THE BIOSYNTHESIS OF CRYPTOSPORIOPSINOL.

by

ALISTAIR MACSWEEN MARR

A Thesis presented for part fulfilment of the requirements for the Degree of Doctor of Philosophy.

Department of Chemistry
University of Glasgow.

October 1990.

I would like to dedicate this thesis to my mother for her love and faith throughout the years.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Robert A. Hill for his help and encouragement throughout this project. The invaluable assistance of Mrs. Pearl Tait and her staff of the mycology department is gratefully acknowledged. The help of my fellow researchers was much appreciated.

I would also like to thank Mrs. Liz Hughes for her patience in the typing of this thesis.

Finance for this work was provided by the European Economic Community.

CONTENTS

Summary

Cryptosporiopsinol is a cyclopentene polyketide metabolite isolated from the fungus *Periconia macrospinosa*. Cryptosporiopsinol is believed to arise via a ring contraction of an isocoumarin precursor. This study is concerned with further details of the biosynthetic pathway to cryptosporiopsinol.

[3,4,9-$^{2}H_{3}$]-5,7-dichloro-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin and [4,9,9,9-$^{2}H_{4}$]-5,7-dichloro-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin were synthesised. On feeding these compounds to *Periconia macrospinosa* it was found that the cryptosporiopsinol was labelled with deuterium. The results indicated that the 4-proS proton of the isocoumarin is cleaved whereas the 4-proR proton is retained. This supports the proposal that stereospecific hydroxylation may occur at the 4-position of an isocoumarin similar to that in perimacol, a 4-hydroxylated isocoumarin co-metabolite.

To further investigate the ring contraction in the biosynthesis of cryptosporiopsinol 5,7-dichloro-3,4-dihydro-6-hydroxy-8-methoxy-3-methylisocoumarin and 2-methoxy-6-prop-1-enyl-1,4-benzoquinone are required. Routes to these compounds were investigated and synthetic strategies to their syntheses were developed.

During the studies of the synthesis of the 1,4-benzoquinone, the successful coupling of heptyne with iodovanillin was achieved using bis(triphenylphosphine)-

dichloropalladium and cuprous iodide as a catalyst. In another route to the 1,4-benzoquinone the condensation of 2,3,5-trimethoxybromobenzene with propanal using n-butyllithium was achieved.

Chapter 1

Introduction

Organic chemistry has its origins in the study of natural products and this still remains its most important role. These natural products have been used by man for thousands of years[1] e.g. pigments, dyes, poisons, medicines etc. The wonder is not only in the discovery of these products but also in the discovery of their properties e.g. antibiotics, analgesics, recreational intoxicants.

The supply of these products has been derived from almost every living organism e.g. fungi, plant, animal and human. As our techniques (e.g. chromatography, n.m.r) for isolating these products and discovering their properties become more refined, the chance factor of our science is being reduced and the challenge factor increased. There is still an undiscovered chemical bank from natural sources which could be used as medicines for so far incurable illnesses or redress of our environmental damage, e.g. acid rain, polluted waterways etc.

The living organisms produce their metabolites by means of interrelated, enzyme-catalysed, chemical reactions (both degradative and synthetic). The primary metabolism, basically the same and essential for survival for all organisms, produces sugars, amino acids, proteins and nucleotides. Secondary metabolism is more species specific and therefore lends itself to a far more extensive array of substances e.g. alkaloids, terpenes, mycotoxins etc. Secondary metabolism

therefore requires more complex functionalisation, in turn requiring a large number of specific enzymes[2].

The reasons as to the amount of secondary metabolites produced still remains a mystery. Some, however, play a vital role in the survival of the species over another (e.g. defence chemicals, sex attractants). One explanation why so many are produced is that they are detoxification products of poisonous or overabundant metabolites, which accounts for the greater production in plants over animals.

Fungi belong to a division of plant organisms with no true roots, stems or leaves, along with algae and bacteria, called Thallophyta[3]. Fungi lack photosynthetic pigments and are therefore unable to biosynthesise organic molecules from carbon dioxode and water. Instead, they use oxidizable organic compounds as energy sources obtained from dead or living plants, animals or micro-organisms. This is a property that can be of great use commercially.

The most important biosynthetic route of secondary metabolites of fungi is the polyketide or acylpolymalonate pathway.

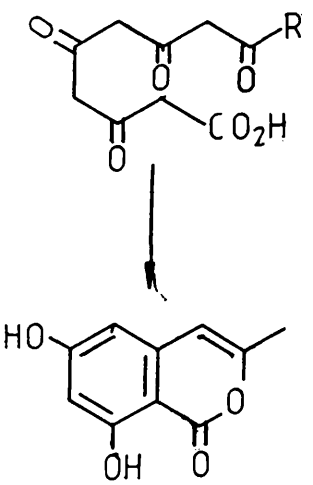
The idea that naturally occurring, highly oxygenated compounds may arive by condensation of acetate units was first suggested by Collie[4] in 1893. This was largely ignored until, with the aid of ^{14}C-labelling, the initial hypothesis was restated by Birch sixty years later. Birch showed ^{14}C-acetate was

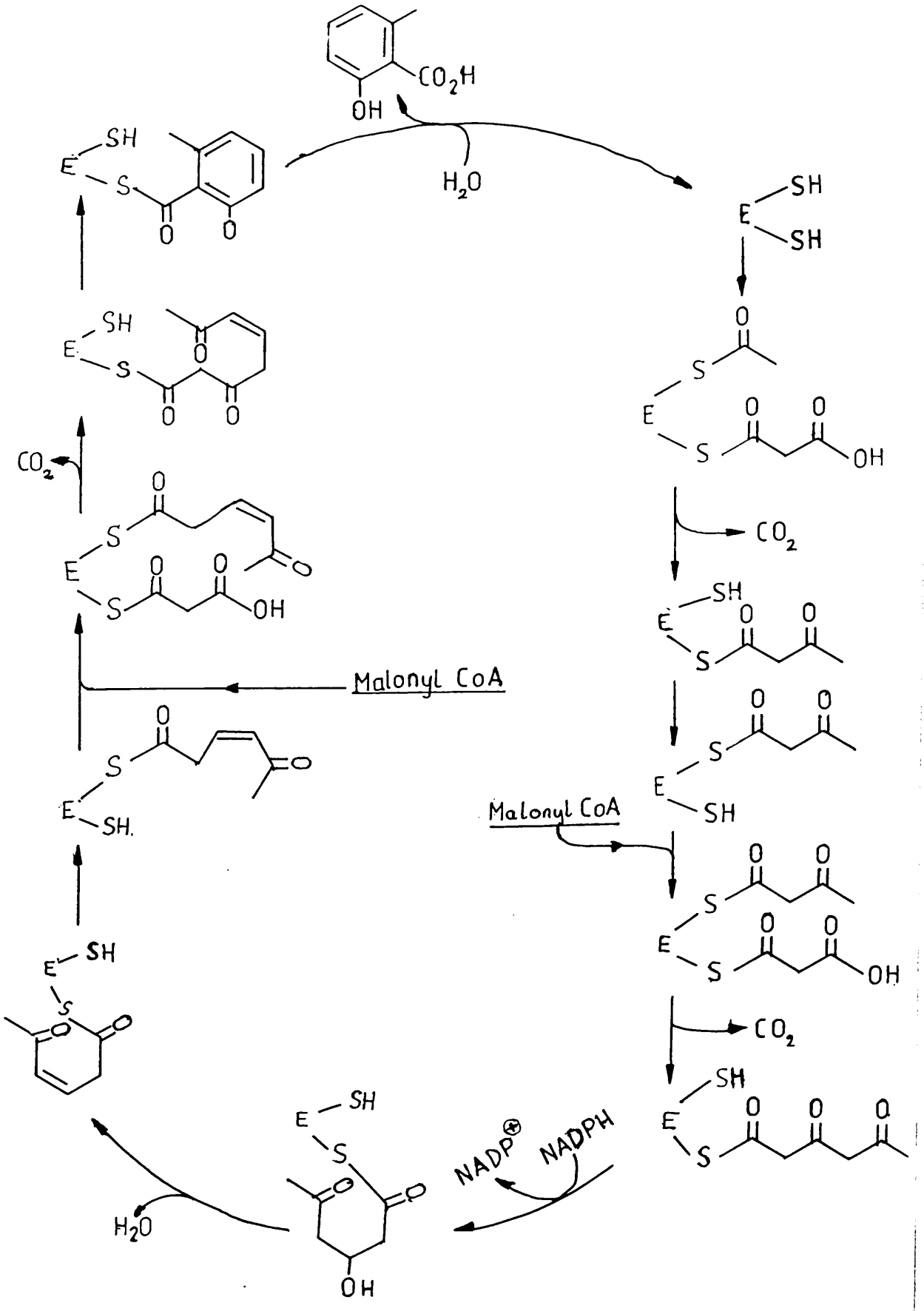
incorporated into 6-methylsalicylic acid (6-MSA), a metabolite of *Penicillium griseofulvum* and other fungi[5,6].

The basic concepts of Birch's polyketide hypothesis are:-

1. The C_2 unit of acetic acid (in its activated forms of acetyl CoA and malonyl CoA) condense in head to tail linkages with each other to form polyketomethylenic chains $[-(CH_2-CO)_m-]$.
2. The polyketomethylenic chains commonly undergo stabilising secondary changes. Particularly favourable are Aldol (crotonic) and Claisen condensations to give aromatic rings. (Scheme 1).
3. The carbon skeleton may be further modified by addition of alkyl groups.
4. Reduction and oxidation may occur before or after cyclisation.
5. Further modification of functions or of mono or polycyclic skeleton resulting from 2.

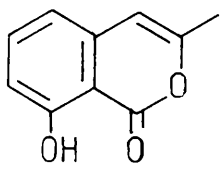
e.g. Lactonisation may also occur to give an isocoumarin which is of particular interest in this work.



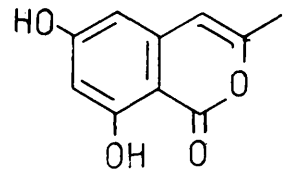


SCHEME 1.

There are many examples of isocoumarins isolated from fungi e.g. 8-hydroxy-3-methylisocoumarin(1) from *Marasmius ramealis*[7], 8-hydroxy-6-methoxy-3-methylisocoumarin(2) from *Ceratocystis fimbriata*[8] and *Streptomyces mobaraensis*[9]

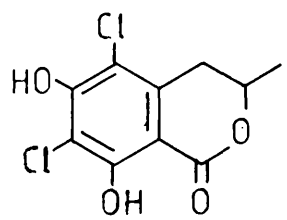


(1)



(2)

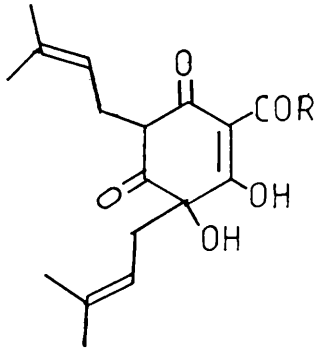
This work involves another isocoumarin namely 5,7-dichloro-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin(3) and its further modification



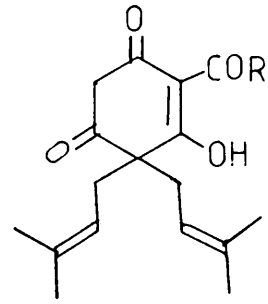
(3)

Most polyketide derived natural products contain aromatic rings. However, a number of non-aromatic polyketides have been isolated and some have been shown to be formed by ring cleavage. This dissertation is concerned with a compound of this type and a brief survey of the biosynthesis of some of these compounds follows.

In the brewing industry a number of metabolites of the hop (*Humulus lupulus*) have been isolated and identified[10]. Of most interest are the α and β acid classification (the bittering components)



(4)

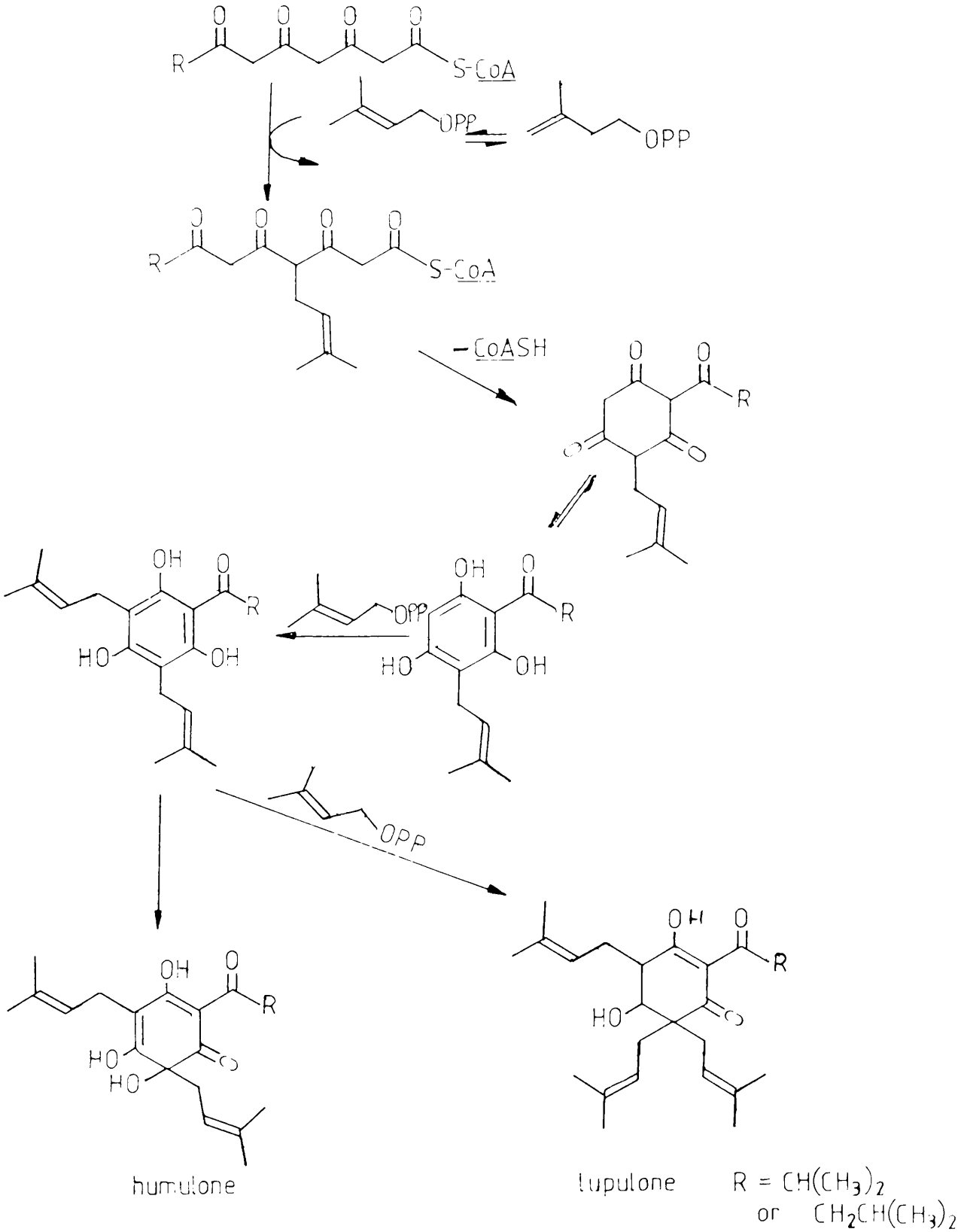


(5)

Work done by Drawert[11-13] has shown that these compounds are derived from polyketide and isovaleryl or isobutyryl residues. Drawert has proposed the following biosynthetic pathway[14] (Scheme 2)

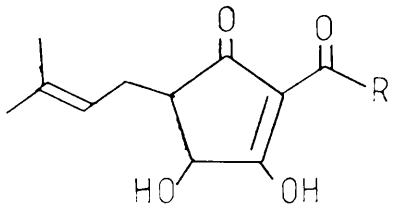
The polyketide forms the six-membered ring (deduced from [^{14}C]acetate experiments) and leucine precursor for isovaleryl and valine and isoleucine precursors for isobutyryl and 2-methylbutyryl residues.

There appears to be a ring contraction to form a series of more bitter 5-membered-ring compounds. These compounds include humulinic acid[15](6) and humulinone(7). There has been speculation as to whether humulinone is a true natural product or an artefact of the isolation

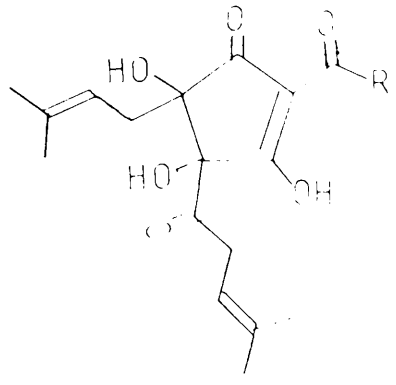

R
S-CoA
OPP
OPP
R
S-CoA
-CoASH
R
OH
R
HO
OH
OPP
OH
R
HO
OH
OPP
OH
R
HO
HO
humulone
OH
R
HO
lupulone
R = CH(CH3)2
or CH2CH(CH3)2

SCHEME 2.

procedure[16-19] but a novel ring contraction none the less.

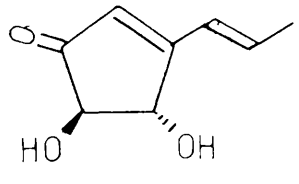


(6)

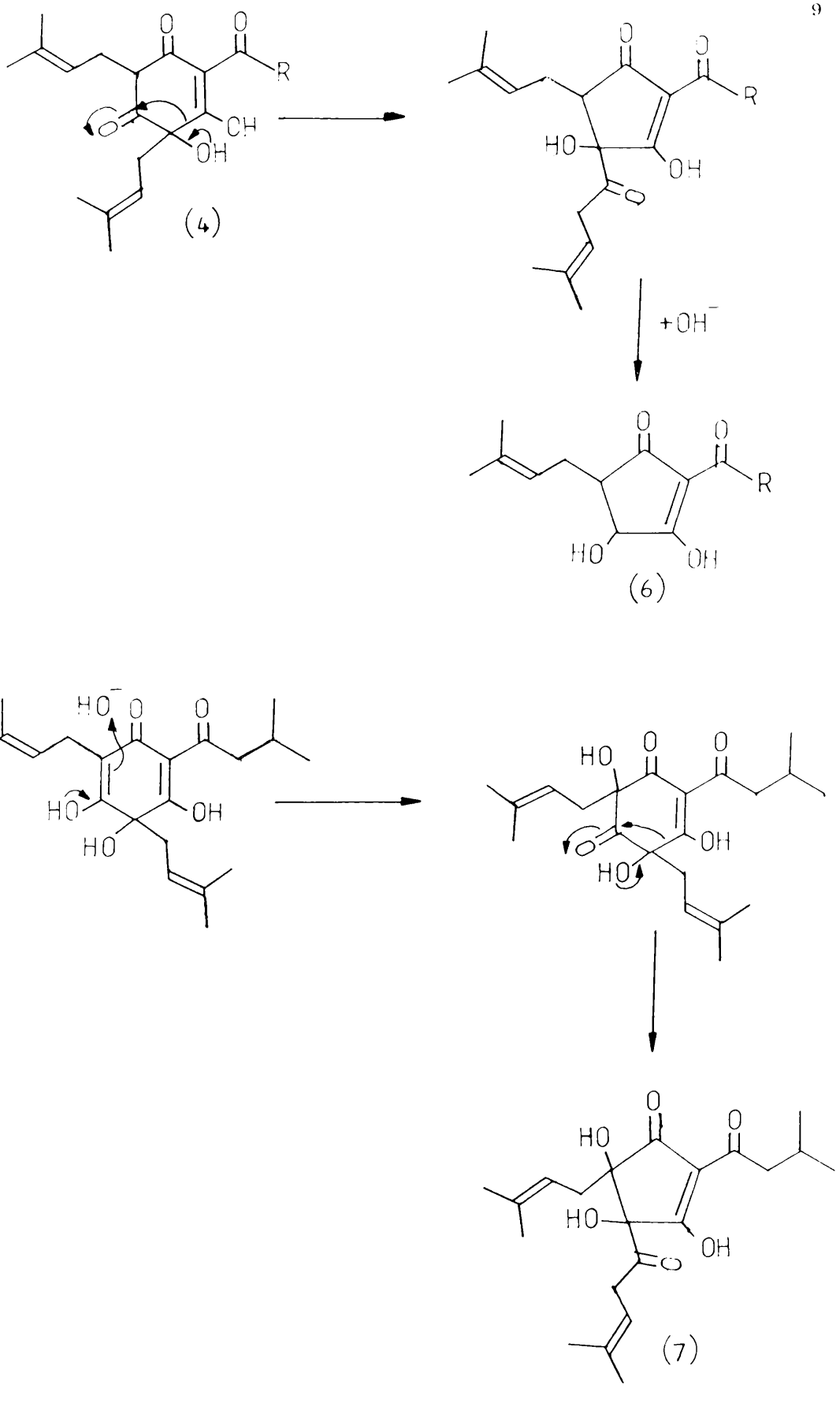


The humulinic acid could be derived either from humulone (α-acid)(4) or lupulone (β-acid)(5). These compounds (6) and (7) are also created chemically using alkaline hydrolysis and hydrogenolysis in the brewing industry[20]. A possible pathway to these 5-membered rings is shown (Scheme 3).

A further example of ring cleavage can be seen in the biosynthesis of terrein(8), a metabolite of *Aspergillus terreus*[21].

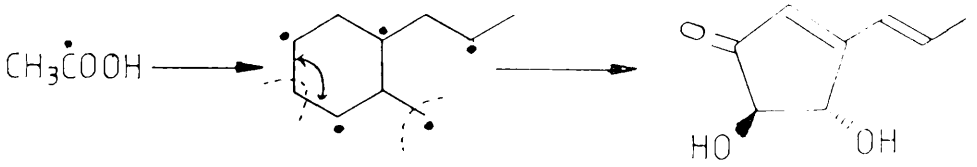


(8)



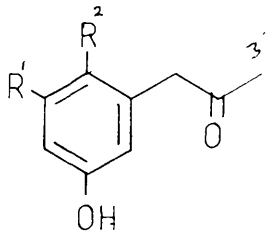
SCHEME 3.

Early work by Birch[22] using [1-^{14}C]- and [2-^{14}C]-acetate showed the radioactivity was incorporated suggesting a polyketide origin. From these results Birch proposed a scheme in which the five membered ring of terrein is formed by contraction of a six membered ring as shown (Scheme 4).

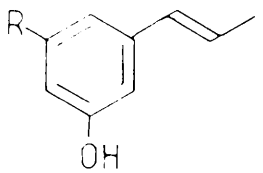


Scheme 4

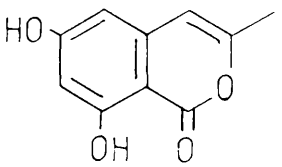
A more recent investigation of terrein biosynthesis using singly and doubly labelled [^{13}C]-acetate has confirmed Birch's original experiments. To test Birch's hypothesis, a number of hypothetical aromatic precursors were synthesised for incorporation studies. Depending on the timing of the various steps (ring contraction, loss of two C_1 units and generation of alkene bond in the side chain) a large number of candidates are possible.



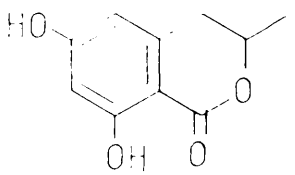
(9) R^1= OH R^2= H
(10) R^1= H R^2= OH



(11) R= OH
(12) R= H



(13)



(14)

The first four correspond to a pathway in which one C_1 unit is lost prior to ring contraction, the remaining two are not dependant on this assumption. These compounds were synthesised with radio-labelled ^{14}C in the positions indicated and gave the results in Table 1.

An intact incorporation should result in terrein being labelled at C-1 and this was checked by Kuhn-Roth oxidation to produce acetic acid from C-1 and C-2. The terrein produced in experiments 2,3 and 6 were found to carry one quarter total activity in the key C_2 unit. This is consistent with prior degradation of administered compound to [2-^{14}C]acetate, which is incorporated in the normal way. It should be noted that in experiment 3 a higher incorporation is achieved compared to acetate incorporation of experiment 1. This might indicate that degradation to acetic acid is occurring at a site close to polyketide synthesis in the organism.

Table 1.

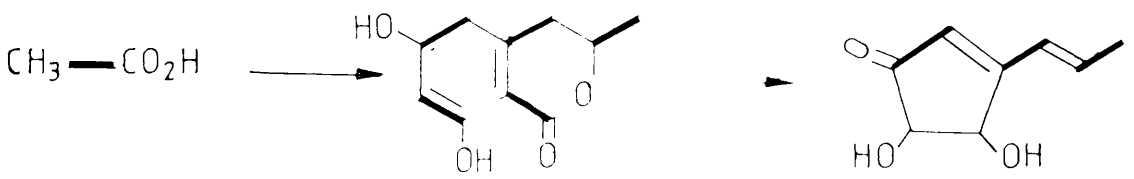
Results of Incorporation Studies

Expt. No.	Administered Compound	Incorporation	% Label at C-1 & C-2
1	[carboxy-^{14}C]$MeCO_2H$	0.59	24.1
2	[3-^{14}C]-(9)	0.19	24.4
3	[3-^{14}C]-(10)	0.75	24.5
4	[3-^{14}C]-(11)	0.01	
5	[3-^{14}C]-(12)	0.01	
6	[Me-^{14}C]-(13)	0.04	24.3
7	[Me-^{14}C]-(14)	2.27	92.3

In contrast, terrein derived from (14) yielded acetic acid which carried 92% of total activity and all of this activity resided in the terminal methyl group from Schmidt degradation. This dihydroisocoumarin (14) is a cometabolite[24] of terrein in *Aspergillus terreus* and therefore it is very likely to be a true biosynthetic intermediate.

The feeding of singly and doubly labelled [^{13}C]acetate reinforced the radiolabelled results and showed terrein

incorporates three intact C_2 units, the remaining two carbons being derived from separate C_2 units. The interpretation of Staunton et al is described in Scheme 5.

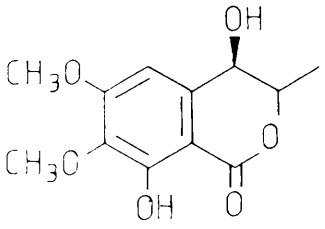


(15)

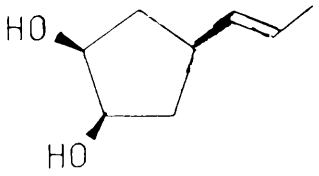
Scheme 5.

It is proposed that intermediate (14) is labelled as shown. These results show that terrein is derived from acetate via a ring contraction of a dihydroisocoumarin precursor with loss of aromatic carbon C-7 and carbonyl carbon C-1.

Recently further related metabolites have been isolated from the fungus *Aspergillus terreus*. The first is a dihydroisocoumarin[25] (15) and the second[26] is a five-membered ring (16) similar to terrein.



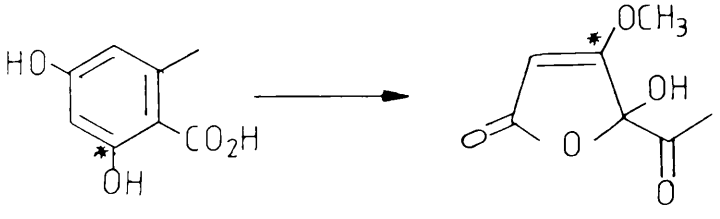
(15)



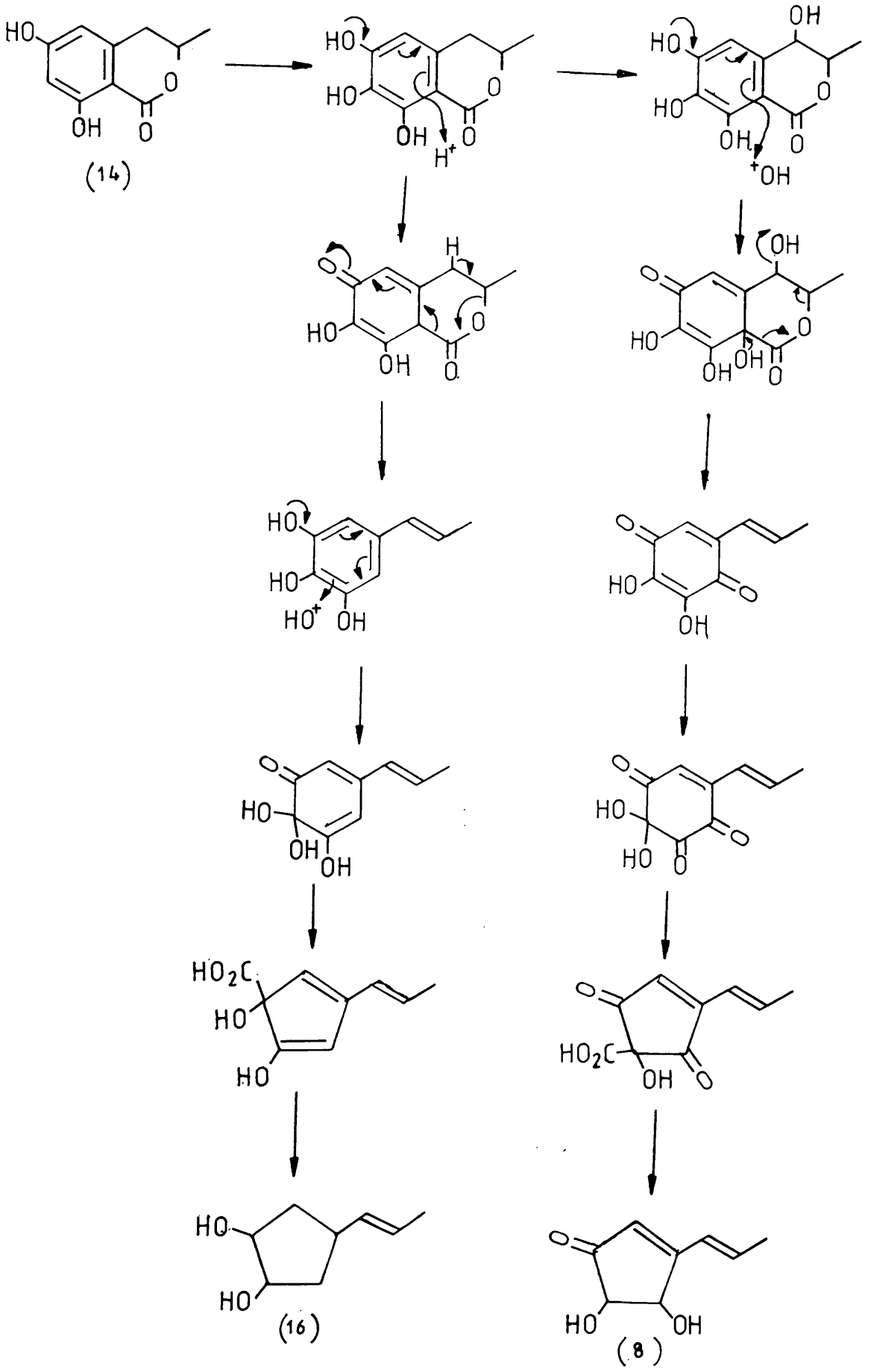
(16)

It is proposed that isocoumarin (14) undergoes hydroxylation at C-4, C-7 and C-8a to give intermediates as indicated in Scheme 5 to eventually yield terrein as product. It is also proposed that if hydroxylation stops at C-7 further ring contraction could lead to the five-membered ring in (16) also shown (Scheme 6).

In the biosynthesis of penicillic acid, produced by various fungi, Mosbach[27] showed that orsellinic acid (17) labelled as shown produces penicillic acid (18) with the label located in the methoxyl bearing carbon.

