vinyl proton. The $^1H$ nmr spectrum of this mixture also showed a doublet at δ 2.65 (*J* 6.86 Hz) corresponding to the (-CH-) proton at the α position of the methyl ester grouping in the desired bromomethylcyclopropane **107**. This characteristic signal for this type of proton on a cyclopropane ring, was overlapped with the AB part of an ABX system (δ 2.6) which corresponds to the ($-CH_2-$) protons also at the α position of a methyl ester in the bromo alkene **114** as deduced from the $^{13}C$ nmr. In a DEPT spectrum signals at δ 40.21 and 31.91 were assigned to the carbons of the cyclopropyl methine (-CH-) groups at C-2 and C-3 respectively and at δ 32.12 for the bromomethyl ($-CH_2Br$) carbon. The IR spectrum showed bands at 1730 and 1740 $cm^{-1}$ for the saturated C=O esters in **107**, together with a conjugated -C=CHBr absorption at 1630 $cm^{-1}$ in compound **114**.

Assuming concerted elimination of $Br^-$ by the α-anion and that the anion is such that α-carbomethoxyl group does not become *cis* to two groupings in the cyclopropane product, cyclisation of isomer **113a** would give cyclopropane **107a** whereas isomer **113b** would give cyclopropane **107b**. Possibly isomer **107a** in which the bulky $-CH_2Br$ group is *cis* to the propyl group might be formed while isomer **107b** in which it is *cis* to the more bulky ester group would not be formed. Isomer **113b** might instead undergo reaction leading to the vinyl bromide **114**. The size of the coupling constant between the protons at C-2 and C-3 in the cyclopropane **107** (*J* 6.86 Hz) are in accord with these hydrogens being *trans* as discussed with various cyclopropanes prepared later in this work. These considerations would favour the relative stereochemistry of this cyclopropane being as in **107a**.



(113 a)

(107 a)



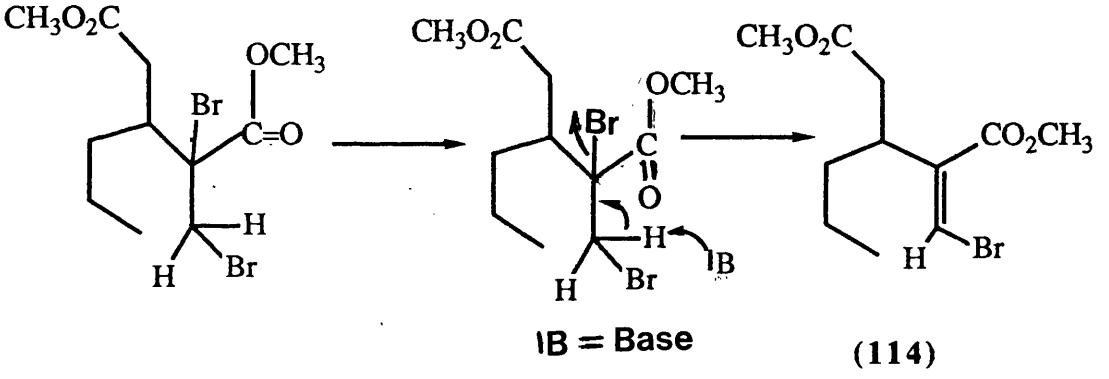
(113 b)

(107 b)

The remaining compound in the more polar fraction was obtained in 55% yield and was deduced to be the vinyl ether **115**. The molecular formulae $C_{12}H_{20}O_5$ was deduced from mass spectroscopy which gave the molecular ion at m/z 244. The $^1H$ nmr spectrum showed In addition to the usual resonances for the methyl esters and propyl groups, the vinyl methine group appeared as a singlet at δ 7.30 while the ethereal methoxyl group appeared as a singlet at δ 3.59. In the $^{13}C$ nmr, multiplicities were established using DEPT and for **115** signals of the expected resonance were found for the methylene grouping α to the methyl ester function (δ 37.83 ), and for the ester methoxyl groupings (δ 51.50 and 51.29), together with appropriate resonances for the propyl group.

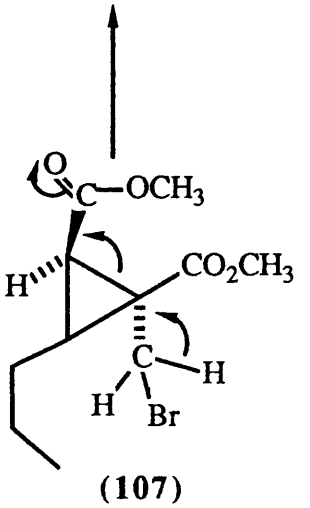
The special features of the spectrum of this compound are at δ 159.91 and 111.62 for the terminal methylene carbons, and at δ 61.43 for the ethereal methoxyl carbon.

The formation of the above alkene , could be via the highly strained cyclopropane **107** in the presence of a nucleophile e.g. the generated $Br^-$ Scheme **19**.




**IB = Base**

**(114)**




**(107)**

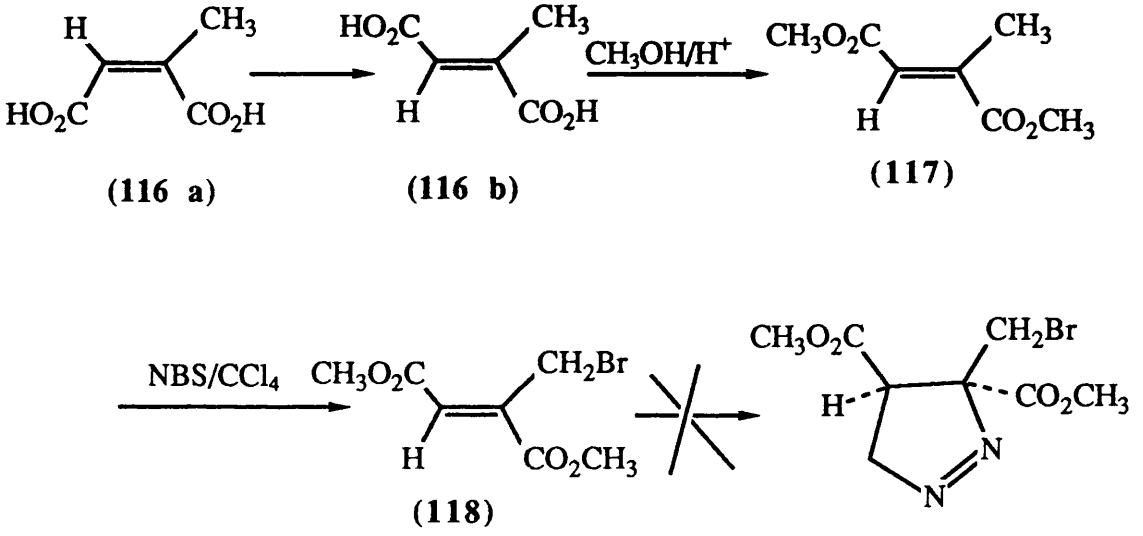
**Scheme 19**

## 2. Preparation of some cyclopropane dicarboxylic acid derivatives via 1-pyrazolines.

The previously described route to cyclopropanes related to the proposed biosynthetic intermediate **79** suffered from the difficulty found in separating the desired compound **107** from the mixture of the byproducts **114** and **115** which were present in larger quantities. It was therefore desirable to find another route which would afford cyclopropanes cleanly, and it was thought that this might be achieved by reaction of diazoalkanes [72] with appropriate alkenes to give pyrazolines, which should undergo loss of nitrogen $N_2$ to produce the corresponding isomeric cyclopropanes.

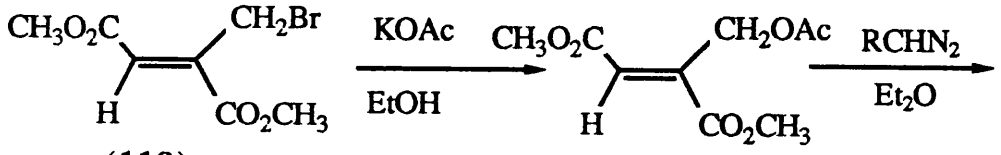
The first alkene required was dimethyl bromomesaconate **118**. This was readily prepared (Scheme **20**) by treating citraconic acid **116a** with hot dilute $HNO_3$ to give its stereoisomer mesaconic acid **116b**, which was esterified by treating with methanolic HCl and then brominated with N-bromosuccinimide to give dimethyl bromomesaconate **118** as a colourless oil.

Model studies of the diazoalkane addition step have been carried out using diazomethane. This was generated from nitrosan by a standard method [73] and was then added as an ethereal solution to dimethyl bromomesaconate **118** in dry ether at 0 $^0$C. TLC showed that the product was a complex mixture which may be due to the presence of the reactive bromomethyl (-$CH_2$Br) grouping. This reaction was attempted a number of times at -78 $^0$C but the desired compound could not be prepared, complex mixtures again being formed (Scheme **20**).

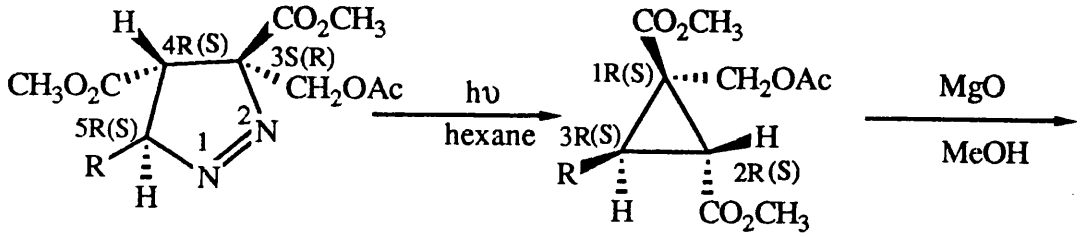



**Scheme 20**

Since the bromomethyl grouping appeared to be too reactive under the conditions of diazoalkane addition, the bromomethyl alkene **118** was converted by treatment with KOAc/EtOH to the corresponding acetoxy derivative **119**. This gave the expected microanalytical and mass spectral data including the molecular ion (m/z 216). The $^1H$ nmr gave sharp singlets at δ 6.78 and 5.05 indicating the presence of the vinyl proton and the oxymethylene group (i.e. $-CH_2O-$) respectively, together with appropriate signals for the carbomethoxyl groupings at δ 3.69, 3.66 and the acetate methyl group at δ 1.89. In the IR, it exhibited a broad C=O bands at ca. 1735 $cm^{-1}$ together with a medium band at 1655 $cm^{-1}$ due to the expected conjugated double bond.




(118) (119)

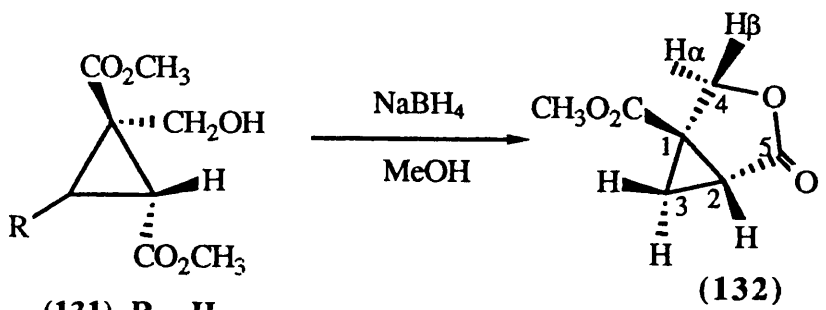



(120) R=H

(128) R=n-Pr

(121) R=H

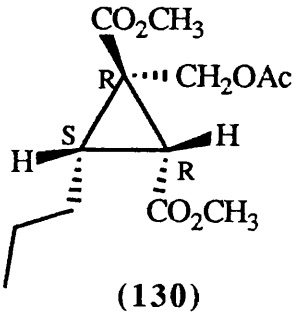
(129) R=n-Pr



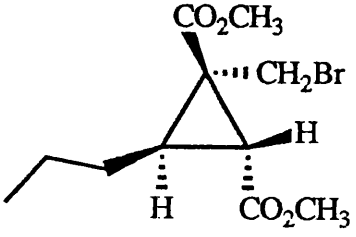

(131) R= H

(132)

## Scheme 21.1




(130)




(107 c)

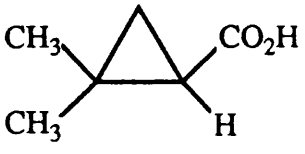
The acetoxy derivative **119** was treated with diazomethane to give an oily product which was chromatographed, using a silica gel column and TLC to remove minor impurities and afford the desired *trans* -3,4-dicarbomethoxy-3-acetoxymethyl-1-pyrazoline **120** in good yield (Scheme **21.1**). This showed the expected molecular ion at m/z 258 corresponding to $C_{10}H_{14}N_2O_6$ and an abundant ion at m/z 199 corresponding to loss of $N_2$ and -$OCH_3$. It was evident that the product was a 1-pyrazoline rather than a 2-pyrazoline from the presence in the IR of a peak at 1438 $cm^{-1}$ corresponding to the azo (-N=N-) grouping together with the absence of -NH- absorption. The structure was confirmed by the presence in the $^1H$ nmr of features corresponding to the $CH_2$ group (C-5) $\alpha$ to the azo grouping. Thus a 2H multiplet centred at $\delta$ 4.97 and a 1H double doublet at $\delta$ 3.55, 3.50 form a typical ABX system corresponding to the C-5 hydrogen atoms and the methine hydrogen at C-4. Also C-5 appeared in the $^{13}C$ nmr spectrum as a signal at $\delta$ 80.84. This corresponded to a methylene carbon from the DEPT spectrum and the chemical shift is appropriate for location adjacent to the diazo system. The -$CH_2O$- grouping gave rise to an AB quartet at $\delta$ 4.76 and 4.70 in the $^1H$ spectrum and to a methylene carbon resonance at $\delta$ 61.95. Appropriate resonances appeared corresponding to the carbomethoxyl and acetoxyl groupings. (Tables **2.1** and **2.2**).

Attempted preparation of the cyclopropane **121** by treatment of **120** with ceric ammonium nitrate [74] in acetone at 0 °C gave, according to TLC, unreacted starting material. When the reaction was allowed to proceed for 2 days, a complex mixture (TLC) was

obtained whose $^1H$ nmr spectrum included signals corresponding to alkenes. It is well known that decomposition (photolysis) of pyrazolines can give rise to alkenes [74-76] as well as cyclopropanes. However, when the pyrazoline **120** was subjected to photolysis in hexane at room temperature, a clean loss of $N_2$ from **120** occurred to give after purification with flash chromatography the desired *trans* 1,2-dicarbomethoxy-1-acetoxymethylcyclopropane **121** as a colourless oil (88% yield) which gave microanalytical data corresponding to $C_{10}H_{14}O_6$ and an appropriate mass spectrum, with molecular ion at m/z 230, abundant ions at m/z 157, 170 due to loss of $-CH_2OAc$ and acetic acid respectively and an ion at m/z 171 due to the loss of a carbomethoxyl group.

Elimination of nitrogen from **120** could have given rise to alkenes as well as cyclopropanes. However the absence of vinyl hydrogen or carbon atoms in the product was evident from the IR and NMR spectra. The ester carbonyl groups in this compound appear as a broad band at ca.1735 $cm^{-1}$ in the IR, very little different from the value for the ester groups of the unsaturated ester **119**. However unlike the latter compound the photolysis product showed no double bond IR absorption in the 1700-1600 $cm^{-1}$ region and no resonances in the NMR due to vinyl hydrogens or carbons. The $^1H$ nmr spectrum also showed that a cyclopropane ring had been formed with the ring methylene group hydrogens ($\delta_A$ 1.60, $\delta_B$ 1.54) and the ring methine hydrogen ($\delta_X$ 2.48) $\alpha$ to a carbomethoxyl group together forming an ABX system. Analysis of this shows that the geminal coupling $J_{AB}$ is 4.64 Hz while $J_{Ax}$ and $J_{BX}$ are 8.58 and 6.84 respectively. The small size of the

geminal coupling is normal for cyclopropanes (an average value has been quoted as 4.51 Hz) [77]. From models, the dihedral angles between *cis*-vicinal protons is $0^0$ while that between *trans*-vicinal protons is ca. $130^0$ and using the Karplus equation the *J cis* would be predicted to be larger than *J trans* i.e. 8.58 and 6.84 Hz respectively. These assignments are in accord with these assigned for the cyclopropane acid **122** [78] $J_{gem}$ = 4.3 Hz, $J_{cis}$ = 8.0 Hz and $J_{trans}$ = 5.6 Hz. The acetoxymethyl group remained intact the methylene hydrogens appearing in the $^1H$ nmr as an AB quartet at δ 4.75 and 4.14 with no sign of long range coupling to the ring hydrogens. $^{13}C$ nmr spectra (PND and DEPT) showed the presence of the ring atoms as a methylene carbon at δ 19.76, methine carbon at δ 25.99 and quaternary carbon at δ 30.34, while the oxymethylene carbon appeared at δ 61.78. Expected signals in $^1H$ and $^{13}C$ nmr spectra for acetoxyl and carbomethoxyl groups were also present. (Tables **3.1** and **3.2**).

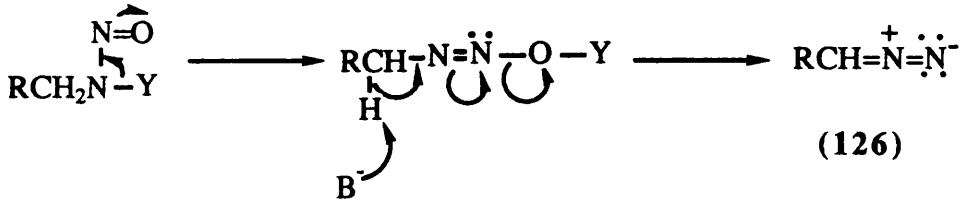



**(122)**

Having established that the addition of diazomethane to **119** gave the pyrazoline **120**, efforts were now directed towards the comparable addition of diazobutane **126**. This can be generated (Scheme **22.1**) from the nitroso compound N-[(N-nitrosobutylamino)methyl]carbamate **125**, which in turn was synthesised using a standard procedure as shown in Scheme **22.2**. Isopropyl carbamate **123** was prepared from isopropanol, NaCNO

and $CF_3CO_2H$. This was treated with the HCl salt of butylamine and $CH_2O$ in EtOH under neutral conditions to give **124** which was treated with nitrous acid to afford the desired crystalline nitroso compound **125**, m.p. 52-54 $^0$C. This was fully characterised and the IR, $^1$H and $^{13}$C nmr spectra were identical to those published in the literature. [79]

When too much acid was used in the preparation of the butylamine hydrochloride, a substantial quantity of a byproduct identified as **127** was obtained as a white crystalline solid m.p.150-152 $^0$C having molecular formula $C_9H_{18}N_2O_4$ on the basis of microanalysis and mass spectroscopy. This was deduced to have structure **127** from spectroscopic evidence including mass spectral cracking pattern. In the IR, it exhibited strong (-CONH-) bands at 1690, 1530 cm$^{-1}$ and -NH- bands at 3325 cm$^{-1}$. It was evident that there were two -NH- groupings from a 2H multiplet in the $^1$H nmr spectrum at $\delta$ 5.78 and that there were two -CHO- groupings from the presence of appropriate signals. A two proton triplet at $\delta$ 4.45 (*J* 6 Hz) was assigned to the (-$NCH_2N$-) and the remaining signals to the methyl groups of the isopropoxycarbonyl groupings. The $^{13}$C nmr spectrum also confirmed the above symmetrical structure by showing a signal for the methylene carbon at $\delta$ 47.80 together with signals for the isopropoxycarbonyl carbons as expected at $\delta$ 156 (-CONH), 68.49 (-CH-) and $\delta$ 22.03 (-$CH_3$). Synthesis of this product was reported and its preparation was achieved under similar conditions as described above. [80]

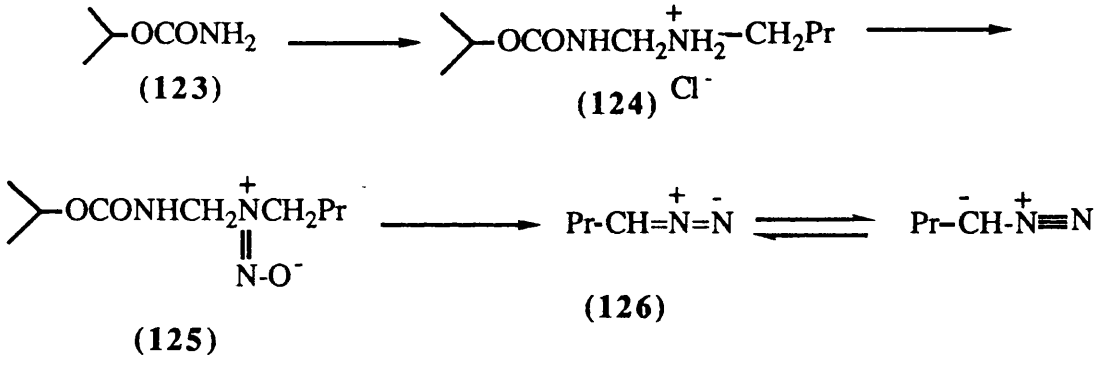


(125) R = n-Pr

Y = $CH_2NHCO_2CH(CH_3)_2$

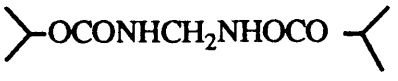
(126)

**Scheme 22.1**



(123) (124) $Cl^-$ (125) (126)

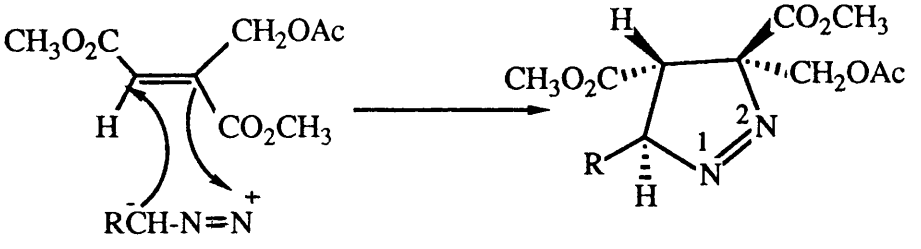
**Scheme 22.2**



(127)

The addition of diazobutane **126** to the unsaturated ester **119** appeared to take a parallel course to the addition of diazomethane (Scheme **22.3**) and afforded a colourless oil in 91% yield. That thus was the desired *trans*-3,4-dicarbomethoxy-3-

acetoxymethyl-5-propyl-1-pyrazoline **128** was confirmed by mass spectroscopy which showed a molecular ion at m/z 300 as expected for $C_{13}H_{20}N_2O_6$, by the presence in the IR of a band at 1455 $cm^{-1}$ corresponding to the azo (-N=N-) grouping and by NMR spectroscopy. Thus the ring hydrogen atom attached to C-5 *i.e.* α to the azo grouping appeared at δ 4.99 as a multiplet owing to coupling with two vicinal hydrogens in the propyl group as well as with the other ring hydrogen (H-4) which appeared as a doublet at δ 3.14. C-5 appeared in the $^{13}C$ nmr spectrum as as a signal at δ 93.36. This corresponds to a methine carbon as shown by the DEPT spectrum and the chemical shift is appropriate for its situation α to the diazo system. The presence of the propyl side chain was shown by a 4H multiplet at δ 1.65 and methylene carbon resonances at δ 35.13 and 19.64 together with a 3H triplet at δ 0.98 and a methyl carbon resonance at δ 13.89. The acetoxymethyl group and methoxycarbonyl groups gave rise to signals similar to those found for those of the diazomethane adduct **120.** (Tables **2.1** and **2.2**).




**(128)** R = n-Pr

**Scheme 22.3**

With regard to the stereochemistry of the adduct **128**, since the methine proton (H-4) α to a carbomethoxyl group appears as a sharp doublet (*J* 8.2 Hz) and there is no evidence of doubling of the carbon NMR signals it is evident that the diazobutane adduct is a single (racemic) substance. If it is presumed that the relative stereochemistry is determined by addition occurring so that there is minimum steric interaction of the propyl group in the transition state with other groupings, then the adduct will be the SRR/RSS racemic pair. Confirmation of this from the size of the coupling between the methine proton (H-4) α to a carbomethoxyl group and the adjacent ring proton (H-5) geminal to the propyl group is not possible since examination of the spectrum of the corresponding diazomethane adduct **120** shows that the proton α to a carbomethoxyl group is coupled almost equally to each of the adjacent ring protons.

In view of the successful synthesis of the cyclopropane **121** the propylpyrazoline **128** was subjected to photolysis in hexane at room temperature resulting in quantitative elimination of nitrogen. (Scheme **21.1**). The molecular formula of the product was shown to be $C_{13}H_{20}O_6$ by microanalysis and mass spectroscopy which gave a molecular ion at m/z 272 together with abundant ions including m/z 198 (loss of methyl acetate or its equivalent) and m/z 139 (loss of $-CO_2CH_3$ from the ion m/z 198). The product was shown to be the desired *trans*-1,2-dicarbomethoxy-1-acetoxymethyl-3-propylcyclopropane **129** rather than an isomeric alkene by the absence of alkene carbon or proton resonances and by presence of features due to the cyclopropyl hydrogens and carbons in the NMR. The ring methine

hydrogen atom i.e. attached to C-2 and α to a carbomethoxyl grouping appeared at δ 2.54 as a doublet (*J* 7 Hz) owing to coupling with the other ring hydrogen atom i.e. attached to C-3 and α to the propyl group. The signal for this second methine hydrogen was upfield at ca. δ 1.29 together with 4H multiplet due to the methylene hydrogens of the propyl group. $^{13}C$ nmr spectra (PND & DEPT) showed the ring atoms as methine carbons (C-2 and C-3) at δ 32.86 and 30.05 and a quaternary carbon at δ 36.71 (C-1). Other features in the NMR were as expected and included an AB quartet at δ 4.91 and 4.09 for the oxymethylene protons and resonances for the ester carbonyl groups at δ 171.27, 170.26 and 170.07 (Tables **3.1** and **3.2**). In the infrared spectrum, this compound showed bands for carbonyl group absorption at ca. 1740 $cm^{-1}$ but no double bond absorption in the 1700-1600 $cm^{-1}$ region.

As with the diazobutane adduct **128**, NMR spectra indicate that the propylcyclopropane **129** is a single (racemic) substance. Thus the ring proton (H-2) α to the carbomethoxyl group appears as a sharp doublet (*J* 7 Hz) and there is no doubling of the methoxyl proton signals or of any of the carbon resonances

The foregoing $^{1}H$ nmr spectral analysis of the three cyclopropane derivatives has established that in the acetoxymethylcyclopropane **121** the ring proton H-2 α to the carbomethoxyl grouping shows coupling constants to H-3β (H *cis*) and H-3α (H *trans*) of 8.58 and 6.84 Hz respectively. Since H-2 in the propylcyclopropane **129** and the proton at H-3 have a coupling constant of 7.0 Hz the propyl group at C-3 is evidently *trans* to the carbomethoxyl group at C-2 as predicted from consideration of the mode of formation of the propylpyrazoline **128** and

propylcyclopropane **129**. The thermal decomposition of pyrazolines to cyclopropanes has been reported to occur in a non-stereospecific manner [81] whereas photolytic decomposition of 1-pyrazolines occurred in a stereospecific manner. [82-86] In keeping with this, loss of nitrogen from **128** upon photolysis occurs with retention of configuration to give the enantiomeric pair of propylcyclopropanes having the relative stereochemistry as in **129** i.e. RRR or SSS. On the other hand, thermal decomposition of **128** in xylene at 160 $^0$C produced an oily product which gave after distillation and TLC material chromatographically identical to the above cyclopropane **129** in 50% yield. This was mainly the previously prepared RRR/SSS diastereoisomers **129** but possibly also contained some of the diastereoisomeric racemate **130** *i.e.* RRS/SSR.

