PHYTOTOXIC METABOLITES OF CERATOCYSTIS FUNGI

by

John Bryce Strachan

A thesis presented in part fulfilment of

the requirements for the Degree of

Doctor of Philosophy.

Department of Chemistry

October 1992

University of Glasgow

"The road of excess leads to the palace of wisdom."

- Blake

CONTENTS

SUMMARY

A number of naturally occurring isocoumarins which have been isolated from <u>Ceratocystis</u> fungi have been synthesised to evaluate their role as phytotoxins and phytoalexins.

The route which was used was a modification of the homophthalate approach, with an improved synthesis of the homophthalate itself. This route was adapted to allow the synthesis of some 4-substituted isocoumarins, including the <u>cis</u> diastereomer of perimacol, a metabolite of <u>Periconia macrospinosa</u>. The stereochemistry of these 3,4-disubstituted dihydroisocoumarins was investigated, and in particular the correlation between ^{1}H nmr chemical shifts and coupling constants and relative stereochemistry was studied.

5-Methylmellein was prepared by the indanone approach in a shorter synthesis than the previously published one. An attempt to use the indanone approach to produce 3-hydroxymethyl isocoumarins failed. It was found that such compounds can be obtained by allylic functionalisation of suitably substituted isocoumarins.

1. BIOLOGICAL PROPERTIES OF ISOCOUMARINS

1.1 Introduction

Isocoumarins (or benzopyran-1-ones) comprise a large class of natural products that contain an aromatic ring fused to a six-membered lactone.
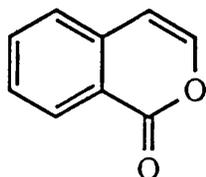
They have been isolated from a wide variety of sources, including fungi, plants and insects. Most of them are polyketides, derived biosynthetically from acetate via the acetate-polymalonate pathway (Scheme 1.1).
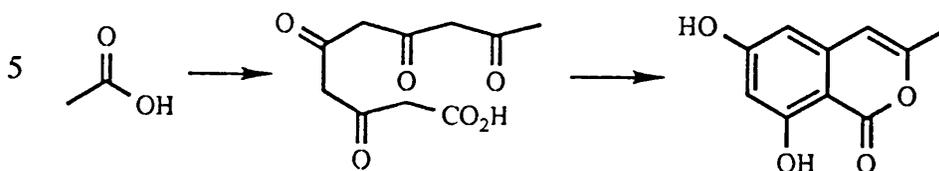
Scheme 1.1

A very broad spectrum of biological activity has been accredited to this group of compounds. They include in their number antibiotics, antifungal agents, plant growth inhibitors, mycotoxins and antitumour agents.

The isolation, structure, properties and synthesis of isocoumarins has been the subject of reviews in 1964[1] and 1986.[2]

The aim of this study was to develop syntheses of certain naturally occurring isocoumarins to obtain sufficient amounts of these compounds for biological testing.

1.2 Wilt Diseases of Trees

Wilt diseases are a class of disorders which affect the vascular system of a plant.[3] They are caused by fungi that are either carried through the bark on an insect or penetrate the roots directly. Once inside the tree, the fungus remains almost exclusively in the vascular system. It spreads through the vessels in the outer rings of xylem, where most of the transport of sap occurs. This causes moisture stress, leading to wilting of leaves, twigs and branches and possibly the death of the tree. The fungus can remain on the dead tree for many years, providing a source of infection for healthy trees.

These diseases are difficult to treat since systemic fungicides require an efficient vascular system to function. Hence the fungicide cannot reach the areas where it is needed most.

Dutch elm disease was first reported in Europe in 1918. The symptoms include yellowing and/or wilting of leaves and the appearance of necrotic lesions between the veins of leaves. The fungus responsible was identified as Ceratocystis ulmi.

In the early years of the disease, little damage was caused, with twig and branch infection but few fatalities. However a severe outbreak in the 1930's resulted in the death of many trees, before declining towards the end of that decade.

Around 1930 the disease spread to North America, probably carried there on imported timber from Europe. It spread rapidly, with American elms proving highly susceptible to the disease.

In the late 1960's an epidemic broke out in Britain and Europe, probably caused by reimportation of the fungus from North America.[4] The source again appears to have been imported logs, since the initial areas of outbreak in Britain were near the ports where they had landed. By 1972 it was estimated that about a quarter of the elm trees in Britain were infected with the disease, with 10% dead or dying.

The fungus is carried to healthy trees by the elm bark beetle; in Europe, Scolytus multistriatus, in North America, Hylurgopinus rufipes. They burrow through the bark of healthy trees, thus infecting them, and laying their eggs in the infected tissue. The eggs hatch in late autumn and the larvae tunnel beneath the bark, emerging as adults in late spring. Their bodies are contaminated with sticky spores of the fungus and the cycle continues.

It became apparent that the epidemic in the 1970's was caused by a virulent strain of C. ulmi.[5] Isolates from British trees fell into two categories - the slow growing, endemic strain described as "waxy", and the fast growing, virulent strain described as "fluffy". In a screening experiment 150 trees were inoculated with the fluffy strain. After four weeks 96% of the leaves had gone, and after fourteen weeks only four of the trees were still alive.

Comparison with isolates of the fungus from North America revealed that the fluffy strain was identical. Hence the epidemic was caused by the virulent strain from North America. In areas where the epidemic had not struck, only the mild, waxy strain was found. This is probably a remnant from the outbreak in the thirties.

It had originally been thought that the action of the fungus was simply to plug the sap vessels. However in 1942 it was shown that C. ulmi produces phytotoxic material.[6] Culture filtrates, freed of the fungus, were able to reproduce the typical symptoms of the disease in healthy elms - namely wilting, formation of necrotic spots and plugging of xylem vessels. The suggestion was made that C. ulmi kills elms primarily by toxin production, with vessel plugging being a secondary factor.

The nature of the toxin was investigated and found to consist of two components.[7] One was a high molecular weight polysaccharide, precipitated by ethanol, which caused leaf curling. The other, ethanol-soluble fraction was highly toxic, producing necrosis on the

leaves.

Further investigation showed that the polysaccharide is not the prime toxic component.[8]   The evidence for this was threefold:

1. There was no correlation between polysaccharide concentration and wilting.

2. Analysis of the culture filtrate showed that the polysaccharide contributed little to the overall wilting.

3. When polysaccharide formation was eliminated by buffering, the culture filtrate still caused wilting.

Following this discovery, attention was concentrated on the ethanol-soluble fraction.   A mixture of high molecular weight glycoproteins was isolated by dialysis and ultracentrifugation.[9]   These induced all of the characteristic symptoms of Dutch elm disease in healthy elm trees.

Extraction of the culture with ethyl acetate yielded three low molecular weight compounds.[10]   The major component was identified as the isocoumarin (1).   It was accompanied by smaller amounts of two other isocoumarins (2) and (3).
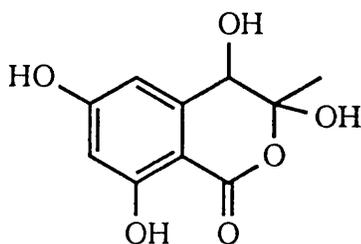
[1]
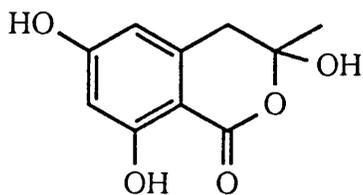
[2]
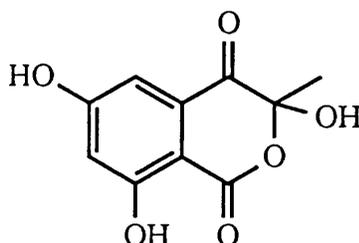
[3]

Isocoumarin (2) had previously been shown to cause necrotic lesions on the leaves of pear trees.[11]

Another devastating wilt disease is blue stain of conifers (or

Southern pine disease). Blue stain is the most destructive disease in the pine forests of North America, estimated in 1984 to kill forty million trees a year in Canada alone. The symptoms are discolouration and wilting of needles and blue staining of the wood.

Blue stain bears many similarities to Dutch elm disease. It is caused by fungal infection, brought about by the action of beetles of the genus Dendroctonus. Particular pests are the mountain pine beetle (D. ponderosae) and the southern pine beetle (D. frontalis).

It was realised early on that the action of the beetle was insufficient to explain the rapid death of attacked trees.[12] This was supported by the observation that blue stain fungi could kill pine trees on their own.

Experiments with dyes showed that the infected trees lose the ability to conduct water successively from the outer rings towards the centre.[13, 14] Eventually the water supply to the leaves is cut off and the tree dies. It is believed that the death of the tree results from the combined effect of the beetle tunneling into the sap vessels and the fungus inhibiting water movement.[15] Reduced water levels, reduced resin production and aeration of the tree tissue also facilitate the development of the young larvae, contributing to the mutual relationship between the beetle and the fungus.

The micro-organisms associated with the beetles have been investigated.[16, 17] They were found to consist of yeasts and fungi, especially of the genera Ceratocystis and Europhium. The principal blue stain fungus, and the one which causes the most rapid staining is Ceratocystis minor.

The metabolites of C. minor were studied by McGraw and Hemingway.[18] The major compound isolated from the liquid culture was the isocoumarin (4). There were also smaller amounts of isocoumarin (5) and scytalone (6).
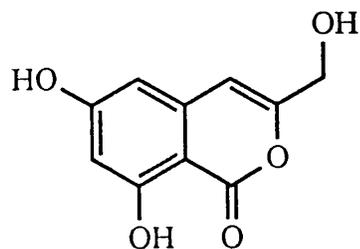
[4]
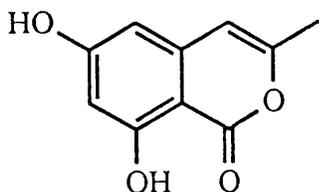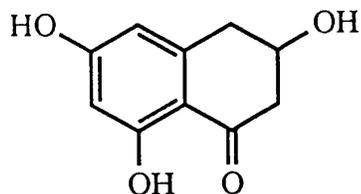
[5]   [6]

C. minor was reinvestigated in 1987 and four new isocoumarins (1), (2), (7) and (8) were isolated.[19]   The novel compound ceratenolone (9) was also discovered.   It forms a blue chelate with ferric ions, and may be responsible for the blue colour of the wood.
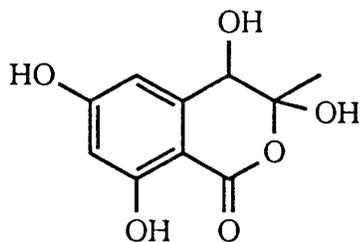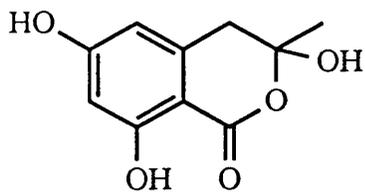
[1]   [2]
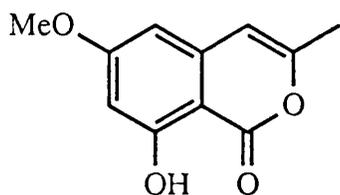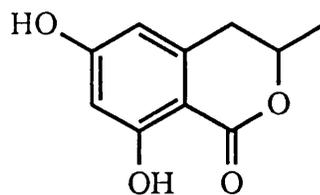
[7]   [8]
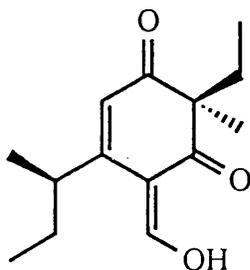
[9]

Three other species of <u>Ceratocystis</u> found in blue stain fungi, namely <u>C. clavigera</u>, <u>C. huntii</u> and <u>C. ips</u> have also been studied.[20]

The broth of <u>C. clavigera</u> yielded several simple aromatic compounds (10)-(14) but no isocoumarins. Similarly, <u>C. huntii</u> produced no isocoumarins, but several aromatic metabolites (11), (15)-(18). <u>C. ips</u> did however produce the same two isocoumarins (4) and (5) which were originally isolated from <u>C. minor</u>. These were accompanied by four other compounds (11), (17), (19) and (20).
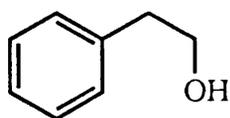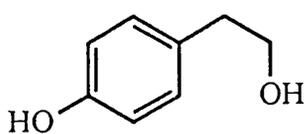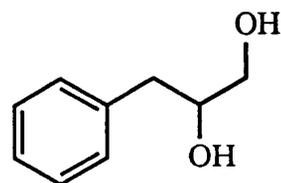
[10]   [11]   [12]
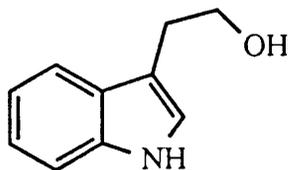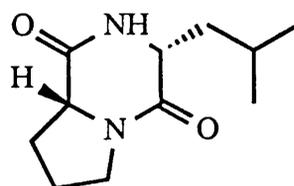
[13]   [14]
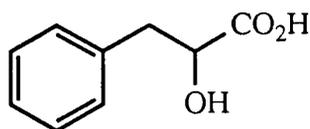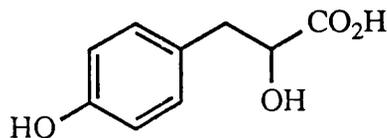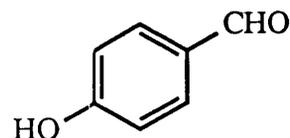
[15]   [16]   [17]
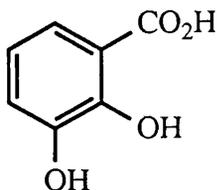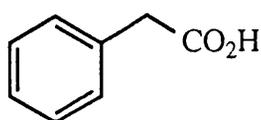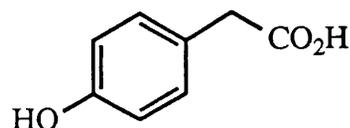
[18]   [19]   [20]

To examine the possible role of isocoumarins in the blue stain disease, solutions of isocoumarins (4) and (5) were fed to healthy trees.[21] It was found that these compounds doubled the rate of water

loss from the trees within one day of inoculation. The suggestion

was made that the isocoumarins are transported to the needles in the

transpiration stream. There they cause ion leakage in the epidermal

cells, opening the pores and allowing water to escape.

Oak wilt is another wilting disease which has much in common with

Dutch elm disease and blue stain.[22] Again the symptoms include leaf

discolouration and loss, and again it is caused by fungal infection.

In this case the fungus involved is C. fagacearum, spread by beetles of

the Nitidulidae family. The mechanism of the disease is believed to

be similar to those discussed above.

Ceratocystis fimbriata is a pathogen of a range of fruit trees

including peach, apricot and almond.[23] The fungus colonizes injured

bark and spreads inwards throughout the tree, causing a disease known as

Ceratocystis canker. This is spread from tree to tree by nitidulid

beetles.

Chestnut blight is a similar canker disease which is caused by

the fungus Endothia parasitica. This micro-organism was investigated

in 1966 and found to produce the isocoumarin, diaporthin (21).[24]
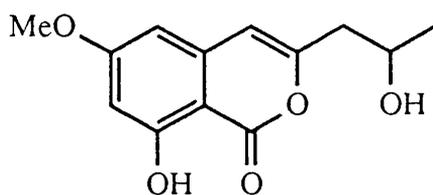
[21]

The fungal pathogen Botryosphearia obtusa is responsible for spots

on apple leaves and black rot of the fruit. Three of its metabolites

were identified as mellein (22), 4-hydroxymellein (23) and

5-hydroxymellein (24).[25] Subsequent testing of the pure substances

revealed that all three were phytotoxic, causing necrosis of the

leaves.[26]

The same workers have also obtained 6-hydroxymellein (8) from

culture filtrates of Discula sp., a pathogen of dogwood in North

America.[27]   The symptoms of the disease (known as anthracnose) are

canker of the stem and the progressive death of leaves and branches.

Pure 6-hydroxymellein (8) was able to induce necrosis on dogwood

leaves.   It is interesting to note that this compound is also a

metabolite of C. minor (see above).
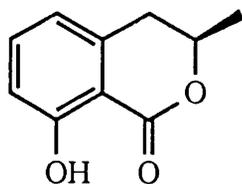
[22]
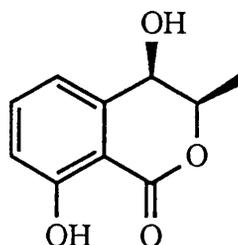
[23]
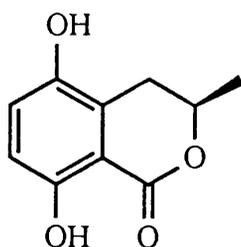
[24]
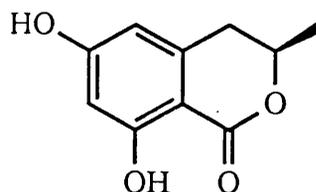
[8]

Obviously isocoumarins are widespread metabolites of fungi which

cause wilt disease in trees.   Though some have been shown to produce

leaf necrosis, their precise role is not yet clear.   The aim of this

project was to synthesise principally those isocoumarins isolated from

Ceratocystis fungi to test their phytotoxicity and perhaps determine

what part they play in the death of host trees.

1.3 Phytoalexins

Since plants have no immune system, the question is raised as to

how they protect themselves against disease.   Several mechanisms of

resistance have been identified, including the use of physical

barriers and detoxification of enzymes and toxins.

One particularly interesting mode of defence is the use of

phytoalexins.[28]   These are antimicrobial compounds of low molecular

weight that are synthesised by, and accumulated in, plants after

exposure to micro-organisms.

Features of the so-called "phytoalexin theory" are as
follows:

1. The phytoalexin is formed only when the host and parasite
    come into contact.

2. The reaction only occurs in living cells.

3. The reaction is confined to the infected area.

4. The phytoalexin is the product of abnormal metabolism in the
    plant.

5. The phytoalexin is non-specific, though different fungi may
    show different sensitivity to it.

6. Whether the plant survives or not depends on how quickly the
    phytoalexin is formed.

7. The defence reaction depends on the genetic constitution of
    the host and the parasite.

Phytoalexins encompass a wide variety of natural products,
including sesquiterpenes, isoflavonoids and polyacetylenes. Some well
known examples include pisatin (25) (peas), ipomeamarone (26) (sweet
potatoes) and orchinol (27) (orchids).
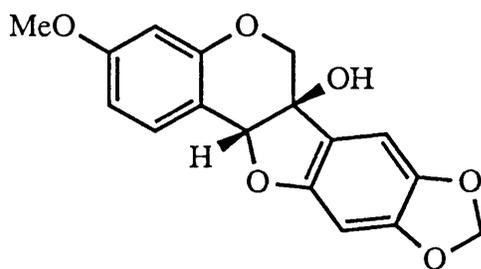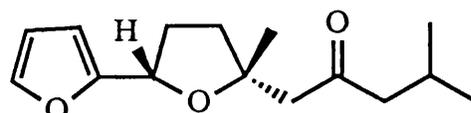
[25]   [26]
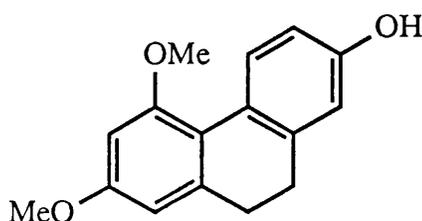
[27]

Induction of phytoalexin formation is normally brought about by

micro-organisms, but can also be caused by microbial metabolites (elicitors), cold, U.V. light or heavy metal salts.

In 1957, Sondheimer isolated the isocoumarin, 6-methoxymellein (28) from carrots which had been held in cold storage.[29]   The carrots had developed a bitter taste during storage, believed to be caused by a build-up of 6-methoxymellein (28).

MeO—⟨structure⟩

OH   O

[28]
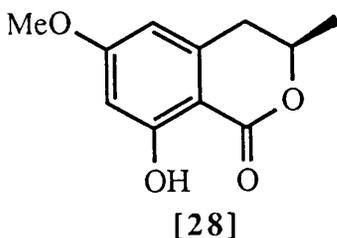
In 1960, Condon and Kuc discovered a fungitoxic compound in carrot roots which had become infected by Ceratocystis fimbriata.[30] It was later identified as 6-methoxymellein (28).[31]   Formation of this substance appeared to be dependent on the interaction between the fungus and the carrot, as it was not detected in fresh, uninfected carrots. This led to the suggestion that this compound is a phytoalexin.

Growth of C. fimbriata was completely inhibited at a methoxymellein concentration of 5×10^{-4}M.[32]   This level was reached in the carrot within one day of inoculation, and coincided with the cessation of fungal growth.

However, doubt was shed on whether 6-methoxymellein is a phytoalexin by Curtis.[33]   He investigated cultures of C. fimbriata grown on a synthetic medium and isolated the related compounds (5) and (7).

HO—⟨structure⟩

OH   O

[5]

MeO—⟨structure⟩

OH   O

[7]
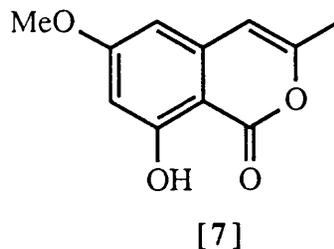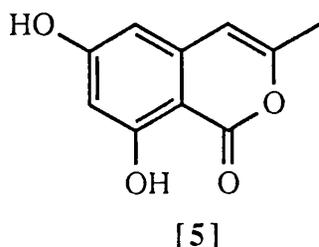
Curtis implied that the ability of the fungus alone to produce such similar compounds made it unlikely that 6-methoxymellein (28) was

being biosynthesised by the carrot in response to fungal infection. This idea was expanded by Stoessl, who suggested that the low levels of the compound discovered in healthy carrots were due to undetected fungal infection.[34]

This notion was refuted by Kuc.[35] He pointed out that the amount of isocoumarin produced by the fungus alone (18 mg from 7 l of culture after 11 days) was far less than that isolated from infected carrots (2.3 g from 1 kg of carrots after 3 days). That observation made it highly unlikely that the fungus was supplying the biosynthetic precursor of 6-methoxymellein.

It was discovered that the compound was accumulated in carrots not only after infection with C. fimbriata, but after treatment with other fungi[36], cold[29], or chemicals such as ethylene.[37] These observations suggest that, as with most phytoalexins, accumulation of 6-methoxymellein is not a specific response to infection, but rather a response to stress.

In a later study, 6-hydroxymellein (8), the actual biosynthetic precursor, was isolated from carrots treated with ethylene.[38] That lent further support to the idea of 6-methoxymellein (28) as a stress metabolite of the carrot.

$$\text{[8]}$$
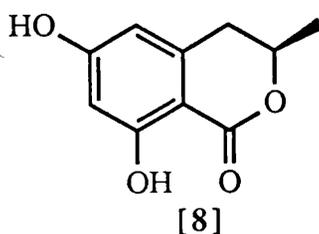
As to the toxicity of these compounds, 6-methoxymellein (28) has been shown to have a broad antimicrobial spectrum.[39] It inhibited the growth of a variety of fungi, yeasts and bacteria at a concentration of 5×10^{-4}M. With many of the fungi this inhibition approached 100%. The toxicity towards animals is very low.[38]

Little is known about the mode of action of 6-methoxymellein (28). It has been shown that the compound inhibits the cyclic nucleotide phosphodiesterase enzyme (the enzyme responsible for

hydrolysis of cyclic AMP).[40]   Since it bears no resemblance to

c AMP it is unlikely to be mimicking that compound.   It was found

that inhibition was inversely proportional to the concentration of

magnesium ions present.   Magnesium is known to be essential for the

activity of the enzyme.

The suggestion made is that the isocoumarin uses its _peri_

hydroxyl and carbonyl groups to bind metal cations such as Mg^{2+}

and Ca^{2+} in a 6-membered chelate (figure 1).   Hence the compound will

inhibit any enzymes which are activated by these metals.
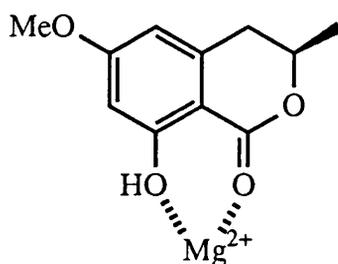
Figure 1

Our intention was to prepare 6-methoxymellein (28) and those

isocoumarins extracted from _C. fimbriata_ cultures to study their

antifungal properties and their role in the phytoalexin mechanism.

The synthetic route used (see later) also yields compounds which are

methylated at the 8-hydroxyl.   These compounds would allow us to

test the proposed mode of action involving the _peri_ hydroxyl group.

1.4   General Activity of Isocoumarins

As mentioned briefly at the start of this chapter, isocoumarins

exhibit a great variety of biological properties.   Mellein (22), one of

the simplest naturally occurring isocoumarins, is a constituent of the

defensive secretion of many types of ant.
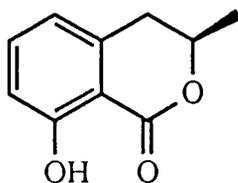
[22]

A number of isocoumarins have been implicated as plant growth regulators. For example, sclerotinins A and B, (29) and (30), from the fungus Sclerotinia sclerotiorum both promoted the growth of rice seedlings at a concentration of 5 ppm.[41] The effect of sclerotinin A (29) was later shown to be synergistic with gibberellic acid.[42]
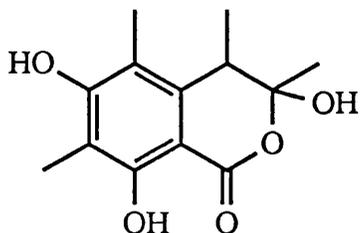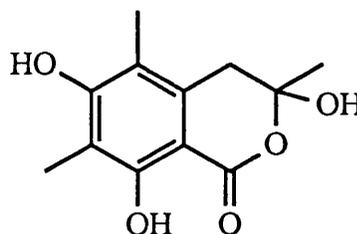
[29]   [30]

A systematic study of several analogues revealed that the essential features were the 8-hydroxyl group and the lactol. In fact compound (31), which contains the minimum structural requirements, showed activity at a level of 10 ppm.
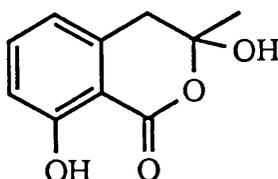
[31]

The 6-hydroxy derivative (2) has been isolated from Alternaria kikuchiana, the pathogen of Japanese pears.[11] It was found to stimulate root elongation of rice seedlings and radishes at a 5 ppm concentration. 6-Hydroxymellein (8), which lacks the 3-hydroxyl group, stimulates root elongation at the higher level of 60 ppm.[43]
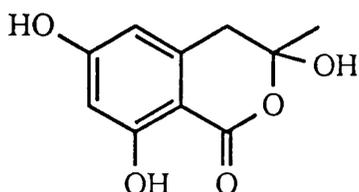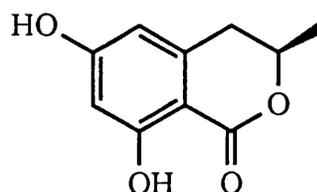
[2]   [8]

There are numerous examples of isocoumarins that display antibiotic or antifungal activity. Nakajima et al. tested the

antifungal properties of the natural products, oosponal (32), oospolactone (33), phyllodulcin (34) and hydrangenol (35).[44]   All four exhibited antifungal activity, inhibiting the growth of various moulds at concentrations of 5-200 ppm.   Methylation or acetylation of the hydroxyl groups decreased activity and optically active isomers were more potent than racemates.
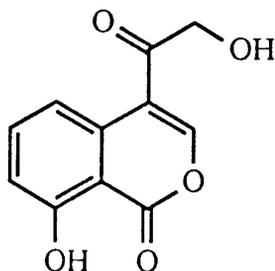
[32]
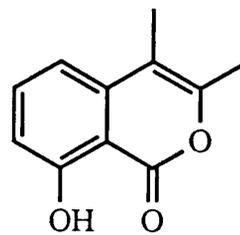
[33]
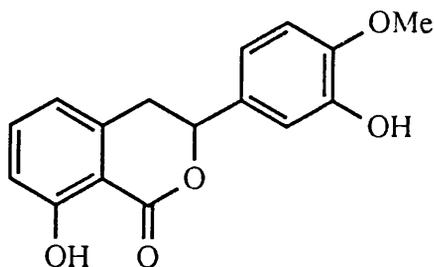
[34]
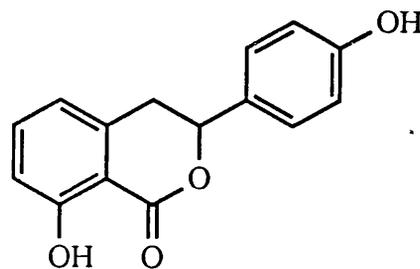
[35]

In a study of synthetic analogues, the same workers found that most 3-aryl-8-hydroxyisocoumarins were antifungal agents.[45]   The most active compound (36) completely inhibited all fungi tested at a level of 12.5 ppm.   Introduction of substituents at the 4-position resulted in complete loss of activity.[46]
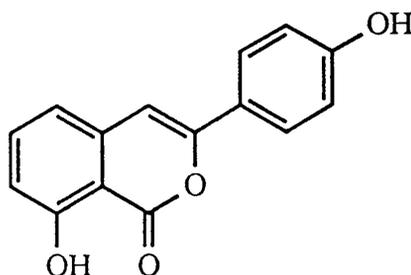
[36]

Phyllodulcin (34) is responsible for the sweet taste of hydrangea leaves, which are used as a type of tea in Japan, or as a sugar substitute for diabetics.

AI-77-B (37) is an extremely potent antiulcer agent isolated

from the soil bacteria, Bacillus pumilus.[47]   Unlike other antiulcer

agents, it has no central nervous system, antihistaminergic or

anticholinergic effects.
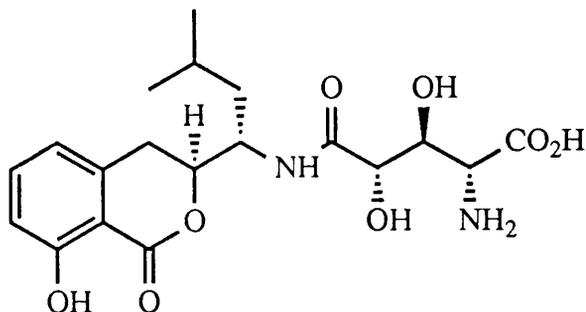
[37]

Synthetic N-alkylspiroisocoumarin-piperidines are known to inhibit

the release of histamine in rats.[48]   They have been investigated with a

view to developing new antiallergic agents.   The benzene ring of the

isocoumarin was essential for activity and is thought to interact with a

receptor.   The most active compound (38) caused nearly complete

inhibition of histamine release at 10^{-4} M concentration.
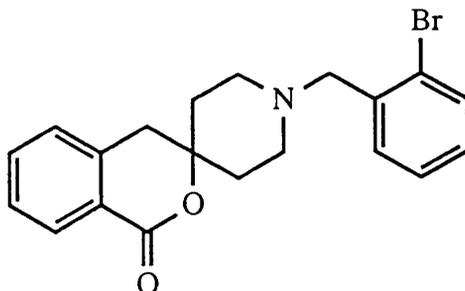
[38]

Serine proteases are enzymes involved in many important

physiological processes and they are believed to be involved in

diseases such as emphysema, arthritis and tumour formation.   In

view of this, efforts have been made to discover irreversible

inhibitors of serine proteases as potential therapeutic agents.   It

has been found that 3,4-dichloroisocoumarin (39) is a potent and

specific inhibitor of various serine proteases.[49]   It is believed

to act as a masked acid chloride, capable of acylating an active

nucleophilic site on the enzyme.   3-Alkoxy-4-chloroisocoumarins were

also effective, and a 7-amino group makes the isocoumarin stable to

spontaneous hydrolysis in aqueous solution.[50]   Hence the best serine

protease inhibitor to date is the isocoumarin (40).

[39]   [40]

2. SYNTHESIS OF ISOCOUMARINS

2.1 Early Forays

The isocoumarin ring system has revealed a resistance to synthesis that belies its apparent simplicity to the extent that, more than a century since the first synthesis, there is still no generally applicable route to these compounds.   The difficulty appears to lie in the fact that the oxygen atom is not directly bonded to the aromatic ring, thus requiring that two separate carbon chains are added to the aromatic nucleus.   In contrast, the coumarin skeleton, which does have the oxygen atom bonded to the aromatic ring, is relatively easy to construct.

The first recorded synthesis of an isocoumarin was reported by Gabriel in 1885.[51]   He condensed phenylnitromethane with phthalic anhydride (41) to give nitrobenzylidene phthalide (42), which upon reduction with phosphorus and hydrogen iodide, yielded 3-phenylisocoumarin (43) (Scheme 2.1).

Scheme 2.1

He later[52] extended this method to prepare isocoumarin itself, but the yields in these syntheses were very poor (2% overall in the case of isocoumarin).

Another early synthesis of interest is that of Bamberger in 1892 (Scheme 2.2).[53]   He prepared o-carboxyphenylglyceric acid (45) by oxidative cleavage of o-naphthoquinone (44) with sodium hypochlorite. The corresponding lactone was dehydrated by heating with concentrated hydrochloric acid at 160°C, giving 3-carboxyisocoumarin (46).

Scheme 2.2

Bamberger subsequently found that isocoumarin could be obtained by heating the silver salt of the carboxylic acid with powdered clay.[54]

Clearly these pioneering syntheses were inadequate for the preparation of naturally occurring isocoumarins. The yields were very low and the harsh conditions and reagents would not tolerate sensitive functional groups.

2.2 The Homophthalate Approach

The problems mentioned above led to many research groups investigating the use of classical condensation reactions, which were emerging at the time. Dieckmann obtained isocoumarin by Claisen condensation of ethyl formate with diethyl homophthalate (47), followed by hydrolysis, cyclisation and decarboxylation of the intermediate diester (48) by heating with sulphuric acid (Scheme 2.3).[55]

Scheme 2.3

The Claisen condensation route proved to be a useful method for preparing isocoumarins and was probably the most popular early procedure.

It was improved in 1948, with the condensation, hydrolysis and

cyclisation being carried out in one pot.[56]   The decarboxylation was

brought about by heating to 300°C with metallic copper, giving an overall

yield for the synthesis of 33%.

Oxalates can also be used in the Claisen condensation, affording

3,4-disubstituted isocoumarins (Scheme 2.4).[57]

Scheme 2.4

As early as 1950 the Claisen method was used to prepare several

substituted isocoumarins.[58]   It was found that the ester hydrolysis,

which gave low yields due to polymerisation, was better effected with

boron trifluoride in acetic acid.

The Stobbe condensation of homophthalates with aldehydes or

ketones has also proved useful.[59]   These condense together in the

presence of sodium hydride to form a half ester (53) which cyclises on

treatment with acetic anhydride and sodium acetate (Scheme 2.5).

Scheme 2.5

However the Stobbe condensation method gives low yields for

aliphatic aldehydes, required to make unsaturated isocoumarins containing

a 3-alkyl group.

Probably the most useful general synthesis of isocoumarins

nowadays involves the reaction of a homophthalic acid with an anhydride

in the presence of an amine catalyst.

Tirodkar and Usgaonkar found that heating 4-methoxyhomophthalic acid (55) with acetic anhydride and pyridine gave the 4-carboxyisocoumarin (56).[60]

[55]   [56]

At room temperature the product obtained was the isochroman-1,3-dione (57).

[57]

This was converted to the isocoumarin (56) on heating with acetic anhydride and pyridine, indicating that it was an intermediate in the reaction.   The mechanism probably involves, therefore, anhydride formation, acylation and rearrangement (Scheme 2.6).
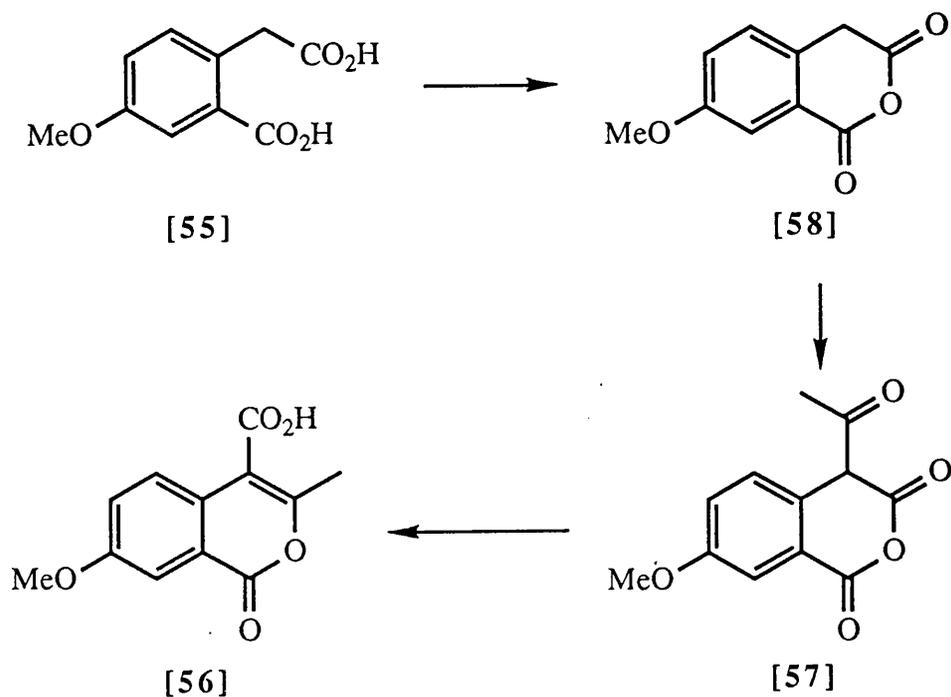
Scheme 2.6

Since homophthalate esters do not undergo such reactions, the electron withdrawing effect of the two carbonyl groups in the anhydride must be necessary to make the hydrogens sufficiently acidic to be removed by pyridine.

Tirodkar and Usgaonkar also found that the product could be decarboxylated either by heating with aqueous sulphuric acid, or by hydrolysis followed by decarboxylation of the intermediate β -ketoacid using aqueous sodium hydroxide (Scheme 2.7).
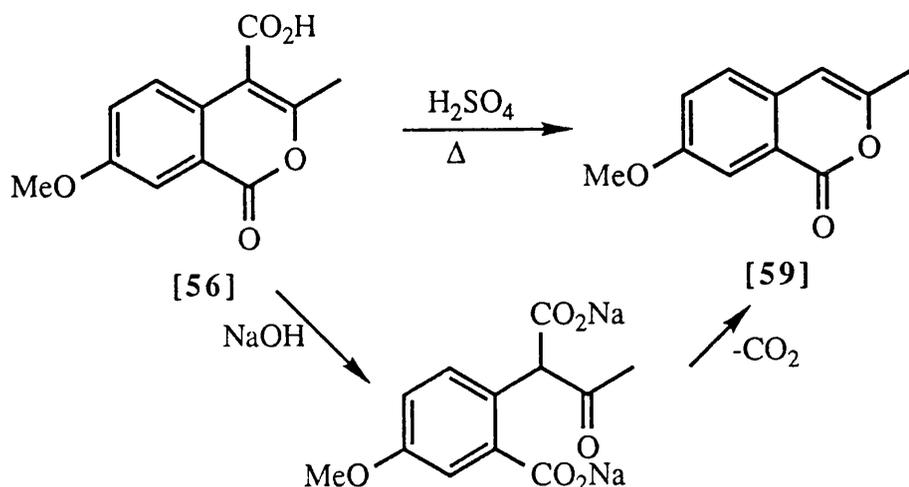
Scheme 2.7

This route was soon found to be extremely general, being applied to different aliphatic[61] and aromatic[62] anhydrides and different homophthalic acids.[63] The conditions were also mild enough to permit the synthesis of several naturally occurring isocoumarins.[64, 65]

In a modification of the above procedure, phosphorus oxychloride and dimethylformamide were used in place of acetic anhydride/pyridine, and the intermediate (60) rearranged and decarboxylated (Scheme 2.8).[66]
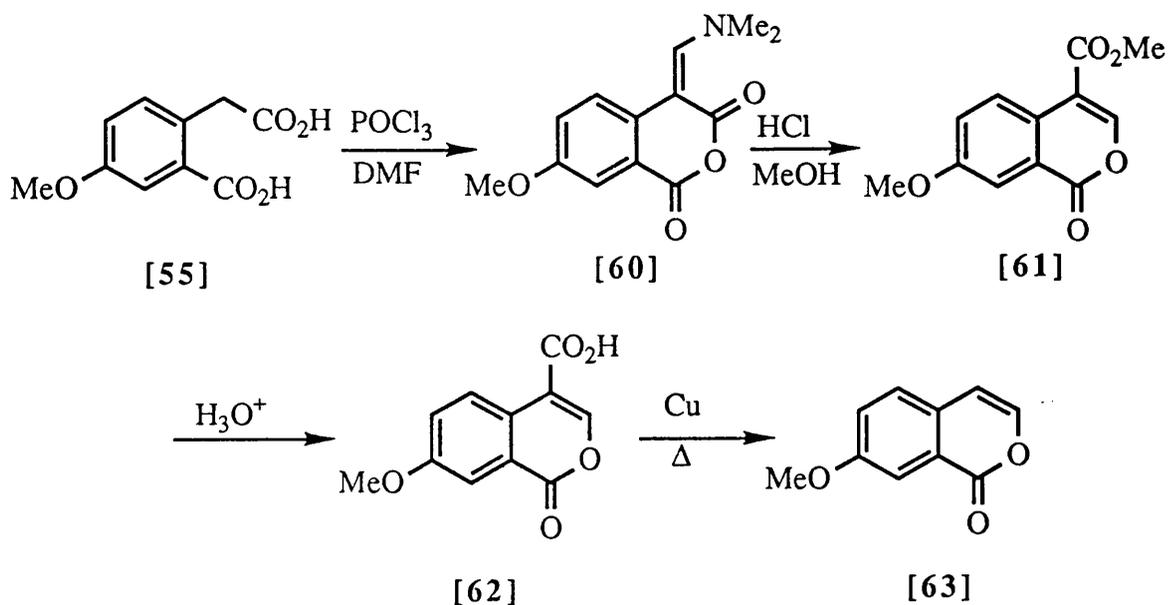
Scheme 2.8

3-Aryl substituted isocoumarins are simply prepared by heating the corresponding homophthalic acid with an aromatic acid

chloride.[45]

[64]   [65]
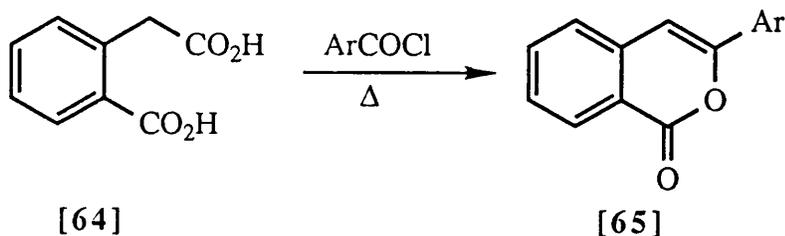
Alternatively they may be obtained by shaking the homophthalic anhydride with an aromatic aldehyde in the presence of sodium carbonate.[46]

[66]   [67]
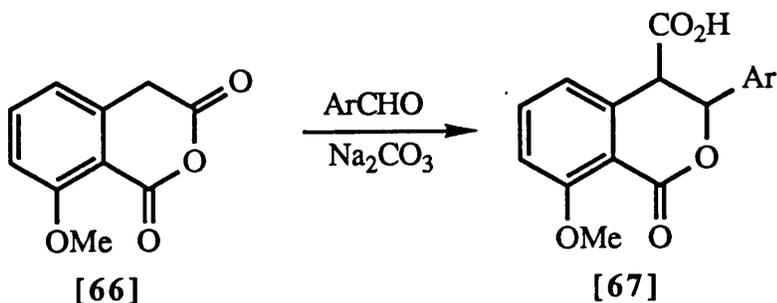
One final synthesis of note utilising a homophthalic acid derivative involved reaction of the half acid chloride with diazomethane.[67] On treatment of the diazoketone (69) with hydriodic acid the isocoumarin (70) was produced (Scheme 2.9).