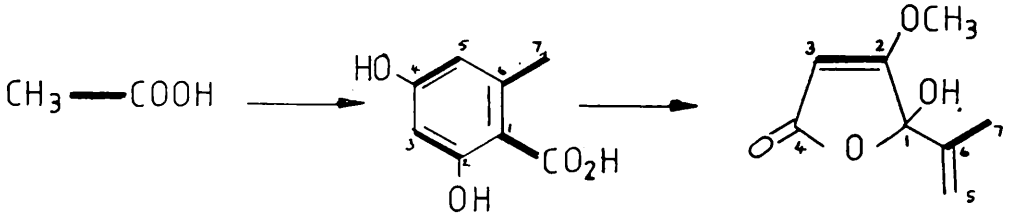


(17) (18)



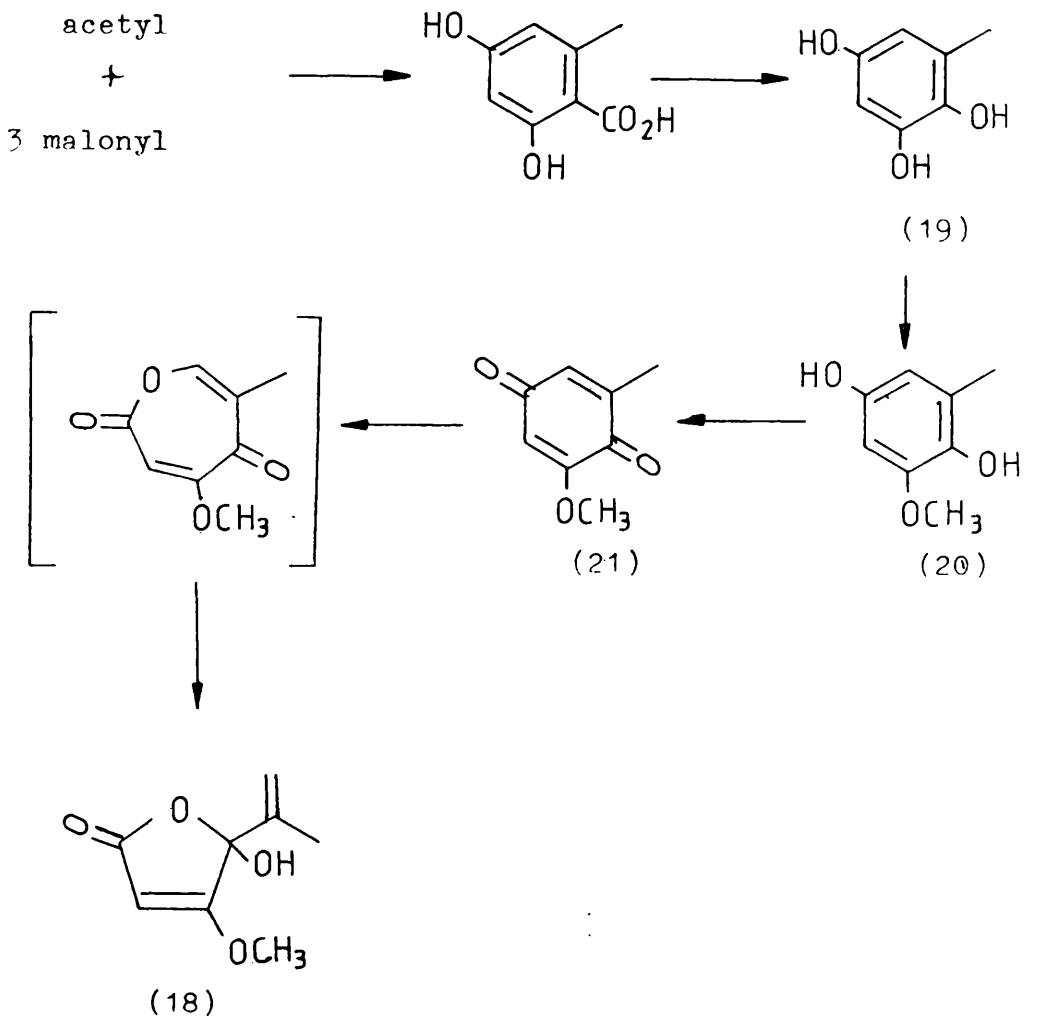
SCHEME 6.

Feeding sodium [1,2-^{13}C]acetate produced[28] penicillic acid whose ^{13}C n.m.r gave coupling between C-2 and C-3 and between C-6 and C-7 showing two intact acetate units were retained, thus confirming previous results.



It has been shown recently[29] that ^{14}C labelled 6-methyl-1,2,4-benzenetriol (19) is incorporated into *Penicillium cyclopium* to produce penicillic acid. This compound (19) was isolated from a mutant of *P. cyclopium* and had previously not been known as a metabolite. The radioactivity of [1-^{14}C]acetate was rapidly incorporated into 6-methyl-1,2,4-benzenetriol in a culture of the *P. cyclopium* mutant.

This metabolite (19), its methyl ether (20) and the benzoquinone (21) are all incorporated into *P. cyclopium* to produce penicillic acid. 1-O-Methylorcinol was found not to be incorporated. These results lead to the proposal of a new pathway for the formation of penicillic acid (Scheme 7) superseding the previous one proposed by Gatenbeck[30,31].

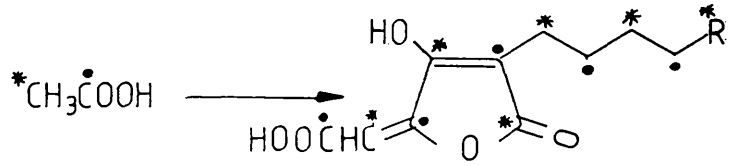


Scheme 7.

The first steps involve oxidative decarboxylation followed by methylation to give hydroquinone (20), which, after further oxidation to quinone (21), is ring cleaved to penicillic acid. The first steps are the opposite to that proposed by Gatenbeck.

In the biosynthesis of the tetronic acids (22), (23) and (24) produced by *Penicillium multicolor*, Holker et al proposed a pathway[32] on evidence gained by incorporation studies with [1-^{13}C]-, [2-^{13}C]- and [1,2-

^{13}C]acetate and ethyl [2-^{14}C]-6-pentylresorcylate. The acetate feeding experiments gave the labelling pattern shown.

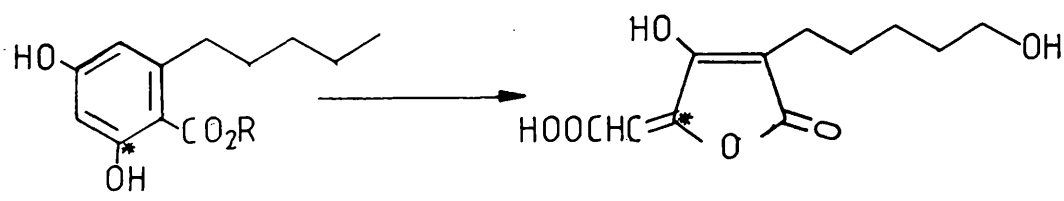


(22) R = $-CO_2H$ multicolosic

(23) R = $=CH_2OH$ multicolic

(24) R = $-CH_3$ multicolanic

The intermediacy of 6-pentylresorcylic acid in the pathway was established by feeding ethyl [2-^{14}C]-6-pentylresorcylic acid to produce the tetronic acids. This indicates oxidative fission of the resorcylic acid to produce the tetronic acids.



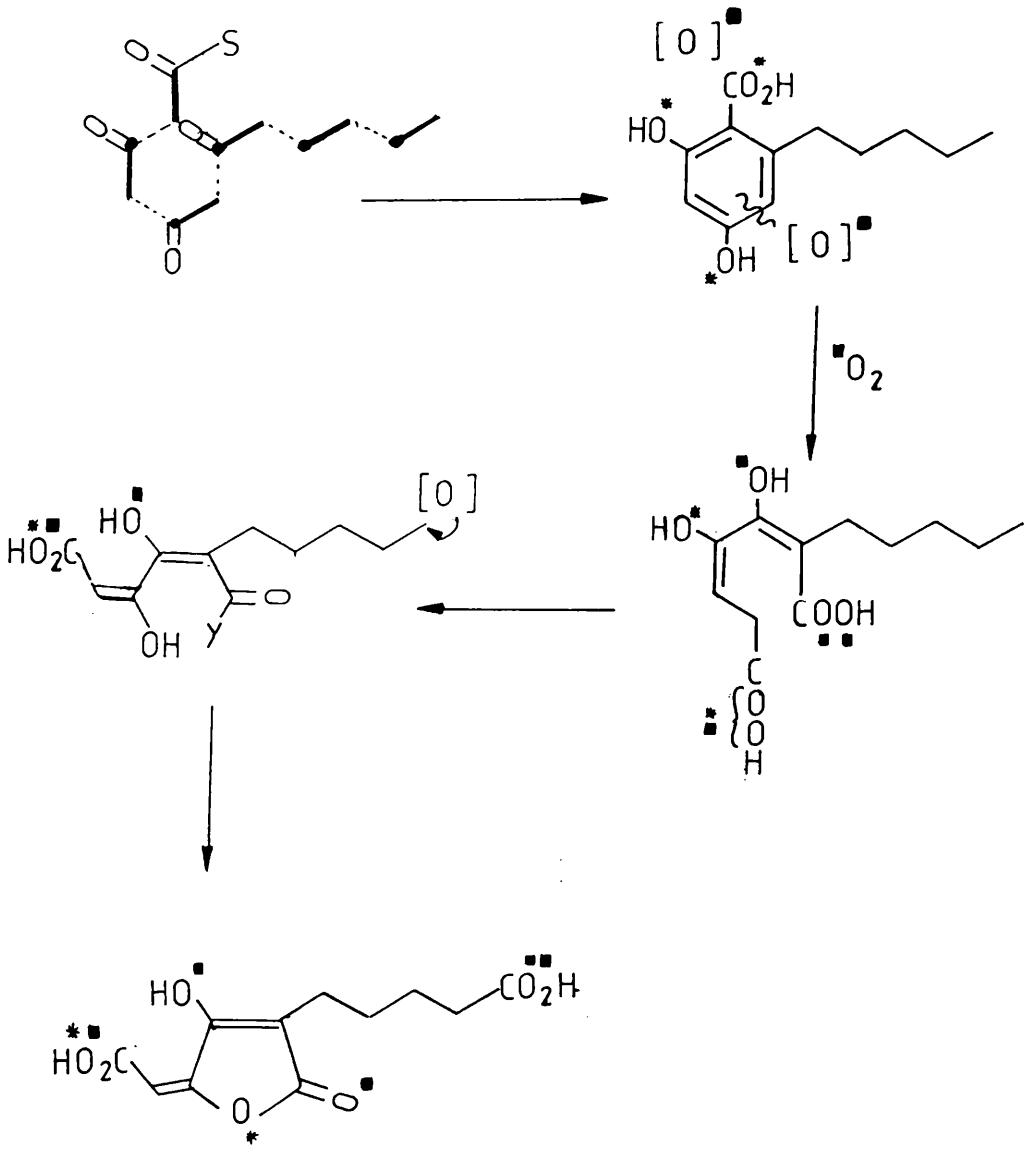
Further evidence was gained by feeding sodium [1-^{13}C,$^{18}O_2$]acetate[33] and fermentation of *Penicillium multicolor* under an atmosphere containing $^{18}O_2$.[34] Incorporation of ^{18}O can be detected by upfield ^{18}O induced shifted signals in the ^{13}C n.m.r.

These shifts occur for directly attached carbons and, in some cases, β carbons. Incorporation of doubly labelled acetate (^{13}C, ^{18}O) into polyketides followed by ^{13}C n.m.r can identify intact carbon-oxygen bonds during biosynthesis.

The tetronic acids isolated (and derivatised) after incubation with [1-^{13}C,$^{18}O_2$]acetate showed upfield shifted signals only for C-4 and C-11. The C-4 had one shifted signal but C-11 had two, one from -C^{18}O.OMe and one from -CO.^{18}OMe. The combined intensities of shifted signals due to C-11 were approximately equal to that due to C-4 indicating equal incorporation of intact [1-^{13}C,$^{18}O_2$]acetate at both sites.

The metabolite multicolosic acid (22) isolated after incubation in an atmosphere containing $^{18}O_2$ gave the following results. All the carbons bearing oxygen showed α-isotope shifts except C-4. The lactone carbonyl (C-1) showed a single shifted satellite which indicates only the carbonyl oxygen is labelled. The same ^{16}O:^{18}O ratio was seen at C-3. C-11 exhibits two upfield isotope shifts as was expected from [1-^{13}C, $^{18}O_2$]acetate results. These ^{18}O results, along with the ^{13}C and ^{14}C results earlier, support the biosynthetic pathway proposed in Scheme 8.

As seen above, labelling with a stable isotope and detection by n.m.r is a useful technique in biosynthetic studies[35]. Another useful isotope in this respect is deuterium. Various n.m.r techniques can be employed



SCHEME 8.

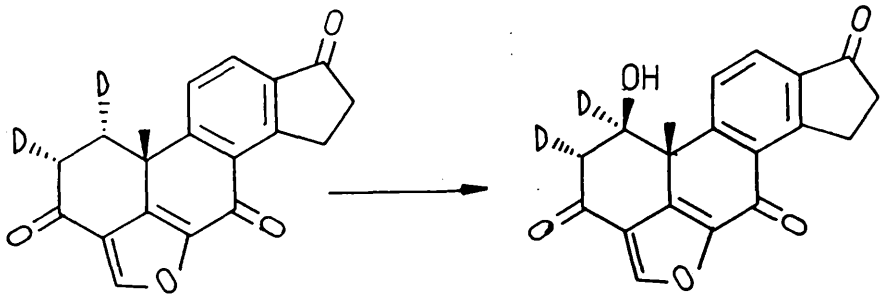
for detection of deuterium. These include direct ^{2}H n.m.r, double labelling with ^{13}C, α,β and γ isotope induced shifts of ^{13}C n.m.r and multi-pulse n.m.r.

Direct ^{2}H n.m.r has both advantages and disadvantages. The major drawbacks are the low sensitivity for detection compared to ^{1}H n.m.r, poor dispersion of chemical shifts (the range is 15% that of ^{1}H n.m.r) and broad signals (due to the nuclear quadruple of ^{2}H). The advantages are that deuterium is inexpensive, non-radioactive and very low levels can be detected (due to its low natural abundance). ^{2}H chemical shifts correspond closely (in p.p.m) to ^{1}H chemical shifts which means a complete assignment of the ^{1}H n.m.r spectrum allows ^{2}H shifts to be identified.

This technique was used in the study of the biosynthesis of the aflatoxin B_1 (25) produced by the fungus *Aspergillus flavus* and *A. parasiticus*. Previous work suggested that aflatoxin B_1 (25) is produced from averufin (26) via versiconal acetate (27) and versicolorin A (28)[36]. This work used averufin biosynthetically labelled from ^{13}C or ^{14}C acetate which could theoretically undergo side chain degradation hence producing labelled acetate which on reincorporation gave identically labelled aflatoxin B_1. This degradation was discounted[37] by using averufin with a specific deuterium label at C-4′. The aflatoxin B_1 produced had the deuterium label on position H-16 shown by ^{2}H n.m.r. It was also shown at this time[38] that averufin labelled entirely with deuterium at C-4′ and C-6′ was incorporated by *A. parasiticus* into versiconal acetate.

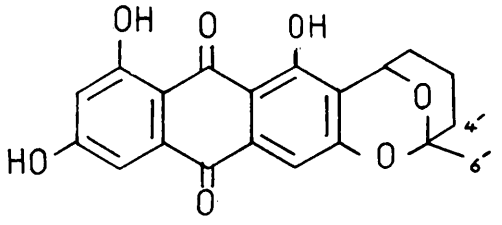
It was thus demonstrated that the whole side chain of averufin becomes the side chain of versiconal acetate. It can be concluded that a Baeyer-Villiger-like oxidation occurs leading to the loss of the two terminal carbons on the averufin side chain (as acetate) in the course of the bisfuran formation of aflatoxin B_1 (Scheme 9).

Biological oxidation can also be studied[35] using ^2H n.m.r as is reported[39] for the formation of demethoxyviridin(29). [1α,2α-$^2H_2$]-Dehydroxydemethoxy-viridin(30) was incorporated by *Nodulisporium hinnuleum* to form demethoxyviridin (29). This showed that the biological oxidation occurred with the normal retention of configuration.

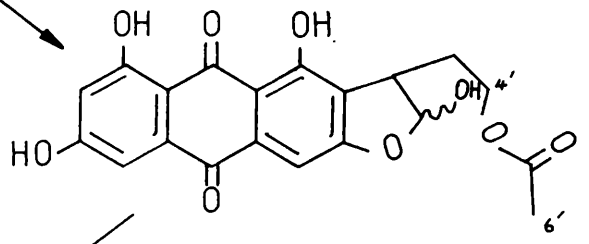


(30)

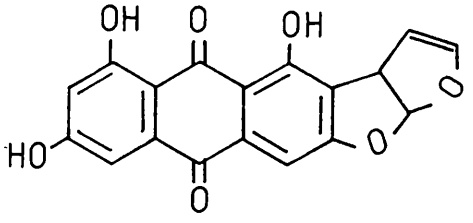
(29)



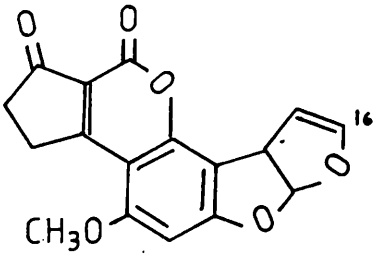
(26)



(27)



(28)



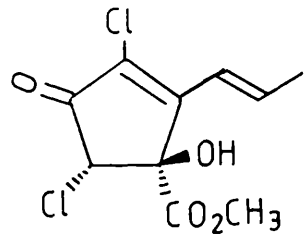
(25)

SCHEME 9.

Chapter 2.

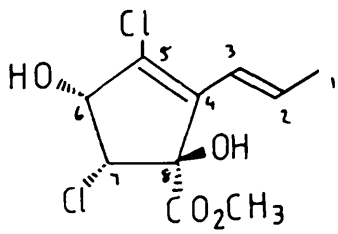
The Biosynthesis of Cryptosporiopsinol

The isolation of cryptosporiopsin(31) was first reported in 1969 as the result of independant studies on the coprophilous fungus *Sporomia affinis*[40] and a *Cryptosporiopsis sp.* isolated from the yellow birch *Betula alleghaniensis*[41]. The structure and absolute configuration were determined using degradative and spectroscopic methods and by X-ray analysis[40].

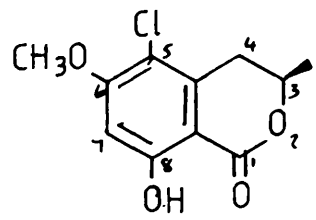


(31)

Shortly afterwards cryptosporiopsin was detected chromatographically in extracts from the fungus *Periconia macrospinosa.* The main metabolites isolated from the fungus are the alcohol, cryptosporiopsinol (32) and the dihydroisocoumarin(33)[42].

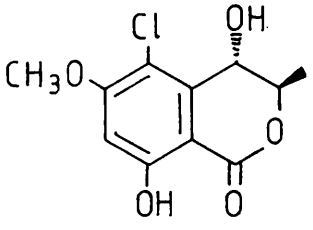


(32)

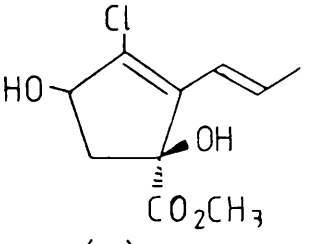


(33)

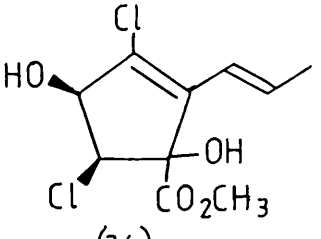
A number of other minor metabolites were also isolated from the fungus[43,44]. These are perimacol(34) and two cyclopentenones. (35) and (36).



(34)

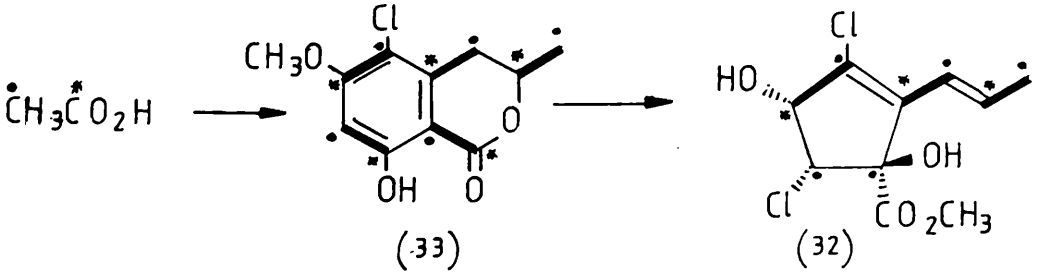


(35)



(36)

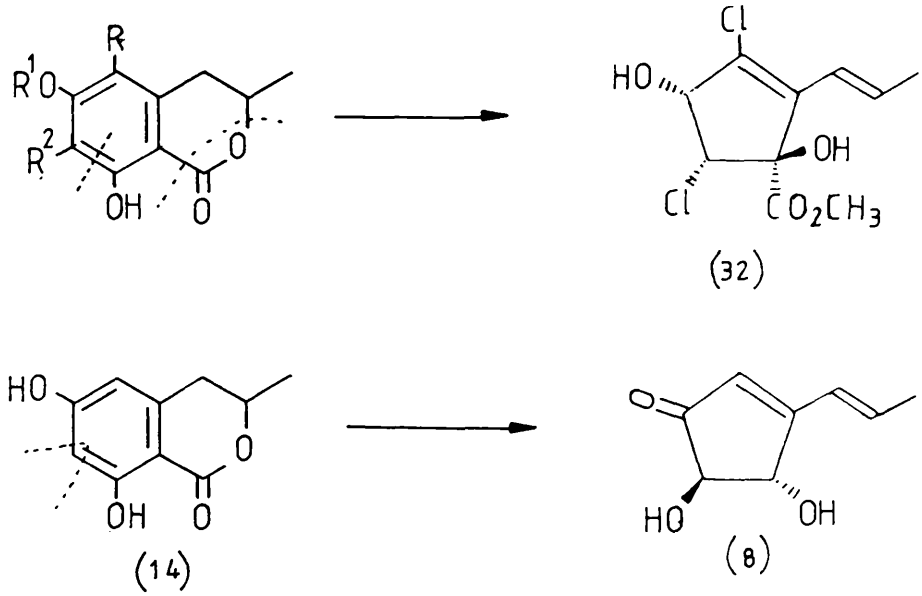
The acetate origin of the metabolites of *P. macrospinosa* was deduced by Holker and Young[45]. Cultures of *P. macrospinosa* were supplemented with [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]-acetate. The ^{13}C n.m.r spectra of the metabolites (32) and (33) were assigned and the compounds were found to be enriched in the positions shown.



(33) (32)

These results suggest that the metabolites (32) and (33) could be derived from a common aromatic precursor, probably closely related to the dihydroisocoumarin metabolite (33). This was investigated further by Henderson[43] who synthesised various dihydroisocoumarins with a ^{14}C label at the C-3 position and studied their incorporation by *P. macrospinosa*. These results are given in Table 2. Kuhn-Roth degradation of the metabolites showed that the compounds were being incorporated intact i.e. without degradation to labelled acetate. Degradation of the precursors to acetate would lead to only 25% of the label being found at C-2 in cryptosporiopsinol (32). The results show some degradation is taking place but the majority of the dihydroisocoumarin is being incorporated intact.

The results show that all the dihydroisocoumarins are incorporated to some extent into cryptosporiopsinol (32). The [^{13}C]acetate results of Holker indicate a ring-cleavage involving the extrusion of C-8 from the aryl ring[45]. The intermediacy of a dihydroisocoumarin is shown to be involved by the work done by Henderson. This contrasts with the cleavage of the dihydroisocoumarin (14) in the case of terrein (8) where C-7 is lost from the aryl ring[46] (see previous chapter).



The question arose as to which dihydroisocoumarin was on the direct pathway to cryptosporiopsinol (32). To elucidate further, various chlorinated and 6-methoxydihydroisocoumarins (3,14,33,37-41) were labelled at C-3 and fed to *P. macrospinosa* and the incorporation into cryptosporiopsinol (32) and the co-metabolite (33) were studied (Tables 2 and 3).

The dihydroisocoumarin (14) and its 5-chloro derivative (40) were incorporated more efficiently than the 6-O-methyl derivative (37) into the dihydroisocoumarin metabolite (33). Thus suggests chlorination occurs before methylation.

Table 2.

Incorporation of [3-^{14}C]Dihydroisocoumarins into Cryptosporiopsinol (32)

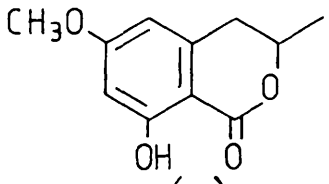
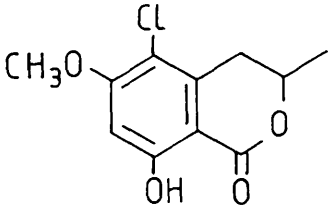
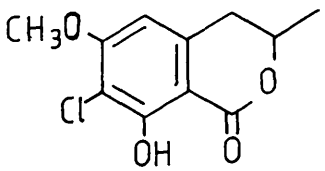
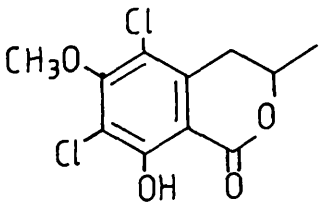
Compound Fed	Incorp. into (32) %	% Activity at C-2
 (37)	0.97	85
 (33)	0.13	-
 (38)	1.75	96
 (39)	2.30	87

Table 2 contd.

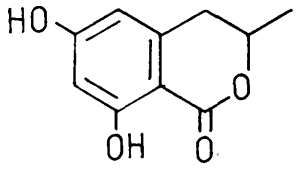
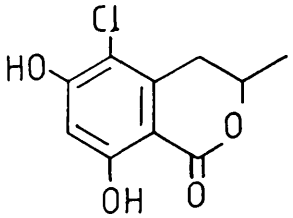
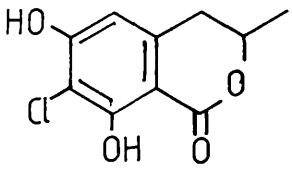
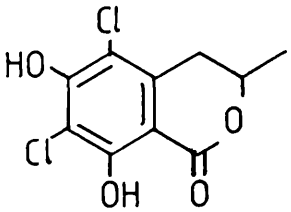
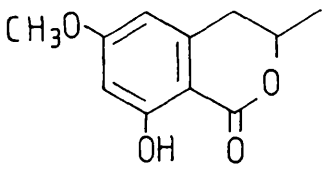
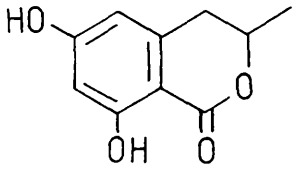
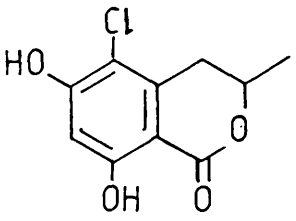
Compound Fed	Incorp. into (32)%	% Activity at C-2
 (14)	**4.60**	**89**
 (40)	**5.0**	**81**
 (41)	**8.0**	**84**
 (3)	**17.6**	**87**

Table 3.

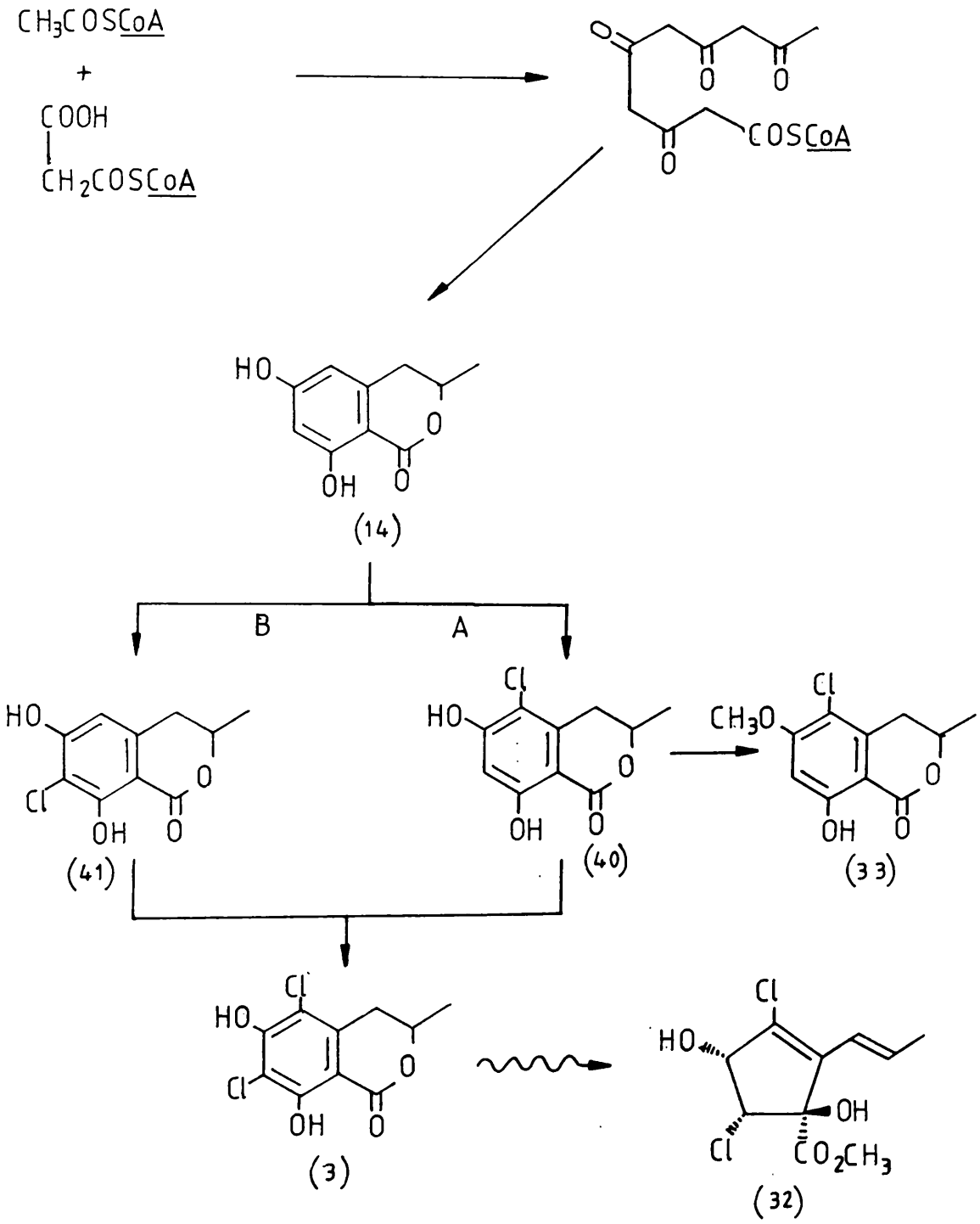
Incorporation of [3-^{14}C]Dihydroisocoumarins into 5-Chloro-3,4-dihydro-6-methoxy-8-hydroxy-3-methylisocoumarin (33)

Compound Fed	Incorp. into (33)%	% Activity at C-3
 (37)	1.66	89
 (14)	17.60	83
 (40)	16.20	85

The 6-methoxydihydroisocoumarins (33), (37), (38) and (39) were incorporated into cryptosporiopsinol (32) to a significantly lower extent than the corresponding 6-hydroxy analogues (3), (14), (40) and (41) suggesting the 6-methoxydihydroisocoumarins were not on the direct pathway. To investigate this further, Henderson carried out studies on chlorine depleted cultures. The dihydroisocoumarins (14) and (37) were isolated from the culture with no ring cleaved products detected. This implies the presence of 5-chloro-6,8-dihydroxydihydro-isocoumarin (40) as a true intermediate. The 7-chloro and 5,7-dichloro-6,8-dihydroxydihydroisocoumarins (41) and (3) were also shown to be true intermediates by precursor trapping experiments. (This was done by feeding radiolabelled acetate along with the proposed precursor, therefore increasing the metabolite pool. If the compounds are precursors they will be isolated in radiolabelled form i.e. trapped).