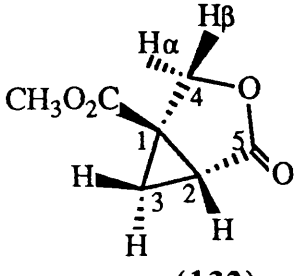
The remaining stages required to prepare the proposed intermediate **79** via the bromomethylcyclopropane *e.g.* **107c**, were selective hydrolysis of the acetate **129** and then conversion of the resulting alcohol to the corresponding bromo compound (*e.g.* with triphenyl phosphine and $CBr_4$) followed by reduction to the radical **79** with $Bu_3SnH$. The relatively accessible cyclopropane **121** was used to investigate the hydrolysis step.

A preliminary attempt was made to selectively hydrolyse the acetoxyl group using $NH_3$ in methanol at -15 $^0$C a reagent used in sugar chemistry [124b], but a complex mixture resulted which could not be separated by TLC. Although a clean reaction occurred when the acetoxy compound **121** was treated with $NaBH_4$ in MeOH, after acidic work up, the product proved to be the lactone **132**. This exhibited in the IR spectrum strong bands at 1790 and 1742 cm$^{-1}$

characteristic for the lactone and the saturated ester carbonyl (C=O) absorptions respectively. High resolution mass spectroscopy established the molecular formula as $C_7H_8O_4$ and gave a recognisable cracking pattern. In the $^1H$ nmr and $^{13}C$ nmr spectra, the formation of the lactone was reflected in the absence of the acetate methyl and carbonyl resonances (i.e. no 3H multiplet at δ 2.00), and presence of only one methoxyl resonance (δ 3.76). The lactone oxymethylene grouping (-$CH_2OCO$-) gave rise to a methylene carbon resonance at δ 68.34 and an AB quartet in which fine coupling of both H-4 β (*J* 0.95 Hz) and H-4 α (*J* 0.9 Hz) was apparent. A complete analysis is shown in the following Table [see below].

Examination of a model shows that for H-2 a large *cis* vicinal coupling to H-3 β (dihedral angle $0^0$) and a smaller *trans* vicinal coupling to H-3α (dihedral angle ca. $130^0$) would be expected along with long range coupling to H-4α, and that H-3β is well situated for long range (W type) coupling to both H-4α and H-4β while H-3α is not to either. $J_{gem}$ for H-3α and H-3β is the relatively small value (4.45 Hz) normal for cyclopropyl hydrogens as discussed earlier.

Facile lactonisation confirms that the -$CH_2OAc$ group is *cis* to a carbomethoxyl group *i.e.* that the carbomethoxyl groups are *trans* as expected from *cis* addition to a *trans* dienoic ester.




**(132)**

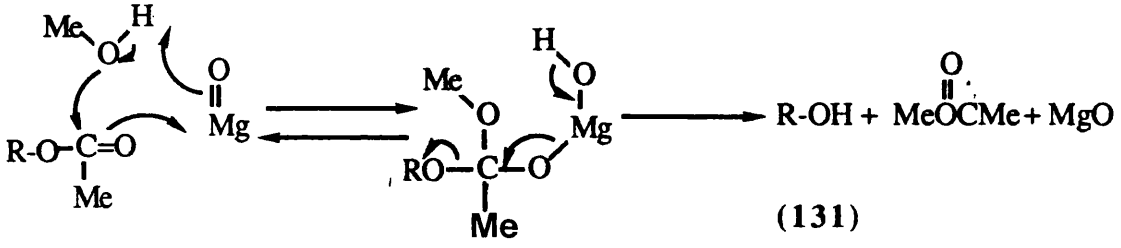
$^1$H nmr data for the lactone **132**: (represented as the enantiomer **132**).

| δ | Assignment | Coupling constant (Hz) |
|---|---|---|
| 4.73 | H-4 β | $J_{4\beta,4\alpha}$ = 9.6; $J_{4\beta,3\beta}$ = 0.95. |
| 4.28 | H-4 α | $J_{4\alpha,4\beta}$ = 9.6; $J_{4\alpha,2}$ = 0.9; $J_{4\alpha,3\beta}$ = 0.3. |
| 2.57 | H-2 | $J_{2,3\alpha}$ = 4.45; $J_{2,3\beta}$ = 9.56; $J_{2,4\alpha}$ = 0.9. |
| 2.05 | H-3 β | $J_{3\beta,2}$ = 9.56; $J_{3\beta,3\alpha}$ = 4.45; $J_{3\beta,4\beta}$ = 0.95; $J_{3\beta,4\alpha}$ = 0.3. |
| 1.33 | H-3 α | $J_{3\alpha,2}$ = 4.45; $J_{3\alpha,3\beta}$ = 4.45. |

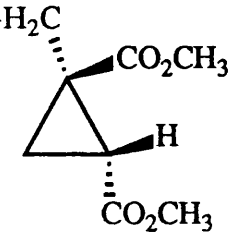
In an attempt to prepare the desired alcohol **131** under milder conditions, the acetate **121** was treated with Analar MgO in MeOH at room temperature. An oily product was obtained which was shown by TLC and $^1$H nmr to be a mixture of fats and several polar components. Similar results were observed when the propylcyclopropane **129** was treated under the same conditions. This is probably due to some contaminants in the Analar MgO used.

When the acetate **121** was treated with special MgO [Merck 5866] (Scheme **21.2**) in MeOH [87, 88] at room temperature for 48

hrs complete deacetylation occurred ($^1$H and $^{13}$C nmr) to give an oily product consisting of two components (analytical TLC). Attempts to separate this mixture by preparative TLC on silica resulted in recovery only of lactone. This mixture consisted mainly of the desired alcohol **131** together with ca. 30% of the lactone **132**, as indicated by TLC and by IR spectroscopy, the properties of the alcohol were deduced from the spectra of the mixture. The presence of primary hydroxyl grouping was indicated by an absorption band in the IR at 3620 $cm^{-1}$ and by a multiplet in the $^1$H nmr at $\delta$ 2.35 which underwent a $D_2O$ exchange. The methylene protons of the primary hydroxyl group appeared as a pair of broad doublets which sharpened upon $D_2O$ exchange of the OH group to an AB system ($H_A$ $\delta$ 4.25, $H_B$ $\delta$ 3.75, $J_{AB}$ 12.0 Hz). The carbon resonance of this grouping appeared at $\delta$ 59.83 which is the position expected [89] ca. 2 ppm upfield from the corresponding resonance in the acetate **121.** The high resolution mass spectrum showed the expected molecular ion (m/z 188) together with fragment ions which included at m/z 170 an abundant ion corresponding to loss of water. The presence of two carbomethoxyl groups was evident from appropriate resonances in the $^1$H and $^{13}$C nmr spectra and the cyclopropyl hydrogens gave rise to the characteristic ABX system with $J_{gem}$= 4.3 Hz, $J_{cis}$ vicinal= 8.57 Hz and $J_{trans}$ vicinal= 6.67 Hz, (Tables **4.1** and **4.2**).




(121) R = -$H_2C$

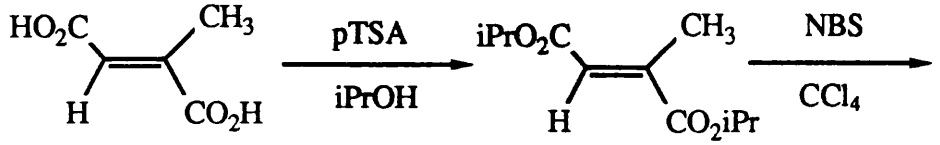



**Scheme 21.2**

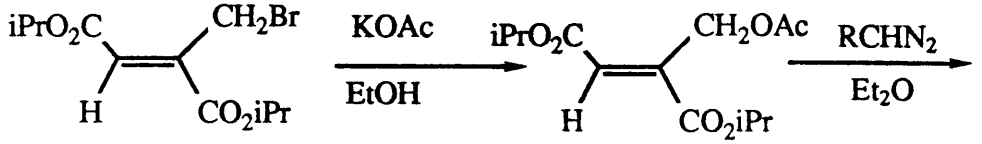
In view of the facile cyclisation which occurred in attempted reactions of the cyclopropane dimethyl esters **121** and **129**, the preparation and properties of the analogous isopropyl esters were studied (Scheme **23.1**), hoping that at least a better yield of the corresponding cyclopropyl methanol would be obtained. For this purpose mesaconic acid **116b** was esterified by treatment with isopropyl alcohol and *p*-toluenesulfonic acid in toluene. The ester gave the expected molecular ion (m/z 214), conjugated ester absorption in the IR (bands at 1720 and 1650 $cm^{-1}$) and $^{1}H$ and $^{13}C$ nmr spectra which included resonances corresponding to two isopropyl ester functions and vinyl hydrogen and vinyl methyl resonances (a fine quartet at δ 6.6 and fine doublet at δ 2.2). Diisopropyl mesaconate **133** prepared in this way was smoothly brominated with N-bromosuccinimide to give diisopropyl bromomesaconate **134** which gave spectra similar to those of the

analogous dimethyl ester **118** with appropriate changes for the different ester function except that the two methoxyl groups in **118** unlike the isopropyl groups in **134** had different chemical shifts. As found for the dimethyl ester, direct treatment with diazomethane at -78 $^0$C gave a complex mixture (TLC, $^1$H nmr) probably due to the presence of the reactive (-$CH_2$Br) grouping. Accordingly the bromo ester **134** was converted into the corresponding acetoxy derivative **135** by treatment with KOAc in EtOH, the product showing the expected features in the IR and NMR. This unsaturated isopropyl ester **135** reacted with diazomethane at room temperature to give the pyrazoline **136** and with diazobutane at -70 $^0$C to give the n-propyl-1-pyrazoline **137**. Mass spectral analysis of these products gave the expected molecular ions and, as for the corresponding methyl esters, only the 1-pyrazolines were formed. The absence of the isomeric imino-2-pyrazolines was evident from $^1$H and $^{13}$C nmr spectra which were similar to the spectra of the methyl esters **120** and **128** discussed earlier (cf. Tables **2.1** and **2.2**). Just as the difference in chemical shift of the methoxyl groups in the pyrazolines **120** or **128** is greater than that of the methoxyl groups in the foregoing alkenes **117**, **118** or **119**, so the two isopropyl group resonances in the pyrazolines **136** or **137** show a wider difference in the NMR than observed with the corresponding alkenes. Both compounds show two 1H septets for the methine proton, and in one or both isopropyl groups, the methyl groups are non equivalent giving rise to three or four doublets respectively. (Table **2.1**).

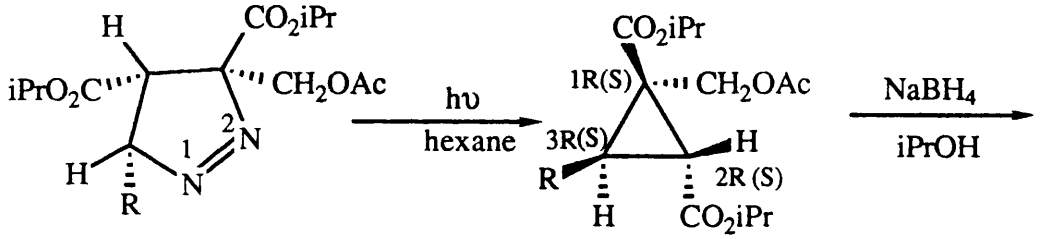
Photolysis of the foregoing pyrazolines **136** and **137** in hexane resulted in quantitative elimination of nitrogen and formation of the desired cyclopropanes **138** and **139** respectively in good yield. Spectroscopic properties of these compounds corresponded closely to those of the corresponding methyl esters except for features due to ester alkyl groups. For example, the isopropyl esters showed fragment ions corresponding to losses of propene. In the $^{1}H$ nmr spectra, the two isopropyl groups gave different signals (Tables **3.1** and **3.2**). In the propylcyclopropane **139**, the methyl groups of both isopropyl groups gave rise to four doublets, this being to non equivalence of methyl groups rather than the sample being a mixture of diastereoisomers. (There was no doubling of signals in the $^{13}C$ nmr spectrum). As discussed earlier for the methyl ester **129**, the isopropyl ester **139** consists of the RRR/SSS enantiomeric pair.




(116b) (133)




(134) (135)

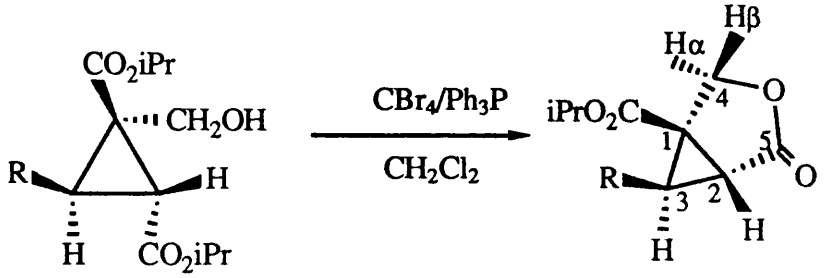



(136) R=H

(137) R=n-Pr

(138) R=H

(139) R=n-Pr

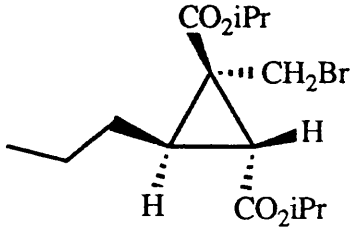



(140) R= H

(141) R= n-Pr

(142) R= Pr

## Scheme 23.1




(143)

**TABLE 2.1 :**

$^1$H nmr data (δ) for some 1-pyrazolines.

| Compound | (120) | (128) | (136) | (137) |
|---|---|---|---|---|
| -$CO_2CH_3$ | 3.83<br>3.63 | 3.86<br>3.64 | - - - - | ---- |
| -$CH_2O$- | 4.76<br>4.70 | 4.69<br>4.58 | 4.71<br>4.66 | 4.64 |
| -$CH_2N$=N-, H-5 | 4.97 | - - - - | 4.95 | - - - - |
| -CHN=N-, H-5 | - - - - | 4.99 | - - - - | 4.95 |
| -$OCOCH_3$ | 1.89 | 1.90 | 1.90 | 1.92 |
| -CH-, H-4 | 3.55<br>3.50 | 3.14 | 3.45 | 3.05 |
| -$(CH_2)_2CH_3$ | - - - - | 1.65 | - - - - | 1.60 |
| -$(CH_2)_2CH_3$ | - - - - | 0.98 | ---- | 1.01 |
| -$CO_2CH(CH_3)_2$ | ---- | - - - - | 5.15<br>4.93 | 5.15<br>4.95 |
| -$CO_2CH(CH_3)_2$ | - - - - | ---- | 1.24, 1.2,<br>1.19 | 1.31, 1.3,<br>1.27,1.2. |

**TABLE 2.2 :**

$^{13}$C nmr data (δ) for some 1-pyrazolines.

| Compound | (120) | (128) | (136) | (137) |
|---|---|---|---|---|
| -$CO_2\underline{C}H_3$ | 53.59<br>52.51 | 53.61<br>52.48 | - - - - | - - - - |
| -$\underline{C}H_2O$- | 61.95 | 61.88 | 62.21 | 62.13 |
| -$\underline{C}H_2N$=N-, (C-5) | 80.84 | ---- | 80.63 | - - - - |
| -$\underline{C}$HN=N-, (C-5) | - - - - | 93.36 | - - - - | 93.01 |
| -OCO$\underline{C}H_3$ | 20.23 | 20.32 | 20.37 | 20.46 |
| -$\underline{C}$H-, (C-4) | 41.35 | 47.24 | 42.2 | 48.17 |
| -($\underline{C}H_2$)$_2$$CH_3$ | ---- | 35.13<br>19.64 | - - - - | 35.21<br>19.67 |
| -($CH_2$)$_2$$\underline{C}H_3$ | ---- | 13.89 | ---- | 13.97 |
| -$CO_2\underline{C}H(CH_3)_2$ | - - - - | ---- | 70.89<br>70.30 | 70.81<br>69.43 |
| -$CO_2CH(\underline{C}H_3)_2$ | - - - - | - - - - | 21.63, 21.51<br>21.41, 21.21 | 21.66<br>21.58, 21.47. |

It was hoped that the alcohols **140** and **141** corresponding to the above cyclopropylmethyl acetates **138** and **139** could be obtained in better yield and be more amenable to further transformation than the methyl esters counterparts. Treatment of the acetate **138** with MgO in methanol at room temperature indeed afforded some of the desired alcohol **140** although spectroscopic data indicated that considerable transesterification had occurred and showed the presence of the methyl ester lactone **132** as a major product. It was also found that when the $CDCl_3$ solution of this mixture was allowed to stand at 37 °C, lactonisation occurred.

Attempted deacetylation of **139** using MgO in isopropanol gave even after 10 days only partial deacetylation and a low yield of product. Efficient deacetylation however was achieved by brief treatment with $NaBH_4$ in refluxing isopropanol followed by careful work up using aqueous $NaH_2PO_4$ (pH=7). Spectroscopic data showed that complete deacetylation had occurred without lactonisation.

The IR spectrum showed strong bands at 3630 and 3220 $cm^{-1}$ characteristic for free and bonded -OH absorptions respectively, together with a band at 1740 $cm^{-1}$ corresponding to the ester carbonyl groups with a weaker absorption at ca. 1700 $cm^{-1}$ perhaps due to H-bonded ester carbonyl group. In the $^1H$ nmr spectrum of **141** run in $CCl_4$ solution (in order to avoid lactonisation), the hydroxyl group was evident as a broad multiplet at δ 9.5 which readily underwent $D_2O$ exchange, and the oxymethylene grouping ($-CH_2O-$) appeared as broad doublets at δ 4.6 and 3.88 which changed after $D_2O$ treatment to an AB quartet

($\delta_A$ 4.30, $\delta_B$ 3.65, $J_{AB}$ 11.0 Hz). A doublet appeared at δ 2.35 corresponding to the ring proton at C-2, which is α to the isopropoxycarbonyl grouping and is coupled to the C-3 proton. Since the coupling constant is 5.7 Hz these hydrogens are evidently *trans* as discussed earlier. In this solvent there was little resolution of the upfield resonances corresponding to the isopropyl and propyl groups and the methine hydrogens of the two isopropoxy groups appeared as a multiplet at δ 5.19. The $^{13}C$ nmr spectrum showed the expected resonances including one at δ 69.15 corresponding to the isopropoxy methine carbons and at δ 62.40 corresponding to the hydroxymethyl carbon (Tables **4.1** and **4.2**).

**TABLE 3.1 :**

$^1$H nmr data (δ) for some acetoxymethylcyclopropane dicarboxylic acid derivatives.

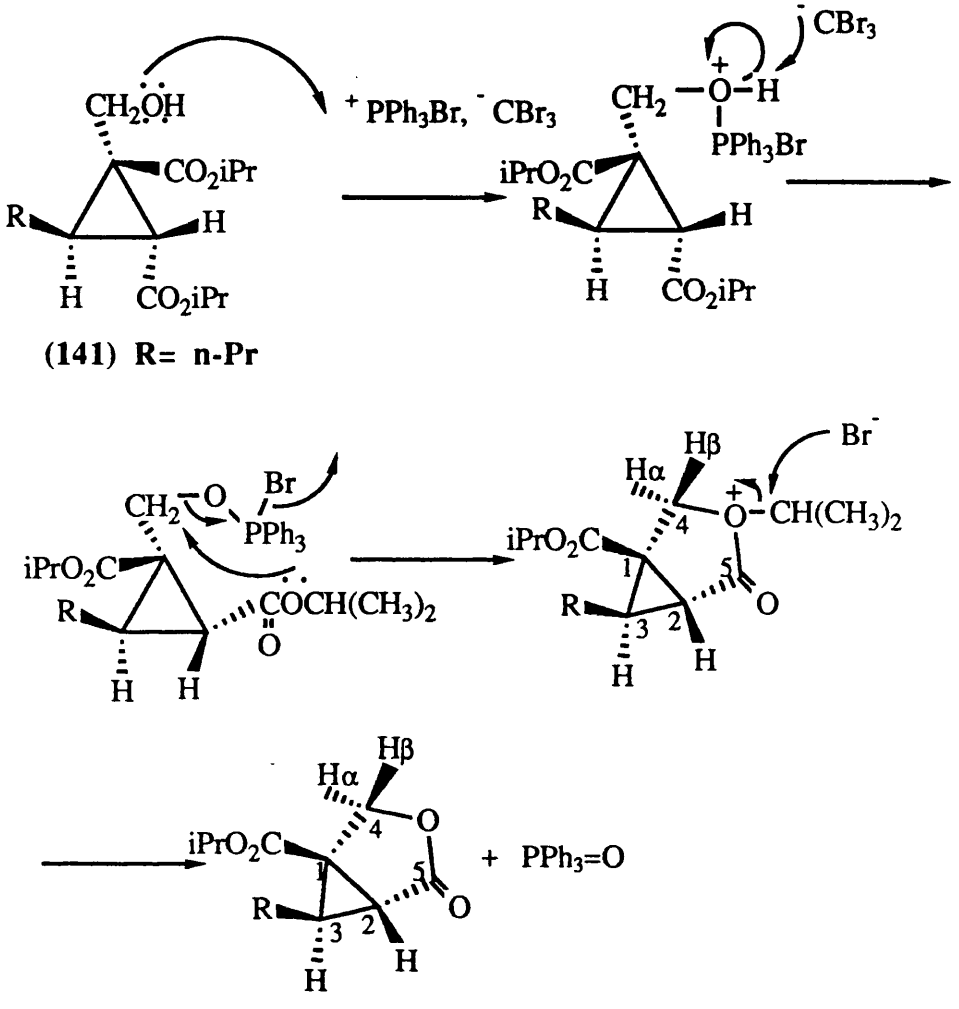
| Compound | (121) | (129) | (138) | (139) |
|---|---|---|---|---|
| -$CO_2CH_3$ | 3.67<br>3.65 | 3.71<br>3.66 | ---- | - - - - |
| -$CH_2O$- | 4.75<br>4.14 | 4.91<br>4.09 | 4.85<br>4.12 | 4.94<br>3.78 |
| -$CH_2$-, (H-3) | 1.57 | - - - - | 1.55 | - - - - |
| -CH-, (H-3) | - - - - | 1.29 | - - - - | 1.25 |
| -$OCOCH_3$ | 1.95 | 1.95 | 1.97 | 1.92 |
| -CH-, (H-2) | 2.48 | 2.54 | 2.45 | 2.48 |
| -$(CH_2)_2CH_3$ | ---- | 1.29 | - - - - | 1.25 |
| -$(CH_2)_2CH_3$ | ---- | 0.85 | - - - - | 0.90 |
| -$CO_2CH(CH_3)_2$ | - - - - | - - - - | 4.99 | 5.00 |
| -$CO_2CH(CH_3)_2$ | ---- | ---- | 1.25, 1.22, 1.20. | 1.215, 1.204, 1.202, 1.181. |

**TABLE 3.2 :**

$^{13}C$ nmr data (δ) for some acetoxymethylcyclopropane dicarboxylic acid derivatives.

| Compound | (121) | (129) | (138) | (139) |
|---|---|---|---|---|
| $-CO_2\underline{C}H_3$ | 52.62<br>52.20 | 52.54<br>52.14 | - - - - | - - - - |
| $-\underline{C}H_2O-$ | 61.78 | 63.10 | 61.82 | 63.03 |
| $-\underline{C}H_2-$, (C-3) | 19.76 | - - - - | 19.60 | ---- |
| $-\underline{C}H-$, (C-3) | - - - - | 32.86 | ---- | 30.33 |
| $-OCO\underline{C}H_3$ | 20.55 | 20.73 | 20.67 | 20.64 |
| $-\underline{C}H-$, (C-2) | 25.99 | 30.05 | 26.35 | 32.33 |
| $-(\underline{C}H_2)_2CH_3$ | - - - - | 28.09<br>21.92 | - - - - | 29.36<br>22.02 |
| $-(CH_2)_2\underline{C}H_3$ | - - - - | 13.45 | - - - - | 13.91 |
| $-CO_2\underline{C}H(CH_3)_2$ | - - - - | - - - - | 69.19<br>68.75 | 69.06<br>68.37 |
| $-CO_2CH(\underline{C}H_3)_2$ | - - - - | - - - - | 21.66<br>21.51 | 21.89, 21.68<br>21.60, 21.43. |

It was now required to convert the above alcohol **141** to a halo derivative and experience with the corresponding dimethyl ester alcohol **131** indicated that mild conditions would be required to avoid lactonisation. However when the alcohol **141** was treated at low temperatures either with thionyl chloride in pyridine [90, 91] or with carbon tetrabromide and triphenylphosphine [92-97] the product in both cases was the new lactone **142** (Scheme **23.2**), as shown by carbonyl absorption in the IR at 1780 and 1720 $cm^{-1}$, and only one isopropyl ester function being apparent in the $^{1}H$ and $^{13}C$ nmr. Comparison of the $^{1}H$ nmr of this propyl substituted cyclopropyl lactone **142** with the unsubstituted cyclopropyl lactone **132** previously discussed shows interesting changes due to the presence of the propyl group. Although the signal for H-3 is obscured by the isopropyl group resonances, H-2 appears as a doublet $J$ =4.31 Hz indicating that H-3 is the *trans* vicinal proton i.e. α. The oxymethylene hydrogens appears as an AB quartet in which H-4β gives a sharp doublet [cf. **132** where long range coupling $J$ =0.95 Hz, with H-3β was evident] and H-4α a slightly broadened doublet due to long range coupling with H-2 [but with $J$ =0.1 Hz which is much less than observed in **132** where $J_{2,\,4\alpha}$ =0.9 Hz].