[68]   [69]   [70]
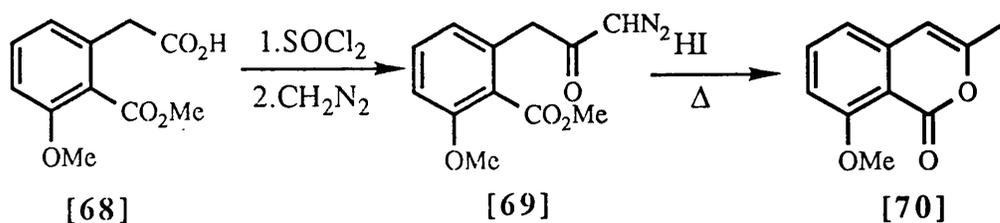
Scheme 2.9

Ethylisocoumarins were obtained using diazoethane instead.[68]

All of the above routes to isocoumarins are useful provided the homophthalic acids are available. In Dieckmann's original Claisen synthesis homophthalic acid (64) was obtained by oxidation of naphthalene (71), followed by reduction of the phthalonic acid (72) (Scheme 2.10).

[71] [72] [73]
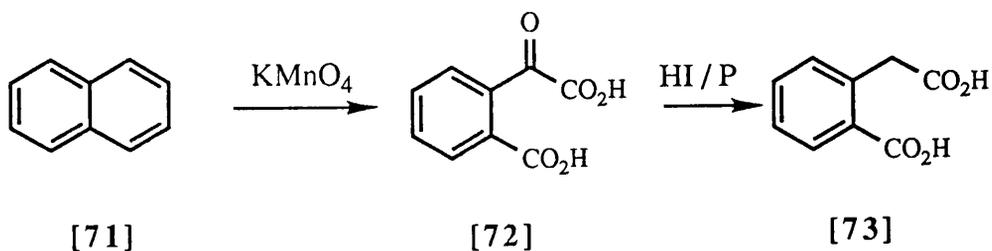
Scheme 2.10

It can also be prepared by vigorous oxidation of indanone

(73)[69] or indene (74)[70].

[73]

[74]

[64]
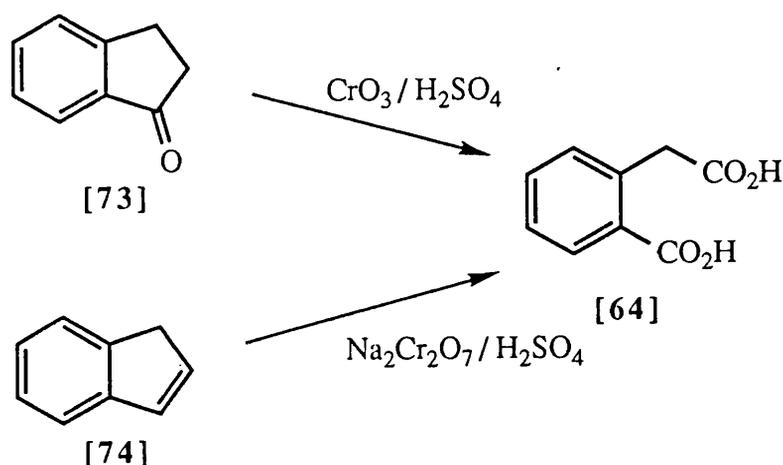
In a novel approach, the Hurtley reaction of o-bromobenzoic

acid (75) with ethyl acetoacetate gives ketoester (76).[71] This

undergoes hydrolysis and a retro-Claisen reaction with sodium

hydroxide (Scheme 2.11).

[75] [76] [64]
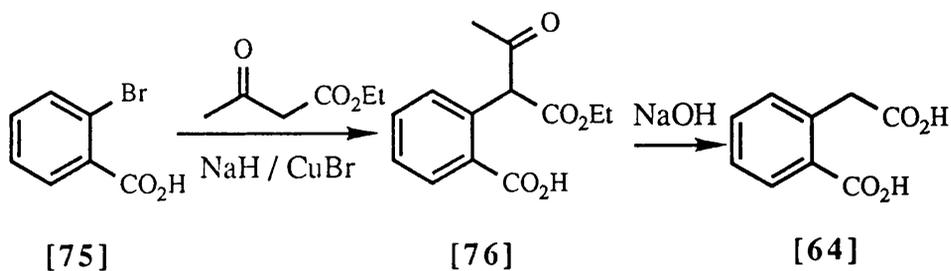
Scheme 2.11

From bromobenzene (77) itself, the anion of diethyl malonate

adds to the benzyne generated by sodium amide.[72] Transfer of a

carboxyl group and hydrolysis of the imide (78), which is the

initial product, yields homophthalic acid (64) (Scheme 2.12).
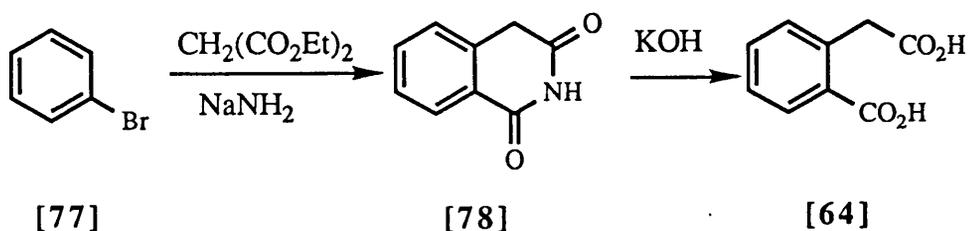
Scheme 2.12

Currently the most widely used route to homophthalic acids involves carboxylation of the dianion of a toluic acid (79).[73]   The dianion is generated by treatment with two equivalents of lithium diisopropylamide and it reacts _in situ_ with dimethyl carbonate.   This method has been used to prepare a variety of substituted homophthalic acids.
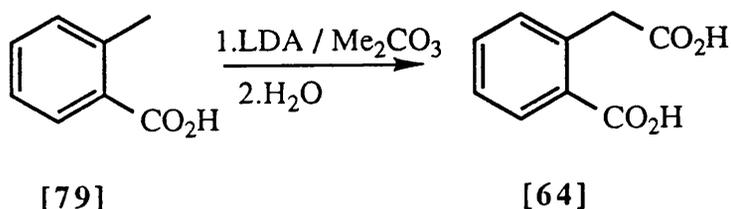
2.3 The Indanone Approach

Aside from condensation reactions involving homophthalates, the most commonly used route to isocoumarins is via the oxidative cleavage of a 5-membered ring.   Shriner reported that ozonolysis of indene (74), followed by reduction and acid catalysed cyclisation yielded dihydroisocoumarin (81) (Scheme 2.13).[74]
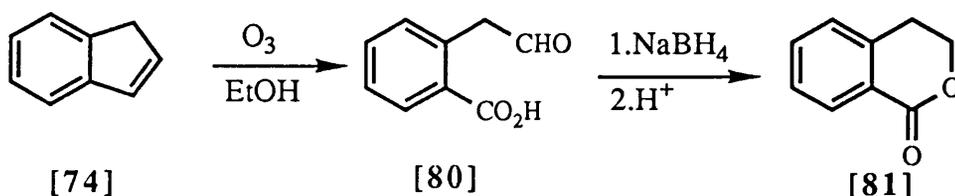
Scheme 2.13

Allen and Gates prepared isocoumarins from indenones by epoxidation and acid catalysed rearrangement (Scheme 2.14).[75]

[82]   [83]   [84]
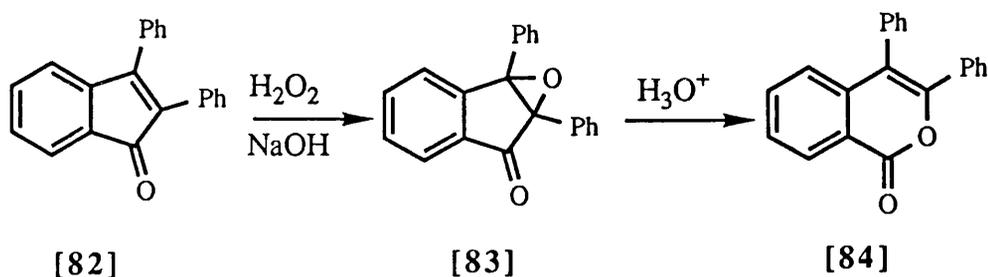
<u>Scheme 2.14</u>

The mechanism of the rearrangement is believed to be as shown below (Scheme 2.15).
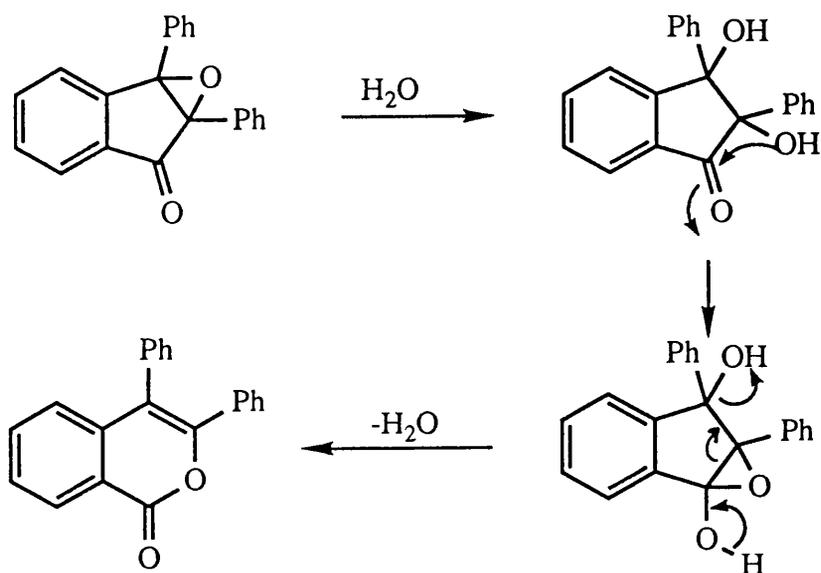
<u>Scheme 2.15</u>

The preferred precursors, however, are indanones since these are the most readily available. Schopf and Kuhne prepared isocoumarin (49) in high yield by cyclisation of o-carboxyphenylacetaldehyde, obtained by periodate oxidation of 2-hydroxyindan-1-one (86).[76] This was synthesised from 2-bromoindan-1-one (85) by acetolysis, followed by hydrolysis of the resulting 2-acetoxyindan-1-one (Scheme 2.16).
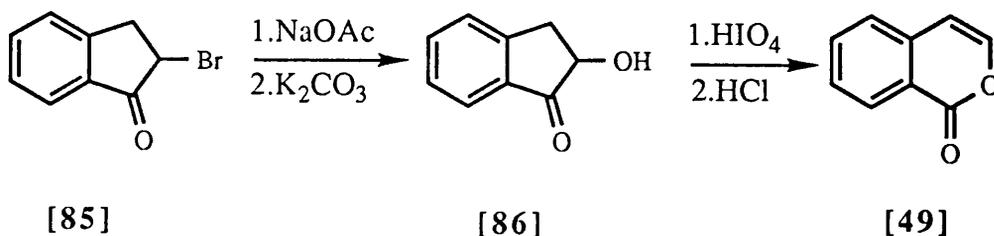
Scheme 2.16

Under the original conditions the acetolysis failed when methoxyl groups were present on the aromatic ring, limiting the usefulness of this pathway for the synthesis of naturally occurring compounds. However, Chaudhury found that the reaction could be carried out in dimethylsulphoxide.[77] He also found that the bromoindanones were readily prepared from indanones using a mixture of cupric bromide and potassium bromide (Scheme 2.17).
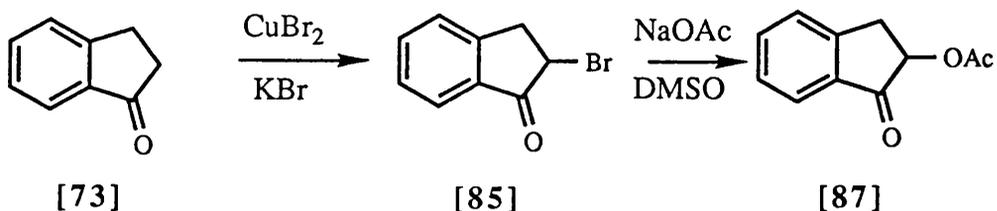
Scheme 2.17

Chaudhury also developed a completely different synthesis of acetoxyindanones, outlined below (Scheme 2.18).[78]

Nitrosation of the indanone (88) and treatment of the sodium salt with hypochlorite and ammonia produced the corresponding diazoketone (90). Addition of the diazoketone (90) to acetic acid furnished the acetate (91), with loss of nitrogen. The excellent yields obtained allowed this method to be adapted for the synthesis of several substituted isocoumarins.[79]
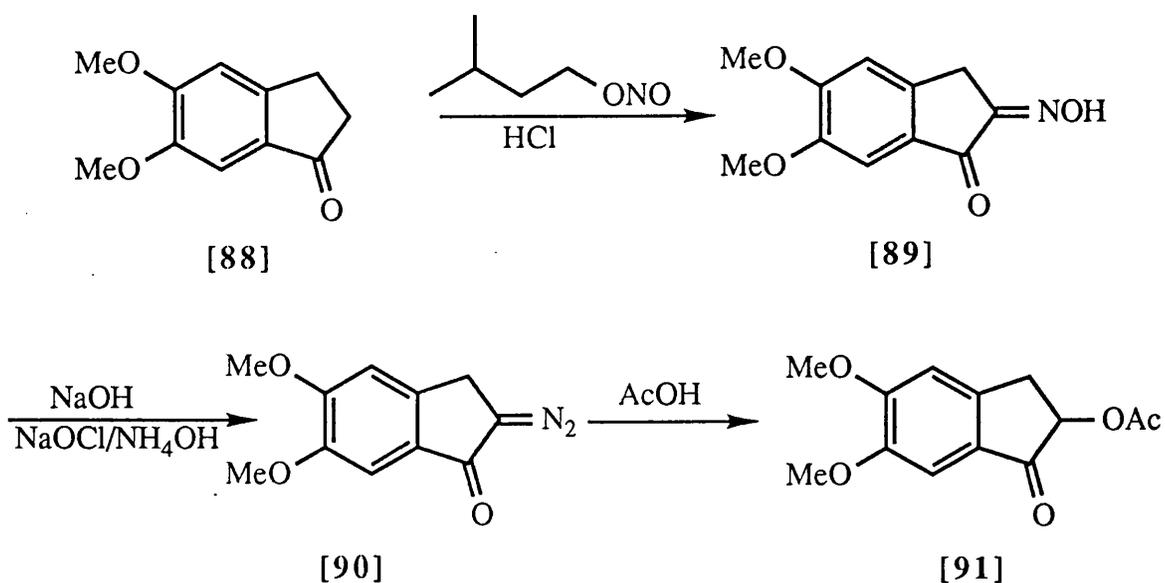
Scheme 2.18

The most convenient indanone to isocoumarin conversion to date was discovered by Staunton and coworkers.[80] They found that indanones are cleanly converted to their enol trifluoroacetates simply by stirring in trifluoroacetic anhydride. These are then cleaved by ozonolysis without purification (Scheme 2.19).
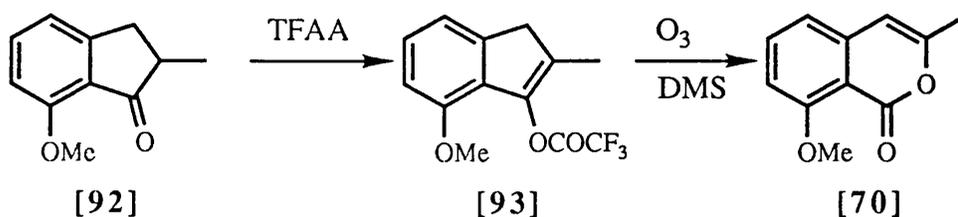
Scheme 2.19

As with the homophthalates, pathways involving indanones as intermediates are useful provided that they themselves are available. Two methods have been used for their preparation - the Friedel-Crafts cyclisation of arylpropanoic acids (94) and the Nazarov reaction of acrylophenones (95) (Scheme 2.20).[81]

[94]   [73]   [95]
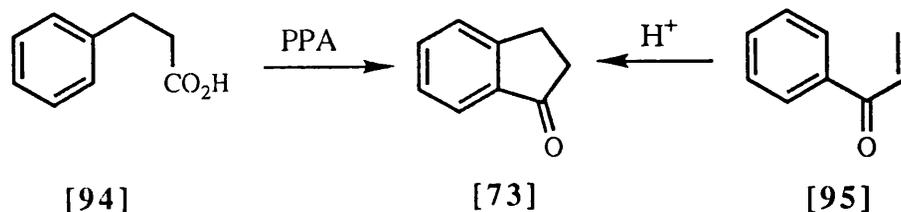
Scheme 2.20

2.4   The Organometallic Approach

In recent years research into isocoumarin synthesis has concentrated on adding the "top" carbons of the skeleton in a single unit.   This would dramatically cut the number of steps required and allow the use of simple aromatic compounds as starting materials. These approaches have invariably involved organometallic chemistry.

The first contribution in this field came from Narasimhan and Bhide.[82]   They made use of the ability of an amide group to direct the ortho lithiation of an aromatic ring.   Thus o-methoxy N-methyl benzamide (96) was lithiated with n-butyllithium, and the lithiated species trapped with propylene oxide.   Hydrolysis and cyclisation of the amide (97) gave mellein methyl ether (98) (Scheme 2.21).

[96]   [97]   [98]
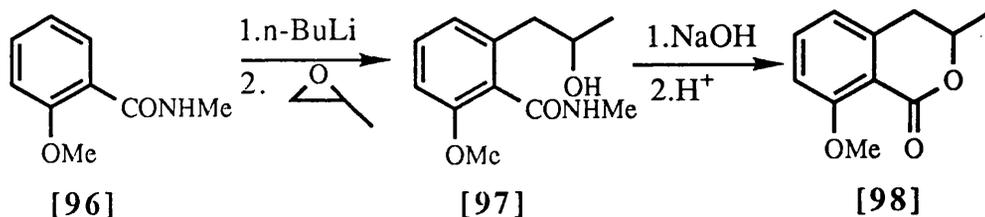
Scheme 2.21

The methoxyl group is also able to control the position of lithiation in such reactions.   The success of the above synthesis suggests that the amide group is more effective at complexing the butyllithium.

The simplicity of this approach can be appreciated in noting that previous syntheses of mellein by conventional methods required eleven and fourteen steps.

Benzamides were also used as starting materials by Snieckus.[83] He showed that lithiation with s-butyllithium followed by transmetallation using magnesium bromide gave Grignard reagents which reacted successfully with a range of electrophiles. In particular, allyl bromide gives 2-allylbenzamides which are converted to isocoumarins by acid treatment (Scheme 2.22).

[99]   [100]   [101]
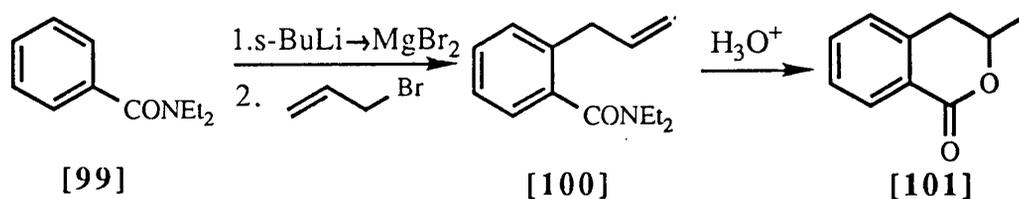
Scheme 2.22

Making use of organocopper chemistry, Stevenson prepared 3-alkylisocoumarins (103) by coupling copper acetylides to iodobenzoic acid (102).[84]

[102]   [103]
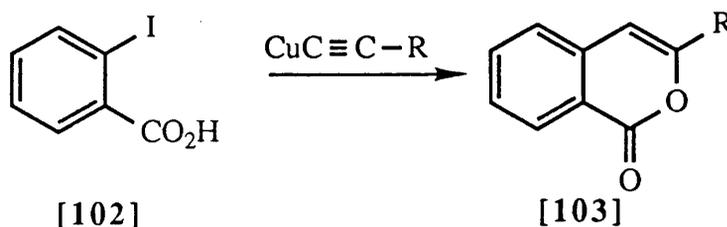
The reaction fails with aromatic R groups; phthalides being formed instead. Although this is a potentially useful procedure, no examples with functionalised benzoic acids have been reported.

When methyl 2-bromobenzoates are treated with the complex π-(2-methoxyallyl)nickel bromide, acetonylbenzoic esters (105) are formed.[85]

[104]   [105]
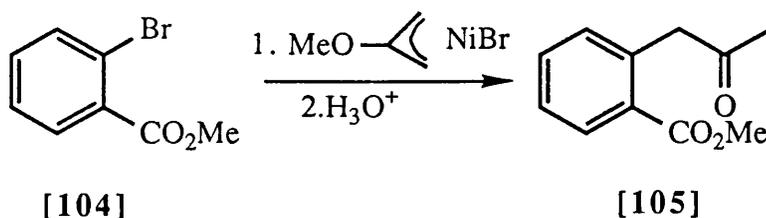
These can be converted to isocoumarins with sodium hydride and a little t-butanol, or to dihydroisocoumarins with sodium borohydride (Scheme 2.23).

[106]   [105]   [101]
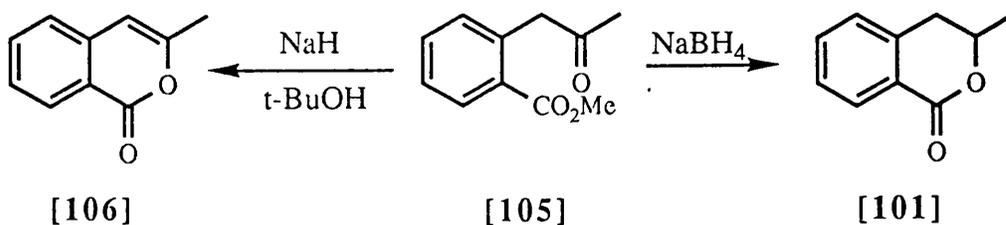
Scheme 2.23

The sodium salts of 2-bromobenzoic acids (107) also react with π-allylnickel halides to produce 2-allylbenzoic acids (108). These cyclise to isocoumarins with palladium chloride (Scheme 2.24).

[107]   [108]   [106]
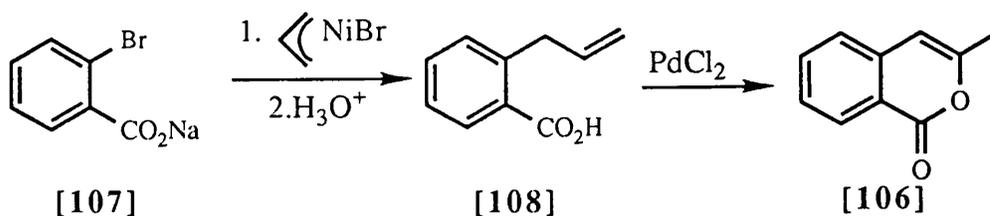
Scheme 2.24

Conditions for these reactions are outstandingly mild. Methoxyl, chloro and pyridyl groups are all tolerated by the reaction, and there is great potential for the synthesis of natural products.

Finally, Larock has used ortho thallated benzoic acids (109) to couple with terminal alkenes in the presence of palladium chloride.[86]

[109]   [103]
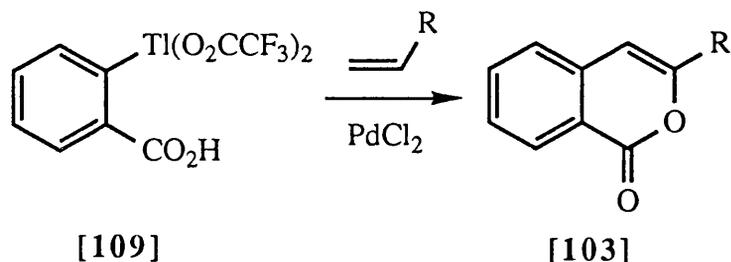
However, the reaction only gave good yields when the olefin

contained no allylic hydrogens (e.g. styrene, dimethylbutene). With

vinyl halides the yields are much better and the reaction is catalytic

in palladium.

[109]   [110]
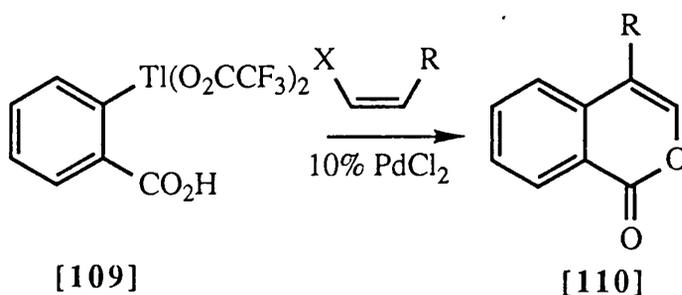
The arylthallium reagents are easily made from benzoic acids by

reaction with thallium trifluoroacetate and trifluoroacetic acid and

are stable for several months. In addition the reaction is highly

regioselective, giving 90-95% of the ortho product.

Clearly the simplicity of the organometallic approaches to

isocoumarins is appealing but they are not as yet completely reliable.

2.5 Miscellaneous Methods

There are many other strategies for synthesising isocoumarins which

are useful in particular cases. One obvious possibility for forming

a δ-lactone would be the halolactonisation of an unsaturated

carboxylic acid. However, such reactions are usually unsuccessful

since the carboxyl group tends to attack the more reactive benzylic

carbon, producing a phthalide. Only when the 3-substituent is an

aromatic ring is the isocoumarin formed.[87]

[111]   [112]
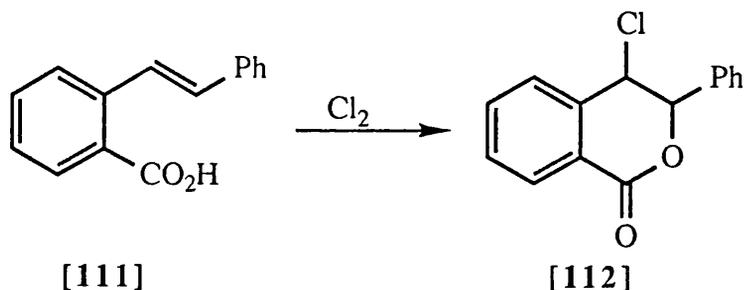
Indeed many intended isocoumarin syntheses yield phthalides instead and the likelihood of their formation must always be considered. A recent publication has stated that phthalides can be converted into isocoumarins using aluminium chloride.[88]

[113]   [22]
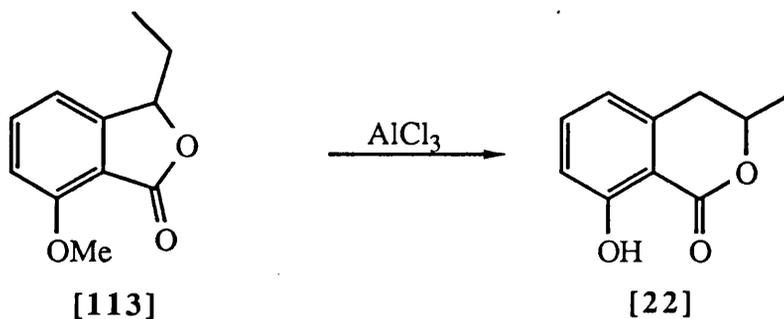
These workers also reported that propenylbenzoic acids (114), which are readily available by Wittig reaction, can be converted directly to isocoumarins (22) by treatment with aluminium chloride.

[114]   [22]
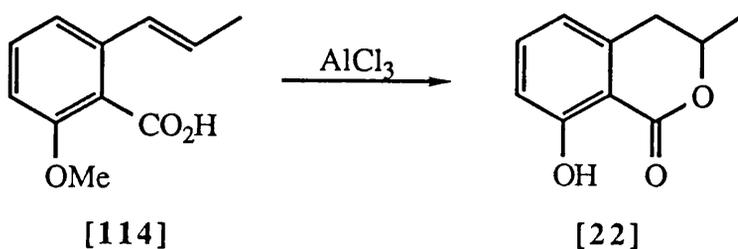
Isochromans (115) can be converted to isocoumarins by oxidation with selenium dioxide.[89] They are themselves available by chloromethylation of phenethyl alcohols (10) (Scheme 2.25).

[10] → [115] → [81]
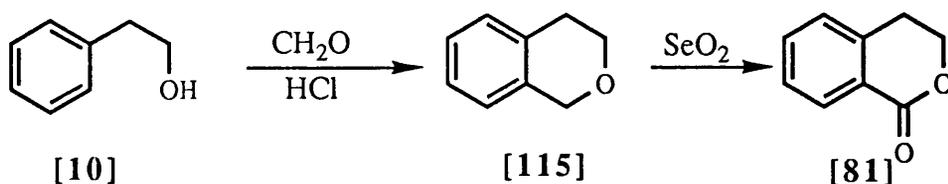
Scheme 2.25

Chromium trioxide/acetic acid is reported to give better results in the oxidation step.[90] A drawback of the isochroman route is that the chloromethylation step does not generally give exclusively the product of *ortho* substitution.

Staunton has shown that the anions of *o*-toluate esters (116) (formed by deprotonation with LDA) condense with N-methoxy-N-methyl amides to form ketoesters (117).[91] These can be converted directly to isocoumarins on treatment with sodium *t*-butoxide (Scheme 2.26). A methoxyl group *ortho* to the ester is essential to prevent self-condensation.

[116] → [117] → [118]
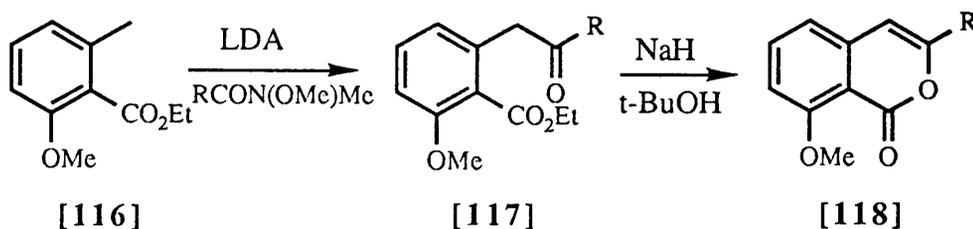
Scheme 2.26

3-Aryldihydroisocoumarins (121) can be prepared using tertiary benzamides (119) and aromatic aldehydes.[92] Hydrolysis of the amide (120) gave the isocoumarin (121) an acidification (Scheme 2.27). In this case a methoxyl group *ortho* to the amide is unnecessary.

[119] → [120] → [121]
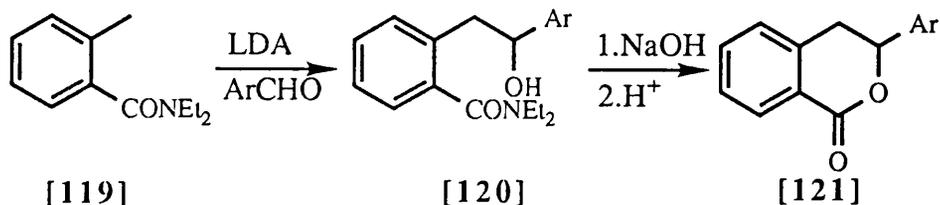
Scheme 2.27

In a further refinement of this process, Staunton used a chiral lithium amide base to prepare mellein methyl ether in 53% enantiomeric excess.[93]

Hauser developed a new general route to isocoumarins based on the Henry condensation of nitroalkanes and phthalaldehydic acids (122).[94] The products (123) of such reactions can be converted to isocoumarins by reductive cleavage of the benzylic oxygen, unmasking of the nitro group by a Nef reaction and cyclisation of the ketoacid (Scheme 2.28).
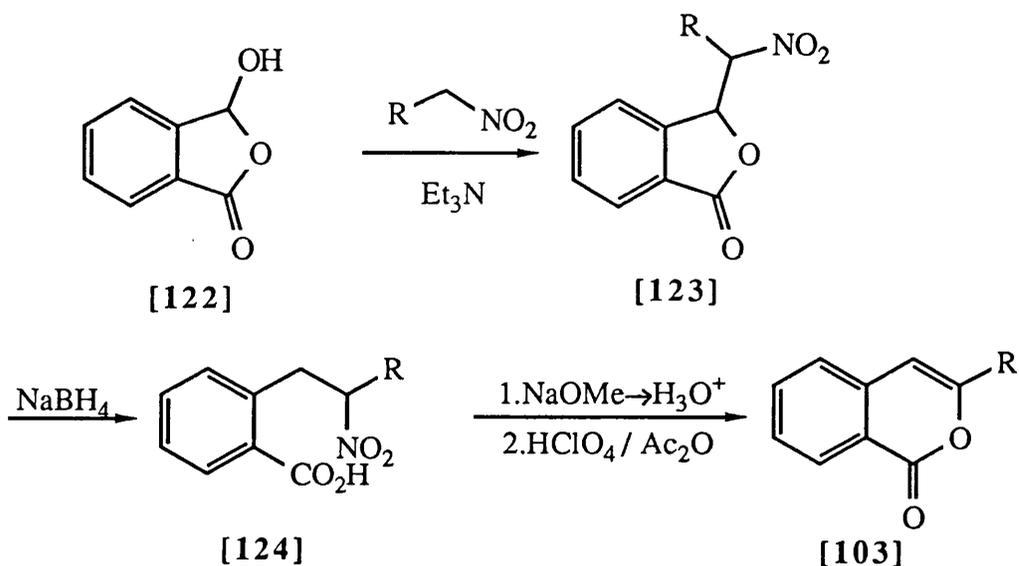
Scheme 2.28

Since the nitroalkane can be varied at will, this provides a good route to 3-substituted isocoumarins. Also the sequence can be shortened so that only the nitroalkylisobenzofuranone (123) requires purification. A limitation to this method is that the phthalaldehydic acids are not trivial to make.

Whenever a six-membered ring is present in a molecule pericyclic reactions can be considered as an option. These have been rarely used in isocoumarin synthesis until recently.

In 1982, Harwood constructed the lactone ring via a regioselective Claisen rearrangement.[95] Since the reaction was carried out in trifluoroacetic acid, the immediate product (127) cyclised spontaneously to the dihydroisocoumarin (24). Mesylation

and hydrogenolysis of of the non-hydrogen bonded hydroxyl yielded

mellein (22) (Scheme 2.29).
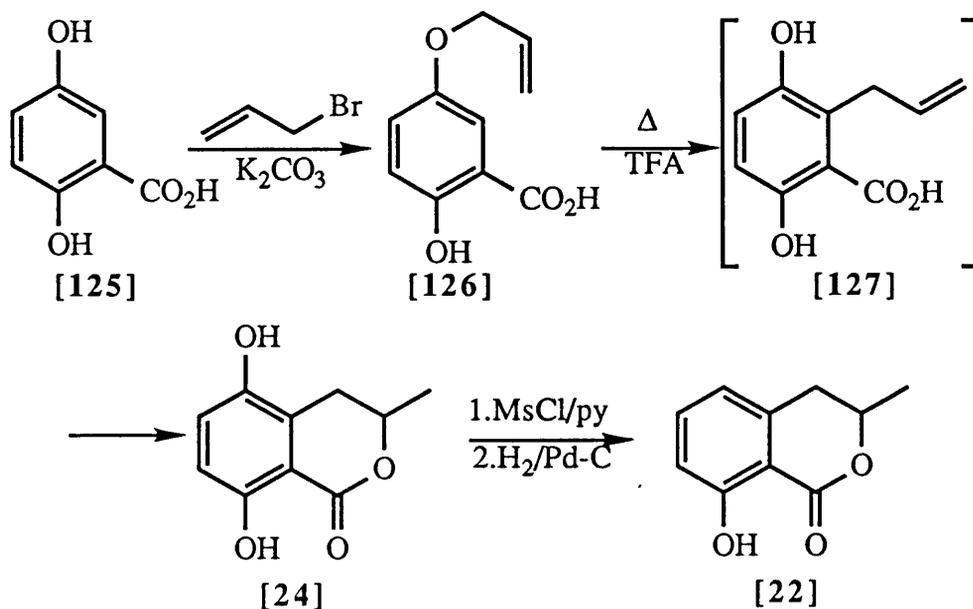
Scheme 2.29

Mellein (22) has also been prepared by Diels-Alder

methodology.[96]   The optically active ester (128) was converted in

four steps to ester (132).   This underwent an intramolecular

Diels-Alder reaction to cyclohexene (133).   Aromatisation and

Baeyer-Villiger oxidation completed this asymmetric synthesis of

mellein (22) (Scheme 2.30).

EtO_2C ⋯⋯ OTHP **[128]** →(DIBALH)→ OHC ⋯⋯ OTHP **[129]**

Ph_3P⋯ → ⋯OTHP **[130]** →(PPTS / MeOH)→ ⋯OH **[131]**

(structure with O, CO_2H) →(DCC / DMAP)→ Ac⋯O⋯O **[132]** →(Δ)→ H⋯Ac⋯H⋯O **[133]**

→(1.NBS / 2.DBU)→ Ac⋯O⋯O **[134]** →(1.CF_3CO_3H / 2.H_2O)→ OH⋯O⋯O **[22]**
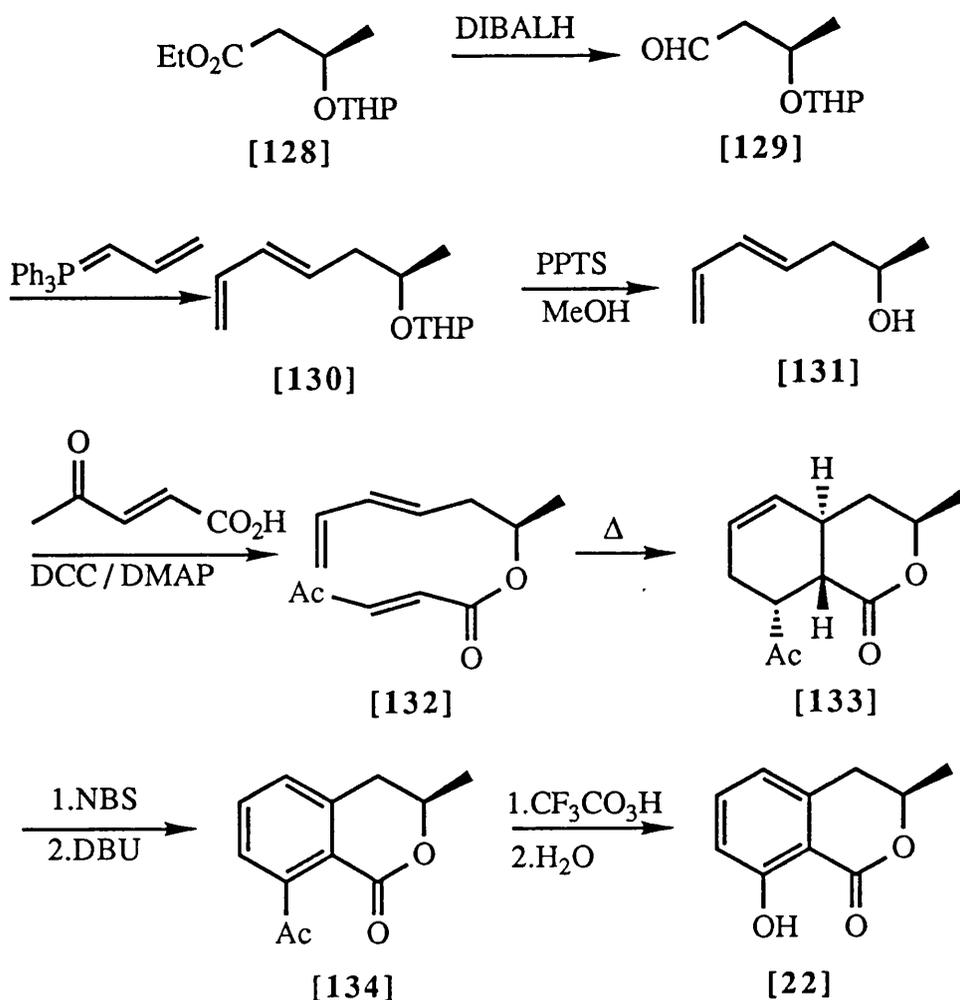
Scheme 2.30

The power of the Diels-Alder reaction has been realised in the convergent one-pot dihydroisocoumarin synthesis shown below (Scheme 2.31).[97] The diene (135) and the alkyne (136) form an adduct (137) which extrudes ethylene in a retro Diels-Alder reaction and cyclises spontaneously.