On the basis of these experiments Henderson proposed a pathway for the early stages of the biosynthesis of cryptosporiopsinol (Scheme 10). The first step involves the cyclisation of a pentaketide chain to give the dihydroxydihydroisocoumarin (14). The intermediate (14) is chlorinated at the 5-position (path A) or at the 7-position (path B). Either of these intermediates can be chlorinated to give the 5,7-dichlorinated species (3) which eventually undergoes ring cleavage to give cryptosporiopsinol (32).

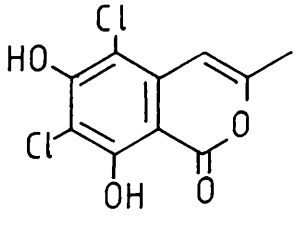
Henderson speculated on the apparent enzymic non-specificity with regard to the site of chlorination of


CH3COSCoA
+
COOH
CH2COSCoA
COSCoA
HO
OH
(14)
B
A
Cl
(41)
(40)
CH3O
(33)
(3)
(32)
OH
CO2CH3

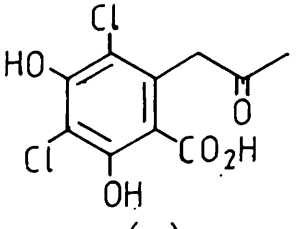
SCHEME 10

the dihydroisocoumarin (14). He suggested a simple model (Scheme 11) that features the initial binding of the enzyme to the 6-hydroxy group of (14). This bonding would bring both sites into approximately equal reactivity towards chlorination, therefore over-riding the reactivity of the substrate at C-5.

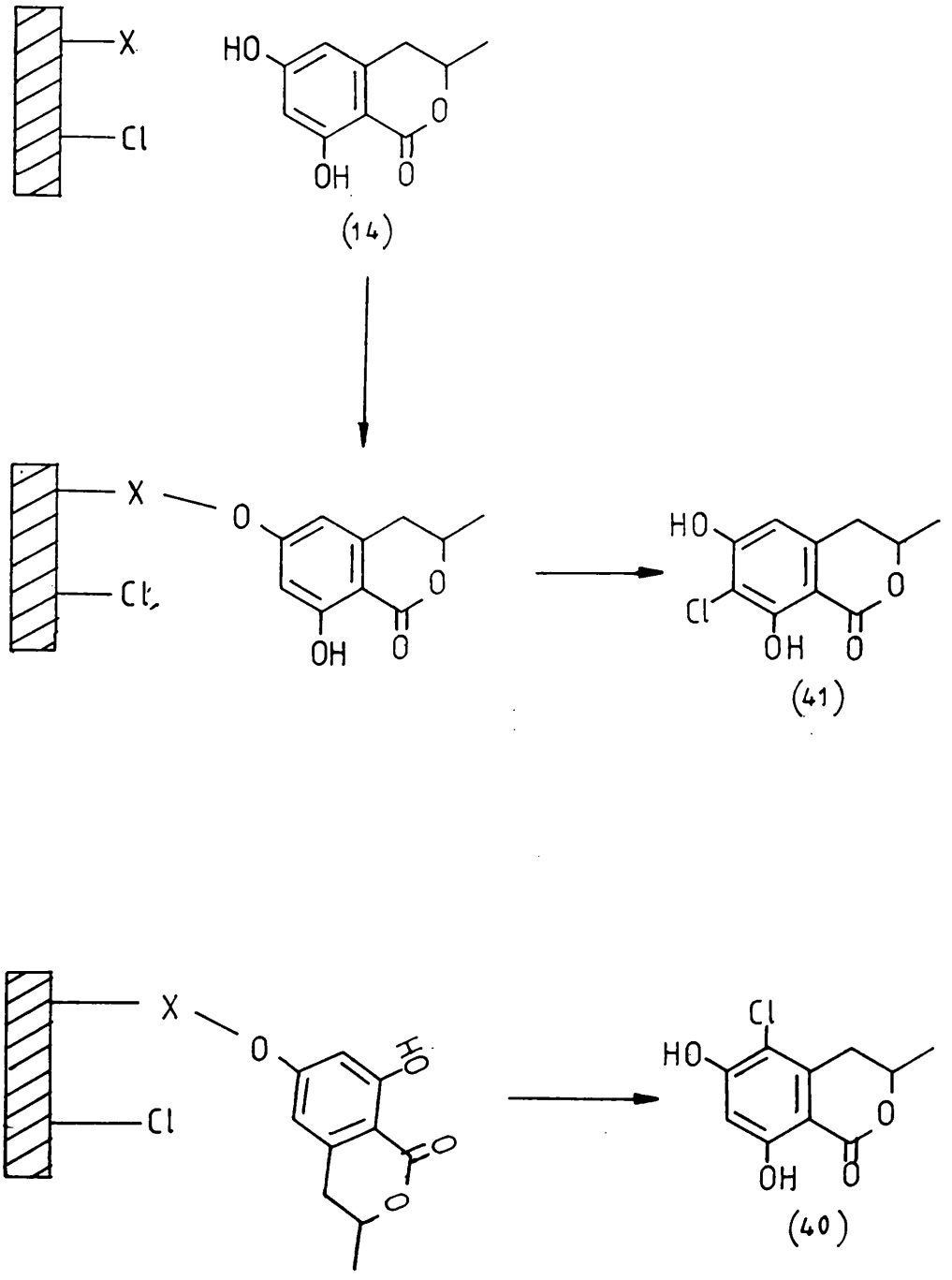
To investigate the biosynthesis of cryptosporiopsinol (32) further it was decided to trace the fate of the three protons at the 3- and 4- positions of the dihydroisocoumarin (3). The fate of the proton at the 3- position of (3) was investigated by Henderson. This position was labelled with deuterium and the [3-^{2}H]-dihydroisocoumarin (42) fed to the fungus. Deuterium labelling was used for the reasons described in Chapter 1. Cryptosporiopsinol (43) was isolated and its ^{13}C n.m.r spectrum recorded. The presence of deuterium was noted by the downfield shift of 0.2 p.p.m at 19.2 and 120.3 p.p.m. due to the carbons C-1 and C-3 which experience β-deuterium shifts. This result shows that the proton at the 3- position in the dihydroisocoumarin (3) is retained throughout its conversion to cryptosporiopsinol (32). These results rule out possible intermediates (44) and (45).



(44)

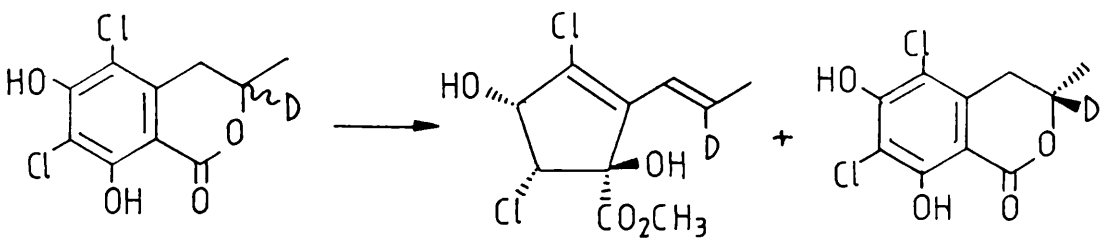


(45)



SCHEME 11

An interesting aside was also obtained from this experiment. Some [3-^{2}H]dihydroisocoumarin (46) which had not been metabolised was recovered and found to be optically active, though the material fed was a racemate. This showed that only the (-)-isomer of the racemate was being incorporated. This (-)-enantiomer corresponds to the 3R-isomer i.e. only [3R]-dihydroisocoumarin (3) is incorporated.

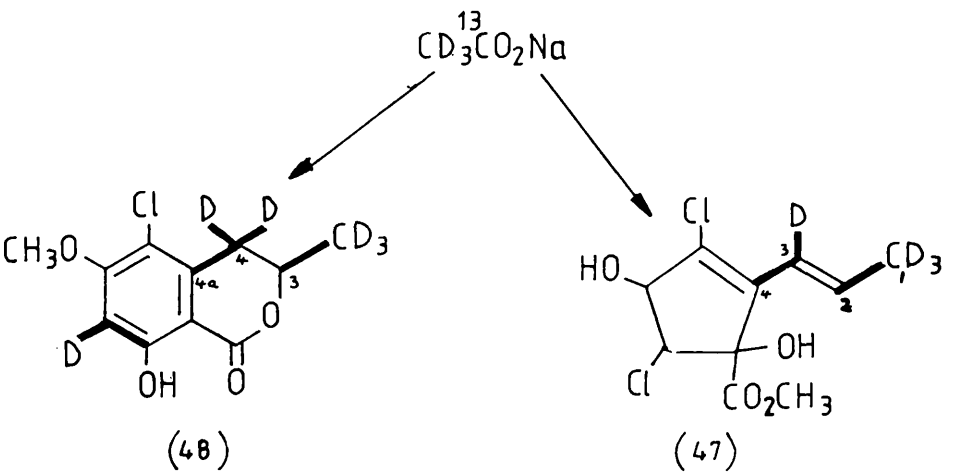


(42) (43) (46)

This piece of information will be made use of further in the present study.

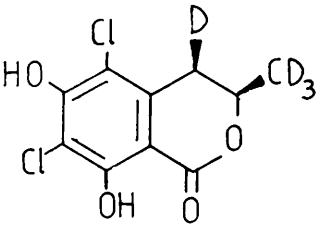
To investigate the fate of the protons at C-3 and C-4 Macaulay[44] fed sodium [1-^{13}C, 2-$^{2}H_3$]acetate to the fungus. The ^{13}C n.m.r spectra of the metabolites cryptosporiopsinol (47) and the dihydroisocoumarin (48) were recorded. In the ^{13}C n.m.r spectrum of dihydroisocoumarin (48) the peak corresponding to C-8 showed a β-isotope shifted signal due to one deuterium at C-7. The peak corresponding to C-4a showed β-isotope shifted signals due to two deuterium atoms being retained at C-4 and the signal due to C-3 showed β-isotope shifts in accordance with three deuterium atoms

being retained in the starter unit methyl group. The cryptosporiopsinol (47) spectrum showed the peak corresponding to C-2 of the side chain with β-isotope shifted signals corresponding to three deuteriums being retained on the methyl group (C-1). The peak corresponding to C-4 showed one β-isotope shifted signal due to one deuterium being retained at C-3.

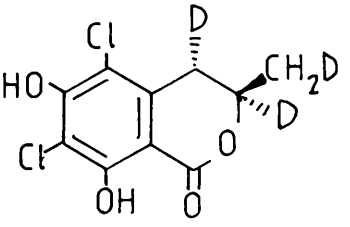


This showed that the deuterium of the original acetate unit has stayed intact in the olefinic group, i.e. has not been cleaved and re-connected due to coupling to ^{13}C-label in the ^{13}C n.m.r spectrum. Again this shows that the pathway does not go through intermediates such as isocoumarin (44) and ketone (45). This also showed that it is likely only one deuterium is removed in the allyl group formation.

The fate of the protons at C-4 was studied. This required the synthesis of the [4-$^{2}H_{proR}$]- and [4-$^{2}H_{proS}$]-labelled dihydroisocoumarins (49) and (50).



(49)

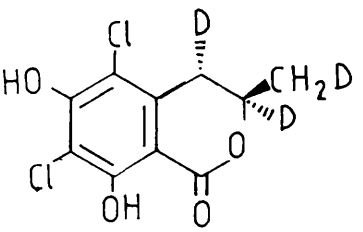
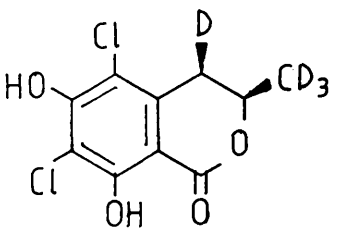


(50)

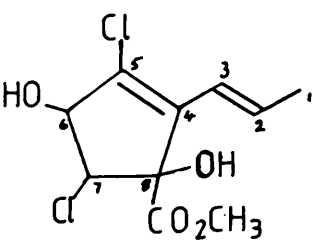
These dihydroisocoumarins (49) and (50) were synthesised and fed in the racemic form. This was not important as the fungus only incorporates the 3-R form of the dihydroisocoumarin (3). This meant that the dihydroisocoumarins are incorporated specifically with etiher 4-R (49) or 4-S (50) configuration. Knowing which proton at C-4 is lost would allow the stereo-chemistry of the allyl-group formation to be determined (assuming the process is in fact stereospecific). The cryptosporiopsinol obtained from the feeding experiments was analysed using ^{2}H n.m.r. This gave the following results in Table 4.

Table 4

Incorporation of [4-^{2}H]Dihydroisocoumarins into Cryptosporiopsinol (32)

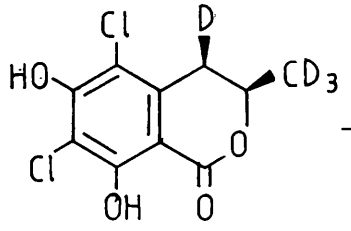
Compound Fed	Chemical shift of ^{2}H signal	Assignment
 (50)	6.23	C-2
	1.78	C-1
 (49)	6.19	C-3
	1.77	C-1

Assignments are given according to structure below:-

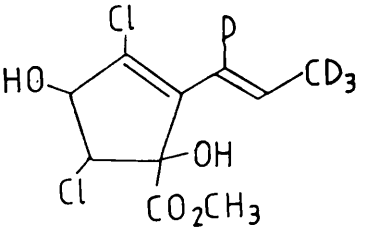


(32)

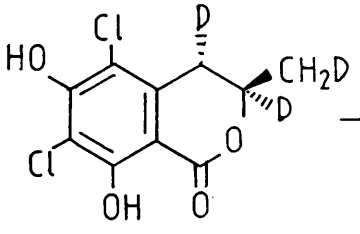
The results indicate the following reactions are taking place.



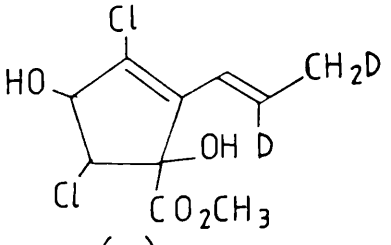
(49)



(51)



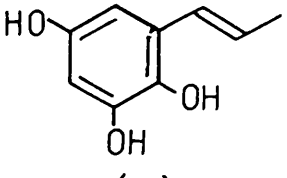
(50)



(52)

These results show that the 4-H_{proS} proton is cleaved specifically from dihydroisocoumarin (3). The deuterium signal at C-1 of cryptosporiopsinol (52) from the feeding of dihydroisocoumarin (50) is due to the methyl of dihydroisocoumarin (50) being labelled during the hydrogenation step (due to allylic scrambling) in the synthesis.

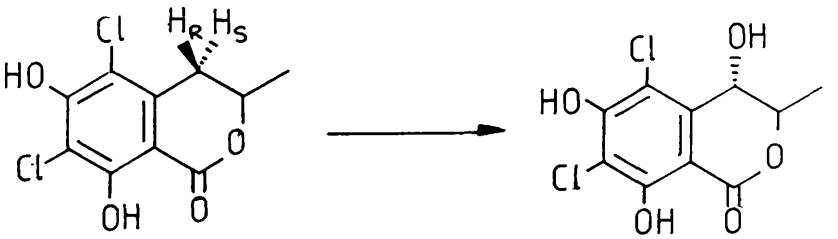
In his study of the biosynthesis, MacLachlan[47] found that on feeding the trihydroxyphenylpropene (53) (as the triacetoxy) to the fungus, the production of cryptosporiopsinol was inhibited and that the metabolite perimacol (34) was formed as the main metabolite.



(53)

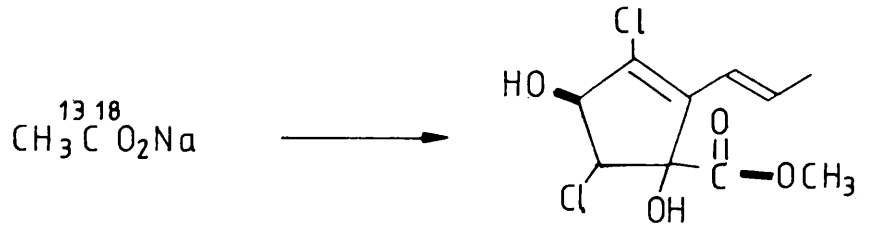
The absolute configuration of perimacol (34) was deduced by Macaulay[44] and was found to have the 3-R, 4-S-configuration. The hydroxy group at C-4 of perimacol (34) takes the position of the 4-H_{proS} proton removed from dihydroisocoumarin (3) (as expected for stereospecific enzymic hydroxylation).

These pieces of evidence lead us to suspect that a 4-hydroxydihydroisocoumarin such as (54) is an intermediate on the direct pathway to cryptosporiopsinol. This can only be verified on the synthesis of a C-4 hydroxylated dihydroisocoumarin.



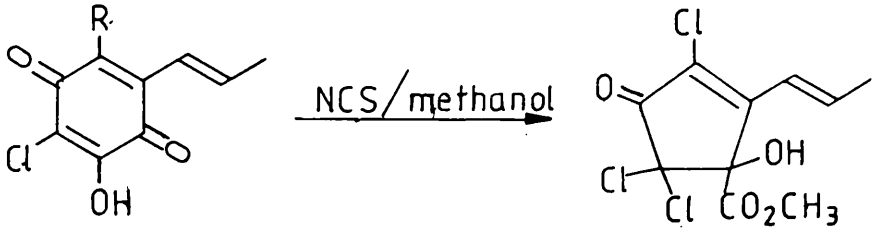
(3) (54)

To investigate the ring contraction further, Macaulay fed [1-^{13}C,$^{18}O_2$]acetate to the fungus. The cryptosporiopsinol (55) obtained was studied using ^{13}C n.m.r. An isotope shift (0.017 p.p.m) of the signal due to the carbonyl carbon of cryptosporiopsinol suggests that the methoxy oxygen is labelled. This, in addition to the intact unit at C-6, gives the following labelling pattern.



(55)

This is in contrast to a proposed[43] biomimetic ring contraction of quinone (56) to the cyclopentenone (57).



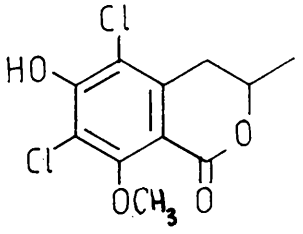
(56)

R= H or Cl

(57)

As can be seen from the acetate feedings, the methoxy oxygen is acetate derived whereas the comparative synthetic cyclopentenone (57) would have the methoxy oxygen derived from the medium.

To investigate this further it was decided to synthesise the dihydroisocoumarin (58) with the C-8 hydroxy group methylated.

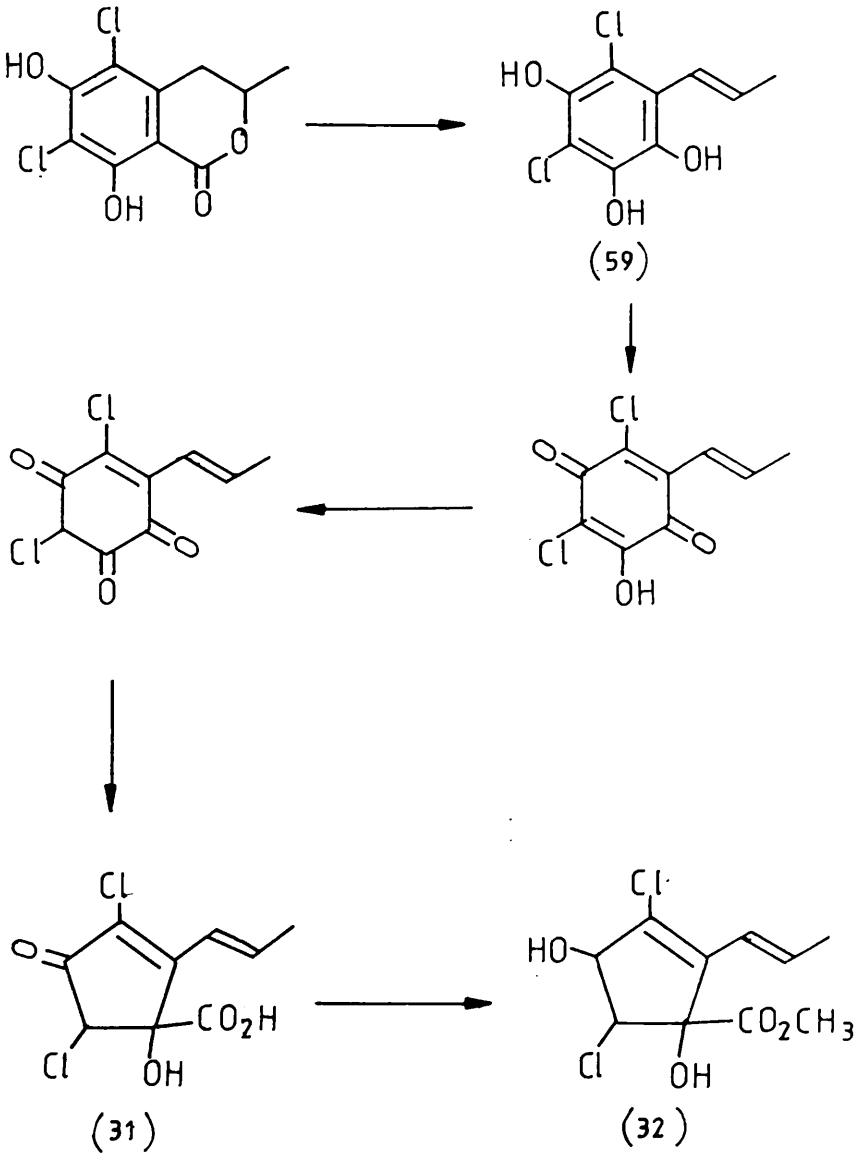


(58)

If the dihydroisocoumarin (58) was incorporated it would indicate how early in the pathway that methylation occurred and verify or discount the [1-^{13}C, $^{18}O_2$]-acetate results. It would probably be best to label the methyl group attached to the hydroxy at C-8 to discount a demethylation, remethylation procedure. However due to lack of time, the dihydroisocoumarin (58) was unable to be synthesised and therefore no feeding experiments could be carried out.

In his study of the final stages of the biosynthesis of cryptosporiopsinol MacLachlan[47] proposed the intermediacy of a trihydroxyphenylpropene (59) (Scheme 12) which involves a similar ring contraction to that of the chemical process of the quinone (56).

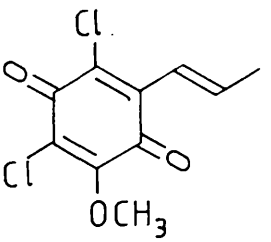
However on feeding the triacetoxy derivative of this compound (59) no incorporation was found to have


Cl
HO
Cl
OH
O
O
Cl
HO
Cl
OH
OH
(59)
Cl
O
Cl
O
O
Cl
O
Cl
OH
Cl
O
Cl
CO2H
OH
(31)
Cl
HO
Cl
CO2CH3
OH
(32)

SCHEME 12

taken place. It could be argued that the acetoxy groups prevented incorporation or that the trihydroxy compound (59) was not an intermediate. The [1-^{13}C, $^{18}O_2$]acetate feedings support the latter solution, that the trihydroxy compound (59) is not an intermediate.

It was thus proposed that the quinone (60) was an intermediate and its synthesis was attempted.



(60)

The quinone (60) could be derived from the 4-hydroxydihydroisocoumarin (54) and would explain the labelling pattern of the [1-^{13}C, $^{18}O_2$]acetate feeding experiments.

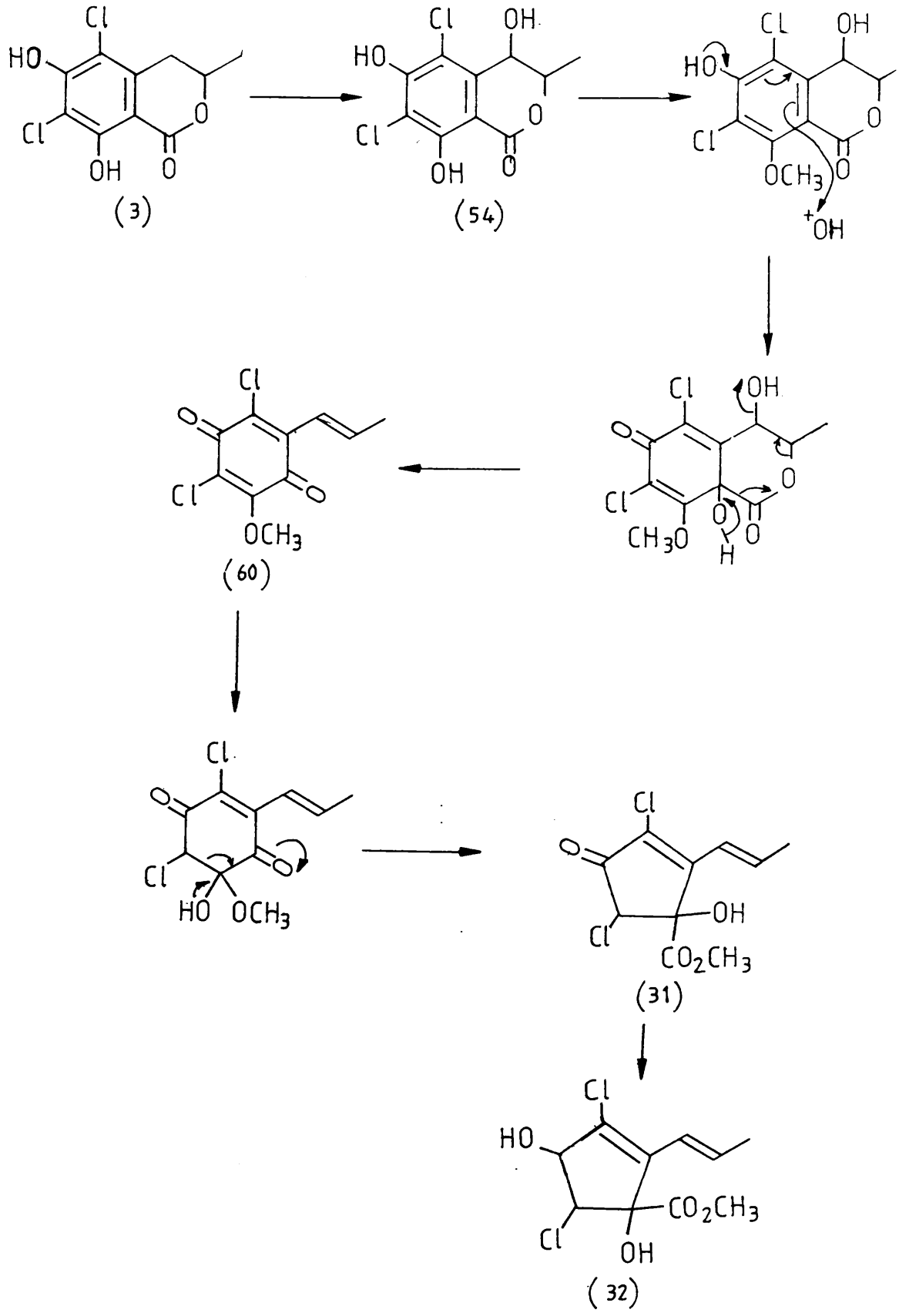
In the time available the synthesis of quinone (60) was not completed and no feeding experiments could be carried out. Therefore, no firm conclusion can be drawn about the final ring contraction pathway.

The following mechanisms are, therefore, being tentatively proposed:

a) Hydroxylation is occurring at C-4 of the dihydroisocoumarin (3).

b) Methylation is occurring at the hydroxy group on C-8 of dihydroisocoumarin (3).
c) Oxidative decarboxylation is occurring at C-8a.
d) Ring cleavage occurs by attack on the quinone intermediate (60).

This leads to the following mechanism (Scheme 13). Stereospecific hydroxylation is occurring at C-4 of the dihydroisocoumarin (3). At some stage methylation of the oxygen at C-8 occurs, which may be followed by enzymic oxidation at C-8a with subsequent loss of C-1 as carbon dioxide and elimination of hydroxyl group to give the quinone (60). The quinone (60) may then undergo hydroxylation at C-2 followed by ring contraction to give cryptosporiopsin (31) which is then reduced to cryptosporiopsinol (32). This mechanism involves the metabolite cryptosporiopsin (31) which has also been isolated from *P. macrospinosa*.


Cl
HO
Cl
OH
O
O
(3)
Cl
OH
HO
Cl
OH
O
O
(54)
Cl
OH
HO
Cl
O
OCH3
O
+OH
Cl
OH
Cl
O
O
CH3O
O
H
Cl
Cl
OCH3
(60)
Cl
Cl
HO
OCH3
Cl
OH
Cl
CO2CH3
(31)
Cl
HO
CO2CH3
Cl
OH
(32)

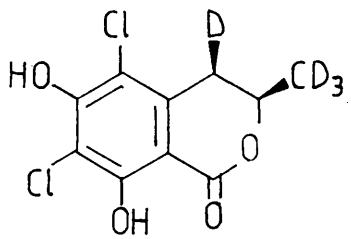
SCHEME 13

Chapter 3

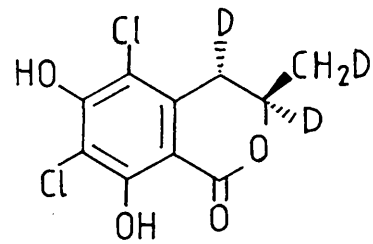
Synthesis of Labelled Compounds

(a) [4-^{2}H]Dihydroisocoumarins.

To investigate the biosynthetic pathway of cryptosporiopsinol (32) in *Periconia macrospinosa* further, the following dihydroisocoumarins (49) and (50) were synthesised.



(49)

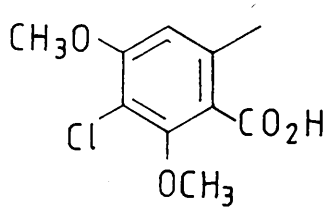


(50)

It was not important if racemic mixtures of these compounds were produced as the fungus only incorporated the 3-R isomer[43] (as described in the previous chapter).