**(141) R= n-Pr**

**(142) R = n-Pr**

**Scheme 23.2**

**TABLE 4.1 :**

$^1$H nmr data (δ) for some hydroxymethylcyclopropane dicarboxylic acid derivatives.

| Compound | (132) | (131) | (142) | (141) |
|---|---|---|---|---|
| $-CO_2CH_3$ | 3.76 | 3.91<br>3.89 | ---- | - - - - |
| $-CH_2O-$ | 4.73<br>4.28 | 4.25<br>3.75 | 4.46<br>4.16 | 4.30<br>3.65 |
| $-CH_2-$, (H-3) | 2.05<br>1.33 | 1.70 | - - - - | - - - - |
| -CH-, (H-3) | - - - - | - - - - | 1.8-1.3 | 1.7-1.35 |
| -CH-, (H-2) | 2.57 | 2.98 | 2.26 | 2.35 |
| $-(CH_2)_2CH_3$ | ---- | - - - - | 1.8-1.3 | 1.7-1.35 |
| $-(CH_2)_2CH_3$ | ---- | - - - - | 0.9 | 0.90 |
| $-CO_2CH(CH_3)_2$ | - - - - | - - - - | 5.1 | 5.1 |
| $-CO_2CH(CH_3)_2$ | ---- | ---- | 1.28-1.1 | 1.27-1.2 |

**TABLE 4.2 :**

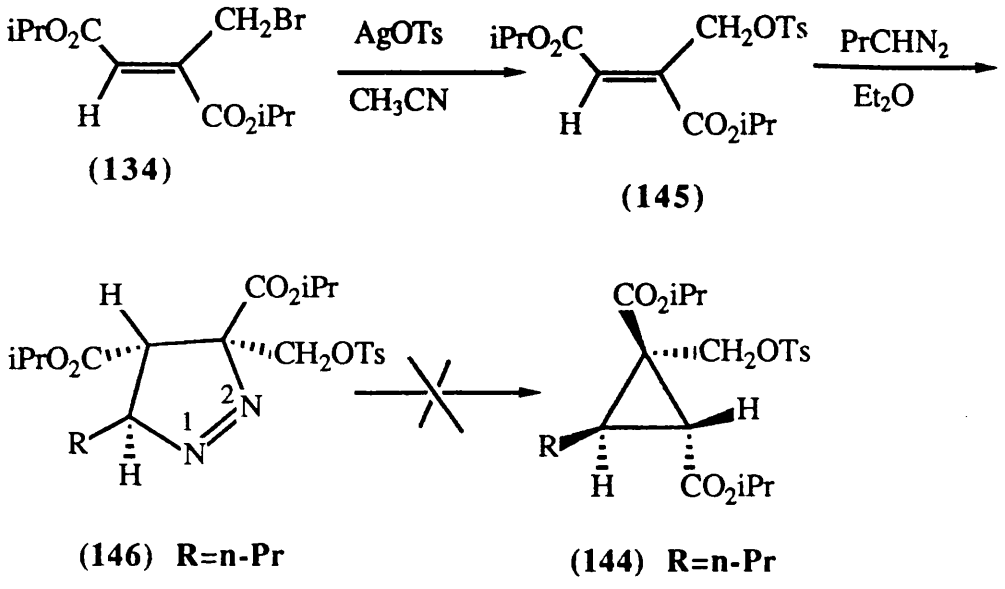
$^{13}C$ nmr data (δ) for some hydroxymethylcyclopropane dicarboxylic acid derivatives.

| Compound | (132) | (131) | (142) | (141) |
|---|---|---|---|---|
| $-CO_2\underline{C}H_3$ | 52.69 | 52.58<br>52.39 | - - - - | - - - - |
| $-\underline{C}H_2O-$ | 68.34 | 59.83 | 68.65 | 62.40 |
| $-\underline{C}H_2-$, (C-3) | 19.34 | 19.12 | - - - - | ---- |
| $-\underline{C}H-$, (C-3) | - - - - | - - - - | 30.64 | 30.24 |
| $-\underline{C}H-$, (C-2) | 26.99 | 26.32 | 33.29 | 34.02 |
| $-(\underline{C}H_2)_2CH_3$ | - - - - | - - - - | 27.89<br>22.14 | 29.92<br>22.69 |
| $-(CH_2)_2\underline{C}H_3$ | - - - - | - - - - | 13.57 | 14.25 |
| $-CO_2\underline{C}H(CH_3)_2$ | - - - - | - - - - | 68.4 | 69.15 |
| $-CO_2CH(\underline{C}H_3)_2$ | - - - - | - - - - | 21.84 | 22.42<br>22.34 |

It is quite apparent that a major difficulty in the conversion of the above alcohol **141** to the bromo derivative **143** lay in the ease with which it undergoes cyclisation, because of the *cis* relationship of the hydroxymethyl and a carbomethoxyl group. It was thought possible that the desired bromo compound might be obtainable via the corresponding tosylate **144** by treatment with LiBr or HBr. [98, 99] Since it seemed likely that the alcohol **141** would lactonise rather than esterify if treated with tosyl chloride and pyridine, an attempt was made to prepare this tosylate by cyclopropanation of diisopropyl tosyloxymesaconate **145** (Scheme **24**). This alkene was prepared from the readily available diisopropyl bromomesaconate **134** by treatment with silver tosylate in refluxing acetonitrile. The $^1H$ nmr spectrum of this was similar to that of the bromo compound **134** but showed the features expected of a tosylate grouping (multiplets at δ 7.7, and 7.25 due to aryl protons and a 3H multiplet at δ 2.38 due to the aryl methyl group) with the oxymethylene grouping appearing as a singlet at δ 5.15.

The tosylate **145** reacted smoothly with diazobutane at -70 $^0C$ to give the tosyl n-propyl-1-pyrazoline **146** which had spectroscopic properties comparable to the acetyl n-propylpyrazoline **128**. Since it has been found that tosylates are cleaved by photolysis [100-104] to give alcohols, photolysis of **146** was not investigated since it seemed likely to give the previously obtained hydroxymethylcyclopropane **141**. Since thermal decomposition of the pyrazoline **146** in xylene was found to give a reasonable yield of acetoxymethyl cyclopropane **129** the tosyl pyrazoline **146** was subjected to similar treatment but this

afforded a complex mixture in which the desired cyclopropane **144** could not be detected by TLC or spectroscopically.

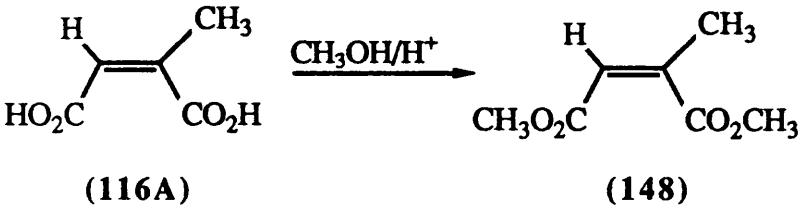



**Scheme 24**

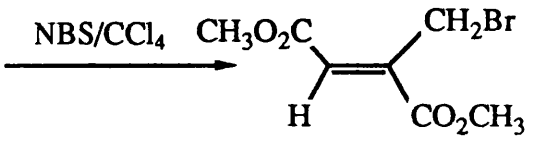
In the proposed reductive rearrangement in the biosynthesis of ethisolide **1**, the relative stereochemistry of the proposed intermediate cyclopropylmethyl radical is a matter of speculation as is the possible importance of such stereochemistry. It was therefore desirable to prepare a cyclopropylmethyl derivative similar to **79** but having the carbomethoxyl *cis* to one another.

The alkenoic ester required for this purpose appeared to be the *Z* isomer **147** of dimethyl bromomesaconate **118**. However attempts to prepare this by bromination of dimethyl citraconate **148** afforded only dimethyl bromomesaconate **118** (Scheme **25**).

(Isomerisation of citraconic acid to mesaconic acid is known to be affected by traces of bromine). [105]




(116A) (148)




(118)

**Scheme 25**

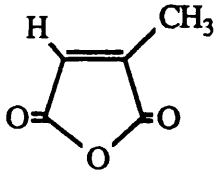
To avoid this isomerisation and any lactonisation in the following reactions, preparation of the bromocitraconic anhydride **149** was attempted by treatment of citraconic anhydride **150a** with $NBS/CCl_4$ but gave according to TLC and $^1H$ nmr analysis a complex mixture. However, **149** was obtained by adding bromine to itaconic anhydride **150b** to give a dibromo compound **151** (Scheme **26**) which underwent loss of HBr by treatment with $Et_3N/Et_2O$. This exhibited in the IR a band at 1642 $cm^{-1}$ due to the conjugated double bond and strong bands at 1840 and 1755 $cm^{-1}$ characteristic for the anhydride carbonyl absorptions. Acetolysis of **149** using the usual method KOAc/EtOH was unsuccessful.

It was also thought that it might be possible to prepare the bromomethylcyclopropane anhydride **152** indirectly via the corresponding tosyl derivative **153** (Scheme **26**). For this reason,

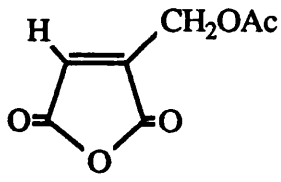
the bromocitraconic anhydride **149** was converted after treatment with silver *p*-toluenesulphonate in refluxing dry acetonitrile to *p*-tolunesulphonyloxycitraconic anhydride **154**. The presence of the tosylate grouping in this was indicated in the $^1H$ nmr by multiplets at δ 7.65, 7.25 and a three proton singlet at δ 2.31. Long range coupling between the protons of the ($-CH_2O-$) grouping (fine doublet at δ 4.80) and the vinyl proton (fine triplet at δ 6.80) was also apparent. This tosylate **154** reacted smoothly with diazobutane to give an oily product, which was initially shown by the $^1H$ nmr spectrum to be the desired pyrazoline **155**. However, after standing, the $^1H$ spectrum of the same sample indicated that decomposition had occurred. TLC also showed the product was a complex mixture and separation of its components using ether/hexane (1:4) was unsuccessful.

The pyrazoline route to the desired bromomethylcyclopropane ester **143** afforded useful model cyclopropane derivatives but was not investigated further.

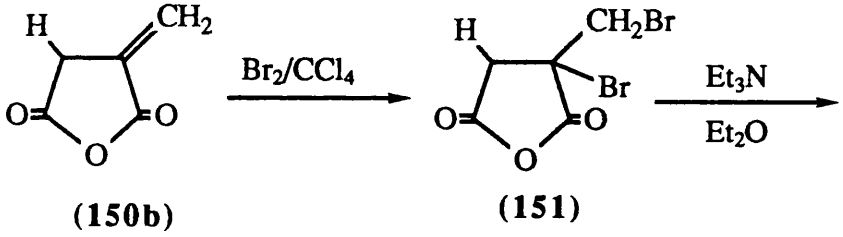
It was thought that the cyclopropylmethyl alcohol **141** having the alcoholic grouping and the isopropoxycarbonyl group on the adjacent carbon *trans* to one another might be more resistant to cyclisation during the $CBr_4/PPh_3$ reaction. Accordingly an attempt was made to generate the corresponding acetate **156** by epimerising the known acetate **139** at C-2. Treatment with LDA in THF and quenching of the anion with aqueous $NaH_2PO_4$ afforded only unreacted acetate **139** (Scheme **27**).



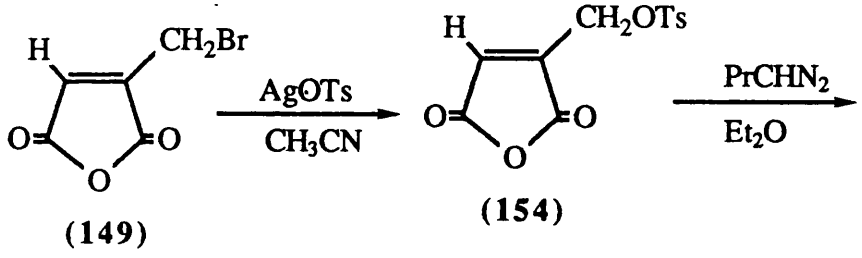
(150a)



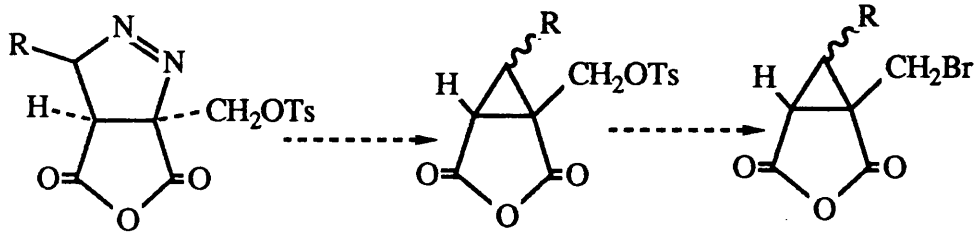
(151)



(150b) (151)

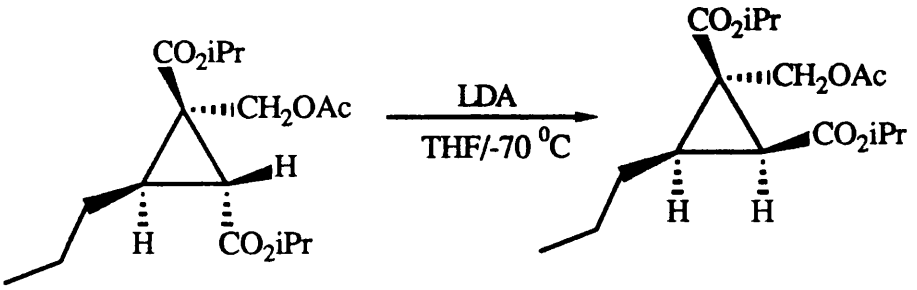


(149) (154)



(155) R= n-Pr (153) (152)

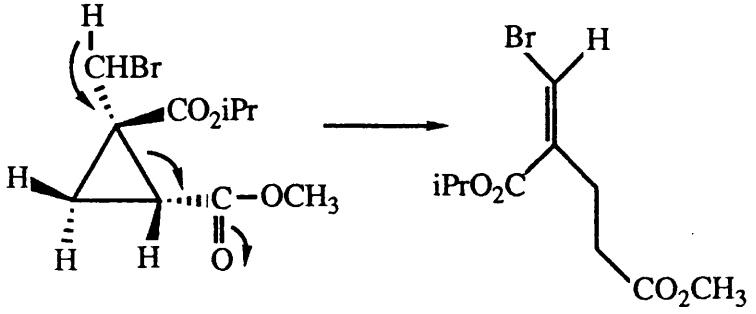
**Scheme 26**




(139) (156)

**Scheme 27**

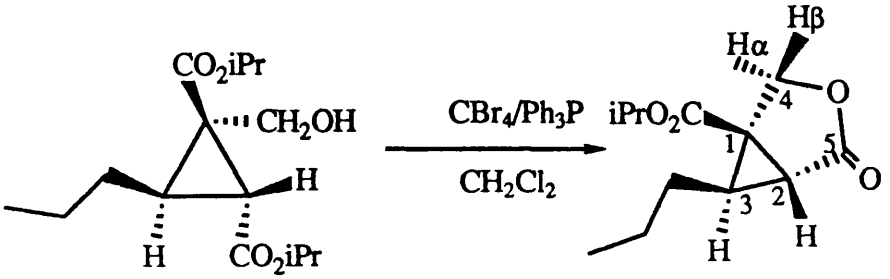
Efforts were also made to prepare the bromo compound **143** using the lactonic products obtained in the previous reactions. It has been reported [106] that treatment of five membered lactones with $BBr_3$ followed by quenching with alcohol will produce a compound with a ($-CH_2Br$) grouping together with a new ester grouping. The lactone **142** was treated with $BBr_3$ in $CH_2Cl_2$ under nitrogen at room temperature for 16 hrs and then quenched with isopropanol in $CH_2Cl_2$. The product was mainly unreacted starting material with a small amount of a new product which could possibly be the vinyl bromo compound **157** in view of the presence in the $^1H$ nmr of a singlet at $\delta$ 7.8 (Scheme **28**).




(157)

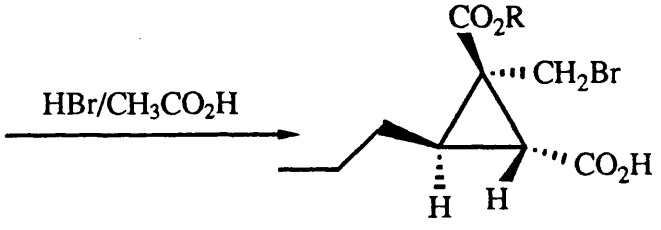
**Scheme 28**

The desired mono isopropyl bromomethylcyclopropane ester **158** was however obtained (Scheme **29**) by treatment of the lactone **142** with HBr in acetic acid. The product also contained some of the corresponding diacid **159**. This was shown by the high resolution mass spectrum in which the most significant fragments are the isomeric ions ($C_9H_{12}BrO_3$) at m/z 248 and 246 due to loss of the (-$OCH(CH_3)_2$) group from the mono ester **158** together with a peak at m/z 185 an abundant ion corresponding to loss of (Br•) from the diacid **159**. The IR spectrum of this mixture showed broad absorptions at 3010 $cm^{-1}$ typical of a carboxylic acid grouping together with strong bands at 1740 and 1680 $cm^{-1}$ corresponding to the free and H- bonded carbonyl absorptions. The $^1H$ nmr of the above mixture also showed that the γ-lactone ring has been opened and the presence of the (-$CH_2Br$) grouping in **158** was evident as an AB quartet (*J* 10.6 Hz) at δ 4.25 and 3.66 and at δ 4.19 and 3.65 (*J* 10.45 Hz) for the diacid **159**. By contrast the signals for the oxymethylene hydrogens in the lactone **142** were closer together and the geminal coupling constant was also smaller (*J* 9.4 Hz). Expected signals in the $^1H$ and $^{13}C$ nmr for the remaining cyclopropane ring, the isopropyl, the carboxylic acid and the propyl functions were also present as described in Tables **5.1** and **5.2**.




(141)

(142)




**(158) R= iPr**

**(159) R= H**

**Scheme 29**

**TABLE 5.1 :**

$^1$H nmr data (δ) for some bromomethylcyclopropane dicarboxylic acid derivatives.

| Compound | (107) | (158) | (159) |
|---|---|---|---|
| -$CO_2CH_3$ | 3.77<br>3.73 | ---- | ---- |
| -$CH_2Br$- | 4.30, 3.75<br>($AB_q$, *J* 10.5 Hz) | 4.25, 3.66<br>($AB_q$, *J* 10.6 Hz) | 4.19,3.65<br>($AB_q$, *J* 10.45 Hz) |
| -CH-, (H-3) | 1.88 | 1.9 | 1.80 |
| -CH-, (H-2) | 2.65 | 2.61 | 2.56 |
| -$(CH_2)_2CH_3$ | 1.29 | 1.50 | 1.50 |
| -$(CH_2)_2CH_3$ | 0.83 | 0.94 | 0.9 |
| -$CO_2CH(CH_3)_2$ | - - - - | 5.10 | ---- |
| -$CO_2CH(CH_3)_2$ | - - - - | 1.32<br>1.30 | ---- |

**TABLE 5.2 :**

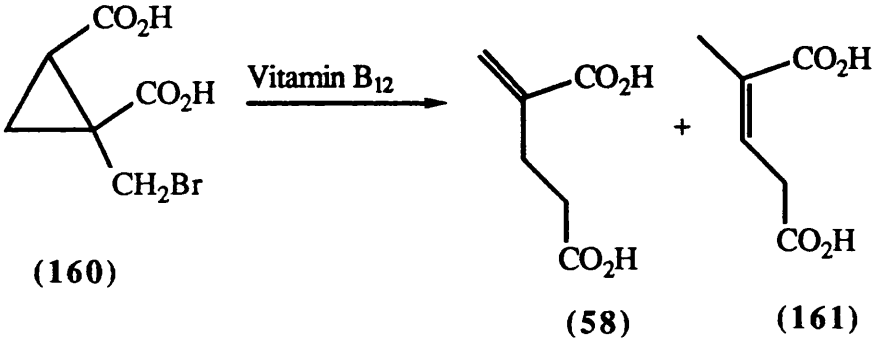
$^{13}C$ nmr data (δ) for some bromomethylcyclopropane dicarboxylic acid derivatives.

| Compound | (107) | (158) | (159) |
|---|---|---|---|
| $-CO_2\underline{C}H_3$ | 52.72<br>52.25 | - - - - | - - - - |
| $-\underline{C}H_2Br-$ | 32.12 | 30.84 | 30.13 |
| -CH-, (C-3) | 31.91 | 32.38 | 31.45 |
| -CH-, (C-2) | 40.21 | 38.37 | 37.58 |
| $-(\underline{C}H_2)_2CH_3$ | 28.771<br>21.98 | 28.44<br>22.07 | 29.83<br>24.51 |
| $-(CH_2)_2\underline{C}H_3$ | 13.65 | 13.46 | 13.46 |
| $-CO_2\underline{C}H(CH_3)_2$ | ---- | - - - - | ---- |
| $-CO_2CH(\underline{C}H_3)_2$ | ---- | 21.81<br>21.77 | ---- |

Regarding the stereochemistry of these molecules, it is quite clear that the $-CH_2Br$ grouping and the carboxylic acid group have the *cis* relationship since the lactone **142** was used in this reaction. Also H-2 in both compounds **158** and **159** appeared in the $^1H$ nmr as doublets at ca. δ 2.6 (*J* 7.0 Hz) indicating that H-3 is the *trans* vicinal proton in both compounds as described previously for **107** and other related cyclopropanes. Having obtained the desired tetrasubstituted bromomethylcyclopropanes **158** and **159**, the preparation of the presumed radical intermediate **79**, in the biosynthesis of the antibiotic ethisolide **1**, should be straight forward by simply esterification of the above compounds under neutral conditions followed by reduction [107-110] with tributyltinhydride.

Alternatively the possible rearrangement of organocobalt derivatives prepared from **158** and **159** could be studied by analogy with the early work on the rearrangement of methylitaconic acid to α-methyleneglutaric acid which involved an organocobalt derivative prepared from α-bromomethylitaconic acid as described in the introduction (Scheme **5**). Powerful evidence for the feasibility of this scheme has in fact just been provided [61] by further studies reported on the methylitaconic acid/α-methyleneglutaric acid system in which some trisubstituted cyclopropyl dicarboxylic acid derivatives (e.g. **160**) were used in a series of experiments involving treatment of these acids and their methyl and tetrahydropyranyl esters with vitamin $B_{12}$. No methylitaconic acid **59** could be detected in any of the reaction mixtures but α-methyleneglutaric acid **58** and

methylglutaconic acid **161** were observed as the reaction products (Scheme **30**).




**(160)** **(58)** **(161)**

**Scheme 30**

It is clear from these results that the tetrasubstituted bromomethylcyclopropanes prepared in this work would be likely to give the corresponding α-methyleneglutaric acid derivatives e.g. **106** via the radical **79** prepared by reduction with tributyltinhydride or using an organocobalt derivative (the cobalt atom of vitamin $B_{12}$). Hence the proposed pathway for ethisolide biosynthesis (Scheme **7**) involving such a rearrangement now seems distinctly feasible.

## CHAPTER 4 : Experimental section.

## GENERAL EXPERIMENTAL .

The cultures studied in this thesis are *Penicillium decumbens* (3903 00T), and *Aspergillus avenaceus* (AGG 4558). The fungi were subcultured on to 2% malt agar slants and thence to agar seed bottles, prior to inoculating Roux surface culture bottles containing culture medium (200 ml) which had previously been sterilised (0.5 hr with steam at 117 °C and 12 p.s.i).

*P. decumbens* and *A. avenaceus* were grown on a culture medium (Czapek-Dox + 0.1% yeast extract) containing glucose (50 g), $NaNO_3$ (2 g), KCl (1 g), $MgSO_4.7H_2O$ (1 g), $K_2HPO_4$ (0.5 g), $FeSO_4.7H_2O$ (0.01 g) and yeast extract (0.1 g) per litre of distilled water. The cultures were allowed to grow undisturbed at 25 °C and 70% relative humidity, artificial illumination being provided by fluorescent tubes for eight hours per day.

Thanks and recognition are due to Mrs. Pearl Tait and her staff of the Mycology Unit who prepared all of the cultures used in this work.

$^1H$ nmr spectra were recorded on a Perkin Elmer R32 90 MHz spectrometer and at 200 MHz on BRUKER AM and WP 200 SY instruments. $^{13}C$ nmr spectra were recorded at 25.15 MHz using a varian XL-100 F.T. spectrometer or at 50.3 MHz on BRUKER AM and WP 200 SY spectrometers. The spectra were determined in $CDCl_3$ solutions, unless otherwise stated. Chemical shifts are expressed in parts per million ($\delta$) downfield from tetramethylsilane as internal reference.

$^2H$ nmr spectra were recorded by Dr D. Rycroft, Mr J. Gall and Mr J. McIver with a BRUKER W. P. 200SY spectrometer. Unless otherwise stated nmr were recorded with $CHCl_3$ as solvent and TMS as internal standard.

Melting points which are uncorrected, were determined on a

Reichert hot-stage apparatus. Microanalysis were performed by Mrs. W. Harkness and her staff.

IR spectra were recorded on a Perkin-Elmer 580 spectrometer on $CHCl_3$ or $CCl_4$ solutions. The peaks of medium to high intensity are reported as $\upsilon_{max}$ in inverse centimetres.

Mass spectra were recorded by Mr. A. Ritchie on a VG upgraded Kratos MS 12 instrument.

Kieselgel $GF_{254}$ (MERCK) or $HF_{254}$ (MERCK) were used for preparative TLC; Kieselgel (MERCK) was used for analytical TLC. Analytical and preparative TLC plates were visualised by ultra-violet light (254 or 350 nm) and by staining with iodine vapour.

Tetrahydrofuran was refluxed with $Cu_2Cl_2$ overnight followed by distillation over KOH pellets. The peroxide possibly present in THF was destroyed by refluxing with sodium metal and benzophenone under nitrogen gas. Methanol or isopropanol were dried by addition of a few grams of magnesium turnings and iodine to 100 ml of alcohol. The mixture was refluxed till the colour of iodine disappeared, then a further 600-800 ml alcohol was added. The mixture was refluxed for 1/2 hr and the alcohol distilled off.

Organic solutions were dried with anhydrous $MgSO_4$ or $Na_2SO_4$ and solvents were removed using a Buchi Rotavapor coupled to a water aspirator. The solvents used for chromatography are expressed in a volume ratio, e.g. ethyl acetate - light petroleum 2 :1.

## ABBREVIATIONS :

The following abbreviations and symbols have been used in this thesis :

| | |
|---|---|
| br | broad |
| d | doublet |
| m | multiplet |
| q | quartet |
| s | singlet |
| sep | septet |
| t | triplet |
| NMR or nmr | nuclear magnetic resonance |
| I.R | Infra-red |
| u.v.. | ultra-violet |
| TLC | Thin Layer Chromatography |
| HPLC | High Performance Liquid Chromatography |
| Hz | Hertz |
| hr | hour |
| hrs | hours |
| min | minute |
| lit. | literature |
| m.p. | melting point |
| b.p. | boiling point |
| m/z | mass to charge ratio |
| med. | medium |
| str. | strong |
| w | weak |
| Ts | 4-toluenesulfonyl ($MeC_6H_4SO_2$) |
| Pr | 1-propyl |
| iPr | 2-propyl |
| Rt | retention time |
| THF | tetrahydrofuran |
| DBP | dibenzoyl peroxide |
| DMF | dimethylformamide |
| DEPT | distortionless enhancement by polarisation transfer |
| DBU | 1,8-diazabicyclo[5.4.0]undec-7-ene |

**Synthesis and feeding of $^2$H-labelled hexanoic acids to *Penicillium implicatum*.** [64]

1. 2,3-$^2H_4$-hexanoic acid **81**.

To Hex-2-ynoic acid **80** (600 mg, 5.35 mmol) in ethanol (25 ml) containing NaOD (1.3 eq) was added $PtO_2/D_2O$ in ethanol and the mixture was treated with $D_2$ gas [produced from adding $D_2O$ to lithium metal]. After the reaction was complete, the mixture was filtered and the filtrate was evaporated under reduced pressure to give 2,3-$^2H_4$-hexanoic acid **81** (400 mg).

$^2$H n.m.r: ($CHCl_3$) δ 2.27 (str), 1.57 (str), 1.26 (str), 0.88 (str).

$^1$H n.m.r: ($CDCl_3$) δ 1.28 (m, 2H, H-4, H-5), 0.87 (t, *J* 2 Hz, 3H, H-6).

2. 3,4-$^2H_2$-hexanoic acid **83**.