The diene is available with a range of substitution patterns by Birch reduction of aromatic compounds. Consequently many substituted isocoumarins were prepared by this route.
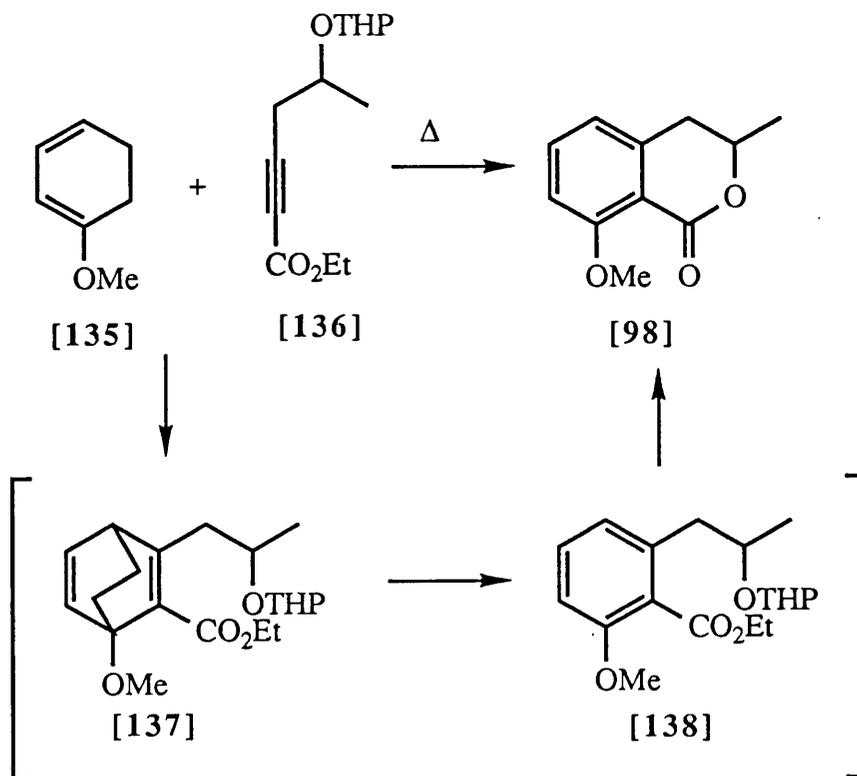
Scheme 2.31

2.6 Synthesis of 6,8-Dioxygenated Isocoumarins

In order to prepare those naturally occurring isocoumarins which we were interested in, we required an efficient synthesis of the 6,8-dioxygenated skeleton. The first synthesis of such a molecule was published by Nogami in 1941 and is shown below (Scheme 2.32).[98]

Diethyl acetonedicarboxylate (139) undergoes Claisen condensation followed by aldol cyclisation in the presence of magnesium. Hydrolysis and decarboxylation of the ester between the two phenols yielded the homophthalate (143). The phenols were protected as their methyl ethers and the unconjugated ester converted to the acid chloride (145). This was then condensed with ethyl acetoacetate, and the product subjected to retro-Claisen reaction and decarboxylation. Finally the ketoester (147)·cyclised to the isocoumarin (148) in formic acid.
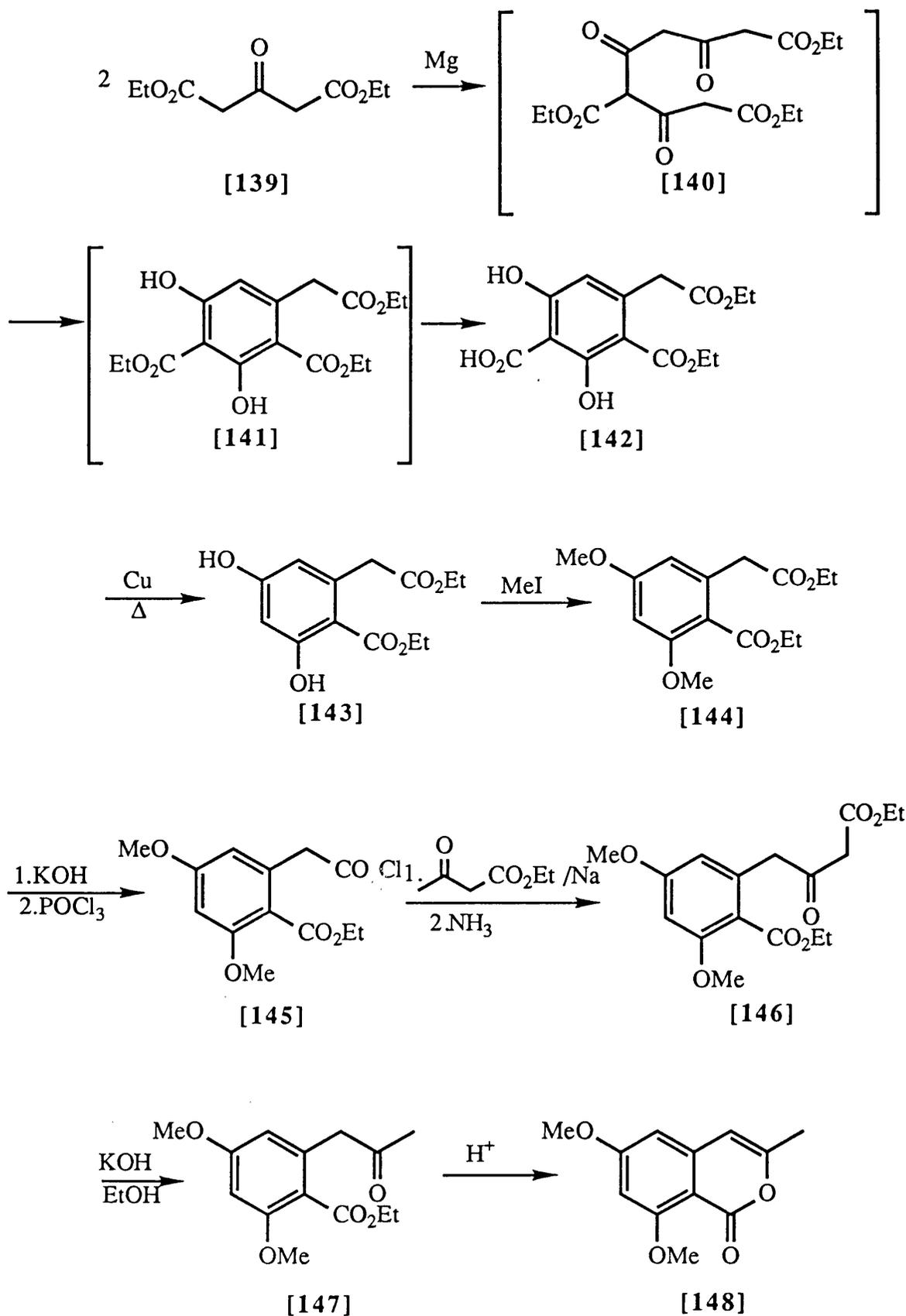
Scheme 2.32

The yield of the homophthalate was poor (only ~30%) and the route involved fairly harsh reagents and high temperatures.

This synthesis was refined by Curtis and coworkers to enable 6,8-dihydroxy-3-methylisocoumarin (5) to be obtained (Scheme 2.33).[99] The non-hydrogen bonded phenol of the homophthalate (143) was selectively benzylated using benzyl chloride and potassium carbonate. Curtis converted the acid chloride (150) to the ketone (151) in one step with dimethyl cadmium. Cyclisation and hydrogenolysis of the benzyl ether completed the synthesis.
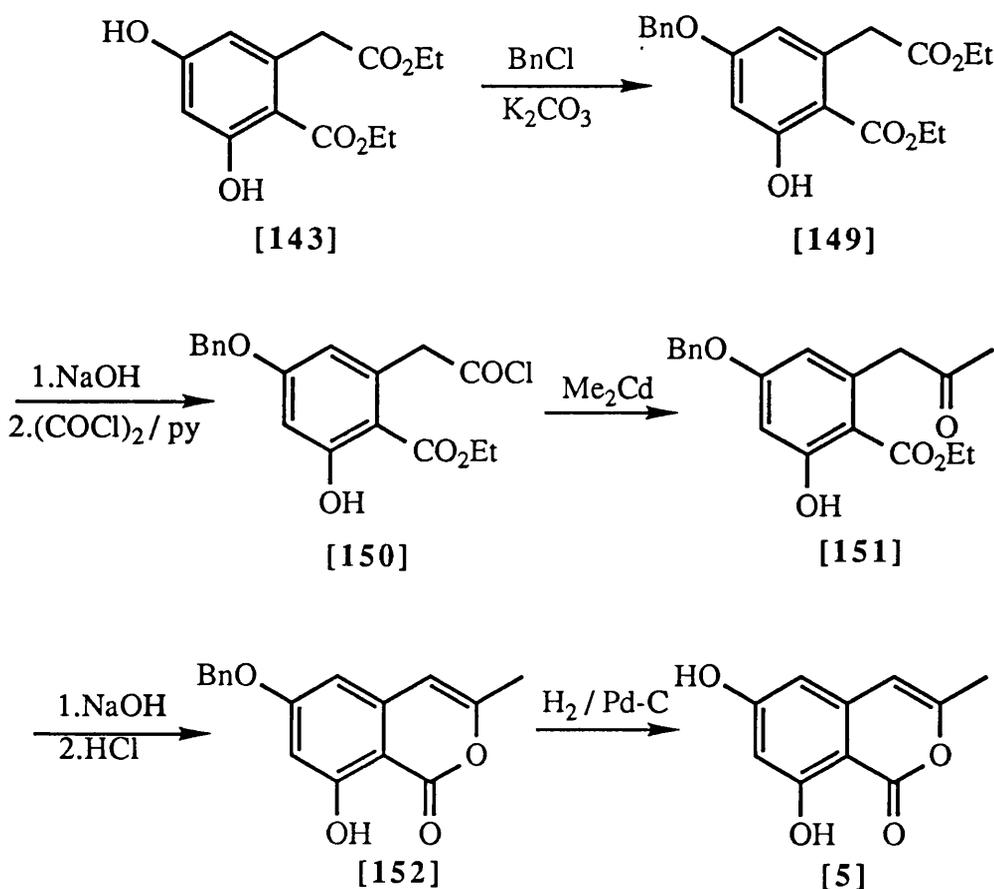
Scheme 2.33

Curtis used Nogami's synthesis of the homophthalate, and the route is still pretty laborious, taking six steps between the homophthalate and the isocoumarin.

A somewhat shorter route was published in 1977, taking only three steps between the homophthalic acid (160) and the isocoumarin (148) (Scheme 2.34).[100] Michael reaction and Dieckmann cyclisation of

ethyl acetoacetate (153) with ethyl crotonate (154) yielded the

diketone (155). This was aromatised by a bromination-debromination

procedure to the orsellinate (157). The dianion of dimethylorsellinic

acid (159) was carboxylated by dimethyl carbonate to yield, after

hydrolysis, the homophthalic acid (160). The last three steps

(acetylation, decarboxylation and cyclisation) were carried out

without isolation of any intermediates.
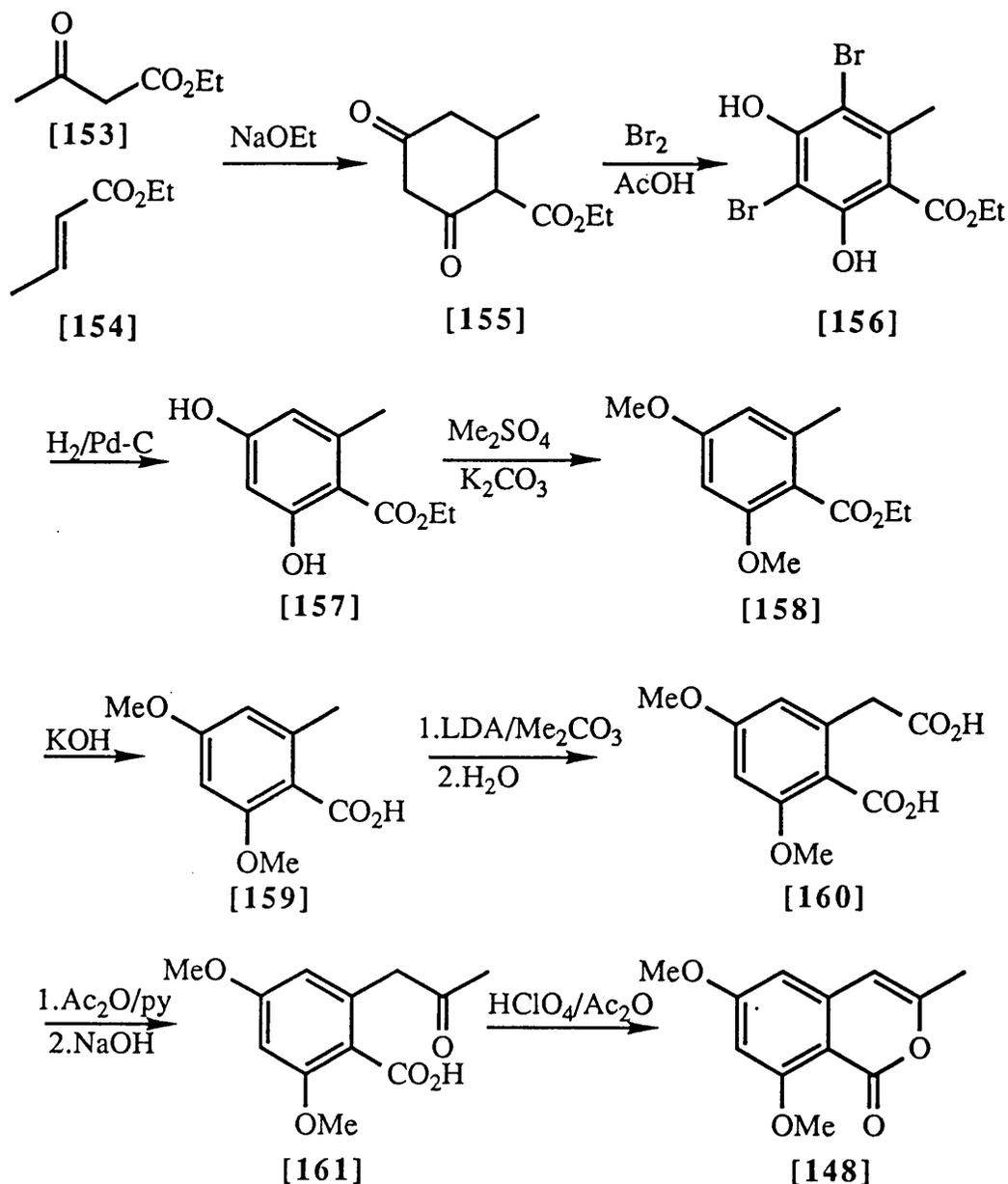
Scheme 2.34

Hill and Henderson used the same route to the homophthalate (160)

but improved the yield in the later steps by isolating the homophthalic

anhydride (162) (Scheme 2.35).[101] However, both these syntheses

suffer from the lengthy and low yielding orsellinate preparation.
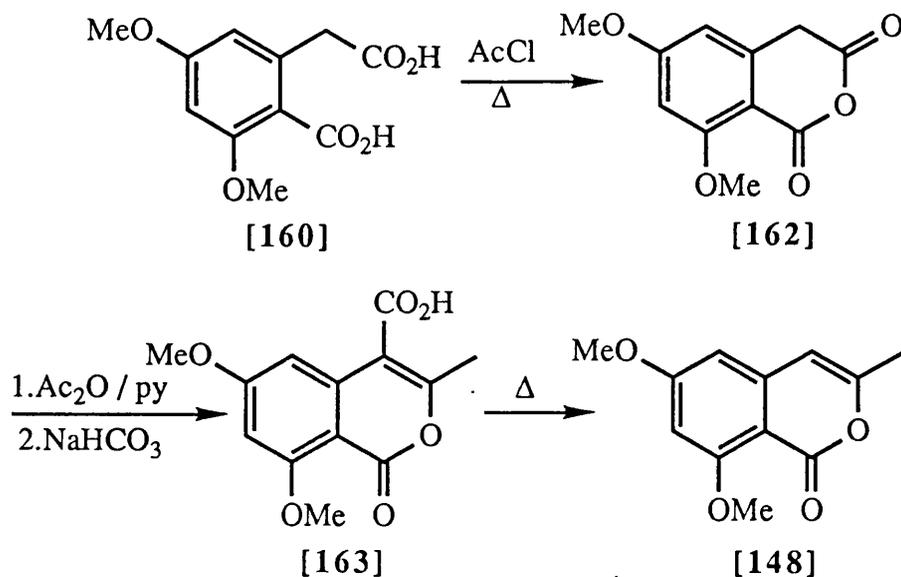
Scheme 2.35

Staunton reported the preparation of the target compound (148) in two steps from the orsellinate (158) via condensation with an N-methoxyamide (Scheme 2.36).[91]
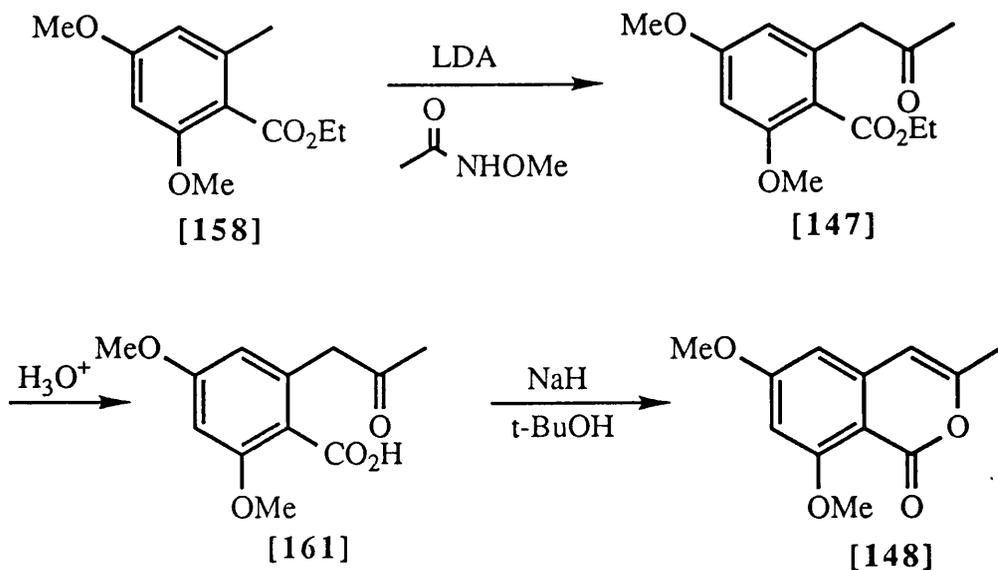
Scheme 2.36

Pathways utilising indanones were first employed in 1967 (Scheme 2.37).[102] The indanone (168) was prepared by cyclisation of the appropriate phenylpropanoic acid (167). This was in turn available by alkylation of diethyl malonate with the bromide (165), followed by hydrolysis and decarboxylation. Cleavage of the indanone was achieved
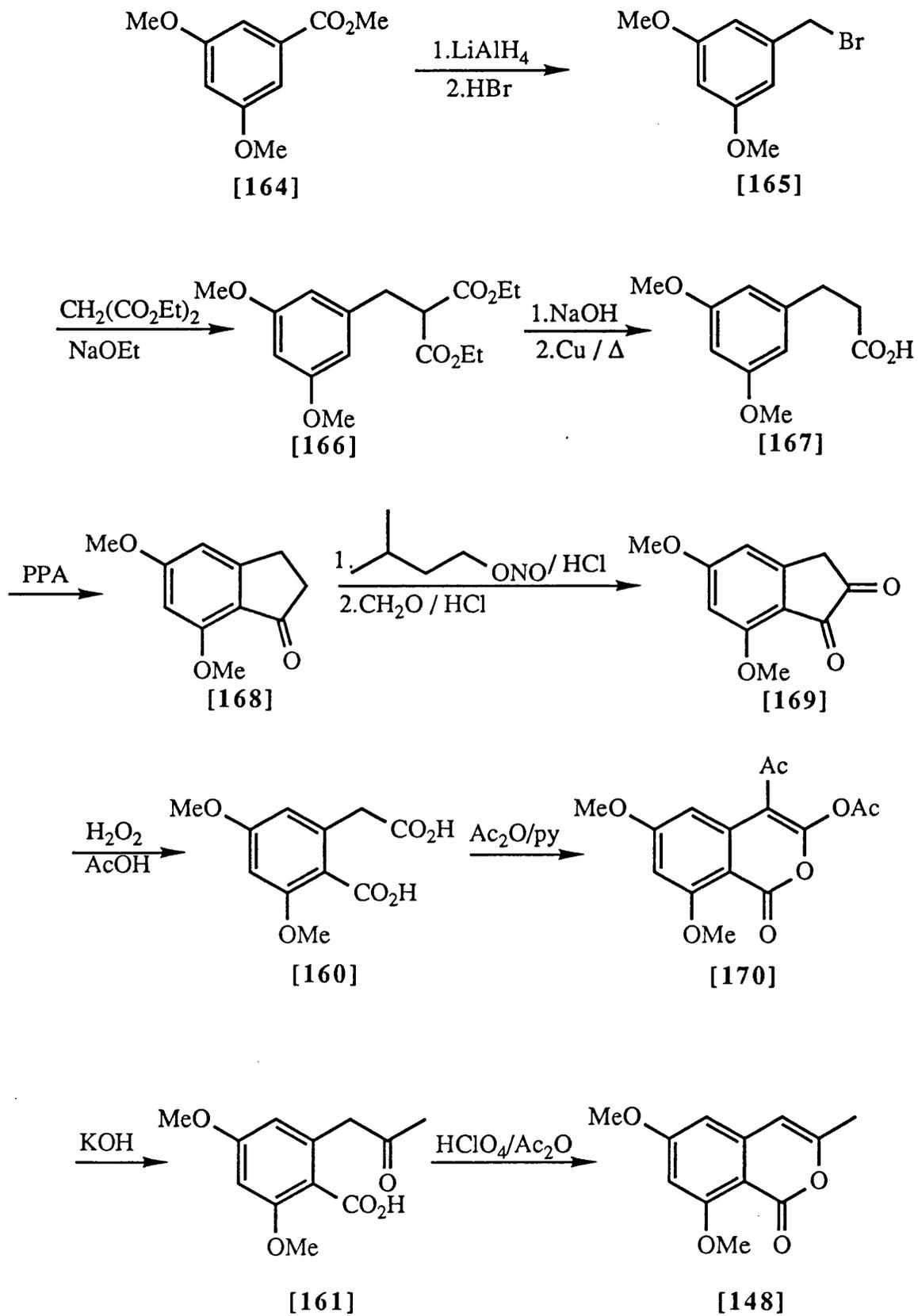
Scheme 2.37

by nitrosation and exchange-hydrolysis to the diketone (169), followed by
peracid oxidation.   From the resulting homophthalate (160), the
standard acylation, decarboxylation, cyclisation procedure was used.

Hill and Staunton shortened the indanone synthesis by carrying out
a Knoevenagel reaction of 3,5-dimethoxybenzaldehyde (171) with diethyl
malonate.[80]   This could be hydrogenated and alkylated to position the
3-substituent before cleavage.   Cyclisation to the indanone (175) was
achieved with a mixture of trifluoroacetic acid and trifluoroacetic
anhydride.   Cleavage was effected by ozonolysis of the enol
trifluoroacetate (Scheme 2.38).
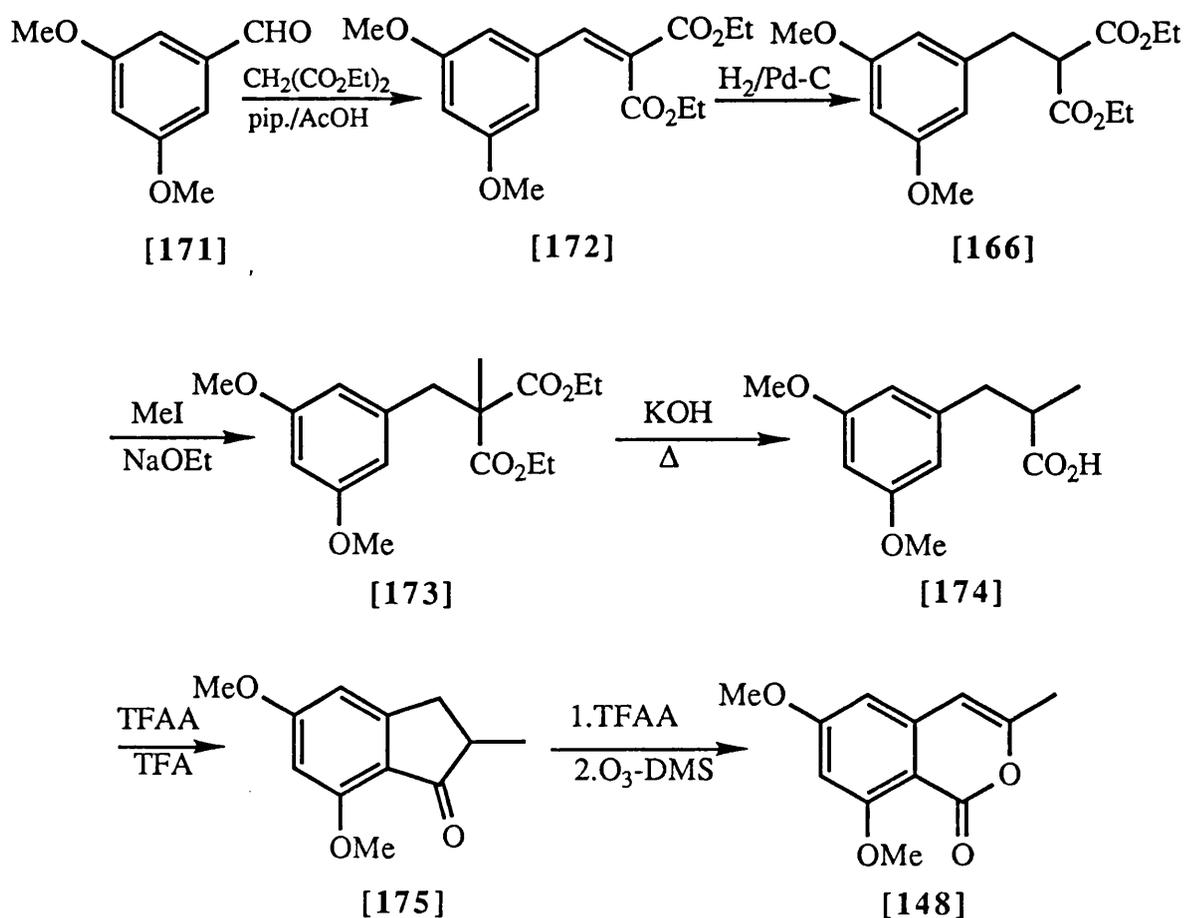
Scheme 2.38

Recently, two steps were cut from the indanone synthesis by using

a Perkin condensation with propanoic anhydride in place of the

Knoevenagel reaction above (Scheme 2.39).[103]   This increased the

overall yield for the synthesis to 35%.
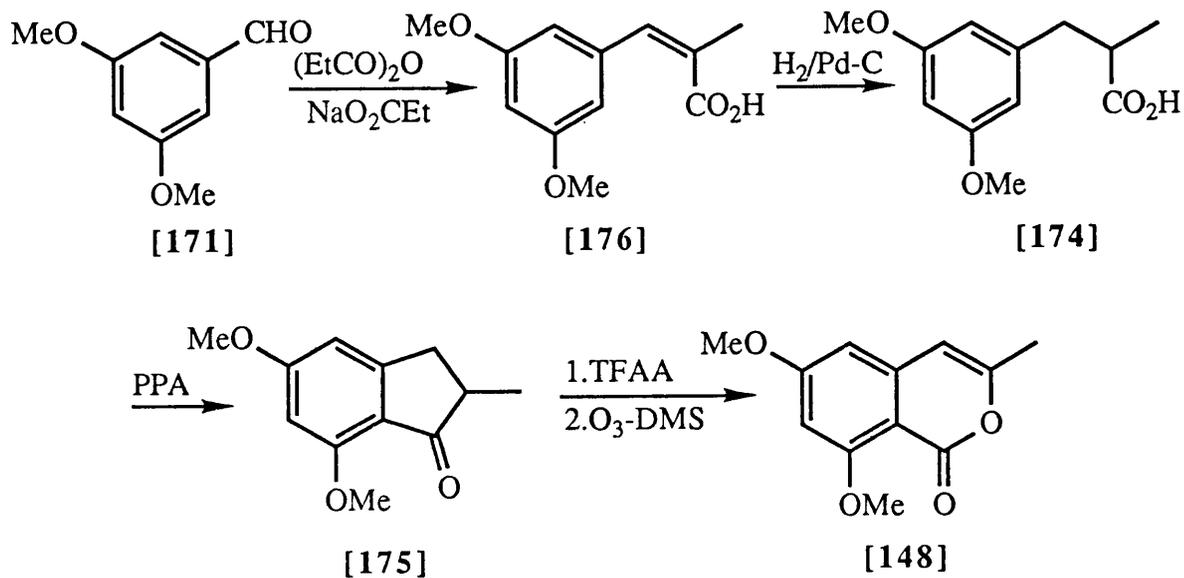
Scheme 2.39

3. RESULTS AND DISCUSSION

3.1 Synthesis of 6,8-Dioxygenated Isocoumarins

The naturally occurring isocoumarins which we set out to synthesise are listed below.

[1]

[2]

[3]

[4]

[5]

[7]

[8]

[28]

[177]

[178]
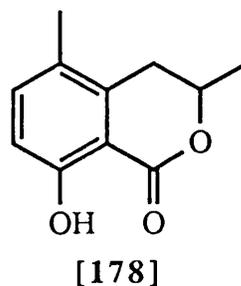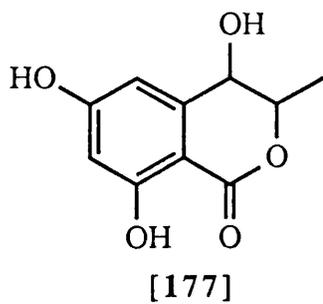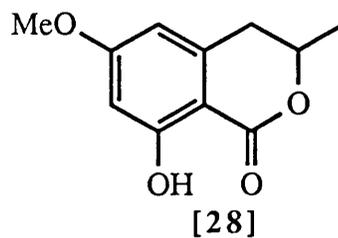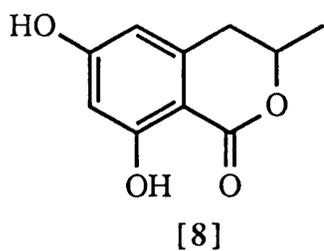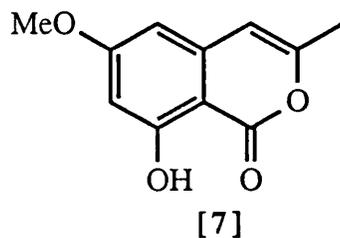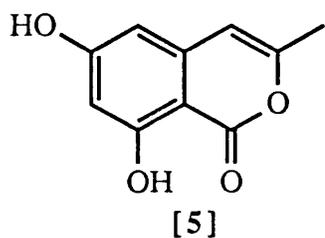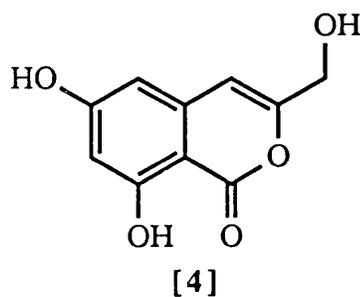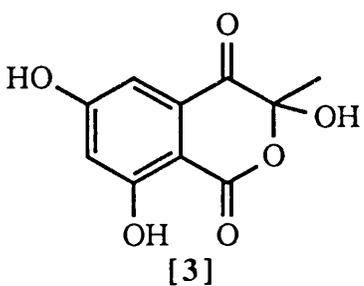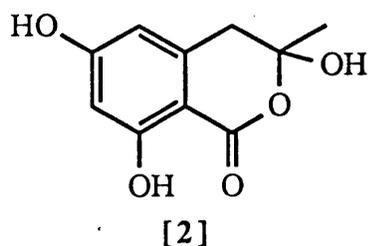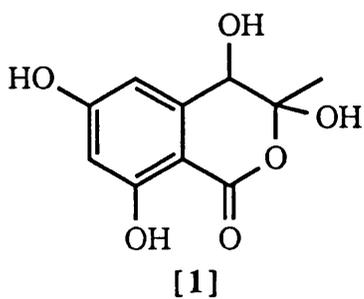
Our first objective was to obtain a sufficient quantity of the key intermediate, 6,8-dimethoxy-3-methylisocoumarin (148). This is the compound which we hoped to transform into most of our targets. The way in which we obtained it is outlined below (Scheme 3.1). This synthesis was developed within our group by Macaulay.[104] Its main advantage over the other published syntheses is the improved approach to the orsellinate (159), prepared in three excellent yielding steps.
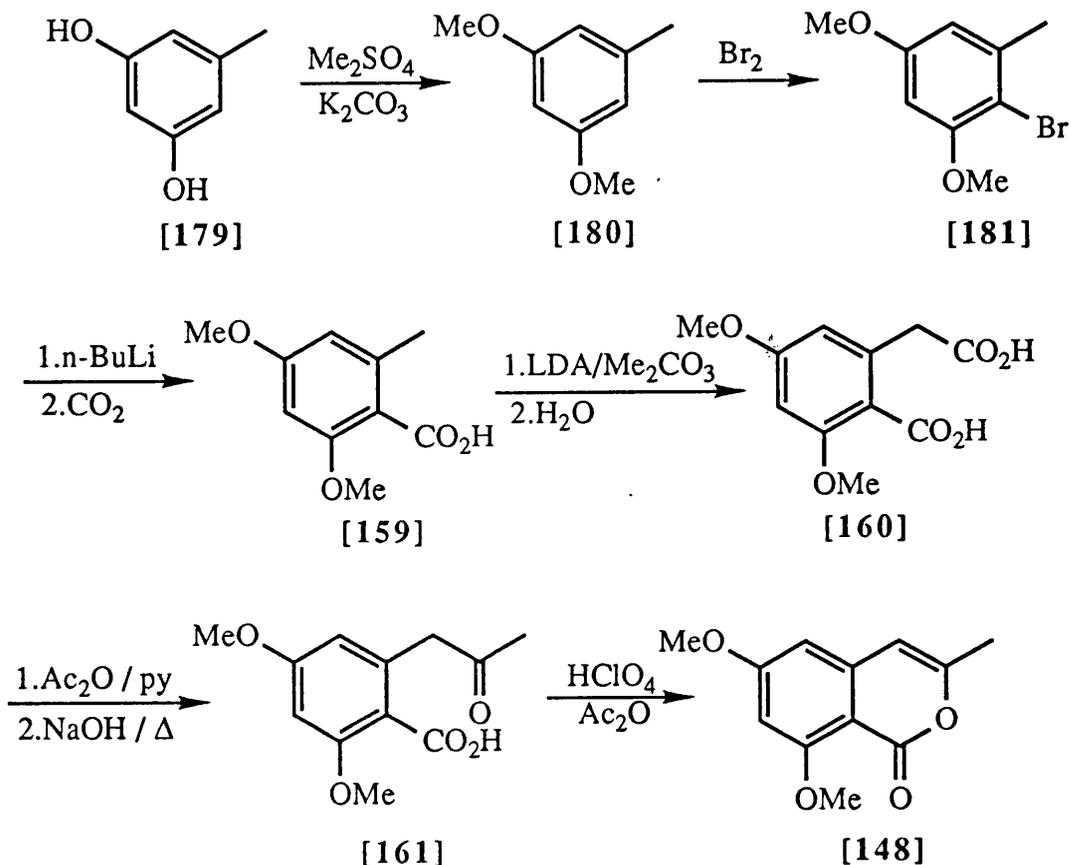
Scheme 3.1

Orcinol (179), a cheap, readily available compound, was used as starting material. The phenolic groups were protected as their methyl ethers on treatment with dimethyl sulphate and potassium carbonate. The two methoxyl groups made the aromatic ring sufficiently activated that no catalyst was required for the bromination.[105] Indeed, a small amount of dibrominated material (182) was formed, removed by fractional crystallisation.
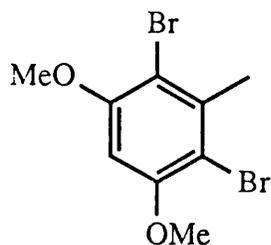
[182]

The regioselectivity of the bromination is interesting.   In this
case steric factors probably favour 2-bromination (methoxyls are more
sterically demanding than methyls) but with larger groups than methyl,
electrophilic attack still occurs at the 2-position.   Subtle electronic
effects are probably the most important factor.   In any case, the
4-bromo compound (183) is known to rearrange to the 2-bromo isomer (181)
in the presence of hydrogen bromide (formed in the reaction).[105]
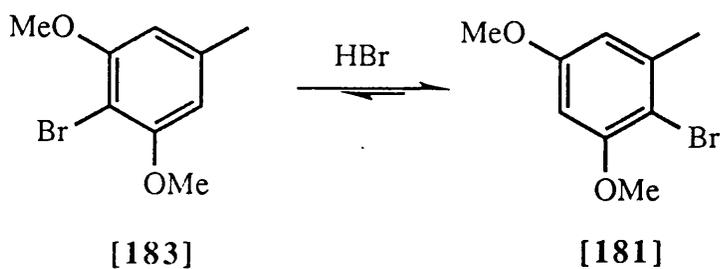
[183]   [181]

The bromine of (181) was replaced by lithium in a metal-halogen
exchange reaction with n-butyllithium.   The resulting lithio species
was quenched with dry ice to give the corresponding carboxylic
acid (159).

A useful method for producing substituted carboxylic acids
involves treatment of the dianion, generated by two equivalents of a
strong, hindered base, with an electrophile.   Thus deprotonation of the
acid (159) with an excess of lithium diisopropylamide produced the
resonance stabilised dianion:
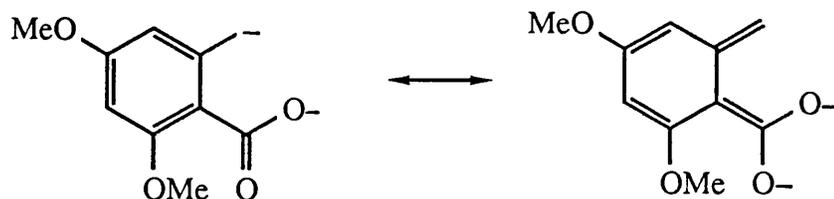
This dianion reacted _in situ_ with dimethyl carbonate to yield, after hydrolysis of the methyl ester, the dicarboxylic acid (160).[100]

In the presence of acetic anhydride/pyridine, the diacid (160) cyclised to the anhydride (162). This was deprotonated and acylated _alpha_ to the carbonyl group. Hydrolysis and decarboxylation of the unisolated β-ketoacid gave the product (185) (Scheme 3.2).[100]

[160] → [162] → [184]

→ [161] → [185]
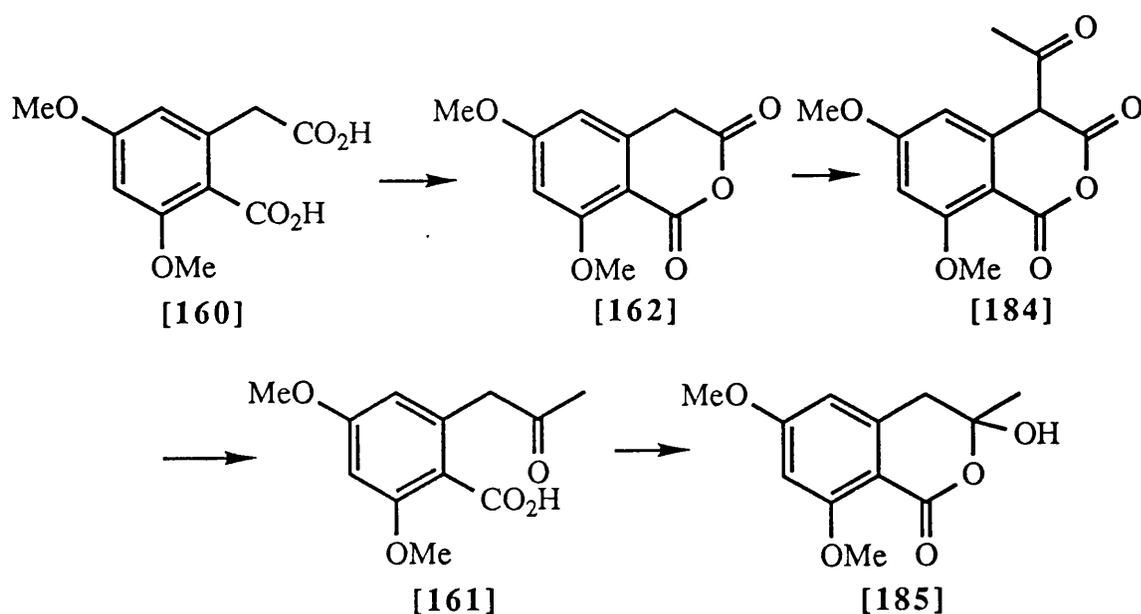
Scheme 3.2

The product exists, not as the ketoacid (161), but as the cyclic lactol (185). The i.r. spectrum of this compound shows a sharp O-H stretch band at 3360 cm^{-1}, rather than the broad band which would be expected of a carboxylic acid. There is also only one carbonyl stretch band at 1690 cm^{-1}.

Dehydration of the alcohol (185) is particularly easy due to the special stability of the 10 π-electron isocoumarin system. It was effected at room temperature using acetic anhydride and a catalytic

amount of perchloric acid under the conditions of Edwards and Rao.[106] This reagent is very useful for the formation of enol esters.

Our isocoumarin synthesis is very reliable, giving good yields at each step (~70%). Several grammes of 6,8-dimethoxy-3-methylisocoumarin (148) can be obtained in this way.

Having constructed the basic isocoumarin structure, all that remained to prepare some of the targeted natural products was to remove the protecting groups. Methyl ethers are seldom used as protecting groups for alcohols since, although they are easily put on and tolerate most conditions, they are difficult to remove. Aryl methyl ethers however are relatively easy to cleave.

The strong Lewis acid boron tribromide is a very powerful demethylating agent and removed both methyl groups.[107] Aluminium chloride is a much milder Lewis acid and removed only the methyl group adjacent to the carbonyl group (Scheme 3.3).
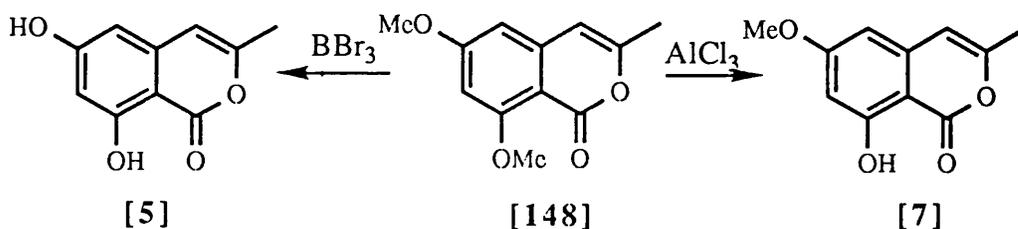
Scheme 3.3

The selectivity of aluminium chloride results from the formation of a six-membered chelate involving the peri carbonyl group (figure 2).
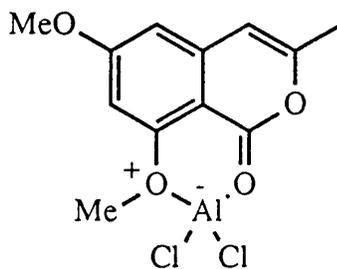
Figure 2

Reduction of the double bond was achieved by hydrogenation over a palladium-charcoal catalyst.[107]

[148]   H$_2$/Pd-C   [186]
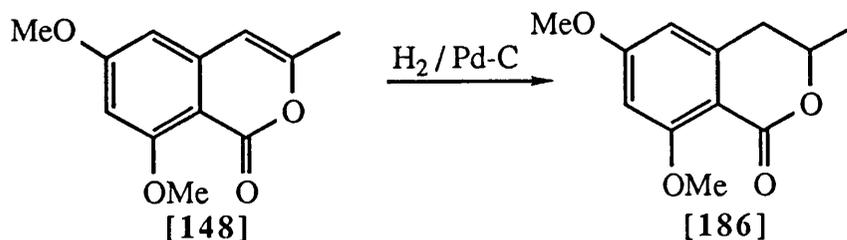
This allowed two more target compounds to be prepared by removal of both methyl ethers with boron tribromide, or only one with aluminium chloride (Scheme 3.4).[101]

[8]   BBr$_3$ [186]   AlCl$_3$ [28]
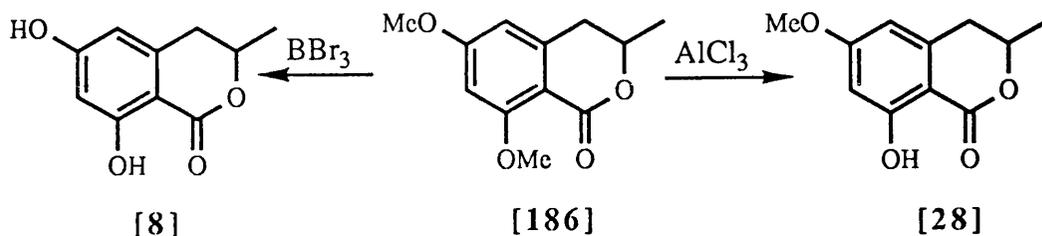
Scheme 3.4

The lactol (185) is the dimethyl ether of one of the target isocoumarins. We therefore envisaged that boron tribromide demethylation would yield the desired triol (2). However this reaction produced a brown tar containing many compounds. Probably the lactol system was too sensitive to the strongly acidic reagent.