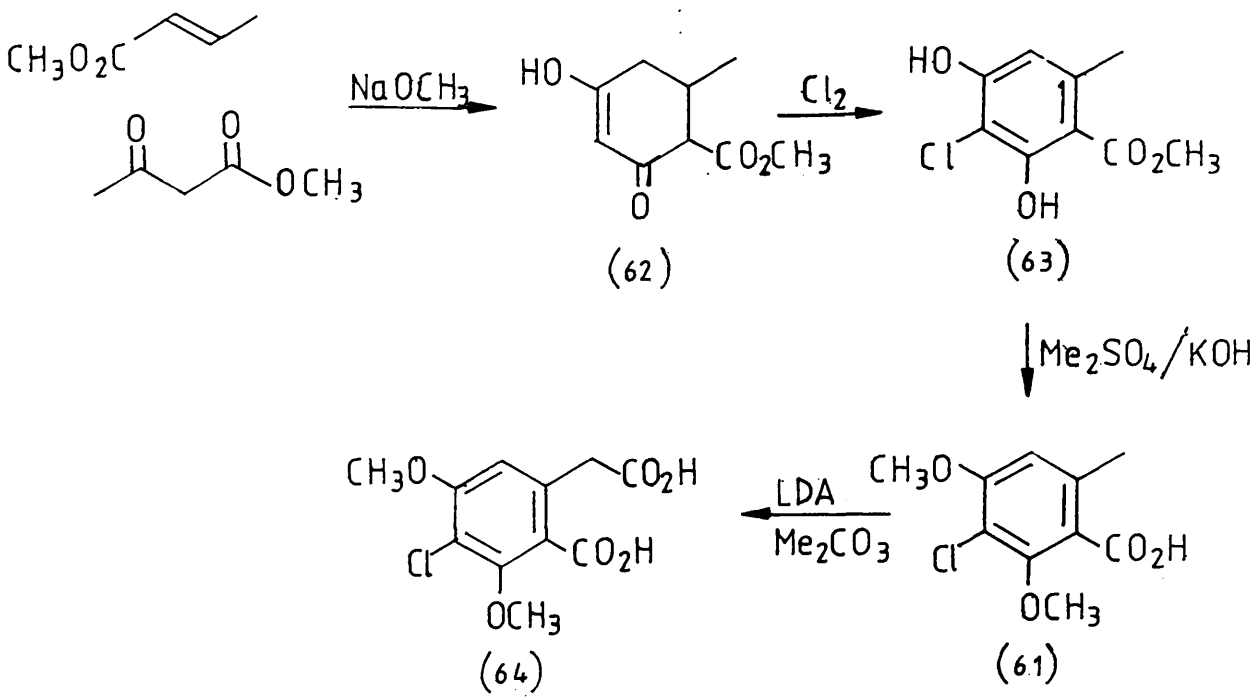
Because isocoumarins occur so widely in nature a number of synthetic routes have been reported.[48,49] A route[50] based on the acylation of homophthalic acid was decided upon. Many slight variations[51] have been employed on this basic route. The synthesis of the homophthalic acid was carried out using orsellinic acid derivatives. The 3-chloro-orsellinic acid (61) could be prepared and further chlorination could occur at a later stage. This was because of better yields[43] for

the mono chloro compounds.



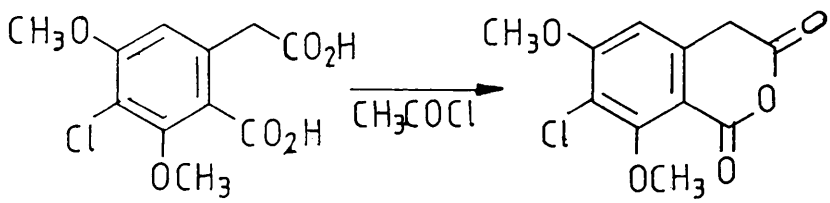
(61)

The 3-chloro-orsellinic acid was prepared[52] by chlorination and aromatisation of the dihydro-orsellinic ester (62) to give orsellinate (63) followed by methylation (with dimethyl sulphate) and, finally, hydrolysis of the ester. This toluic acid (61) was converted into the corresponding homophthalic acid (64) by carboxylation of the dianion produced by lithium di-isopropylamide[53,54] (Scheme 14).



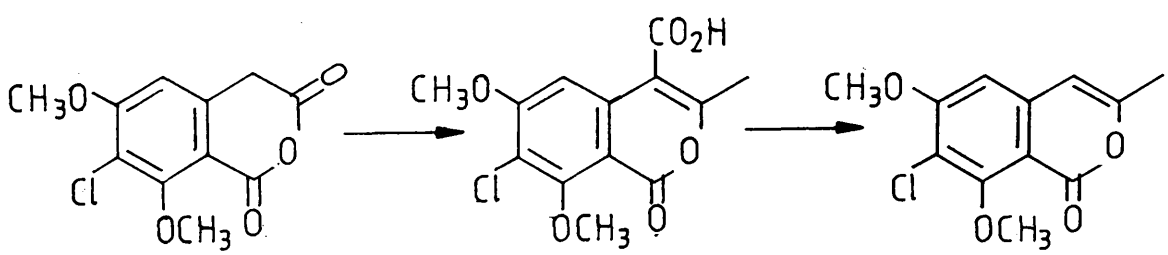
Scheme 14

It was then proposed to acylate the homophthalic acid (64) using acetic anhydride and pyridine.[55] However, yields of product using this route were erratic. It was then decided to investigate other routes. A similar route[43] to that above was attempted which involved the homophthalic anhydride (65) in place of homophthalic acid (64).



(64) (65)

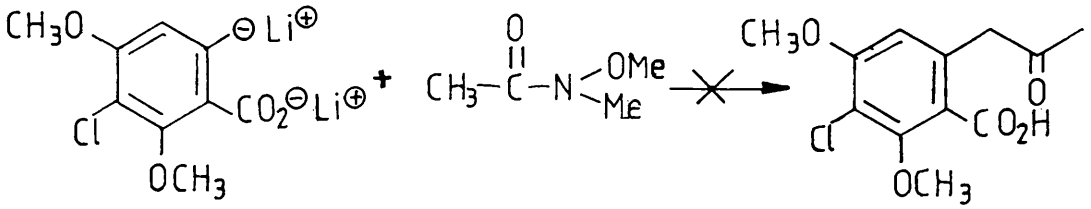
This homophthalic anhydride (65) was acylated, using acetic anhydride and pyridine to give the corresponding 4-carboxyisocoumarin (66). The 4-carboxyisocoumarin decarboxylated on heating to give the isocoumarin (67).



(65) (66) (67)

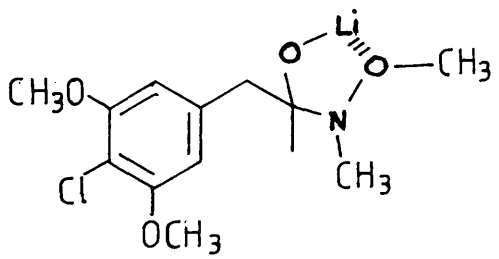
The isocoumarin (67) could be labelled with deuterium at the C-4 position by exchanging the carboxylic proton of 4-carboxyisocoumarin (66) with deuterium oxide and then heating.

This route, however, gave lower yields than was reported[43] and therefore another more recent route was attempted.[56] This involved the formation of a toluate dianion (68) and its condensation with N-methoxy-N-methylacetamide (69).



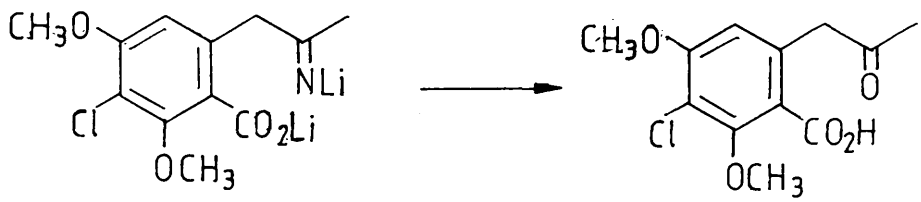
(68) (69) (70)

This route proved unsuccessful as only starting material was recovered. The reaction proceeds through a proposed[57] intermediate (71) which might be destabilised by the chlorine.



(71)

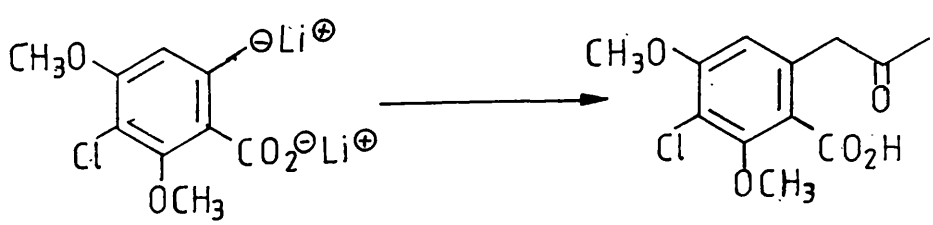
Further acylating agents were tried under the same conditions as before. The first was acetonitrile. It was hoped the toluic anion would attack the nitrile moiety to give the ketimine salt (72) which could be hydrolysed to give the ketone (70).



(72) (70)

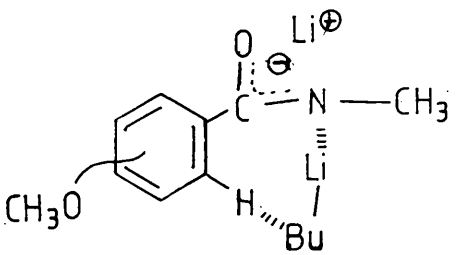
Again, only starting material was recovered. This could be explained if a metal-hydrogen exchange occurred between the toluic acid anion and the acetonitrile. The protons on the benzyllic position of the ketimine salt (72) are activated therefore exchanging with any toluic anion formed. These competing reactions would both give back starting material.

It was reported[58] that controlled acylation of 2-ethoxycarbonyl-3,5-dimethoxybenzyllithium with acetyl chloride under inverse conditions gave the corresponding ketone in moderate yields. This was attempted using the toluate anion (68) under the same conditions. This gave the desired compound (70) but in a poor yield.



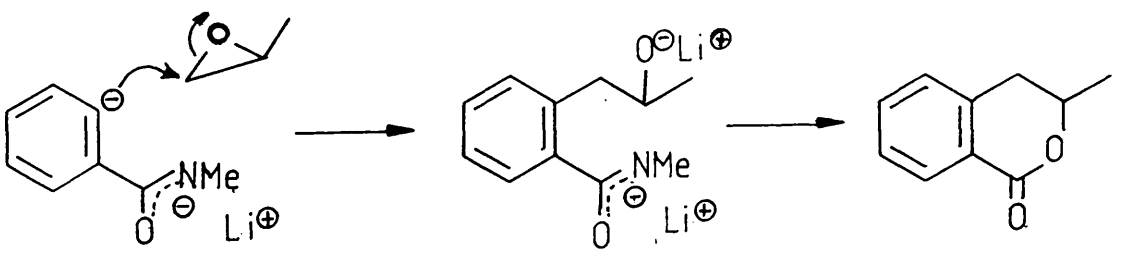
(68) (70)

Another interesting route to isocoumarins involves the lithiation of N-methylbenzamides at the ortho position to the amide group. A number of isocoumarins have been synthesised in this way.[59] The reaction is thought to proceed through the complex (73),[59] in which the acidic proton on the nitrogen is replaced by lithium.

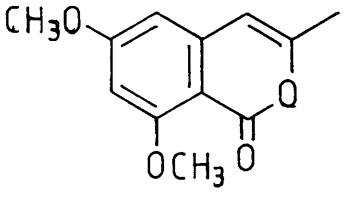


(73)

This complex is then reacted with propylene oxide followed by acidic work up to give directly a dihydro-isocoumarin.

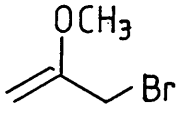


Dihydroisocoumarins can be converted into isocoumarins by reacting with N-bromosuccinimide followed by elimination using triethylamine. This type of reaction has been used to synthesise[60] the isocoumarin with the correct oxygenation pattern on the aromatic ring, namely 5,8-dimethoxy-3-methylisocoumarin (74).



(74)

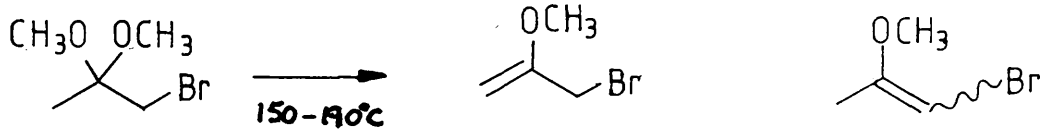
However, when this route was attempted only starting material was recovered. It was decided to try other acylating agents. It was reported[61] that 2-methoxyallylbromide (75), "a superior acetonyl alkylating agent", has been successfully used as a masked acylating agent.



(75)

Acids, esters, amides, nitriles and β-keto esters were reported[61] to be monoalkylated successfully using 2-methoxyallylbromide (75) and lithium di-isopropylamide at -78°C.

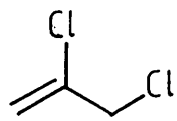
The 2-methoxyallylbromide (75) was reported[61] to be prepared by pyrolytic cracking of 1-bromo-2,2-dimethoxypropane (76) in the presence of diisopropylethylammonium tosylate which leads to a mixture of 2-methoxyallylbromide (75), 1-bromo-2-methoxy-1-propene (77) and starting material which could not be separated.



(76) (75) (77)

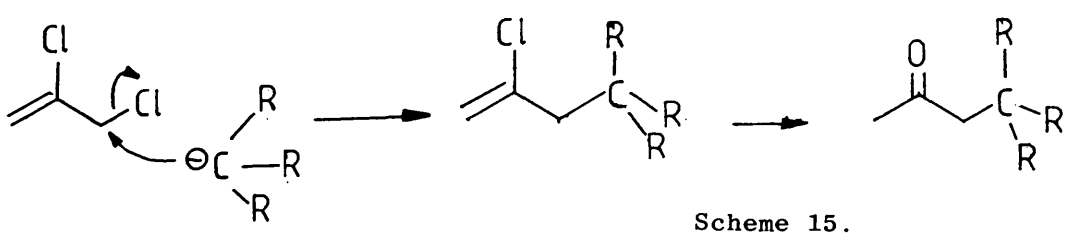
When this was attempted under these conditions, only decomposition products were obtained. This was the problem encountered in the report[61] while trying to purify the products by distillation.

The use of this masked agent was abandoned and another masked agent, 2,3-dichloropropene (78), was used. This reagent has been used in the synthesis of cyclic ketones[62] and substituted furans.[63]



(78)

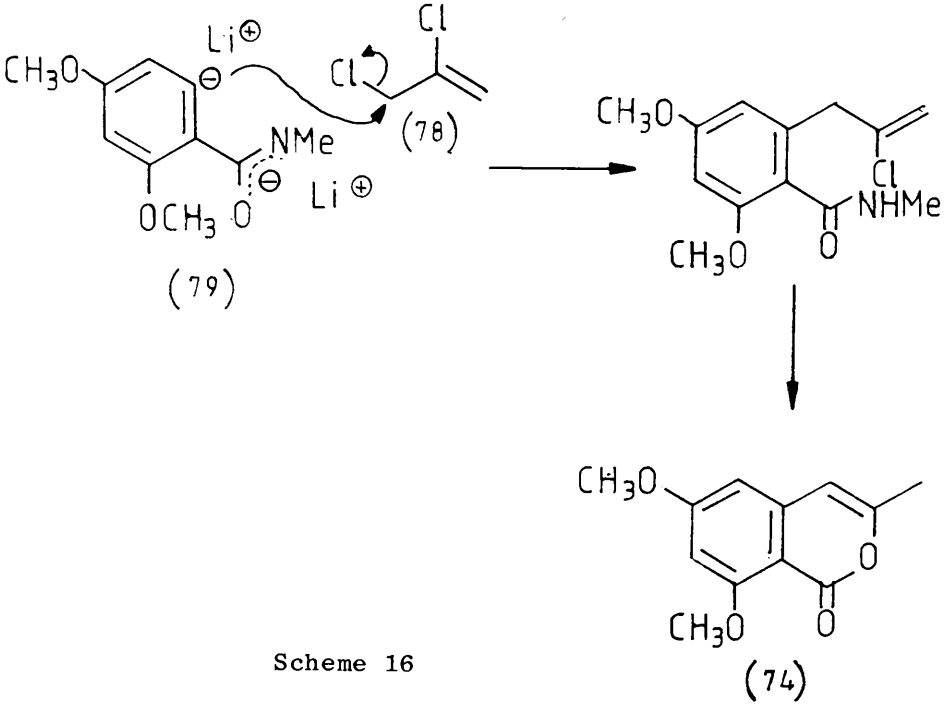
The 2,3-dichloropropene (78) was allowed to react with a nucleophillic reagent to produce a β-chloroallyl derivative (79) followed by hydrolysis of the masked ketone (Scheme 15).



Scheme 15.

It was hoped that in a similar way the o-lithiated

benzamide would give the desired product (74) (Scheme 16).



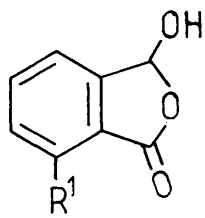
Scheme 16

When the reaction was carried out it was found that the dichloropropene (78) had polymerised and only starting dimethoxybenzamide was recovered, even after using very high dilution and syringe pump techniques.

On further investigation of these acylation reactions it was discovered that the anion (79) was not formed. This was achieved using deuterium labelling techniques by quenching the dianion (79) with deuterium oxide. No aromatic proton had exchanged with deuterium, therefore no anion could have been formed. This was probably due to the reactions being carried out at lower temperature than reported[60] for the anion (73) formation.

A new route was recently reported[64] that produced

isocoumarins with various substitution patterns in higher yields than previously reported. The route is achieved from phthalaldehydic acids (80) and nitroalkanes (81).

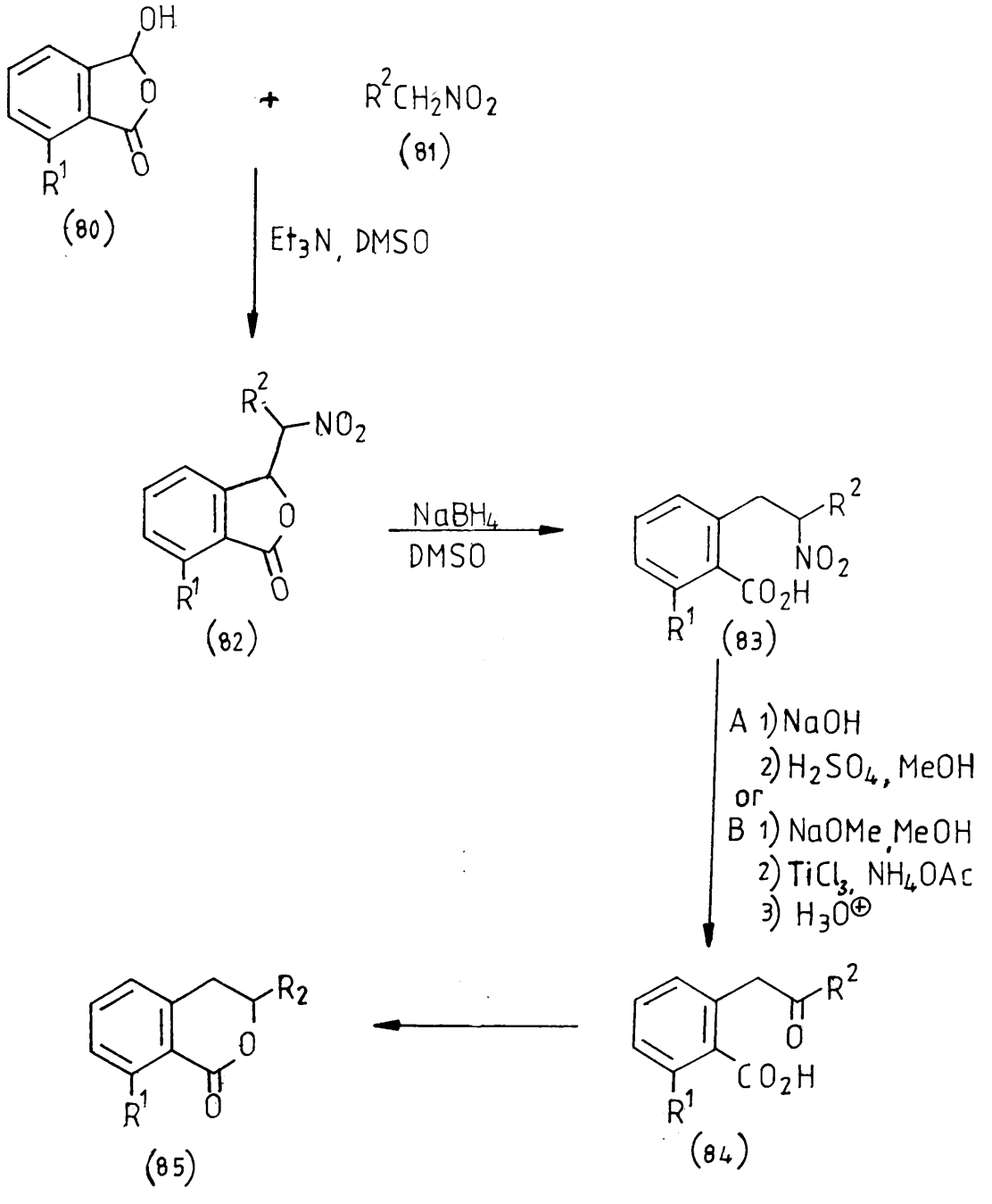


(80)

$R^2CH_2NO_2$

(81)

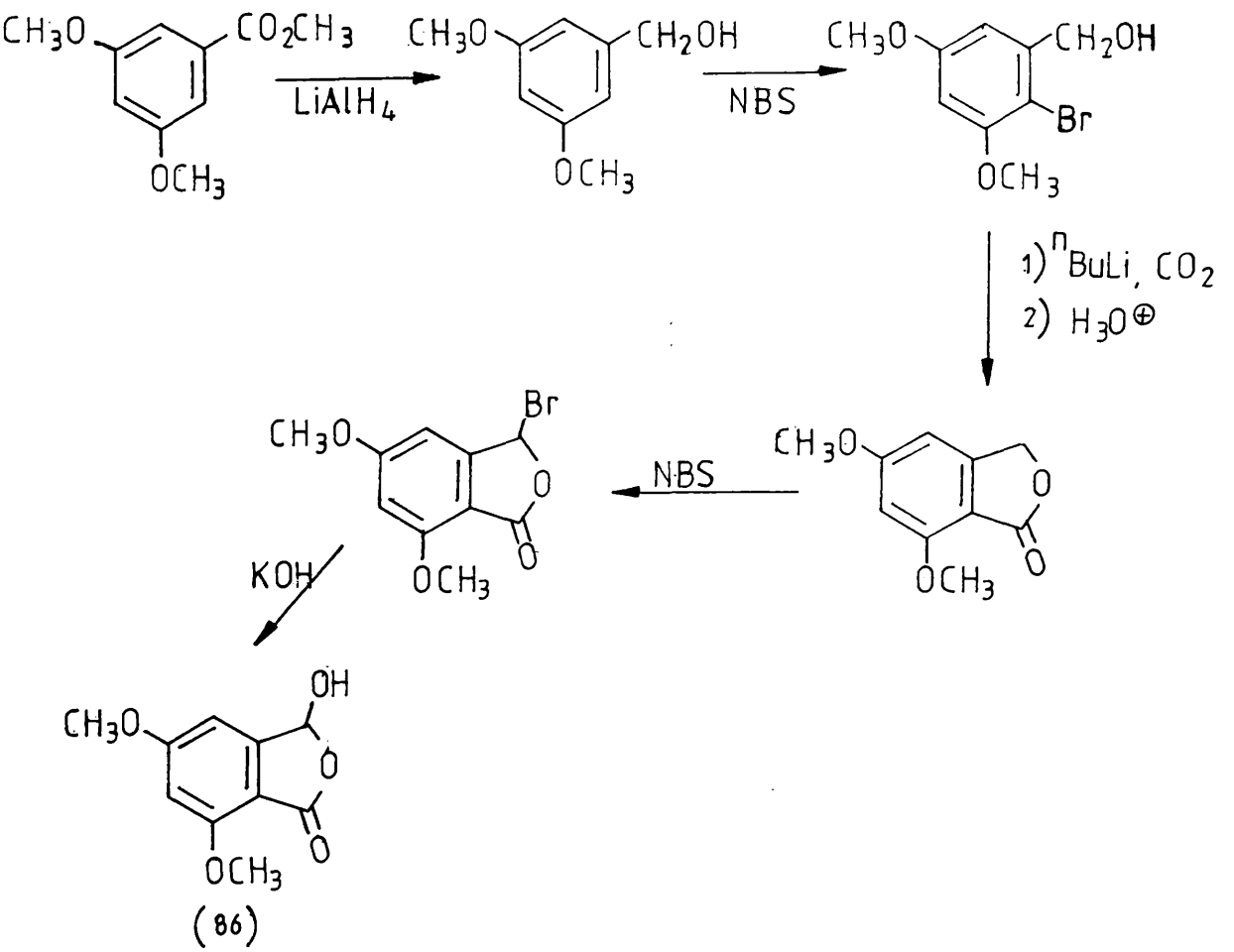
The first step involves the Henry condensation of the phthalaldehydic acid and the nitroalkane to form nitroalkyl-substituted isobenzofuranones (82) using generally triethylamine as base. The nitroalkyliso-benzofuranones (82) underwent reductive cleavage using sodium borohydride in dimethylsulfoxide, to give the corresponding nitroalkylbenzoic acid (83). Two routes were then used. The first involved adding sodium hydroxide to form the nitronate anion, which was added to sulfuric acid in methanol to give the corresponding keto acid (84). This could also be achieved by using McMurry's titanium trichloride procedure. The keto acid (84) was cyclised to isocoumarin (85) with acetic anhydride, ethyl acetate and perchloric acid as catalyst (Scheme 17).


OH
O
O
R1
(80)
+
R2CH2NO2
(81)
Et3N, DMSO
R2
NO2
O
O
R1
(82)
NaBH4
DMSO
R2
NO2
CO2H
R1
(83)
A 1) NaOH
2) H2SO4, MeOH
or
B 1) NaOMe, MeOH
2) TiCl3, NH4OAc
3) H3O⊕
R2
O
CO2H
R1
(84)
R2
O
O
R1
(85)

SCHEME 17

At first this route seemed very promising as it gave a method of preparing the desired keto acid (84) which could be conveniently used to give the desired labelled dihydroisocoumarins (49) and (50).

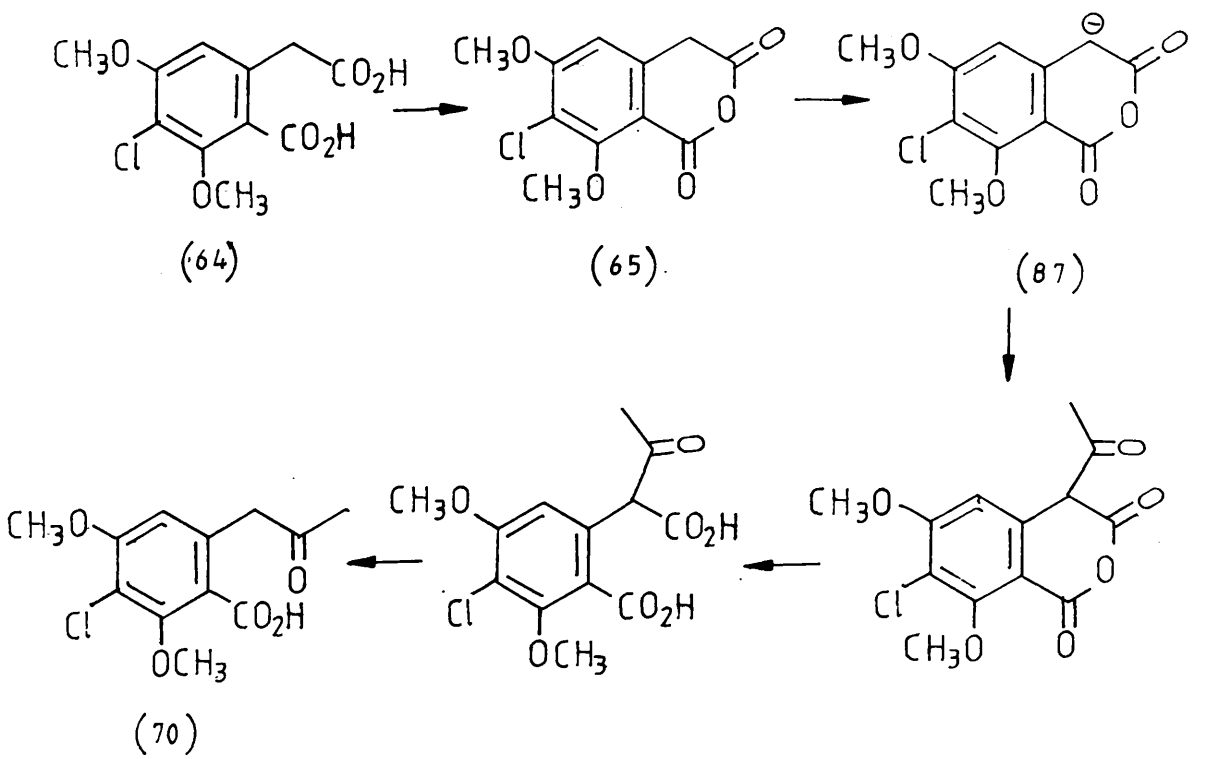
To investigate this route it was decided to prepare a phthalaldehydic acid with the desired oxygenation pattern, but without the chlorine substituent as this would be easier to prepare. It was decided to prepare the dimethoxyphthalaldehydic acid (86) by literature methods[65] (Scheme 18).



Scheme 18

The yields achieved in the synthesis of the dimethoxyphthalaldehydic acid were less than those reported. In consideration of this, the number of steps involved to the isocoumarin and the introduction of chlorine to the benzene ring system this scheme was abandoned.

It was thus decided to attempt the initial route[55] again but varying the conditions involved. To follow this route homophthalic acid (64) initially forms the homophthalic anhydride (65). This will then lose a proton from the benzylic position (C-4) and in turn can be acylated at that position then decarboxylated under alkaline hydrolysis to give the keto acid (70) (Scheme 19).



Scheme 19

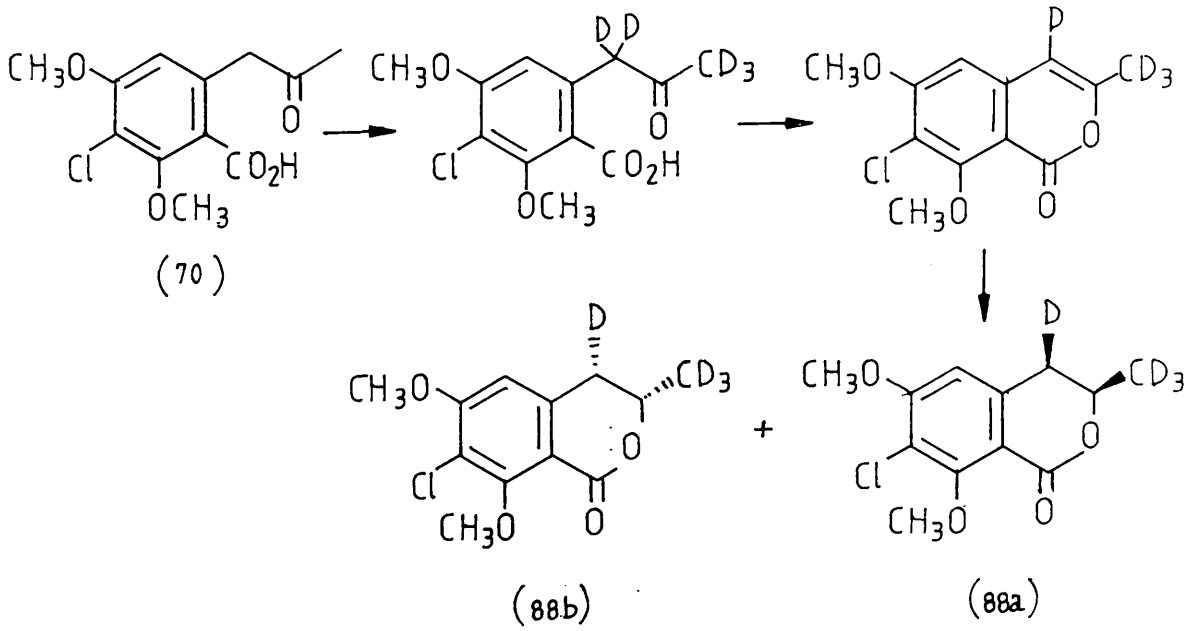
Using reported conditions[55] this reaction did not proceed. It was thought that the anion (87) did not form and therefore stronger bases, e.g. triethylamine, were used. This proved unsuccessful. The reaction was carried out at higher temperatures using initial conditions and stronger bases. Again this proved unsuccessful and only starting material was recovered.