To Hex-3-enoic acid **82** (500 mg, 4.38 mmol) in dry and degassed benzene (25 ml) was added tris-(triphenylphosphine)-rhodium chloride (120 mg). The reaction mixture was treated with $D_2$ gas produced from adding $D_2O$ to lithium metal] and left stirring for two days. After the reaction was complete, the solvent was removed and the residue was washed with saturated $NaHCO_3$ to form a slurry. This was filtered, acidified with aqueous HCl and

then thoroughly with ether to give after evaporation under reduced pressure 3,4-$^2H_2$-hexanoic acid **83** (450 mg).

$^2$H n.m.r: ($CHCl_3$) δ 1.31 (str), 1.61 (str), 1.26 (str), 2.31 (w).

$^1$H n.m.r: ($CDCl_3$) δ 2.32 (t, *J* 3.5 Hz, 2H, H-2), 1.28 (m, 1H, H-5), 0.87 (m, 2H, H-6).

**3.** 4,5-$^2H_2$-hexanoic acid **85.**

To hex-4-enoic acid **84** (500 mg, 4.38 mmol) in ethanol (25 ml) containing NaOD (1.3 eq) was added $PtO_2/D_2O$ in ethanol and the mixture was treated with $D_2$ gas [produced from adding $D_2O$ to lithium metal]. After the reaction was complete, the mixture was filtered and the filtrate was evaporated under reduced pressure to give 4,5-$^2H_2$-hexanoic acid **85** (450 mg).

$^2$H n.m.r: ($CHCl_3$) δ 2.27 (w), 1.57 (m), 1.26 (str), 0.83 (str).

**4.** Feeding of 2,3-$^2H_4$-hexanoic acid **81** to surface cultures of *P. implicatum*.

2,3-$^2H_4$-Hexanoic acid **81** (380 mg), converted to its sodium salt in sterile water, was administered in 5 x 12 hrs pulses to two (02) Roux bottles containing seven (07) day old surface cultures of *P. implicatum.* After fourteen (14) days of growth the

cultures were harvested by decanting off the aqueous broth, which was extracted at end pH with EtOAc for 12 hrs. The ethyl acetate was dried over anhydrous $MgSO_4$, filtered and evaporated to give deuterium enriched ethisolide **86** (36 mg) as colourless needles m.p. 121-123 $^0$C from ethanol. [lit.[4] m.p. 122-123 $^0$C]

$^1$H n.m.r: ($CDCl_3$) δ 6.49 (d, *J* 3.0 Hz, 1H, H-9a), 5.78 (d, *J* 3.0 Hz, 1H, H-9b), 5.14 (d, *J* 7.2 Hz, 1H, H-2), 4.70 (m, 1H, H-4), 4.06 (m, 1H, H-3), 1.70 (m, 1H, H-5a), 1.52 (m, 1H, H-5b), 1.04 (t, *J* 6.0 Hz, 3H, H-6).

$^2$H n.m.r: ($CHCl_3$) δ 4.67 (str), 4.007 (str), 3.7(w).

**5.** Feeding of 3,4-$^2H_2$-hexanoic acid **83** to surface cultures of *P. implicatum*.

3,4-$^2H_2$-Hexanoic acid **83** (400 mg), converted to its sodium salt in sterile water, was administered to two (02) Roux bottles containing seven (07) day old surface cultures of *P. implicatum* in 5 X 12 hrs. After fourteen (14) days of growth the cultures were harvested by decanting off the aqueous broth, which was extracted at end pH with EtOAc for 12 hrs. The ethyl acetate was dried over anhydrous $MgSO_4$, filtered and evaporated to give deuterium enriched ethisolide **87** (16 mg) as colourless needles m.p. 120-122.5 $^0$C from ethanol. [lit.[4] m.p. 122-123 $^0$C]

$^2$H n.m.r: ($CHCl_3$) δ 4.78 (w), 4.00 (str), 3.78 (str), 1.25 (w).

6. Feeding of 4,5-$^2H_2$-hexanoic acid **85** to surface cultures of *P. implicatum*.

4,5-$^2H_2$-Hexanoic acid **85** (400 mg), converted to its sodium salt in sterile water, was administered in 5 x 12 hrs pulses to two (02) Roux bottles containing seven (07) day old surface cultures of *P.implicatum.* After fourteen (14) days of growth the cultures were harvested by decanting off the aqueous broth, which was extracted at end $_p$H with EtOAc for 12 hrs. The ethyl acetate solution was dried over anhydrous $MgSO_4$, filtered and the filtrate was evaporated under reduced pressure to give deuterium enriched ethisolide **88** (36 mg) as colourless needles m.p. 122-123 $^0$C from ethanol. [lit.[4] m.p. 122-123 $^0$C]

$^2$H n.m.r: ($CHCl_3$) δ 4.66 (str), 3.99 (str), 1.06 (str).

## Synthesis and feeding of 3,4-$^2H_2$-lauric acid 90 to *A.avenaceus*.

### 1. decanoyl chloride 92.

To thionyl chloride (100 ml), while stirring at room temperature decanoic acid (60 g, 0.34 mol) was added in small portions. After the addition was complete, the reaction mixture was heated to 60 $^0$C for 1 hr and concentrated under reduced pressure to a yellow oil. Distillation of the crude oil yielded decanoyl chloride **92** as a colourless oil (50 g, 75%) b.p. 80 $^0$C/ 0.1 mm.

LR $\upsilon_{max}$ ($CCl_4$) 2940, 2862, 1805, 1720 $cm^{-1}$.

### 2. 1-$^2H_2$-Decanol 93.

To a stirred solution of LAD (5 g, 0.12 mol) in ether (200 ml) was added decanoyl chloride **92** (22.7 g, 0.12 mol) in ether (50 ml) at - 5 $^0$C. The mixture was stirred under reflux for 24 hrs, after which time aqueous $Na_2SO_4$ (25 ml) was added at 0 $^0$C and the reaction mixture was allowed to stand at room temperature for another 24 hrs. The clear ether solution was decanted and the residue was washed with ether (200 ml). The combined ether extracts were dried over anhydrous $MgSO_4$, filtered and the filtrate was evaporated under reduced pressure to give 1-$^2H_2$-Decanol **93** as a colourless oil (18 g, 94 %).

LR $\upsilon_{max}$ ($CCl_4$) 3630 (OH), 2930, 2855 $cm^{-1}$.

$^1$H n.m.r. (90 MHz, $CDCl_3$) δ 1.98 (s, 2H, H-2), 1-1.5 (br s, 14H, H-3 - H-9), 0.9 (br t, 3H, H-10).

**3.** 1-$^2H_2$-Decyl-*p*-nitrobenzenesulfonate **94.**

*p*-Nitrobenzenesulfonyl chloride (50 g, 0.23 mol) in $CH_2Cl_2$ (250 ml) under an atmosphere of nitrogen was added dropwise to a solution of $^2H_2$-Decanol **93** (12 g, 0.07 mol) and $Et_3N$ (25 ml) in $CH_2Cl_2$ (100 ml). The resulting yellow solution was stirred for 12 hrs and then treated with ice-water, 5 % aqueous HCl, aqueous $NaHCO_3$ and brine. The $CH_2Cl_2$ phase was dried over $MgSO_4$, filtered and the filtrate was evaporated under reduced pressure to give the *p*-nitrobenzenesulfonate **94** (23 g, 89%) m.p 51- 53 $^0$C from hexane.

I.R $\upsilon_{max}$ ($CCl_4$) 2920, 2850, 1610 $cm^{-1}$.

$^1$H n.m.r. ($CDCl_3$) δ 8.40 (d, *J* 12.0 Hz, 2H, aromatic H), 8.1 (d, *J* 12.0 Hz, 2H, aromatic H), 1.63 (m, 2H, H-2, i.e. -$CH_2CD_2$-), 1.2-1.27 (m, 14H, -$CH_2$-), 0.85 (br t, 3H, -$CH_3$).

$^{13}$C n.m.r. ( $CDCl_3$) δ 129.13, 124.4 (aromatic CH), 31.78, 29.38, 29.29, 29.18, 28.84, 28.57, 25.17, 22.60 ($CH_2$), 14.05 ($CH_3$).

M.S (m/z) 345 ($M^+$), 159 ( $M^+$ - $C_6H_4NO_4S$, 0.2 %), 143 ($M^+$-$C_6H_4NO_5S$, 11.6 %), 142 ($M^+$- $C_6H_5NO_5S$, 100 %).

Mass measurement: Found $M^+$ 345.1576, $C_{16}H_{23}D_2NO_5S$ requires 345.4283.

4. Dimethyl 1-$^2H_2$-Decylmalonate **95** .

To sodium hydride (60% dispersion) (0.9 g, 22.7 mmol) in dry THF (50 ml), under an atmosphere of nitrogen, was added dimethyl malonate (3 g, 22.7 mmol) at 0-5 $^0$C and the mixture was then stirred at room temperature for 45 min. To the resulting solution 1-$^2H_2$-Decyl-*p*-nitrobenzenesulfonate **94** (7.6 g, 22.3 mmol) in dry THF (50 ml) was added during 30 min. with cooling to 20 $^0$C. After allowing the reaction mixture to reflux for 48 hrs, the solvent was removed by evaporation under reduced pressure. The residue was extracted with ether (250 ml), washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered and the filtrate was evaporated to give a brown oil (8 g). This was treated with n-heptane (2x25 ml) to separate the excess of dimethyl malonate as an insoluble oil. The heptane solution was evaporated under reduced pressure to give the dimethyl ester **95** (3.5 g, 58%) as a colourless oil b.p. 120 $^0$C/ 0.2 mm.

I.R $\upsilon_{max}$ ($CCl_4$) 2925, 2850, 1755, 1740 $cm^{-1}$.

$^1$H n.m.r. ($CDCl_3$) $\delta$ 3.7 (s, 3H, -$OCH_3$), 3.36 (s, 1H, -OCCHCO-), 1.21-1.24 (m, 16H, -$CH_2$-), 0.83 (br t, 3H, -$CH_3$).

$^{13}$C n.m.r. ( $CDCl_3$) $\delta$ 129.92, 166.88 (C=O), 52.34 ($CH_3$), 51.49 (CH), 31.85, 29.66, 29.51, 29.46, 29.26, 29.09, 27.09, 22.63, ($CH_2$), 14.05 ($CH_3$).

M.S (m/z) 274 ($M^+$), 242 ( $M^+$ - MeOH), 132 ($M^+$- $C_{10}H_8D_2$, 100%).

Mass measurement: Found $M^+$ 274.2129, $C_{15}H_{26}D_2O_4$ requires 274.3690.

**5.** 3-$^2H_2$-dodecanoic acid **90** .

A solution of 1M NaOH (15 ml) was added to the diester **95** (0.8 g, 2.9 mmol) in distilled water (5 ml). The mixture was stirred at room temperature for a period of 24 hrs and then heated under reflux for 2-3 hrs. The reaction mixture was thoroughly extracted with $Et_2O$ (50 ml) to remove any unreacted ester and the aqueous phase was acidified at 0 $^0$C using conc. HCl, then saturated with solid NaCl. The solution was extracted with EtOAc (2x50 ml) and the extract was washed with brine (30 ml), dried and evaporated under reduced pressure to give the diacid as a white solid. This was heated to 125-130 $^0$C for a period of 3 hrs, after which time $CO_2$ evolution has completely ceased and after cooling to room temperature a white solid (0.7 g) was obtained. This was shown by $^1$H nmr and TLC in EtOAc:petroleum ether to be a mixture. Preparative TLC gave 3-$^2H_2$-dodecanoic acid **90** Rf. 0.7 (0.35 g, 60 %) as a white crystalline product m.p. 43-45 $^0$C.

I.R $\upsilon_{max}$ ($CCl_4$) 3000, 2920, 2845, 1708 $cm^{-1}$.

$^1$H n.m.r. ($CDCl_3$) δ 11.5 (br s, 1H, -OH), 2.32 (s, 2H, H-2), 1.24 (m, 16H, $-CH_2-$), 0.87 (br t, 3H, $-CH_3$).

$^2$H n.m.r. ($CHCl_3$) δ 1.60 (str).

**Table 6 :** $^{13}$C n.m.r. data (δ).

| | Dodecanoic acid [54a] | 3-$^2H_2$-dodecanoic acid **90** |
|---|---|---|
| C-1 | 180.69 | 180.55 |
| C-2 | 34.27 | 34.02 |
| C-3 | 24.83 | ----- |
| C-4 | 29.24 | 28.95 |
| C-5 | 29.41 | 29.29 |
| C-6 | 25.58 | 29.54 |
| C-7 and C-8 | 29.74 | 29.69 |
| C-9 | 29.47 | 29.43 |
| C-10 | 32.07 | 32.00 |
| C-11 | 22.80 | 22.79 |
| C-12 | 14.12 | 14.21 |

M.S (m/z) 202 ($M^+$, 42.1 %), 173 ( $M^+ - C_2H_5$, 6.5 %), 131 ($M^+ - C_5H_{11}$, 45.1 %), 75 ($M^+ - C_9H_{19}$, 100 %).

Mass measurement: Found $M^+$ 202.1885, $C_{12}H_{22}D_2O_2$ requires 202.3054.

**6.** Feeding of 3-$^2H_2$-dodecanoic acid **90** to surface cultures of *A. avenaceus*.

3-$^2H_2$-Dodecanoic acid **90** (300 mg), converted to its sodium salt in sterile water, was administered to eight (08) Roux bottles containing seven (07) day old surface cultures of *A avenaceus* on three consecutive days. After a further two days growth the cultures were harvested by decanting off the aqueous broth, which was then acidified to around pH 2 using conc. HCl. This was followed by continuous extraction with EtOAc for 48 hrs after which time the ethyl acetate solution was dried over anhydrous $MgSO_4$, filtered and evaporated to give an oily product. This was treated with aqueous $NaHCO_3$, extracted with EtOAc, dried and evaporated to give crude avenaciolide . Four, (20x20) $HF_{254}$ Merck silica plates (0.75 mm thick) were loaded with the crude extract (400 mg) and eluted once with $CHCl_3$. The relevant band was located under UV (254 nm), removed and extracted with EtOAc (3x50 ml) to give deuterium enriched avenaciolide **96**, Rf 0.45 (50 mg) m.p. 54-55 $^0$C from ether:hexane [lit.[5] m.p. 54-56 $^0$C]

$^1$H n.m.r: ($CDCl_3$) δ 6.45 (d, *J* 2.50 Hz, 1H, H-15a), 5.88 (d, *J* 2.50 Hz, 1H, H-15b), 5.05 (d, *J* 7.5 Hz, 1H, H-2), 4.45 (m, 1H, H-4), 3.54 (m, 1H, H-3), 1.85 (m, 2H, H-5), 1.2-1.4 (m, 12H, -$CH_2$-), 0.92 (m, 3H, H-12).

$^2$H n.m.r: ($CHCl_3$) δ 3.59 (str).

**Isolation of ethisolide 1, trimethyl citrate 98, methyl ethisate 76, trimethyl butylcitrate 100 and/or dimethyl-α-butyl-α-hydroxyitaconate 99 from cultures of *P.decumbens*.** [20]

In a typical feeding experiment [2-$^{13}$C] labelled NaOAc (100-250 mg) in 12 hrs pulses was administered to two (02) Roux bottles containing seven (07) day old surface cultures of *P. decumbens* on three consecutive days. The fungus was harvested after a further two (02) days undisturbed growth. The aqueous broth (400 ml) was decanted off and extracted at end pH with $CHCl_3$ (2x100 ml). The chloroform solution was dried over anhydrous $MgSO_4$, filtered and the filtrate was evaporated under reduced pressure to give crude ethisolide (400 mg). This was recrystallised from acetone/petroleum ether to afford ethisolide **1** as colourless needles m.p. 121-125 $^0$C [lit.[4] m.p. 122-123 $^0$C].

The extracted aqueous phase was concentrated under reduced pressure to give a mixture of acids. The residue was treated without delay with excess ethereal diazomethane [generated from 25 g of nitrosan in 10 g batches]. Solvents were removed and the residue was partitioned between $CHCl_3$ (100 ml) and $H_2O$ (100 ml). The aqueous phase was further extracted with $CHCl_3$ (2x50 ml), the combined extracts dried over $Na_2SO_4$, filtered and the filtrate was evaporated under reduced pressure to give a brown oil. On cooling and seeding most of the trimethyl citrate **98** crystallised. After overnight standing at 0 $^0$C the paste was triturated with ether (3x5 ml). The crude trimethyl citrate (3 g)

was dissolved in benzene (15 ml) and filtered through a short column of silica gel ($HF_{254}$) (3x1.5 cm). Elution with EtOAc:$C_6H_6$ (1:1) gave colourless product from yellow impurities. Recrystallisation from $iPr_2O$ gave trimethyl citrate **98** (2.3 g) as white crystals m.p. 76-77 $^0$C [lit.[20] m.p. 75-76$^0$].

$^1$H n.m.r: ($CDCl_3$) δ 4.1 (s, 1H, -OH), 3.82 (S, 3H, -$OCH_3$), 3.7 (S, 6H, -$OCH_3$), 2.85 (d, *J* 0.3 Hz, 4H, -$CH_2$),

$^{13}$C n.m.r: ($CDCl_3$) δ 173.9, 170.3, 73.3, 53.1, 51.9, 43.1.

The ether solution containing the minor metabolites was evaporated with a stream of nitrogen and the residue dissolved in benzene. Initial isolation of these metabolites was achieved by column chromatography on silica gel. Elution with EtOAc:$C_6H_6$ (1:9) gave a mixture of products almost free from trimethy citrate **98** and other impurities which were remained on the column. The fractions were assayed by TLC using acetone:light petroleum (1:4) and the appropriate fractions were combined and rechromatographed on neutral alumina. Elution with benzene gave an oil (500 - 600 g), which was further purified by preparative TLC using acetone:light petroleum (1:1) as eluent. The separated bands were located under UV (254 nm) and eluted with $CHCl_3$ to give :

*(i).* Methyl ethisate **76** Rf 0.45 as a colourless oil (50 mg).

$^1$H n.m.r: ($CDCl_3$) δ 6.15 (d, *J* 3.0 Hz, 1H, H-9a), 5.52 (d, *J* 3.0 Hz, 1H, H-9b), 4.93 (d, *J* 7.2 Hz, 1H, H-2), 3.77 (s, 3H, -$OCH_3$),

3.20 (m, 1H, H-3), 1.1-1.80 (m, 4H, -CH2), 0.95 (t, *J* 6.0 Hz, 3H, H-6).

$^{13}$C n.m.r: ($CDCl_3$) δ 169.3, 136.9, 121.5, 77.7, 52.3, 42.5, 31.0, 20.1, 13.9..

*(ii)*. Trimethyl butylcitrate **100** Rf 0.35 which was invisible under UV, as a colourless oil (150-200 mg in early cultures, later not detected).

$^{1}$H n.m.r: ($CDCl_3$) δ 3.88 (m, 1H, H-4), 3.80 (S, 3H, $-OCH_3$), 3.7 (S, 6H, $-OCH_3$), 3.69 (S, 3H, $-OCH_3$), 3.15 & 2.7 (ABq, $J_{AB}$ 16.0 Hz, 2H, H-9), 0.9-1.8 (m, 6H, $-CH_2$), 0.83 (m,3H, H-8).

$^{13}$C n.m.r: ($CDCl_3$) δ 173.9, 173.1, 171.4, 76.1, 52.9, 52.7, 51.9, 39.6, 29.4, 26.2, 22.4,13.7.

*(iii)*. Dimethyl 2-butylhydroxyitaconate **99** Rf 0.35 which was visible under UV, as a colourless oil (ca. 100 mg in earlier cultures but subsequently not detected).

$^{1}$H n.m.r: ($CDCl_3$) δ 6.38 (s, 1H, H-9), 5.72 (s, 1H, H-9), 3.80 (S, 3H, $-OCH_3$), 3.72 (S, 6H, $-OCH_3$), 3.00 (m,3H, -OH), 1.2-1.6 (m, 4H, $-CH_2$), 0.83 (br t,3H, H-6).

$^{13}$C n.m.r: ($CDCl_3$) δ 174.7, 167.8, 139.6, 127.3, 73.00, 52.1, 44.6, 30.2, 20.3, 14.00.

**Trapping experiments designed to detect n-decylitaconic acid 66 and α-methylene-β,n-nonylglutaric acid 101 in *A. avenaceus*.**

1. Preparation of α-methylene-β,n-nonylglutaric acid **101** :

Methyl *trans*-dodec-2-enoate **102** :

Redistilled n-decanal (50 g), was added over 1 hr to a cooled (4 $^0$C) mixture of malonic acid (16 g), methanol (50 ml) and pyridine (20 ml). The mixture was then gently warmed to dissolve the malonic acid and stirred at room temperature for 2 hrs. After refluxing for a further 3 hrs, the mixture was acidified with conc. HCl to pH 1 and extracted thoroughly with EtOAc. The resulting crude acid was esterified by refluxing with methanol (80 ml) containing conc, $H_2SO_4$ (6 ml) overnight. Ether and water were then added and the ether layer was separated to give the methyl ester **102** (22 g, 32%) b.p. 100-110 $^0$C/0.08 mm [lit.[111] b.p. 89-91 $^0$C/0.63 mm].

I.R. $\upsilon_{max}$ ($CCl_4$) 1720 ( ester C=O), 1660 (med, C=C), 987 (med, -CH=CH) $cm^{-1}$

$^1$H n.m.r. ($CDCl_3$) δ 6.94 (d-t, *J* 15 Hz, 7 Hz, 1H, H-3), 5.78 (d-t, *J* 15 Hz, 1.5 Hz, 1H, H-2), 3.71 (s, 3H, $-OCH_3$), 2.15 (m, 2H, H-4), 1.25 (br s, 14H, $-CH_2-$), 0.84 (BR t, 3H, $-CH_3$).

Methyl-3-(dicarbomethoxymethyl)dodecanoate **103** .

Methyl *trans* dodec-2-enoate **104** (18 g) in dry DMF (25 ml) was added dropwise to a solution of dimethyl sodiomalonate [prepared from sodium hydride (3.80 g) and dimethyl malonate (10 ml) in dry DMF (50 ml)] and the mixture stirred and heated under reflux at 70 $^0$C overnight. The solution was evaporated and the residue taken up in water and thoroughly extracted with ether to give methyl-3-(dicarbomethoxymethyl) dodecanoate **103** (22 g, 75%) as a yellow oil.

I.R. $\upsilon_{max}$ ($CCl_4$) 1730 (C=O), no peak at 1600 (C=C) cm-1

$^1$H n.m.r. ($CDCl_3$) δ 3.66 (s, 6H, $OCH_3$), 3.60 (s, 3H, -$OCH_3$), 3.55 (d, *J* 7.5 Hz, -OCC<u>H</u>CO-), 2.5 (d, *J* 17.0 Hz, -COC<u>H</u>$_2$-), 2.4 (m, 1H, H-3), 1.55 (m, 2H, H-4), 0.81 (br t, 3H, -$CH_3$)

$^{13}$C n.m.r. ($CDCl_3$) δ 172.76, 169.017, 168.79 (C=O), 53.94 (CH), 52.23, 52.16, 51.42 ($OCH_3$), 35.77 ($CH_2$), 34.88 (CH), 31.78, 31.72, 29.59, 29.39, 29.34, 29.18, 26.68, 22.56 ($CH_2$), 13.96 ($CH_3$).

This was spectroscopically identical with a sample of authentic material. [69]

2-Carboxy-3-n.nonylglutaric acid **104** .

To the trimethyl ester **103** (10 g) and 1M aqueous NaOH (150 ml) were stirred overnight at room temperature. The mixture was

then heated at 80 $^0$C for 3 hrs and after cooling, the solution was extracted with ether to give unreacted ester (3 g). The aqueous layer was acidified at 0 $^0$C with conc. HCl, saturated with solid NaCl and thoroughly extracted with EtOAc to give 2-Carboxy-3-n,nonylglutaric acid **104** (3.9 g, 44%). m.p. 115-117 $^0$C [lit. [69] m.p. 115-117 $^0$C]. (Found C, 60.14, H, 8.80, calculated for $C_{15}H_{26}O_6$ C, 59.60, H, 8.66%).

I.R $\upsilon_{max}$ (nujol) 3600-2400 (-OH), 1700 (acid C=O) cm-1

$^1$H n.m.r. ($C_5D_5N$) $\delta$ 8.5 (br s, 3H, -OH), 4.2 (d, *J* 5.00 Hz, 1H, H-2), 3.16 (br m, 3H, H-3, H-4), 1.15-2.0 (m, 16H, -$CH_2$), 0.9 (br t, 3H, $CH_3$).

M.S (m/z) $M^+$ 302 (1%), 284 ($M^+$ - $H_2O$, 3%), 240 ($M^+$ -18-44, 26%), 199 ($M^+$ - $CH(CO_2H)$, 100%).

α-Methylene-β-n.nonylglutaric acid **101** .

2-Carboxy-3-n,nonylglutaric acid **104** (6 g, 20 mmol) in methanol (15 ml) at -20 $^0$C was treated with 40% aqueous diethylamine (14 ml) in methanol (15 ml) and the solution stirred for 30 min. 37% Aqueous formaldehyde (15 ml) in methanol (20 ml) was added dropwise and the mixture was stirred at room temperature overnight. After heating to 85 $^0$C for 1 hr, the solvent was removed by evaporation under reduced pressure and the residue was taken up in $H_2O$, acidified at 0 $^0$C with dilute HCl, saturated with solid NaCl and thoroughly extracted with EtOAc to give α-

methylene-β-n,nonylglutaric acid **101** (3.8 g, 71%) m.p. 46-48 $^{0}$C from light petroleum [lit.[69] m.p. 46-48 $^{0}$C]. (Found C, 66.95, H, 9.67, calculated for $C_{15}H_{26}O_4$ C, 66.64, H, 9.69%).

I.R. $\upsilon_{max}$ (nujol) 3500-2400 (-OH), 1695 (C=O), 1625 (med, C=C), 920 (med, $C=CH_2$) $cm^{-1}$

$^{1}$H n.m.r. ($CDCl_3$) δ 11.85 (br s, 2H, -OH), 6.39 (s, 1H, H-15), 5.69 (s, H, H-15), 3.02 (m, 1H, H-3), 2.57 (m, 2H, H-2), 1.52 (m, 4H, H-4), 1.28 (br s, 14H, $-CH_2-$), 0.85 (br t, 3H, H-12).

$^{13}$C n.m.r. ($CDCl_3$) δ 179.23, 172.46 (C=O), 141.86 (C-14), 127.96 (C-15), 38.84 ($CH_2$), 37.39 (CH), 33.68, 31.83, 29.49, 29.45, 29.40, 29.25, 27.01, 22.62 ($CH_2$), 14.04 ($CH_3$).

M.S. (m/z) 270 ($M^+$, 20%), 252 ($M^+$- H2O, 100%), 234 ($M^+$ - 36, 32%), 224 ($M^+$ - 18-28, 34%).

**2.** Feeding of 2,3-[$^{2}H_4$]-succinic, 2,3-[$^{2}H_2$]-fumaric, 2-[$^{3}H_3$]-acetic acids and α-methylene-β,n-nonylglutaric acid **101** to surface cultures of *A. avenaceus.*

2-[$^{3}H_3$]-Acetic acid (1.14 μci), 2,3-[$^{2}H_4$]-succinic acid (192 mg), 2,3-[$^{2}H_2$]-fumaric acid (420 mg) and α-methylene-β,n-nonylglutaric acid **101** (3.7 g), converted to their sodium salt in sterile water, were fed in 5 x 12 hrs pulses, on the 7, 8 and 9$^{th}$ days after inoculation, to thirty (30) Roux bottles containing cultures of *A. avenaceus.* On the 10 th day the aqueous broth was

separated by decantation, acidified to pH 3 with conc. HCl and continuously extracted with EtOAc for 48 hrs. The organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated to give a brown oil (7.25 g), which was taken up in EtOAc (25 ml) and extracted with saturated aqueous $NaHCO_3$. The organic phase gave after evaporation and preparative TLC ($CHCl_3$), avenaciolide **105** (50 mg) m.p. 54-55 $^0$C from ether/petroleum ether [lit.[5] m.p. 54-56 $^0$C].