The triol (2) was eventually obtained by alkaline hydrolysis of the previously prepared isocoumarin (5), followed by mild acidification.[108]

[5]   1.NaOH 2.H$_3$O$^+$ [2]
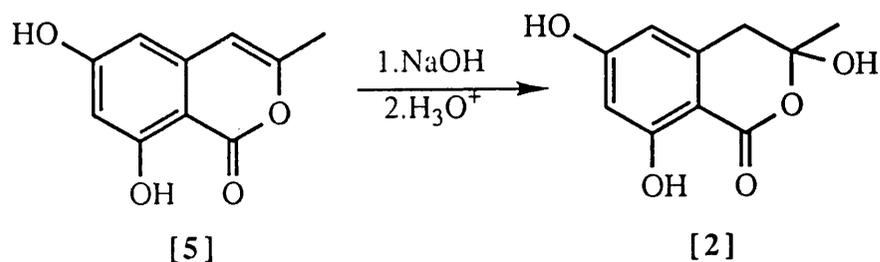
3.2 Synthesis of 4-Substituted Isocoumarins

Having synthesised the simplest of the isocoumarins, we turned our attention to those which were functionalised at the 4-position. Very little synthetic work had been done in this area despite the fact that many such compounds have been discovered. We hoped that a useful general method could be developed.

It had been reported by Grove that the dimethyl ether (187) of one of our targets could be prepared by oxidation of the lactol (185) with selenium dioxide.[108] He also found that the dimethyl ether (188) of another of the targets could be synthesised from the ketone (187) by hydrogenation in basic solution (Scheme 3.5).

[185]   [187]   [188]
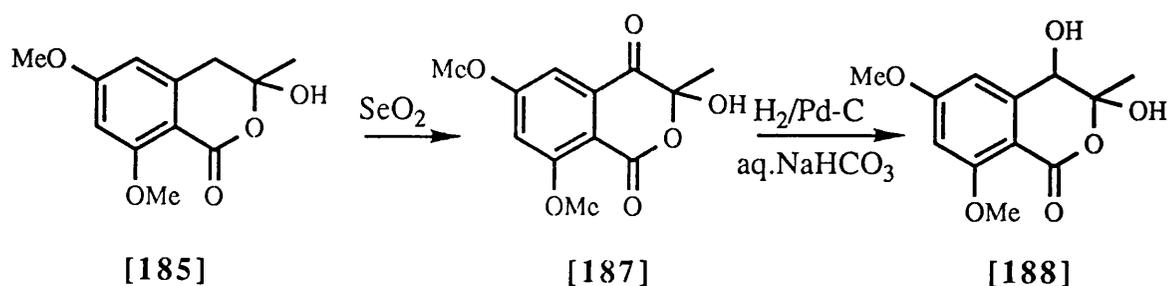
Scheme 3.5

Working with fermentation-derived material, Grove showed that the alcohol (1) could be oxidised to the ketone (3) with copper (II) sulphate.

[1]   [3]
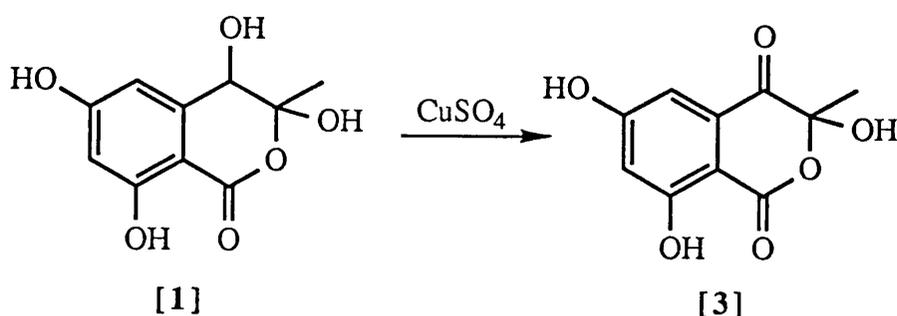
When the selenium dioxide reaction was attempted by us, no trace of the desired compound was observed and the products were heavily polluted with malodorous selenium compounds. A catalytic method, using t-butylhydroperoxide to recycle the selenium dioxide, resulted in recovery of starting material.[109]

Other methods for the α-oxidation of ketones were also investigated. The most successful of these appears to be t-butylhydroperoxide with a catalytic amount of chromium trioxide.[110] However in this case (as in some of the examples in the original paper) the reagent was destroyed before it could react. A sudden colour change from purple to green was observed.

We next decided to attempt to oxidise the 4-position in two steps by a bromination/hydroxylation procedure. We anticipated that it would be possible to brominate the benzylic carbon of the lactol (185) using N-bromosuccinimide under radical conditions.[111] The reactive benzylic bromide (189) could then be hydrolysed to the alcohol (188) (Scheme 3.6).

[185]   [189]   [188]
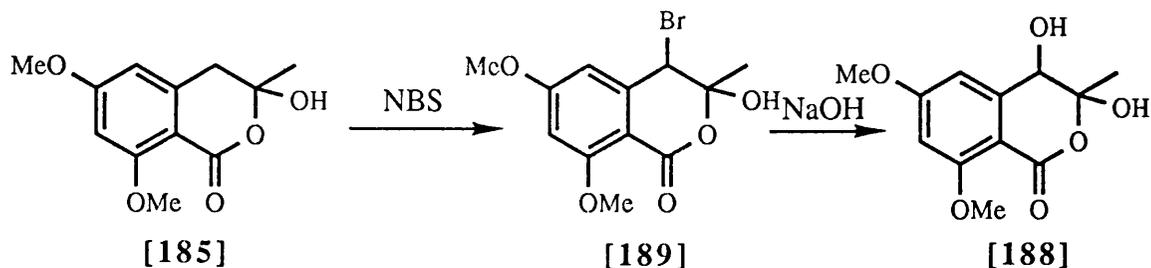
Scheme 3.6

Despite repeated attempts we failed to bring about radical bromination. Different solvents, different temperatures, light, radical initiators (AIBN or benzoyl peroxide), different stoichiometric amounts of reagents and order of addition all failed to produce the desired compound. Instead varying amounts of the product of aromatic bromination (190) were obtained. Similarly, the dihydroisocoumarin yielded the aromatic bromide (191).

[190]   [191]
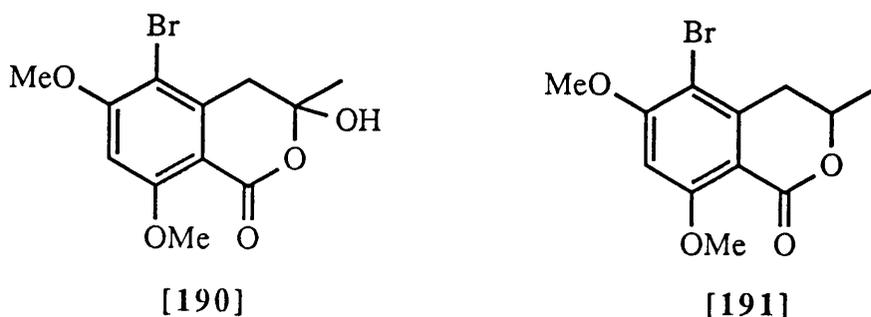
These results suggest that the two methoxyl groups in the ortho/para positions make the aromatic ring too activated to allow radical bromination to take place.   The ionic reaction appeared to take place very quickly.

Attempts to functionalise the alkene (148) were not successful either.   Epoxidation with mcpba in dichloromethane and dihydroxylation with osmium tetroxide in dichloromethane resulted in recovery of starting material (Scheme 3.7).
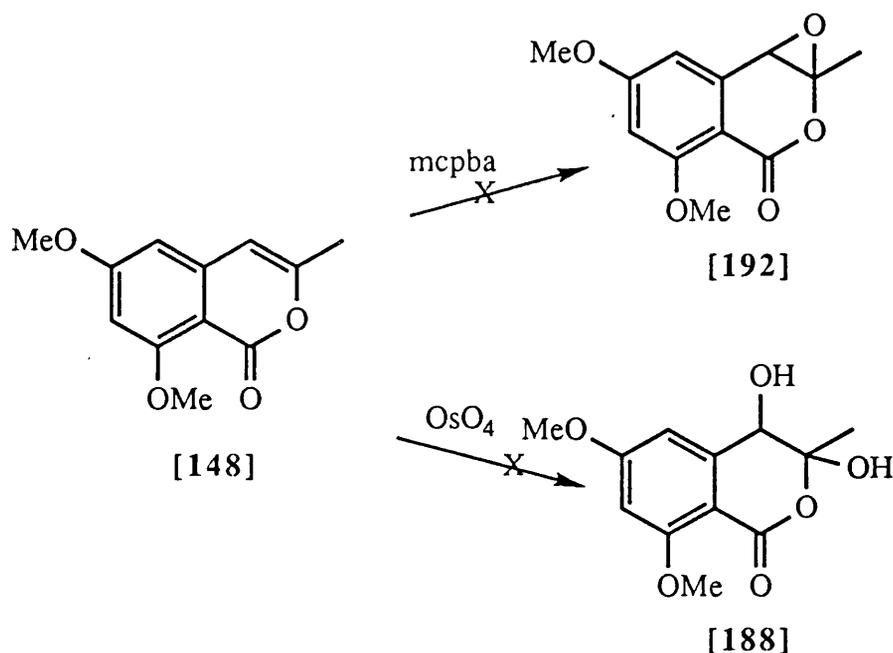
Scheme 3.7

The failure of these reactions is probably due to unreactivity of the double bond.   The special stability of the 10 π-electron isocoumarin system gives the double bond a degree of aromaticity.

Having repeatedly failed to functionalise the 4-position of the isocoumarin, we decided to change direction and carry the oxygen through the synthesis rather than introducing it at the end.   The problem then became finding a suitable protecting group.   We decided to use a methyl ether as the protecting group since it would be easy to put on and was already known to withstand all reaction conditions.   An obvious problem with the methyl ether could be its removal at the end of the synthesis.

The route used (analogous to the previous one) is shown below

(Scheme 3.8).
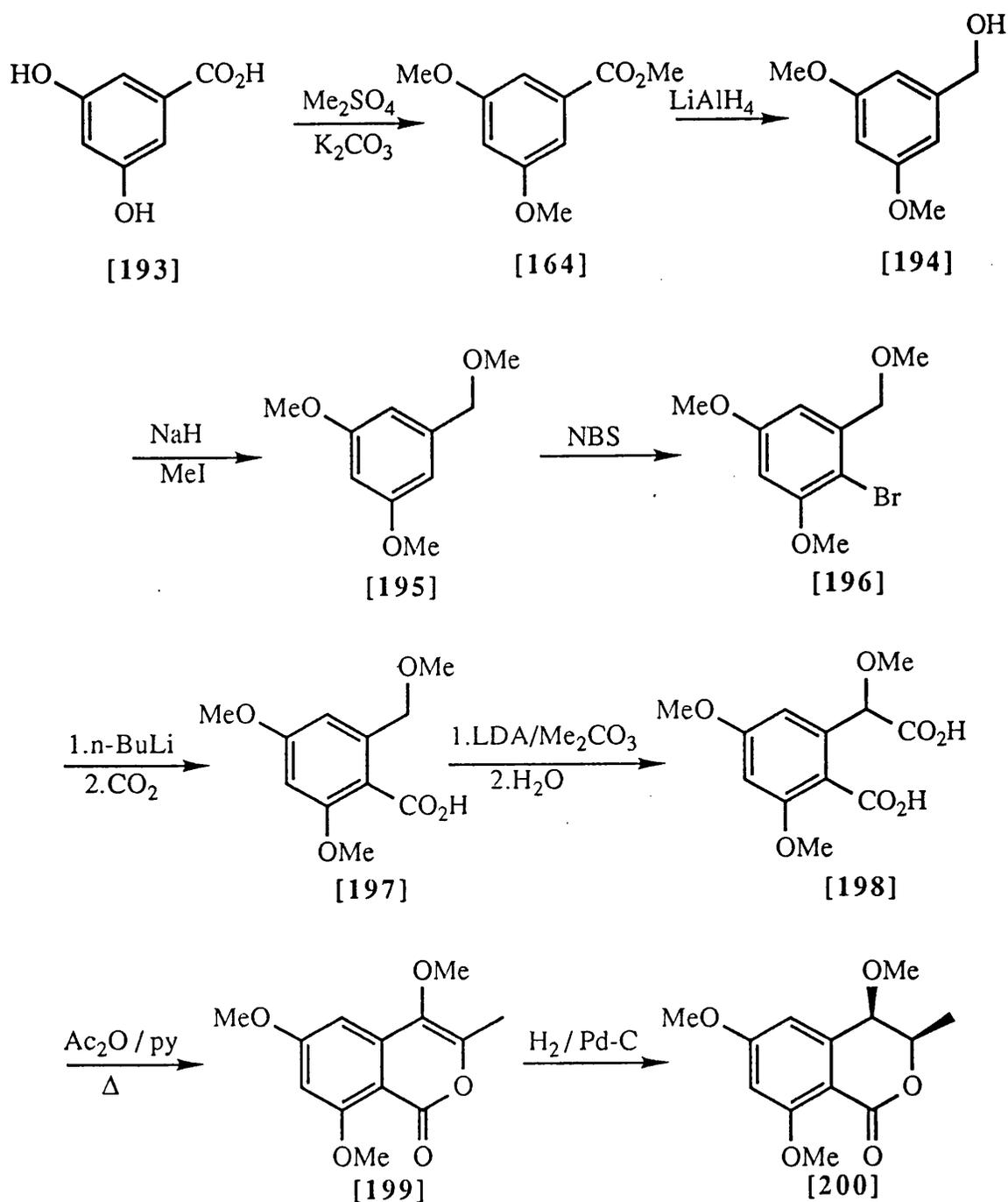
Scheme 3.8

Readily available 3,5-dihydroxybenzoic acid (193) was methylated with dimethyl sulphate/potassium carbonate to give methyl 3,5-dimethoxybenzoate (164).[112] The ester was then reduced to the benzyl alcohol (194) with lithium aluminium hydride. This was then methylated in excellent yield using Barton's sodium hydride/methyl iodide method.[113]

For the bromination, N-bromosuccinimide was used instead of bromine. Apart from being easier to handle, NBS is also a milder

reagent and gave very little dibromination.   Both carboxylations were carried out as before, using n-butyllithium/dry ice and lithium diisopropylamide/dimethyl carbonate respectively.

In our previous synthesis we proceeded from the homophthalic acid to the isocoumarin in two steps.   First the homophthalate was acylated with acetic anhydride/pyridine and the product decarboxylated. The dehydration was then carried out using perchloric acid and acetic anhydride.   On this occasion the first step failed, only starting material being recovered.   Fortuitously however, the whole transformation could be achieved in one step upon refluxing the diacid (198) in acetic anhydride with a few drops of pyridine.

Hydrogenation of the isocoumarin (199) was very sluggish, requiring a weekend at atmospheric pressure for complete reaction.   This is probably a consequence of the aromaticity of the isocoumarin and the tetrasubstituted nature of the double bond.

Having constructed the desired isocoumarin framework, all that remained was to remove the methyl protecting groups.   Treatment of the isocoumarin (199) with boron tribromide not only cleaved the methyl ethers but also two carbons from the backbone, giving the anhydride (201).

[199]   [201]

No precedent for such a reaction being induced by boron tribromide has been found in the literature and the mechanism is puzzling.   One speculative mechanistic explanation is outlined on the next page (Scheme 3.9).

Scheme 3.9

The dihydroisocoumarin (200) was completely demethylated by boron tribromide without loss of any other carbons. However the mass spectrum revealed the presence of a bromine atom, and the product turned out to be the bromide (202).

[200]   [202]
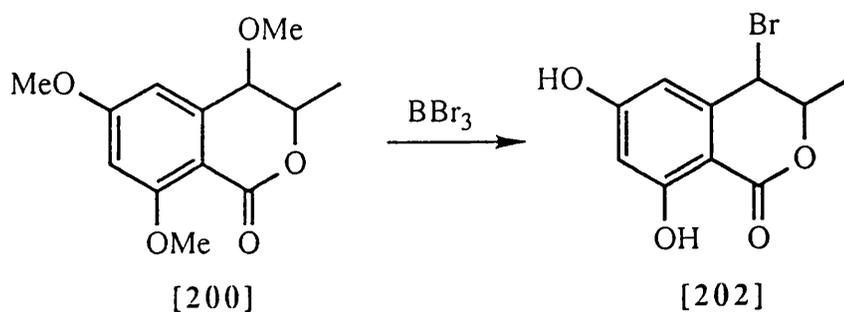
Bromination can be understood by considering the mechanism of boron tribromide demethylation.[114] The Lewis acid coordinates to the oxygen lone pair and bromide ion attacks the adjacent carbon (figure 3).
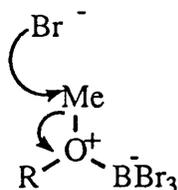
Figure 3

Normally the bromide ion attacks the methyl carbon rather than the aryl carbon. In this case the benzylic carbon is more reactive, giving the observed product.

When problems arose with the methyl ether as a protecting group we investigated alternatives. The t-butyldimethylsilyl ether (203) of the benzyl alcohol (194) was easily formed using t-butyldimethylsilyl chloride, 4-dimethylaminopyridine and triethylamine.[115] The TBDMS ether withstood the bromination but fell off in the carboxylation reaction (Scheme 3.10).

[194]   [203]   [204]
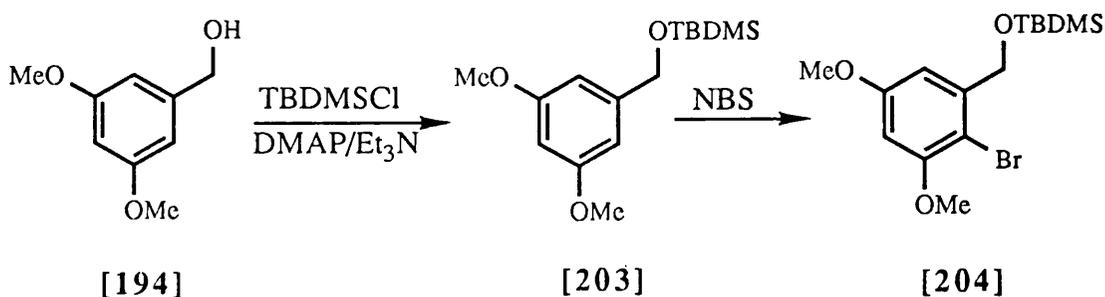
Scheme 3.10

A report by Trost that 3,5-dimethoxybenzyl alcohol (194) can be converted directly to the phthalide (206) by metallation-carboxylation prompted us to investigate the possibility of avoiding a protecting group altogether.[116] Better yields of the phthalide (206) can be obtained by initial bromination with NBS (Scheme 3.11).[117] The success of this route is attributed to the faster rate of metal-halogen exchange compared with metal-hydrogen exchange.

[194]   **[205]**   **[206]**
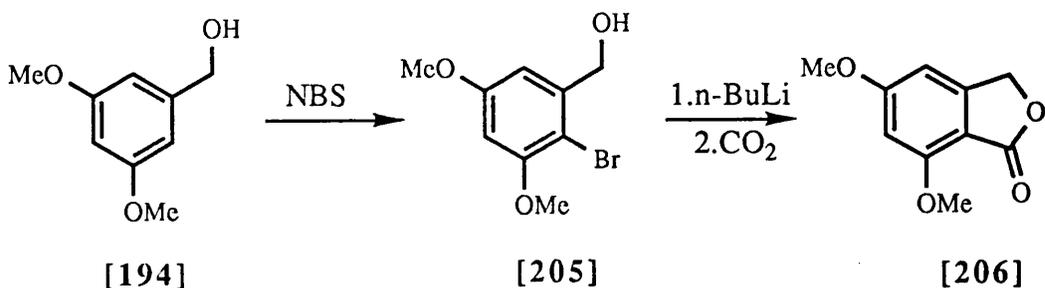
Scheme 3.11

Carboxylation of the phthalide with lithium diisopropylamide/dry ice proceeded in low yield to give the carboxylic acid (207). Treatment of the acid with acetic anhydride/pyridine produced the phthalide (208) (Scheme 3.12). This forced us to abandon the phthalide route.

[206]   **[207]**   **[208]**
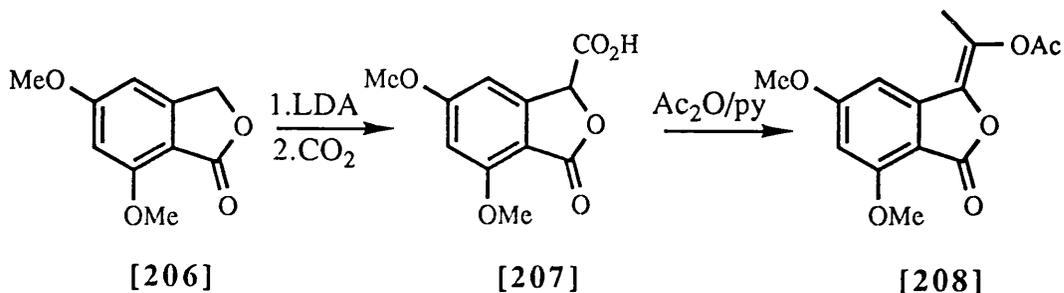
Scheme 3.12

Returning to the benzylic bromide (202), we still expected that it would be fairly easy to convert it to the desired alcohol. The hydrolysis was carried out under mild conditions (dilute sodium hydroxide, room temperature) to avoid the facile dehydrobromination. Although the product had the correct molecular weight, the ^{1}H nmr spectrum was complicated. The ^{13}C nmr spectrum showed that the alcohol (177) had been formed as a 60:40 mixture of diastereomers.

[202]   **[177]**
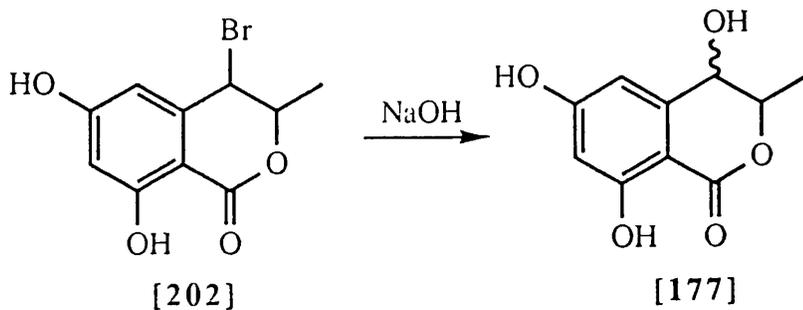
The stereoisomers could not be separated by chromatography or recrystallisation. The use of other nucleophiles, such as acetate, did not improve the diastereoselectivity.

A recent paper reported that a similar secondary benzylic bromide (209) could be hydrolysed simply by stirring in aqueous tetrahydrofuran at room temperature.[118]
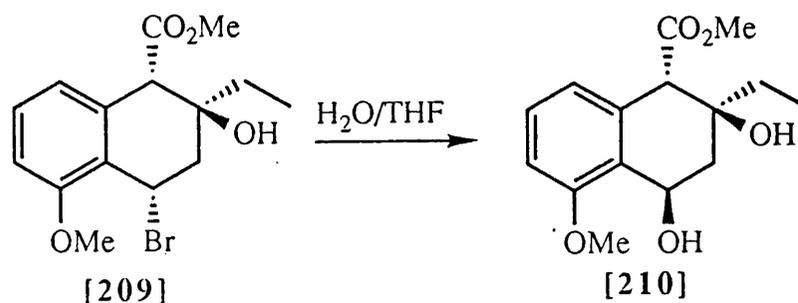
However our bromide remained unchanged when stirred in a 1:1 water:THF mixture at room temperature or at reflux. We decided to try adding silver nitrate to the reaction to enhance the reactivity of the bromide. This approach was successful, giving a 90:10 mixture of diastereomers which on recrystallisation gave exclusively one compound. This proved to be the natural stereoisomer by comparison of its melting point and ^{1}H nmr spectrum.[119]

The methyl ether (200), bromide (202) and alcohol (177) can exist as cis or trans diastereomers, (figure 4) but only one was formed in each case.
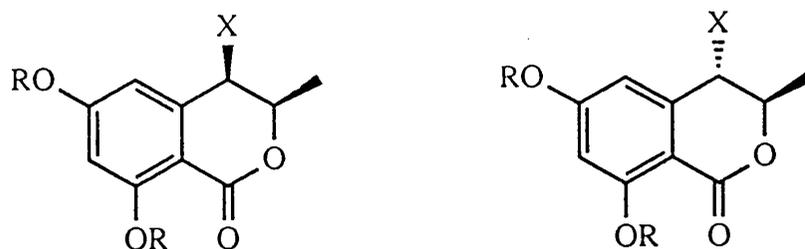
R = H, Me   X = OMe, Br, OH

Figure 4

Dihydroisocoumarins are known to adopt a half-chair conformation, with substituents in pseudoaxial and pseudoequatorial positions.[120]

In the case of the <u>cis</u> diastereomer, one substituent must be pseudoaxial and one pseudoequatorial. The hydrogens then have a dihedral angle of approximately 60°, and the Karplus relationship predicts a coupling constant of ~2 Hz (figure 5).
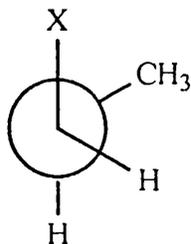
Cis , $\emptyset = 60^{\circ}$

Figure 5

This prediction is borne out by the ^{1}H nmr spectra of naturally occurring <u>cis</u> 3,4-disubstituted dihydroisocoumarins. The H3-H4 coupling constant in the compounds (23)-(217) is consistently in the range 1.5 to 3 Hz.[121-126]

In the case of the <u>trans</u> diastereomers, coupling constants need to be treated with a little more caution. The substituents can be either both pseudoaxial or both pseudoequatorial, giving the hydrogens dihedral angles of 60° or 180° respectively (figure 6).
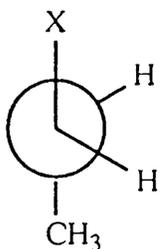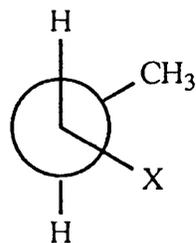
Trans diaxial , $\emptyset = 60^{\circ}$ *Trans* diequatorial , $\emptyset = 180^{\circ}$

Figure 6

63
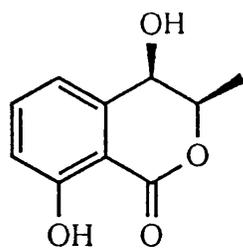
[23] *2 Hz*
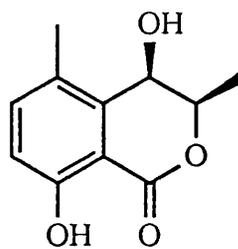
[211] *1.5 Hz*
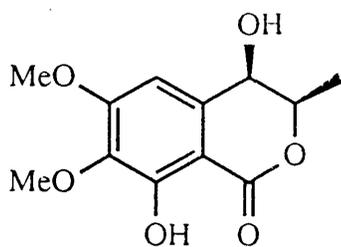
[212] *2 Hz*
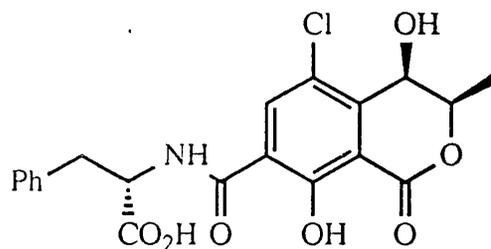
[213] *2 Hz*
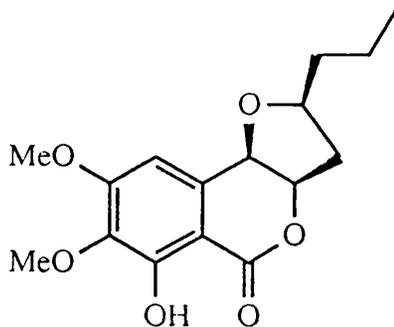
[214] *3 Hz*
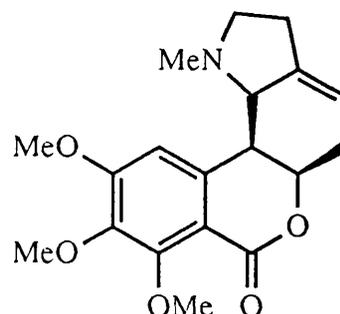
[215] *2 Hz*
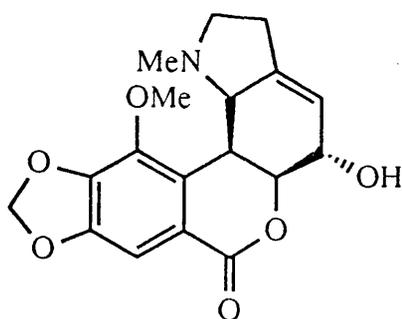
[216] *2 Hz*
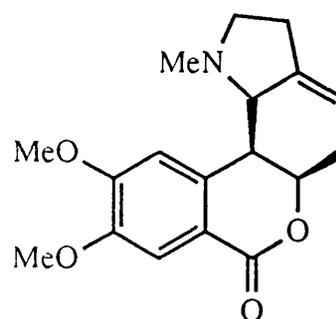
[217] *2.5 Hz*

When the dihedral angle is 180°, large coupling constants of around 10 Hz are predicted and observed. Examples of this class include the alkaloid clivonine (218), which has a 12 Hz coupling constant[127] and the C-glucoside norbergenin (219) which has a coupling constant of 10.3 Hz.[128]
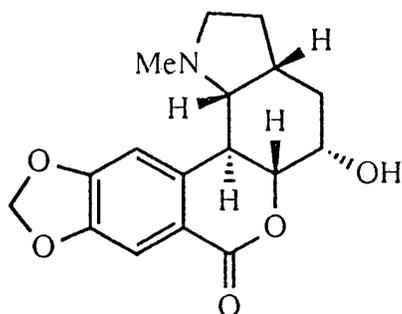
[218]
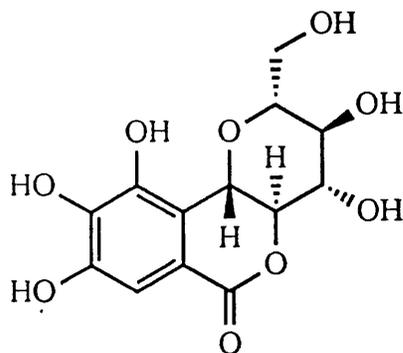
[219]

However when the substituents are pseudoaxial the dihedral angle is 60° and the coupling constant is only about 2 Hz. This situation is present in chebulic acid (220) which has a trans ring fusion, but a coupling constant of 1.5 Hz.[129] The nmr data is supported by an x-ray crystallographic analysis, which shows that the two substituents are pseudoaxial.

The trans isomer of 4-hydroxy-5-methyl mellein (221) also exhibits a small (1.5 Hz) coupling constant.[122] Here again the substituents have adopted the pseudoaxial position.
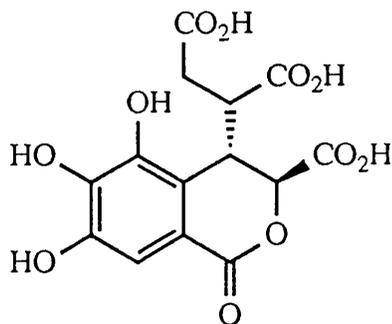
[220]
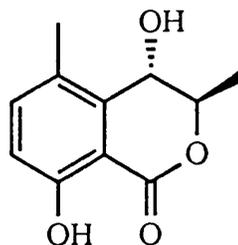
[221]

It would appear that trans 3,4-disubstituted dihydroisocoumarins

prefer to adopt the conformation with the substituents pseudoequatorial unless there are bulky groups at C-4 or C-5. Then an adverse <u>peri</u> interaction forces the substituents into pseudoaxial positions (figure 7).[104]
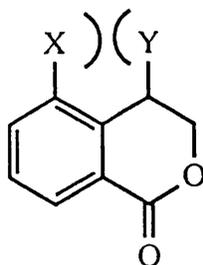
<u>Figure 7</u>

When the H3-H4 coupling constant is around 2 Hz it is difficult to tell from this information alone whether the compound is <u>cis</u> or <u>trans</u>. A better indication of the stereochemistry can be obtained from the position of the methyl doublet. This typically occurs at 1.4-1.7 ppm for <u>cis</u> stereoisomers and at 1.1-1.4 ppm for <u>trans</u> stereoisomers. The upfield shift in the <u>trans</u> isomer has been attributed to the fact that the pseudoaxial methyl group is above the shielding zone of the aromatic ring and the carbonyl group (figure 8).[122]

Trans   *Cis*
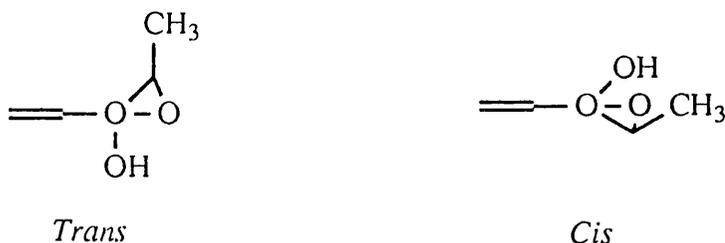
<u>Figure 8</u>

To investigate the stereochemistry of our compounds we needed to obtain the <u>cis</u> and <u>trans</u> isomers of the methyl ether (200), bromide (202) and alcohol (177). The methyl ether (200), produced by a <u>syn</u> hydrogenation must have <u>cis</u> stereochemistry. This is borne out by the 2.4 Hz coupling constant and the methyl doublet at 1.43 ppm.

Carrying out the reduction with alkaline sodium borohydride yields a 2:1 mixture of <u>cis</u>:<u>trans</u> diastereomers (Scheme 3.13). The <u>trans</u>

diastereomer shows a 7 Hz coupling constant and a methyl doublet at 1.24 ppm.
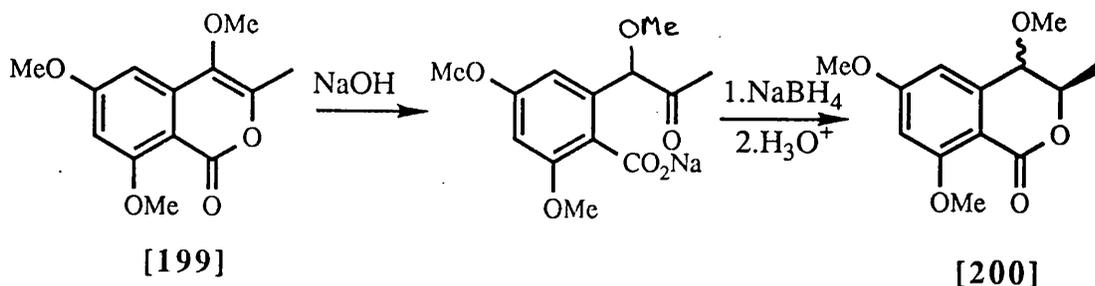
Scheme 3.13

On treatment with boron tribromide, a 2:1 mixture of <u>trans</u>:<u>cis</u> diastereomers was produced, with coupling constants of 2.6 Hz and 1.9 Hz respectively. Here the substituents are diaxial due to the bulky bromine atom at C-4. Comparison of the methyl doublets revealed that the bromide prepared previously was the <u>trans</u> isomer. Hence the demethylation had occurred with inversion of stereochemistry, supporting the proposed S_N2 mechanism.

The <u>cis</u> and <u>trans</u> alcohols had H3-H4 coupling constants of 2.3 Hz and 8.5 Hz respectively. In the absence of the bromine atom the <u>trans</u> diastereomer has reverted to the diequatorial conformation, giving an appropriately large <u>J</u> value. The triol (177) which we had made before had the <u>cis</u> relative stereochemistry, as assigned in the original paper.[119] The results of our stereochemical studies are summarised below (Scheme 3.14).
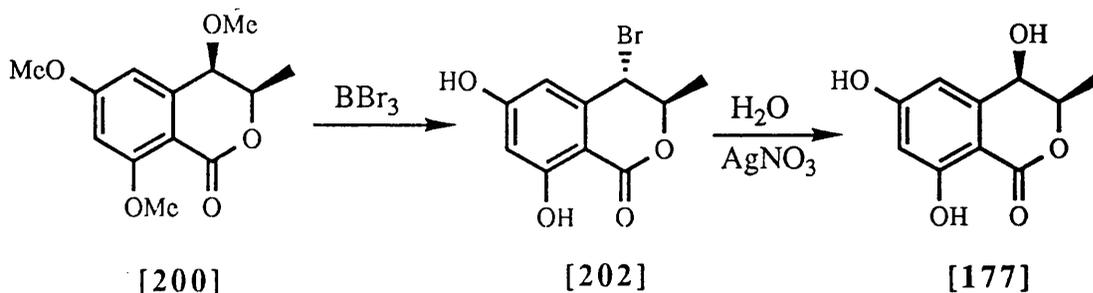
Scheme 3.14

The fact that the sodium hydroxide reaction yields a 60:40 mixture of diastereomers is curious. A possible explanation is that in

67

base the lactone (202) is hydrolysed to the bromohydrin (222) which

cyclises to the epoxide (223). This epoxide is then opened to give the

diol (224), which cyclises to the isocoumarin (225) on acidification

(Scheme 3.15). The two inversions in this mechanism would be

equivalent to retention of stereochemistry. The cis diastereomer

(177) is formed by a straight S_N2 reaction.
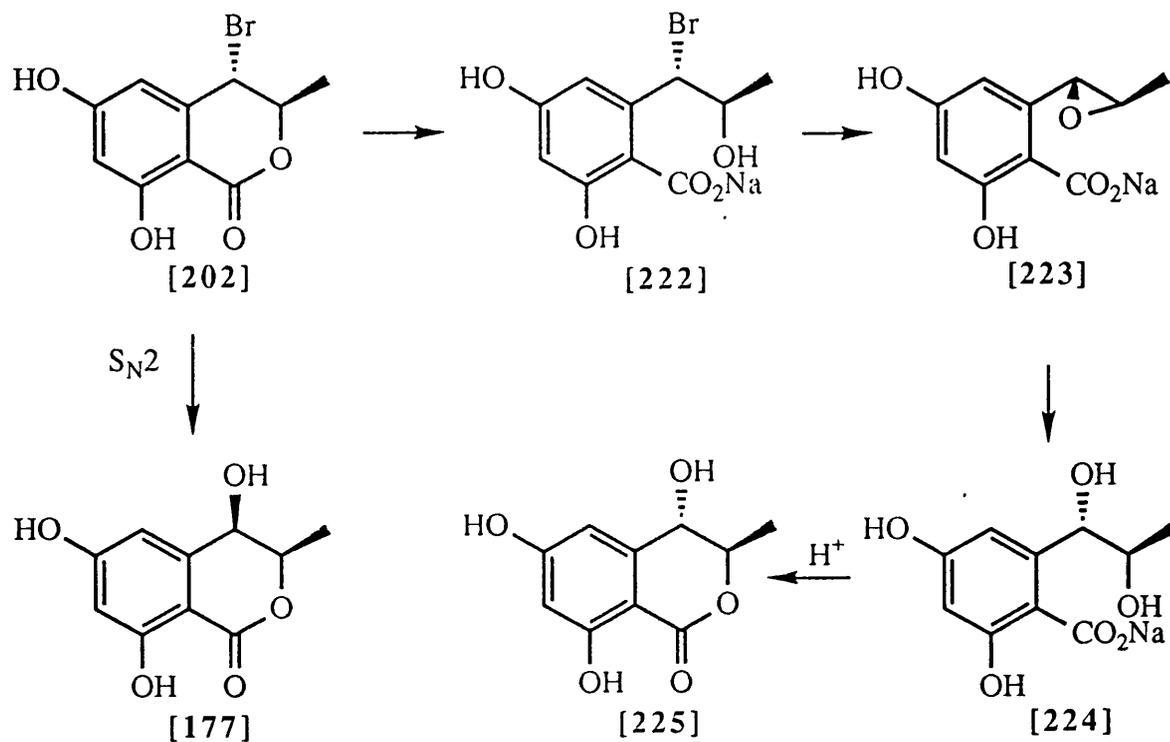
Scheme 3.15

In order to study the generality of this route to

4-hydroxydihydroisocoumarins and to further investigate the

stereochemistry we decided to attempt to synthesise perimacol (226).

Perimacol is a metabolite of Periconia macrospinosa first isolated by

Henderson.[130] He assigned it the cis stereochemistry at the

3,4-positions, but Macaulay later showed that it was in fact the trans

isomer.[104]

[226]
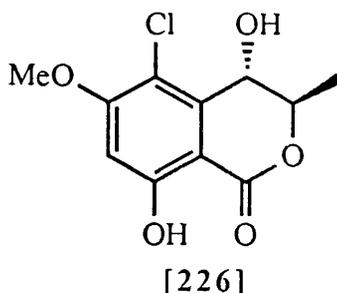
The methyl ether (200) was chlorinated at the 5-position using sulphuryl chloride. The substitution pattern in the product (227) was confirmed by examining the ^{13}C nmr signal of the chlorine-bearing carbon, which appeared at 113 ppm, in agreement with prediction. The 7-chloro isomer (228) would show a singlet at ~104 ppm.

[227]

[228]
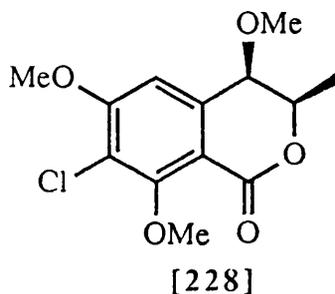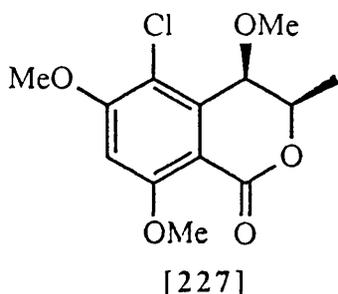
In order to cleave the 8-methyl ether only, aluminium chloride was required for the deprotection. However this reaction yielded a mixture of three products (229), (230) and (231).

Apparently the most reactive methyl ether, _peri_ to the carbonyl group, is cleaved first (since this is absent in all three products). Then the benzyl methyl ether is cleaved, possibly assisted by complexation of the Lewis acid to the aromatic chlorine. Finally some loss of HCl occurs to give the alkene (230).