These previous reactions were carried out in diethylether which appeared to be a poor solvent for the mixture as a substance probably the pyridinium salt (87) precipitated out of solution. It was decided to use a more polar solvent that would not react with the anion (87) if formed. Tetrahydrofuran met these conditions and when used the reaction produced the keto acid (70) in good yields.

It is likely that the chloro-substituent at the 4-position of the homophthalic acid plays a part in the ability of the pyridinium salt (87) to dissolve in the ether. Because these compounds will be more polar and bulky, a more polar solvent is required in the form of tetrahydrofuran. Trouble has also been encountered with a methoxy group at the 4-position of homophthalic acid which may destabilise the anion formed. This may be caused by an increase in electron density of the para-position to the methoxy group.

The keto acid (70) can be ring closed using an acid catalyst[66] to give the isocoumarin (67). To achieve the correct stereochemistry of dihydroisocoumarins (49) and (50) the following

procedure was adopted. For compound (49) the benzylic protons of the keto acid (70) were exchanged with deuterium using sodium deuteroxide. Deuterium also replaced the methyl group protons. The corresponding dihydroisocoumarin (88a) was formed by ring closure, described above, followed by hydrogenation using hydrogen on a palladium/carbon catalyst. This gave the deuterium label on the same face as the methyl group (Scheme 20).

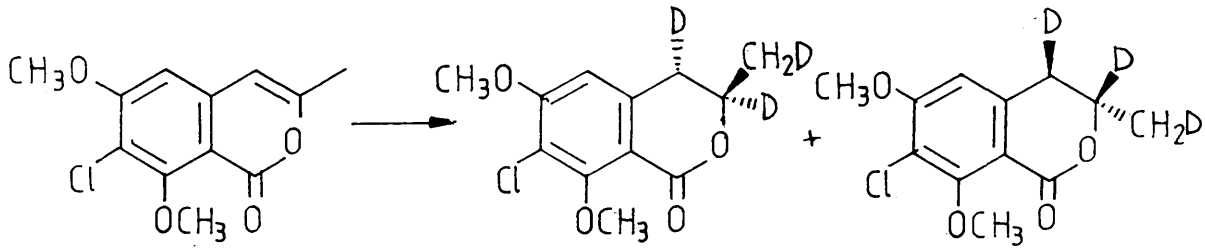


Scheme 20

The corresponding stereoisomer (88b) would also be produced, but this was not important in the feeding experiments for the reasons described in chapter 2.

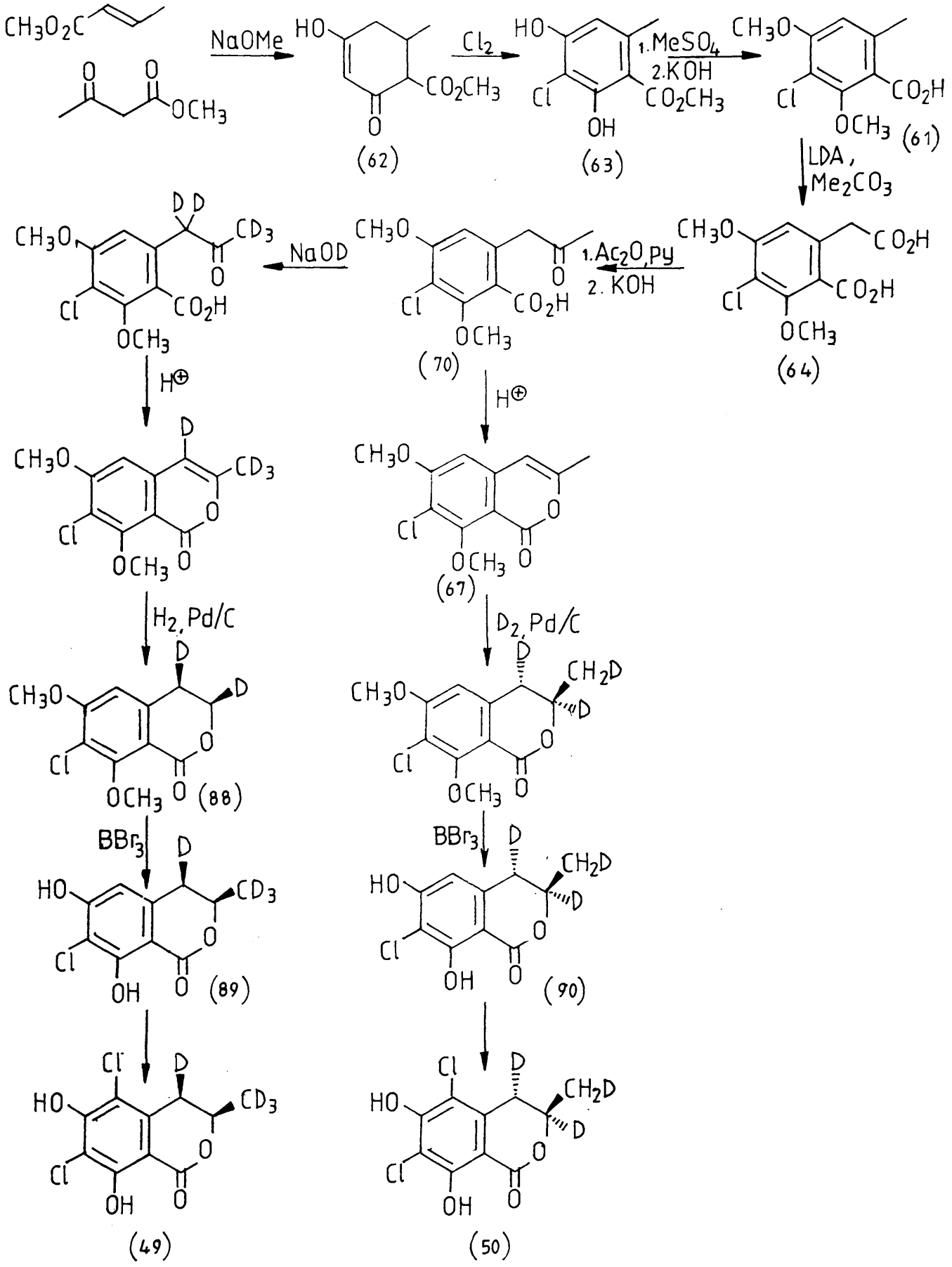
The other dihydroisocoumarin (50) was obtained by forming the isocoumarin (67), then deuterating using deuterium gas in place of hydrogen on a palladium/carbon

catalyst. This gave the deuterium label on the opposite face to the methyl group. Again both stereoisomers would be produced.



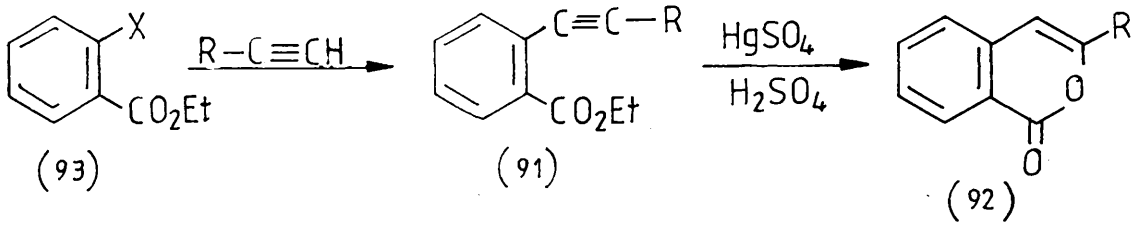
(67)

These dihydroisocoumarins were then demethylated using boron tribromide to give the dihydroxy compounds (89) and (90) and then chlorinated at the 5-position using sulphuryl chloride. This gave the dichloro-dihydroxydihydroisocoumarin which gave the best incorporation results reported[43] earlier. The overall route is described in Scheme 21.



SCHEME 21

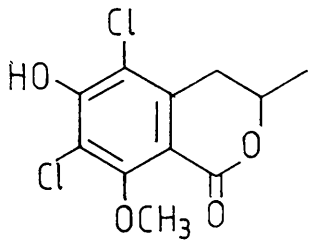
A new and very interesting route[67] to isocoumarins involves the hydration of o-ethynylbenzoic acid derivatives (91). The derivatives, when heated with mercuric sulfate in dilute sulfuric acid, gave the corresponding 3-substituted isocoumarins (92). The o-ethynylbenzoic acid derivatives are produced from the (93) using terminal acetylenes and a palladium catalyst. This type of acetylinic coupling will be discussed in greater detail in the continuing sections.



(93) (91) (92)

(b)8-methoxydihydroisocoumarins.

To investigate the biosynthetic pathway to cryptosporiopsinol (32) further it was necessary to prepare the 8-methoxydihydroisocoumarin (94).



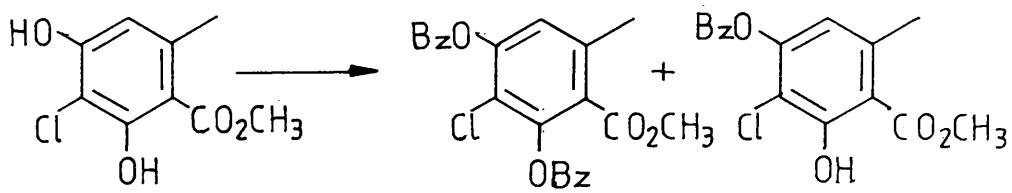
(94)

The 8-methoxydihydroisocoumarin (94) would have a radio-label incorporated before feeding. The major problem in the synthesis was the preferential methylation of the 8-hydroxy position. This could be achieved in two ways; either preferentially protect the 6-position with an easily removable group or protect both positions and remove preferentially the group at the 8-position. The choice of protecting group was also important. This had to be able to withstand the various conditions required in the synthetic route to the dihydroisocoumarin (94) and be removed without great difficulty under conditions that would not affect the rest of the molecule.

It was decided to attempt the route used in the previous synthesis of the deuterium labelled dihydro-isocoumarins (49) and (50). The benzyl protecting group was considered best as this could easily be formed

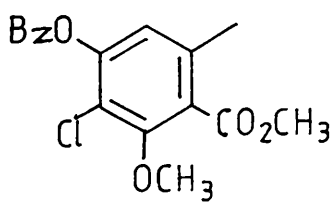
and cleavage occurs under catalytic hydrogenation. This is useful as a hydrogenation step is required in the late stages of the synthesis.

The first step of the synthesis involved the benzylation of the orsellinate (63) to give the corresponding 4-O-benzylorsellinate (95). This procedure was also used in the preparation of 6-benzyloxy-8-hydroxy-3-methylisocoumarin[68] and by Sargent and co-workers[69] in the synthesis of the non-chlorinated derivative of the orsellinate (95). When this procedure was attempted the major product isolated was the di-O-benzylorsellinate (96) along with the 4-O-benzylorsellinate (95).



(63) (96) (95)

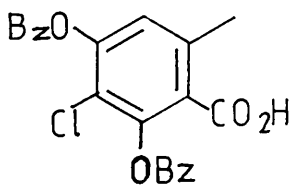
The orsellinate (95) was then methylated at the 2-position to give the desired compound (97) for the next stages of the synthesis. These two stages, however, gave the O-benzyl-O-methylorsellinate (97) in a poor overall yield.



(97)

It was therefore decided to continue with the di-O-benzylorsellinate (96), the major product, in the next stages of the synthesis. This was due to the fact that the hydroxy at the 8-position of isocoumarins can be preferentially deprotected using aluminium trichloride[43] and could then be methylated at this position.

When the di-O-benzylorsellinate (96) was hydrolysed with sodium hydroxide it gave a crystalline material that could not be dissolved in any solvent except sodium hydroxide. Due to this lack of solubility the proposed orsellinic acid (98) could not be fully characterised.

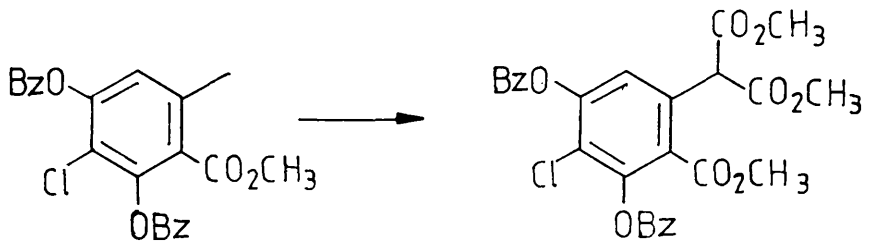


(98)

It was decided to carboxylate the di-O-benzyl-orsellinate (96) instead of the acid (98). Because of the amount of benzylic protons in the molecule a more sterically hindered base than lithium di-isopropylamide (LDA) was used. The base lithium dicyclohexylamide was prepared and used in the same way as the LDA by replacing di-isopropylamine with dicyclohexylamine. It was hoped this base would deprotonate the less hindered toluic methyl in preference to the benzylic protons of the protecting groups.

When the reaction was carried out using dimethyl

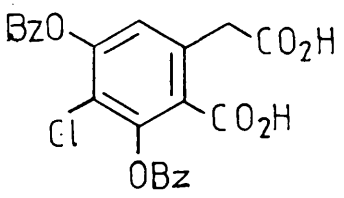
carbonate as the carboxylating agent it was found that the di-O-benzylorsellinate (96) had carboxylated twice at the 6-methyl position to give a dimethyl malonate ester (99) as a crystalline solid.



(96) (99)

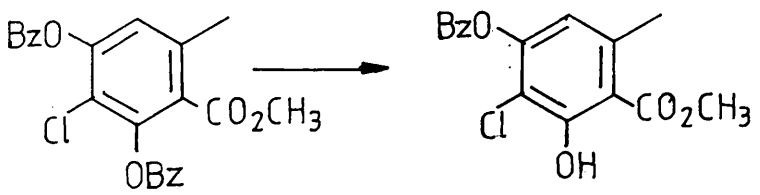
The reaction of LDA on the dimethoxyorsellinic acid (61) to give the corresponding homophthalic acid (64), described previously, probably goes through the malonate ester also. This would undergo β-decarboxylation on the addition of water to the reaction mixture (base hydrolysis). The ester of the dibenzyloxy compound (99) is isolated without basic work up.

When this malonate ester (99) was hydrolysed by heating with aqueous sodium hydroxide a white, crystalline solid was obtained. This had similar properties to that of the proposed di-O-benzylorsellinic acid (98) in that it would not dissolve in any solvent and could not be characterised fully. The compound was thought to be the dibenzyloxyhomophthalic acid (100).



(100)

Due to the solubility problems it was decided to abandon the use of the dibenzyl protected compounds and return to the O-benzyl-O-methylorsellinate (97). Because of the low yield in producing this compound it was proposed to partially deprotect the di-O-benzyl-orsellinate (96) at the 2-position using aluminium trichloride.[43] When this reaction was carried out the expected 2-hydroxyorsellinate (95) was obtained.

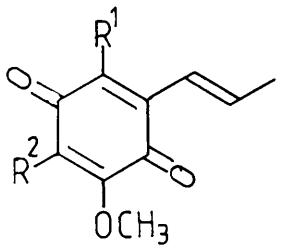


(96) (95)

Due to time constraints this route was not investigated further.

(c). 2-Methoxy-6-prop-1-enyl-1,4-benzoquinone.

As described in the previous chapter, the synthesis of the benzoquinone compounds (60, 101-103) was required to investigate the biosynthesis of cryptosporiopsinol (32) further.



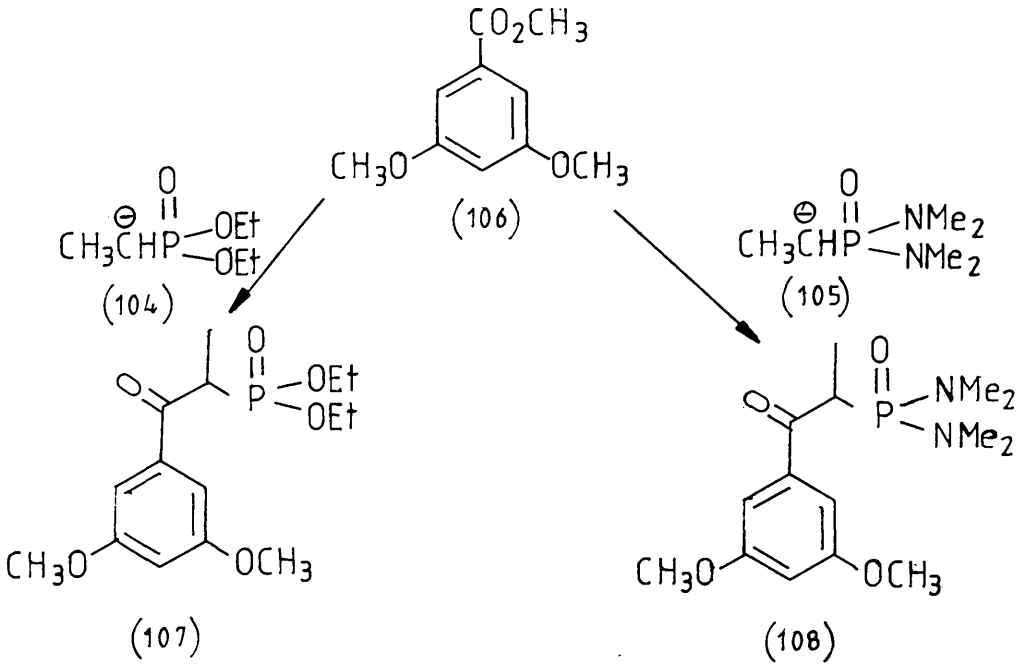
$R^1 = R^2 = H$ (101)
$R^1 = R^2 = Cl$ (60)
$R^1 = H, R^2 = Cl$ (102)
$R^1 = Cl, R^2 = H$ (103)

It was preferable that the dichlorinated species (60) could be synthesised, but it was felt that a basic route to the benzoquinones be produced and that chlorination could be achieved at some point in the synthesis. Various routes have been attempted[43,47] to these compounds without much success.

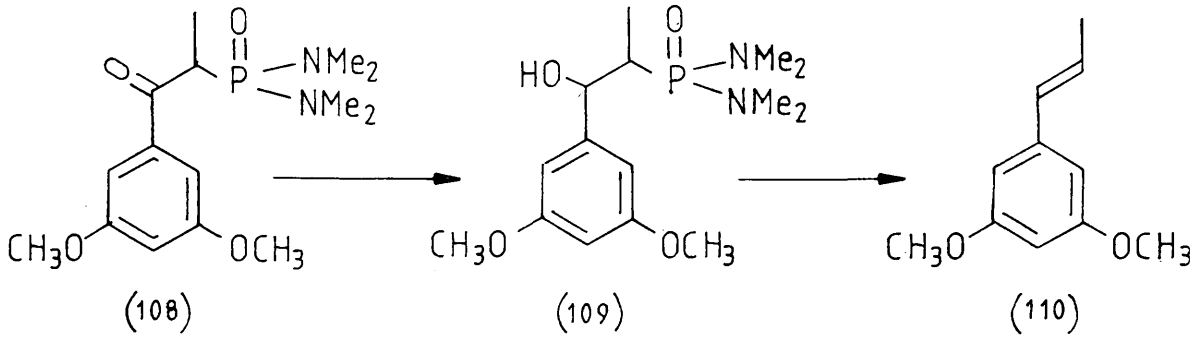
Various factors such as the high degree of oxygenation, the number of substituents (steric problems) and the tendency of the double bond to undergo polymerisation made the synthesis of these compounds difficult.

Three different routes were attempted concurrently. The first route involved the use of an ethyl phosphonate anion (104) or the anion of ethyl

phosphoric acid bis(dimethylamide) (105). Both of these were reacted with methyl 3,5-dimethoxybenzoate (106) to give either the corresponding β-ketophosphonate (107) or the β-ketophosphonamide (108).

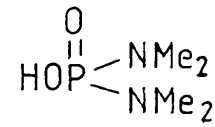


It was reported[47] that the reduction of the carbonyl of compound (108) with sodium borohydride was totally stereospecific giving the β-hydroxyphosphonamide (109) with one configuration at the hydroxyl-bearing carbon. This β-hydroxyphosphonamide undergoes acid catalysed cyclo-elimination in a <u>syn</u>-manner <u>via</u> a postulated four membered cyclic transition state to give specifically (2<u>E</u>)-1-(3,5-dimethoxyphenyl)prop-1-ene (110) and tetramethyl phosphorodiamidic acid (111). When this was investigated as a model reaction it was found to be the case.



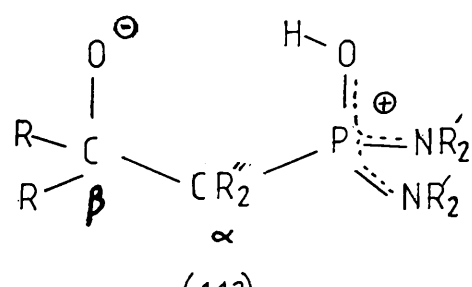
(108) (109) (110)

+

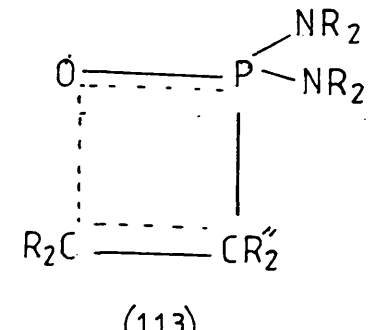


(111)

This type of reaction was first used by Corey[70] who proposed that the formation of olefins by thermal decomposition of β-hydroxyphosphonic acid bisamides probably involves a zwitterionic intermediate of type (112) which undergoes <u>syn</u>-cycloelimination. The marked dependence of the rate of elimination on the degree of substitution on the α and β carbon atoms are consistent with a transition state (113) in which CO and CP bonds are well advanced.[70]

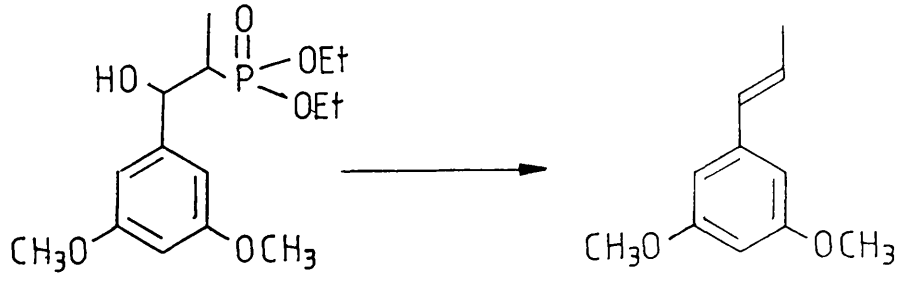


(112)



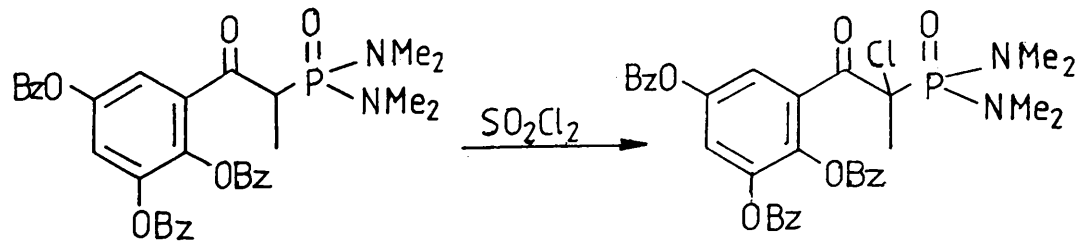
(113)

The β-hydroxyphosphonate (114) corresponding to the reduced β-ketophosphonate (107) requires heating at 160°C for fifteen hours to affect cycloelimination.[71] The olefin was reported to be obtained in a 2:1 ratio of cis:trans stereoisomers.[47] Though these were harsh conditions and the trans isomer was the minor product it was decided to continue with this route to investigate any advantage over the phosphonic acid bisamide in the subsequent chlorination and quinone producing steps.



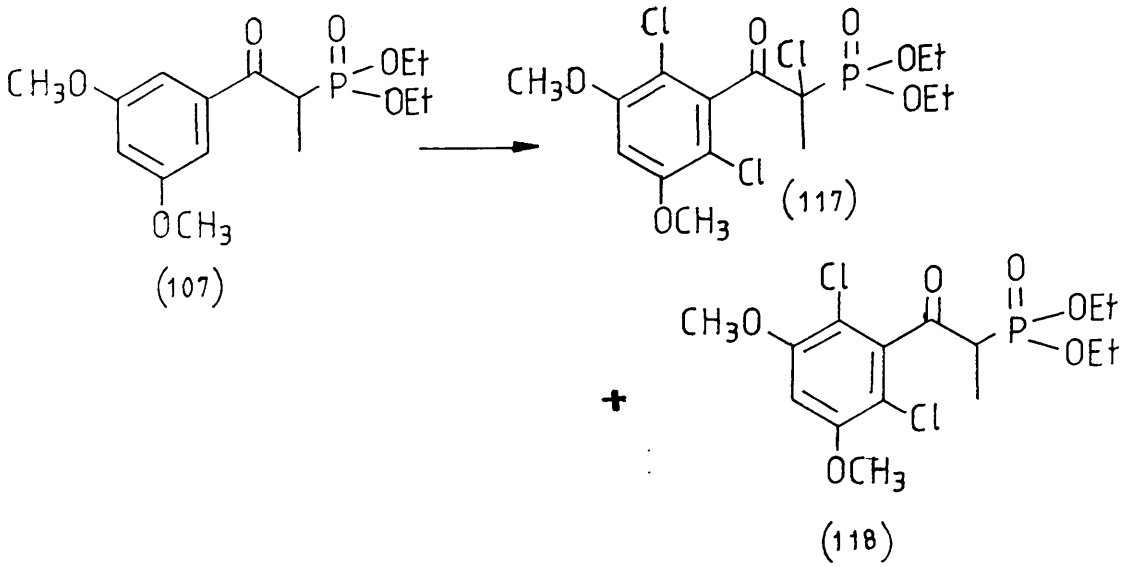
(114) (110)

Work done by MacLachlan[47] showed that if chlorination preceded the addition of the anion then the addition was blocked due to steric crowding. He also showed that treatment of the β-ketophosphonamide (115) with sulphuryl chloride led to chlorination α to the ketone via enol formation to give compound (116). All attempts at chlorination using sulphuryl chloride on the β-hydroxyphosphonamide led to cyclo-elimination which was expected due to the presence of acid.



(115) (116)

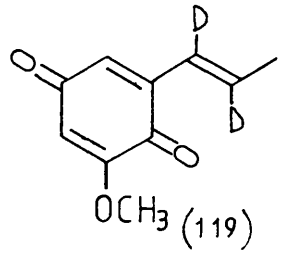
For these reasons it was decided to investigate the chlorination using the β-ketophosphonate (107). On treatment of the β-ketophosphonate (107) with sulphuryl chloride two products were isolated. In the first product (117) chlorination had occurred twice on the ring at positions ortho to the keto group and once at the position α to the ketone group. In the second product (118) chlorination had only occurred on the ring at positions ortho to the keto group.



This suggests that chlorination occurs on the ring first, followed by chlorination via enol formation α to the carbonyl. As an excess of sulphuryl chloride was used conditions may be altered so that only two equivalents of sulphuryl chloride are present and different parameters used to optimise chlorination only on the ring. This chlorination study was discontinued due to lack of time.

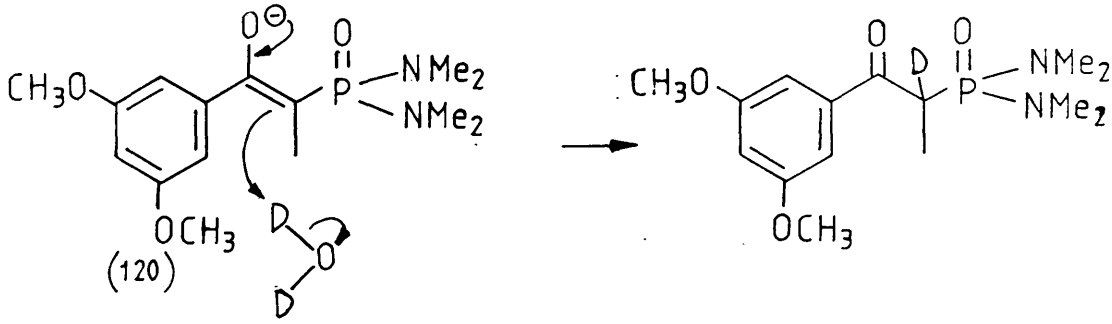
The biosynthetic study required the synthesis of the quinones (60,101-103) with a deuterium label or

labels attached to the propene system e.g. (119)

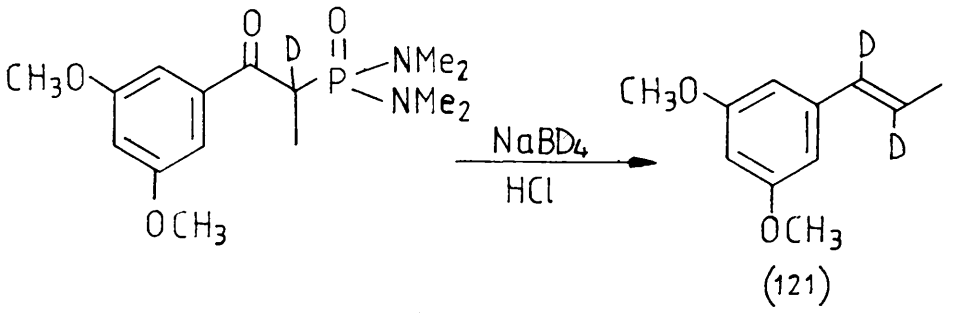


(119)

MacLachlan[47] traced the origin of the proton of the C-2 carbon. On reaction with methyl benzoate two equivalents of the anion of ethyl phosphonic acid bis(dimethylamide) (105) are required due to enolate formation caused by the removal of the acidic proton α to the newly formed ketone (108) by one mole of base. Once the reaction was completed the β-ketophosphonamide was in its enolate form and was quenched by the addition of water. Therefore, deuterium was introduced by quenching of the enolate (120) with deuterium oxide.

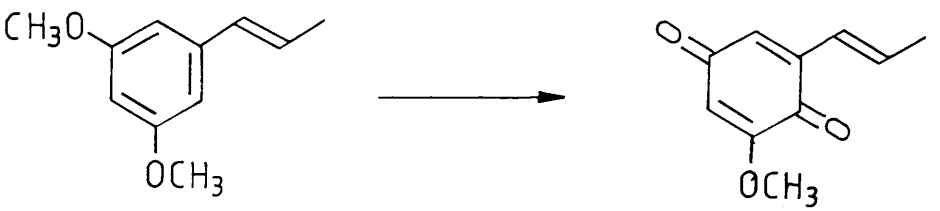


The deuterium at the C-1 carbon can be introduced by reducing the carbonyl with sodium borodeuteride, followed by the same cyclo-elimination described previously. This produced a crystalline product (121) instead of an oil for the non-deuterated quinone (110).