$^1$H n.m.r: ($CDCl_3$) δ 6.45 (d, *J* 2.50 Hz, 1H, H-15a), 5.78 (d, *J* 2.50 Hz, 1H, H-15b), 5.04 (d, *J* 7.5 Hz, 1H, H-2), 4.39 (m, 1H, H-4), 3.54 (m, 1H, H-3), 1.80 (m, 2H, H-5), 1.25 (m, 12H, -$CH_2$-), 1.04 (br t, 3H, H-12).

$^2$H n.m.r: ($CHCl_3$) δ 2.58 (str), 1.28 (m).

The activity isolated as [$^3$H]-avenaciolide **105** was (50 mg, 6528 dpm/mg, giving an incorporation of 12.89% from [$^3$H]-acetate).

The aqueous $NaHCO_3$ layer was acidified with dilute HCl and extracted with ethyl acetate to give an oily solid which was dissolved in $CHCl_3$ and filtered to give crude α-methylene-β,n-nonylglutaric acid **101** (1.98 g). Extensive preparative TLC ($HF_{254}$, 0.75 mm thick) in light petroleum/ ether/acetic acid with the ratios 28:12:1 respectively, separated the mixture into two main fractions. The first fraction, Rf. 0.30, contained a small amount of an olefinic compound yet to be identified. [$^1$H nmr ($CDCl_3$) δ 7.15 (d, *J* 9 Hz), 6.8 (d, *J* 9 Hz)].
The second component, corresponded ($^1$H nmr) to α-methylene-

β,n-nonylglutaric acid **101** (300 mg) m.p. 48-49 $^{0}$C from light petroleum. The activity isolated as [$^{3}$H]-isolated α-methylene-β,n-nonylglutaric acid **101** (300 mg, 437.77 dpm/mg, giving an incorporation of 5.2% from [$^{3}$H]-acetate).

3. Feeding of 2,3-[$^{2}H_4$]-succinic, and α-methylene-β,n-nonylglutaric acid **101** to *A. avenaceus*.

2,3-[$^{2}H_4$]-Succinic acid (350 mg) and α-methylene-β,n-nonylglutaric acid **101** (300 mg), converted to their sodium salt in sterile water, were fed in 5x12 hrs pulses, on the 7, 8 and 9 th days after inoculation, to twelve (12) Roux bottles containing cultures of *A. avenaceus*. On day 10, the aqueous broth was separated by decantation, acidified to pH 3 with conc.HCl and continuously extracted with EtOAc for 48 hrs. The organic phase was dried over anhydrous $MgSO_4$, filtered and the filtrate was evaporated to a brown oil (3 g). This was taken up in EtOAc (10 ml) and extracted with saturated aqueous $NaHCO_3$. The organic phase gave after evaporation and preparative TLC in $CHCl_3$, avenaciolide **2** (45 mg) m.p. 54-55 $^{0}$C from ether/petroleum ether [lit.[5] m.p.54-56 $^{0}$C]..

$^{1}$H n.m.r: as described for previously obtained samples.

$^{2}$H n.m.r: ($CHCl_3$) δ 1.28 (str).
(10% of natural abundance $CDCl_3$)

The aqueous $NaHCO_3$ layer was acidified with dilute HCl and extracted with ethyl acetate to give an oily solid which was dissolved in $CHCl_3$ and filtered to give crude α-methylene-β,n-nonylglutaric acid **101** (0.6 g). Extensive preparative TLC ($HF_{254}$, 0.75 mm thick) in light petroleum/ether/acetic acid with the ratios 28:12:1 respectively, separated the mixture into two main bands. The first band, Rf. 0.20, was removed, extracted with EtOAc, evaporated to give a mixture of olefinic components [$^1H$ nmr ($CDCl_3$) δ 7.90 (m, -OH), 6.9 (s), 6.8 (d, *J* 3 Hz), 5.85 (d, *J* 3 Hz)]; with no sign of n-decylitaconic acid **66**.

The second band Rf. 0.35, contained ($^1H$ nmr) α-methylene-β,n-nonylglutaric acid **101** (170 mg) m.p. 48-49 $^0C$ from light petroleum.

**Feeding of 2,3-$^2H_4$-succinic acid to (10) day old surface cultures of *P. decumbens*.**

**(1).** 2,3-$^2H_4$-Succinic acid (1.5 g), converted to its sodium salt in sterile water, was administered to thirty (30) Roux bottles containing seven (07) day old surface cultures of *P.decumbens* on two (02) consecutive days. Ten days after inoculation the cultures were harvested by decanting off the aqueous broth, which was extracted at end with EtOAc for 24 hrs after. The ethyl acetate solution was dried over anhydrous $MgSO_4$, filtered and evaporated to give ethisolide **1** (1.2 g) m.p. 121-122 $^0$C from ethanol [lit.[4] m.p. 122-123 $^0$C]

$^1$H n.m.r: as described for previously obtained samples.

$^2$H n.m.r: ($CHCl_3$) δ 6.68 (med.), 5.95 (med.).

The extracted aqueous phase was concentrated using a Buchi-evaporator equipped with an acetone /'DRIKOLD' cooling bath on receiver flask, to give a semi solid residue. Elution with cold ether separated ethisolide **1** from a mixture of acids (107 mg), which was shown by TLC in $CHCl_3$:acetone:acetic acid (9:4:1) to be a mixture of three components. Preparative TLC in the same solvent gave :

(*i*) further crystalline ethisolide **1** m.p. 121-122 $^0$C, Rf. 0.7 (20 mg).

(*ii*) Decumbic acid **77** [$^1$H nmr signals at δ 5.15 (m, 1H, H-3), 2.2 (d, *J* 3 Hz, C=CH3), 1.4 (m, 4H,H-4, H-5), 0.95 (m, 3H, H-6)] and deuterium enriched ethisic acid **5** Rf. 0.3 (75 mg) as a colourless

oil. $^{1}$H n.m.r.: ($CDCl_3$) δ 1219.9 (br s, 1H, OH), 6.31 (d, *J* 3 Hz, 1H, H-9a), 5.61 (d, *J* 3 Hz, 1H, H-9b), 5.04 (d, *J* 8 Hz, 1H, H-2), 3.30 (m, 1H, H-3), 1.75 (m, 2H, H-4), 1.5 (m, 2H, H-5), 0.85(br t, 3H, H-6).

$^{13}$C n.m.r: ($CDCl_3$) δ 173.25, 169.64, 136.03, 122.53, 77.62, 41.86, 30.54, 19.90, 13.87.

$^{2}$H n.m.r.: ($CHCl_3$) δ 6.4 (w), 5.6 (w).

**(2)**. Similar results were obtained from an experiment in which, $^{2}$H-succinic acid (310 mg) was fed to (08) Roux bottles containing 10 day old surface cultures of the above fungus in 2 consecutive days. Usual work-up afforded ethisolide **1** and concentration of the aqueous phase, extraction with ether gave a mixture of ethisic acid **5** and decumbic acid **77** (20 mg) together with traces of olefinic compounds [terminal methylene doublets ca. *J* 2.5 Hz, at δ 6.44, 5.88, 6.02, 6.45 and a singlet at δ 6.56].

$^{2}$H n.m.r.: ($CHCl_3$) δ 2.67 (str.), no signals at δ 6.5 and 5.8.

**Feeding of 2,3-$^2H_4$-succinic acid to (09) day old surface cultures of *P. decumbens*.**

2,3-$^2H_4$-Succinic acid (500 mg), converted to its sodium salt in sterile water, was administered to thirty (30) Roux bottles containing seven (07) day old surface cultures of *P decumbens* on two (02) consecutive days. Nine days after inoculation the cultures were harvested by decanting off the aqueous broth, which was extracted at end with EtOAc for 24 hrs. The ethyl acetate solution was dried over anhydrous $MgSO_4$, filtered and evaporated to give ethisolide **1** (0.5 g) m.p. 121-122 $^0C$ from ethanol, identical (m.p. and $^1H$ nmr) to an authentic sample.

$^2H$ n.m.r; ($CHCl_3$) δ 6.70 (str.), 5.92 (str.).

The extracted aqueous phase was concentrated using an acetone/'DRIKOLD' cooling bath on receiver flask, to give a semi solid residue. Elution with cold ether separated ethisolide **1** from a mixture of acids (52.5 mg), which was shown by TLC in $CHCl_3$:acetone:acetic acid (9:4:1) to be a mixture of two fractions. Preparative TLC in the same solvent gave : (*i*) further crystalline ethisolide **1**, (*ii*) decumbic acid **77** (*iii*) ethisic acid **5** [identified by comparison with the spectra of previously obtained samples], and traces of olefinic compounds (terminal methylene doublets ca. *J* 1.5 Hz, at δ 6.4, 6.3, 6.1 and 5.8). The above extracted residue was further extracted with EtOAc for 24 hrs to give more ethisolide **1** and $^1H$ nmr showed no sign of the minor metabolite n-butylitaconic acid **65**.

**The minor metabolites of unfed cultures of *P. decumbens*.**

Twenty Roux bottles containing 10 day old surface cultures of *P. decumbens*, were harvested by decanting off the aqueous broth, which was concentrated using a Buchi evaporator equipped with an acetone/ 'DRIKOLD' cooling bath on receiver flask to give a mixture of acids. This was thoroughly extracted with EtOAc (5x250 ml) and was then washed with aqueous $NaHCO_3$ to remove acids, dried and evaporated to give ethisolide **1** (2.6 g). The aqueous phase was acidified with conc. HCl, extracted with EtOAc, washed with brine, dried over $MgSO_4$, filtered and the filtrate was evaporated under reduced pressure to give a mixture mainly containing decumbic acid **77** together with a small amount of ethisic acid **5**. Initial isolation of these metabolites was achieved by column chromatography on silica gel $HF_{254}$. Elution with $CHCl_3$ gave the mixture of the above minor metabolites (0.3 g) almost free from acetic acid and other impurities which remained on the column. [$^1H$ nmr as described for previously obtained samples]. The more polar fractions were assayed by TLC using $CHCl_3$: acetone: acetic acid (9:4:1) and the appropriate fractions were combined. $^1H$ nmr showed no sign of n-butylitaconic acid **65** in these fractions too.

## HPLC EXPERIMENTAL :

A Perkin-Elmer High-performance liquid chromatograph with pumps, a variable-wavelength LC90 UV detector, a two components solvent system (A= $H_2O$, B= $CH_3OH$) was developed after several experiments with different programmes. The solvent temperature was held at room temperature. The UV detector was set to monitor at $\lambda$ = 210 nm. Injections were made with LSS 100 autosampler injector system (10 µl). Analysis were performed on an ODS (5 µm particles) Perkin-Elmer Pecosphere-3x3C C-18 [112] and Pecosphere-3CR C-8 reversed phase columns.

The final programme was set as follows :

Using a C-8 column; $H_2O$:MeOH with the ratio 80:20 as solvent system; UV $\lambda$ =210 nm; the flow rate was 1.5 ml $min^{-1}$ and with attenuations 128 and 256. The solvents were HPLC grade, degassed.

The retention time (Rt) in minutes of the injected standard compounds was as shown below in Table 1.1:

**Table 1.1**

| Compound | Rt (min.). |
|---|---|
| Citric acid **7** | 0.23. |
| α-Methylene-β,n-propylglutaric acid **106** | 1.00. |
| Butylitaconic acid **65** | 1.43. |
| Ethisolide **1** | 2.32. |
| Trimethyl citrate **98** | 1.54. |
| $CH_3OH$ | 0.36. |
| Ethisic and decumbic acids **5** &**77** | 0.31 |

When the compounds **7**, **106**, **65** and **1** were dissolved in methanol and the mixture was injected the retention times obtained were as the following Table :

**Table 1.2**

| Compound | Rt (min.). |
|---|---|
| **7** | 0.23. |
| **106** | 1.03. |
| **65** | 1.43. |
| **1** | 2.38. |

Under these conditions the retention times (Rt, min.), for a sample obtained from fed [$^2$H]-succinate aqueous broth of *P.decumbens*, were shown as the following: 0.31, 1.11, 1.48 min.

The chromatograms from elution with $H_2O$:MeOH 80:20 containing 0.01 mM of $H_3PO_4$ (pH 4.4) were good for the distribution of the retention times but the peaks were slightly broad and not symmetrical. The retention times (Rt) under these conditions for the previous standard compounds and some secondary metabolites from *P. decumbens* are listed in Table 1.3 :

**Table 1.3**

| Compound | Rt (min.). |
|---|---|
| **1** | 1.50. |
| **65** | 5.79. |
| **106** | 7.65. |
| **$CH_3OH$** | 0.37. |

A mixture of **1**, **65** and **106** was dissolved in methanol, injected and the retention times obtained were 1.50, 5.74 and 7.44 min. respectively.

## Isolation of the minor metabolites from unfed cultures of *P. decumbens* as their methyl esters.

Twenty Roux bottles containing 09 day old surface cultures of *P. decumbens*, were harvested by decanting off the aqueous broth, which was extracted at end pH with EtOAc (5x100 ml). The EtOAc solution was washed with aqueous $NaHCO_3$ (3x20 ml), dried over $MgSO_4$, filtered and the filtrate evaporated to give ethisolide **1** (2.5 g) in colourless needles from ethanol (m.p. and $^1H$ nmr) identical to an authentic sample. The extracted aqueous phase was concentrated using a Buchi evaporator equipped with an acetone/ 'DRIKOLD' cooling bath on receiver flask to give a mixture of acids. This was treated with $NaHCO_3$ (50 g) in distilled water (30 ml) and combined with the above $NaHCO_3$ extract and the solution was stirred with $Me_2SO_4$ (50 ml) at 60 $^0C$ for 3 days. The resulting solution was then treated with $NaOAc.3H_2O$ (20 g) to destroy any unreacted $Me_2SO_4$. After overnight stirring at room temperature, the reaction mixture was extracted with EtOAc (3x100 ml), dried over $MgSO_4$, filtered and the filtrate evaporated to give an oil (2 g). This was shown by TLC in acetone/light petroleum 1:4 to be a mixture of two compounds. Preparative TLC on silica gel $HF_{254}$ in the same solvent gave methyl ethisate **76** Rf. 0.45 as a colourless oil (60

mg). [Taking into account that only a portion of the mixture was chromatographed].

$^1$H n.m.r: ($CDCl_3$) δ 6.15 (d, *J* 3.0 Hz, 1H, H-9a), 5.52 (d, *J* 3.0 Hz, 1H, H-9b), 4.93 (d, *J* 8.0 Hz, 1H, H-2), 3.77 (s, 3H, $-OCH_3$), 3.20 (m, 1H, H-3), 1.1-1.8 (m, 4H, H-4, H-5), 0.95 (t, *J* 6.0 Hz, 3H, H-6), identified by comparison with the spectra of an authentic sample as methyl decumbate **56** (65 mg) Rf. 0.55.

$^1$H n.m.r: ($CDCl_3$) δ 5.15 (br m, 1H, H-3), 3.88 (s, 3H, $-OCH_3$), 2.20 (d, *J* 3.0 Hz, 3H, $-C=CH_3$), 1.40 (m, 4H, H-4, H-5), 0.95 (t, *J* 7.0 Hz, 3H, H-6).

**Feeding of 2-[$^2H_3$]-acetic acid to surface cultures of *A. avenaceus*.**

2-[$^2H_3$]-Acetic acid (310 mg), converted to its sodium salt in sterile water, was administered to seven (07) Roux bottles containing seven (07) day old surface cultures of *A. avenaceus* on three consecutive days. After a further five days growth the cultures were harvested by decanting off the aqueous broth, which was then cautiously acidified to around pH 2 using conc. HCl. This was followed by continuous extraction with EtOAc for 48 hrs after which time the ethyl acetate solution was dried over anhydrous $MgSO_4$, filtered and evaporated to give an oily product. Three, one metre $HF_{254}$ Merck silica plates (0.75 mm thick) were loaded with the crude extract (81.50 mg) and eluted once with $CHCl_3$. The relevant band was located under UV (254 nm), removed and extracted with EtOAc (3x20 ml) to give deuterium enriched avenaciolide **2**, Rf 0.45 (43 mg) m.p. 54-55 $^0$C from ether:hexane [lit.[5] m.p. 54-56 $^0$C]

$^1$H n.m.r: ($CDCl_3$) δ 6.45 (d, *J* 2.50 Hz, 1H, H-15a), 5.88 (d, *J* 2.50 Hz, 1H, H-15b), 5.05 (d, *J* 7.5 Hz, 1H, H-2), 4.45 (m, 1H, H-4), 3.54 (m, 1H, H-3), 1.85 (m, 2H, H-5), 1.2-1.4 (m, 12H, -CH2-), 0.92 (m, 3H, H-12).

$^2$H n.m.r: ($CHCl_3$) δ 1.29 (med.), 0.85 (str).

(Peaks 10% of natural abundance $CDCl_3$ peak).

**Feeding of 2,3-[$^2H_2$]-fumaric acid to surface cultures of *A. avenaceus*.**

2,3-[$^2H_2$]-fumaric acid (310 mg), converted to its sodium salt in sterile water, was administered to eight (08) Roux bottles containing seven (07) day old surface cultures of *A. avenaceus* on three consecutive days. After a further five days growth the cultures were harvested by decanting off the aqueous broth, which was then cautiously acidified to around pH 2 using conc. HCl. This was followed by continuous extraction with EtOAc for 48 hrs after which time the ethyl acetate solution was dried over anhydrous $MgSO_4$, filtered and evaporated. Three, one metre $HF_{254}$ Merck silica plates (0.75 mm thick) were loaded with the crude extract (980 mg) and eluted once with $CHCl_3$. The relevant band was located under UV (254 nm), removed and extracted with EtOAc (3x20 ml) to give deuterium enriched avenaciolide **2**, Rf 0.45 (72.1 mg) m.p. 54-55 $^0C$ from ether:hexane [lit.[5] m.p. 54-56 $^0C$]

$^2H$ n.m.r: ($CHCl_3$) $\delta$ 1.27 (str.), 0.9 (med.).

(Peaks < 1% of natural abundance $CDCl_3$ peak).

The above experiment was repeated and again no incorporation of deuterium was observed at the terminal methylene positions at C-15.

**Feeding of 2,3-[$^2H_4$]-succinic acid to surface cultures of *A. avenaceus*.**

2,3-[$^2H_4$]-Succinic acid (310 mg), converted to its sodium salt in sterile water, was administered to eight (08) Roux bottles containing seven (07) day old surface cultures of A. avenaceus on three consecutive days. After a further five days growth the cultures were harvested by decanting off the aqueous broth, which was then cautiously acidified to around pH 2 using conc. HCl. This was followed by continuous extraction with EtOAc for 48 hrs after which time the ethyl acetate solution was dried over anhydrous $MgSO_4$, filtered and evaporated. Three, one metre $HF_{254}$ Merck silica plates (0.75 mm thick) were loaded with the crude extract (980 mg) and eluted once with $CHCl_3$. The relevant band was located under UV (254 nm), removed and extracted with EtOAc (3x20 ml) to give deuterium enriched avenaciolide **2**, Rf 0.45 (147.3 mg) m.p. 54-55 $^0C$ from ether/hexane [lit.[5] m.p. 54-56 $^0C$]

$^2H$ n.m.r: ($CHCl_3$) δ 6.46 (str.), 5.82 (str).
(Peaks 8% of natural abundance $CDCl_3$ peak).

$^{13}C$ n.m.r: ($CDCl_3$) δ 169.82, 167.53 (C=O), 134.5 (C-14), 126.29 (C-15), 85.19, 74.26, 44.03 (-CH-), 35.96, 31.69, 29.61, 29.24, 29.04, 24.75, 22.54 (-$CH_2$-), 14.01 (-$CH_3$).

# EXPERIMENTAL (PART 2).

**Methyl *trans* hex-2-enoate 109 .**

To *trans* 2-hexenoic acid **108** (50 g, 0.44 mol) in methanol (70 ml) was added dropwise while stirring acetyl chloride (17 ml) and the mixture stirred at 0 $^0$C for an period of 2 hrs. After overnight stirring at room temperature the reaction mixture was refluxed at 45-50 $^0$C for 3 hrs and the methanol was evaporated under reduced pressure to give the methyl ester **109** ( 53.8 g, 96 %) b.p. 35 $^0$/0.2 mm [lit.[113] b.p. 32 $^0$C/0.2 mm].

I.R $\upsilon_{max}$ ($CCl_4$) 1720 (C=O), 1660 (C=C), 980 (med, *trans* -CH=CH) $cm^{-1}$

$^1$H n.m.r. ($CDCl_3$) $\delta$ 7.0 (d-t, *J* 15 Hz, 7 Hz, 1H, H-3), 5.8 (d, 2H, *J* 15 Hz, 1H, H-2), 3.56 (s, 3H, -$OCH_3$), 2.0 (m, 2H, H-4), 1.32 (m, 2H, H-5), 0.77 (t, *J* 5 Hz, 3H, H-6).

$^{13}$C n.m.r. ($CDCl_3$) 166.66 (C=O), 149.01, 120.69 (CH), 50.87 ($CH_3$), 55.86, 20.96 ($CH_2$), 13.22 ($CH_3$).

**Methyl-3-(dicarbomethoxymethyl)hexanoate 110 .**

Methyl *trans*-hex-2-enoate **33** (9.65 g, 0.08 mol) in dry DMF (10 ml) was added dropwise to a solution of dimethyl sodiomalonate [prepared from sodium hydride (2.01 g) and dimethyl malonate (10 ml) in dry DMF (20 ml)] and the solution stirred and heated under reflux overnight. The solution was evaporated and the residue

taken up in water and extracted with ether to give an oily product (20 g). Distillation gave methyl-3-(dicarbomethoxymethyl) hexanoate **110** (12 g, 61%) b.p. 120 $^0$C/0.8 mm. (Found C, 55.59, H, 7.88, $C_{12}H_{20}O_6$ requires C, 55.38, H, 7.69%).

I.R $\upsilon_{max}(CCl_4)$ 1735, (C=O) $cm^{-1}$

$^1$H n.m.r. ($CDCl_3$) $\delta$ 3.78 (s, 6H, $OCH_3$), 3.66 (s, 4H, H-2, $OCH_3$), 2.6-2.4 (m, 3H, H-2, H-3), 1.4 (m, 4H, H-4, H-5), 0.9 (m, 3H, $CH_3$)

$^{13}$C n.m.r. ($CDCl_3$) 172.86, 169.06, 168.84 (C=O), 53.90 (CH), 52.26, 52.19, 51.45 ($OCH_3$), 36.12 ($CH_2$), 35.52 (CH), 34.58 ($CH_2$), 19.59 ($CH_2$), 13.79 ($CH_3$).

M.S. (m/z) 260 ($M^+$), 229 ($M^+$- $OCH_3$, 18%), 197 (26%), 187 ($M^+$ - $CH_2CO_2CH_3$, 12.5%), 132 ($CH_2(CO_2CH_3)_2$, 100%), 129 ($M^+$ - $CH(CO_2CH_3)_2$, 48.1%).

**2-Carboxy-3-n,propylglutaric acid 111 .**

To the trimethyl ester **110** (10 g, 0.04 mol) and 1M aqueous NaOH (50 ml) were stirred overnight at room temperature. The mixture was then heated at 70 $^0$C for 3 hrs and after cooling, acidified at 0 $^0$C with dilute HCl, saturated with solid NaCl and thoroughly extracted with EtOAc to give 2-Carboxy-3-n,propylglutaric acid **111** (6.42 g, 76%). m.p. 128-130 $^0$C from ethyl acetate /light

petroleum. (Found C, 49.68, H, 6.56, calculated for $C_9H_{14}O_6$ C, 49.54, H, 6.42%).

I.R $\upsilon_{max}(CCl_4)$ 5500-2400 (-OH), 1710 (acid C=O) $cm^{-1}$

$^1$H n.m.r. ($C_5D_5N$) $\delta$ 10.2 (br m, 3H, -OH), 4.4 (d, *J* 6.25 Hz, 1H, H-8), 3.4 (m, 2H, H-2), 3.11 (m, 1H, H-3), 2.05 (m, 2H, H-4), 1.65 (m, 2H, H-5), 0.9 (t, *J* 7.25 Hz, 3H, $CH_3$).

$^{13}$C n.m.r. ($C_5D_5N$) 175.58, 172.72, 172.52 (C=O), 56.19, 35.51 (CH), 37.23, 34.83, 20.61 ($CH_2$), 14.46 ($CH_3$).

**α-Methylene-β-n,propylglutaric acid 106 .** [69]

A solution of 2-carboxy-3-n,propylglutaric acid **111** (5 g, 0.02 mol) in methanol (20 ml) was treated with 40% aqueous diethylamine (20 ml) stirred at -10 $^0$C for 10 min. and 37% aqueous formaldehyde (45 ml) in methanol (15 ml) was added dropwise. The mixture was stirred overnight at room temperature, refluxed for 2 hrs and evaporated under reduced pressure. The residue was taken up in $H_2O$, acidified at 0 $^0$C with dilute HCl, saturated with solid NaCl and thoroughly extracted with EtOAc. Removal of the solvent gave α-methylene-β-n,propylglutaric acid **106** (3.19 g, 76%) m.p. 55-57 $^0$C from ethyl acetate /light petroleum.

I.R $\upsilon_{max}(CCl_4)$ 3200-2400 (-OH), 1700 (C=O), 1620 (C=C), 920 ($C=CH_2$) $cm^{-1}$

$^1$H n.m.r. ($CDCl_3$) δ 12.08 (s, 2H, -OH), 6.5 (s, 1H, H-9a), 5.8 (s, H, H-9b), 3.0 (m, 1H, H-3), 2.6 (d, *J* 7.0 Hz, 2H, H-2), 1.5 (m, 4H, H-4, H-5), 0.9 (m, 3H, H-6).

M.S. (m/z) 186 ($M^+$), 229 ($M^+$- $H_2O$, 20%), 143 ($M^+$ - $C_3H_7$, 31%), 140 ($M^+$ - 18-28, 47%), 127 ($M^+$- $CH_2CO_2H$, 73%), 109 (63%), 98 (100%).

**Esterification of α-methylene-β-n.propylglutaric acid 106 .**

To the acid **106** (4.5 g, 0.02 mol) in methanol (25 ml) was added dropwise while stirring, acetyl chloride (10 ml) and the mixture stirred at 0 $^0$C for a period of 2 hrs. After overnight stirring at room temperature the reaction mixture was refluxed at 45-50 $^0$C for 3 hrs and the methanol was evaporated under reduced pressure to give a yellow oil. This was distilled to afford the dimethyl ester **112** as a colourless oil (3.0 g, 58%) b.p. 110 $^0$C/0.05 mm. (Found C, 61.66, H, 8.60, $C_{12}H_{20}O_6$ requires C, 61.68, H, 8.41%).