Interestingly, the dichloride (231) was obtained as a 50:50 mixture of diastereomers, in contrast to the bromide obtained from the boron tribromide demethylation. It could be that the change in conditions for the aluminium chloride reaction (more polar solvent, higher temperature) caused a change in mechanism from S_N2 to S_N1. This would also explain the formation of the isocoumarin (230), by loss

of a proton from the intermediate carbonium ion.

[229]

[230]

[231]
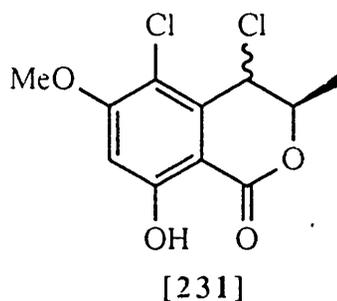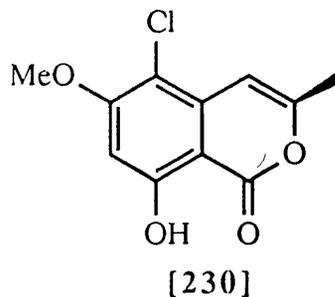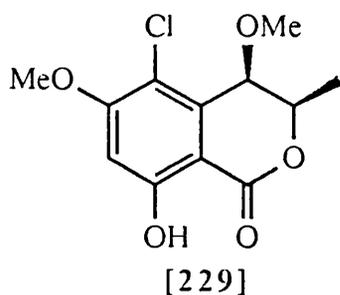
To investigate what role the aromatic chlorine might have played, the aluminium chloride reaction was carried out in its absence. This time no chloride was obtained, only methyl ether (232) and olefin (7) in a 1:2 ratio.

[200]   AlCl₃   [232]   +   [7]
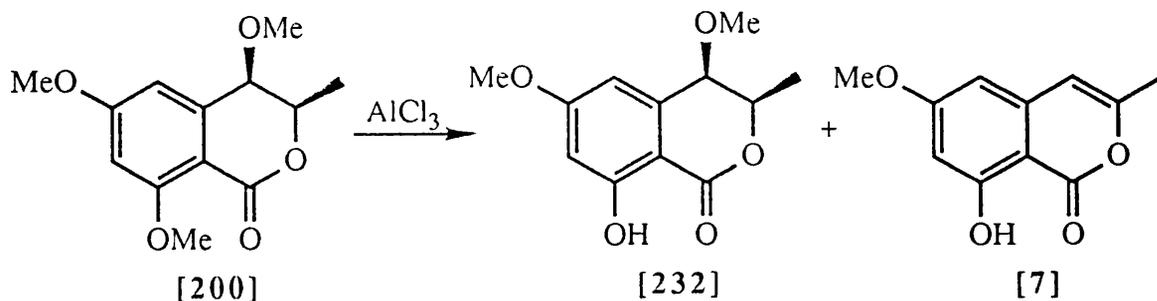
Whatever the case, the aluminium chloride reaction was never going to be a viable step in a synthesis of perimacol, due to the low yield of the dichloride (30%) and the lack of diastereoselectivity. What it did suggest was that the two most reactive methyl ethers might be selectively cleaved by using two equivalents of boron tribromide. This indeed turned out to be the case.

[200]   BBr₃   [233]
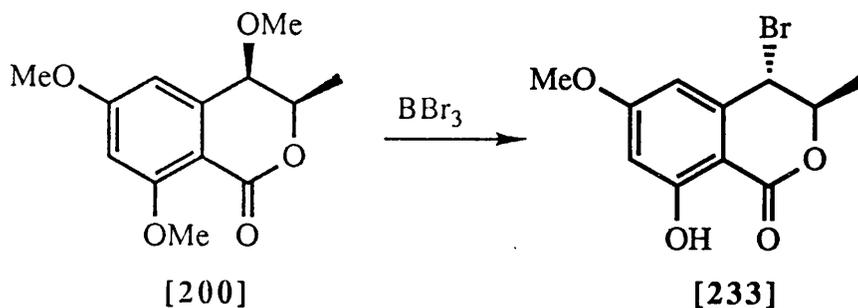
The yield was pretty good (56%) and the reaction gave one diastereomer only. Chlorination of the product was successful, but the silver nitrate catalysed hydrolysis would not take place. Presumably the aromatic chlorine hindered the reaction.

The hydrolysis of the bromide (233) was successful, and chlorination with sulphuryl chloride produced the cis diastereomer of perimacol (235) (Scheme 3.16).

[233]   [234]   [235]
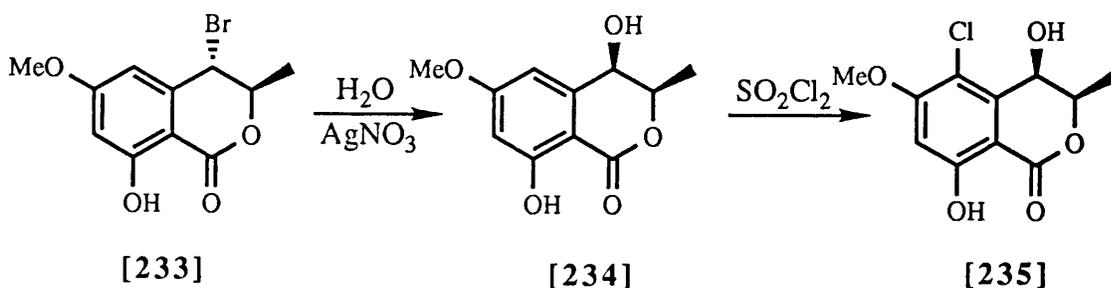
Scheme 3.16

Our final task was to oxidise the alcohol (177) to the target natural product (3).

[177]   [3]
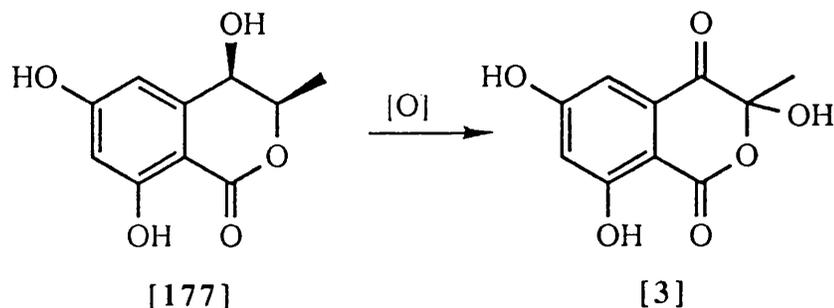
Initially we attempted to oxidise both the benzylic alcohol and the lactone alcohol using an aqueous acidic or basic oxidising agent. However, alkaline permanganate, chromic acid and Jones reagent were all

far too harsh for the triol (177). Next we attempted to selectively

oxidise the benzyl alcohol using manganese dioxide or silver carbonate

on celite, but both of these reagents yielded only starting material.

Even Swern oxidation proved too harsh for our compound, and the

best result which we obtained was a low yield of the ketone (236) using

pyridinium chlorochromate.[131]

[177]   [236]
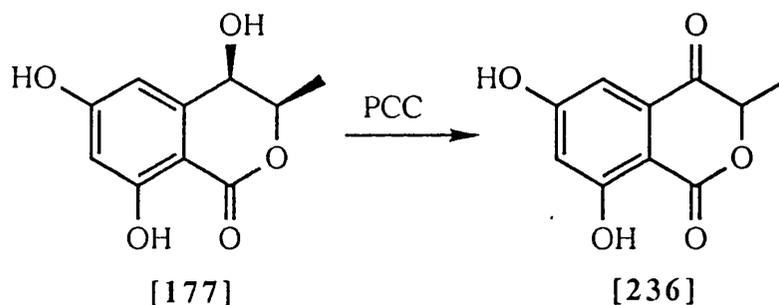
It would appear that the free phenolic groups are the stumbling

block to successful oxidation. They will probably have to be protected

before the oxidation.

3.3 Synthesis of 5-Methylmellein

5-Methylmellein (178) had previously been synthesised by a very

long and laborious route (Scheme 3.17).[132] The homophthalate (242) was

prepared by oxidative cleavage of the appropriate indanone (240).

Reaction of the half acid chloride with diethyl malonate, followed by

hydrolysis, decarboxylation and hydrogenation completed the synthesis.
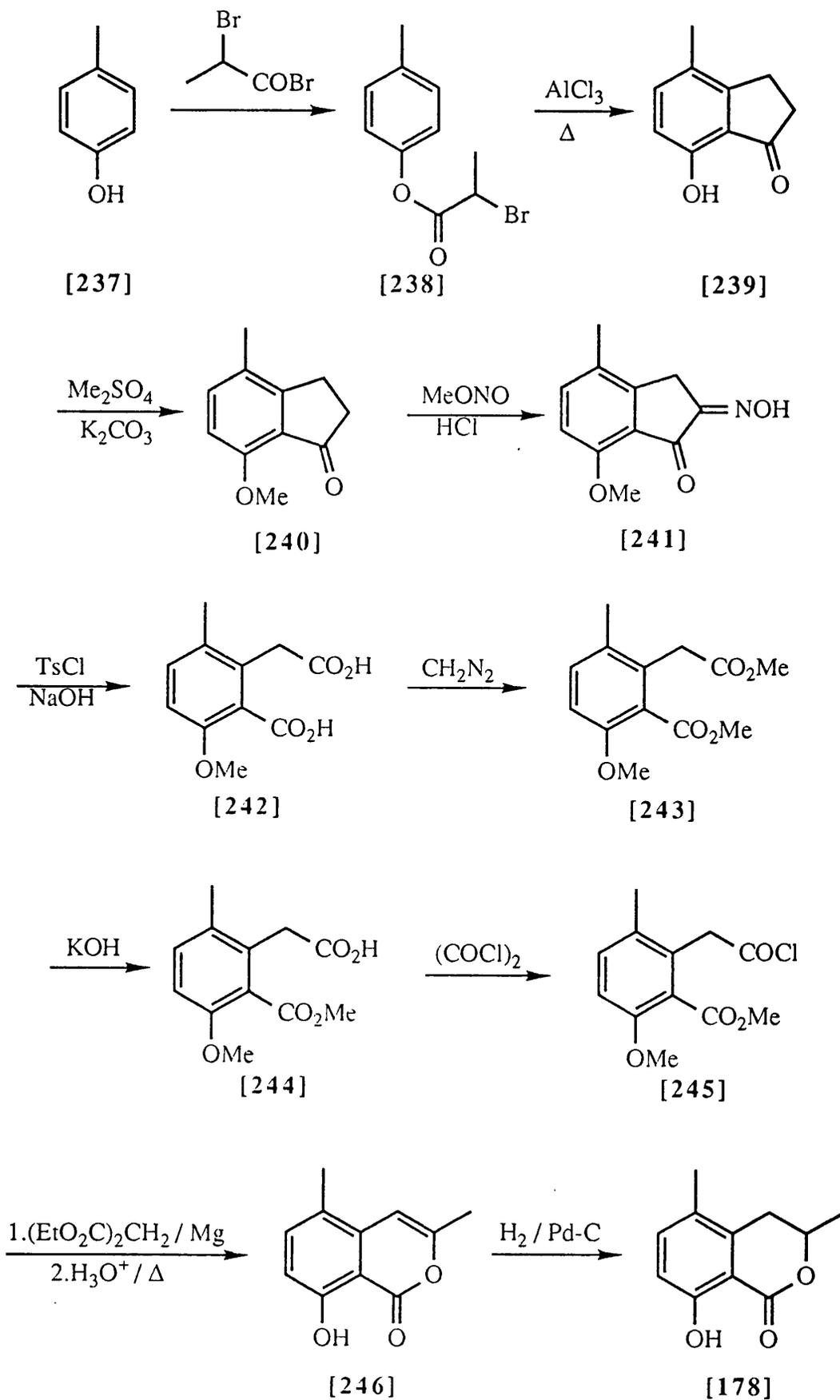
Scheme 3.17

We decided to develop a far shorter synthesis of this compound. The methods used by us previously, based on the homophthalic acid approach, were of no use here, since the required bromination would take place at the wrong position.[133]

[247]   [248]
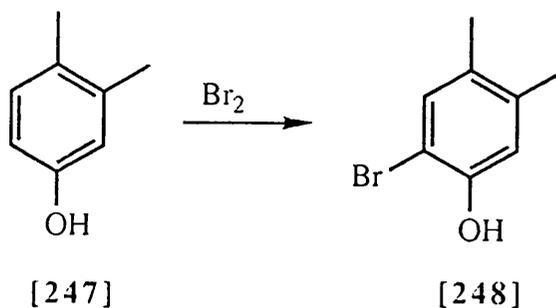
In this instance the indanone approach was more attractive. The completed synthesis is shown below (Scheme 3.18).

[237]   [238]   [239]

[240]   [249]

[250]   [178]
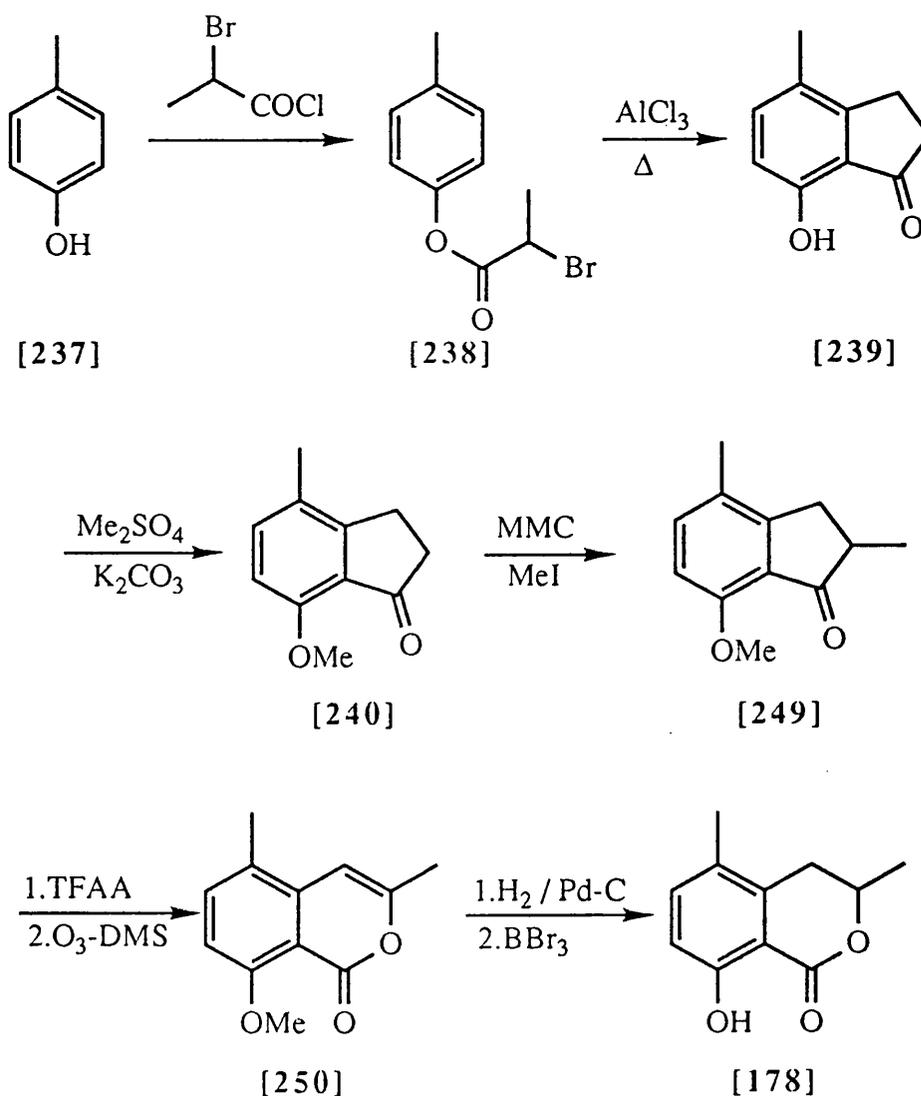
Scheme 3.18

The indanone (239) was made by the route used in the original synthesis.[134]   2-Bromopropanoyl chloride (252) was prepared by chlorination of propanoic acid (251) with thionyl chloride followed by Hell-Volhard-Zelinsky bromination using bromine and red phosphorus.[135]

$$\text{[251]} \qquad \xrightarrow[\text{2.Br}_2\text{/P}]{\text{1.SOCl}_2} \qquad \text{[252]}$$
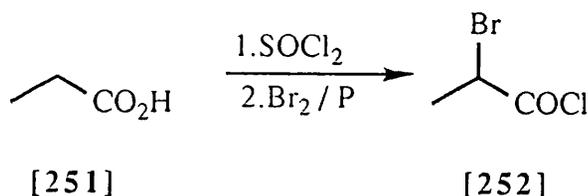
The acid chloride (252) reacted with p-cresol (237) yielding the bromoester (238).   This underwent a Fries rearrangement on heating with aluminium chloride, giving only the ortho product since the para position is blocked.   The immediate product (253) lost HBr and then Nazarov cyclisation gave the indanone (239) as the ultimate product (Scheme 3.19).

[238]   [253]   [254]   [239]
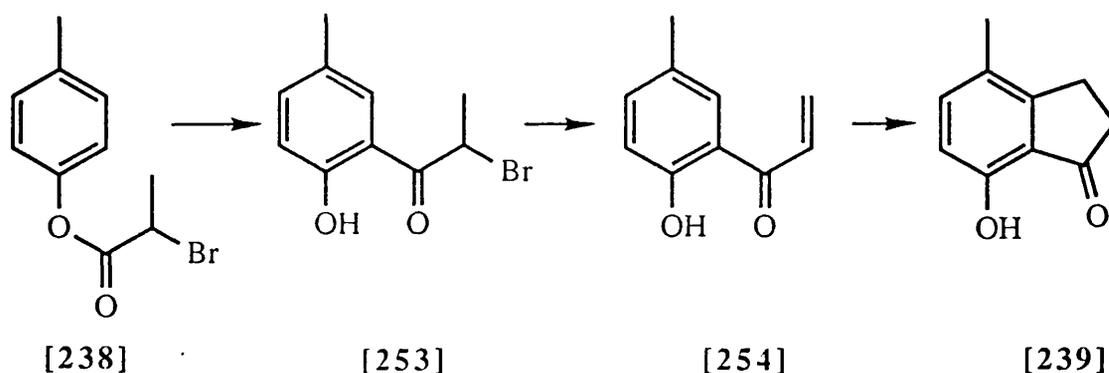
Scheme 3.19

The yield of the indanone (239) was not very high (21%) and it required a good deal of purification.   However this was tolerable since this was only the second step and the reagents were very cheap.   The phenol was protected in the usual way using dimethyl sulphate and potassium carbonate.

Attempted methylation of the indanone (240) alpha to the carbonyl group using LDA/methyl iodide resulted in significant dialkylation. This problem was overcome using magnesium methyl carbonate as base, which

carboxylates first so that only monoalkylation can occur <u>via</u> a stable magnesium enolate (255).[136] The β-ketoacid decarboxylated spontaneously on acidification. Any unreacted starting material could be recycled (Scheme 3.20).
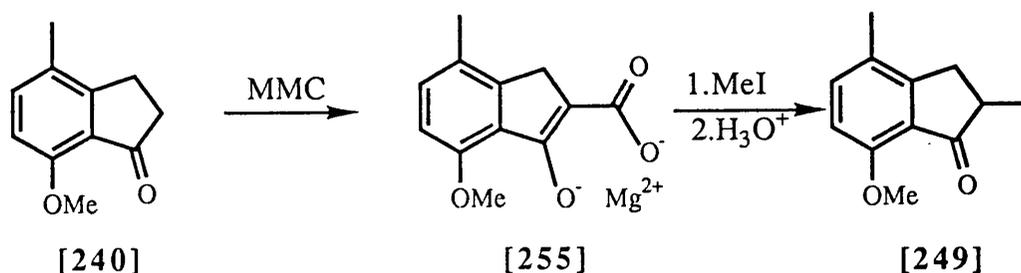
Scheme 3.20

Oxidative cleavage of the indanone (249) was achieved by ozonolysis of the enol trifluoroacetate. Hydrogenation over a palladium catalyst and demethylation with boron tribromide finished the synthesis.

3.4 Synthesis of 3-Hydroxymethyl Isocoumarins

Since the enolate of an indanone can react with a range of electrophiles, the indanone approach provides a versatile method for functionalising the 3-position of the isocoumarin. In light of this, we anticipated that another of our targets (4) might be prepared by this route.

[4]
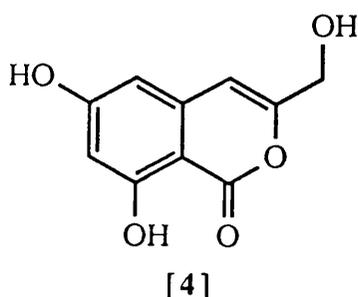
The required indanone (168) was obtained in the following manner (Scheme 3.21).[81] Knoevenagel condensation of 3,5-dimethoxy-benzaldehyde (171) with malonic acid followed by loss of water and carbon

dioxide produced the cinnamic acid (256) as exclusively the trans

isomer.   The product was hydrogenated to the propanoic acid (167)

which underwent smooth cyclisation in hot polyphosphoric acid.

MeO—CHO $\xrightarrow[\text{py / pip.}]{\text{CH}_2(\text{CO}_2\text{H})_2}$ MeO—CH=CH—CO$_2$H

[171]   [256]

$\xrightarrow{\text{H}_2 / \text{Pd-C}}$ MeO—CH$_2$CH$_2$—CO$_2$H $\xrightarrow{\text{PPA}}$ MeO—[indanone]—OMe O

[167]   [168]
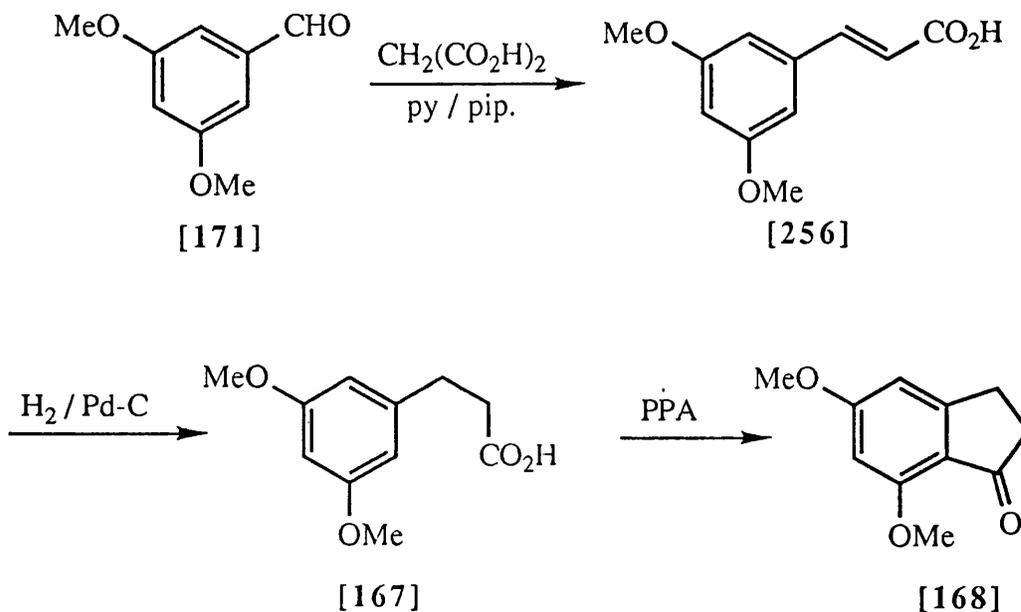
Scheme 3.21

Ideally the enolate of the indanone (168) should be reacted with

formaldehyde to add the desired hydroxymethylene group.   However

formaldehyde presents several problems as an electrophile.   First of

all it is a gas, although polymeric (paraformaldehyde) and trimeric

(trioxane) forms are available which are more suitable for synthesis.

Secondly it is very reactive and tends to add more than once to

ketones.   Finally, it is prone to Cannizzaro reactions, giving rise to

unwanted side products.

We decided instead to carry out a Claisen condensation using

sodium hydride and dimethyl carbonate.   This produced the β-ketoester

(257).

[168]   [257]
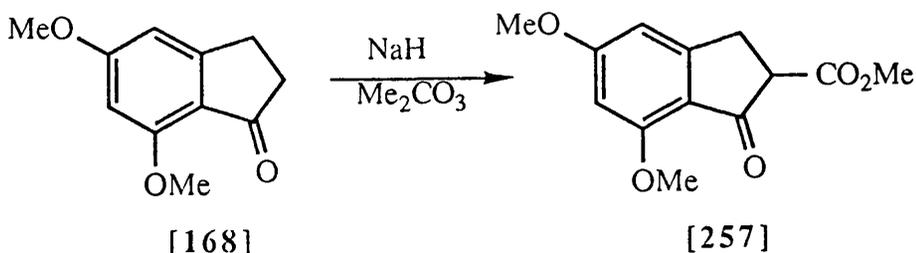
Our original plan from here had been to protect the ketone as its ethylene acetal to allow selective reduction of the ester group. This would furnish the desired hydroxymethylene group. Treatment of the β-ketoester (257) with ethylene glycol and p-toluenesulphonic acid under Dean-Stark conditions or with molecular sieves simply caused trans-esterification. Both the monomer (258) and the dimer (259) were obtained. Using boron trifluoride as the catalyst led to recovery of starting material.

[258]

[259]
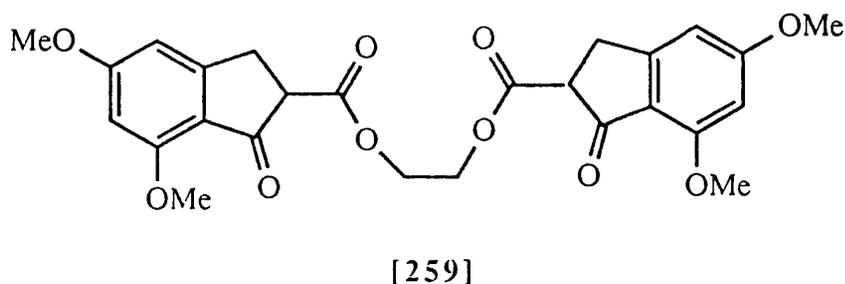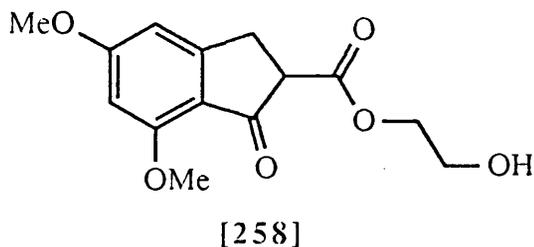
The unreactivity of the ketone could be due to steric effects, as the carbonyl group is fairly hindered. It could also be caused by conjugation with the phenyl and carboxyl groups which lowers the electrophilicity of the carbonyl carbon.

In view of this problem, we decided to reduce both the ketone and the ester. Then a selective oxidation of the benzyl alcohol or a selective protection of the primary alcohol could be used to obtain the

desired compound.   However, attempted reduction with either lithium

aluminium hydride or diisobutylaluminium hydride led to loss of most of

the material.   Only a mixture of products was recovered.

Our next approach was to reduce the ketone first with sodium

borohydride.   Dehydration would then allow the ester to be reduced.

Although this would mean the loss of the ketone, ozonolysis of the alkene

would be no more difficult.   In fact the dehydration occurred

spontaneously after the reduction, probably due to the extensive

conjugation in the product (260).
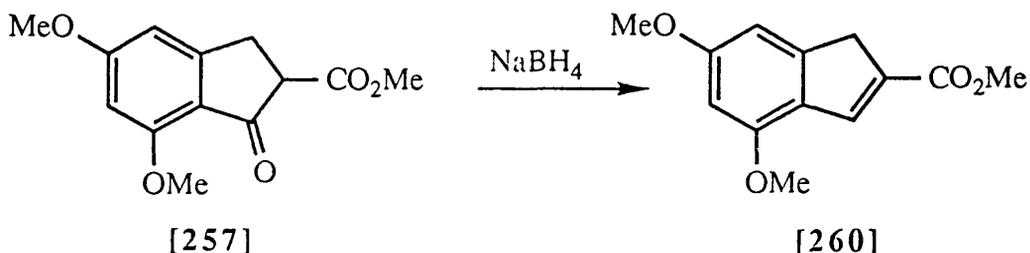
Although a white solid was initially obtained from the reaction,

it gradually turned a dark green colour.   Eventually, after chromatography,

the compound appears to stabilise but the yield is reduced.   The cause

of this green colour could not be determined.

The ester was reduced in good yield with diisobutylaluminium

hydride.   Lithium aluminium hydride was not used since it tends to also

reduce the double bond of β-phenylacrylic esters.   The primary alcohol

(261) was protected as its acetate (262) using acetic anhydride/pyridine

(Scheme 3.22).
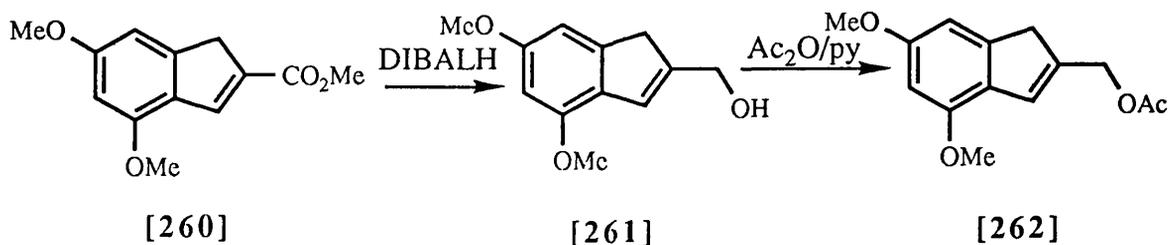
Scheme 3.22

Having at last reached a stage where the oxidative cleavage could be attempted, the ozonolysis failed. The mass spectrum and the ^{1}H nmr showed that extensive fragmentation had occurred, probably be ozonolysis of the aromatic ring. Ruthenium tetroxide also destroyed the molecule.

At this point we abandoned the indanone route altogether and considered the allylic functionalisation of our previously prepared isocoumarin (148).

[148]
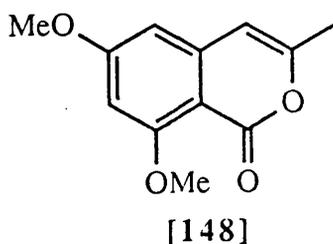
3-Formylisocoumarin (263) has been prepared in moderate yield by selenium dioxide oxidation of 3-methylisocoumarin (106).[137]

[106]   [263]
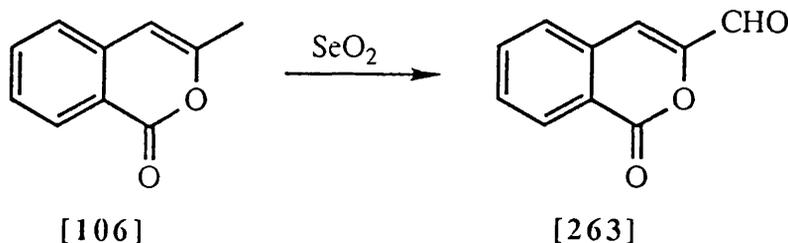
Selenium dioxide oxidation of our molecule gave not the aldehyde but the alcohol (264).

[148]   [264]
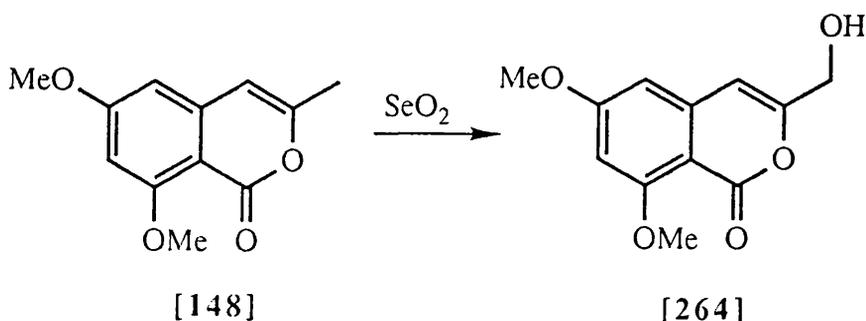
The yield was poor (~20%) but the bulk of the material was unreacted starting material which could be recycled.

Another report stated that 3-methylisocoumarin (106) could be brominated at the benzylic position with NBS and benzoyl peroxide.[138]

The yield was much better than that from the selenium dioxide reaction.
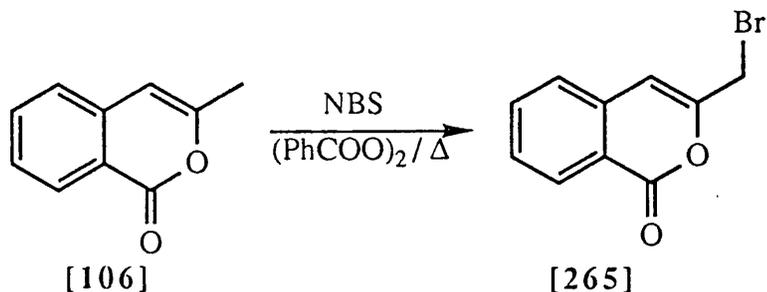
In fact the bromination of our compound was reported very recently to proceed in 80% yield.[139, 140]
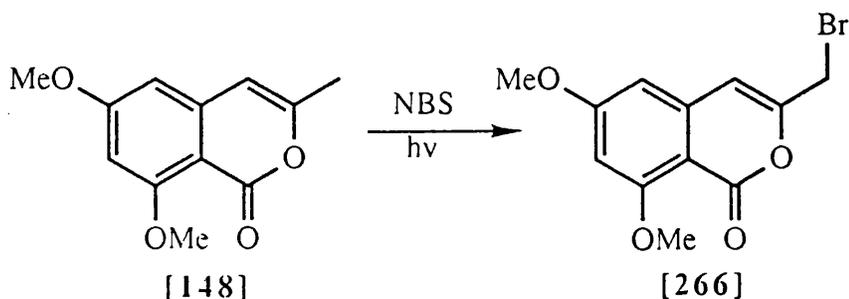
The far better yield in the bromination made this reaction more appealing for our synthesis than the selenium dioxide oxidation.   In any case, both compounds gave the same allylic bromide (267) on demethylation with boron tribromide (Scheme 3.23).
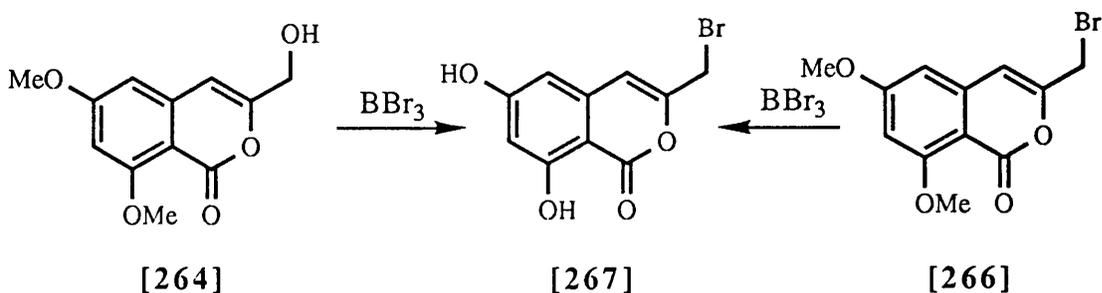
Scheme 3.23

Hydrolysis of the bromide (267) was achieved by refluxing it in aqueous tetrahydrofuran, without disturbing the lactone.   This completed the synthesis of our target compound (4).

[267]   [4]
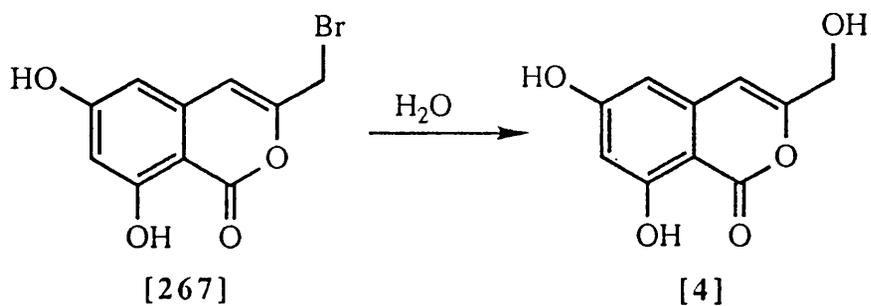
The 6-methyl ether of compound (4) has been isolated from the soil

bacteria, Streptoverticillium eurocidicum.[141]   This compound, known as

cytogenin (268), is a fairly potent anti-tumour agent and it may be

worth investigating the tumour inhibiting properties of our

3-substituted isocoumarins.

[268]
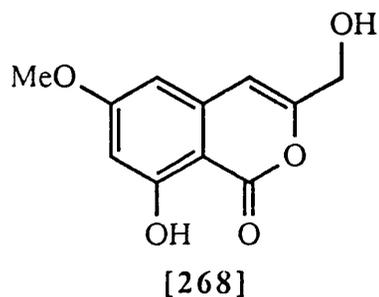
4 EXPERIMENTAL

General Procedure

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

Infra-red spectra were recorded for potassium bromide discs (unless otherwise stated) 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 measured at 200 MHz on a Bruker AM200 SY or a Bruker NP 200 SY spectrometer and at 90 MHz on a Perkin-Elmer R32 or a Varian EM390 spectrometer. Carbon nuclear magnetic resonance spectra were determined at 50 MHz on a Bruker AM200 SY or a Bruker WP200 SY spectrometer in the pulsed FT mode. DEPT editing was used to assist peak assignment. Spectra were recorded in deuteriochloroform (unless otherwise stated) with tetramethylsilane as internal standard or using residual undeuteriated solvent as reference. The following abbreviations are used: s - singlet, d - doublet, t - triplet, q - quartet, m - multiplet and br - broad.

Mass spectra were obtained with a MS12 or MS 902 mass spectrometer.

Flash chromatography was carried out on silica gel HF_{254} and TLC on 0.2 mm silica gel 60 F_{254}.

Tetrahydrofuran was freshly distilled from sodium/benzophenone. Dichloromethane was distilled from phosphorus pentoxide, acetone from anhydrous potassium carbonate and pyridine from potassium hydroxide pellets. Diethyl ether was dried over sodium wire.

Organic solutions were evaporated on a rotary evaporator under reduced pressure; solutions in organic solvents were dried over anhydrous magnesium sulphate.

3,5-Dimethoxytoluene[105]
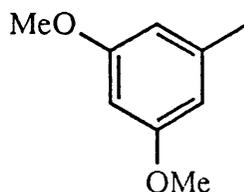
[180]

Orcinol hydrate (20 g, 0.14 mol.) dissolved in dry acetone (200 ml) with anhydrous potassium carbonate (88 g, 0.89 mol) and dimethyl sulphate (32 ml, 0.34 mol) was heated at reflux for 8 hours with stirring. After cooling the solution was filtered and the residue washed with acetone. The combined organic solutions were evaporated to give an oil which was dissolved in ether (200 ml). The organic layer was washed with ammonia solution (10%, 4 x 50 ml), sodium hydroxide solution (10%, 3 x 50 ml) and water (3 x 50 ml). The organics were dried and evaporated to give a red oil which was purified by distillation under reduced pressure (b.p. 38°C/0.1 mm Hg, lit.[105] 111-112°C/13 mm Hg) to give a colourless oil (20.6 g, 96%) $v_{max.}$ (film) 1 600 s, 1 210 s and 1 150 s cm^{-1}; δ_H 2.32 (3 H, s, Me), 3.78 (6 H, s, 2 x OMe), 6.31 (1 H, t, J 2 Hz, Ar-H), 6.35 (2 H, d, J 2 Hz, 2 x Ar-H); δ_C 21.8 (q, Me), 55.1 (q, 2 x OMe), 97.4 (d, C-4), 107.0 (d, C-2, C-6), 140.1 (s, C-1), 160.7 (s, C-3, C-5); Found M$^+$, 152.0833, $C_9H_{12}O_2$ requires M, 152.0837.

2-Bromo-3,5-dimethoxytoluene[105]
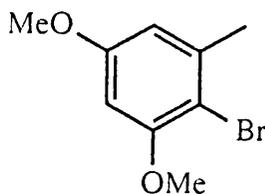
[181]

Bromine (0.8 ml, 0.016 mol) in 1,2-dichloroethane (5 ml) was

added with stirring over 5 minutes to a solution of 3,5-dimethoxy-toluene (2.4 g, 0.016 mol) in 1,2-dichloroethane (25 ml). Stirring was continued for a further 20 minutes at room temperature. The organic phase was washed with water (3 x 20 ml), dried and evaporated to give an oil which crystallised on standing. The product was recrystallised from methanol to yield the desired compound as white needles (2.9 g, 80%), m.p. 54-56°C (lit.[105] 56-58°C); $\nu_{max.}$ 1 590 s, 820 s and 610 s cm^{-1}; δ_H 2.37 (3 H, s, Me), 3.77 (3 H, s, OMe), 3.84 (3 H, s, OMe), 6.33 (1 H, d, $\underline{J}$ 2.7 Hz, Ar-H), 6.41 (1 H, d, $\underline{J}$ 2.7 Hz, Ar-H); δ_C 23.5 (q, Me), 55.4 (q, OMe), 56.2 (q, OMe), 97.1 (d, C-4), 105.0 (s, C-2), 107.1 (d, C-6), 139.7 (s, C-1), 156.5 (s, C-5), 159.2 (s, C-3); Found M$^+$, 231.9932, $C_9H_{11}O_2Br$ requires M, 231.9923.

2,6-Dibromo-3,5-dimethoxytoluene - m.p. 168°C (lit.[105] 171-172°C); $\nu_{max.}$ 1 580 s, 800 s and 680 m cm^{-1}; δ_H 2.60 (3 H, s, Me), 3.88 (6 H, s, 2 x OMe), 6.40 (1 H, s, Ar-H); δ_C 24.1 (q, Me), 56.5 (q, 2 x OMe), 94.7 (d, C-4), 105.6 (s, C-2, C-6), 139.1 (s, C-1), 155.7 (s, C-3, C-5); Found M$^+$ 309.9019, $C_9H_{10}O_2Br_2$ requires M, 309.9029.

<u>2,4-Dimethoxy-6-methylbenzoic Acid</u>
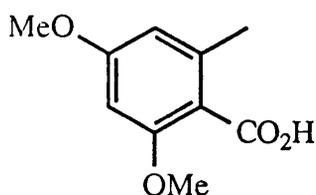
[159]

<u>n</u>-Butyllithium (54.6 ml/1.6 M in hexanes, 0.087 mol) was added to a stirred solution of 2-bromo-3,5-dimethoxytoluene (20 g, 0.087 mol) in dry THF (500 ml) at -78°C under nitrogen. The solution was stirred at this temperature for a further 15 minutes and then poured onto an excess of crushed dry ice. The solution was allowed to warm to room

temperature and water (200 ml) was added.   The organic phase was

evaporated and the aqueous solution washed with ether (3 x 50 ml).