(121)

If the phenylpropene (110) could be oxidised preferentially across the ring <u>para</u> to one of the methoxy groups this would give directly the desired quinone (101).



(110) (101)

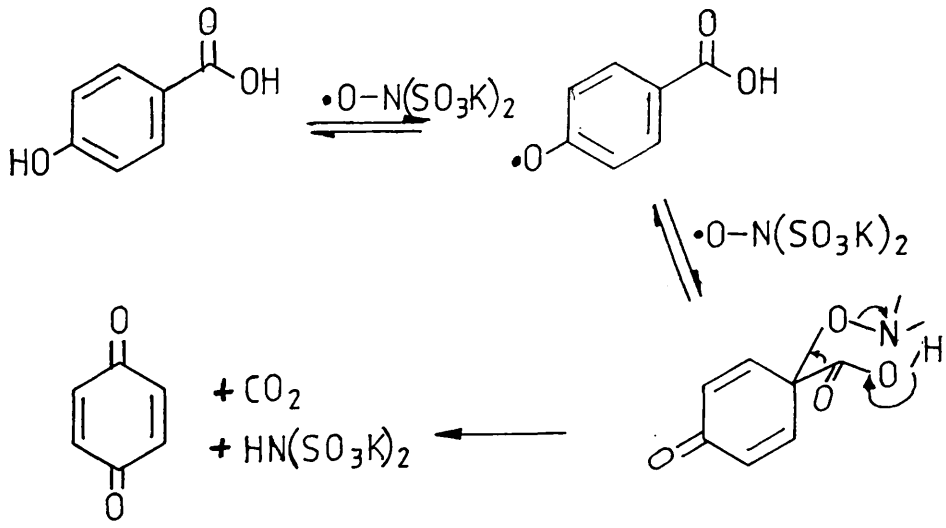
It has been reported[72] that trimethoxybenzenes have been oxidised to dimethoxy-<u>p</u>-benzoquinones using hydrogen peroxide and a hexacyanoferrate catalyst. The hydrogen peroxide generates hydroxyl radicals by catalysis by iron salts. This can lead to complex mixtures of products due to the high reactivity of the primary product (hydroxylated arene). In addition other iron salts complex with the phenol produced. The hexacyanoferrate, however, has little ability to complex with ligands other than the cyanide ion.

When the reaction was caried out on the dimethoxy-phenylpropene (121) a very complex mixture of products was obtained. This was probably due to a combination

of the hydroxyl radicals attacking the olefin and also dimerisation, polymerisation of the double bond and the ring due to delocalisation of radicals.

As an exploratory experiment the oxidation of the dimethoxyphenylpropene (121) was attempted using Fremy's salt. This, however, did not affect the ring system. Two products were isolated. The first was starting material and the other an unidentifiable substance containing a tetra-butylammonium moiety (probably from the phase transfer catalyst) which had a dark red/black crystalline appearance. This compound was soluble in organic solvents.

The oxidation of the dimethoxyphenylpropene (121) did not occur because there was no hydroxy group present in the aryl ring. This is required for the following type of mechanism[73] to take place.



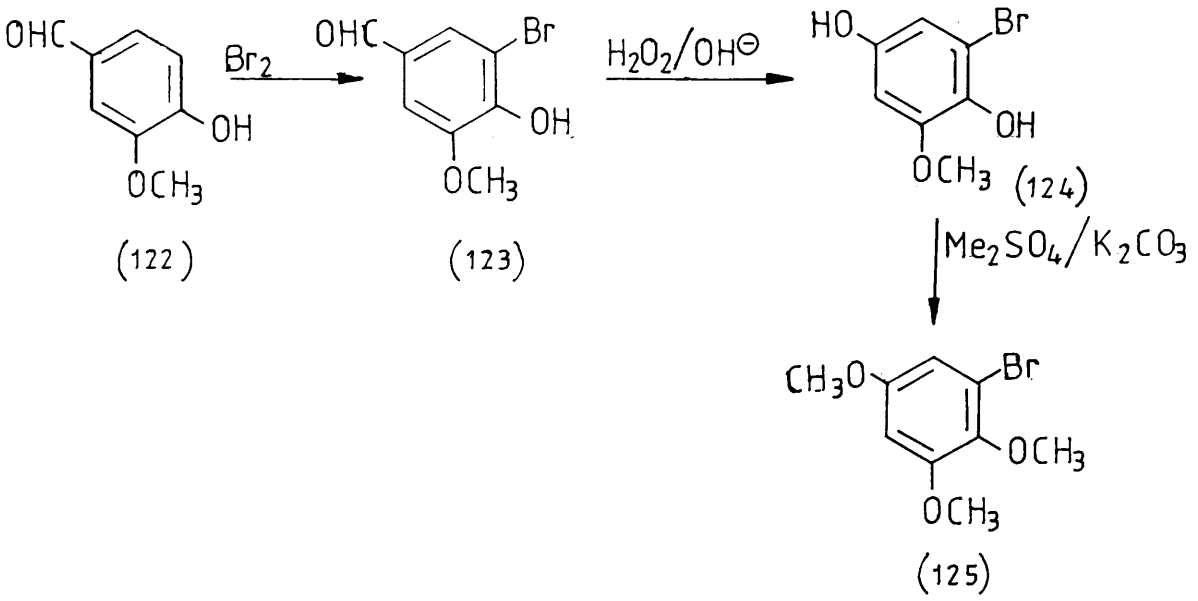
The hydroxyl proton is easily removed as a hydrogen radical by the Fremy's salt. The radical produced is delocalised by the ring. Further oxidation

then occurs either at the para or ortho positions with the para position being favoured.

To overcome these problems the olefin group would be protected as the phosphonamide which would eliminate the problem of polymerisation of the double bond. Chlorination of this protected species could be affected before the oxidaion step which would eliminate the possibility of dimerisation[47] of the ring system. This route was not investigated further due to lack of time.

Another route was attempted which introduced the correct oxygenation pattern at an earlier stage and would leave the introduction of the olefinic group to nearer the final stages. The correct oxygenation pattern can be achieved simply from vanillin (122) using the following route.[74]

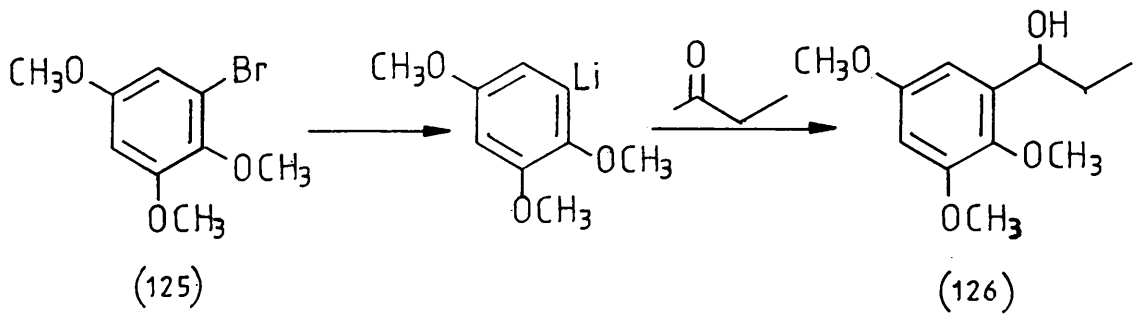
Vanillin was brominated at the 5 position using bromine in acetic acid to give compound (123). This underwent a Dakin reaction of the aldehyde moiety to give compound (124) with the correct oxygenation pattern. The hydroxyl groups were then protected as the methyl ethers (125) to investigate the route further (Scheme 22).



SCHEME 22

If the following route proved successful different protecting groups such as THP could be used to leave the methoxy group <u>meta</u> to the bromine intact.

A propyl moiety was required to be formed next at the bromine site. This was achieved by carrying out a metal-halogen exchange to give the lithiated trimethoxybenzene. This was then reacted with propanal to give the corresponding phenylpropanol (126).

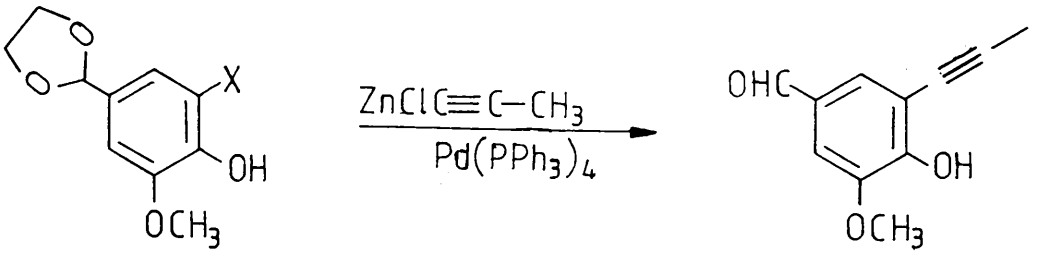


The next stages would involve the chlorination of the ring, deprotection of the methyl ethers, elimination to form the propene and oxidation to the quinone. This route was discontinued due to lack of time.

The final route attempted involved the investigation of palladium catalysed alkynylations and alkenylations. It was envisaged that this method could introduce the propene moiety either directly as propene or as propyne followed by reduction.

It has been known that copper promoted aryl alkynyl coupling[75] involving either the reaction of alkynyl coppers with aryl halides or that of aryl coppers with alkynyl halides can be achieved. These methods sometimes, however, are unsatisfactory due to the ethynyl copper being unstable with respect to disproportionation. These reagents also tend to be explosive.

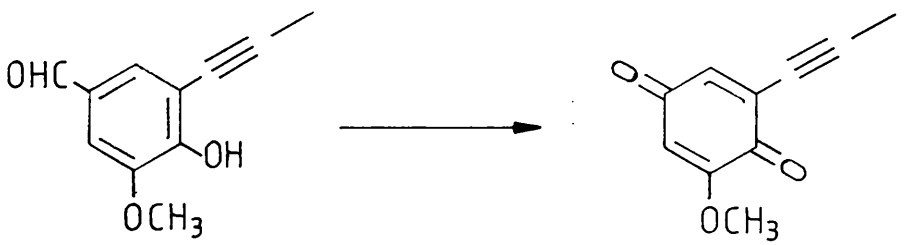
It has been reported[76] that organo-zinc compounds react rapidly with various aryl and alkenyl halides to produce cross-coupled products in the presence of a palladium or nickel catalyst. It was thus envisaged that a protected halovanillin (127) could be coupled to propynylzinc chloride to give the corresponding alkynylated arene (128).



(127) (128)

X = I or Br

The propynylarene (128) could then be oxidised[73], using Fremy's salt, para to the hydroxyl group, to give the corresponding 1,4-benzoquinone (129).



(128) (129)

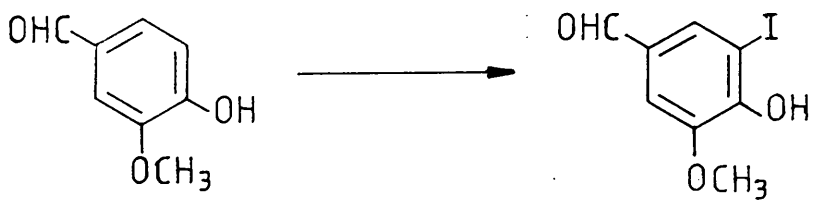
To investigate this as a possible route the alkynylation was attempted on bromo- and iodo- anisoles. Heptynylzinc chloride was used as the alkynyl moiety. This was because heptyne is a liquid at room temperature and is therefore more easily handled than the gaseous propyne. These reactions proved unsuccessful and only starting mateial was recovered. The reasons for this failure will be discussed later in this chapter.

Recently it has been reported[77] that alkynylations

occur with terminal alkynes using a palladium and cuprous iodide catalyst. It was thus decided to investigate this as a possible route. This removed the requirement of an acetal protecting group for the aldehyde substituent of the aromatic ring which may have reacted with heptynylzinc chloride.

The chosen route involved bromovanillin, tetrakis-(triphenylphosphine)palladium, cuprous iodide and triethylamine. This again proved unsuccessful. It was decided to attempt this reaction again using an iodo group in place of the bromo substituent.

Iodovanillin (130) was prepared as reported[78] using chloroamine T and sodium iodide which supplies iodine in the form of iodine monochloride.



(122) (130)

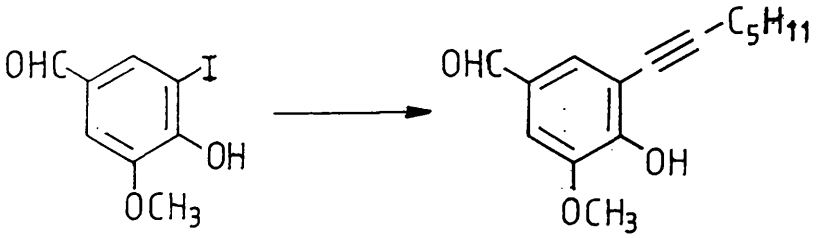
When the iodovanillin (130) was reacted under the same conditions using the same catalyst again only starting material was recovered.

It was decided that another palladium catalyst should be employed. This was because the tetrakis-(triphenylphosphine)palladium is unstable and appears to be oxidised in air to the green oxygen complex bis(triphenylphosphine)oxygenopalladium(II).[79] This

could cause the palladium complex to lose its catalytic activity. This would account for the fact that iodoanisole did not undergo alkynylation as described previously (although reported[76] successful).

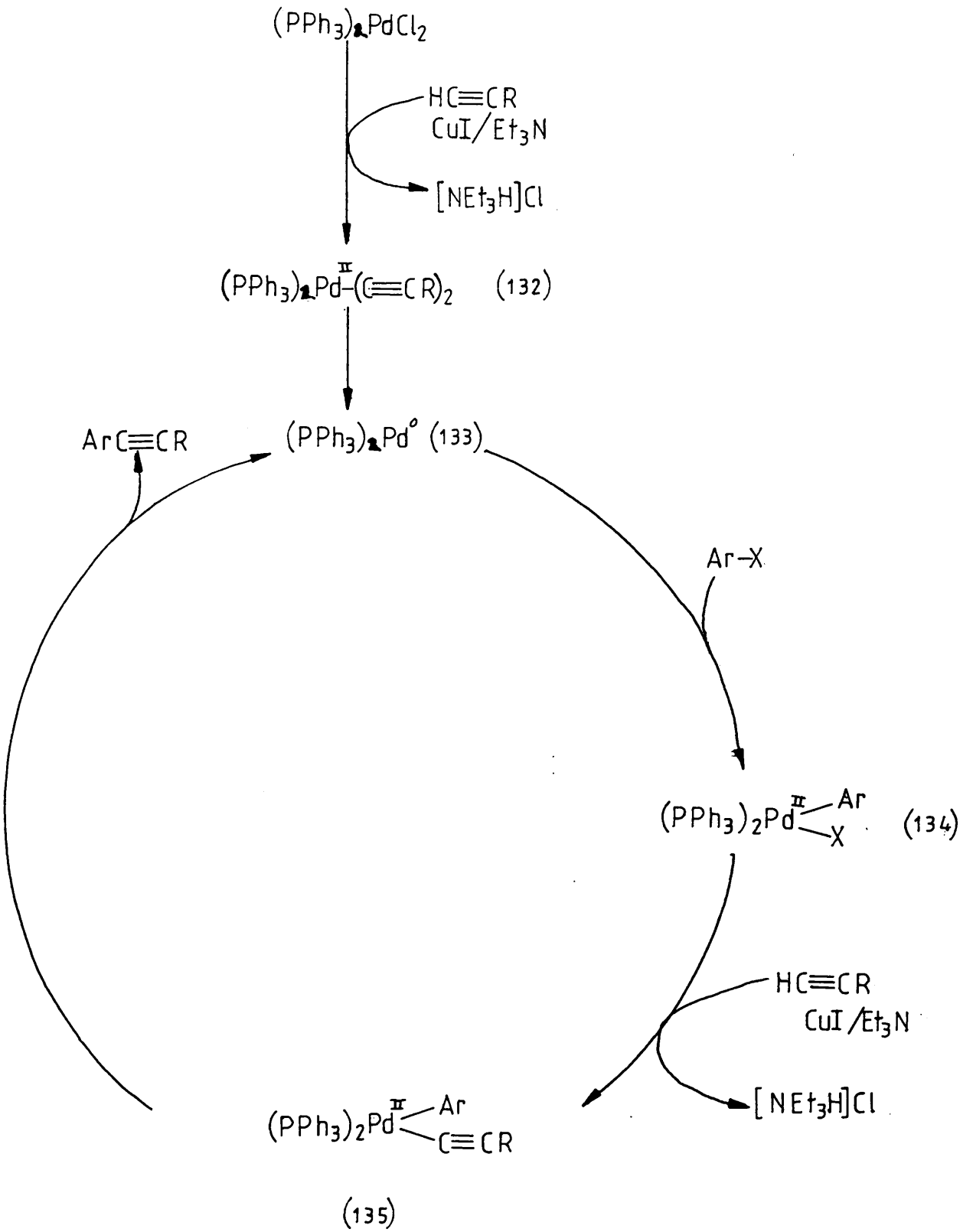
It was decided to use the palladium complex bis(triphenylphosphine)dichloropalladium which appears to be more stable in air and has been used successfully in previous alkynylation.[77,80]

When iodovanillin (130) was reacted with this catalyst, heptyne, cuprous iodide and triethylamine in tetrahydrofuran, alkynylation was found to have occurred, to give the alkynylated arene (131).



(130) (131)

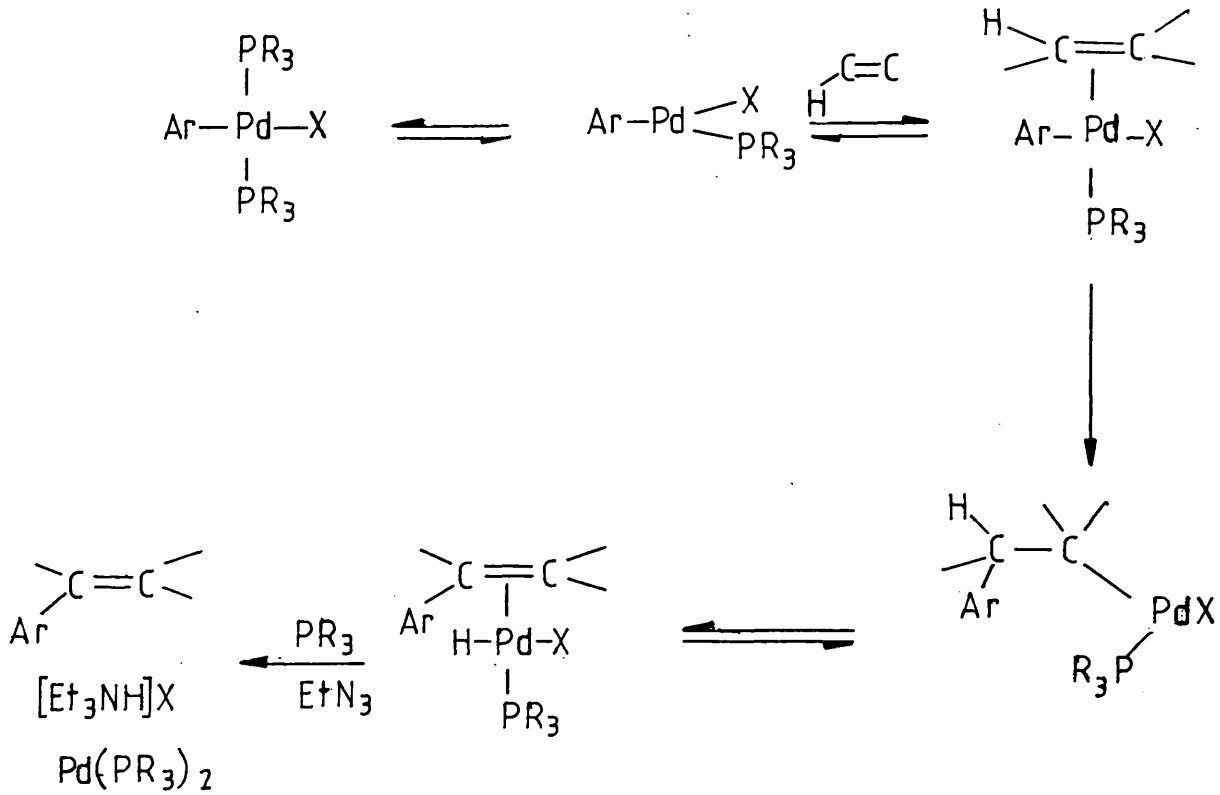
Various factors could account for alkynylation occurring with the iodoarene (130) using bis(triphenylphosphine)dichloropalladium and the lack of success with other catalysts and bromoarenes. This mainly involves the stability of the intermediate palladium complex and its possible subsequent reactions. The detailed mechanism of the proposed[80] pathway is not yet clarified but previous studies suggest the route in Scheme 23.


(PPh3)2PdCl2
HC≡CR
CuI/Et3N
[NEt3H]Cl
(PPh3)2Pd(C≡CR)2 (132)
ArC≡CR
(PPh3)2Pd0 (133)
Ar–X
(PPh3)2PdArX (134)
HC≡CR
CuI/Et3N
[NEt3H]Cl
(PPh3)2PdArC≡CR
(135)

SCHEME 23

The first step involves the formation of bis(triphenylphosphine)dialkynylpalladium(II) (132) which gives the catalytic species, bis(triphenylphosphine) palladium (O) (133), through reductive elimination of the diyne. Subsequent oxidative addition of the aryl halide to (133) followed by alkynylation of (134) gives (135) which regenerates the original bis(triphenylphosphine)palladium (133) through reductive elimination of the substitution products.

The reaction seems to involve attack by an acetylide anion as the presence of cuprous iodide is required.[80] This is supported by a similar reaction involving sodium methoxide in place of cuprous iodide and triethylamine.[81] This is in comparison to the olefinic addition to the palladium complex which seems to bond through a π-complex[82] (Scheme 24).



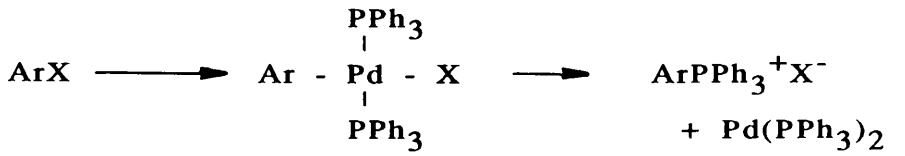
Scheme 24

The catalyst initially undergoes the same oxidative addition of the haloarene. Dissociation of one triphenylphosphine from the halo(bistriphenylphosphine)arylpalladium(II) species (136) then occurs, followed by the olefin coordinating at the vacant site. This is then followed by rearrangement with addition of the aryl and formation of a sigma bond to the palladium. A proton shift could occur to give the arylolefin π complex (137). Reductive elimination through loss of halide ion and proton with base and substitution of a triphenylphosphine unit could give back the catalyst and the alkenylated arene.

There are various explanations why this pathway might not be followed. As explained earlier, the tetrakis(triphenylphosphine)palladium (O) is quite unstable and is easily oxidised by molecular oxygen to bis(triphenylphosphine)oxygenopalladium(II). If this occurred the bis(triphenylphosphine)palladium (O) (133) could not be formed and oxidative addition of the aryl halide could not occur.

A competing reaction[82] also occurs and its rate is dependent on the ligands attached to the palladium. This is a quaternization reaction which involves the oxidative addition of the aryl halide as described previously. This complex undergoes reductive elimination of the phosphonium ion and final loss of the

halide ion from the metal. The reductive elimination may require the coordinaion of a third phosphine or another ligand.

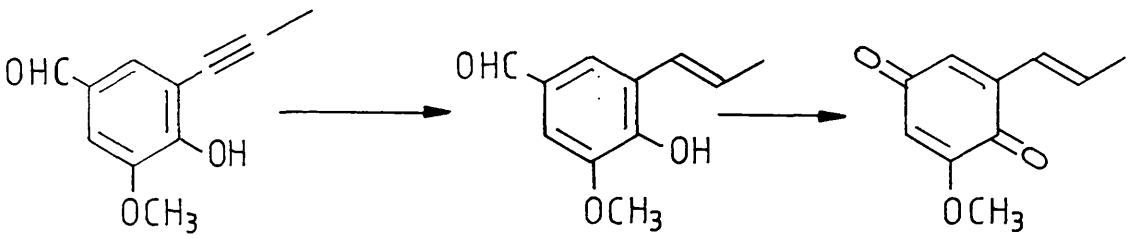


Various factors affect this reaction.[82] These include the electronic effects of substituents on the aromatic ring particularly p-substituted electron donating groups and steric factors, with bulkier groups hindering the attack of the third phosphine ligand so reducing the rate of quaternization. The steric effect has been demonstrated by using tri-o-tolylphosphine instead of triphenylphosphine. Substitution of bromoarenes are promoted by using the tri-o-tolylphosphine in the case of olefinic coupling to aryls. Alternatively, the iodo group which is bulkier can be used. No substitution of chloroarenes has been reported for olefins.[77] This is probably due to the size of the chlorine making the quaternization reaction rate such that it outweighs the reaction pathway of Scheme 24. The fact that coupling occurs for alkynes and not alkenes may be explained by the rates of each of their respective pathways in comparison to the rates of the other competing reacions. This would also explain why bromoarenes react with alkynes without the need for bulkier tri-o-tolylphosphine ligands on the palladium.

Another competing reaction is the precipitation of

palladium metal. This was noted, to some extent in all the reactions attempted. This is dependent on any complex formed throughout the pathway, i.e. any particular complex may be so unstable that palladium metal is deposited. The triphenylphosphine ligand may be replaced by any other ligand (e.g. solvent) and would be another factor to consider in any of the above reactions.

It was proposed to alkynylate the iodovanillin with propyne which could be reduced to the trans-olefin using a variety of methods[83] or to the cis-olefin by catalytic hydrogenaion followed by olefinic inversion.[84] This resulting compound could then be oxidised using Fremy's salt[73] as described earlier.

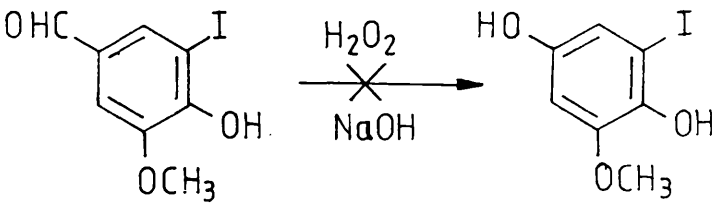


(128) (101)

Alternatively the olefin could be coupled[85] directly to the iodovanillin using the catalytic method described earlier, but this would involve a pre-labelled propene and substitution would have to occur at the 1-position of propene.

To facilitate chlorination it was proposed to oxidise the iodovanillin to the dihydroquinone (138).

However, subjecting the compound to Dakin oxidation produced only starting material.



(130) (138)

The reasons for this lack of reativity is probably due to steric factors caused by the iodine group. Similar difficulty has been reported[86] in the Baeyer-Villiger oxidation of hexa-substituted arenes containing iodine.

If this dihydroquinone (138) could be produced, chlorination would be relatively easy due to the number of activating groups. The alkynylation or alkenylation would then occur specifically at the iodo-position for the reasons described earlier. The bromovanillin can be oxidised under the same conditions and if chlorination can be effected this would give a dihydroquinone suitable for alkynylation. The catalyst, however, would have to have bulkier phosphine ligands e.g. tri-o-tolylphosphine as discussed earlier. This procedure could not be investigated further due to lack of time.

Chapter 4

Experimental

General Procedures

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Ultra violet spectra were measured on a Pye Unicam SP8-100 spectrophotometer, Infra-red spectra were recorded for potassium bromide discs on a Perkin-Elmer 983 spectrometer. The following abbreviations are used: s-strong; m-medium; w-weak and br-broad.

Proton nuclear magnetic resonance spectra were recorded on a Perkin-Elmer R32 (90MHz) spectrometer using deuterochloroform as solvent (unless otherwise stated). Tetramethylsilane was used as an internal standard. ^1H n.m.r spectra were also recorded at 200 MHz on a Bruker WP200SY instrument, employing a deuterium lock system, setting chloroform ($C\underline{H}Cl_3$) in $CDCl_3$ at δ7.25 p.p.m., as internal standard. The following abbreviations are used: s-singlet; d-doublet; t-triplet; q-quartet; m-multiplet; dd-double-doublet; dt-double-triplet; dq-double quartet and br-broad. Carbon nuclear magnetic resonance spectra were determined on a Bruker WP200SY spectrometer in $CDCl_3$, setting the reference $CDCl_3$ signal at δ77.0 p.p.m. A Bruker WH-360 instrument was used to determine the deuterium nuclear magnetic resonance spectra of isolated metabolites.

Mass spectra were obtained using a VG/Kratos MS12 spectrometer or a VG/Kratos MS90S spectrometer for high

resolution.

All apparatus and solutions involved in the growth of the micro-organism were sterilised before and after use in an autoclave.

Separation of fungal metabolites was carried out on a positive pressure flash chromatography column using Merck Kieselgel 60 230-400 mesh and preparative layer chromatography (plc) using Merck Kieselgel GF254.

Separation of chemical components was carried out either by plc described above or using negative pressure flash column chromatography using Merck Kieselgel GF254,

The solvents were dried as follows: tetrahydrofuran (THF) was distilled from sodium/benzophenone; methanol was dried using magnesium activated with iodine and stored over 3A molecular sieves; and ether was dried using sodium wire.

Organic solutions were dried over magnesium sulphate and evaporated on a rotary evaporator under reduced pressure.

Culture and harvesting of *Periconia macrospinosa*

Stock cultures of *P. macrospinosa* (CMI24411) were maintained on potato-dextrose agar slopes. The slopes were inoculated with a spore inoculum from the master strain (or from earlier sub-cultures) and incubated at 25°C for 14 days after which time they were used as inocula for large-scale cultures or stored at 4°C.

Large-scale cultures of *P. macrospinosa* were grown as surface cultures in Roux bottles on a solution of glucose (50g) sodium nitrate (2g), potassium chloride (1g), magnesium sulphate (1g), dipotassium hydrogen ortho-phosphate (0.5g), ferrous sulphate (0.01g), yeast extract (1g) in distilled water (1l). After 21 days incubation at 25°C (during which time the pH of the culture medium increased from 5 to 7.5) the mould had formed a thick black surface mat and the medium had become cloudy and dark brown in colour. The mycelium was separated from the culture medium and dried over P_2O_5. The culture medium was continuously extracted with ethyl acetate for 24 hours.

Isolation of fungal metabolites.

The ethyl acetate extract of *P. macrospinosa* culture medium was dried and evaporated to leave a brown gum (typically 1g/L of medium). The extract was first passed through a short column of silica gel (2.5 x 1cm) under suction, using ethyl acetate as eluant, to remove highly polar resinous material. This was then subjected to separation by positive pressure flash column chromatography using chloroform:ether, 95:5 as eluant.

Methyl 2-allyl-3,5-dichloro-1,4-dihydroxy-cyclopent-2-eneoate (32).

m.p. 121-122°C (lit.[42] 121;122°C);

δ6.21 (2H,m, H_a, and H_b), 4.48 (2H,m, H_4 and H_5),

3.91 (3H,s, $-OCH_3$), 2.95 (1H, br, D_2O ex, 6-OH),

1.78 (3H, m, $-CH_3$) p.p.m.

5-Chloro-3,4-dihydro-8-hydroxy-6.methoxy-3-methyl-isocoumarin (33).

m.p. 123-124 (lit.[42] 123-124°C)

δ12.34 (1H,s, -OH), 6.43 (1H, s, Ar-H), 4.65 (1H,m,3-H) 3.92 (3H,s, $-OCH_3$), 3.23 (1H, dd, J 4Hz and 18Hz, $-CH_2-$) 2.72 (1H, dd, J 12Hz and 18Hz, $-CH_2-$), 1.53 (3H,d, J 7Hz, $-CH_3$) p.p.m.