I.R $\upsilon_{max}(CCl_4)$ 3500-2300, 1730, 1720, 1690 (C=O), 1620 (C=C) $cm^{-1}$

$^1$H n.m.r. ($CDCl_3$) δ 6.17 (s, 1H, H-9a), 5.52 (s, 1H, H-9b), 3.71 (s, 3H, $OCH_3$), 3.59 (s, 3H, $OCH_3$), 3.0 (m, 1H, H-3), 2.5 (ABX, $J_{AB}$ 15.3 Hz, $J_{AX}$ 7.18 Hz, $J_{BX}$ 7.6 Hz, 2H, H-2), 1.2-1.4 (m, 4H, H-4,

H-5), 0.85 (t, *J* 7.13 Hz, 3H, H-6).

$^{13}$C n.m.r. ($CDCl_3$) 172.87, 167.10 (C=O), 142.37 (C=C), 125.19 ($CH_2$), 51.73, 51.41 (-$OCH_3$), 38.95 ($CH_2$), 37.84 (CH), 35.99, 20.16 ($CH_2$), 13.87 ($CH_3$).

M.S. (m/z) 214 ($M^+$), 183 ($M^+$- $OCH_3$, 44.9%), 171 ($M^+$- $(CH_2)_2CH_3$, 40.4%), 155 ($M^+$- $CO_2CH_3$, 17.3%), 141 ($M^+$- $CH_2CO_2CH_3$, 66.3%), 129 ($M^+$ - $CH_2$=C($CO_2CH_3$), 11.7%), 125 (168- $(CH_2)_2CH_3$, 27.8%), 81 (168- $CO_2$ - $(CH_2)_2CH_3$, 100%).

**The addition of bromine to dimethyl α-methylene-β-n.propylglutarate 112 .**

To a solution of the diester **112** (12.5 g, 5.6 mmol) in $CCl_4$ (12 ml) was added while stirring, bromine (0.897 g, 0.28 ml) in $CCl_4$ (14 ml). The reaction mixture was stirred at room temperature overnight and then at 40 $^0$C for a period of 3 hrs. The $CCl_4$ was evaporated under reduced pressure to give a reddish oil. Short path distillation gave the dibromo compound **113** (2.05 g, 98%) as a colourless oil b.p. 130 $^0$C/ 0.05 mm. This was separated from minor impurities by TLC Rf 0.8 in EtOAc/ light petroleum (2:1) to give the dibromo compound **113** as inseparable *threo* and *erythro* isomers.

I.R $\upsilon_{max}$($CCl_4$) 2958, 2865, 1750, 1735 (C=O), no peak at 1620 (C=C) $cm^{-1}$

$^1$H n.m.r. ($CDCl_3$) δ 4.1 & 3.88 (ABq, $J_{AB}$ 10.5 Hz) and 3.98 & 3.85 (ABq, $J_{AB}$ 11.0 Hz), [2H, H-9], 3.80, 3.78, 3.69 and 3.67 (s, each 3H, $OCH_3$), 2.5-2.8 (m, 2H, H-2), 2.2-2.4 (m, 2H, H-2), 1.75 (m, 1H, H-3), 1.35 (m, 4H, H-4, H-5), 0.9 (br t, 3H, H-6).

$^{13}$C n.m.r. ($CDCl_3$) 172.84, 172.78, 168.61 (C=O), 71.98, 69.37 (-CBr), 53.60, 53.55, 52.02, 51.9 ($OCH_3$), 41.8, 39.15, (-CH-), 38.82, 38.28, 37.68, 36.35, 36.19, 55.54, 20.63, 20.41 ($-CH_2-$), 14.28, 13.98 ($-CH_3$).

M.S. (m/z) 376 and 372 ($M^+$, 0%), 295 & 293 ($M^+$- Br, 11%), 236 ($M^+$- HBr - $OCH_3$, 12.9%), 261 (372- HBr - $OCH_3$, 24.6%), 235 &255 ($M^+$- Br - $CO_2CH_3$, 6.0%), 214 ($M^+$- $Br_2$, 1.7%), 183 (214- $OCH_3$, 22.0%), 171 (214- $(CH_2)_2CH_3$, 19.90%), 155 ($M^+$- $CO_2CH_3$, 16.1%), 154 (214- $CH_2$=CH$(OH)_2$, 32.4%), 129 ($M^+$ - $CH_2$=C$(CO_2CH_3)$, 81.7%), 81 ($^{\cdot}$Br, 100%).

**Treatment of the dibromo ester 113 with:**

**1. $d_5$-Pyridine.**

The dibromo compound **113** (75 mg) was treated with pyridine (3 ml) and the mixture was allowed to stand at room temperature for 20 to 30 min. with occasional shaking. The solvent was removed in *vacuo* to give unchanged starting material **113**.

**2. NaH/ THF.**

To the dibromo compound **55** (0.2 g, 0.53 mmol) in dry THF (5 ml) at room temperature under an atmosphere of nitrogen, was added NaH [114] (0.013 g, 0.53 mmol) with vigorous stirring. The reaction mixture was then heated under reflux for 1 hr after which time the mixture was cooled to 0 $^0$C and acidified with aq. HCl (1M). The solution was extracted with EtOAc (25 ml) and the organic layer was then washed with brine, dried over anhydrous $MgSO_4$, filtration and solvent removal in *vacuo* gave mainly the debrominated ester **112** [identified by comparison of the $^1$H nmr spectrum of an authentic sample] together with minor components yet to be identified [$^1$H nmr signals at $\delta$ 5.1 (m), 3.45 (m)].

**3. KOAc/ EtOH.**

To a solution of the dibromo compound **113** (0.1 g, 0.26 mmol) in absolute ethanol, was added with vigorous stirring KOAc (0.07 g,

0.78 mmol) in ethanol (10 ml). [115] The reaction mixture was refluxed for 15 hrs and then stirred at room temperature for 15 min. After evaporation of the ethanol, the semi-solid residue was treated with water, extracted with $CHCl_3$ (20 ml), dried over anhydrous $MgSO_4$, filtration and solvent removal in *vacuo* gave an oil (85 mg) containing unchanged starting material 55 together with the debrominated alkene 55 [identified by comparison of the $^1H$ nmr spectrum of an authentic sample].

**4. $K_2CO_3$/ $CH_3OH$.**

The dibromo compound **113** (0.3 g, 0.79 mmol) and $K_2CO_3$ in dry methanol, were stirred at room temperature for 48 hrs. [67, 68, 116] Dilution with $H_2O$ and extraction with $CHCl_3$ (30 ml) gave an oily product, which was shown by TLC and $^1H$ nmr to be unreacted starting material **113**. When the reaction mixture was refluxed, a complex mixture was obtained. This was not investigated any further.

**5. $Ag_2O$/DMF.**

A mixture of the ester **113** (0.1 g, 0.26 mmol) in DMF (10 ml) and $Ag_2O$ (0.06 g, 0.26 mmol) was stirred at room temperature for 24 hrs. The reaction mixture was eluted with $CHCl_3$ (10 ml), the solution filtered and the filtrate evaporated under reduced pressure to give unreacted starting material ($^1H$ nmr).

Similar results were obtained when $Al_2O_3$ was used instead of silver oxide.

**6. t-BuLi/ THF:**

To the dibromo compound **113** (0.1 g, 0.26 mmol) in dry THF (10 ml) at -78 $^0$C under an atmosphere of nitrogen, was added dropwise *tert*-butyllithium (0.012 ml, 2.6 M) in THF. When the addition was complete, the resulting mixture was stirred at the above temperature for an additional 30 min. after which time aqueous HCl (1M) was rapidly added to the solution. This was extracted with $CHCl_3$ (20 ml) and the organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$. Filtration and solvent removal *in vacuo* gave unreacted starting material ($^1$H nmr spectrum).

**7. DBU/ THF. (The vinyl bromide 114).**

To the dibromo compound **113** (0.3 g, 0.8 mmol) in dry THF (5 ml) at -78 $^0$C under an atmosphere of nitrogen, was added dropwise DBU [116] (0.12 g, 0.8 mmol) in THF. When the addition was complete, the resulting mixture was stirred at the same temperature for an additional 1 hr. after which time aqueous HCl (1M) was rapidly added to the solution. Stirring was continued for a few minutes during which time the reaction mixture was warmed to room temperature. The solution was extracted with $CHCl_3$ (30 ml) and the organic layer was then separated, washed with brine, dried over anhydrous $MgSO_4$, filtration and solvent removal *in vacuo* gave the vinyl bromide **114** as a colourless oil (0.21 g, 89% ).

I.R $\upsilon_{max}(CCl_4)$ 3420; 1740, 1725 (C=O); 1630 (C=C); 1595, 1435, 1230 $cm^{-1}$

$^1$H n.m.r. ($CDCl_3$) $\delta$ 7.65 (s, 1H, H-9), 3.72 (s, 3H, $OCH_3$), 3.60 (s, 3H, $OCH_3$), 3.5 (m, 3H, H-3), 2.6 (ABX, $J_{AB}$ 15.3 Hz, $J_{AX}$ 6.5 Hz, $J_{BX}$ 8.5 Hz, 2H, H-2), 1.2-1.5 (m, 4H, H-4, H-5), 0.85 (br t, 3H, H-6). [characteristic signals for **112** were also apparent eg. $\delta$ 6.2, 5.52 and $\delta$ 3.0].

$^{13}$C n.m.r. ($CDCl_3$) 172.68, 164.26 (C=O), 138.55 (C=C), 124.31 (CH), 51.90, 51.62 (-$OCH_3$), 37.59 (CH), 37.50, 34.87, 20.22 ($CH_2$), 13.95 ($CH_3$).

M.S. (m/z) 294 and 292 ($M^+$), 263 and 261 ($M^+$- $OCH_3$, 5.5%), 213 ($M^+$- Br, 100%), 129 ($M^+$- $C_4H_4BrO_2$, 7.9%).

**8. NaOMe/MeOH**. (Formation of the bromomethycyclopropane **107**).

To a stirring solution of the dibromo compound **113** (0.35 g, 0.94 mmol) in dry methanol (10 ml) under an atmosphere of nitrogen, was added at 0 $^0$C NaOMe (0.05 g, 0.94 mmol). [117] The reaction mixture was then heated under reflux for 1 hr after which time the mixture was cooled to room temperature for 30 min. After evaporation of the methanol, the semi-solid was treated with $H_2O$, extracted with $CHCl_3$ (25 ml), dried over anhydrous $MgSO_4$, filtered and the filtrate was evaporated under reduced pressure to give an oil. This was shown by TLC in EtOAc/ petroleum ether

(1:2) to be a mixture of at least three (3) components. Preparative TLC in the same solvent system gave:

(*a*) Fraction (1), Rf.0.7 (0.12 g) containing a mixture of the vinyl bromide **114**, traces of the alkene **112** [$^1$H and $^{13}$C nmr as described before] and the bromomethylcyclopropane **107**.

I.R $\upsilon_{max}(CCl_4)$ 2950; 1730, 1740 (C=O), 1595 (C=C) $cm^{-1}$

$^1$H n.m.r. ($CDCl_3$) δ 4.3 & 3.75 (ABq, $J_{AB}$ 10.5 Hz, 2H, -C$H_2$Br), 3.77, 3.73 (s, each 3H, -$OCH_3$), 2.65 (d, *J* 6.86 Hz, 1H, H-2), 1.88 (m, 1H, H-3), 1.29 (m, 4H, $CH_2$), 0.83 (br t, 3H, -$CH_3$).

$^{13}$C n.m.r. ($CDCl_3$) 172.20, 170.59 (C=O), 137.81 (-C-), 52.72, 52.25 ($OCH_3$), 32.12 (-$CH_2$Br-), 40.21, 31.91 (-CH-), 28.77, 21.98 (-$CH_2$-), 13.65 (-$CH_3$-).

M.S. (m/z) 294 and 292 ($M^+$), 213 ($M^+$- Br, 100%), 182 ($M^+$- Br - $OCH_3$, 3.0%), 181 (182.- H, 23.3% ).

(*b*) Fraction (2), Rf. 0.45 consisting mainly of the vinyl ether compound **115** (0.12 g, 52% ) as a colourless oil.

I.R $\upsilon_{max}(CCl_4)$ 2950; 1740, 1710 (C=O); 1640 (C=C) $cm^{-1}$

$^1$H n.m.r. ($CDCl_3$) δ 7.30 (s, 1H, H-9), 3.77 (s, 3H, $OCH_3$), 3.66 (s, 3H, $OCH_3$), 3.59 (s, 3H, $OCH_3$), 3.0 (m, 3H, H-3), 2.6 (ABX, $J_{AB}$ 15.2 Hz, $J_{AX}$ 6.5 Hz, $J_{BX}$ 8.1 Hz, 2H, H-2), 1.1-1.5 (m, 4H, H-4, H-5), 0.85 (t, *J* 7.0 Hz, 3H, H-6).

$^{13}$C n.m.r. ($CDCl_3$) 173.54, 168.17 (C=O), 159.91 (CH), 111.62 (C=C), 124.31 (CH), 61.43, 51.50, 51.29 (-$OCH_3$), 37.83, 35.09 ($CH_2$), 31.83 (CH), 20.43 ($CH_2$), 13.73 ($CH_3$).

M.S. (m/z) 244 ($M^+$), 213 ($M^+$- $OCH_3$, 18.7%), 212 ($M^+$- $CH_3OH$, 5.60%), 184 ($M^+$- $CH_3OH$- CO, 34.5%), 152 (184- $CH_3OH$, 24.5%), 75 ($M^+$- $C_3H_7O_2$, 100%).

Mass measurement: Found $M^+$ 244.1309 $C_{12}H_{20}O_5$ requires 244.2878.

**Preparation of mesaconic acid 116b .** [118]

A mixture of citraconic anhydride (100 g, 0.89 mol), water (100 ml) and dilute $HNO_3$ (150 ml) is evaporated in 500 ml Erlenmeyer flask until the appearance of red fumes. The solution is cooled and the mesaconic acid is collected on a filter. The mother liquor is evaporated to 150 ml, cooled and crystalline solid which separates is collected on a filter. Further concentration of the mother liquor to 50 ml yields more product. The entire product is recrystallised from $H_2O$ (100 ml), the yield of mesaconic acid **116b** is (75 g, 64%) 204-205 $^0C$.

**Esterification of mesaconic acid 116b .**

Mesaconic acid **116** (65 g, 0.5 ml) was treated with dry $CH_3OH$ (100 ml) and $CH_3COCl$ (17 ml) and the mixture was stirred at 0 $^0C$ for 2 hrs. After stirring overnight at room temperature, the solution was refluxed at 50 $^0C$ for 5 hrs and $CH_3OH$ evaporated under reduced pressure to give a yellow oil. Distillation gave dimethyl mesaconate **117** (62 g, 78%) as a colourless oil b.p. 80 $^0C$/0.01 mm.

$^1$H n.m.r. ($CDCl_3$) 90 MHz δ 6.75 (q, *J* 1.5 Hz, 1H, -C=CH-), 3.80 (s, 3H, $-OCH_3$), 3.75 (s, 3H, $-OCH_3$), 2.27 (01, *J* 1.5 Hz, 3H, $C=CCH_3$).

**Dimethyl bromomesaconate 118.**

Dimethyl mesaconate **117** (62 g, 0.39 mol) was refluxed with N-bromo Succinimide [119] (65 g, 0.42 mol) and $CCl_4$ (250 ml) for 42 hrs. The reaction mixture was then cooled, filtered, and the filtrate was evaporated under reduced pressure to give a semi-solid residue. This was treated with light petroleum (200 ml) and more succinimide was obtained as white precipitate. After filtration and removal of solvent, the product was distilled b.p. 120-127 $^0$C/0.1 mm to afford dimethyl bromomesaconate **118** (68 g, 83%) as a colourless oil.

$^1$H n.m.r. ($CDCl_3$) δ 6.8 (s, 1H, -C=CH-), 4.65 (s,2H, $-CH_2Br$), 3.84 (s, 3H, $CO_2CH_3$), 3.80 (s, 3H, $-CO_2CH_3$).

**Preparation of diazomethane .** [73]

In a flat bottom flask set for distillation was placed a solution of 30% aqueous NaOH and 2-ethoxyethanol (2.11 g) in dry ether (25 ml) and nitrosan (8 g, Ca.10 eq) in dry ether (50 ml) was slowly added at 0 $^0$C. The mixture was then magnetically stirred and warmed gradually to 50 $^0$C and the yellow distillate was collected in dry ice/acetone cooled receiver flask containing dry ether. The diazomethane (30%) in the distilled ethereal solution was dried over KOH pellets and used in the following reaction without any further determination.

**Addition of diazomethane to dimethyl bromomesaconate 118 .**

A solution of diazomethane (10 eq) in dry ether (50 ml) was added in small portions with occasional shaking, to dimethyl bromomesaconate 55 (0.2 g, 0,85 mmol) in ether (10 ml) at -70 $^{0}$C. The reaction mixture was allowed to warm to room temperature and was then left stirring overnight. Excess of diazomethane was removed with nitrogen and removal of the colourless solution by evaporation under reduced pressure gave an oily product (0.22 g). This was shown by TLC and $^{1}$H nmr to be a complex mixture. Extensive preparative TLC failed to separate the components and the reaction was not investigated further.

**Preparation of dimethyl acetoxymethylmesaconate 119 .**

Dimethyl bromomesaconate **118** (66 g, 0.28 mol) was stirred under reflux with KOAc (35 g, 0.36 mol) in absolute ethanol (150 ml) for a period of 1 hr. [107] The mixture was then cooled and ethanol was evaporated under reduced pressure to give a brown residue. This was treated with $CHCl_3$ to give a precipitate, which was filtered off and the filtrate was distilled off under reduced pressure. The resulting oily product was chromatographed on a silica gel column eluting with light petrol/EtOAc using the ratio 9:1, 7:1, 5:1, 3:1, and 1:1. The fractions were assayed by TLC using petrol/EtOAc (1:1) and the appropriate fractions with the ratio (3:1) were combined and the solvents evaporated to afford the acetoxy derivative **119** as a colourless oil (35 g, 58%) (Found

C,50.19, H, 5.55, $C_9H_{12}O_6$ requires C, 50.00, H, 5.55%).

I.R $\upsilon_{max}(CCl_4)$ 2980, 1744, 1735, 1655, (C=C), 1035 $cm^{-1}$

$^1$H n.m.r. ($CDCl_3$) $\delta$ 6.78 (s, 1H, -C=CH-), 5.05 (s, 2H, $-OCH_2-$), 3,69 (s, 3H, $-OCH_3$), 3.66 (s, 3H, $-OCH_3$), 1.89 (s, 3H, $-COCH_3$).

$^{13}$C n.m.r. ($CDCl_3$) 169.84, 165.27, 164.61 (C=O), 139.58 (C=CH), 130.58 (CH), 57.45 ($CH_2$), 52.41, 58.86 ($OCH_3$), 20.25 ($CH_3$).

M.S. (m/z) 216 ($M^+$), 173 ($M^+$- $COCH_3$, 11.3%), 156($M^+$ - $CH_3CO_2H$, 35.6%), 142 ($M^+$ - $CH_3CO_2CH_3$, 37.1%), 112 ($M^+$ - $CH_2OCOCH_3$ - $OCH_3$, 11.3%), 42 (100%).

**Preparation of *trans*-3,4-dicarbomethoxy-3-acetoxymethyl-1-pyrazoline 120 .**

A solution of diazomethane (10 eq) in ether (50 ml) was added with occasional shaking dimethyl acetoxymesaconate 55 (0.45 g, 2.08 mmol) in dry ether at 0 $^0$C. The reaction mixture was allowed to warm to room temperature and was then left stirring overnight. Excess of diazomethane was removed with nitrogen and the ether solution was evaporated under reduced pressure to give an oil. This was shown by TLC in EtOAc:Petrol (3:1) to be a mixture of three components. Preparative TLC in the same solvent gave *trans*-3,4- dicarbomethoxy-3-acetoxymethyl-1-pyrazoline **120** Rf 0.55 (0.46 g, 85%) as a colourless oil.

I.R. $\upsilon_{max}(CCl_4)$ 2958, 1760, 1750, 1438, 1222, 1040 $cm^{-1}$

$^1$H n.m.r. ($CDCl_3$) δ 4.97 (ABX, 2H, H-5), 4.76 & 4.70 ($AB_q$, $J_{AB}$ 10.2 Hz, 2H, $-OCH_2-$), 3.83 (s, 3H, $-OCH_3$), 3.63 (s, 3H, $-OCH_3$), 3.55 & 3.50 (dd, *J* 8.03 Hz, J 8.05 Hz, 1H, -COCH-), 1.89 (s, 3H, $-COCH_3$).

$^{13}$C n.m.r. ( $CDCl_3$) δ 169.56, 167.26 , (C=O), 98.56 (=C-), 80.84, 61,95 ($CH_2$), 53.59, 52.51 ($OCH_3$), 41.35 (-CH-), 20.23 ($CH_3$).

M.S. (m/z) 258 ($M^+$), 227 ($M^+ - OCH_3$, 1.9%), 258 ($M^+$), 199 ($M^+ - N_2 - OCH_3$, 4.2%), 188 ( $M^+ - N_2 - CH_2=C=O$, 4.7%), 185 ($M^+ - CH_2OCOCH_3$, 30.9%), 171 ($M^+ - N_2 - CO_2CH_3$, 87.5%), 170 ($M^+ - N_2 - CH_3CO_2H$, 72.7%), 157 (185 - $N_2$, 79%), 129 (188 - $CO_2CH_3$, 100%).

## Treatment of the pyrazoline 120 with CAN in acetone.

The pyrazoline **120** (0.1 g, 0.39 mmol) in acetone was treated with a small amount of ceric ammonium nitrate [74] (CAN) at 0 $^0$C. The reaction mixture was stirred at this temperature for a period of 3 days, after which time the solution was filtered and the filtrate was evaporated under reduced pressure to give unchanged starting material **120**. When the reaction mixture was refluxed a

complex mixture was obtained, distillation and thin layer chromatography both failed to yield identifiable products and the behaviour of the residue indicated decomposition. This reaction was not investigated further.

**Preparation of 1,2-dicarbomethoxy - 2 - acetoxymethyl--cyclopropane 121 .**

*trans*- 3,4- Dicarbomethoxy -3- acetoxymethyl-1- pyrazoline **120** (0.5 g, 1.9 mmol) was irradiated in n-hexane (50 ml) at room temperature using a cylindrical reaction vessel consisting of concentrical pyrex tubes. The inner part contained the $H_2O$ condenser, the outer (250 ml size) contained the reaction mixture. A (125 W, 360 nm) Hanovia lamp was placed in the centre of the vessel. The reaction mixture was stirred at room temperature and the nitrogen evolution was monitored and was complete after 1.5 hrs. The hexane was evaporated under reduced pressure, and the residue was purified by flash chromatography eluting with EtOAc: Petrol with the ratio (1:4) respectively to give *trans* 1,2-dicarbomethoxy-1-acetoxymethylcyclopropane **121** (0.39 g, 88%) as a colourless oil. (Found C, 52.39; H, 6.25 $C_{10}H_{14}O_6$ requires C, 52.17; H, 6.08%).

I.R $\upsilon_{max}$ ($CCl_4$) 2930,1742,1735,1250,1040 $cm^{-1}$.

$^1$H n.m.r. ($CDCl_3$) $\delta$ 4.75 & 4.14 ($AB_q$, $J_{AB}$ 11.9 Hz,2H,-$CH_2O$-), 3.67 (s, 3H, -$OCH_3$), 3.65 (s, 3H, -$OCH_3$), 2.48 (dd, *J*8.5 Hz, 6.9

Hz,1H, H-2), 1.95 (s, 3H, -$OCOCH_3$), 1.57 (ABX, $J_{AB}$4.64Hz, $J_{AX}$8.58 Hz, $J_{BX}$6.83Hz, 2H, H-3).

$^{13}$Cnmr ($CDCl_3$) δ 171.27,170.28,170.07 (C=O), 61.78 ($OCH_2$), 52.62,52.20 (-$OCH_3$), 30.34 (-C-), 25.99 (CH), 20.55 (-$CH_3$), 19.76 ($CH_2$).

M.S (m/z) 230 ($M^+$), 171 ($M^+$- $OCOCH_3$, 10%), 157 ($M^+$- $CH_2OCOCH_3$, 20.1%), 170 ($M^+$- $CH_3CO_2H$, 100%), 156 ($M^+$- $CH_3CO_2CH_3$, 6.7%), 125 (156 - $OCH_3$, 2.6%), 97(156 - $CO_2CH_3$, 9.9%).

**Preparation of Isopropyl Carbamate 123 .** [120]

A solution of isopropyl alcohol (12.02 g, 15.3 ml, 0.2 mol) in $CH_2Cl_2$ (125 ml) is placed in 500 ml three-necked flask equipped with a stirrer, a thermometer and an addition funnel, and sodium cynate (26 g, 0.4 mol) is added. The suspension is stirred as slowly as possible (Ca. 120 r.p.m); while $CF_3CO_2H$ (48 g, 31.2 ml, 0.42 mol) is added dropwise at a rapid rate. The temperature slowly rises to about 37 $^0$C after three quarters of the $CF_3CO_2H$ has been added (Ca 7 min.). At this point, the mixture is cooled to 55-35 $^0$C by brief immersion in an ice-water bath, then the addition is continued. When the addition of the acid is completed (10-12 mins total time), the temperature slowly rises to 40 $^0$C and then gradually subsides. Slow stirring is continued overnight at room temperature. The mixture is treated with $H_2O$ (35 ml) and stirred slowly for a few minutes.

The $CH_2Cl_2$ layer is decanted and the aqueous slurry is further extracted with two 150 ml portions of $CH_2Cl_2$. The combined organic extracts are washed with 5M NaOH (100 ml) and $H_2O$ (100 ml), dried over $MgSO_4$ and filtered. The solvent is removed by distillation at 30 $^0$C under reduced pressure to give isopropyl carbamate **123** as white needles (17 g, 82%) m. p. 91-93 $^0$C [lit. [121] m.p. 92-93 $^0$C].

$^1$H n.m.r. ($CDCl_3$) 90 MHz $\delta$ 4.85 (m, 3H, -OCH-, -$NH_2$), 1.20 (d, *J* 9 Hz, 6H, -$C(CH_3)_2$ ).

**Attempted preparation of Isopropyl-N-(butylaminomethyl)Carbamate hydrochloride 124 .**

Into a solution of butylamine hydrochloride (15.53 g, 0.14 mol) [prepared from the addition of HCl aq to $BuNH_2$ at 0 $^0C$, pH acidic] in $H_2O$ (30 ml), $CH_2O$ (38%, 14.2 g, 0.47 mol) and then a solution of isopropylcarbamate (14.46 g, 0.14 mol) in EtOH (30 ml) were added. The reaction mixture was heated at 30-40 $^0C$ with occasional shaking. After 30 min the reaction was concentrated under reduced pressure to give a white solid. Recrystallisation of the resulting product in petroleum ether gave N,N'-methylenebis-isopropylcarbamate **127** as white leaflets [80] (25 g, 81%), m.p. 150-151 $^0C$ (Found C, 49.90; H, 8.13; N, 12.76 $C_9H_{18}N_2O_4$ requires C, 50-00; H, 8.25; N, 12.84%).

LR $\upsilon_{max}$(KBr) 5525 (-NH), 2980, 1680, 1530 (-CONH) $cm^{-1}$

$^1$H n.m.r. ($CDCl_3$) $\delta$ 5.78 (m, 2H, -NH-), 4.95 (sep, 2H, -OCH-), 4.45 (t, *J* 6.0 Hz, 2H, $-NCH_2N-$), 1.21 (s, 6H, $CH_3$), 1.18 (s, 6H, $CH_3$).