Acidification with dilute hydrochloric acid, followed by extraction

with ethyl acetate (3 x 50 ml), drying and evaporating yielded the

desired acid.   The crude product was crystallised from dichloromethane/

hexane as white cubes (14.0 g, 83%), m.p. 139-141°C (lit.[130] 140°C);

$\nu_{max.}$ 2 920 br and 1 680 s cm^{-1}; δ_H 2.52 (3 H, s, Me), 3.82 (3 H, s,

OMe), 3.91 (3 H, s, OMe), 6.36 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H), 6.40 (1 H, d,

$\underline{J}$ 2.3 Hz, Ar-H); δ_C 22.3 (q, Me), 55.4 (q, OMe), 56.4 (q, OMe), 96.4

(d, C-3), 108.8 (d, C-5), 112.5 (s, C-1), 143.4 (s, C-6), 159.5 (s, C-2),

162.2 (s, C-4), 169.2 (s, CO$_2$H);   Found M$^+$ 196.0726, C$_{10}$H$_{12}$O$_4$ requires M,

196.0735.

3,5-Dimethoxyhomophthalic Acid[100]

[160]
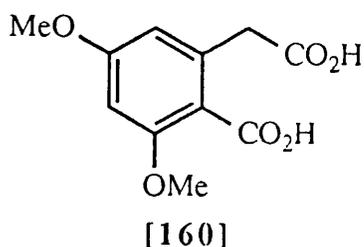
$\underline{n}$-Butyllithium (152 ml/1.6 M in hexanes, 0.24 mol) was added to a

solution of diisopropylamine (34.1 ml, 0.24 mol) in dry THF (75 ml) under

nitrogen at 0°C with stirring.   After 10 minutes the solution was

cooled to -78°C and a solution of 2,4-dimethoxy-6-methylbenzoic acid

(12 g, 0.06 mol) and dimethyl carbonate (12.3 ml, 0.15 mol) in dry THF

(150 ml) was added dropwise over half an hour.   The cooling bath was

removed and the solution allowed to warm to room temperature.   After 4

hours, water (100 ml) was added and the suspension stirred overnight.

The organic solvents were removed by evaporation and the resulting

aqueous solution washed with ether (2 x 50 ml).   After acidification,

the solution was extracted with ethyl acetate (3 x 50 ml).   The

combined extracts were dried and evaporated to give a white solid.

This was recrystallised from acetone/hexane as colourless needles

(11.1 g, 76%), m.p. 172-173°C (lit.[100] 171-173°C); $V_{max.}$ 3 200 br,

1 720 s and 1 700 s cm^{-1}; δ_H (d$_6$-acetone) 3.77 (2 H, s, CH$_2$), 3.83

(3 H, s, OMe), 3.87 (3 H, s, OMe), 6.55 (1 H, d, $\underline{J}$ 2.5 Hz, Ar-H), 6.58

(1 H, d, $\underline{J}$ 2.5 Hz, Ar-H); δ_C (d$_6$-DMSO) 38.8 (t, CH$_2$), 55.4 (q, OMe),

55.8 (q, OMe), 97.2 (d, C-4), 108.0 (d, C-6), 117.4 (s, C-2), 134.9

(s, C-1), 157.8 (s, C-3), 160.7 (s, C-5), 168.2 (s, CO$_2$H), 171.8 (s,

CO$_2$H);   Found M$^+$ 240.0620, C$_{11}$H$_{12}$O$_6$ requires M, 240.0634.

4-Hydro-3-hydroxy-6,8-dimethoxy-3-methylisocoumarin

[185]
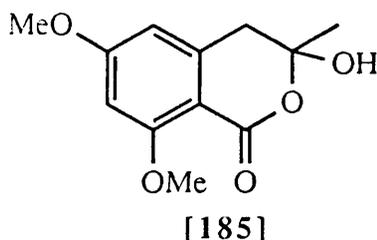
3,5-Dimethoxyhomophthalic acid (1.5 g, 6.25 mmol) was added

portionwise to a mixture of acetic anhydride (3 ml, 31.8 mmol) and dry

pyridine (0.75 ml, 9.3 mmol) at such a rate that it all dissolved.

After 5 minutes stirring, when a thick precipitate started to form,

dry ether (15 ml) was added, and stirring continued for 2 hours.

Sodium hydroxide solution (125 ml, 4 M) was cautiously added and the

mixture refluxed until all the solid material had dissolved.   The

solution was cooled, washed with an equal volume of dichloromethane,

then carefully acidified.   The acidic solution was extracted with

ethyl acetate (3 x 50 ml), dried and evaporated and the product

recrystallised from acetone (1.12 g, 75%), m.p. 141-143°C (lit.[24]

139-140°C); $\nu_{max.}$ 3 360 s and 1 690 s cm^{-1}; δ_H (d$_6$-acetone) 2.12

(3 H, s, Me), 3.84 (6 H, s, 2 x OMe), 3.89 (2 H, s, CH$_2$), 6.49 (1 H, d,

$\underline{J}$ 2 Hz, Ar-H), 6.61 (1 H, d, $\underline{J}$ 2 Hz, Ar-H); Found M$^+$ 238.0835,

C$_{12}$H$_{14}$O$_5$ requires M, 238.0841.

6,8-Dimethoxy-3-methylisocoumarin[102]
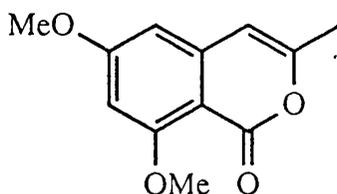
[148]

Perchloric acid (0.05 ml/72%, 0.36 mmol) was added to a portion of

ethyl acetate (50 ml). 10 ml of this solution was then added to a

mixture of ethyl acetate (30 ml) and acetic anhydride (4.8 ml, 50 mmol)

and the solution made up to 50 ml with further ethyl acetate. To this

reagent was added 4-hydro-3-hydroxy-6,8-dimethoxy-3-methylisocoumarin

(500 mg, 2.1 mmol) and the flask allowed to stand at room temperature

for 10 minutes. The solution was then washed with water (3 x 50 ml)

and sodium bicarbonate solution (3 x 30 ml), dried and evaporated.

The crude isocoumarin was purified by flash chromatography (ethyl acetate

as eluent) and recrystallised from ethanol (330 mg, 71%), m.p. 156-157°C

(lit.[80] 157-158°C); $\nu_{max.}$ 1 710 s cm^{-1}; δ_H 2.18 (3 H, d, $\underline{J}$ 0.9 Hz,

Me), 3.85 (3 H, s, OMe), 3.92 (3 H, s, OMe), 6.05 (1 H, q, $\underline{J}$ 0.9 Hz,

H-4), 6.26 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H), 6.38 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H);

δ_C 19.4 (q, Me), 55.5 (q, OMe), 56.2 (q, OMe), 98.1 (d, C-7), 99.3 (d,

C-5), 102.7 (s, C-8a), 103.6 (d, C-4), 142.4 (s, C-4a), 155.4 (s, C-3),

159.6 (s, C-8), 163.2 (s, C-6), 165.3 (s, C-1); Found M$^+$ 220.0727,

C$_{12}$H$_{12}$O$_4$ requires M, 220.0735.

6,8-Dihydroxy-3-Methylisocoumarin[107]
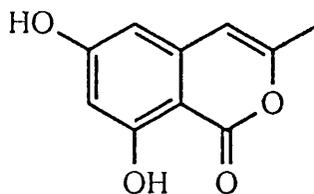
[5]

6,8-Dimethoxy-3-methylisocoumarin (110 mg, 0.5 mmol) in dry dichloromethane (10 ml) was cooled to -78°C and boron tribromide (1 ml, 10.6 mmol) was added, under nitrogen with stirring. The mixture was allowed to warm to room temperature overnight, then diluted with ether (20 ml) and water (20 ml). The organic layer was separated, washed with water (3 x 20 ml), dried and evaporated. The product was sublimed (200°C/0.2 mm Hg, lit.[107] 180°C/0.05 mm Hg) to a white powder (80 mg, 83%), m.p. 250-253°C (lit.[107] 250-252°C); $V_{max.}$ 3 250 br and 1 680 s cm^{-1}; δ_H (d$_6$-acetone) 2.21 (3 H, d, $\underline{J}$ 0.99 Hz, Me), 6.36 (3 H, m, 2 x Ar-H and CH), 11.13 (1 H, br s, OH); δ_C (d$_6$-acetone) 19.2 (q, Me), 99.6 (s, C-8a), 102.1 (d, C-7), 103.1 (d, C-5), 105.0 (d, C-4), 141.0 (s, C-4a), 155.3 (s, C-3), 164.6 (s, C-8), 166.3 (s, C-6), 167.0 (s, C-1); Found M$^+$ 192.0415, $C_{10}H_8O_4$ requires M, 192.0422.

8-Hydroxy-6-methoxy-3-methylisocoumarin

[7]
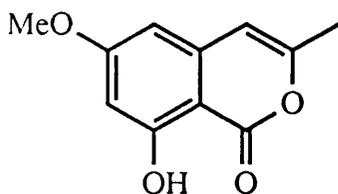
Aluminium chloride (300 mg, 2.25 mmol) was added to a solution of 6,8-dimethoxy-3-methylisocoumarin (100 mg, 0.45 mmol) in freshly distilled nitrobenzene (8 ml). The solution was stirred at 50-60°C for 6 hours,

then poured into ice-water and acidified with dilute hydrochloric acid. The acidic solution was extracted with ether (3 x 20 ml), then the combined organic solutions were extracted with aqueous sodium hydroxide (10%, 2 x 10 ml). This basic solution was washed with ether (20 ml), acidified, and finally extracted with ether (3 x 20 ml). The last extract was dried and evaporated to give a white powder which was recrystallised from ether/hexane as needles (63 mg, 67%), m.p. 125-127°C (lit.[33] 129°C); $\nu_{max.}$ 3 450 br and 1 680 s cm^{-1}; δ_H 2.24 (3 H, d, $\underline{J}$ 0.9 Hz, Me), 3.85 (3 H, s, OMe), 6.16 (1 H, q $\underline{J}$ 0.9 Hz, H-4), 6.27 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H), 6.42 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H), 11.08 (1 H, s, OH); δ_C 19.4 (q, Me), 55.6 (q, OMe), 99.7 (s, C-8a), 100.2 (d, C-7), 100.9 (d, C-5), 104.6 (d, C-4), 139.4 (s, C-4a), 154.3 (s, C-3), 163.5 (s, C-8), 166.3 (s, C-6), 166.8 (s, C-1); Found M$^+$ 206.0570, $C_{11}H_{10}O_4$ requires M, 206.0580.

3,4-Dihydro-6,8-dimethoxy-3-methylisocoumarin[107]
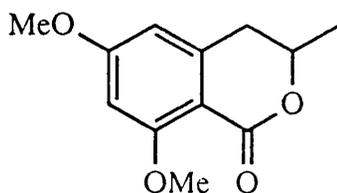
[186]

Palladium on charcoal (10%, 80 mg) was added to a stirred solution of 6,8-dimethoxy-3-methylisocoumarin (230 mg, 1.0 mmol) in ethyl acetate (40 ml) and the mixture hydrogenated overnight at room temperature. The catalyst was removed by filtering through celite and the solvent evaporated to leave an oil which crystallised on standing. The crude product was recrystallised from ethyl acetate/ hexane (220 mg, 95%), m.p. 108-110°C (lit.[107] 125-126°C); $\nu_{max.}$ 1 710 s cm^{-1}; δ_H 1.41 (3 H, d, $\underline{J}$ 6.3 Hz, Me), 2.77 (2 H, m, CH$_2$), 3.81

(3 H, s, OMe), 3.87 (3 H, s, OMe), 4.46 (1 H, m, CH), 6.26 (1 H, d,

$\underline{J}$ 2.3 Hz, Ar-H), 6.36 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H); δ_C 20.6 (q, Me), 36.4

(t, C-4), 55.4 (q, OMe), 56.0 (q, OMe), 73.4 (d, C-3), 97.6 (d, C-7),

103.7 (d, C-5), 106.6 (s, C-8a), 143.8 (s, C-4a), 162.6 (s, C-8), 163.0

(s, C-6), 164.2 (s, C-1); Found M$^+$ 222.0891, $C_{12}H_{14}O_4$ requires M,

222.0892.

3,4-Dihydro-6,8-dihydroxy-3-methylisocoumarin[107]

[8]
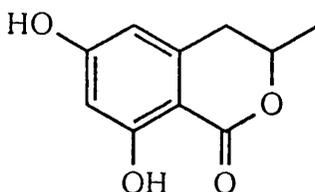
A solution of 3,4-dihydro-6,8-dimethoxy-3-methylisocoumarin

(184 mg, 0.83 mmol) in dry dichloromethane (15 ml) was cooled to -78°C

and boron tribromide (1 ml, 10.6 mmol) was added under nitrogen with

stirring. The mixture was allowed to warm to room temperature over

16 hours, diluted with ether (30 ml) and washed with water (3 x 30 ml).

The ethereal solution was dried and evaporated to give a powder which was

recrystallised from acetone/hexane as needles (102 mg, 63%), m.p.

220-221.5°C (lit.[107] 214-215°C); $v_{max.}$ 3 220 br and 1 650 s cm^{-1}; δ_H

(d_6-acetone) 1.44 (3 H, d, $\underline{J}$ 6.3 Hz, Me), 2.85 (1 H, dd, $\underline{J}$ 16 Hz, $\underline{J}^1$

11 Hz, H-4), 2.96 (1 H, dd, $\underline{J}$ 16 Hz, $\underline{J}^1$ 4.0 Hz, H-4), 4.69 (1 H, m, H-3),

6.25 (1 H, m, Ar-H), 6.29 (1 H, m, Ar-H), 11.29 (1 H, s, OH); δ_C

(d_6-acetone) 20.8 (q, Me), 35.0 (t, C-4), 76.3 (d, C-3), 101.7 (s, C-8a),

101.9 (d, C-7), 107.4 (d, C-5), 143.2 (s, C-4a), 165.1 (s, C-8), 165.2

(s, C-6), 170.7 (s, C-1); Found M$^+$ 194.0576, $C_{10}H_{10}O_4$ requires M,

194.0579.

3,4-Dihydro-8-hydroxy-6-methoxy-3-methylisocoumarin[101]
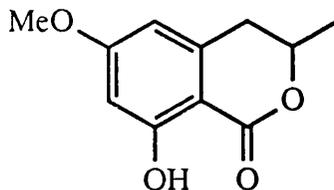
[28]

Aluminium chloride (600 mg, 4.5 mmol) was added to a solution of 3,4-dihydro-6,8-dimethoxy-3-methylisocoumarin (200 mg, 0.9 mmol) in freshly distilled nitrobenzene (16 ml). The solution was stirred at 50-60°C for 6 hours, then poured into ice-water and acidified with hydrochloric acid. The acidic solution was extracted with ether (3 x 40 ml) and the ethereal solution extracted with sodium hydroxide (10%, 2 x 40 ml). The basic solution was washed with ether (40 ml), acidified and extracted with ether (3 x 40 ml). The last extract was dried and evaporated to leave a brown solid which was recrystallised from ether/hexane as needles (152 mg, 87%), m.p. 95-97°C (lit.[101] 96°C); $\nu_{max.}$ 3 440 br and 1 660 s cm^{-1}; δ_H (d$_6$-acetone) 1.45 (3 H, d, $\underline{J}$ 6.3 Hz, Me), 2.88 (1 H, dd, $\underline{J}$ 18 Hz, $\underline{J}^1$ 11 Hz, H-4), 3.00 (1 H, dd, $\underline{J}$ 18 Hz, $\underline{J}^1$ 4.0 Hz, H-4), 3.85 (3 H, s, OMe), 4.71 (1 H, m, H-3), 6.37 (2 H, m, 2 x Ar-H), 11.33 (1 H, s, OH); δ_C (d$_6$-acetone) 20.8 (q, Me), 35.0 (t, C-4), 56.0 (q, OMe), 76.4 (d, C-3), 100.0 (d, C-7), 102.4 (s, C-8a), 106.6 (d, C-5), 142.8 (s, C-4a), 165.2 (s, C-8), 166.7 (s, C-6), 170.6 (s, C-1); Found M$^+$ 208.0730, $C_{11}H_{12}O_4$ requires M, 208.0736.

4-Hydro-3,6,8-trihydroxy-3-methylisocoumarin[108]
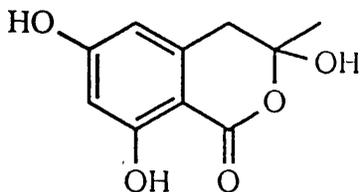
[2]

6,8-Dihydroxy-3-methylisocoumarin (70 mg, 0.36 mmol) was stirred

at 100°C for one hour with 0.1 M sodium hydroxide solution (20 ml, 2.0

mmol) under nitrogen.   The cooled solution was acidified to pH 3 with

dilute hydrochloric acid and extracted with ethyl acetate (3 x 20 ml),

dried and evaporated to leave an oil (63 mg, 82%); $V_{max.}$ 3 300 br and

1 700 s cm^{-1}; δ_H (d_6-acetone) 2.07 (3 H, s, Me), 3.51 (2 H, s, CH_2),

6.25 (2 H, br s, 2 x Ar-H);   Found M^+ 210.0521, $C_{10}H_{10}O_5$ requires M,

210.0548.

5-Bromo-4-hydro-3-hydroxy-6,8-dimethoxy-3-methylisocoumarin
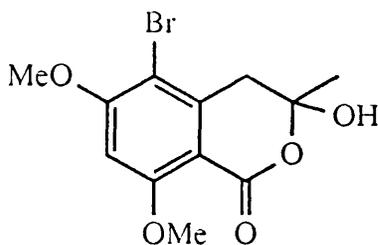
[190]

N-bromosuccinimide (74 mg, 0.42 mmol) in dichloromethane (10 ml)

was added dropwise over 5 minutes to a stirred solution of 4-hydro-3-

hydroxy-6,8-dimethoxy-3-methylisocoumarin (100 mg, 0.42 mmol) in

dichloromethane (10 ml) under irradiation by a 150 W lamp.   After 15

minutes, when all the solid had dissolved, the solvent was evaporated

and the crude product purified by flash chromatography (9:1

dichloromethane:methanol as eluent) as a white solid (102 mg, 76%);

$V_{max.}$ 3 340 br and 1 690 s cm^{-1}; δ_H (d_6-DMSO) 2.11 (3 H, s, Me),

2.55 (2 H, s, CH$_2$), 3.83 (3 H, s, OMe), 3.91 (3 H, s, OMe), 6.74 (1 H, s, Ar-H); δ_C (d$_6$-DMSO) 19.9 (q, Me), 48.3 (t, C-4), 56.6 (q, OMe), 57.0 (q, OMe), 96.7 (d, C-7), 100.4 (s, C-5), 105.6 (s, C-8a), 118.4 (s, C-3), 134.2 (s, C-4a), 156.8 (s, C-8), 157.3 (s, C-6), 168.4 (s, C-1); Found M$^+$ 317.9920, C$_{12}$H$_{13}$O$_5$Br requires M, 317.9927.

5-Bromo-3,4-dihydro-6,8-dimethoxy-3-methylisocoumarin
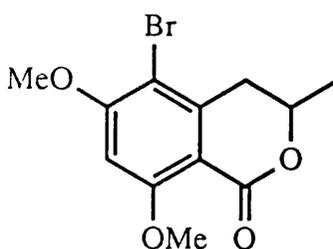
[191]

<u>N</u>-bromosuccinimide (50 mg, 0.28 mmol) was added to a stirred solution of 3,4-dihydro-6,8-dimethoxy-3-methylisocoumarin (50 mg, 0.23 mmol) and benzoyl peroxide (7 mg, 0.03 mmol) in carbon tetrachloride (15 ml) at reflux, under irradiation by a 150 W lamp. After half an hour the reaction was stopped and the solvent evaporated. The residue was dissolved in dichloromethane (20 ml), washed with sodium bicarbonate solution (3 x 10 ml), water (3 x 10 ml) and dried and evaporated. The product recrystallised from ethyl acetate/hexane as needles (52 mg, 77%), m.p. 143-145°C; $\nu_{max.}$ 1 710 s cm^{-1}; δ_H 1.50 (3 H, d, $\underline{J}$ 6.3 Hz, Me), 2.74 (1 H, dd, $\underline{J}$ 17 Hz, $\underline{J}^1$ 12 Hz, H-4), 3.23 (1 H, dd, $\underline{J}$ 17 Hz, $\underline{J}^1$ 2.8 Hz, H-4), 3.98 (3 H, s, OMe), 3.99 (3 H, s, OMe), 4.47 (1 H, m, H-3), 6.47 (1 H, s, Ar-H); δ_C 20.7 (q, Me), 36.5 (t, C-4), 56.4 (q, 2 x OMe), 73.0 (d, C-3), 95.2 (d, C-7), 102.5 (s, C-5), 108.0 (s, C-8a), 142.5 (s, C-4a), 160.4 (s, C-8), 162.2 (s, C-6), 162.5 (s, C-1); Found M$^+$ 301.9948, C$_{12}$H$_{13}$O$_4$Br requires M, 301.9978.

Methyl 3,5-Dimethoxybenzoate[112]

MeO — CO$_2$Me

OMe
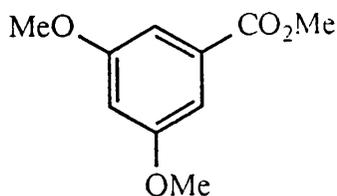
[164]

3,5-Dihydroxybenzoic acid (30 g, 0.19 mol) dissolved in dry acetone (300 ml) with anhydrous potassium carbonate (130 g, 1.3 mol) and dimethyl sulphate (60 ml, 0.62 mol) was stirred at reflux for 8 hours. After cooling, the solution was filtered and the residue washed with acetone. The combined acetone solutions were evaporated and the golden brown residue dissolved in ether (200 ml), washed with ammonia solution (10%, 3 x 50 ml), sodium hydroxide (10%, 3 x 50 ml) and water (3 x 50 ml). After drying and evaporating, the residue solidified on cooling and was recrystallised from methanol as white needles m.p. 42°C (lit.[112] 42°C); $\nu_{max.}$ 1 720 s cm^{-1}; δ_H 3.81 (6 H, s, 2 x OMe), 3.89 (3 H, s, CO$_2$Me), 6.63 (1 H, t, J 2.4 Hz, Ar-H), 7.17 (2 H, d, J 2.4 Hz, 2 x Ar-H); δ_C 52.2 (q, Me), 55.5 (q, 2 x OMe), 105.7 (d, C-4), 107.0 (d, C-2, C-6), 131.9 (s, C-1), 160.6 (s, C-3, C-5), 166.8 (s, CO); Found M$^+$ 196.0729, C$_{10}$H$_{12}$O$_4$ requires M, 196.0735.

3,5-Dimethoxybenzyl Alcohol

OH

MeO

OMe
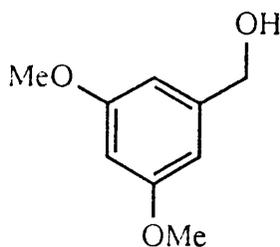
[194]

Methyl 3,5-dimethoxybenzoate (20 g, 0.10 mol) in dry

tetrahydrofuran (150 ml) was added dropwise to lithium aluminium

hydride (5 g, 0.13 mol) in tetrahydrofuran (80 ml) and the mixture

stirred at reflux for 8 hours.   After cooling, water (10 ml) was added

cautiously, followed by 15% sodium hydroxide solution (10 ml) and more

water (30 ml) with stirring.   The granular aluminium hydroxide was

filtered and washed with ether and the organic solutions evaporated.

The residue was dissolved in ether (100 ml), the aqueous layer

separated and the organic layer dried and evaporated.   The alcohol was

recrystallised from diisopropyl ether as needles (17.0 g, 99%), m.p.

46-47°C (lit.[112] 48-48.5°C); $\nu_{max.}$ 3 350 m cm^{-1}; δ_H 2.03 (1 H, br

s, OH), 3.77 (6 H, s, 2 x OMe), 4.60 (2 H, br s, CH$_2$), 6.36 (1 H, t, $\underline{J}$

2.3 Hz, Ar-H), 6.50 (2 H, d, $\underline{J}$ 2.3 Hz, 2 x Ar-H); δ_C 55.3 (q, 2 x OMe),

65.2 (t, CH$_2$), 99.6 (d, C-4), 104.5 (d, C-2, C-6), 143.4 (s, C-1),

160.9 (s, C-3, C-5);   Found M$^+$ 168.0781, C$_9$H$_{12}$O$_3$ requires M, 168.0786.

3,5-Dimethoxybenzyl Methyl Ether
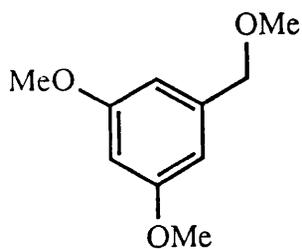
[195]

In a dry round bottom flask was placed sodium hydride (3.2 g/60%

dispersion in oil, 80 mmol).   The flask was purged with nitrogen and

dry tetrahydrofuran (60 ml) was added with a syringe.   With stirring,

the slurry was heated at 45-50°C in an oil bath and methyl iodide (9 ml,

145 mmol) was added.   3,5-Dimethoxybenzyl alcohol (7 g, 42 mmol) in

tetrahydrofuran (20 ml) was added dropwise over 10 minutes;   hydrogen

evolution accompanied the addition.   After a further 30 minutes

heating, the reaction mixture was cooled and hydrolysed by dropwise

addition of sufficient water to dissolve any precipitate.   The

aqueous layer was separated and extracted twice with ether (30 ml),

then the combined organic solutions were washed with brine (30 ml) and

dried and evaporated.   The product was purified by distillation

(80 °C/0.1 mm Hg); $\nu_{max.}$ (film) 1 600 s and 835 m cm^{-1}; δ_H 3.38 (3 H,

s, OMe), 3.78 (6 H, s, 2 x OMe), 4.39 (2 H, s, CH$_2$), 6.38 (1 H, t, $\underline{J}$

2.3 Hz, Ar-H), 6.49 (2 H, d, $\underline{J}$ 2.3 Hz, 2 x Ar-H); δ_C 55.3 (q, 2 x OMe),

58.1 (q, OMe), 74.6 (t, CH$_2$), 99.7 (d, C-4), 105.2 (d, C-2, C-6), 140.6

(s, C-1), 160.9 (s, C-3, C-5);   Found M$^+$ 182.0945, C$_{10}$H$_{14}$O$_3$ requires M,

182.0943.

2-Bromo-3,5-dimethoxybenzyl Methyl Ether
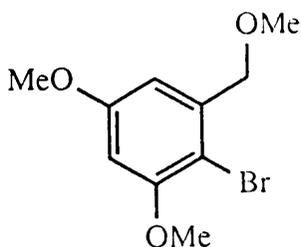
[196]

Recrystallised $\underline{N}$-bromosuccinimide (9.8 g, 55 mmol) was added in

small portions over 2 hours to a refluxing solution of 3,5-dimethoxy-

benzyl methyl ether (10 g, 55 mmol) in carbon tetrachloride (300 ml)

with stirring.   After a further hour the solution was cooled and the

solvent evaporated.   The residue was dissolved in dichloromethane

(200 ml), washed with sodium bicarbonate solution (2 x 50 ml), dried

and evaporated.   The bromide crystallised from methanol as cubes (12.67

g, 88%), m.p. 60-62 °C; $\nu_{max.}$ 1 590 s, 1 075 s and 865 m cm^{-1}; δ_H

3.45 (3 H, s, OMe), 3.79 (3 H, s, OMe), 3.84 (3 H, s, OMe), 4.49

(2 H, s, CH$_2$), 6.40 (1 H, d, $\underline{J}$ 2.8 Hz, Ar-H), 6.66 (1 H, d, $\underline{J}$ 2.8 Hz,

Ar-H); δ_C 55.5 (q, OMe), 56.2 (q, OMe), 58.6 (q, OMe), 73.9 (t, CH$_2$), 98.7 (d, C-4), 102.2 (s, C-2), 104.2 (d, C-6), 139.5 (s, C-1), 156.3 (s, C-5), 159.8 (s, C-3); Found M$^+$ 262.0021, C$_{10}$H$_{13}$O$_3$Br requires M, 262.0029.

2,4-Dimethoxy-6-methoxymethyl benzoic Acid
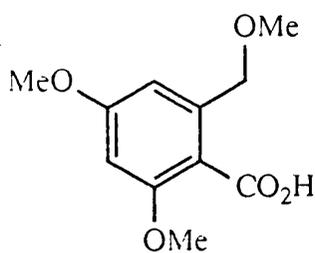
[197]

n-Butyllithium (10 ml/1.6 M in hexanes, 16 mmol) was added to a stirred solution of 2-bromo-3,5-dimethoxybenzyl methyl ether (2.3 g, 8.8 mmol) in THF (50 ml) at -78°C under nitrogen. The solution was stirred at this temperature for a further 15 minutes and then poured onto an excess of crushed dry ice. The solution was allowed to warm to room temperature and water (25 ml) added. The organic phase was evaporated and the aqueous layer acidified. This was then extracted with ether (3 x 25 ml) and the ethereal layer extracted with sodium hydroxide solution (10%, 2 x 30 ml). Finally the aqueous layer was acidified, extracted with ethyl acetate (3 x 40 ml), dried and evaporated to leave a brown solid. This was recrystallised from ethyl acetate/hexane as needles (1.64 g, 82%), m.p. 105°C; $V_{max.}$ 2 950 br and 1 660 s cm^{-1}; δ_H 3.47 (3 H, s, OMe), 3.87 (3 H, s, OMe), 3.97 (3 H, s, OMe), 4.81 (2 H, s, CH$_2$), 6.45 (1 H, d, J 2.4 Hz, Ar-H), 6.95 (1 H, d, J 2.4 Hz, Ar-H); δ_C 55.6 (q, OMe), 56.8 (q, OMe), 58.7 (q, OMe), 73.0 (t, CH$_2$), 97.7 (d, C-3), 104.7 (d, C-5), 109.3 (s, C-1), 146.5 (s, C-6), 159.7 (s, C-2), 163.4 (s, C-4),

166.3 (s, CO_2H); Found M^+ 226.0835, $C_{11}H_{14}O_5$ requires M, 226.0841.

α,3,5-Trimethoxyhomophthalic Acid
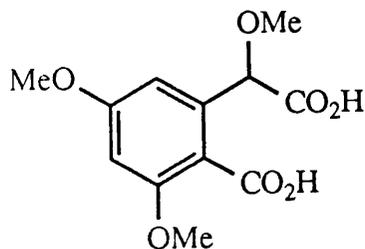
[198]

n-Butyllithium (43.9 ml/1.6 M in hexanes, 70 mmol) was added to a solution of diisopropylamine (9.85 ml, 70 mmol) in THF (25 ml) under nitrogen at 0°C with stirring.   After 10 minutes, the solution was cooled to -78°C and a solution of 2,4-dimethoxy-6-methoxymethylbenzoic acid (4 g, 18 mmol) and dimethyl carbonate (3.56 ml, 42 mmol) in THF (25 ml) was added dropwise over 10 minutes.   The cooling bath was removed and the solution allowed to warm to room temperature.   After 4 hours, water (75 ml) was added and the suspension stirred overnight. The organic solvents were evaporated and more water (75 ml) added.   The resulting aqueous solution was washed with ether (2 x 30 ml).   After acidification the solution was extracted with ethyl acetate (3 x 50 ml). The combined extracts were dried and evaporated to leave a yellow oil which solidified on standing.   This was recrystallised from dichloromethane/hexane as needles (3.98 g, 83%), m.p. 151-153°C; $v_{max.}$ 3 100 s and 1 740 s cm^{-1}; δ_H (d_6-acetone) 3.36 (3 H, s, OMe), 3.83 (3 H, s, OMe), 3.87 (3 H, s, OMe), 5.12 (1 H, s, CH), 6.63 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H), 6.69 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H); δ_C (d_6-acetone) 55.9 (q, OMe), 56.5 (q, OMe), 57.7 (q, OMe), 79.8 (d, CH), 99.2 (d, C-4), 104.8 (d, C-6), 116.6 (s, C-2), 139.1 (s, C-1), 159.5 (s, C-3), 163.0 (s, C-5), 169.2 (s, CO_2H), 170.7 (s, CO_2H); Found M^+ 270.0745,

$C_{12}H_{14}O_7$ requires M, 270.0740.

4,6,8-Trimethoxy-3-methylisocoumarin

[199]
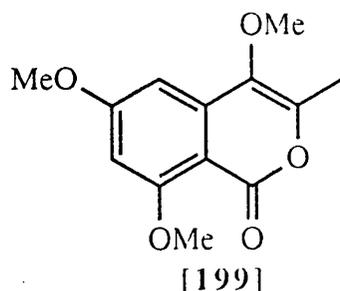
α,3,5-Trimethoxyhomophthalic acid (1 g, 3.7 mmol) was added to acetic anhydride (3 ml, 32 mmol) and pyridine (0.5 ml, 6.2 mmol) and the mixture refluxed for 2 hours. The acetic anhydride and pyridine were evaporated and water (50 ml) and ethyl acetate (50 ml) was added. The suspension was stirred for half an hour and the organic phase separated, dried and evaporated to give a brown solid. This was submitted to flash chromatography (ethyl acetate as eluent) and recrystallisation from ethyl acetate/hexane as plates (0.8 g, 86%), m.p. 125-126°C; $V_{max.}$ 1 730 s and 1 720 s cm^{-1}; δ_H 2.26 (3 H, s, Me), 3.74 (3 H, s, OMe), 3.92 (3 H, s, OMe), 3.96 (3 H, s, OMe), 6.45 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H), 6.60 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H); δ_C 14.4 (q, Me), 55.7 (q, OMe), 56.4 (q, OMe), 61.2 (q, OMe), 95.0 (d, C-7), 98.3 (d, C-5), 103.0 (s, C-8a), 136.1 (s, C-4), 139.5 (s, C-4a), 147.7 (s, C-3), 158.4 (s, C-8), 163.7 (s, C-6), 165.8 (s, C-1); Found M$^+$ 250.0854, $C_{13}H_{14}O_5$ requires M, 250.0841.

3,4-Dihydro-4,6,8-trimethoxy-3-methylisocoumarin

[200]
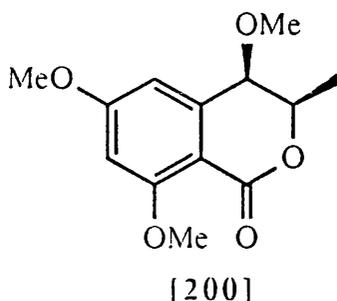
Palladium on charcoal (10%, 50 mg) was added to 4,6,8-trimethoxy-3-methylisocoumarin (500 mg, 2 mmol) in ethyl acetate (30 ml) and the mixture hydrogenated at room temperature for 3 days. The catalyst was removed by filtering through celite and the solvent evaporated. Recrystallisation from ethyl acetate/hexane gave needles (500 mg, 99%), m.p. 107°C; $\nu_{max.}$ 1 710 s cm^{-1}; δ_H 1.44 (3 H, d, $\underline{J}$ 6.6 Hz, Me), 3.33 (3 H, s, OMe), 3.88 (3 H, s, OMe), 3.92 (3 H, s, OMe), 4.06 (1 H, d, $\underline{J}$ 2.4 Hz, H-4), 4.56 (1 H, dq, $\underline{J}$ 2.4 Hz, $\underline{J}^1$ 6.6 Hz, H-3), 6.46 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H), 6.51 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H); δ_C 15.7 (q, Me), 55.6 (q, OMe), 56.2 (q, OMe), 56.7 (q, OMe), 75.0 (d, C-4), 76.4 (d, C-3), 99.0 (d, C-7), 104.8 (d, C-5), 106.0 (s, C-8a), 142.0 (s, C-4a), 161.5 (s, C-8), 163.0 (s, C-6), 164.1 (s, C-1); Found M$^+$, 252.1002, $C_{13}H_{16}O_5$ requires M, 252.0997.

A 2:1 cis:trans mixture was obtained in the following manner. A solution of 4,6,8-trimethoxy-3-methylisocoumarin (250 mg, 1 mmol) in aqueous sodium hydroxide (15%, 25 ml) was heated at reflux for an hour. Sodium borohydride (50 mg, 1.3 mmol) was added portionwise and the solution stirred at reflux for 30 minutes. A further portion of sodium borohydride (50 mg, 1.3 mmol) was added and the solution refluxed for another 30 minutes. On cooling, the aqueous solution was acidified and extracted with ethyl acetate (3 x 50 ml). The organics were dried and evaporated to leave an oil which was purified by flash chromatography (2:1 ethyl acetate:light petrol as eluent) as a white solid (150 mg, 60%). Data for the trans isomer:

δ_H 1.24 (3 H, d, $\underline{J}$ 7 Hz,Me), 3.35 (3 H, s, OMe), 3.88 (3 H, s, OMe), 3.92 (3 H, s, OMe), 4.54 (2 H, m, H-3, H-4), 6.50 (2 H, m, 2 x Ar-H).

4-Bromo-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin
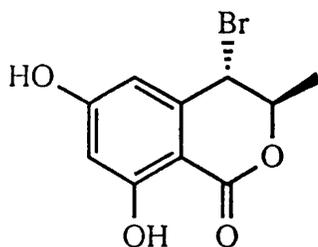
[202]

A solution of 3,4-dihydro-4,6,8-trimethoxy-3-methylisocoumarin (300 mg, 1.2 mmol) in dry dichloromethane (15 ml) was cooled to -78°C and boron tribromide (2 ml, 20 mmol) was added, under nitrogen with stirring. The mixture was allowed to warm to room temperature over 16 hours. The solution was then diluted with ether (20 ml) and washed with water (3 x 20 ml). The ethereal solution was dried and evaporated to give a brown solid. This was purified by flash chromatography (1:1 ethyl acetate:light petrol as eluent) and recrystallised from ethyl acetate/hexane (250 mg, 77%), m.p. dec. > 225°C; $\nu_{max.}$ 3 260 br and 1 660 s cm^{-1}; δ_H (d$_6$-acetone) 1.43 (3 H, d, $\underline{J}$ 6.7 Hz, Me), 5.03 (1 H, dq, $\underline{J}$ 2.6 Hz, $\underline{J}^1$ 6.7 Hz, H-3), 5.47 (1 H, d, $\underline{J}$ 2.6 Hz, H-4), 6.39 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H), 6.59 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H), 11.21 (1 H, s, OH); δ_C (d$_6$-acetone) 19.2 (q, Me), 47.1 (d, C-4), 80.9 (d, C-3), 100.0 (s, C-8a), 104.1 (d, C-7), 109.1 (d, C-5), 141.2 (s, C-4a), 165.1 (s, C-8), 165.4 (s, C-6), 167.9 (s, C-1); Found M$^+$ 273.9666, C$_{10}$H$_9$O$_4$Br requires M, 273.9665.

A 2:1 $\underline{trans}$:$\underline{cis}$ mixture was obtained by treatment of the mixture of methyl ethers by the foregoing method. Data for the $\underline{cis}$ isomer: δ_H (d$_6$-acetone) 1.53 (3 H, d, $\underline{J}$ 6.2 Hz, Me), 4.69 (1 H, dq, $\underline{J}$

1.8 Hz, $\underline{J}^1$ 6.2 Hz, H-3), 5.46 (1 H, d, $\underline{J}$ 1.8 Hz, H-4), 6.40 (1 H, d, $\underline{J}$ 2.2 Hz, Ar-H), 6.54 (1 H, d, $\underline{J}$ 2.2 Hz, Ar-H).

3,4-Dihydro-4,6,8-trihydroxy-3-methylisocoumarin

[177]
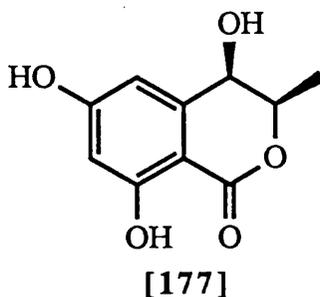
4-Bromo-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin (1 g, 3.7 mmol) was dissolved in THF (50 ml) and 5% silver nitrate solution (50 ml, 14.7 mmol) added. The mixture was stirred in the dark for ten days then saturated with sodium chloride and filtered through celite. The aqueous layer was separated and extracted with ethyl acetate (3 x 50 ml) and the combined organic solutions were dried and evaporated. The crude product was purified by flash chromatography (1:1 ethyl acetate: light petrol as eluent) and recrystallised from ethyl acetate/hexane as needles (0.6 g, 77%), m.p. 182-184°C (lit.[119] 183-185°C); $\nu_{max.}$ 3 500 br and 1 660 s cm^{-1}; δ_H (d$_6$-acetone) 1.46 (3 H, d, $\underline{J}$ 6.6 Hz, Me), 4.55 (1 H, d, $\underline{J}$ 2.2 Hz, H-4), 4.68 (1 H, dq, $\underline{J}$ 2.2 Hz, $\underline{J}^1$ 6.6 Hz, H-3), 6.34 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H), 6.48 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H), 11.27 (1 H, s, OH); δ_C (d$_6$-acetone) 16.1 (q, Me), 67.2 (d, C-4), 78.7 (d, C-3), 100.6 (s, C-8a), 103.1 (d, C-7), 107.8 (d, C-5), 145.1 (s, C-4a), 164.8 (s, C-8), 165.2 (s, C-6), 170.1 (s, C-1); Found M$^+$ 210.0528, C$_{10}$H$_{10}$O$_5$ requires M, 210.0528.

A cis:trans mixture was obtained by treatment of the mixture of bromides by the foregoing method. Data for the trans isomer: δ_H (d$_6$-acetone) 1.45 (3 H, d, $\underline{J}$ 6.1 Hz, Me), 4.45 (1 H, dq, $\underline{J}$ 8.5 Hz, $\underline{J}^1$

6.1 Hz, H-3), 4.55 (1 H, m, H-4), 6.30 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H),

6.62 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H), 11.18 (1 H, s, OH).

3,5-Dihydroxyphthalic Anhydride
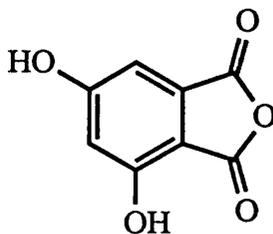
[201]

4,6,8-Trimethoxy-3-methylisocoumarin (400 mg, 1.6 mmol) in

dry dichloromethane (15 ml) was cooled to -78°C and boron tribromide

(3 ml, 31 mmol) was added, under nitrogen with stirring.   The mixture

was allowed to warm to room temperature over 16 hours.   The solution was

then diluted with ether (20 ml) and ethyl acetate (50 ml), then washed

with water (3 x 20 ml).   The organic layer was dried and evaporated

and the crude product submitted to flash chromatography (1:1 ethyl

acetate:light petrol as eluent).   Recrystallisation from acetone/

hexane yielded white needles (120 mg, 42%); $\nu_{max.}$ 3 570 m, 3 490 m,

3 340 br, 1 820 m and 1 740 s cm^{-1}; δ_H (d_6-acetone) 6.74 (1 H, d, $\underline{J}$

1.9 Hz, Ar-H), 6.87 (1 H, d, $\underline{J}$ 1.9 Hz, Ar-H); δ_C (d_6-acetone) 105.6

(d, C-4), 107.8 (s, C-2), 109.9 (d, C-6), 135.8 (s, C-1), 159.2 (s, C-3),

161.6 (s, C-5), 164.2 (s, CO), 167.1 (s, CO);   Found M$^+$ 180.0056,

$C_8H_4O_5$ requires M, 180.0058.