Methyl-2-allyl-3,5-dichloro-1-hydroxy-4-oxocyclopent-2-enoate (31).

m.p. 134-136°C (lit.[42] 134-136°C);

δ6.78 (1H,dq,J 7Hz and 16Hz, H_6), 6.45 (1H,dq,J 1Hz and 16Hz, H_9), 4.63 (1H,s,5-H), 4.40 (1H,br, D_2O ex, -OH), 3.79 (3H,s,$-OCH_3$), 1.95 (3H, dd, J 1Hz and 7Hz $-CH_3$) p.p.m.

5-Chloro-3,4-dihydro-4,8-dihydroxy-6-methoxy-3-methyl-isocoumarin (34).

δ11.41 (1H, s, D_2O ex, -OH), 6.55 (1H, s, Ar-H), 4.95 (2H, m, 3H and 4H), 3.94 (3H, s, $-OCH_3$), 1.31 (3H, d, J 7Hz, $-CH_3$) p.p.m.

Methyl 1,2-dihydro-o-orsellinate (62).

A solution of sodium methoxide was prepared from sodium (23g) and dry methanol (300ml) under nitrogen. Methyl acetoacetate (126g) was added refluxed for 30 min, then methyl crotonate (102g) added dropwise over 90 min. Reaction mixture was refluxed for 20h, cooled on ice, filtered and washed with very little ethanol and petroleum ether, air dried then dried in a drying

cupboard overnight. This formed the sodium salt. This was dissolved in minimum ice cold water, then ice cold concentrated hydrochloric acid was added and ethyl dihydro-orsellinate crystallised out on ice. The product was collected by filtration and recrystallised from benzene/light petroleum (40-60) (113g, 62%), m.p. 121-124°C (lit.[87] 122-124°C), δ9.5 (1H, s, -OH), 5.5 (1H, s, 3-H), 3.8 (3H, s, $-OCH_3$), 3.1 (1H, d, J 10Hz, 1-H), 2.9-2.0 (3H, m, 5-H and 6-H), 1.05 (3H, d, $-CH_3$) p.p.m.

Methyl 3-chloro-o-orsellinate (63).

A solution of chlorine (35g) in glacial acetic acid (200ml) was added with stirring and ice cooling, to a solution of methyl 1,2-dihydro-o-orsellinate (49.25g) in glacial acetic acid (150ml) in a closed system. The reaction mixture was stirred at room temperature for 0.5h then at 60°C for 3h then poured onto iced water (1L). The precipitate was separated by filtration, washed with water and air dried. This was recrystallised from dichloromethane/hexane (23g, 40%) m.p. 128-130°C (lit.[62] 129-130°C) δ11.29 (1H,s,-OH), 6.36 (1H,s, Ar-H), 5.89 (1H, s, -OH), 3.90 (3H,s, $-OCH_3$) 2.43 (3H,s, $-C\underline{H}_3$) p.p.m.

3-Chloro-2,4-dimethoxy-6-methylbenzoic acid[62](61).

To a solution of methyl 3-chloro-o-orsellinate (17g) in anhydrous acetone (250ml) was added dimethylsulphate (25.5ml) and anhydrous potassium carbonate (42.5g). The mixture was refluxed for 8h.

On cooling, the reaction mixture was filtered and evaporated to leave a dark brown oily residue. The residue was dissolved in ether (75ml) and washed with ammonium hydroxide solution. The ether solution was evaporated to give a pale yellow oil (which crystallised on standing). The oil was suspended in 10% potassium hydroxide solution (200ml) and heated at 100°C for 6h. On cooling, the basic solution was washed with ether. On acidification with concentrated hydrochloric acid the product precipitated from solution. The precipitate was collected, washed with water and air dried. Recrystallised from dichloromethane/hexane (15g, 83%) m.p. 152°C (lit.[88] 154-156°C), δ[$(CD_3)_2CO$], 6.81 (1H, s, Ar-H), 3.91 (3H, s, $-OCH_3$), 3.86 (3H,s, $-OCH_3$), 8.31 (3H,s, Ar-CH_3) p.p.m.

4-Chloro-3,5-dimethoxyhomophthalic acid[43] (64)

n-Butyllithium (54ml; 1.5M in hexane) was added to a solution of di-isopropylamine (8.2g) in tetrahydrofuran (THF) (25ml) under nitrogen at 0°C with stirring. After 10 mins, the solution was cooled to -78°C and a solution of 2,4-dimethoxy-6-methylbenzoic acid (4g) and dimethyl carbonate (4.4g) in THF (25ml) was added dropwise during 15 mins. The cooling bath was removed and the solution allowed to warm to room temperature. After 4h, water (30ml) was added and the suspension stirred for 16h. The organic solvents were removed by evaporation and the resulting aqueous solution was washed with ether. After acidification with 1N hydrochloric acid, the solution was extracted with ethyl acetate. The combined extracts were dried

and evaporated to give 4 chloro-3,5-dimethoxyhomophthalic acid which was recrystallised from dichloromethane/hexane as prisms (4.2g, 88%), m.p. 141°C (lit.[43] 141-146°C) δ($(CD_3)_2CO$) 7.04 (1H, s, Ar-H), 4.00 (3H, s, $-OCH_3$), 3.94 (3H, s, $-OCH_3$), 3.87 (2H, s, $-CH_2-$) p.p.m.

Attempted synthesis of 3-chloro-2,4-dimethoxy-6-(2-oxopropyl)benzoic acid

3-Chloro-2,4-dimethoxyhomophthalic acid (0.25g) was added portionwise to a mixture of acetic anhydride (0.5ml) and dry pyridine (0.125ml) in a flask equipped with stirrer. Dry ether (15ml) was added after 5 min and stirring continued for 2h. Sodium hydroxide (10ml, 4M) was slowly added and mixture refluxed until all the solid dissolved. The solution was cooled washed with dichloromethane and then acidified with concentrated hydrochloric acid. The acidic solution was extracted with ethyl acetate dried and evaporated. This method gave erratic yields of product.

The above procedure was attempted using triethylamine as base in place of pyridine. Only starting material recovered.

Acylating Agent - CH_3CN

Di-isopropylamine (1.4ml) in THF was stirred at 0°C under nitrogen. n-Butyl-lithium (6.1ml, 1.6M) was added and stirred for 10 min. This was cooled to -78°C and 3-chloro-2,4-dimethoxy-6-methylbenzoic acid (1g) and

acetonitrile (1ml) in THF added dropwise. The solution was allowed to warm to room temperature and stirred for 3h. Water was added and aqueous layer separated and acidified with hydrochloric acid (1M). This was extracted with ethyl acetate, dried and evaporated to dryness. Starting material only was recovered.

N-methoxy-N-methyl acetamide[57] (69)

Pyridine (14.26ml) was slowly added to a well stirred slurry of O,N-dimethylhydroxylamine hydrochloride (10g) and acetyl chloride (5.70ml) in CH_2Cl_2 (120ml) under N_2 at 0^oC. The mixture was warmed to room temperature and stirred for 2h at which time brine (80ml) and ether (120ml) were added. Separation of layers, extraction with ether (60ml) and evaporation of combined organic layers followed by distillation under reduced pressure (b.p. 40-44°C 20mmHg) gave the product, δ3.73 (3H, s, $-OCH_3$), 3.21 (3H, s, $-NCH_3$), 2.13 (3H, s, $-COCH_3$).

Attempted synthesis of 3-chloro-2,4-dimethoxy-6 (2 oxopropyl)benzoic acid (70)

N-methoxy-N methyl acetamide (1.7ml) was injected into a solution containing the toluic anion of 3-chloro-2,4-dimethoxy-6-methylbenzoic acid(described previously) and mixture stirred for 10 min at -78^oC. The cooling bath was removed and stirring continued for 2 h. The solution was added to dilute sodium hydroxide and the layers separated. The aqueous layer was washed with dichloromethane. The organic solutions were combined and back extracted with more sodium hydroxide. The

combined aqueous layers were acidified and extracted with dichloromethane, dried, and evaporated to yield only starting material.

4-Chloro-3,5-dimethoxyhomophthalic anhydride[43] (65)

A solution of 4-chloro-3,5-dimethoxyhomophthalic acid (1g) in acetyl chloride (25ml) was heated at reflux for 1 h. After cooling the solution was diluted with light petrol and product crystallised out. This was collected by filtration and washed with light petrol (0.75g), m.p. 142°C (lit.[43] 141-146°C). $\delta((CD_3)_2CO)$ 7.04 (1H,s,Ar-H), 4.00 (3H,s,-OCH_3), 3.94 (3H,s, -OCH_3), 3.87 (2H,s,-CH_2-) p.p.m.

4-carboxy-7 chloro-6,8-dimethoxy-3-methyl-isocoumarin (66)[43]

To a solution of 4-chloro-3,5-dimethoxyhomophthalic anhydride (1g) in anhydrous THF (30ml) was added acetic anhydride (0.6ml) and pyridine (0.8ml). The solution was stirred at room temperature for 40 min then more acetic anhydride (0.8ml) was added and this was heated at gentle reflux for 1h. After cooling the solution was evaporated and residue dissolved in dilute aqueous sodium bicarbonate. The basic solution was washed with ether, then acidified with dilute HCl and extracted with ethyl acetate, dried and evaporated (0.58g, 50%) m.p. 164°C decomp. (lit. m.p.[43] 164°C decomp.) $\delta((CD_3)CO)$ 7.25 (1H,s,Ar-H), 4.10 (3H,s,-OCH_3), 3.91 (3H,s, -OCH_3) 2.72 (3H, s, -CH_3) p.p.m.

7-Chloro-6,8-dimethoxy-3-methylisocoumarin (67)[43]

4-Carboxy-7-chloro-6,8-dimethoxy-3-methyliso-coumarin (100mg) was heated to just below its melting point for 20 min under vacuum by which time carbon dioxide evolution had ceased. The residue was recrystallised from ethanol. (30mg, 40%). m.p. 187-200°C (decomp.) (lit[43] m.p. 187-200°C (decomp.)) δ6.53 (1H,s, 5-H), 6.12 (1H,s, 4-H), 3.97 (6H, s, 2xOCH_3) 2.20 (3H, s, -CH_3) p.p.m.

3,5-Dimethoxy benzyl alcohol

Methyl 3,5-dimethoxybenzoate (2g) was dissolved in dry THF (60ml). Lithium aluminium hydride (0.5g) was added and mixture heated with stirring under gentle reflux for 5h. The mixture was allowed to cool and excess lithium aluminium hydride destroyed by adding water (0.5ml) with stirring followed by sodium hydroxide (1.5ml, 15%). and a further 0.5ml of water. The pecipitate formed was filtered and washed with ether. The organic solution was evaporated. Recrystallised from diisopropyl ether (0.98g, 60%) m.p. 48°C (lit.[88] m.p. 48-48.5°C) δ 6.25 (3H,m,Ar-H), 4.47 (2H,s,-CH_2-), 3.70 (6H,s,-OCH_3x2), 2.16 (1H,s,-OH).

2-Bromo-3,5-dimethoxybenzyl alcohol[89]

3,5-Dimethoxybenzyl alcohol (1g) was dissolved in carbon tetrachloride (50ml). N-Bromosuccinimide (1.08g) was added in small portions over 5 minutes. The mixture was refluxed with stirring at -70°C for 40 min. The reaction mixture was filtered while still warm and ether (25ml) added to the filtrate. The organic solution was washed twice with water, dried and evaporated. Recrystallised from acetone/pet.ether (1,1g, 72%) m.p. 85-89°C (lit.[89] 91-92°C) δ 6.78 (1H,d,ArH), 6.41 (1H,d, ArH), 4.70 (2H,s,-CH_2-), 3.85 (3H,s, -OCH_3), 3.80 (3H,s, -OCH_3), 2.00 (1H,s,br,-OH).

5,7-Dimethoxyphthalide

2-Bromo-3,5-dimethoxybenzyl alcohol (1g) in THF (40ml) under nitrogen was stirred at -78°C. n-Butyl-lithium (4.7ml, 2.5M) was added over 10 min. This was stirred for a further 30 min. Carbon dioxide was then passed through the reaction mixture. The flask was allowed to warm to room temperature after 1h. Hydrochloric acid (20ml, 2M) was then added. This was extracted with ether and the organic layer washed with water, dried and evaporated to give product. Recrystallised from acetone/pet.ether (0.47g, 60%), m.p. 147-148°C (lit.[90] 146-148°C) δ 6.50 (1H,s,ArH) 6.44 (1H,s,ArH), 5.16 (2H,s,-CH_3), 3.96 (3H,s,-OCH_3), 3.89 (3H,s, -OCH_3) p.p.m.

N-Methyl-2,4-dimethoxybenzamide

Methyl 2,4-dimethoxybenzoate (1.4g) is placed in a round bottom flask and to this was added methylamine (25ml, 30% solution in water). This was shaken for a few minutes by which time the ester had gone into solution. This was allowed to stand for 20 min., then extracted with dichloromethane, dried and evaporated to give product. Recrystallised from ether (98%) m.p. 69-70°C (lit.[60] m.p 71°C), δ 8.15(1H,d,J 9Hz, 6-H), 6.60(1H,d, J 4Hz, 3-H), 6.47 (1H,dd,J 9Hz,3Hz, 5-H), 3.89 (3H,s,-OCH_3), 3.81 (3H,s, -OCH_3), 2.84 (3H,d,J 6Hz, N-CH_3).

Attempted synthesis of N-Methyl-2,4-dimethoxy 6(-2-chloro-prop-2-enyl)benzamide

N-Methyl 2,4-dimethoxybenzamide (0.2g) in THF was cooled to -78°C under nitrogen. To this was slowly added, with stirring, n-butyllithium (2ml, 1.6M). After 15 min 2,3-dichloro-1-propene (0.2ml) was added. This was stirred for 5 h, after which time dilute hydrochloric acid (10ml) was added. This was extracted with ether, dried and evaporated. Only N-methyl-2,4-dimethoxybenzamide (starting material) and a black polymeric substance were recovered.

Diisopropylethylammonium p-toluenesulfonate[61]

To p-toluenesulfonic acid monohydrate (3.8g) in anhydrous methanol (10ml) was added di-isopropylethylamine (3.8ml). The resulting solution was

concentrated in vacuo yielding an oil which crystallised on standing. The resulting solid was crushed and dried in vacuum. m.p. 87-88°C (lit.[61] m.p. 87-88°C). δ 9.18 (1H,br,s), 7.82 (2H,d), 7.17 (2H, d), 3.3-3.9 (2H,m), 2.8-3.3 (2H,m), 2.35 (3H,s), 1.37 (15H, m).

Attempted synthesis of 3-Bromo-2-methoxypropene[61] (75)

A mixture of 1-bromo-2,2-dimethoxypropane (2.5g) and diisopropylethylammonium p-toluenesulphonate (0.04g) was heated at 140-150°C while distilling off the methanol through a 12 inch 1.5cm vigreoux fractionating column. The rate of distillation was kept at 1 drop/s. After distillation was complete a black unidentifiable residue was left.

Attempted metalation of N-methyl 2,4-dimethoxybenzamide

N-Methyl 2,4-dimethoxybenzamide (0.2g) in THF was cooled to 0°C under nitrogen. To this was slowly added with stirring n-butyllithium (2ml, 1.6M). This was stirred at this temperature for 10 min. Deuterium oxide (0.1ml) in THF was then added stirring was continued for 5 minutes. The resulting solution was allowed to warm to room temperature and water added. The organics were evaporated off and the aqueous layer extracted with ether. This was dried and evaporated to furnish only starting material (98% recovery).

3-Chloro-2,4-dimethoxy-6(2-oxopropyl)benzoic acid (70)

4-Chloro-3,5-dimethoxyhomophthalic acid (2.5g) was added portionwise to a mixture of acetic anhydride (10ml) and dry pyridine (2.5ml) in a flask equipped with a stirrer. A precipitate formed after a few minutes and THF (25ml) was added, stirring was continued for 2 hours. Sodium hydoxide (50ml, 5M) was slowly added and mixture stirred until all mateial dissolved. The resulting solution was washed with dichloromethane and then acidified with concentrated hydrochloric acid. The acidic solution was extracted with ethyl acetate, dried and evaporated. Product was recrystallised from acetone (1.5g, 60%). Double m.p. 136-138°C and 150-152°C. (Found $\underline{M}^+$ 272.0454 $C_{12}H_{13}O_5Cl$ requires 272.0452). ν_{max} 3 360 m, 1 695 s, 1 587 s, 1 241 s, 1 1025cm^{-1}. $\delta((CD_3)_2CO)$ 6.62(1H,s,Ar-H), 4.02(2H,s,-CH_2-), 3.88(3H,s,-OCH_3), 3.85(3H,s,-OCH_3), 1.98(3H,s,-CH_3)p.p.m.

3-Chloro-2,4-dimethoxy-6(2-oxopropyl)benzoic acid

Diisopropylamine (1.9ml) in THF (25ml) under nitrogen was stirred at room temperature. To this was added n-[illegible] in hexane (3.1ml, 1.6M) and stirred for 15 min. The solution was cooled to -78°C and 3-chloro-2,4-dimethoxy-6-methylbenzoic acid (1g) in THF added dropwise. This was further stirred for 30 min. The anion solution, at -78°C was transferred using a syringe to a flask containing freshly distilled acetyl chloride (4.3ml). This was allowed to warm to room temperature and stirred for 4 h. Water (25ml) was added the mixture was

acidified with dilute hydrochloric acid and extracted with ethyl acetate and ether. The organic solution was washed with brine, dried and evaporated to dryness. The 3-chloro-2,4-dimethoxy-6(2-oxopropyl)benzoic acid was separated from residue using plc with ether:hexane, 2:1 as eluant. Recrystallised from ethanol (110mg, 9%). Data as above.

7-Chloro-6,8-dimethoxy-3-methylisocoumarin (67)

3-Chloro-2,4-dimethoxy-6-(2-oxopropyl)benzoic acid (0.22g) was dissolved in a solution of acetic anhydride (4.8ml), perchloric acid (0.375ml, 72%) in ethyl acetate made up to 25ml and allowed to stand at room temperature for 10 min. The solution was washed with sodium bicarbonate, dried and evaporated to dryness. The product was recrystallised from ethanol, (0.18g, 88%), m.p. 187-200°C (decomp).) (lit.[43] 187-200°C (decomp.))
δ 6.52 (1H,s,5-H), 6.12(1H,s,4-H), 3.96(3H,s,-OCH_3), 3.94(3H,s,-OCH_3), 2.18(3H,s,-CH_3) p.p.m.

[3,4,9-$^2H_3$]-7-Chloro-3,4-dihydro-6,8-dimethoxy-3-methylisocoumarin

7-Chloro-6,8-dimethoxy-3-methylisocoumarin (250mg) in ethyl acetate was stirred under deuterium in the presence of 5% palladium/charcoal catalyst overnight. The resulting mixture was passed through a column of celite and the ethyl acetate evaporated off to yield product (240mg, 98%) m.p. 155°C (lit.[43] 154-157°C) δ 6.55 (1H,s,5-H), 3.90 (3H,s,-OCH_3), 3.87 (3H,s,-OCH_3) 2.80 (1H,br,s,4-H) 1.38 (2H,br,s,-CH_2D).

[3,4,9-$^2H_3$]-7-Chloro-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin

To a solution of[3,4,9-$^2H_3$]-7-chloro-3,4-dihydro-6,8-dimethoxy-3-methylisocoumarin (100mg) in dichloromethane (25ml) was added boron tribromide (0.5ml) at -70°C. The solution was allowed to warm gradually, while stirring over 40 h. Ether was added, then water (cautiously!). The organic layer was dried and evaporated. The residue was purified by plc (ethanol/hexane (1:5) as eluant) to give the product (59mg, 67%). This was recrystallised from ethyl acetate/hexane m.p. 194-196°C (lit.[43] 193-196°C) δ (CD_3OH) 6.30 (1H,s,5-H), 2.78 (1H,s,4-H), 1.40 (2H,s,-CH_2D) p.p.m.

[3,4,9-$^2H_3$]-5,7-Dichloro-3,4-dihydro-6,8-dihydroxy-3-methyl-isocoumarin (50)

To a solution of[3,4,9-$^2H_3$]-7-chloro-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin (100mg) in anhydrous ether (25ml) was added sulphuryl chloride (0.6ml). The solution was kept in the dark for 1 h at room temperature, then the excess sulphuryl chloride was destroyed with water. The organic layer was dried and evaporated. The product was recrystallised from acetone (100mg, 87%), m.p. 232-234°C (lit.[43] 230-234°C) δ ($(CD_3)_2CO$) 2.71 (1H,m,br,4-H), 1.50 (2H,s,-CH_2D) p.p.m.

[1′,1′,3′,3′,3′-$^2H_5$]-3-chloro-2,4-dimethoxy-6-(2′-oxopropyl)benzoic acid

3-Chloro-2,4-dimethoxy-6-(2-oxopropyl)benzoic acid (0.9g) was added to sodium (0.4g) in deuterium oxide (~5ml). This was stirred till all material dissolved. This was acidified extracted with ethyl acetate dried and evaporated to give product (0.54g, 60%), m.p. 138-140°C recrystallises remelts 152°C. δ 6.68 (1H,s,Ar-H), 3.92(6H,s,-OCH_3 x 2) p.p.m.

[4,9,9,9-$^2H_4$]-7-Chloro-6,8-dimethoxy-3-[4-^2H,$^2H_3$]methyl isocoumarin.

[1′,1′,3′,3′,3′-$^2H_5$]-3-Chloro-2,4-dimethoxy-6-(2′-oxopropyl) benzoic acid (0.2g) was dissolved in a solution of acetic anhydride (4.8ml), perchloric acid

(0.375ml,72%) in ethyl acetate made up to 25ml and allowed to stand at room temperature for 10 min. The solution was washed with sodium bicarbonate, dried and evaporated to dryness. Recrystallised from ethanol (0.18g,88%) m.p. 187-200°C (decomp.) (lit.[43] 187-200°C decomp.) δ 6.51 (1H,s,5-H), 3.96 (6H,s,-OCH_3 x 2) p.p.m.

[4,9,9,9-$^2H_4$]-7-Chloro-3,4-dihydro-6,8-dimethoxy-3-methyl-isocoumarin

To[4,9,9,9-$^2H_4$]-7-chloro-6,8-dimethoxy-3-methyl isocoumarin (0.25g) in ethyl acetate (25ml) was added 10% palladium on charcoal catalyst (0.05g). This was allowed to stir under a blanket of hydrogen for 8 h. The residue was passed through a celite column and filtrate evaporated to dryness. The product was recrystallised from dichloromethane/hexane (0.22g, 88%), m.p. 155° (lit.[43] 154-157°C) δ 6.62 (1H,s,5-H), 4.50 (1H,br,3-H), 3.95 (6H,s,-OCH_3 x 2), 2.80 (1H,br,4-H) p.p.m.

[4,9,9,9-$^2H_4$]-7-Chloro-3,4-dihydro-6,8-dihydroxy-3-methyl-isocoumarin

To a solution of [4,9,9,9-$^2H_4$]-7-chloro-3,4-dihydro-6,8-dimethoxy-3-methylisocoumarin (100mg) in dichloromethane (25ml) was added boron tribromide (0.5ml) at -70°C. The solution was allowed to warm gradually while stirring over 40 h. Ether was added, then water (cautiously!). The organic layer was dried and evaporated. The residue was purified by plc (ethanol/hexane (1:5) as eluant) to give the product

(55mg, 62%). This was recrystallised from ethyl acetate/hexane m.p. 195°C(lit.[43] 193-196°). δ ($(CD_3)_2CO$) 6.50 (1H,s,5-H), 4.70 (1H,br,3-H), 2.93 (1H,br,4-H). p.p.m.

[4,9,9,9-$^2H_4$]-5,7-dichloro-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin (49)

Toa solution of [4,9,9,9-$^2H_4$]-7-chloro-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin (100mg) in anhydrous ether (25ml) was added sulphuryl chloride (0.6ml). The solution was kept in the dark for 1 h at room temperature, then the excess sulphuryl chloride was destroyed with water. The organic layer was dried and evaporated. The product was recrystallised from acetone (100mg, 87%), m.p. 232-234°C (lit.[43] 230-234°C) δ ($(CD_3)_2CO$), 4.80 (1H,s,br,3-H), 3.26 (1H,s,br,4-H), p.p.m.

Methyl 4-benzyloxy-3-chloro-2-hydroxy-6-methyl-benzoate(95)

Methyl-3-chloro-o-orsellinate (7.5g), potassium carbonate (15g) and benzyl chloride (5.3g) were stirred and heated under reflux in acetone (100ml) for 22 h. This was allowed to cool and filtered. The filtrate was evaporated to leave an oil. This was dissolved in ether and ammonium hydroxide added. The aqueous layer was acidified with hydrochloric acid. This was extracted with ethyl acetate, dried and evaporated. The product crystallised on standing. This was recrystallised from methanol (2.6g,25%) m.p. 122°C

(Found: C, 62.66; H,4.93; Cl, 12.00% requires C, 62.65; H, 4.93; Cl, 11.56%); ν_{max} 2 950 br, 1 704 s, 1 595 s, 1 372 s, 1 225 s, 1 101 s cm^{-1}; δ ($(CD_3)_2CO$), 12.10 (1H,s,-OH), 7.37 (5H,m,Ph-H), 6.15 (1H,s,Ar-H), 5.22 (2H,s,$-CH_2-$), 3.88 (3H,s,$-OCH_3$), 2.47 (3H,s,$-CH_3$), ppm; m/e 306/308 (M^+).

Methyl 3-chloro-2,4-dibenzyloxy-6-methylbenzoate (96)

The ether layer from experiment described above is dried and evaporated to give product. This was recrystallised from methanol. (8.9g, 65%), m.p. 96-98°C. (Found: C, 69.65; H, 5.36; Cl, 8.51% requires C, 69.61; H, 5.33; Cl 8.93); ν_{max} 2 940 m, 1 732 s, 1 592 s, 1 225 s, 1 202 s, 1 100 s cm^{-1}; δ 7.38 (10H,m,Ph-H), 6.61 (1H,s,ArH), 5.13 (2H,s,$-CH_2-$), 5.07 (2H,s,$-CH_2-$), 3.76 (3H,s,$-OCH_3$), 2.26 (3H,s,$-CH_3$), p.p.m. m/e 396, 398 (M^+).

Methyl 3-chloro-2-methoxy-4-benzyloxy-6-methyl benzoate(97)

Methyl 4-benzyloxy-3-chloro-4-hydroxy-6-methylbenzoate (1g), dimethyl sulphate (2ml), and anhydrous potassium carbonate (3g) in acetone were heated under reflux for 8 h. On cooling, this was filtered and evaporated to leave an oil. This was dissolved in ether and washed with ammonium hydroxide. The ether solution was dried and evaporated to give an oil which crystallised on standing. Product was recrystallised from ethanol. (0.42g, 40%), m.p. 70-73°C (Found: C, 63.66; H, 5.38; Cl 10.93 requires C, 63.65;

H, 5.34; Cl 11.05%); ν_{max} 2 940 w, 1 725 s, 1 587 s, 1 280 s, 1 224 s, 1 208 s, 1 093 s cm^{-1}; δ 7.70 (5H,m,Ph-H), 6.92 (1H,s,Ar-H), 5.44 (2H,s,$-CH_2-$), 4.18 (6H,s, $-OCH_3$ x 2), 2.58 (3H,s, $-CH_3$) p.p.m; m/e 320/322 (M^+).

3-Chloro-2,4-dibenzyloxy-6-methylbenzoic acid (98)

Methyl 3-chloro-2,4-dibenzyloxy-6-methylbenzoate (1g) was refluxed overnight in sodium hydroxide (5M). The mixture was cooled, washed with ether and acidified with hydrochloric acid. This produced a white precipitate which was collected by filtration. The white solid produced was insoluble in all solvents.

Dimethyl(3,5-dibenzyloxy-4-chloro-2-methoxy-carbonylphenyl)malonate(99)

Dicyclohexylamine (10ml) in dry THF under nitrogen was cooled to $0^{o}C$. To this was added n-butyllithium (31.6ml, 1.6M) and the mixture stirred for 10 min. The mixture was then cooled to $-78^{o}C$ and a solution of methyl 3-chloro-2,4-dibenzyloxy-6-methylbenzoate (4g) and dimethyl carbonate (8.5ml) in THF added slowly. The mixture was allowed to warm to room temperature and stirring continued for 4 h. At this time dilute hydrochloric acid was added and dicyclo hexylamine hydrochloride precipitated out. This was removed by filtration. The layers were separated and organic layer dried and evaporated to give an oil. This was passed through a column (eluant 20% chloroform in pet. ether) to give desired product. Recrystallised from ether (3.7g, 72%), m.p. 100-101^{o}C (Found: C, 63.24; H, 4.77; Cl, 6.72% required C, 63.22; H, 4.91; Cl, 6.91%) ν_{max} 2 950 w, 1 758 s, 1 719 s, 1 590 s, 1 295 s, 1 225 s, 1 100 s cm^{-1}; δ_H 7.43 (10H,m,Ph-H), 7.03 (1H,s,Ar-H), 5.23 (2H,s,-CH_2-), 5.11 (2H,s,-CH_2), 4.88 (1H,s,-CH-), 3.78 (3H,s,-OCH_3), 3.72 (6H,s,-OCH_3 x 2) p.p.m.

δ_C (200MHz) 167.79 (s), 166.42 (s), 156.27(s), 154.06(s), 136.56(s), 135.63(s), 130.481(s), 127.15-128.54 (d), 122.70(s), 118.15(s), 110.20(d), 96.02(t), 70.89(t), 54.02(d), 52.96(q), 52.40(q) p.p.m. m/e 512,514 (M^+).

3-Chloro-2,4-dibenzyloxyhomophthalic acid(100)

Dimethyl(3,5-dibenzyloxy-4-chloro-2methoxycarbonyl phenyl)malonate (1g) was heated under reflux with sodium hydroxide (25ml, 5M). This was cooled and washed with ether. The aqueous portion was acidified using dilute hydrochloric acid, extracted with ethyl acetate, dried and evaporated to leave a white solid. Recrystallised from ethyl acetate m.p. 177-181°C

δ_H (methanol, chloroform) 7.37(10H,m,Ph-H),6.92(1H,s,Ar-H), 5.16(2H,s,-CH_2-), 5.05(2H,s,-CH_2), 3.71(2H,s,-CH_2-CO_2H).

Methyl 4-benzyloxy-3-chloro-2-hydroxy-6-methylbenzoate (95)

To a solution of methyl 3-chloro-2,4-dibenzyloxy-6-methylisocoumarin (1g) in freshly distilled nitrobenzene (20ml) was added powdered aluminium chloride (1.7g). The solution was stirred at 60°C for 6 h, then poured onto iced water and acidified with dilute hydrochloric acid. The acidic solution was extracted with ether. The extracts were extracted with sodium bicarbonate solution. The basic solution was washed with ether, acidified and the acidic solution was extracted with ethyl acetate. This was dried and evaporated to give product. (0.52g, 68%) m.p. 122°C, δ 7.4 (5H,m,Bz-H), 6.65 (1H,s,Ar-H), 5.21 (2H,s,-CH_2-), 3.88 (3H,s,-OCH_3), 2.47 (3H,s,-CH_3) p.p.m.