$^{13}$C n.m.r. ($CDCl_3$) $\delta$ 156.42 (C=O), 68.49 (CH), 47.80 ($CH_2$), 22.03 ($CH_3$).

M.S. (m/z) 218 ($M^+$), 175 ($M^+$- ipr, 32.9%), 155 ($M^+ - C_3H_7 - CO_2$, 18.4%), 116 ($M^+ - CH_2{=}CHCH_3 - 42 - H_2O$, 17.2%), 89 ($M^+ -116 - CO_2$, 36%), 42 ($M^+$-176, 100%).

**Preparation of isopropyl N-(butylaminomethyl)Carbamate hydrochloride 124 .** [79]

Into a solution of butylamine hydrochloride (15.53 g, 0.14 mol) [prepared from the addition of HCl aq to $BuNH_2$ at 0 $^0C$, pH neutral] in EtOH (30 ml), $CH_2O$ (38%, 14.2, 0,47 mol) and then a solution of isopropylcarbamate (14.46 g, 0.14 mol) in EtOH (30 ml) were added with stirring at 35-40 $^0C$. The mixture was further stirred for 1 hr, followed by concentration under reduced pressure. Recrystallisation of the resulting residue from EtOH gave isopropyl N-[butylamino-methyl] Carbamate hydrochloride **124** (26.1 g, 83%) as white plates m.p. 147-149 $^0C$ [lit. [79] m.p 146-148 $^0C$].

**Preparation of isopropyl N-[(N-nitrosobutyl amino)methyl] carbamate 125.** [79]

Into a saturated aqueous solution of the previous obtained hydrochloride salt **124** (26.1 g, 0.17 mol), (35%) HCl (5 ml) was added at 20-25 $^0C$ and then a solution of $NaNO_2$ (10 g, 0.15 mol) in $H_2O$ (30 ml) was added dropwise with vigorous stirring. This was continued at the above temperature for additional 30 min. After cooling, the product was isolated from the reaction mixture by filtration as yellowish solid. This was recrystallised from petroleum ether to afford isopropyl N-[(N-nitrosobutylamino)methyl]Carbamate **125** as white needles

(20.68 g, 82%) 52-54 $^{0}$C [lit. [79] m.p. 53-55 $^{0}$C]. (Found C, 49.72; H, 8.83; N, 19.37 $C_9H_{19}N_3O_3$ requires C, 49.75; H, 8.81; N, 19.34%).

I.R. $\upsilon_{max}$ (KBr) 5514 (-NH-), 1690, 1546 (-CONH) $cm^{-1}$

$^1$H n.m.r. ($CDCl_3$) δ 5.9 (m, 1H, *syn* -NH-), 5.7 ( m, 1H, *anti* -NH), 5.5 (d, *J* 7.5 Hz, 2H, *syn* -$NHCH_2N$=), 4.9 (m, 2H, *syn* & *anti* -$OCH(CH_3)_2$), 4.71 (d, *J* 5 Hz, 2H, *anti* -$NHCH_2N$=), 4.28 (t, *J* 7 Hz, 2H, *syn* -$NCH_2$-), 3.61 (t, *J* 7.5 Hz, 2H, *anti* -$NCH_2$-), 1.75 (m, 2H, *syn* $CH_2$), 1.4 (m, 4H, *syn* & *anti* $CH_2$), 1.18 (m, 4H, *syn* & *anti* $CH_2$), 1.17 (br d, 6H, -$CH_3$), 1.16 (br d, 6H, -$CH_3$), 0.90 (t, *J* 7.5 Hz, 3H, *syn* -$CH_3$), 0.87 (t, *J* 7.5 Hz, 3H, *anti* -$CH_3$).

$^{13}$C n.m.r. ( $CDCl_3$):

*Syn* Compound :

δ 156.06 (C=O), 69.1 (OCH), 58.02 (-$NHCH_2N$-), 52.2 (-$NH\underline{C}H_2CH_2$-), 30.3 (-$NHCH_2\underline{C}H_2$- ), 21.86 (-$CH(\underline{C}H_3)_2$), 20.23 (-$CH_2$-) , 13.46 (-$CH_3$).

*Anti* Compound :

δ 155.86 (C=O), 68.9 (-OCH-), 49.1 (-$NHCH_2N$-), 42.6 (-$NH\underline{C}H_2CH_2$- ), 28.1 (-$NHCH_2\underline{C}H_2$- ), 21.56 (-$CH(\underline{C}H_3)_2$), 19.52 (-$CH_2$-) , 13.37 (-$CH_3$).

M.S. (m/z) 217 ($M^+$), 116 ($M^+$ - $CH_2$=$CCH_3$ - $CO_2$ - $CH_3$, 53.5%), 57 (Bu, 13.9%), 43 (100%).

**Preparation of diazobutane 126 .**[79]

In a flat bottom flask set for distillation was placed a solution of KOH (5 g) and n-butanol (20 ml) in dry ether (25 ml). Isopropyl N-[(N-nitrosopropylamino)methyl] Carbamate **125** (10 g, ca. 10 eq) in dry ether (50 ml) was then added at 0 $^0$C and the mixture was warmed gradually to 60 $^0$C. The yellow distillate was collected in a dry ice /acetone cooled receiver flask containing dry ether. The diazobutane (30%) in the distilled ethereal solution was dried over KOH pellets and used in the next reaction without any further determination.

***trans* 3,4-Dicarbomethoxy-3- acetoxymethyl - 5- propyl -1- pyrazoline 128 .**

A solution of diazobutane **126** (8 eq) in dry ether (50 ml) was added [76] with occasional shaking to dimethyl acetoxymesaconate 55 (0.5 g, 2.31 mmol) in dry ether at 0 $^0$C. The reaction mixture was allowed to warm to room temperature and was then left stirring overnight. Excess of diazobutane was removed with a stream of nitrogen and the ether solution was evaporated under reduced pressure to give the desired pyrazoline **128** (0.63 g, 91%) as a colourless oil.

I.R. $\upsilon_{max}(CCl_4)$ 2960, 1750, 1745, 1455, 1432, 1340 $cm^{-1}$

$^1$H n.m.r. ($CDCl_3$) $\delta$ 4.99 (m, 1H, H-5) , 4.69 & 4.58 ($AB_q$, $J_{AB}$ 12.07 Hz, 2H, -$OCH_2$-), 3.86 (s, 3H, -$OCH_3$), 3.64 (s, 3H, -

$OCH_3$), 3.14 (d, *J* 8.2 Hz, 1H, H-4), 1.90 (s, 3H, $-OCOCH_3$), 1.65 (m, 4H, $-CH_2-$), 0.98 (t, *J*7 Hz, 3H, $-CH_3$).

$^{13}$C n.m.r. ( $CDCl_3$) δ 169.69, 169.55, 167.55 , (C=O), 98.84 (=C-), 93.36 (CH), 61.88 ($CH_2$), 53.61, 52.48 ($OCH_3$), 47.24 (CH), 35.13 ($CH_2$), 20.32 ($CH_3$), 19.64 ($CH_2$), 13.89 ($CH_3$).

M.S. (m/z) 300 ($M^+$), 272 ($M^+ - N_2$, 0.2%), 269 ($M^+ - OCH_3$, 0%), ($M^+ - N_2 - C_3H_6$, 1.0%), 229 ($M^+ - N_2 - C_3H_7$, 0.8%), 227 ($M^+ - CH_2OCOCH_3$, 9.3%), 213 ($M^+ - N_2 - CO_2CH_3$, 29.4%), 199 (272 - $CH_2CO_2CH_3$, 9.0%), 198 (272 - $CH_3CO_2CH_3$, 6.4%), 167 (198 - $OCH_3$, 5.1%), 139 (198 - $CO_2CH_3$, 19.6%), 93 (100%).

Mass measurement: Found $M^+$ 300.1357 $C_{13}H_2N_2O_6$ requires 300.3117.

**Preparation of 1,2-dicarbomethoxy-2-acetoxymethyl-3-n.propylcyclopropane 129 .**

**1. By photolysis.**

*trans*-3,4-Dicarbomethoxy-3-acetoxymethyl-5-propyl-1-pyrazoline **128** (0.5 g, 1.66 mmol) was irradiated in n-hexane (50 ml) at room temperature using a cylindrical reaction vessel consisting of concentrical pyrex tubes. The inner part contained the $H_2O$ condenser, the outer (250 ml size) contained the reaction mixture. A (125 W, 360 nm) Hanovia lamp [76] was placed in the centre of the vessel. The reaction mixture was stirred at room temperature and the nitrogen evolution was monitored and was complete after 1 hr 45 min. The hexane was evaporated under reduced pressure to give *trans* 1,2-dicarbomethoxy-1-acetoxymethylcyclopropane **129** (0.43 g, 95 %) as a colourless oil. (Found C, 57.46; H, 7.58 $C_{13}H_{20}O_6$ requires C, 57.35; H, 7.35%).

I.R $\upsilon_{max}$ ($CCl_4$) 2960,1744,1755, 1435, 1250,1035 $cm^{-1}$.

$^1$H n.m.r. ($CDCl_3$) $\delta$ 4.91 & 4.09 ($AB_q$, $J_{AB}$ 11.85 Hz, 2H, $-CH_2O-$), 3.71 (s, 3H, $-OCH_3$), 3.66 (s, 3H, $-OCH_3$), 2.54 (d, *J* 7 Hz, 1H, H-2), 1.95 (s, 3H, $-OCOCH_3$), 1.29 (m, 5H, $-CCH_2C-$, H-3), 0.85 (t, *J* 7 Hz, 3H, $-CH_3$).

$^{13}$Cnmr ($CDCl_3$) δ 170.71,170.46,169.65 (C=O), 63.10 (-$CH_2O$-), 52.54, 52.14 (-$OCH_3$), 36.71 (-C-), 32.86, 30.05 (-CH-), 28.09, 21.92 (-$CH_2$-), 20.73, 13.45 (-$CH_3$).

M.S (m/z) 272 ($M^+$), 213 ($M^+$- $OCOCH_3$, 7.0%), 199 ($M^+$- $CH_2OCOCH_3$, 8.4%), 198 ($M^+$- $CH_3CO_2CH_3$, 2.3%), 180 (198 - $H_2O$, 12.4%), 139 (198 - $CO_2CH_3$, 10%), 155 (198 - $(CH_2)_2CH_3$, 5.3%), 43 (100%).

**2. By thermal decomposition in xylene.**

The pyrazoline **128** (0.1 g, 0.55 mmol) in xylene [75] (15 ml) was stirred under nitrogen for 12 hrs at 120 $^0$C. Stirring was continued at 160 $^0$C for 3 hrs and xylene was evaporated under reduced pressure to give an oily residue. This was shown by $^1$H nmr and TLC in EtOAc:light petroleum (1:1) to be a mixture of the cyclopropane **129** and other components. Distillation followed by preparative TLC gave the desired cyclopropane **129**, Rf. 0.7 (45 mg, 50%).

IR and $^1$H nmr spectra are identical to those of the photolysis product described above.

**Deacetylation of the acetate 121 .**

**1.** Using $NH_3$/MeOH :

*trans* -1,2-Dicarbomethoxy-1-acetoxymethylcyclopropane **121** (0.2 g, 0.86 mmol) in methanol (25 ml) was cooled in an ice/salt bath and a fairly rapid stream of dry ammonia (through soda lime) was passed into the solution for 15 min. The reaction mixture was left standing at 10 $^0$C for 40 hrs and the solution was concentrated to give an oily product. This was shown by TLC and $^1$H nmr to be a complex mixture containing traces of the starting material and other unidentified components.

**2.** Using $NaBH_4$/MeOH :

To a stirred solution of the acetoxy compound **121** (80 mg, 0.35 mmol) in methanol (20 ml) under nitrogen, was added a solution of $NaBH_4$ (15 mg, 0.39 mmol) [124] in methanol (10 ml). After stirring at 60 $^0$C for 30 min., the reaction mixture was allowed to cool and the methanol was evaporated under reduced pressure at room temperature. The residue was treated with (1M) HCl and then extracted with EtOAc (30 ml). The combined extracts were washed with brine, dried over $MgSO_4$, filtered and the filtrate was evaporated under reduced pressure to give the lactone **132** (47.7 mg, 88%).

I.R $\upsilon_{max}$ ($CCl_4$) 2960,1790,1742, 1440, 1035 $cm^{-1}$.

$^1$H n.m.r. ($CDCl_3$) δ 4.73 (dd, *J* 9.6 Hz, *J* 0.95 Hz, 1H, H-4β), 4.28 (ddd, *J* 9.6 Hz, *J* 0.9 Hz, *J* 0.3 Hz, 1H, H-4α), 3.76 (s, 3H, -$OCH_3$), 2.57 (ddd, *J* 9.56 Hz, *J* 4.45 Hz, *J* 0.9 Hz, 1H, H-2), 2.05 (dddd, *J* 9.56 Hz, *J* 4.45 Hz, *J* 0.95 Hz, *J* 0.3 Hz, 1H, H-3β), 1.33 (dd, $J_1 = J_2 = 4.45$ Hz, 1H, H-3α).

$^{13}$C nmr ($CDCl_3$) δ 170.11,168.13 (C=O), 68.34 (-$CH_2O$-), 52.69 (-$OCH_3$), 30.34 (-C-), 26.99 (-CH-),19.34 (-$CH_2$-).

M.S (m/z) 156 ($M^+$), 128 ($M^+$- CO, 94.6%), 125 ($M^+$- $OCH_3$, 51.6%), 112 ($M^+$- $CO_2$, 15.3%), 97 ($M^+$ - $CO_2CH_3$, 20.9%), 56 (128 - $CH_3OH$, 100%).

Mass measurement: Found $M^+$ 156.0432, $C_7H_8O_4$ requires 156.1381.

**3.** Using analar MgO/MeOH :

To a stirred solution of the acetoxy compound **121** (120 mg, 0.5 mmol) in methanol (15 ml) was added analar MgO (150 mg, 3.7 mmol). The reaction mixture was stirred at room temperature for 24 hrs, MgO was filtered and concentrated to dryness. The residue was slurried in methanol, filtered and the filtrate was evaporated under reduced pressure to give an oily product. This was shown by TLC to be a mixture of fats and several polar components.

Similar results were obtained when *trans* 1,2-dicarbomethoxy-1-acetoxymethyl-3-n,propylcyclopropane **129** was used under the same conditions.

**4.** Using MgO [Merck 5866] /MeOH : [87, 88]

To a stirred solution of *trans*-1,2-dicarbomethoxy-1-acetoxymethylcyclopropane **121** (0.26 g, 1.1 mmol) in methanol (20 ml) was added MgO [Merck 5866] (0.5 g, 11 mmol). Stirring was continued at room temperature for 48 hrs after which time TLC in EtOAc/light petroleum (1:1) indicated the complete disappearance of the starting material. MgO was filtered and the filtrate was evaporated under reduced pressure to give a semi-solid product (0.25 g) containing the alcohol **131** together with the lactone **132**.

I.R $\upsilon_{max}$ ($CCl_4$) 3620 (free OH), 2960, 2930, 1792 (lactone C=O), 1740,1250,1040 $cm^{-1}$.

The alcohol **131**:

$^1$H n.m.r. ($CCl_4$) δ 4.25 (br d, *J* 12 Hz, 1H, -CHOH-), 3.91 (s, 3H, $-OCH_3$), 3.89 (s, 3H, $-OCH_3$), 3.75 (br d, *J* 12 Hz, 1H, -CHOH), 2.98 (dd, *J* 8.5 Hz, *J* 6.7 Hz, 1H, H-2), 2.35 (m, 1H, $-CH_2OH$), 1.70 (ABX, $J_{AB}$ 4.3 Hz, $J_{AX}$ 8.57 Hz, $J_{BX}$ 6.67 Hz, 2H, H-3).

$^{13}$C nmr ($CCl_4$) δ 172.51,170.16 (C=O), 59.83 ($-CH_2O-$), 52.58, 52.39 ($-OCH_3$), 34.29 (-C-,singlet), 26.32 (-CH-), 19.12 ($-CH_2$).

M.S (m/z) 188 ($M^+$,1.5%), 170 ($M^+$- $H_2O$, 14.3%), 157 ($M^+$- $CH_2OH$, 46%), 156 ($M^+$- $CH_3OH$, 10.2%), 129 ($M^+$- $CO_2CH_3$, 71.3%), 128 (156- CO, 100%), 111(170 - $CO_2CH_3$, 32.5%).

Mass measurement: Found $M^+$ 188.0683, $C_8H_{12}O_5$ requires 188.1803.

The lactone **132** :

$^1$H n.m.r. ($CCl_4$) δ 4.85 (dd, *J* 9.5 Hz, 0.94 HZ, IH, H-4β), 4.38 (ddd, *J* 9.6 Hz, *J* 0.9 Hz, *J* 0.3 Hz, 1H, H-4α), 3.83 (s, 3H, -$OCH_3$), 2.65 (ddd, *J* 9.5 Hz, *J* 4.4 Hz,*J* 0.9 Hz, 1H, H-2), 2.05 (dddd, *J* 9.54 Hz, *J* 4.6 Hz, *J* 0.95 Hz, *J* 0.3 Hz, 1H, H-3β), 1.47 (dd, $J_1$ = $J_2$ = 4.45 Hz, 1H, H-3α).

$^{13}$Cnmr ($CCl_4$) δ 170.11,168.13 (C=O), 67.76 (-$CH_2O$-), 52.65 (-$OCH_3$), 30.29 (-C-, singlet), 27.12 (-CH-),19.37 (-$CH_2$-).

M.S (m/z) The peaks for **132** are included above and are identical to the previously obtained lactone.

Mass measurement: Found $M^+$ 156.0410, $C_7H_8O_4$ requires 156.1381.

**Diisopropyl mesaconate 133 .**

To a mixture of mesaconic acid **116** (10 g, 0.08 mol) and *p*TSA (0.1 g) in toluene (35 ml), was added dropwise while stirring at room temperature, isopropanol [122] (14 g, 0.23 mol). The reaction mixture was refluxed for 48 hrs and after cooling the solution was washed with $H_2O$ (25 ml), then with aqueous $Na_2CO_3$ and finally with two (25 ml) portion of $H_2O$. The organic phase (pH neutral) was dried over anhydrous $MgSO_4$, filtered and the filtrate was evaporated under reduced pressure to give the diisopropyl ester **133** (9.1 g, 55%) as a colourless oil.

I.R $\upsilon_{max}$ ($CCl_4$) 2990, 1720, 1650, 1260, 1105, 1038 $cm^{-1}$.

$^1$H n.m.r. ($CDCl_3$) δ 6.6 (q, *J* 1.6 Hz, 1H, -C=CH-), 5.03 (m, 2H, -OCH-), 2.2 (d, *J* 1.6 Hz, 3H, -C=CC$\underline{H}_3$), 1.23 (d, *J* 6.25 Hz, 6H, -O($CH_3$)$_2$), 1.21 (d, *J* 6.25 Hz, 6H, -O($CH_3$)$_2$).

$^{13}$C nmr ($CDCl_3$) δ 166.53, 165.41 (C=O), 143.66 (-$\underline{C}$=CH-), 126.75 (-C=$\underline{C}$H-), 68.95, 67.94 (-CHO-), 21.68, 21.58 (-$CH_3$), 14.08 (-$CH_3$).

M.S (m/z) 214 ($M^+$), 155 ($M^+$- $OCH(CH_3)_2$, 0.2%), 127 ($M^+$-$CO_2CH(CH_3)_2$, 0.1%), 98 (100%).

**Diisopropyl bromomesaconate 134.**

Diisopropyl mesaconate **133** (4.3 g, 20 mmol) was refluxed with N-bromosuccinimide (7 g, 4 mmol), DBP (0.1 g) [123] and $CCl_4$ (35 ml) for 46 hrs. The reaction mixture was then cooled, filtered, and the filtrate was evaporated under reduced pressure to give a semi-solid residue. This was treated with light petroleum (50 ml) and more succinimide was obtained as white precipitate. After filtration and removal of solvent, the product was distilled b.p. 130 $^0$C/0.08 mm to afford diisopropyl bromomesaconate **134** (5.85 g, 99%) as a colourless oil.

I.R $\upsilon_{max}$ ($CCl_4$) 3020, 2690, 2400, 1725 (br), 1650 $cm^{-1}$.

$^1$H n.m.r. ($CDCl_3$) 90 MHz $\delta$ 6.75 (S, 1H, -C=CH-), 5.1 (m, 2H, -OCH-), 4.68 (s, 2H, -$CH_2$Br ), 1.35 (d, *J* 8.5 Hz, 12H, -O($CH_3$)$_2$)

M.S (m/z) 294 & 292 ($M^+$), 250 (292 - $CH{=}CH_2CH_3$, 1.8%), 233 (292- $OCH(CH_3)_2$, 2.2%), 170 ($M^+$- $(CH_3)_2CHBr$, 0.1%), 111 (170 - $OCH(CH_3)_2$, 12.2%), 83 (170 - $CO_2CH(CH_3)_2$, 5.2%), 43 (100%).

**Addition of diazomethane to diisopropyl bromomesaconate 134.**

A solution of diazomethane (10 eq) in dry ether (75 ml) was added in small portions with occasional shaking, to diisopropyl bromomesaconate **229** (0.1 g, 0.34 mmol) in dry ether (10 ml) at -70 $^{0}$C. The reaction mixture was allowed to warm to room temperature and was then left stirring overnight. Excess of diazomethane was removed with nitrogen and removal of the colourless solution by evaporation under reduced pressure gave an oily product (0.1 g). After standing for a short time at room temperature the product was shown by TLC and $^{1}$H n.m.r. to be a complex mixture. Extensive preparative TLC failed to separate the components and the reaction was not investigated further.

**Diisopropyl acetoxymethylmesaconate 135.**

Diisopropyl bromomesaconate **134** (5.9 g, 19 mmol) was stirred under reflux with KOAc (2.3 g, 23 mmol) in absolute ethanol (50 ml) for a period of 1hr. The mixture was then cooled and ethanol was evaporated under reduced pressure to give a brown residue. This was treated with EtOAc to give a precipitate, which was filtered off through a short column of silica gel ($HF_{254}$) and the filtrate was evaporated under reduced pressure to give the acetoxy derivative **135** (3.5 g, 68%) as a colourless oil.

I.R $\upsilon_{max}$ ($CHCl_3$) 3020, 2990, 1730 (br C=O), 1660, 1250, 1100 $cm^{-1}$

$^1$H n.m.r. ($CDCl_3$) 90 MHz $\delta$ 6.8 (S, 1H, -C=CH-), 5.2 (S, 2H, -$OCH_2$-), 5.00 (m, 2H, -OCH-), 2.05 (S 3H, -$OCH_3$), 1.3 (d, *J* 7 Hz, 12H, -$C(CH_3)_2$).

***trans*-3,4-Dicarboisopropoxy-3-acetoxymethyl-1--pyrazoline 136 .**

A solution of diazomethane (10 eq) in ether (70 ml) was added with occasional shaking to diisopropyl acetoxymesaconate **135** (1.22 g, 0.45 mmol) in dry ether at -70 $^0$C. The reaction mixture was allowed to warm to room temperature and was then left stirring overnight. Excess of diazomethane was removed with nitrogen and the ether solution was evaporated under reduced

pressure to give *trans*-3,4-dicarboisopropoxy-3-acetoxymethyl-1-pyrazoline **136** (1.05 g, 74%) as a colourless oil.

I.R. $\upsilon_{max}(CCl_4)$ 3960, 3030, 2405, 1785, 1740, (br), 1680, 1520, 1220, 1105 $cm^{-1}$

$^1$H n.m.r. ($CDCl_3$) δ 5.15 (sep, 1H, -OCH-), 4.95 (d, *J* 8.9 Hz, 2H, H-5), 4.93 (septet, 1H, -OCH-), 4.71 & 4.66 ($AB_q$, $J_{AB}$ 12.0 Hz, 2H, $-OCH_2-$), 3.45 (t, *J* 8.92 Hz, 1H, H-5), 1.90 (s, 3H, $-COCH_3$), 1.24 (d, *J* 6.28 Hz, 6H, $-OCH(CH_3)_2$), 1.20 (d, *J* 6.26 Hz, 3H, $-OCH(CH_3)_2$), 1.19 (d, *J* 6.28 Hz, 3H, $-OCH(CH_3)_2$).

$^{13}$C n.m.r. ($CDCl_3$) δ 169.60, 168.53, 166.44 (C=O), 98.01 (=C-), 80.63 ($CH_2$), 70.89, 70.30 (CH), 62.21 ($CH_2$), 42.2 (CH), 21.63, 21.51, 21.41, 21.21, 20.37 ($CH_3$).

M.S. (m/z) 314 ($M^+$), 271 ($M^+ - CH(CH_3)_2$, 0.9%), 227 199 ($M^+ - N_2 - OCOCH_3$, 1.1%), 125 ( 184 - $OCHCH(CH_3)_2$, 8.6%), 43 ( 100%).

**Preparation of 1,2-dicarboisopropoxy-1-acetoxymethyl--cyclopropane 138 .**

*trans* 3,4-Dicarboisopropoxy-3-acetoxymethyl-1-pyrazoline **136** (1 g, 3.18 mmol) was irradiated in n-hexane (100 ml) at room temperature using a cylindrical reaction vessel consisting of concentrical pyrex tubes. The inner part contained the $H_2O$ condenser, the outer (250 ml size) contained the reaction mixture. A (125 W, 360 nm) Hanovia lamp was placed in the centre of the vessel. The reaction mixture was stirred at room temperature and the nitrogen evolution was monitored and was complete after 1 hr 30 min. The hexane was evaporated under reduced pressure and the residue was purified by flash chromatography eluting with light petroleum/EtOAc with the ratios 9:1, 8:1, 7:1, 5:1, 3:1, 2:1 and 1:1. The fractions were assayed by TLC using light petroleum/EtOAc (1:1) and the appropriate fractions eluted with the solvent ratios (3:1, 2:1) were combined and the solvents evaporated to give *trans* 1,2-Dicarboisopropoxy-1-acetoxymethylcyclopropane **138** (0.84 g, 93%) as a colourless oil.

I.R $\upsilon_{max}$ ($CCl_4$) 2980, 2920, 1740, 1720, 1245, 1105 $cm^{-1}$.

$^1$H n.m.r. ($CDCl_3$) $\delta$ 4.99 (septet, 2H, -OCH-), 4.85 & 4.12 ($AB_q$, $J_{AB}$ 11.8 Hz, 2H, $-OCH_2-$), 2.45 (m, 1H, H-2), 1.97 (s, 3H, $-COCH_3$), 1.55 (ABX, $J_{AB}$ 4.51 Hz, $J_{Ax}$ 8.47 Hz, $J_{Bx}$ 6.83 Hz, 2H, H-3), 1.25 (d, $J$ 6.34 Hz, 3H, $-OCH(CH_3)_2$), 1.22 (d, $J$ 6.23 Hz, 6H, $-OCH(CH_3)_2$), 1.20 (d, $J$ 6.39 Hz, 3H, $-OCH(CH_3)_2$).

$^{13}$C n.m.r. ( $CDCl_3$) δ 170.42, 170.35, 169.39 (C=O), 69.19, 68.75 (-OCH-), 61.82 (-$OCH_2$), 30.61, 26.35 (-CH-), 21.66, 21.51, 20.67 ($CH_3$), 19.60 ($CH_2$).