3,5-Dimethoxybenzyl t-Butyldimethylsilyl Ether

MeO

OTBDMS

OMe

[203]
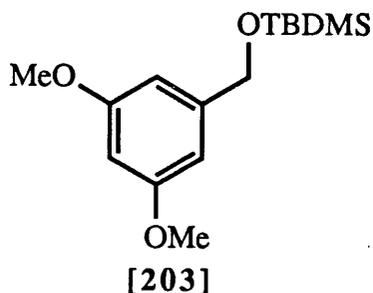
3,5-Dimethoxybenzyl alcohol (3.4 g, 20 mmol), t-butyldimethylsilyl chloride (3.4 g, 23 mmol), triethylamine (3.4 ml, 25 mmol) and dimethylaminopyridine (100 mg, 0.8 mmol) in dry dichloromethane (100 ml) were stirred at room temperature for 5 hours. The mixture was then washed with water (50 ml) and saturated ammonium chloride solution (50 ml), dried and evaporated. The product was purified by flash chromatography (3:1 ether:light petrol as eluent) as a yellow oil (5.2 g, 91%); $v_{max.}$ (film) 1 590 s cm^{-1}; δ_H 0.00 (6 H, s, 2 x Me), 0.85 (9 H, s, t-Bu), 3.67 (6 H, s, 2 x OMe), 4.59 (2 H, s, CH_2), 6.24 (1 H, t, J 2.3 Hz, Ar-H), 6.40 (2 H, d, J 2.3 Hz, 2 x Ar-H); δ_C -5.3 (q, 2 x Me), 18.4 (s, $\underline{C}(CH_3)_3$), 25.9 (q, $C(\underline{C}H_3)_3$), 55.2 (q, 2 x OMe), 64.8 (t, CH_2), 98.9 (d, C-4), 103.7 (d, C-2, C-6), 144.1 (s, C-1), 160.8 (s, C-3, C-5); Found M^+ 282.1663, $C_{15}H_{26}O_3Si$ requires M, 282.1652.

2-Bromo-3,5-dimethoxybenzyl t-Butyldimethylsilyl Ether

MeO

OTBDMS

Br

OMe

[204]
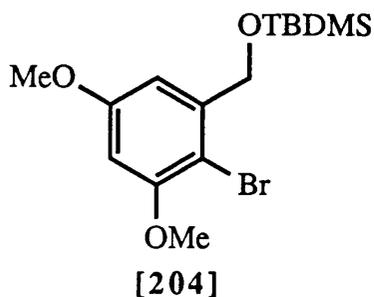
3,5-Dimethoxybenzyl t-butyldimethylsilyl ether (2 g, 7.1 mmol) in

carbon tetrachloride (100 ml) was stirred at reflux and N-bromosuccinimide (1.3 g, 7.3 mmol) added in small portions over one hour. The cooled solution was washed with water (3 x 50 ml), dried and evaporated. Flash chromatography (3:1 ether:light petrol as eluent) gave the bromide as an oil (2 g, 78%); $v_{max.}$ (film) 1 590 s cm^{-1}; δ_H 0.00 (6 H, s, 2 x Me), 0.84 (9 H, s, t-Bu), 3.67 (3 H, s, OMe), 3.72 (3 H, s, OMe), 4.59 (2 H, s, CH$_2$), 6.26 (1 H, d, J 2.8 Hz, Ar-H), 6.68 (1 H, d, J 2.8 Hz, Ar-H); δ_C -5.3 (q, 2 x Me), 18.4 (s, C (CH$_3$)$_3$), 25.9 (q, C (CH$_3$)$_3$), 55.4 (q, OMe), 56.3 (q, OMe), 64.8 (t, CH$_2$), 98.2 (d, C-4), 100.6 (s, C-2), 103.5 (d, C-6), 142.5 (s, C-1), 156.1 (s, C-5), 159.8 (s, C-3); Found M$^+$ 362.0712, C$_{15}$H$_{25}$O$_3$BrSi requires M, 362.0737.

2-Bromo-3,5-dimethoxybenzyl Alcohol[117]

[205]
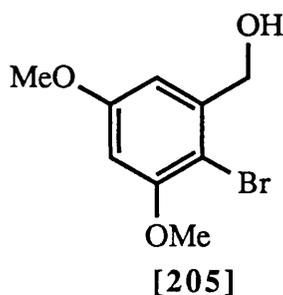
Recrystallised N-bromosuccinimide (0.53 g, 3 mmol) was added in small portions over 30 minutes to a refluxing solution of 3,5-dimethoxy-benzyl alcohol (0.5 g, 3 mmol) in carbon tetrachloride (20 ml) with stirring. After a further 15 minutes the solution was cooled and the solvent evaporated. The residue was dissolved in dichloromethane (20 ml), washed with sodium bicarbonate solution (2 x 10 ml), dried and evaporated to give a white solid which crystallised from diisopropyl ether as needles (0.64 g, 87%), m.p. 92-94°C (lit.[117] 95-96°C); $v_{max.}$ 3 400 br cm^{-1}; δ_H 2.14 (1 H, t, J 5.7 Hz, OH), 3.81 (3 H, s, OMe), 3.86 (3 H, s, OMe), 4.71 (2 H, d, J 5.7 Hz, CH$_2$),

6.42 (1 H, d, $\underline{J}$ 2.7 Hz, Ar-H), 6.68 (1 H, d, $\underline{J}$ 2.7 Hz, Ar-H);

δ_C 55.6 (q, OMe), 56.3 (q, OMe), 65.3 (t, CH_2), 98.8 (d, C-4),

102.2 (s, C-2), 104.7 (d, C-6), 141.7 (s, C-1), 156.5 (s, C-5),

160.0 (s, C-3); Found M^+ 247.9864, $C_9H_{11}O_3Br$ requires M, 247.9873.

5,7-Dimethoxyphthalide[117]

[206]
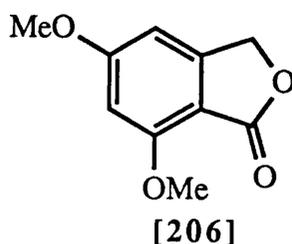
2-Bromo-3,5-dimethoxybenzyl alcohol (2 g, 8.1 mmol) in dry THF

(80 ml) was stirred under nitrogen at -78°C and $\underline{n}$-butyllithium (14 ml/

1.6 M in hexanes, 22 mmol) was slowly added. Stirring was continued for

30 minutes, after which carbon dioxide was passed through the flask.

When addition was complete, the flask was allowed to warm to room

temperature and hydrochloric acid added cautiously. The acidic solution

was extracted with ether (3 x 30 ml) and the combined extracts washed

with water (20 ml), dried and evaporated. The product crystallised

from acetone/hexane as prisms (0.92 g, 59%), m.p. 150-152°C (lit.[117]

149-150°C); $\nu_{max.}$ 1 750 s cm^{-1}; δ_H 3.86 (3 H, s, OMe), 3.92 (3 H, s,

OMe), 5.14 (2 H, s, CH_2), 6.39 (1 H, d, $\underline{J}$ 1.8 Hz, Ar-H), 6.46 (1 H, d,

$\underline{J}$ 1.8 Hz, Ar-H); δ_C 55.9 (q, 2 x OMe), 68.6 (t, C-3), 97.5 (d, C-6),

98.7 (d, C-4), 106.5 (s, C-7a), 151.6 (s, C-3a), 159.6 (s, C-7), 166.7

(s, C-5), 169.0 (s, C-1); Found M^+ 194.0577, $C_{10}H_{10}O_3$ requires M,

194.0579.

3-Carboxy-5,7-dimethoxyphthalide
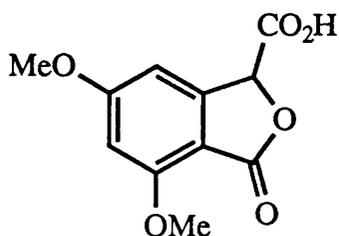
[207]

$\underline{n}$-Butyllithium (2.5 ml/1.6 M in hexanes, 4 mmol) was added to a solution of diisopropylamine (0.55 ml, 4 mmol) in dry THF (10 ml) under nitrogen at 0°C with stirring. After 10 minutes the solution was cooled to -78°C and a solution of 5,7-dimethoxyphthalide (700 mg, 3.6 mmol) in THF (50 ml) was added dropwise over 10 minutes. Stirring was continued for a further 10 minutes, then carbon dioxide was bubbled through the solution. When addition was complete, water (40 ml) was added and the organic phase removed by evaporation. The aqueous solution was washed with ether (2 x 30 ml), acidified and extracted with dichloromethane (3 x 30 ml). The organic solution was dried and evaporated and the crude product crystallised from acetone/hexane (250 mg, 29%), m.p. 178-180°C dec.; $V_{max.}$ 3 200 br, 1 770 s and 1 740 s cm^{-1}; δ_H 3.90 (3 H, s, OMe), 3.94 (3 H, s, OMe), 5.70 (1 H, s, H-3), 6.47 (1 H, d, $\underline{J}$ 1.8 Hz, Ar-H), 6.75 (1 H, d, $\underline{J}$ 1.8 Hz, Ar-H); δ_C 55.8 (q, OMe), 55.9 (q, OMe), 76.4 (d, C-3), 98.3 (d, C-6), 99.6 (d, C-4), 105.2 (s, C-7a), 149.2 (s, C-3a), 159.2 (s, C-7), 166.8 (s, C-5), 167.4 (s, C-1), 168.5 (s, CO$_2$H); Found M$^+$ 238.0462, $C_{11}H_{10}O_6$ requires M, 238.0477.

3-Acetyl-5,7-dimethoxyphthalide Enol Acetate
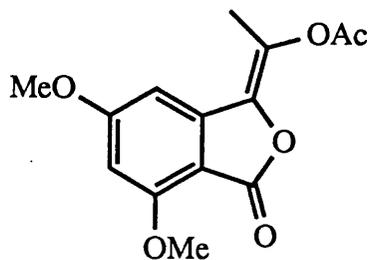
[208]

2-Carboxy-5,7-dimethoxyphthalide (200 mg, 0.84 mmol) was added to acetic anhydride (0.8 ml, 6.5 mmol) and pyridine (5 drops) and the mixture refluxed for 1½ hours.   The acetic anhydride and pyridine were evaporated and water (5 ml) and ethyl acetate (20 ml) added.   The aqueous layer was separated and washed with ethyl acetate (20 ml). The combined organic solutions were then dried and evaporated to yield a brown oil.   This was purified by flash chromatography (1:1 ethyl acetate:light petrol as eluent) to leave an off-white solid (160 mg, 60%); $\nu_{max.}$ 1 765 s cm^{-1}; δ_H 2.28 (3 H, s, Me), 2.36 (3 H, s, Me), 6.44 (1 H, d, $\underline{J}$ 1.8 Hz, Ar-H), 6.67 (1 H, d, $\underline{J}$ 1.8 Hz, Ar-H);   Found M^+ 278.0769, $C_{14}H_{14}O_6$ requires M, 278.0790.

8-Hydroxy-6-benzyloxy-3-methylisocoumarin
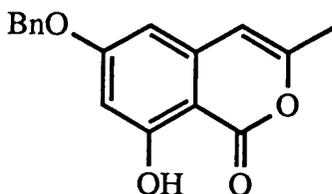
[152]

4-Bromo-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin (100 mg, 0.37 mmol) dissolved in dry acetone (20 ml) with anhydrous potassium carbonate (0.5 g, 5 mmol) and benzyl chloride (0.25 ml, 2 mmol) was

heated at reflux for 7 hours with stirring. On cooling, the solution was filtered and the residue washed with acetone. The combined organic solutions were evaporated, leaving an oil which was dissolved in dichloromethane (80 ml). The organic phase was washed with ammonia (10%, 2 x 30 ml), sodium hydroxide (10%, 2 x 20 ml) and water (30 ml), then dried and evaporated. The crude product was purified by flash chromatography (1:1 ethyl acetate:light petrol as eluent) and recrystallised from ethyl acetate/hexane as needles (90 mg, 87%), m.p. 134-136°C (lit.[99] 134-137°C); $\nu_{max.}$ 3 440 br and 1 690 s cm^{-1}; δ_H 2.23 (3 H, d, $\underline{J}$ 0.9 Hz, Me), 5.10 (2 H, s, CH$_2$), 6.15 (1 H, q, $\underline{J}$ 0.9 Hz, H-4), 6.35 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H), 6.51 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H), 7.39 (5 H, m, Ph), 11.10 (1 H, s, OH); δ_C 19.4 (q, Me), 70.2 (t, CH$_2$), 99.9 (s, C-8a), 100.9 (d, C-7), 101.6 (d, C-5), 104.5 (d, C-4), 127.5 (d, 2 x Ar C-H), 128.3 (d, Ar C-H), 128.7 (d, 2 x Ar C-H), 135.7 (s, Ar $\underline{C}$-CH$_2$), 139.4 (s, C-4a), 154.3 (s, C-3), 163.5 (s, C-8), 165.8 (s, C-6), 166.3 (s, C-1); Found M$^+$ 282.0870, C$_{17}$H$_{14}$O$_4$ requires M, 282.0892.

3-Hydro-6,8-dihydroxy-4-keto-3-methylisocoumarin
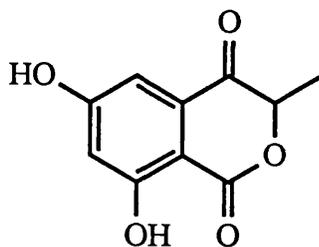
[236]

3,4-Dihydro-4,6,8-trihydroxy-3-methylisocoumarin (100 mg, 0.5 mmol) was dissolved in dichloromethane (20 ml) and pyridinium chlorochromate (400 mg, 1.9 mmol) added. The suspension was stirred overnight at room temperature, then diluted with ether (100 ml) and filtered through celite. Evaporation of the solvent gave the ketone as an oil

(30 mg, 30%); $\nu_{max.}$ (film) 3 200 br, 1 720 s and 1 670 s cm^{-1}; δ_H

(d$_6$-acetone) 1.62 (3 H, d, $\underline{J}$ 7.0 Hz, Me), 5.33 (1 H, q, $\underline{J}$ 7.0 Hz, H-3),

6.73 (1 H, d, $\underline{J}$ 2 Hz, Ar-H), 6.97 (1 H, d, $\underline{J}$ 2 Hz, Ar-H), 11.47 (1 H,

s,OH), δ_C (d$_6$-acetone) 18.4 (q, Me), 81.6 (d, C-3), 104.3 (s, C-8a),

105.6 (d, C-7), 109.4 (d, C-5), 133.9 (s, C-4a), 164.8 (s, C-8), 165.6

(s, C-6), 167.7 (s, C-1), 192.8 (s, C-4); ^{m}/z 208.

5-Chloro-3,4-dihydro-4,6,8-trimethoxy-3-methylisocoumarin
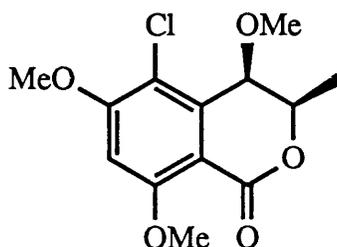
[227]

Sulphuryl chloride (0.8 ml, 10 mmol) was added to a solution of

3,4-dihydro-4,6,8-trimethoxy-3-methylisocoumarin (100 mg, 0.4 mmol) in

dichloromethane (20 ml) and the mixture left to stand in the dark for one

hour. Water (50 ml) was added and the organic layer separated, washed

with water (30 ml), dried and evaporated. The product was

recrystallised from ethyl acetate/hexane as needles (90 mg, 79%), m.p.

155-157 °C; $\nu_{max.}$ 1 720 s cm^{-1}; δ_H 1.53 (3 H, d, $\underline{J}$ 6.6 Hz, Me), 3.37

(3 H, s, OMe), 3.95 (3 H, s, OMe), 3.99 (3 H, s, OMe), 4.39 (1 H, dq,

$\underline{J}$ 1.4 Hz, $\underline{J}^1$ 6.6 Hz, H-3), 4.62 (1 H, d, $\underline{J}$ 1.4 Hz, H-4), 6.57 (1 H, s,

Ar-H); δ_C 16.6 (q, Me.), 56.4 (q, OMe), 56.5 (q, OMe), 57.3 (q, OMe),

72.3 (d, C-4), 75.7 (d, C-3), 96.9 (d, C-7), 107.1 (s, C-8a), 112.9 (s,

C-5), 139.2 (s, C-4a), 159.3 (s, C-8), 161.2 (s, C-6), 161.5 (s, C-1);

Found M$^+$ 286.0583, C$_{13}$H$_{15}$O$_5$Cl requires M, 286.0608.

Demethylation of

5-Chloro-3,4-dihydro-4,6,8-trimethoxy-3-methylisocoumarin
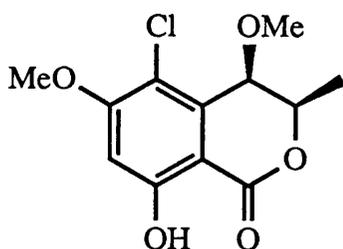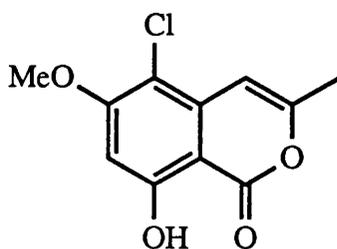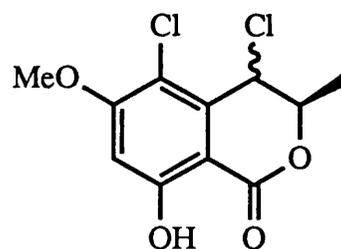
[229]   [230]   [231]

Aluminium chloride (400 mg, 3 mmol) and 5-chloro-3,4-dihydro-4,6,8-trimethoxy-3-methylisocoumarin (100 mg, 0.35 mmol) in freshly distilled nitrobenzene (8 ml) were stirred at 50-60°C for 6 hours. After this time, the mixture was poured into ice-water and acidified with dilute hydrochloric acid. The acidic solution was extracted with ether (3 x 50 ml), then the ethereal solution was extracted with 10% aqueous sodium hydroxide (2 x 30 ml). The basic extracts were acidified and extracted again with ethyl acetate (3 x 50 ml). Drying and evaporating yielded three products which were separated by flash chromatography (1:1 ethyl acetate:light petrol as eluent).

5-Chloro-3,4-dihydro-8-hydroxy-4,6-dimethoxy-3-methylisocoumarin (40 mg, 42%); $V_{max.}$ 3 440 br and 1 660 s cm^{-1}; δ_H 1.62 (3 H, d, J 6.6 Hz, Me), 3.42 (3 H, s, OMe), 3.96 (3 H, s, OMe), 4.57 (1 H, dq, J 1.6 Hz, J^1 6.6 Hz, H-3), 4.62 (d, J 1.6 Hz, H-4), 6.57 (1 H, s, Ar-H), 11.41 (1 H, s, OH); δ_C 16.5 (q, Me), 56.6 (q, OMe), 57.7 (q, OMe), 71.5 (d, C-4), 77.8 (d, C-3), 100.8 (d, C-7), 101.4 (s, C-8a), 112.9 (s, C-5), 136.9 (s, C-4a), 160.9 (s, C-8), 163.0 (s, C-6), 168.7 (s, C-1); Found M$^+$ 274.0400, $C_{12}H_{13}O_5Cl$ requires M, 274.0422.

4,5-Dichloro-3,4-dihydro-8-hydroxy-6-methoxy-3-methylisocoumarin (30 mg, 30%); $V_{max.}$ (film) 3 440 br and 1 660 s cm^{-1}; δ_H (cis isomer) 1.66 (3 H, d, J 6.4 Hz, Me), 3.96 (3 H, s, OMe), 4.78 (1 H, dq, J 1.9 Hz, J^1 6.4 Hz, H-3), 5.25 (1 H, d, J 1.9 Hz, H-4), 6.58 (1 H, s, Ar-H), 11.31

(1 H, s, OH).

δ_H (<u>trans</u> isomer) 1.41 (3 H, d, <u>J</u> 6.9 Hz, Me), 3.96 (3 H, s, OMe), 5.06 (1 H, dq, <u>J</u> 1.2 Hz, <u>J</u>^{1} 6.9 Hz, H-3), 5.27 (1 H, d, <u>J</u> 1.2 Hz, H-4), 6.58 (1 H, s, Ar-H), 11.39 (1 H, s, OH).

Found M$^+$ 275.9945, $C_{11}H_{10}O_4Cl_2$ requires M, 275.9956.

5-Chloro-8-hydroxy-6-methoxy-3-methylisocoumarin (24 mg, 29%); 3 440 br and 1 700 s cm^{-1}; δ_H 2.30 (3 H, d, <u>J</u> 1 Hz, Me), 3.96 (3 H, s, OMe), 6.50 (1 H, s, Ar-H), 6.59 (1 H, q, <u>J</u> 1 Hz, H-4), 11.24 (1 H, s, OH); δ_C 19.7 (q, Me), 56.6 (q, OMe), 98.4 (d, C-7), 99.5 (s, C-8a), 101.0 (d, C-4), 106.8 (s, C-5), 135.7 (s, C-4a), 155.3 (s, C-3), 161.8 (s, C-8), 162.4 (s, C-6), 165.9 (s, C-1); Found M$^+$ 240.0192, $C_{11}H_9O_4Cl$ requires M, 240.0189.

Demethylation of 3,4-dihydro-4,6,8-trimethoxy-3-methylisocoumarin

[7]   [232]
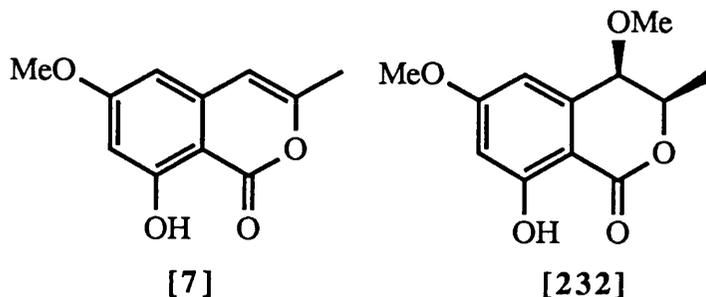
Treatment of 3,4-dihydro-4,6,8-trimethoxy-3-methylisocoumarin (180 mg, 0.7 mmol) with aluminium chloride (500 mg, 3.75 mmol) by the foregoing method yielded two products which were separated by flash chromatography (2:1 hexane:chloroform as eluent).

8-Hydroxy-6-methoxy-3-methylisocoumarin (65 mg, 44%); same data as above

3,4-Dihydro-8-hydroxy-4,6-dimethoxy-3-methylisocoumarin (32 mg,

19%); $V_{max.}$ 3 440 br and 1 660 s cm^{-1}; δ_H 1.54 (3 H, d, $\underline{J}$ 6.6 Hz, Me), 3.32 (3 H, s, OMe), 3.86 (3 H, s, OMe), 4.01 (1 H, d, $\underline{J}$ 2.2 Hz, H-4), 4.66 (1 H, dq, $\underline{J}$ 2.2 Hz, $\underline{J}^1$ 6.6 Hz, H-3), 6.42 (1 H, d, $\underline{J}$ 2.4 Hz, Ar-H), 6.48 (1 H, d, $\underline{J}$ 2.4 Hz, Ar-H); δ_C 15.9 (q, Me), 55.7 (q, OMe), 56.7 (q, OMe), 75.4 (d, C-4), 77.3 (d, C-3), 100.5 (s, C-8a), 100.8 (d, C-7), 107.7 (d, C-5), 139.2 (s, C-4a), 164.7 (s, C-8), 165.3 (s, C-6), 169.0 (s, C-1); Found M$^+$ 238.0815, $C_{12}H_{14}O_5$ requires M, 238.0842

4-Bromo-3,4-dihydro-8-hydroxy-6-methoxy-3-methylisocoumarin
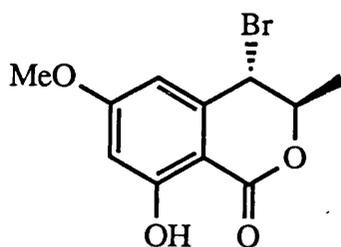
[233]

To a stirred solution of 3,4-dihydro-4,6,8-trimethoxy-3-methyl isocoumarin (1.5 g, 6 mmol) in dry dichloromethane (50 ml) at -78°C was added boron tribromide (1.2 ml, 13 mmol) and the mixture stirred under nitrogen for 16 hours. The solution was quenched with ether (10 ml) and water (20 ml) and the phases separated. The aqueous layer was extracted with dichloromethane (3 x 50 ml) and the combined organic solutions dried and evaporated. Flash chromatography (2:1 light petrol:ethyl acetate as eluent) and recrystallisation from ethyl acetate/hexane yielded the product as white needles (0.95 g, 56%); m.p. 125-127°C; $V_{max.}$ 3 420 br and 1 655 s cm^{-1}; δ_H 1.50 (3 H, d, $\underline{J}$ 6.6 Hz, Me), 3.86 (3 H, s, OMe), 4.96 (1 H, dq, $\underline{J}$ 3.9 Hz, $\underline{J}^1$ 6.6 Hz, H-3), 5.02 (1 H, d, $\underline{J}$ 3.9 Hz, H-4), 6.46 (1 H, d, $\underline{J}$ 2.4 Hz, Ar-H), 6.55 (1 H, d, $\underline{J}$ 2.4 Hz, Ar-H), 11.25 (1 H, s, OH); δ_C 19.5 (q, Me), 46.0 (d, C-4), 55.8 (q, OMe), 79.7 (d, C-3), 99.6 (s, C-8a), 101.4 (d,

C-7), 108.1 (d, C-5), 139.3 (s, C-4a), 164.5 (s, C-8), 166.0 (s, C-6),

167.2 (s, C-1); Found M^+ 287.9796, $C_{11}H_{11}O_4Br$ requires M, 287.9821.

4-Bromo-5-chloro-3,4-dihydro-8-hydroxy-6-methoxy-3-methylisocoumarin
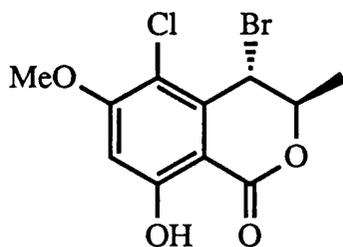
[269]

4-Bromo-3,4-dihydro-8-hydroxy-6-methoxy-3-methylisocoumarin (200

mg, 0.7 mmol) and sulphuryl chloride (1 ml, 12 mmol) in dichloromethane

(25 ml) were left in the dark for one hour. The solution was poured

into water (50 ml) and the resulting aqueous layer extracted with

dichloromethane (3 x 50 ml). The combined organic fractions were

rinsed with brine (2 x 50 ml), dried and evaporated. The product was

purified by flash chromatography (20% ethyl acetate/light petrol as

eluent) and recrystallised from ethyl acetate/light petrol as needles

(160 mg, 71%), m.p. 160-163°C; $V_{max.}$ 3 440 br and 1 660 s cm^{-1}; δ_H

1.42 (3 H, d, $\underline{J}$ 6.9 Hz, Me), 3.96 (3 H, s, OMe), 5.11 (1 H, dq, $\underline{J}$

1.2 Hz, $\underline{J}^1$ 6.9 Hz, H-3), 5.35 (1 H, d, $\underline{J}$ 1.2 Hz, H-4), 6.56 (1 H, s,

Ar-H), 11.41 (1 H, s, OH); δ_C 19.4 (q, Me), 41.8 (d, C-4), 56.7 (q,

OMe), 80.1 (d, C-3), 99.9 (s, C-8a), 101.2 (d, C-7), 113.0 (s, C-5),

135.8 (s, C-4a), 161.6 (s, C-8), 163.1 (s, C-6), 166.5 (s, C-1);

Found M^+ 321.9410, $C_{11}H_{10}O_4BrCl$ requires M, 321.9422.

3,4-Dihydro-4,8-dihydroxy-6-methoxy-3-methylisocoumarin
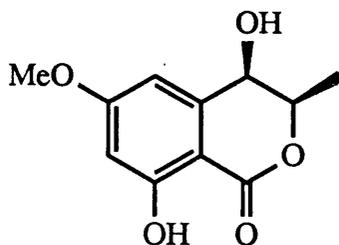
[234]

4-Bromo-3,4-dihydro-8-hydroxy-6-methoxy-3-methylisocoumarin

(500 mg, 1.7 mmol) was dissolved in THF (10 ml) and a solution of silver

nitrate (500 mg, 2.9 mmol) in water (10 ml) added.   The mixture was

stirred at room temperature for one week, then saturated with sodium

chloride and filtered through celite, the residue being washed with

ethyl acetate.   The aqueous layer was separated and extracted with

ethyl acetate (2 x 20 ml).   The combined organic solutions were dried

and evaporated.   Flash chromatography (2:1 light petrol:ethyl acetate)

and recrystallisation from ethyl acetate/light petrol gave the pure

product (200 mg, 51%), m.p. 95-100°C; $\nu_{max.}$ 3 410 br and 1 650 s cm^{-1};

δ_H 1.54 (3 H, d, $\underline{J}$ 6.6 Hz, Me), 3.64 (1 H, d, $\underline{J}$ 6.3 Hz, OH), 3.84 (3 H,

s, OMe), 4.52 (1 H, dd, $\underline{J}$ 6.3 H, $\underline{J}^1$ 2.3 Hz, H-4), 4.64 (1 H, dq, $\underline{J}$

2.3 Hz, $\underline{J}^1$ 6.6 Hz, H-3), 6.42 (1 H, d, $\underline{J}$ 2.4 Hz, Ar-H), 6.50 (1 H, d, $\underline{J}$

2.4 Hz, Ar-H), 11.16 (1 H, s, OH); δ_C 15.7 (q, Me), 55.6 (q, OMe), 67.0

(d, C-4), 77.9 (d, C-3), 100.0 (s, C-8a), 100.9 (d, C-7), 106.5 (d, C-5),

142.4 (s, C-4a), 164.1 (s, C-8), 166.0 (s, C-6), 169.2 (s, C-1);

Found M$^+$ 224.0673, $C_{11}H_{12}O_5$ requires M, 224.0685.

5-Chloro-3,4-dihydro-4,8-dihydroxy-6-methoxy-3-methylisocoumarin
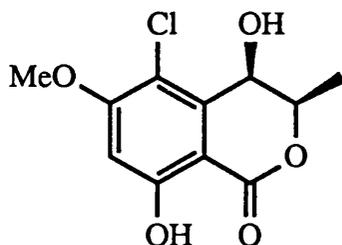
[235]

A solution of 3,4-dihydro-4,8-dihydroxy-6-methoxy-3-methyl
isocoumarin (100 mg, 0.45 mmol) and sulphuryl chloride (0.05 ml, 0.6
mmol) in dichloromethane (20 ml) was left to stand in the dark for $1\frac{1}{2}$
hours. The reaction mixture was then washed with water (3 x 20 ml),
dried and evaporated. The crude product was purified by flash
chromatography (2:1 light petrol:ethyl acetate as eluent) as an oil
(80 mg, 69%); $v_{max.}$ (film) 3 400 br and 1 665 s cm^{-1}; δ_H 1.61 (3 H,
d, J 6.6 Hz, Me), 3.93 (3 H, s, OMe), 4.59 (1 H, dq, J 1.9 Hz, J^1 6.6 Hz,
H-3), 4.89 (1 H, d, J 1.9 Hz, H-4), 6.50 (1 H, s, Ar-H), 11.34 (1 H, s,
OH); δ_C 16.1 (q, Me), 56.7 (q, OMe), 64.3 (d, C-4), 77.8 (d, C-3),
100.6 (s, C-8a), 100.8 (d, C-7), 112.3 (s, C-5), 138.6 (s, C-4a), 161.3
(s, C-6), 162.9 (s, C-8), 169.1 (s, C-1); Found M$^+$ 258.0267,
$C_{11}H_{11}O_5Cl$ requires M, 258.0295.

2-Bromopropanoyl Chloride[135]
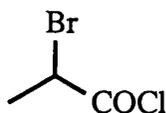
[252]

In a three-necked round-bottomed flask, equipped with a dropping
funnel and a reflux condenser, to which was attached a gas absorption
trap, was placed thionyl chloride (13.5 ml, 0.19 mol) which was heated

to boiling. Propanoic acid (12.6 ml, 0.17 mol) was added at such a rate that the mixture refluxed gently (ca 10 minutes). The mixture was refluxed for a further 30 minutes to expel the dissolved sulphur dioxide, allowed to cool and red phosphorus (50 mg, 1.6 mmol) was added. Dry bromine (10 ml, 0.19 mol) was introduced over 30 minutes to the gently boiling propanoyl chloride, and the mixture refluxed for 7 hours, by which time the evolution of hydrogen bromide had ceased. The remaining bromine and hydrogen bromide was blown off using nitrogen, and the product isolated by distillation at water pump pressure, b.p. 70°C, as an oil (28 g, 97%).

p-Methylphenyl 2-Bromopropanoate
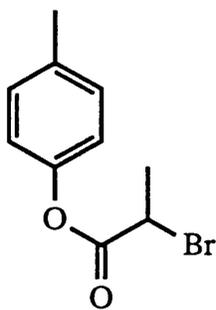
[238]

p-Cresol (17.8 g, 0.16 mol) and 2-bromopropanoyl chloride (28 g, 0.16 mol) were stirred at 140°C until no more hydrogen chloride was given off (~2 hours). The crude product was distilled in vacuo (100°C/1 mm Hg;lit.[134] 145-150°C/18 mm Hg) to give a clear oil (27 g, 68% which solidified as white needles, m.p. 31-33°C (lit.[134] 33°C); $\nu_{max.}$ (film) 1 760 s cm^{-1}; δ_H 1.94 (3 H, d, $\underline{J}$ 6.9 Hz, Me), 2.35 (3 H, s, Me), 4.58 (1 H, q, $\underline{J}$ 6.9 Hz, CH), 7.00 (2 H, d, $\underline{J}$ 8.5 Hz, 2 x Ar-H), 7.19 (2 H, d, $\underline{J}$ 8.9 Hz, 2 x Ar-H); δ_C 20.8 (q, Me), 21.4 (q, Me), 39.7 (d, CH), 120.6 (d, 2 x Ar CH), 130.0 (d, 2 x ArCH), 135.9 (s, Ar C-Me), 148.2 (s, Ar C-O), 168.9 (s, CO); Found M$^+$ 243.9915, $C_{10}H_{11}O_2Br$

requires M, 243.9923.

7-Hydroxy-4-methylindan-1-one[134]
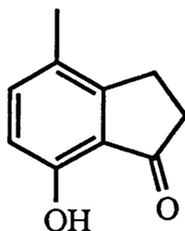
[239]

p-Methylphenyl 2-bromopropanoate (25 g, 0.1 mol) was heated

with aluminium chloride (50 g, 0.37 mol) at 150°C for 4 hours.   The

black solid produced was cooled in ice and dilute hydrochloric acid was

added carefully until no more hydrogen chloride was evolved.   The crude

product was extracted with ethyl acetate, dried and evaporated to leave a

black solid.   This was distilled under reduced pressure (b.p. 120°C

at 1 mm Hg) to give a yellow solid which still contained starting

material.   The solid was dissolved in chloroform and excess sodium

hydroxide added.   The salt produced was filtered off and dissolved in

hydrochloric acid/ethyl acetate.   The aqueous layer was separated,

extracted with ethyl acetate (3 x 50 ml) and the organic solutions

dried and evaporated.   Recrystallisation from ethanol gave white

needles (3.5 g, 21%), m.p. 110-112°C (lit.[134] 110-111°C); $v_{max.}$ 3 290

br and 1 670 s cm^{-1}; δ_H 2.23 (3 H, s, Me), 2.70 (2 H, m, CH$_2$), 2.98

(2 H, m, CH$_2$), 6.68 (1 H, d, $\underline{J}$ 8.2 Hz, Ar-H), 7.27 (1 H, d, $\underline{J}$ 8.2 Hz,

Ar-H), 8.92 (1 H, s, OH); δ_C 16.8 (q, Me), 24.9 (t, C-3), 35.9 (t, C-2),

113.4 (d, C-6), 122.3 (s, C-7a), 126.1 (s, C-4), 138.1 (d, C-5), 153.4

(s, C-3a), 155.4 (s, C-7), 210.4 (s, C-1);   Found M$^+$, 162.0662, C$_{10}$H$_{10}$O$_2$

requires M, 162.0681.

7-Methoxy-4-methylindan-1-one
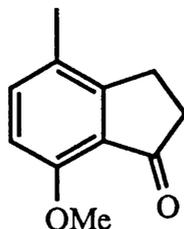
[240]

7-Hydroxy-4-methylindan-1-one (2.8 g, 17 mmol), dimethyl

sulphate (2 ml, 21 mmol) and anhydrous potassium carbonate (10 g, 72

mmol) in dry acetone (100 ml) was refluxed for 7 hours with stirring.

After cooling, the solution was filtered and.the residue washed with

acetone.   The acetone was removed by evaporation and the resulting oil

dissolved in dichloromethane (200 ml).   This solution was then washed

with ammonia (10%, 3 x 50 ml), sodium hydroxide (10%, 2 x 50 ml) and

water (2 x 50 ml), dried and evaporated to dryness.   The product

crystallised from ethanol as needles (2.7 g, 89%), m.p. 108-110°C

(lit.[134] 112-113°C); $\nu_{max.}$ 1 700 s cm^{-1}; δ_H 2.25 (3 H, s, Me), 2.67 (2

H, m, CH$_2$), 2.94 (2 H, m, CH$_2$), 3.91 (3 H, s, OMe), 6.71 (1 H, d, J 8.3

Hz, Ar-H), 7.31 (1 H, d, J 8.3 Hz, Ar-H); δ_C 16.9 (q, Me), 24.6 (t,

C-3), 36.7 (t, C-2), 55.7 (q, OMe), 108.8 (d, C-6), 124.9 (s, C-7a),

127.0 (s, C-4), 136.6 (d, C-5), 156.3 (s, C-3a), 156.4 (s, C-7),

205.3 (s, C-1);   Found M$^+$ 176.0833, C$_{11}$H$_{12}$O$_2$ requires M, 176.0837.

7-Methoxy-2,4-dimethylindan-1-one
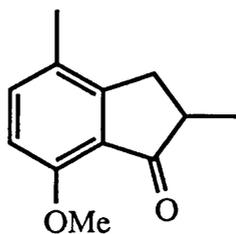
[249]

A suspension of magnesium methoxide (43 g, 0.5 mol) in dry DMF

(100 ml) was saturated with carbon dioxide until no more heat was evolved and all the solid had dissolved. To this solution was added 7-methoxy-4-methylindan-1-one (0.8 g, 4.5 mmol) and the mixture refluxed for 1½ hours. After cooling, methyl iodide (5 ml, 0.08 mol) was added and heating continued for a further 2 hours. The solvent was evaporated and the residue treated with hydrochloric acid until carbon dioxide evolution ceased. The acidic solution was extracted with ether (3 x 50 ml) and the combined extracts dried and evaporated. Purification was achieved by flash chromatography (3:2 light petrol:ether as eluent) and recrystallisation from ethyl acetate/hexane as prisms (0.5 g, 58%), m.p. 77-79 °C; $V_{max.}$ 1 700 s cm^{-1}; δ_H 1.27 (3 H, d, $\underline{J}$ 7.3 Hz, Me), 2.22 (3 H, s, Me), 2.49 (1 H, dd, $\underline{J}$ 17 Hz, $\underline{J}^1$ 3.7 Hz, H-3), 2.64 (1 H, m, H-2), 3.19 (1 H, dd, $\underline{J}$ 17 Hz, $\underline{J}^1$ 7.7 Hz, H-3), 3.89 (3 H, s, OMe), 6.68 (1 H, d, $\underline{J}$ 8.3 Hz, Ar-H), 7.29 (1 H, d, $\underline{J}$ 8.3 Hz, Ar-H); δ_C 16.9 (q, Me), 33.6 (t, C-3), 42.0 (d, C-2), 55.6 (q, OMe), 108.8 (d, C-6), 124.0 (s, C-7a), 126.7 (s, C-4), 136.5 (d, C-5), 154.5 (s, C-3a), 156.3 (s, C-7), 207.6 (s, C-1); Found M^+ 190.0988, $C_{12}H_{14}O_2$ requires M, 190.0994.

7-Methoxy-2,2,4-trimethylindan-1-one, m.p. 85-86 °C; $V_{max.}$ 1 700 s cm^{-1}; δ_H 1.21 (6 H, s, 2 x Me), 2.22 (3 H, s, Me), 2.80 (2 H, s, CH_2), 3.90 (3 H, s, OMe), 6.70 (1 H, d, $\underline{J}$ 8.3 Hz, Ar-H), 7.31 (1 H, d, $\underline{J}$ 8.3 Hz, Ar-H); δ_C 16.9 (q, Me), 25.6 (q, 2 x Me), 41.6 (t, C-3), 45.3 (s, C-2), 55.7 (q, OMe), 108.9 (d, C-6), 122.9 (s, C-7a), 126.8 (s, C-4), 136.6 (d, C-5), 153.2 (s, C-3a), 156.6 (s, C-7), 209.6 (s, C-1); Found M^+ 204.1132, $C_{13}H_{16}O_2$ requires M, 204.1150.

8-Methoxy-3,5-dimethylisocoumarin
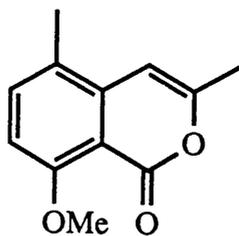
[250]

A solution of 7-methoxy-2,4-dimethylindan-1-one (0.4 g, 2.1

mmol) in trifluoroacetic anhydride (10 ml) was stirred at room

temperature for an hour.   The solvent was evaporated to leave a brown

solid which was dissolved in ethyl acetate (50 ml) and cooled to

-78°C.   Ozonised oxygen was passed through the solution until a blue

colour developed.   Nitrogen was bubbled through the solution until the

blue colour had been removed, then dimethyl sulphide (2 ml, 27 mmol) was

added.   The mixture was allowed to warm up and stirred at room temperature

overnight.   The reaction mixture was washed with water (2 x 20 ml),

dried and evaporated.   Flash chromatography (dichloromethane as eluent)

and recrystallisation from ethyl acetate/hexane yielded prisms (0.25 g,

58%), m.p. 153°C; $v_{max.}$ 1 720 s cm^{-1}; δ_H 2.25 (3 H, s, Me), 2.31 (3 H,

s, Me), 3.94 (3 H, s, OMe), 6.24 (1 H, s, H-4), 6.78 (1 H, d, $\underline{J}$ 8.5 Hz,

Ar-H), 7.39 (1 H, d, $\underline{J}$ 8.5 Hz, Ar-H); δ_C 18.4 (q, Me), 19.8 (q, Me),

56.1 (q, OMe), 100.3 (d, C-4), 108.6 (s, C-8a), 108.8 (d, C-7), 123.6

(s, C-5), 136.6 (d, C-6), 138.7 (s, C-4a), 154.7 (s, C-3), 159.9 (s,

C-8), 160.1 (s, C-1);   Found M$^+$ 204.0773, $C_{12}H_{12}O_3$ requires M, 204.0786.