Diethyl ethylphosphonate[91]

A mixture of triethylphosphite (10g), ethylbromide (2g) and sodium iodide (0.1g), was refluxed for 3h. The product was distilled at reduced pressure to give a colourless oil (9g,90%) b.p.$_{15}$ 89-90°C (lit.[91] b$_{11}$ 80-83°C) δ 4.08(4H,qs,J_{HH} 7Hz and $^{3}J_{PH}$ 7Hz-OCH_2-), 1.70 (2H,dq,J_{HH} 7Hz and $^{2}J_{Ph}$ 18Hz,-CH_2-) 1.32 (6H,t,J 7Hz OCH_2-CH_3), 1.04(3H,t,J 7Hz,CH_3-).

Diethyl-2-[1-oxo-1-(3,5-dimethoxyphenyl) propyl] phosphonate

To a solution of diethyl phosphonate (1g) in dry THF (80ml) under nitrogen at -78°C was added a solution of n-BuLi (3.8ml, 1.6M). The mixture was stirred for 1 h and a solution of methyl 3,5-dimethoxybenzoate (1g) in THF was added. After stirring for 1 h, at -78°C the cooling bath was removed and temperature allowed to rise to 25°C over 30 min. Water (30ml) was added and the organic evaporated under vacuum. The aqueous layer was extracted with ether and extracts dried and evaporated to give an oil (1.26g, 75%). δ 7.14(2H,d,J 2Hz,Ar-H), 6.67(1H,d,J2Hz,Ar-H), 4.11(5H,d.sept,J3Hz and 8Hz,-CH and -OCH_2-), 3.83 (6H,s,OCH_3 x 2), 1.50-1.80(9H,m,-CH_2CH_3 and -CH-CH_3)p.p.m. m/e 330 (M^+).

Diethyl-2-[1-oxo-1-(2,4-dichloro-3,5-dimethoxyphenyl)-propyl]phosphonate (118)

To a solution of diethyl-2-[1-oxo-1-(3,5-dimethoxyphenyl)propyl]phosphonate (100mg) in anhydrous ether was added sulphuryl chloride (1ml). The solution was kept in the dark for 1 h at room temperature. Then, the excess sulphuryl chloride was destroyed with water. The organic layer was separated and evaporated to dryness. This was separated on a silica column using chloroform as eluant. (52mg,43%), m.p. 87-92°C (Found $\underline{M}^+$ 398.0448 $C_{15}H_{21}Cl_2O_6P$ requires $\underline{M}^+$ 398.0453): ν_{max} 2 990m 1 714 s, 1 580 s, 1 345 s, 1 250 s, 1 020 s cm^{-1}; δ 6.85 (1H,s,Ar-H), 4.52 (5H,m,$-OCH_2-$ and -CH-), 4.13 (6H,s,$-OCH_3$ x 2), 1.72-1.27 (9H,m, $-CH_2$ CH_3 and $-CH-CH_3$) p.p.m.

Ethylphosphonic dichloride[92]

Firstly, anhydrous aluminium trichloride (13.3g) was weighed into a glass stoppered conical flask, followed by phosphorous trichloride (13.7g). To this mixture was added ethyl bromide (32.7g) and the flask was stoppered and clamped on a mechanical shaker. (All reactants and reaction flasks were cooled to 4°C just prior to weighing).

After shaking for 15 min the temperature had risen to 23°C. At this temperature the solid aluminium trichloride dissolved accompanied by a sudden rise in temperature to about 35°C. The solution became pale yellow in colour. After a further 12 min the temperature fell to 25°C accompanied by the deposit of a

white crystalline precipitate. The shaking was continued for 1 hour. The reaction flask was then placed in a refrigerator at 4°C for 24 h. The supernatant liquid was poured off leaving a dry, crystalline material which was thought to be complex $C_2H_5PX_3^+AlX_4^-$, where X = Br or Cl. This crystalline complex was dissolved in methylene chloride (200ml) transferred to a 3-necked flask, cooled to 0°C and hydrolysed by adding dropwise, over 20 minutes, cold, concentrated hydrochloric acid (25.3ml). The mixture was stirred for 2 h. The mixture was kept below 7°C throughout the hydrolysis. The cold mixture was filtered and the filtrate was distilled to remove excess solvent, followed by vacuum distillation of residue at 120mm (4.6g, 32%) $b.p._{15}$ 60°C (lit.bp.[92] 174.5) δ 2.61 (2H,dq,$\underline{J}_{HH}$ and $^2\underline{J}_{PH}$ 16Hz $-CH_2-$), 1.39 (3H,dt,$\underline{J}_{HH}$ and $^3\underline{J}_{PH}$ 31 Hz, $-CH_3$).

Ethylphosphonic acid bis(dimethylamide)

To a stirred solution of dimethylamine (18g) in ether (250ml) was added, while at 0°C and under nitrogen, ethylphosphonic dichloride (11g). The resulting solution was stirred at 0°C for 1 h and at room temperature for 3 h. The precipitate of dimethylamine hydrochloride was removed by filtration and this filtrate evaporated under vacuum. Distillation of the residue afforded a colourless oil (6.1g, 50%) $b.p._{1.0}$ 75°C (lit.[93] $b.p._{31}$ 142°C) δ 2.61 (12H,d,$^3\underline{J}_{PH}$ 9Hz $-N-(CH_3)_2$ x 2), 1.77 (2H,dq,$\underline{J}_{HH}$ 5Hz and $^2\underline{J}_{PH}$ 14Hz, $-CH_2-$), 1.09 (3H,dt, $\underline{J}_{HH}$, 7.5Hz and $^3\underline{J}_{PH}$ 18.5 Hz, $-CH_3$).

2-[1-(3,5-Dimethoxyphenyl)-1-oxopropyl]-phosphonic acid bis(dimethylamide) [47] (108)

A stirred solution containing ethylphosphonic acid bis(dimethylamide) (1g) in dry THF (15ml) was treated, while at -78°C under nitrogen with a solution of n-butyllithium in hexane (3.9ml, 1.6M). The mixture was stirred at -50°C for 3 h. After cooling to -78°C a solution of methyl 3,5-dimethoxybenzoate (0.59g) in dry THF (10ml) was added. Stirring was continued for 2h at -70°C and 0.5h at -70° - 25°C. Water was then added and the THF removed by evaporation. The aqueous solution was extracted with ethyl acetate and combined extracts were washed extensively with sodium chloride solution (1M) to remove the unreacted ethylphosphonic acid bis-(dimethylamide). The organic layer was dried and evaporated to give an oil (0.72g, 73%).
δ 7.17 (2H,d,J 2Hz, Ar-H), 6.64 (1H,t,J 2Hz, Ar-H), 4.27 (1H,dq,J_{HH} 7Hz and $^{3}J_{PH}$ 18 Hz, -CH-) 3.83 (6H,s,-OCH_3 x 2), 2.64 (6H,d,$^{3}J_{PH}$ 9 Hz, N-CH_3 x 2) 2.59 (6H,d, $^{3}J_{PH}$ 9 Hz -NCH_3 x 2). 1.49 (5H,dt, J_{HH} 7Hz and $^{3}J_{PH}$ 17Hz, -CH_3) p.p.m.

(2E)-1-(3,5-Dimethoxyphenyl)-prop-1-ene[47] (110)

To a stirred solution of β-ketophosphonic acid bis(dimethylamide) (0.5g) in methanol (15ml) was slowly added while at 0°C, sodium borohydride (0.5g). After stirring for 1 h the reaction mixture was acidified with hydrochloric acid to decompose the excess sodium borohydride and to affect cycloelimination to give the

olefin. The acidic aqueous solution was extracted with ethyl acetate and the organics washed thoroughly with brine (1M), dried and evaporated to yield a colourless oil (0.22g, 81%). δ 6.29 (2H,d,J 2Hz, Ar-H), 6.12 (1H,t,J 2Hz, Ar-H), 6.03-6.16 (2H,m,olefinic), 3.56 (6H,s,-OCH_3 x 2), 1.66 (3H,d, J 5Hz, -CH_3) p.p.m.

Attempted synthesis of 2-methoxy-6-prop-1-enyl-1,4-benzoquinone

To a solution of potassium hexacyanoferrate (50mg) in water (0.2ml) was added a solution of (2E)-1-(3,5-dimethoxy-phenyl)prop-1-ene (70mg) in acetone and hydrogen peroxide (100 vol. 140mg) successively. The solution was stirred for 15 h at room temperature. This was then diluted with dichloromethane, washed with water and dried. The solution was evaporated to dryness to give a complex mixture of components.

Potassium nitrosodisulphonate (Fremy's salt)[94]

To a solution of sodium nitrite (5M,100ml) in ice (200g) was added sodium bisulphite (35% w/v,100ml) followed by glacial acetic acid (20ml). The mixture was stirred for 5 min then ammonia (sp.grav.0.88, 25ml) was added. The pale brown mixture was maintained at $O^{o}C$ while potassium permanganate (0.2M,400ml) was added dropwise over 1 h. The resulting mixture was filtered through two sheets of Whatman No.1 filter paper (using several filter funnels to speed up the process).* The ice cold filtrate was maintained at $O^{o}C$ while a saturated solution of potassium chloride (300ml) was added dropwise over 45 min with stirring. Stirring was continued for a further 1 h by which time the salt had crystallised from solution. The beautiful, orange crystals were collected by filtration and washed with ammoniacal saturated potassium chloride (sat. KCl containing 5% conc. ammonia) (100ml) ammoniacal methanol (methanol containing conc. ammonia 5%) (100ml) and acetone; the filtered salt was not allowed to come into direct contact with air until this washing procedure was complete. The salt was stored in a dessicator which contained calcium oxide and an ammoniacal atmosphere supplied by ammonium carbonate (moist).

*It is essential that the mixture containing precipitated manganese dioxide does not warm up during filtration.

Attempted synthesis of 2-methoxy-6-prop-1-enyl-1,4-benzoquinone

(2E)-1-(3,5-dimethoxyphenyl)prop-1-ene (60mg) was suspended in a solution of dipotassium hydrogenphosphate and tetrabutylammonium iodide in aqueous methanol. A solution of freshly prepared Fremy's salt (50mg) and dipotassium hydrogenphosphate in aqueous methanol was added and stirred at room temperature for 24 h. The mixture was acidified and extracted with chloroform. The chloroform was evaporated to leave a dark, crystalline solid. Separation by plc (chloroform) led to starting material and an unidentifiable component which recrystallised from ethanol m.p. 69-70°C (Found: C, 31.17; H, 6.38; N, 2.46); λ_{max} (Et-OH) 459, 392, 318, 298 nm; ν_{max} 2 955(m) 2 922(m), 2 866(m), 2 361(w), 1 470(m), 1 450(m), 1 370(m) cm^{-1}; δ 3.3-3.1(m), 1.8-1.1(m), 0.95(t,J 8Hz) p.p.m; m/e 242/244.

3-Bromo-4-hydroxy-5-methoxybenzaldehyde[74] (123)

Vanillin (100g) was dissolved in glacial acetic acid (180ml) Bromine (34.5ml) in glacial acetic acid was added with rapid stirring. The mixture was stirred at 15°C for a further 15 min then poured onto ice water (1300ml). The resulting precipitate was filtered and air dried. Recrystallised from ethanol (120g, 79%) m.p. 163-164°C (lit.[74] 163-164°C); δ ($(CD_3)_2CO$) 9.75 (1H,s,-CHO) 7.65 (1H,d,J 2Hz, Ar-H), 7.36 (1H,d,J 2Hz, ArH), 6.60 (1H,br, D_2O ex -OH), 4.00 (3H,s,-OCH_3) p.p.m.

2,5-Dihydroxy-3-methoxybromobenzene[74] (124)

To a solution of 3-bromo-4-hydroxy-5-methoxybenzaldehyde (10g) in potassium hydroxide (40ml, 1M), cooled to 0°C was added a solution of hydrogen peroxide (100 vol. 10ml) in water (65ml) over 3 h. The mixture was stirred for a further 1 h by which time a purple solid had precipitated from solution. The precipitate was filtered washed with water and air dried. Recrystallised from water (6.7g,76%), m.p. 141°C (lit.[74] 141°C), δ ($(CD_3)_2CO$), 6.60 (1H,d,J 3Hz,Ar-H), 6.40 (1H,d, J 3Hz Ar-H), 3.78 (3H,s,-OCH_3) p.p.m.

2,3,5-Trimethoxybromobenzene[43] (125)

A solution of 2,5-dihydroxy-3-methoxybromobenzene (5g), dimethyl sulphate (5ml), anhydrous potassium carbonate (12.5g) in acetone (75ml) was refluxed for 6 h with stirring. On cooling, the mixture was filtered

and the acetone solution was evaporated to leave a brown oily residue. The residue was boiled with 30% sodium hydroxide solution for 1 h and the product was extracted with ether (3 x 50ml). The ether solution was dried and evaporated to give product as a pale yellow oil (52%) which was used without further purification in the next step. δ 6.60 (1H,d,J,3Hz ArH), 6.43 (1H,d,J 3Hz, Ar-H), 4.85 (3H,s,-OCH_3) 4.80 (3H,s,-OCH_3), 4.75 (3H,s,-OCH_3), p.p.m.

6-(1-Hydroxypropyl)-1,2,4-trimethoxybenzene

2,3,5-Trimethoxybromobenzene (1g) in THF (25ml) was cooled to -78°C and a solution of n-butyllithium in hexane (2.8ml,1.6M) was added with stirring. The solution was stirred for several minutes and propanal (0.62ml) in THF was added. The solution was stirred for 2 h and allowed to warm to room temperature. Water was added and the organic layer evaporated off. The aqueous layer was acidified and extracted with ether, dried and evaporated to leave an oil (0.52g, 57%), b.p.$_{0.2}$ 250°C (Found: C, 63.85; H, 8.22 requires C,63.70; H, 8.02); ν_{max} 3 450 br, 2 960 s, 2 940 s, 1 600 s, 1 490 s, 1 465 s, 1 200 s, 1 150 s cm^{-1}; δ 6.52 (1H,d,J 4Hz,Ar-H), 6.43 (1H,d,J 4Hz,Ar-H), 4.84 (1H,t, J 7 Hz, -CH-), 3.88 (3H,s,-OCH_3), 3.79 (3H,s,-OCH_3), 3.76 (3H,s,-OCH_3), 2.98 (1H,s,br, -OH), 1.77 (2H,q, J 8Hz -CH_2-), 0.94 (3H,t, J 8Hz, -CH_3), m/e 226(M^+).

Tetrakis(triphenylphosphine)palladium [95]

A mixture of palladium dichloride (1.77g) triphenylphosphine (13.1g) and 120ml of dimethyl sulfoxide was placed in a flask with magnetic stirrer, a rubber septum and vacuum nitrogen system. The system was placed under nitrogen. The yellow mixture was heated by means of an oil bath with stirring until complete solution occurred at 140°C. The bath was taken away and solution stirred rapidly for 15 min. Hydrazine hydrate (2g) was then rapidly added over approximately 1 min from a hypodermic syringe. A vigorous reaction took place with evolution of nitrogen. The dark solution was then cooled with a water bath. Crystallisation occurred at ~ 125°C. At this point the mixture was allowed to cool normally till room temperature. It was then filtered under nitrogen on a coarse sintered glass funnel. The solid was washed successively with ethanol then ether and dried by passing nitrogen through the funnel overnight. The product was stored under nitrogen (4.8g, 89%) (m.p.[95] lit. 116°C, not very reproducible).

p-Bromoanisole

p-Bromophenol (4g), dimethylsulphate (11ml) and potassium carbonate (16g) were refluxed in acetone for 6 h. This was filtered and filtrate evaporated. This residue was dissolved in ether and washed with ammonium hydroxide. The organic layer was dried and evaporated (3.5g, 82%) b.p. 223 (lit.[96] b.p.223) δ 7.43 (2H,d,J9Hz, Ar-H), 6.75 (2H,d, J 9Hz, Ar-H), 3.70 (3H,s,-OCH_3).

1-Heptynylzinc chloride [76]

To a solution of 1-heptyne (1.9ml) in THF (10ml) at $O^{o}C$ was added n-butyllithium (9.8ml, 1.6M). The solution was stirred for 5 min followed by addition of anhydrous zinc chloride (2g) dissolved in THF (20ml). The mixture was stirred for an additional 15 min at room temperature.

Attempted synthesis of 1-(4-methoxyphenyl)heptyne

Heptynylzinc chloride was prepared as above and to this was added sequentially at $O^{o}C$ p-bromoanisole (3.4g) dissolved in THF (20ml) and tetrakis(triphenyl phosphine)palladium (0.8g) in THF. The reaction mixture was stirred for 1 hour at room temperature. This was then quenched with hydrochloric acid (1N, 50ml). After adding pet. ether (50ml) the two layers were separated and the aqueous layer was extracted with pet. ether. The combined organic layers were washed with saturated sodium bicarbonate, dried, filtered through a short alumina column and evaporated. This left a black residue a portion of which was separated by plc (chloroform) to yield only starting material.

Attempted synthesis of 1-(4-methoxyphenyl)heptyne

The above procedure was repeated using p.iodoanisole. This gave only starting materials.

Attempted synthesis of 4-hydroxy-3-methoxy-5-(hept-1-ynyl)benzaldehyde (131)

A mixture of bromovanillin (250mg), heptyne (0.26ml) tetrakis(triphenylphosphine)palladium (50mg) copper(I) iodide (50mg), triethylamine (5ml) and dimethylsulphoxide (3ml) was heated at 100°C with stirring for 20 hours. After cooling, the reaction mixture was stirred with ice and hydrochloric acid and products extracted with dichloromethane, dried and evaporated. The residue was examined by mass spectroscopy. No alkynylation was found to have occurred. Only starting material recovered.

4-Hydroxy-3-iodo-5-methoxybenzaldehyde[78] (130)

To a solution of vanillin (10g) and sodium iodide (11.8g) in dimethylformamide (250ml) at room temperature was added chloramine T (22.2g). This resulting solution was stirred for 4 h. The solution was then diluted with water, acidified with hydrochloric acid and extracted with ethyl acetate. The organic layer was washed with sodium thiosulphate (5%) and brine, dried and evaporated to yield desired product. Recrystallised from ethanol (16.5g, 90%), m.p. 180°C (lit.m.p.[10] 179-180°C δ 3.95 (3H,s,-OCH_3), 7.39 (1H,d,J 2Hz, Ar-H), 7.83 (1H,d, J 2Hz, Ar_2-H), 9.77 (1H,s,-CHO).

Attempted synthesis of 4-hydroxy-3-methoxy-5(hept-1-ynyl)benzaldehyde

This was attempted using the same reagents as described previously but carrying out the reaction on iodovanillin in place of bromovanillin. This method produced only starting material when examined by mass spectroscopy.

Bis(triphenylphosphine)dichloropalladium[97]

Triphenylphosphine (20g) and palladium(II) chloride (1g) were placed in a flask. This was heated till the triphenylphosphine melted, with stirring. Stirring was continued for 20 min and heat removed. The product and excess triphenylphosphine were extracted with chloroform. The extract was treated with hexane and a yellow product precipitated out. This was filtered and washed with hexane (0.63g, 35%) m.p. 298-300^{o} (decomp) (lit.[97] m.p. 298-300 (decomp.).

4-Hydroxy-3-methoxy-5(hept-1-ynyl)benzaldehyde

To a round bottom flask was added iodovanillin (280mg) bis(triphenylphosphine)dichloropalladium (25mg), heptyne (1.5ml), cuprous iodide (50mg) triethylamine (5ml) and THF (5ml). This was stirred and heated at 100^{o}C for 20 hours. After cooling the reaction mixture was stirred with ice and hydrochloric acid and products extracted with dichloromethane, dried and evaporated. Products were separated using plc (chloroform) (100mg,

43%), b.p.$_{0.2}$ 230°C. (Found: C, 73.11; H, 7.52% requires C, 73.15: H, 7.37%) ν_{max} 2 930m, 1 690s, 1 390s, 1 192 s cm^{-1}; δ 9.97 (1H,s,-CHO), 7.62 (1H,d,J 2Hz, Ar-H), 7.32 (1H,d, J 2Hz, Ar-H), 6.50 (1H,s,-OH), 4.05 (3H,s,-OCH_3), 2.83 (2H,t, J 8Hz -CH_2-), 1.79 (2H,m,-CH_2-), 1.40 (4H,m, -CH_2- x 2), 1.92 (3H,t, J 8Hz, -CH_2), m/e 246 (M^+).

Attempted synthesis of 2,4-dihydroxy-4-methoxy-iodobenzene

To a solution of 4-hydroxy-3-iodo-5-methoxybenzaldehyde (2g) in potassium hydroxide (9.6ml, 1M) cooled to 0°C was added a solution of hydrogen peroxide (100 vol, 2.6ml) in water (15.4ml) over 3 h. The mixture was stirred for a further hour by which time a precipitate appeared. The precipitate was filtered, washed with water and air dried. This yielded only starting material (96% recovery).

References

1. 'Burger's Medical Chemistry' Part 1, Chapter 1 Ed. M.E.Wolff, John Wiley and Sons, 1980.
2. P. Manitto, 'Biosynthesis of Natural Products', John Wiley and Sons, 1981.
3. W.B.Turner, 'Fungal Metabolites I', Academic Press, London, 1971.
4. J.N.Collie, J.Chem.Soc, 1893, 122, 329; ibid, 1907, 1806.
5. A.J.Birch and F.W.Donovan, Aust.J.Chem, 1953, 6, 360.
6. A.J.Birch, R.A.Massey-Westropp and C.J.Moye, Aust.J.Chem, 1955, 8, 539.
7. M.Y.Jarrah and V.Thaller, J.Chem.Soc, Perkin Trans 1, 1983, 1719.
8. A.Stoessl, Biochem.Biophys.Res.Comm, 1978, 35, 186
9. M.A.W.Eaton and D.W.Hutchison, Tetrahedron Letts, 1971, 1337.
10. 'Brewing Science',vol.1, Chapter 6, Ed. J.R.A. Pollock, Academic Press, London, 1979.
11. F. Drawert and J.Beier, Phytochemistry, 1976, 15, 1689.
12. F.Drawert and J.Beier, Phytochemistry, 1976, 15, 1691.
13. F.Drawert and J.Beier, Phytochemistry, 1976, 15, 1693.
14. F.Drawert and J.Beier, Phytochemistry, 1976, 15, 1695.
15. J.Dierckens et al, J.Inst.Brewing, 1969, 75, 391.
16. A.H.Cook and G.Harris, J.Chem.Soc, 1950, 1873.
17. M.Vezele and F.Govaert, J.Chem.Soc, 1952, 3313.

18. F.L.Rigby and J.L.Bethune, J.Am.Chem.Soc, 1952, 74, 6118.

19. G.A.Howard and C.A.Slate, J.Chem.Soc, 1958,1460.

20. 'Brewing Science' vol.1, Chapter 4, Ed. J.R.A. Polock, Academic Press, London 1979.

21. H.Raistrick and G.Smith, Biochem.J., 1935, 29, 606.

22. A.J.Birch, A. Cassero and A.R.Jones, J.Chem.Soc, Chem.Commun, 1965, 167.

23. R.H.Carter, R.A.Hill and J.Staunton, J.Chem.Soc, Perkin Trans 1, 1981, 2570.

24. R.F.Curtis, P.C.Harries, C.H.Hassall and J.D. Levi, Biochem.J, 1964, 90, 43.

25. K.Arai, T.Yoshimura, Y.Itatani and Y.Yamamoto, Chem.Pharm.Bull, 1983, 31, 925.

26. E.L.Ghisalberti, M.J.Narbey and C.Y.Rowland, Journal of Natural Products, 1990,53, 520.

27. K.Mosbach, Acta.Chem.Scand, 1960,14, 457.

28. H.Seto, W.L.Cary and M.Tanabe, J.Antibiot, 1974, 27, 558.

29. J.Sekiguchi, S.Katayama and Y.Yamada, Appl. Environ.Microbiol, 1987, 53, 1531.

30. K.Axberg and S. Gatenbeck, Acta.Chem.Scand. Ser.B, 1975, 29, 749; also F.E.B.S.Lett, 1975, 54, 18.

31. J.Better and S.Gatenbeck, Acta.Chem.Scand.Ser.B, 1977, 31, 391.

32. J.A.Gudgeon, J.S.E.Holker, T.J.Simpson and K. Young, Bioorganic Chem, 1979, 8, 311.

33. J.S.E.Holker, E.O'Brien, R.N.Moore and J.C. Vederas, J.Chem.Soc, Chem.Commun, 1983, 192.

34. J.S.E.Holker, M.Kaneda, S.E.Ramer and J.C. Vederas, J.Chem.Soc, Chem.Commun, 1987, 1099.

35. J.C.Vederas, Nat.Prod.Rep, 1987, 4, 277.

36. T.J.Simpson, 'Biosynthesis-Specialist Periodical Reports', vol.5, pp.21-24, Chemical Society, London, 1977.

37. T.J.Simpson, A.E.DeJesus, P.S.Steyn and R. Vleggaar, J.Chem.Soc, Chem.Commun, 1982, 631.

38. C.A.Townsend, S.B.Christensen and S.G.Davis, J.Am.Chem.Soc, 1982, 104, 6154.

39. J.R.Hanson, M.A.O'Leary and H.J.Wadsworth, J.Chem.Soc, Perkin Trans.1, 1983, 867.

40. W.J.M'Gahren, J.H.Van den Hende and L.A. Mitscher, J.Am.Chem.Soc, 1969, 91, 157.

41. G.M.Stranz, A.S.Court, J.Komlassy and M.A. Stillwell, Can.J.Chem, 1969, 2087 and 3700.

42. D.Giles and W.B.Turner, J.Chem.Soc.C, 1969, 2187.

43. G.B.Henderson, Ph.D. Thesis, Glasgow 1982.

44. G.S.Macaulay, Ph.D. Thesis, Glasgow, in print.

45. J.S.E.Holker and K.Young, J.Chem.Soc, Chem. Commun, 1975, 525.

46. R.H.Carter, R.A.Hill and J.Staunton, J.Chem. Soc., Chem.Commun, 1965, 167.

47. W.S.MacLachlan, Ph.D. Thesis, Glasgow 1985.

48. R.D.Barry, Chem.Rev, 1964, 64, 229.

49. R.Livingstone, 'Rodd's Chemistry of Carbon Compounds', vol.IV, part E, pp 290-297, Ed. S. Coffey, Elsevier, New York, 1977.

50. R.B.Tirodkar and R.N.Usgaonkar, J.Ind. Chem.Soc, 1969, 46, 10; R.B.Tirodkar and R. N.Usgaonkar, Curr.Sci, 1972, 41, 701;

F.M.Hauser and R.J.Rhee, J.Am.Chem.Soc, 1977, 99, 4533.

51. R.B.Tirodkar and R.N.Usgaonkar, J.Ind.Chem Soc, 1969, 46, 935.

52. L.Fitzpatrick, T.Sala and M.V.Sargent, J.Chem. Soc Perkin Trans.1, 1980, 85.

53. F.M.Hauser and R.P.Rhee, Synthesis, 1977, 245.

54. F.M.Hauser, R.P.Rhee, S.Prasanna, S.M.Weinreb and J.H.Dodd, Synthesis, 1980, 72.

55. D.R.Nadkarne, R.N.Usgaonkar, Indian J.Chem, 1978, 16B, 320.

56. C.N.Lewis, P.L.Spargo and J.Staunton, Synthesis, 1986, 944.

57. S.Nahm and S.M.Weinreb, Tetrahedron Lett, 1981, 22, 3815.

58. T.A.Carpenter, G.E.Evans, F.J.Leeper, J.Staunton and M.R. Wilkinson, J.Chem.Soc, Perkin Trans 1, 1984, 1043.

59. N.S.Narasimhan and B.H.Bhide, Tetrahedron, 1971, 27, 6171; Gschwend, Org.React, 1979, 26, 1.

60. B.H.Bhide, D.I.Brahmbatt, Proc.Indian.Acad.Sci, 1980, 89, 525.

61. R.M.Jacobson, R.A.Raths and J.H.MacDonald, J.Org.Chem, 1977, 42, 2545.

62. P.T.Lansbury and E.J.Nienhouse, J.Am.Chem.Soc, 1966, 88, 4290.

63. E.J.Nienhouse, R.M.Irwin and G.R.Finni, J.Am.Chem.Soc, 1967, 89, 4557.

64. F.M.Hauser and V.M.Baghdanov, J.Org.Chem, 1988, 53, 4676.

65. P.L.Noire and R.W.Franck, Synthesis, 1980, 882; B.M.Trust, G.T.Rivers and J.M.Gold, J.Org.Chem, 1980, 45, 1835.

66. B.E.Edwards and P.N.Rao, J.Org.Chem, 1966, 31, 324.

67. T.Sakamoto, M.An-naka, Y.Kondo and H.Yamanaka, Chem.Pharm.Bull, 1986, 34, 2754.

68. R.F.Curtis, P.C.Harries and C.H.Hassall, J.Chem.Soc, 1964, 5382.

69. T.M.Cresp, M.V.Sargent, J.A.Elix and D.P.H. Murphy, J.Chem.Soc.,Perkin Trans, 1973, 340.

70. E.J.Corey and G.T.Kwiatkowski, J.Am.Chem. Soc, 1968, 90, 6816.

71. W.S.Wadsworth.Jr, Org.React, 1977, 25,86.

72. M.Matsumoto and H. Kobayashi, J.Org.Chem, 1985, 50, 1766.

73. J.M.Soa, J.Morey and C.Rubido, J.Org.Chem, 1990 55, 63.

74. H.W.Dorn, W.H.Warren and J.L.Bullock, J.Am. Chem.Soc, 1939, 61, 144.

75. G.H.Posner, Org. React, 1975, 22, 253.

76. A.O.King and E.Negishi, J.Org.Chem, 1978, 43, 358.

77. W.Tao, S.Nesbitt and R.F.Heck, J.Org.Chem, 1990, 55, 63.

78. T.Kometani, D.S.Watt and T.Ji, Tetrahedron Letts, 1985, 26, 2043.

79. C.J.Wyman, C.T.Wymore and G.Wilkinson, J.Chem.Soc (A), 1968, 561.

80. K.Sonogashira, Y.Tohda and N.Hogihara, Tetrahedron Letts, 1975, 50, 4467.

81. L.Cassar, J.Organometal.Chem, 1975, 93, 253.

82. C.B.Ziegler and R.F.Heck, J.Org.Chem, 1978 43, 2941.

83. R.K.Mackie and D.M.Smith, 'Guidebook to Organic Synthesis', Longman, New York, 1982.

84. P.E.Sonnet, Tetrahedron, 1980, 36, 557.

85. R.F.Heck Org.React, 1982, 27, 345; R.F.Heck, 'Palladium Reagents in Organic Synthesis', Academic Press, London, 1985.

86. A.A.Finnie, Ph.D. Thesis, Glasgow 1989.

87. M.V.Sargent, P.Vogel and J.A.Elix, J.Chem.Soc., Perkin Trans. 1, 1975, 1986.

88. D.D.Ridley, E.Ritchie and W.C.Taylor, Aust.J. Chem, 1968, 21, 2979.

89. P.D.Noire and R.W.Franck, Synthesis, 1980,882.

90. B.M.Trost, C.T.Rivers and J.M.Gold, J.Org.Chem, 1980, 45, 1835.

91. H.Coates and P.C.Crofts, British Patent No. 713,669, 1954.

92. J.P.Clay, J.Org.Chem, 1951, 16, 892.

93. G.M.Kosolapoff and L.B.Payne, J.Org.Chem, 1956, 21, 413.

94. H.Zimmer, D.C.Lankin and S.W.Morgan, Chemical Reviews, 1971, 71, 229.

95. D.R.Coulson, Inorg. Synth, 1972, 13, 121.

96. W.Szalecki, Synth. Commun, 1976, 6, 243.

97. H.A.Tayim, A.Bouldoukian and F.Awad, J.Inorg. Nucl. Chem, 1970, 32, 3799.