M.S (m/z) 286 ($M^+$, 1.5%), 244 ($M^+$- $CH_2$=$CHCH_3$, 5.2%), 199 ($M^+$- $CO_2CH(CH_3)_2$, 11.0%), 185 (244 - $OCH(CH_3)_2$, 57.2%), 184 ($M^+$ - $COCH_3$ - $OCH(CH_3)_2$, 17.8%), 157 ($M^+$- 42-$CO_2CH(CH_3)_2$, 9.4%), 142 (184 - $CH_2$=$CHCH_3$, 100%).

Mass measurement: Found 286.1404, $C_{14}H_{22}O_6$ requires 286.3251.

***trans*-3,4-Dicarboisopropoxy-3-acetoxy-methyl-5-n--propyl-1-pyrazoline 137 .**

A solution of diazobutane (10 eq) in ether (75 ml) was added with occasional shaking to diisopropyl acetoxymesaconate **136** (0.77 g, 2.83 mmol) in dry ether (10 ml) at -70 $^0$C. The reaction mixture was allowed to warm to room temperature and was then left stirring overnight. Excess of diazobutane was removed with nitrogen and the ether solution was evaporated under reduced pressure to give *trans*-3,4-dicarboisopropoxy-3-acetoxymethyl-5-n.propyl-1-pyrazoline **137** (0.8 g, 79%) as a colourless oil.

I.R. $\upsilon_{max}(CCl_4)$ 3060, 3040, 3020, 2950, 1820, 1780 (br), 1720, 1500, 1420, 1260 $cm^{-1}$

$^1$H n.m.r. ($CDCl_3$) δ 5.15 (septet, 1 H, -OCH-), 4.95 (m, 2 H, -OCH-, H-5), 4.64 (s, 2H, $-OCH_2-$), 3.05 (d, *J* 12.0. Hz, 1H, H-4), 1.92 (s, 3H, $-COCH_3$), 1.6 (br m, 4H, $-CH_2-$), 1.31 (d, *J* 6.25 Hz, 3H, $-OCH(CH_3)_2$), 1.30 (d, *J* 6.24 Hz, 3H, $-OCH(CH_3)_2$), 1.27 (d, *J* 6.26 Hz, 3H, $-OCH(CH_3)_2$), 1.20 (d, *J* 6.24 Hz, 3H, $-OCH(CH_3)_2$), 1.01 (t, *J* 7.2 Hz, 3H, $-CH_3$).

$^{13}$C n.m.r. ( $CDCl_3$) δ 169.67, 168.72, 166.82 (C=O), 98.31 (-C-), 93.01, 70.81, 69.43 (CH), 62.13 ($CH_2$), 48.17 (CH), 35.21 ($CH_2$), 21.66, 21.58, 21.47, 20.46 ($CH_3$), 19.67 ($CH_2$), 13.97 ($CH_3$).

M.S. (m/z) 356 ($M^+$, 2.31%), 296 ($M^+$ - $(CH_3)_2OH$, 41.2%), 254 ($M^+$ - $COCH_3$ - $OCH(CH_3)_2$, 100%), 212 (254 - $CH_2$=$CHCH_3$, 18.3%), 184 (254-42 - $N_2$, 14.9%), 183 (254 - $N_2$ - $CH_2CH_2CH_3$, 11.6%), 140 (184 - $CO_2$, 43.3%).

Mass measurement: Found 356.1955, $C_{17}H_{28}N_2O_6$ requires 356.4192.

**Preparation of *trans*-1,2-Dicarboisopropoxy-1-acetoxy--methyl-3-n-propylcyclopropane 139 .**

*trans*-3,4-Dicarboisopropoxy-3-acetoxymethyl-5-n,propyl-1-pyrazoline **137** (0.7 g, 1.96 mmol) was irradiated in n-hexane (100 ml) at room temperature using a cylindrical reaction vessel consisting of concentrical pyrex tubes. The inner part contained

the $H_2O$ condenser, the outer (250 ml size) contained the reaction mixture. A (125 W, 360 nm) Hanovia lamp was placed in the centre of the vessel. The reaction mixture was stirred at room temperature and the nitrogen evolution was monitored and was complete after 1 hr 35 min. The hexane was evaporated under reduced pressure and the residue was purified by flash chromatography eluting with light petroleum/EtOAc with the ratios 9:1, 8:1, 7:1, 5:1, 3:1, 2:1 and 1:1. The fractions were assayed by TLC using light petroleum/EtOAc (1:1) and the appropriate fractions with the ratios (9:1) were combined and the solvents evaporated to give *trans* 1,2-Dicarbo-isopropoxy-1-acetoxymethyl-3-n.propylcyclopropane **139** ( 0.52 g, 80%) as a colourless oil.

I.R $\upsilon_{max}$ ($CCl_4$) 3000, 2950, 1750, 1730, 1250, 1110 $cm^{-1}$.

$^1$H n.m.r. ($CDCl_3$) $\delta$ 5.00 (m, 2H, -OCH-), 4.94 & 3.78 ($AB_q$, $J_{AB}$ 11.8 Hz, 2H, $-OCH_2-$), 2.48 (d, *J* 6.78 Hz, 1H, H-2), 1.92 (s, 3H, $-COCH_3$), 1.25 (br m, 5H, H-3, $-(CH_2)_2-$), 1.215 (d, *J* 6.42 Hz, 3H, $-OCH(C\underline{H}_3)_2$), 1.204 (d, *J* 6.13 Hz, 3H, $-OCH(C\underline{H}_3)_2$), 1.202 (d, *J* 6.33 Hz, 3H, $-OCH(C\underline{H}_3)_2$), 1.18 (d, *J* 6.27 Hz, 3H, $-OCH(C\underline{H}_3)_2$), 0.9 (t, *J* 7.0 Hz, 3H, $-CH_3$).

$^{13}$C n.m.r. ( $CDCl_3$) $\delta$ 170.29, 169.87, 168.56 (C=O), 69.06, 68.37 (-OCH), 63.03 ($-OCH_2$), 36.77 (-C-); 32.33, 30.33 (-CH-), 29.36, 22.02 ($CH_2$), 21.89, 21.68, 21.60, 21.43, 20.64, 13.91 ($CH_3$).

M.S (m/z) 328 ($M^+$, 0.6%), 269 ( $M^+$ - $OCOCH_3$, 31.8%), 268 ($M^+$-42-18, 29.6%), 241 ($M^+$- $CO_2CH(CH_3)_2$, 17.4%), 227 ($M^+$- 42-

$OCH(CH_3)_2$, 45.8%), 199 ($M^+$- 42- $CO_2CH(CH_3)_2$, 100%), 184 ($M^+$ - 59-43-43, 35.2%), 139 (226- $CO_2CH(CH_3)_2$, 80.5%),

Mass measurement: Found 328.1904, $C_{17}H_{28}O_6$ requires 328.4057.

**Treatment of the cyclopropane 138 with MgO/MeOH .**

To a stirred solution of trans 1,2-dicarboisopropoxy-1-acetoxymethylcyclopropane **138** (0.2 g, 0.69 mmol) in methanol (20 ml) was added MgO [Merck 5866] (1 g, 25 mmol). Stirring was continued at room temperature for 10 days, MgO was filtered and the filtrate was evaporated under reduced pressure to give a semi-solid product (0.22 g) containing a mixture of the alcohol **131** together with the lactone **132**. After standing in $CDCl_3$ the product was shown by $^1H$ nmr to be converted quantitatively to the lactone **132**.

$^1H$ nmr, IR as described for previously obtained sample **132**.

**Treatment of 139 with MgO/iPrOH .**

To a stirred solution of trans 1,2-dicarboisopropoxy-1-acetoxymethyl-3-n,propylcyclopropane **139** (0.2 g, 0.61 mmol) in isopropanol (20 ml) was added MgO [Merck 5866] (0.6 g, 15 mmol). Stirring was continued at room temperature for 10 days, MgO was filtered and the filtrate was evaporated under reduced pressure

to give unreacted starting material. When the reaction mixture was refluxed at 60 $^0$C for a period of 12 hrs, MgO was filtered and evaporation of the solvent gave the alcohol **141** (43 mg, 25%) as an oily product.

$^1$H nmr, IR as described below.

**Treatment of 139 with $NaBH_4$/iPrOH. (The alcohol 141).**

To a stirred solution of trans 1,2-dicarboisopropoxy-1-acetoxymethyl-3-n,propylcyclopropane **139** (80 mg, 0.24 mmol) in isopropanol (20 ml) under nitrogen, was added a solution of $NaBH_4$ (14.5 mg, 0.38 mmol) [124] in isopropanol (10 ml). After stirring at 60 $^0$C for 10-15 min., the reaction mixture was allowed to cool and the solvent was evaporated under reduced pressure at room temperature. The residue was treated with saturated aqueous $NaH_2PO_4$ (pH neutral) and then extracted with EtOAc (3x20 ml. The combined extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was evaporated under reduced pressure at room temperature to afford the alcohol **141** as a colourless oil (69.5 mg, 99%).

I.R $\upsilon_{max}$ ($CCl_4$) 3630 (s, free OH), 3220 (br, bonded OH), 2970, 1740, 1357 (sharp, OH bending), 1050 (C-O) $cm^{-1}$.

$^1$H n.m.r. ($CCl_4$) $\delta$ 9.5 (m, 1H, -OH), 5.10 (m, 2H, -OCH-), 4.6 (br d, 1H, -OCH-), 3.85 (br d, 1H, -OCH-), 2.45 (br d, *J* 5.7 Hz,1H, H-2), 1.7-1.35 (m, 5H, H-3, -$(CH_2)_2$-), 1.27-1.20 (br s, 12H, -

$OCH(C\underline{H}_3)_2$), 0.9 (br t, 3H, $-CH_3$).

$^1$H n.m.r. ($CCl_4 + D_2O$) δ 5.10 (septet, 2H, -OCH-), 4.3 & 3.65 ($AB_q$, $J_{AB}$ 11 Hz, 2H, $-C\underline{H}_2OH$-), 2.35 (d, *J* 5.2 Hz, 1H, H-2), 1.35-1.7 (m, 5H, H-3, $-(CH_2)_2$-), 1.2-1.27 (br s, 12H, $-OCH(C\underline{H}_3)_2$), 0.9 (br t, 3H, $-CH_3$).

$^{13}$C n.m.r. ($CCl_4$) δ 169.8 (C=O), 69.15 (-OCH-), 62.4 ($-OCH_2$), 41.5 (-C-); 34.02, 30.24 (-CH-), 29.92, 22.69 ($CH_2$), 22.42, 22.34, 14.25 ($CH_3$).

**Attempted halogenation of 141 with:**

**1. $SOCl_2$/pyridine .**

To a stirred solution of the alcohol **141** (69.5 mg, 0.24 mmol) in pyridine (30 ml) at room temperature was added thionyl chloride (42.8 mg, 0.36 mmol). [90, 91] After stirring 15 min. at the same temperature, the reaction mixture was treated with saturated aqueous $NaH_2PO_4$ and extracted with EtOAc (100 ml). The combined extracts were dried over $MgSO_4$, filtered and the filtrate evaporated under reduced pressure to give the lactone **142** (54.8 mg, 99%) as a white oil.

I.R $\upsilon_{max}$ ($CCl_4$) 2980, 2960, 2860, 1780 (C=O), 1720 (C=O), 1030 (C-O) $cm^{-1}$.

$^1$H n.m.r. ($CCl_4$) δ 5.10 (septet, 2H, -OCH-), 4.46 & 4.16 ($AB_q$, $J_{AB}$ 9.4 Hz, 2H, $-C\underline{H}_2O$-), 2.26 (d, *J* 4.3 Hz, 1H, H-2), 1.3-1.8 (m, 5H, H-3, $-(CH_2)_2$-), 1.1-1.28 (br d, 12H, $-OCH(C\underline{H}_3)_2$), 0.9 (br t, 3H, $-CH_3$).

$^{13}$C n.m.r. ( $CCl_4$) δ 170.65, 166.81 (C=O), 68.65 (-$OCH_2$-), 68.4 (-OCH-), 34.10 (-C-); 33.29, 30.64 (-CH-), 27.89, 22.14 ($CH_2$), 21.84, 13.57 ($CH_3$).

M.S (m/z) 226 ($M^+$, 7.9%), 184 ( $M^+$ - $CH_2$=$CHCH_3$, 7.5%), 188 ($M^+$- $CO_2$, 2.8%), 167 ($M^+$- $OCH(CH_3)_2$, 10.80%), 139 ($M^+$ - $CO_2CH(CH_3)_2$, 13.0%), 123 ($M^+$- $CO_2$- $OCH(CH_3)_2$, 8.40%), 111 ($M^+$- CO - 87, 45.7%), 95 ($M^+$- $CO_2$-87, 38.80%), 43 (100%).

Mass measurement: Found $M^+$ 226.1202, $C_{12}H_{18}O_4$ requires 226.2725.

**2. $CBr_4/PPh_3$.**

To a stirred solution of the cyclopropyl alcohol **141** (80 mg, 0.28 mmol) in anhydrous $CH_2Cl_2$ (15 ml) at -20 $^0$C, were added carbon tetrabromide (115 mg, 0.34 mmol) and triphenyl phosphine (107 mg, 0.41 mmol). [92-96] After stirring 10 min. at the same temperature, the reaction mixture was allowed to warm to room temperature and concentrated under reduced pressure. The residue was treated with dry ether to give a precipitate, which was filtered and the filtrate was evaporated to give the lactone **142** (62.6 mg, 99%) as a white oil.

$^1$H, $^{13}$C nmr and IR spectra are identical to those of the previously obtained compound **142**.

**Diisopropyl *p*-toluenesulfonylmesaconate 145.**

To a stirred solution of diisopropyl bromomesaconate **134** (0.22 g, 0.75 mmol) in acetonitrile (15 ml) was added dropwise under nitrogen, silver *p*-toluenesulphonate (0.6 g, 2.15 mmol) in acetonitrile (10 ml) at 0 $^0$C (protected from light). [125, 126] The reaction mixture was allowed to warm to room temperature and stirring was continued under reflux at 50 $^0$C for 12 hrs. The solvent was evaporated to give a semi-solid product. This was extracted with $CHCl_3$ and the extract was filtered and evaporated under reduced pressure to give an oil. Preparative TLC in EtOAc/light petroleum (1:5) gave the tosylate derivative **145** (0.15 g, 52%) as a colourless oil.

$^1$H n.m.r. ($CDCl_3$) $\delta$ 7.7 (m, 2H, aromatic H), 7.25 (m, 2H, aromatic H), 6.78 (s, 1H, vinyl H), 5.15 (s, 2H, -$OCH_2$-), 5.00 (m, 2H, -OCH-), 2.38 (s, 3H, aromatic -$CH_3$), 1.25 (br s, 6H, -OCH($C\underline{H}_3$)$_2$), 1.18 (br s, 6H, -OCH($C\underline{H}_3$)$_2$).

M.S (m/z) 384 ($M^+$, 0.3%), 229 ($M^+$- $SO_2C_6H_4CH_3$, 54.8%), 170 ($M^+$- 59 - $SO_2CHC_6H_4CH_3$, 2.6%), 155 (170 - $CH_3$, 78.9%), 91 ($C_7H_7$, 100%).

Mass measurement: Found $M^+$ 384.1249, $C_{18}H_{24}O_7S$ requires 384.4504.

**3,4-Dicarboisopropoxy-3-*p*-toluenesulfonoxymethyl-5-n-propyl-1-pyrazoline 146 .**

A solution of butane (8 eq) in dry ether (50 ml) was added with occasional shaking to diisopropyl *p*-toluenesulfonyl--mesaconate **145** (0.12 g, 0.31 mmol) in dry ether at -70 °C. The reaction mixture was allowed to warm to room temperature and was then left stirring overnight. Excess of diazobutane was removed with a stream of nitrogen and the ether solution was evaporated under reduced pressure to give the *trans* pyrazoline **146** (100 mg, 68%) as a colourless oil. This compound was used in the following reaction without further purification or characterisation.

$^1$H n.m.r. ($CDCl_3$) δ 7.62 (m, 2H, aromatic H), 7.25 (m, 2H, aromatic H), 4.9 (m, 3H, -OCH-, H-5), 4.5 ($AB_q$, $J_{AB}$ 11.0 Hz, 2H, -$OCH_2$-), 2.95 (d, *J* 9.0 Hz, 1H, -COCH-), 2.34 (S, 3H, aromatic $CH_3$), 1.8 (m, 2H, -$CH_2$-), 1.18 (d, *J* 6.3 Hz, 6H, -OCH($CH_3$)$_2$), 1.1 (d, *J* 6.3 Hz, 6H, -OCH($CH_3$)$_2$), 0.9 (m, 3H, -$CH_3$).

**Pyrolysis of the pyrazoline 146 .**

The pyrazoline **146** (100 mg, 0.21 mmol) in xylene (10 ml) was stirred under an atmosphere of nitrogen at 120 °C for a period of 12 hrs. Stirring was continued for an additional 2 hrs at 160 °C and the solvent was evaporated under reduced pressure to give an oily residue (100 mg). This was shown by TLC in light petroleum/EtOAc (3:1) to be a mixture of several components.

Extensive preparative TLC in the same solvent system failed to separate them and the reaction was not investigated further.

**2-Bromo-2-(bromomethyl)succinic anhydride 151 .** [127]

To a solution of itaconic anhydride (5 g, 45 mmol) in $CCl_4$ (20 ml), was added dropwise while stirring, bromine (7.14 g, 45 mmol) in $CCl_4$ (15 ml). The reaction mixture was stirred at room temperature for a period of 12 hrs and then at 60 $^0$C for 1 hr. The $CCl_4$ was evaporated under reduced pressure to give a reddish-semi solid, which was recrystallised from hexane-chloroform and 2-Bromo-2-(bromomethyl)succinic anhydride **151** (11.8 g, 97%) was obtained as white needles m.p. 56-58$^0$ (lit.[127] m.p. 58-60$^0$)

I.R. $\upsilon_{max}$ ($CCl_4$) 3680, 3010, 2400, 1870, 1800, 1220, 1200, 790 $cm^{-1}$.

$^1$H n.m.r. ($CDCl_3$) $\delta$ 4.2 & 3.8 ($AB_q$, $J_{AB}$ 11.5 Hz, 2H, $-CH_2Br$), 3.9 & 3.4 ($AB_q$, $J_{AB}$ 15.8 Hz, 2H, $-COCH_2-$).

**Bromocitraconic anhydride 149 .** [127, 128]

A solution of dry $Et_3N$ (1.72 g, 17 mmol) in dry ether (10 ml) was added dropwise over a period of 45 min. to a stirred solution of 2-Bromo-2-(bromomethyl)succinic anhydride **151** (4 g, 14.7 mmol) in ether (25 ml) at room temperature. Stirring was continued for an additional 1.5 hr and the resulting black mixture

was filtered to remove $Et_3N.HBr$. Evaporation of the ether under reduced pressure gave a brown oil, which on distillation yielded bromocitraconic anhydride **149** (2.5 g, 89%) as a pure oil b.p. 110-112 $^0C$/0.8 mm (lit. [127] b.p. 116-117 $^0C$/1.2 mm).

I.R. $\upsilon_{max}$ ($CCl_4$) 1840, 1755 (anhydride C=O), 1642 (C=C) $cm^{-1}$.

$^1H$ n.m.r. ($CDCl_3$) $\delta$ 6.9 (t, *J* 1.5 Hz, 1H, vinyl H), 4.23 (d, *J* 1.5 Hz, 2H, $-CH_2Br$).

**Treatment of 149 with :**

**1. KOAc/EtOH.**

Bromocitraconic anhydride **149** (2.1 g, 10.9 mmol) was stirred under reflux with fused KOAc (1.3 g, 13 mmol) in absolute ethanol (30 ml) for a period of 1 hr. The mixture was then cooled and ethanol was evaporated under reduced pressure to give a brown residue. This was treated with EtOAc to give a precipitate, which was filtered off through a short column of silica gel ($HF_{254}$) and the ethyl acetate was distilled off under reduced pressure. The resulting oil was shown by TLC and $^1H$ nmr to be a complex mixture of several components and their separation using preparative TLC was unsuccessful.

**2. AgOTs/$CH_3CN$. (Preparation of *p*-toluenesulfonylcitraconic anhydride 153).**

To a stirred solution of bromocitraconic anhydride **149** (0.3 g, 1.57 mmol) in acetonitrile (20 ml) was added dropwise, silver *p*-toluenesulfonate 55 (0.6 g, 2.15 mmol) in acetonitrile (10 ml) at

0-5 $^0$C (protected from light). [125] The reaction mixture was allowed to warm to room temperature and stirring was continued under reflux at 80 $^0$C for 12 hrs. The solvent was evaporated to give a semi-solid product. This was extracted with $CHCl_3$ (30 ml) and the extract was filtered and evaporated under reduced pressure to give *p*-toluenesulfonylcitraconic anhydride **153** (0.25 g, 57%) as a colourless oil.

$^1$H n.m.r. ($CDCl_3$) δ 7.65 (m, 2H, aromatic H), 7.25 (m, 2H, aromatic H), 6.8 (t, *J* 3 Hz, 1H, vinyl H), 4.8 (d, *J* 3 Hz, 2H, -$OCH_2$-), 2.31 (s, 3H, aromatic -$CH_3$).

**Addition of diazobutane to 153 .**

A solution ofdiazobutane (8 eq) in dry ether (50 ml) was added with occasional shaking to *p*-toluenesulfonylcitraconic anhydride **153** (0.2 g, 0.71 mmol) in dry ether at -70 $^o$C. The reaction mixture was allowed to warm to room temperature and was then left stirring overnight. Excess of diazobutane was removed with a stream of nitrogen and the ether solution was evaporated under reduced pressure to give an oily product (0.25 g). This was initially shown by $^1$H nmr to be the corresponding pyrazoline **155** but after standing the $^1$H spectrum of the same sample indicated decomposition. Simultaneously, TLC in ether-hexane (1:4) showed the product to be a complex mixture.

**Attempted epimerisation of the cyclopropane 139 using LDA .**

To diisopropylamine (0.136 ml, 0.98 mmol) in dry THF (5 ml) at -78 $^0$C under an atmosphere of nitrogen, was added n-butyllithium (0.09 ml, 1.1 mmol). The temperature was kept at -78 $^0$C and the acetoxy cyclopropane **139** (80 mg, 0.24 mmol) in THF (10 ml) was slowly added with stirring. When the addition was complete, the resulting mixture was stirred for an additional 30 min. and was then added dropwise at -15 $^0$C to a stirring solution of aqueous $NaH_2PO_4$. This was extracted with EtOAc (60 ml), dried over anhydrous $MgSO_4$, filtered and the filtrate was evaporated under reduced pressure to give unchanged starting material **139**.

**Treatment of the lactone 142 with $BBr_3$.**

The lactone **142** (200 mg, 1.28 mmol) in $CH_2Cl_2$ (10 ml) was added under nitrogen to a stirring solution of $BBr_3$ (1.30 mmol) in $CH_2Cl_2$ (15 ml). [96] Stirring was continued at room temperature for 16 hrs and the reaction mixture was then quenched with isopropanol (15 ml) and $CH_2Cl_2$ (25 ml) was added. The reaction mixture was washed with aqueous solutions of $NaHCO_3$ (30 ml), sodium thiosulphate (30 ml) and water (30 ml). The organic phase was dried over anhydrous $MgSO_4$ and the was removed under reduced pressure to give an oily residue (210 mg). This was shown by TLC in EtOAc/light petroleum (1:1) to be a mixture of at least three components. Preparative TLC in the same solvent system gave mainly unreacted starting material Rf. 0.6 (86 mg), contaminated with traces of another product thought to be the vinyl bromo compound **157** as concluded from the $^1H$ spectrum.

$^1H$ n.m.r. ($CCl_4$) 90 MHz $\delta$ 7.8 (s, 1H, vinyl H), 5.0 (m, 1H, -OCH-), 3.68 (S, 3H, $-OCH_3$), 2.3 (m, 4H, $-CH_2-$), 1.35 (d, *J* 8.0 Hz, 6H, $-CH(CH_3)_2$).

***trans* 1-Bromomethyl-1-carbo-isopropoxy-2-carboxy-3-n.propylcyclopropane 158.**

The γ-lactone **142** (100 mg, 15 mmol) was placed under nitrogen and treated with (4 g) of a 32 % solution of HBr in acetic acid. [61] The mixture was stirred for 40 hrs at 45 $^0C$. The hydrogen

The mixture was stirred for 40 hrs at 45 $^{0}$C. The hydrogen bromide -acetic acid was evaporated and the residue was flushed by the addition and evaporation of benzene (15 ml) to give a colourless oil (89.5 mg, 76%). This contained the mono isopropyl ester **158** and the diacid **159** with the ratio 2:1 respectively as deduced from $^{1}$H nmr.

**Mono isopropyl 158.**

$^{1}$H n.m.r. ($CCl_4$) δ 11.95 (-$CO_2$H), 5.10 (septet, 1H, -OCH-), 4.25 & 3.66 ($AB_q$, $J_{AB}$ 10.61 Hz, 2H, -C$H_2$Br-), 2.61 (d, *J* 7.03 Hz, 1H, H-2), 1.9 (m, 1H, H-3), 1.5 (m, 4H, -$CH_2$), 1.32 (d, *J* 6.2 Hz, 3H, -OCH(C$H_3$)$_2$), 1.30 (d, *J* 6.18 Hz, 3H, -OCH(C$H_3$)$_2$), 0.94 (br t, 3H, -$CH_3$).

$^{13}$C n.m.r. ( $CCl_4$) δ 174.7 (acid C=O), 172.95 (ester C=O), 68.83 (-OCH-), 40.32 (-C-), 38.37, 32.38 (-CH-), 30.84 (-$CH_2$Br), 28.44, 22.07 (-$CH_2$), 21.81, 21.77, 13.46 (-$CH_3$).

**Diacid 159 .**

$^{1}$H n.m.r. ($CCl_4$) δ 11.95 (-$CO_2$H), 4.19 & 3.65 ($AB_q$, $J_{AB}$ 10.45 Hz, 2H, -C$H_2$Br), 2.56 (d, *J* 7.03 Hz, 1H, H-2), 1.8 (m, 1H, H-3), 1.5 (m, 4H, -$CH_2$), 0.94 (br t, 3H, -$CH_3$).

$^{13}$C n.m.r. ( $CCl_4$) δ 177.24 (acid C=O), 40.81 (-C-), 37.58, 31.45 (-CH-), 30.13 (-$CH_2$Br), 29.83, 24.51 (-$CH_2$), 13.46 (-$CH_3$).

**Acids 158 and 159 :**

I.R $\upsilon_{max}$ ($CCl_4$) 3010-2850 (broad), 1740, 1680, 1425, 1300, 1040, 940 $cm^{-1}$.

M.S (m/z) 308 ($M_1^+$), 266 ($M_2^+$), 185 ($M_2^+$ - Br, 32.8%), 184 ($M_1^+$ - HBr-$CH_2$=$CHCH_3$, 3.5%), 167 ($M_1^+$ - HBr - $OCH(CH_3)_2$, 62.9%), 139 ($M_1^+$ - HBr - $CO_2CH(CH_3)_2$, 41.7%), 129 ($C_5H_5O_4$, 100%).

Exact mass for **158** :

1. calculated for $C_9H_{12}{}^{81}BrO_3$ 248.9930 Found 249.19248.
2. calculated for $C_9H_{12}{}^{79}BrO_3$ 246.9943 Found 247.19248.

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