3,4-Dihydro-8-methoxy-3,5-dimethylisocoumarin
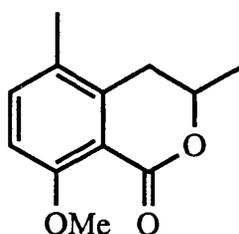
[270]

Palladium on charcoal (10%, 100 mg) was added to a solution of 8-methoxy-3,5-dimethylisocoumarin (250 mg, 1.2 mmol) in ethyl acetate (50 ml) and the mixture hydrogenated at room temperature for 16 hours. The catalyst was removed by filtering through celite and the solvent evaporated. The product was recrystallised from methanol as plates (200 mg, 80%), m.p. 108-110°C; $\nu_{max.}$ 1 710 s cm^{-1}; δ_H 1.48 (3 H, d, $\underline{J}$ 6.3 Hz, Me), 2.21 (3 H, s, Me), 2.66 (1 H, dd, $\underline{J}$ 15 Hz, $\underline{J}^1$ 10 Hz, H-4), 2.86 (1 H, dd, $\underline{J}$ 15 Hz, $\underline{J}^1$ 3.1 Hz, H-4), 3.90 (3 H, s, OMe), 4.48 (1 H, m, H-3), 6.81 (1 H, d, $\underline{J}$ 8.6 Hz, Ar-H), 7.30 (1 H, d, $\underline{J}$ 8.6 Hz, Ar-H); δ_C 18.5 (q, Me), 20.8 (q, Me), 33.1 (t, C-4), 56.1 (q, OMe), 73.4 (d, C-3), 110.4 (d, C-7), 113.7 (s, C-8a), 126.0 (s, C-5), 135.7 (d, C-6), 140.1 (s, C-4a), 159.5 (s, C-8), 163.2 (s, C-1); Found M$^+$ 206.0924, $C_{12}H_{14}O_3$ requires M, 206.0943.

3,4-Dihydro-8-hydroxy-3,5-dimethylisocoumarin
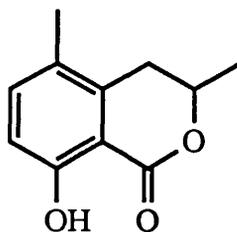
[178]

A solution of 3,4-dihydro-8-methoxy-3,5-dimethylisocoumarin (100 mg,

0.5 mmol) in dry dichloromethane (20 ml) was cooled to -78°C and boron tribromide (1 ml, 10 mmol) was added under nitrogen with stirring. The mixture was allowed to warm to room temperature over 16 hours and diluted with ether (10 ml). The organic solution was washed with water (3 x 20 ml), dried and evaporated. The product was purified by flash chromatography (1:1 dichloromethane:light petrol as eluent) and recrystallisation from ethyl acetate/hexane as needles (74 mg, 79%), m.p. 142-144°C (lit.[132] 140-141°C); $\nu_{max.}$ 3 440 br and 1 665 s cm^{-1}; δ_H 1.54 (3 H, d, $\underline{J}$ 6.3 Hz, Me), 2.18 (3 H, s, Me), 2.70 (1 H, dd, $\underline{J}$ 17 Hz, $\underline{J}^1$ 11 Hz, H-4), 2.94 (1 H, dd, $\underline{J}$ 17 Hz, $\underline{J}^1$ 3.6 Hz, H-4), 4.67 (1 H, m, H-3), 6.80 (1 H, d, $\underline{J}$ 8.8 Hz, Ar-H), 7.27 (1 H, d, $\underline{J}$ 8.8 Hz, Ar-H), 10.98 (1 H, s, OH); δ_C 18.1 (q, Me), 20.9 (q, Me), 31.9 (t, C-4), 75.4 (d, C-3), 108.1 (s, C-8a), 115.7 (d, C-7), 124.9 (s, C-5), 137.0 (s, C-4a), 137.9 (d, C-6), 160.5 (s, C-8), 170.3 (s, C-1); Found M$^+$ 192.0769, $C_{11}H_{12}O_3$ requires M, 192.0786.

3-(3,5-Dimethoxyphenyl)propenoic Acid[81]
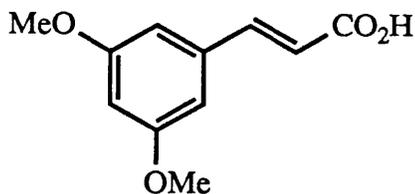
[256]

3,5-Dimethoxybenzaldehyde (4.6 g, 28 mmol), malonic acid (5.5 g, 53 mmol) and piperidine (2.5 ml, 25 mmol) were dissolved in pyridine (100 ml) and the mixture heated with stirring for 5 hours at 110°C. On cooling, the solution was poured onto crushed ice (300 g) and concentrated hydrochloric acid (150 ml) and the solid which precipitated was removed by filtration and dried. Recrystallisation from 95% ethanol gave white needles (5.4 g, 94%), m.p. 177-179°C (lit.[81]

174-176°C); $v_{max.}$ 2 940 br, 1 685 s and 1 630 s cm^{-1}; δ_H 3.81 (6 H,

s, 2 x OMe), 6.41 (1 H, d, $\underline{J}$ 16 Hz, CH), 6.51 (1 H, t, $\underline{J}$ 2.2 Hz, Ar-H),

6.69 (2 H, d, $\underline{J}$ 2.2 Hz, 2 x Ar-H), 7.71 (1 H, d, $\underline{J}$ 16 Hz, CH); δ_C

55.4 (q, 2 x OMe), 103.0 (d, C-4), 106.2 (d, C-2, C-6), 117.7 (d, CH),

135.8 (s, C-1), 147.1 (d, CH), 161.0 (s, C-3, C-5), 172.1 (s, CO_2H);

Found M^+ 208.0727, $C_{11}H_{12}O_4$ requires M, 208.0735.

3-(3,5-Dimethoxyphenyl)propanoic Acid [81]

[167]
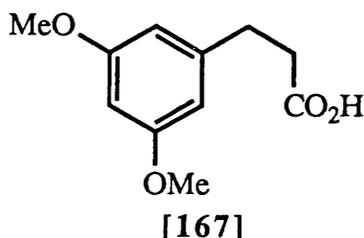
3-(3,5-Dimethoxyphenyl)propenoic acid (2.8 g, 13 mmol) was

dissolved in absolute ethanol (100 ml) and 10% palladium on charcoal

(100 mg) added.   The mixture was hydrogenated at room temperature and

atmospheric pressure until hydrogen uptake ceased.   After filtration

through celite, the solution was evaporated in vacuo to leave a white

solid.   Recrystallisation from cyclohexane gave needles (2.8 g, 99%),

m.p. 60-62°C (lit.[81] 59-61°C); $v_{max.}$ 1 710 s cm^{-1}; δ_H 2.67 (2 H, m,

CH_2), 2.90 (2 H, m, CH_2), 3.77 (6 H, s, 2 x OMe), 6.32 (1 H, t, $\underline{J}$ 2.1

Hz, Ar-H), 6.36 (2 H, d, $\underline{J}$ 2.1 Hz, 2 x Ar-H); δ_C 30.8 (t, CH_2), 35.4

(t, CH_2), 55.2 (q, 2 x OMe), 98.2 (d, C-4), 106.3 (d, C-2, C-6), 142.5

(s, C-1), 160.8 (s, C-3, C-5), 179.1 (s, CO_2H); Found M^+ 210.0889,

$C_{11}H_{14}O_4$ requires M, 210.0892.

5,7-Dimethoxyindan-1-one[81]
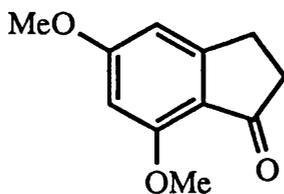
[168]

3-(3,5-Dimethoxyphenyl)propanoic acid (0.9 g, 4.3 mmol) was added with stirring to polyphosphoric acid (30 ml) at 100°C. The mixture was stirred at this temperature for 3 hours, then poured into ice-water (100 ml) with vigorous stirring. After adjusting the solution to pH 6 with 10% sodium hydroxide it was extracted with ethyl acetate (3 x 100 ml). The organic extracts were washed with aqueous sodium hydroxide (10%, 2 x 50 ml) and water (100 ml), then dried and evaporated. Flash chromatography (ethyl acetate as eluent) and recrystallisation from toluene/hexane yielded needles (0.7 g, 85%), m.p. 101-103°C (lit.[81] 98-99°C); $\nu_{max.}$ 1 690 s cm^{-1}; δ_H 2.62 (2 H, m, CH$_2$), 3.00 (2 H, m, CH$_2$), 3.85 (3 H, s, OMe), 3.89 (3 H, s OMe), 6.28 (1 H, d, J 1.8 Hz, Ar-H), 6.46 (1 H, d, J 1.8 Hz, Ar-H); δ_C 25.9 (t, C-3), 36.9 (t, C-2), 55.7 (q, 2 x OMe), 97.3 (d, C-6), 101.6 (d, C-4), 119.4 (s, C-7a), 159.3 (s, C-3a), 160.4 (s, C-7), 166.9 (s, C-5), 203.1 (s, C-1); Found M$^+$ 192.0775, C$_{11}$H$_{12}$O$_3$ requires M, 192.0787.

5,7-Dimethoxy-2-methoxycarbonylindan-1-one
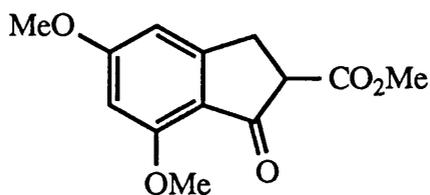
[257]

To a warm suspension (60°C) of sodium hydride (4 g/60% dispersion

in oil, 0.1 mol) and dimethyl carbonate (30 ml, 0.36 mol) in dry THF (100 ml) was added dropwise, with stirring, over half an hour, a solution of 5,7-dimethoxyindan-1-one (5.8 g, 0.03 mol) in THF (100 ml). Stirring was maintained at this temperature for an hour, then the solution was cooled to 0°C and methanol added cautiously.   The mixture was poured into dilute hydrochloric acid, extracted with ethyl acetate (3 x 50 ml) and dried and evaporated.   The crude product was purified by flash chromatography (2:1 ethyl acetate:light petrol as eluent) and recrystallised from ethyl acetate/light petrol as needles (6.4 g, 85%), m.p. 104-106°C; $\nu_{max.}$ 1 730 s and 1 700 s cm^{-1}; δ_H 3.20 (1 H, dd, $\underline{J}$ 19 Hz, $\underline{J}^1$ 8.2 Hz, H-3), 3.41 (1 H, dd, $\underline{J}$ 19 Hz, $\underline{J}^1$ 3.8 Hz, H-3), 3.66 (1 H, dd, $\underline{J}$ 8.2 Hz, $\underline{J}^1$ 3.8 Hz, H-2), 3.73 (3 H, s, CO_2Me), 3.86 (3 H, s, OMe), 3.87 (3 H, s, OMe), 6.28 (1 H, d, $\underline{J}$ 1.8 Hz, Ar-H), 6.48 (1 H, d, $\underline{J}$ 1.8 Hz, Ar-H); δ_C 30.1 (t, C-3), 52.6 (q, OMe), 53.6 (d, C-2), 55.8 (q, 2 x OMe), 97.7 (d, C-6), 101.5 (d, C-4), 117.5 (s, C-7a), 158.7 (s, C-3a), 160.0 (s, C-7), 167.6 (s, C-5), 170.0 (s, CO_2), 195.0 (s, C-1);   Found M^+ 250.0831, $C_{13}H_{14}O_5$ requires M, 250.0841.

Attempted Acetalisation of

5,7-dimethoxy-2-methoxycarbonylindan-1-one
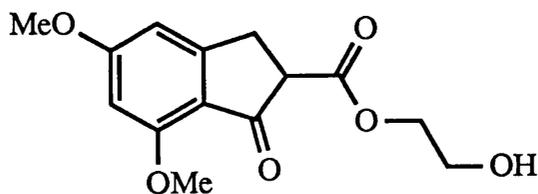
[258]
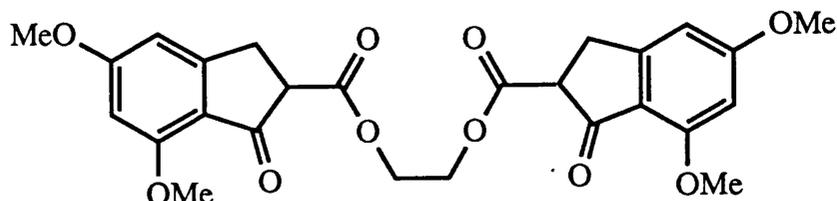
[259]

2-Carboxymethyl-5,7-dimethoxyindan-1-one (2.3 g, 9.2 mmol), ethylene glycol (0.7 ml, 12.5 mmol), and a crystal of p-toluenesulphonic acid were refluxed in toluene (100 ml) for 6 hours under Dean-Stark conditions. On cooling, the solution was washed with sodium bicarbonate solution (2 x 30 ml), dried and the solvent evaporated. The products were separated by flash chromatography (dichloromethane as eluent) to yield the monomer (258) and dimer (259).

Monomer (0.75 g, 29%), m.p. 112-113°C; $\nu_{max.}$ 3 520 br, 1 715 s and 1 690 s cm^{-1}; δ_H 3.15 (1 H, dd, $\underline{J}$ 17 Hz, $\underline{J}^1$ 8.2 Hz, H-3), 3.43 (1 H, dd, $\underline{J}$ 17 Hz, $\underline{J}^1$ 4.3 Hz, H-3), 3.69 (1 H, dd, $\underline{J}$ 8.2 Hz, $\underline{J}^1$ 4.3 Hz, H-2), 3.77 (2 H, t, $\underline{J}$ 4.6 Hz, CH$_2$OH), 3.83 (6 H, 2 x s, 2 x OMe), 4.10 (1 H, dt, $\underline{J}$ 12 Hz, $\underline{J}^1$ 4.6 Hz, CH$_2$OCO), 4.45 (1 H, dt, $\underline{J}$ 12 Hz, $\underline{J}^1$ 4.6 Hz, CH$_2$OCO), 6.24 (1 H, d, $\underline{J}$ 1.9 Hz, Ar-H), 6.46 (1 H, d, $\underline{J}$ 1.9 Hz, Ar-H); δ_C 29.5 (t, C-3), 53.8 (d, C-2), 55.7 (q, OMe), 55.8 (q, OMe), 60.4 (t, CH$_2$OH), 66.9 (t, $\underline{C}$H$_2$OCO), 97.7 (d, C-6), 101.6 (d, C-4), 117.0 (s, C-7a), 158.8 (s, C-3a), 159.9 (s, C-7), 167.8 (s, C-5), 169.3 (s, CO$_2$), 195.6 (s, C-1); Found M$^+$ 280.0942, C$_{14}$H$_{16}$O$_6$ requires M, 280.0947.

Dimer (0.9 g, 20%), m.p. 167-170°C; $V_{max.}$ 1 740 s and 1 700 s

cm^{-1}; δ_H 3.21 (1 H, dd, $\underline{J}$ 17 Hz, $\underline{J}^1$ 8.2 Hz, H-3), 3.41 (1 H, dd, $\underline{J}$ 17

Hz, $\underline{J}^1$ 4.0 Hz, H-3), 3.67 (1 H, m, H-2), 3.86 (6 H, s, 2 x OMe), 4.36

(2 H, s, CH$_2$O), 6.28 (1 H, d, $\underline{J}$ 1.9 Hz, Ar-H), 6.48 (1 H, d, $\underline{J}$ 1.9 Hz,

Ar-H); δ_C 30.2 (t, C-3), 53.7 (d, C-2), 55.7 (q, OMe), 55.8 (q, OMe),

62.8 (t, CH$_2$O), 97.7 (d, C-6), 101.6 (d, C-4), 117.5 (s, C-7a), 158.7

(s, C-3a), 159.9 (s, C-7), 167.5 (s, C-5), 169.4 (s, CO$_2$), 194.7

(s, C-1); Found M - C$_3$H$_6$O$_3$ 408.1208, C$_{23}$H$_{20}$O$_7$ requires M, 408.1209.

5,7-Dimethoxy-2-methoxycarbonylind-1-ene
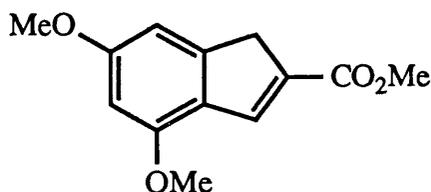
[260]

To a solution of 2-carboxymethyl-5,7-dimethoxyindan-1-one (1.2 g,

4.8 mmol) in methanol (100 ml) was added sodium borohydride (1 g, 26

mmol) with stirring.   After 3 hours the reaction mixture was quenched

with dilute hydrochloric acid and saturated with salt.   The resulting

solution was extracted with ethyl acetate (3 x 50 ml), dried and

evaporated.   The crude product was purified by flash chromatography

(20% ethyl acetate: light petrol as eluent) and recrystallised from

ethyl acetate/hexane as needles (0.6 g, 53%), m.p. 107-110°C; $V_{max.}$

1 690 s cm^{-1}; δ_H 3.63 (2 H, d, $\underline{J}$ 1.5 Hz, CH$_2$), 3.80 (3 H, s, CO$_2$Me),

3.84 (3 H, s, OMe), 3.85 (3 H, s, OMe), 6.38 (1 H, d, $\underline{J}$ 1.9 Hz, Ar-H),

6.66 (1 H, d, $\underline{J}$ 1.9 Hz), 7.81 (1 H, t, $\underline{J}$ 1.5 Hz, H-1); δ_C 38.8 (t,

C-3), 51.4 (q, OMe), 55.4 (q, OMe), 55.6 (q, OMe), 97.1 (d, C-6), 101.3

(d, C-4), 125.1 (s, C-7a), 132.2 (s, C-2), 138.1 (d, C-1), 148.4 (s,

C-3a), 155.3 (s, C-5), 161.9 (s, C-7), 165.5 (s, CO$_2$); Found M$^+$

234.0876, $C_{13}H_{14}O_4$ requires M, 234.0892.

2-Hydroxymethyl-5,7-dimethoxyind-1-ene
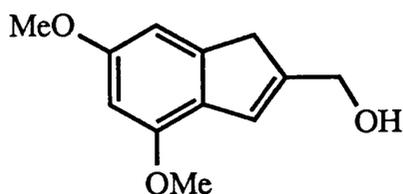
[261]

2-Carboxymethyl-5,7-dimethoxyind-1-ene (100 mg, 0.43 mmol) in dry THF (20 ml) was cooled to -78°C under nitrogen and diisobutylaluminium hydride (1 ml/1 M in hexanes, 1 mmol) was added dropwise with stirring. The cooling bath was removed and stirring continued for 2½ hours at room temperature. The reaction was quenched with excess methanol, celite added and the mixture filtered under vacuum. The residue was washed with acetone and the organic solvents evaporated. Flash chromatography (20% ethyl acetate:light petrol as eluent) and recrystallisation from ethyl acetate/hexane gave white needles (80 mg, 91%), m.p. 92-94°C; $V_{max.}$ 3 340 br cm^{-1}; δ_H 3.40 (2 H, d, $\underline{J}$ 0.7 Hz, H-3), 3.81 (3 H, s, OMe), 3.83 (3 H, s, OMe), 4.50 (2 H, d, $\underline{J}$ 0.6 Hz, CH$_2$OH), 6.37 (1 H, d, $\underline{J}$ 2.0 Hz, Ar-H), 6.65 (1 H, d, $\underline{J}$ 2.0 Hz, Ar-H), 6.79 (1 H, m, H-1); δ_C 39.5 (t, C-3), 55.4 (q, OMe), 55.6 (q, OMe), 61.8 (t, CH$_2$OH), 96.8 (d, C-6), 101.6 (d, C-4), 124.1 (d, C-1), 126.2 (s, C-7a), 144.2 (s, C-2), 146.5 (s, C-3a), 153.3 (s, C-5), 159.2 (s, C-7); Found M$^+$ 206.0926, $C_{12}H_{14}O_3$ requires M, 206.0943.

2-Acetoxymethyl-5,7-dimethoxyind-1-ene
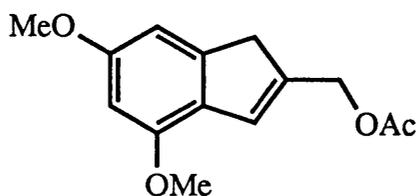
[262]

2-Hydroxymethyl-5,7-dimethoxyind-1-ene (0.5 g, 2.4 mmol) was dissolved in acetic anhydride (10 ml) with a few drops of pyridine and the solution stirred at room temperature overnight.   The reaction mixture was poured into water (100 ml) and the resulting aqueous solution extracted with dichloromethane (3 x 50 ml).   The combined organic fractions were washed with brine (2 x 50 ml), dried and evaporated.   After flash chromatography (20% ethyl acetate:light petrol as eluent) the acetate was recrystallised from light petrol and a few drops of ether (0.5 g, 83%), m.p. 73-75°C; $V_{max.}$ 1 730 s cm^{-1}; δ_H 2.09 (3 H, s, Me), 3.38 (2 H, s, H-3), 3.81 (3 H, s, OMe), 3.83 (3 H, s, OMe), 4.94 (2 H, s, CH_2OAc), 6.37 (1 H, d, $\underline{J}$ 2.0 Hz, Ar-H), 6.64 (1 H, d, $\underline{J}$ 2.0 Hz, Ar-H), 6.87 (1 H, s, H-1); δ_C 21.0 (q, Me), 40.1 (t, C-3), 55.3 (q, OMe), 55.6 (q, OMe), 62.8 (t, CH_2OAc), 96.7 (d, C-6), 101.4 (d, C-4), 125.9 (s, C-7a), 126.8 (d, C-1), 138.3 (s, C-2), 146.5 (s, C-3a), 153.4 (s, C-5), 159.5 (s, C-7), 170.9 (s, CO_2);   Found M^+ 248.1033, $C_{14}H_{16}O_4$ requires M, 248.1049.

3-Hydroxymethyl-6,8-dimethoxyisocoumarin
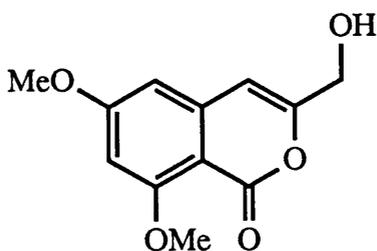
[264]

6,8-Dimethoxy-3-methylisocoumarin (500 mg, 2.3 mmol) and selenium dioxide (700 mg, 6.3 mmol) were dissolved in dioxane (20 ml) and water (5 ml) and the mixture stirred at reflux for one day. More selenium dioxide (400 mg, 3.6 mmol) was added and heating was continued for a second day. The solution was filtered hot and the dioxane evaporated to leave a yellow oil. Water (30 ml) was added and the aqueous solution extracted with ethyl acetate (3 x 30 ml). The combined organic layers were washed with sodium bicarbonate solution (2 x 20 ml), then dried and evaporated. The product was freed of starting material by flash chromatography (1:1 ethyl acetate:light petrol as eluent) and recrystallised from methanol as fine needles (100 mg, 19%), m.p. 183-185°C; $\nu_{max.}$ 3 380 m and 1 700 s cm^{-1}; δ_H (d$_6$-DMSO) 3.84 (3 H, s, OMe), 3.86 (3 H, s, OMe), 4.19 (2 H, br s, CH$_2$OH), 6.49 (1 H, s, H-4), 6.57 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H), 6.66 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H); δ_C 55.9 (q, OMe), 56.1 (q, OMe), 59.5 (t, CH$_2$OH), 98.5 (d, C-7), 100.9 (d, C-5), 101.8 (d, C-4), 101.9 (s, C-8a), 141.6 (s, C-4a), 157.6 (s, C-3), 158.2 (s, C-8), 162.9 (s, C-6), 165.3 (s, C-1); Found M$^+$ 236.0666, C$_{12}$H$_{12}$O$_5$ requires M, 236.0684.

3-Bromomethyl-6,8-dimethoxyisocoumarin[140]
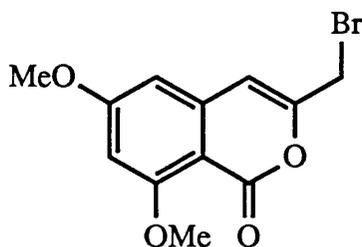
[266]

To a flask containing 6,8-dimethoxy-3-methylisocoumarin (620 mg, 2.8 mmol) dissolved in refluxing carbon tetrachloride (100 ml) was added azobisisobutyronitrile (20 mg, 0.1 mmol) followed by N-bromosuccinimide (510 mg, 2.9 mmol) in small portions under irradiation by a 150 W lamp. Stirring was continued under irradiation for half an hour, then the solution was allowed to cool.   The carbon tetrachloride was evaporated in vacuo and the residue dissolved in dichloromethane (50 ml). This solution was washed with saturated sodium bicarbonate solution (3 x 30 ml), dried and evaporated.   The product crystallised from ethyl acetate as needles (600 mg, 71%), m.p. 191-193°C (lit.[140] 192-193.5°C); $\nu_{max.}$ 1 720 s cm^{-1}; δ_H 3.91 (3 H, s, OMe), 3.96 (3 H, s, OMe), 4.24 (2 H, s, CH_2Br), 6.45 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H), 6.51 (1 H, s, H-4), 6.53 (1 H, d, $\underline{J}$ 2.3 Hz, Ar-H); δ_C 28.1 (t, CH_2Br), 55.7 (q, OMe), 56.3 (q, OMe), 99.4 (d, C-7), 101.0 (d, C-5), 103.0 (s, C-8a), 106.2 (d, C-4), 140.9 (s, C-4a), 152.3 (s, C-3), 158.1 (s, C-8), 163.2 (s, C-6), 165.5 (s, C-1);   Found M^+ 299.9809, $C_{12}H_{11}O_4Br$ requires M, 299.9821.

3-Bromomethyl-6,8-dihydroxyisocoumarin[140]
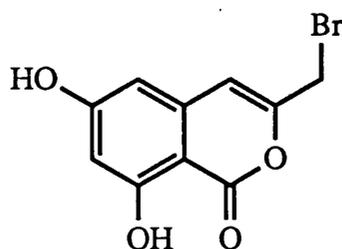
[267]

To a solution of 3-bromomethyl-6,8-dimethoxyisocoumarin (550 mg,
1.84 mmol) in dry dichloromethane (20 ml), stirred at -78°C under
nitrogen, was added boron tribromide (3 ml, 30 mmol). The cooling bath
was removed and the mixture stirred at room temperature overnight.
Ether (10 ml) was cautiously added, followed by water (10 ml) and then
more dichloromethane (50 ml). The organic layer was separated, washed
with water (2 x 20 ml), then dried and evaporated. The crude
product was purified by flash chromatography (1:1 ethyl acetate:light
petrol as eluent) and recrystallised from ethyl acetate/light petrol
as needles (500 mg, 100%), m.p. dec. > 180°C (lit.[140] 181-182.5°C
dec.); $\nu_{max.}$ 3 260 br, 1 675 s and 1 665 s cm^{-1}; δ_H (d$_6$-acetone)
4.47 (2 H, s, CH$_2$Br), 6.46 (1 H, d, J 2.2 Hz, Ar-H), 6.52 (1 H, d, J
2.2 Hz, Ar-H), 6.83 (1 H, s, H-4), 11.01 (1 H, s, OH); δ_C (d$_6$-acetone)
29.1 (t, CH$_2$Br), 100.0 (s, C-8a), 103.5 (d, C-7), 104.8 (d, C-5), 107.7
(d, C-4), 139.6 (s, C-4a), 152.7 (s, C-3), 164.6 (s, C-8), 166.2 (s, C-6),
166.5 (s, C-1); Found M$^+$ 271.9503, C$_{10}$H$_7$O$_4$Br requires M, 271.9508.

Treatment of 3-hydroxymethyl-6,8-dimethoxyisocoumarin (80 mg, 0.34
mmol) with boron tribromide by the foregoing method yielded the same
product (75 mg, 82%).

6,8-Dihydroxy-3-hydroxymethylisocoumarin

[4]
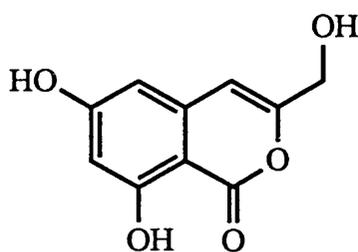
3-Bromomethyl-6,8-dihydroxyisocoumarin (100 mg, 0.37 mmol) was dissolved in a mixture of THF (10 ml) and water (10 ml) and stirred at reflux for 2 days.   On cooling, the solution was saturated with sodium chloride and extracted with ethyl acetate (3 x 20 ml).   The combined organic extracts were dried and evaporated and the crude product purified by flash chromatography (2:1 ethyl acetate:hexane as eluent). The alcohol crystallised from ethyl acetate/hexane as needles (65 mg, 85%), m.p. dec. > 220°C (lit.[18] 220-225°C dec.); $\nu_{max.}$ 3 230 br, 1 630. s and 1 620 s cm^{-1}; δ_H (d$_6$-acetone) 4.39 (2 H, s, CH$_2$OH), 6.39 (1 H, d, $\underline{J}$ 2.2 Hz, Ar-H), 6.46 (1 H, d, $\underline{J}$ 2.2 Hz, Ar-H), 6.58 (1 H, s, H-4), 11.10 (1 H, s, OH); δ_C (d$_6$-acetone) 60.9 (t, CH$_2$OH), 99.9 (s, C-8a), 102.5 (d, C-7), 103.7 (d, C-5), 103.9 (d, C-4), 140.4 (s, C-4a), 157.9 (s, C-3), 164.5 (s, C-8), 166.3 (s, C-6), 166.6 (s, C-1);   Found M$^+$ 208.0365, C$_{10}$H$_8$O$_5$ requires M, 208.0372.

REFERENCES

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

2.   R.A. Hill, Prog. Chem. Org. Nat. Prods., 1986, 49, 1.

3.   T.A. Tattar, Diseases of Shade Trees, Academic Press, 1978.

4.   C.M. Brashier and J.N. Gibbs, Nature, 1973, 242, 607.

5.   J.N. Gibbs and C.M. Brashier, Nature, 1973, 241, 381.

6.   G.A. Zentmyer, Science, 1942, 95, 512.

7.   A.E. Dimond, Phytopathology, 1947, 37, 7.

8.   A.W. Feldman, N.E. Caroselli and F.L. Howard, Phytopathology,

      1950, 40, 341.

9.   C.A. Salemink, H. Rebel, L.C.P. Kerling and V. Tchernoff,

      Science, 1965, 149, 202.

10.   N. Claydon, J.F. Grove and M. Hosken, Phytochemistry, 1974,

      13, 2567.

11.   K. Kameda, H. Aoki, H. Tanaka and M. Namiki, Agric. Biol. Chem.,

      1973, 37, 2137.

12.   R.M. Nelson and J.A. Beal, Phytopathology, 1929, 19, 1101.

13.   R.M. Nelson, Phytopathol. Z., 1934, 7, 327.

14.   R.W. Caird, Bot. Gaz., 1935, 96, 709.

15.   T.D. Paine, Can. J. Bot., 1984, 62, 556.

16.   R.C. Robinson, Can. J. Bot., 1962, 40, 609.

17.   R.C. Robinson-Jeffrey and R.W. Davidson, Can. J. Bot., 1968, 46,

      1523.

18.   G.W. McGraw and R.W. Hemingway, Phytochemistry, 1977, 16, 1315.

19.   W.A. Ayer, S.K. Attah-Poku, L.M. Browne and H. Orszanska,

      Can. J. Chem., 1987, 65, 765.

20.   W.A. Ayer, L.M. Browne, M. Feng, H. Orszanska and

      H. Saeedi-Ghomi, Can. J. Chem., 1986, 64, 904.

21.   J.D. DeAngelis, J.D. Hodges and T.E. Nebeker, Can. J. Bot.,

      1986, 64, 151.

22.   F.F. Jewell, Phytopathology, 1956, 46, 244.

23.   W.J. Moller and J.E. DeVay, Phytopathology, 1968, 58, 1499.

24.   E. Hardegger, W. Rieder, A. Walser and F. Kugler,

      Helv. Chim. Acta, 1966, 49, 1283.

25.   P. Venkatasubbaiah and W.S. Chilton, J. Nat. Prod., 1990, 53,

      1628.

26.   P. Venkatasubbaiah, T.B. Sutton and W.S. Chilton,

      Phytopathology, 1991, 81, 243.

27.   P. Venkatasubbaiah and W.S. Chilton, J. Nat. Prod., 1991, 54,

      1293.

28.   H. Grisebach and J. Ebel, Angew. Chem., Int. Ed. Engl., 1978,

      17, 635.

29.   E. Sondheimer, J. Am. Chem. Soc., 1957, 79, 5036.

30.   P. Condon and J. Kuc, Phytopathology, 1960, 50, 267.

31.   P. Condon and J. Kuc, Phytopathology, 1962, 52, 182.

32.   B.A. Herndon, J. Kuc and E.B. Williams, Phytopathology, 1966,

      56, 187.

33.   R.F. Curtis, Experientia, 1968, 24, 1187.

34.   A. Stoessl, Biochem. Biophys. Res. Commun., 1969, 35, 186.

35.   J. Kuc, A. Rev. Phytopathology, 1972, 10, 207.

36.   P. Condon, J. Kuc and H.N. Draudt, Phytopathology, 1963, 53,

      1244.

37.   B.C. Carlton, C.E. Peterson and N.E. Tolbert, Plant physiol.,

      1961, 36, 550.

38.   D.T. Coxon, R.F. Curtis, K.R. Price and G. Levett, Phytochemistry,

      1973, 12, 1881.

39.   F. Kurosaki and A. Nishi, Phytochemistry, 1983, 22, 669.

40.   M. Amin, F. Kurosaki and A. Nishi, Phytochemistry, 1986, 25,

      2305.

41.   T. Sassa, H. Aoki, M. Namiki and K. Munakata, Agric. Biol. Chem.,

      1968, 32, 1432.

42.   A.K. Tanaka, C. Sato, Y. Shibata, A. Kobayashi and K. Yamashita,

Agric. Biol. Chem., 1974, 38, 1311.

43.   S. Iwasaki, H. Muro, K. Sasaki, S. Nozoe and S. Okuda,

Tetrahedron Lett., 1973, 3537.

44.   K. Nozawa, M. Yamada, Y. Tsuda, K. Kawai and S. Nakajima,

Chem. Pharm. Bull., 1981, 29, 2689.

45.   K. Nozawa, M. Yamada, Y. Tsuda, K. Kawai and S. Nakajima,

Chem. Pharm. Bull., 1981, 29, 2491.

46.   K. Nozawa, M. Yamada, Y. Tsuda, K. Kawai and S. Nakajima,

Chem. Pharm. Bull., 1981, 29, 3486.

47.   Y. Shimojima, H. Hayashi, T. Ooka, M. Shibukawa and Y. Iitaka,

Tetrahedron, 1984, 40, 2519.

48.   K. Hashigaki, K. Hiramatsu, M. Yamato and K. Tasaka,

Chem. Pharm. Bull., 1984, 32, 3561.

49.   J.W. Harper, K. Hemmi and J.C. Powers, Biochemistry, 1985, 24,

1831.

50.   J.W. Harper and J.C. Powers, Biochemistry, 1985, 24, 7200.

51.   S. Gabriel, Ber., 1885, 18, 2433.

52.   S. Gabriel, Ber., 1903, 36, 570.

53.   E. Bamberger and M. Kitschelt, Ber., 1892, 25, 888.

54.   E. Bamberger and W. Frew, Ber., 1894, 27, 198.

55.   W. Dieckmann and W. Meiser, Ber., 1908, 41, 3253.

56.   H.W. Johnston, C.E. Kaslow, A. Langsjoen and R.L. Shriner,

J. Org. Chem., 1948, 13, 477.

57.   N.N. Vorozhtsov Jr. and L.N. Bogusevich, J. Gen. Chem. (USSR),

1940, 10, 2014.

58.   A. Kamal, A. Robertson and E. Tittensor, J. Chem. Soc., 1950, 3375.

59.   H.J.E. Lowenthal and R. Pappo, J. Chem. Soc., 1952, 4799.

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

935.

61.   R.B. Tirodkar and R.N. Usgaonkar, Ind. J. Chem., 1970, 8, 123.

62.   P.G. Deshmukh, U. Usgaonkar and R.N. Usgaonkar, Ind. J. Chem.,

       1973, 11, 413.

63.   R.B. Tirodkar and R.N. Usgaonkar, J. Ind. Chem. Soc., 1971, 48,

       192.

64.   D.R. Nadkarni and R.N. Usgaonkar, Ind. J. Chem., 1978, 16B, 320.

65.   J.N. Chatterjea, S.K. Mukherjee, C. Bhakta, H.C. Jha and

       F. Zilliken, Chem. Ber., 1980, 113, 3927.

66.   V.H. Belgaonkar and R.N. Usgaonkar, J. Chem. Soc., Perkin Trans

       1, 1977, 702.

67.   J.N. Chatterjea, C. Bhakta and T. Radha Vakula, J. Ind. Chem. Soc.,

       1972, 49, 1161.

68.   J.N. Chatterjea, C. Bhakta and J. Mukherjee, Ind. J. Chem., 1974,

       12, 1259.

69.   C.K. Ingold and H.A. Piggott, J. Chem. Soc., 1923, 1469.

70.   W.F. Whitmore and R.C. Cooney, J. Am. Chem. Soc., 1944, 66, 1237.

71.   A. Bruggink and A. McKillop, Tetrahedron, 1975, 31, 2607.

72.   M. Guyot and D. Molho, Tetrahedron Lett., 1973, 3433.

73.   F.M. Hauser and R. Rhee, Synthesis, 1977, 245.

74.   J.L. Warnell and R.L. Shriner, J. Am. Chem. Soc., 1957, 79, 3165.

75.   C.F.H. Allen and J.W. Gates Jr., J. Am. Chem. Soc., 1943, 65,

       1230.

76.   C. Schopf and R. Kuhne, Chem. Ber., 1950, 83, 390.

77.   D. Mukhopadhya and D.N. Chaudhury, J. Ind. Chem. Soc., 1970, 47,

       450.

78.   N.K. Bose and D.N. Chaudhury, J. Ind. Chem. Soc., 1965, 42, 211.

79.   N.K. Bose and D.N. Chaudhury, J. Ind. Chem. Soc., 1966, 43, 411.

80.   R.H. Carter, R.M. Colyer, R.A. Hill and J. Staunton, J. Chem. Soc.,

       Perkin Trans. 1, 1976, 1438.

81.   R.H. Carter, M.J. Garson, R.A. Hill, J. Staunton and D.C. Sunter,

       J. Chem. Soc., Perkin Trans. 1, 1981, 471.

82.   N.S. Narasimhan and B.H. Bhide, Tetrahedron, 1971, 27, 6171.

83. M.P. Sibi, M.A. Jalil Miah and V. Snieckus, J. Org. Chem., 1984, 49, 737.

84. G. Batu and R. Stevenson, J. Org. Chem., 1980, 45, 1532.

85. D.E. Korte, L.S. Hegedus and R.K. Wirth, J. Org. Chem., 1977, 42, 1329.

86. R.C. Larock, S. Varaprath, H.H. Lau and C.A. Fellows, J. Am. Chem. Soc., 1984, 106, 5274.

87. G. Berti, Tetrahedron, 1958, 4, 393.

88. R.S. Mali, P.G. Jagtap, S.R. Patil and P.N. Pawar, J. Chem. Soc., Chem. Commun., 1992, 883.

89. J. Colonge and P. Boisde, Bull. Soc. Chim. France, 1956, 5, 1337.

90. J.N. Srivastava and D.N. Chaudhury, J. Org. Chem., 1962, 27, 4337.

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

92. M. Watanabe, M. Sahara, M. Kubo, S. Furukawa, R.J. Billedeau and V. Snieckus, J. Org. Chem., 1984, 49, 742.

93. A.C. Regan and J. Staunton, J. Chem. Soc., Chem. Commun., 1983, 764.

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

95. L.M. Harwood, J. Chem. Soc., Chem. Commun., 1982, 1120.

96. K. Mori and A.K. Gupta, Tetrahedron, 1985, 41, 5295.

97. C.C.K. Kanakam, N.S. Mani, H. Ramanathan and G.S.R. Subba Rao, J. Chem. Soc., Perkin Trans. 1, 1989, 1907.

98. H. Nogami, J. Pharm. Soc. Japan, 1941, 61, 56.

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

100. F.M. Hauser and R.P. Rhee, J. Org. Chem., 1977, 42, 4155.

101. G.B. Henderson and R.A. Hill, J. Chem. Soc., Perkin Trans. 1, 1982 , 1111.

102. H.L. Slates, S. Weber and N.L. Wendler, Chimia, 1967, 21, 468.

103. J.K. Kendall, T.H. Fisher, H.P. Schultz and T.P. Schultz, J. Org. Chem., 1989, 54, 4218.

104. G.S. Macaulay, Ph.D. Thesis, Glasgow, 1990.

105. J.R. Cannon, T.M. Cresp, B.W. Metcalf, M.V. Sargent,
      G. Vinciguerra and J.A. Elix, J. Chem. Soc., 1971, 3495.

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

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

108. J.F. Grove and M. Pople, J. Chem. Soc., Perkin Trans. 1, 1979,
      337.

109. M.A. Umbreit and K.B. Sharpless, J. Am. Chem. Soc., 1977, 99,
      5526.

110. J. Muzart, Tetrahedron Lett., 1987, 2131.

111. C. Djerassi, Chem. Rev., 1948, 43, 271.

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

113. C.A. Brown and D. Barton, Synthesis, 1974, 434.

114. J.F.W. McOmie, M.L. Watts and D.E. West, Tetrahedron, 1968, 24,
      2289.

115. E.J. Corey and A. Venkateswarlu, J. Am. Chem. Soc., 1972, 94,
      6190.

116. B.M. Trost, G.T. Rivers and J.M. Gold, J. Org. Chem., 1980, 45,
      1835.

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

118. A.I. Meyers and K. Higashiyama, J. Org. Chem., 1987, 52, 4592.

119. G. Assante, R. Locci, L. Camarda, L. Merlini and G. Nasini,
      Phytochemistry, 1977, 16, 243.

120. M.G. Hutchings, A.M. Chippendale, R. Shukla and M. McPartlin,
      Tetrahedron, 1991, 47, 7869.

121. D.C. Aldridge, S. Galt, D. Giles and W.B. Turner, J. Chem. Soc. C.,
      1971, 1623.

122. T. Okuno, S. Oikawa, T. Goto, K. Sawai, H. Shirahama and
      T. Matsumoto, Agric. Biol. Chem., 1986, 50, 997.

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

124. R.D. Hutchison, P.S. Steyn and D.L. Thompson, Tetrahedron Lett., 1971, 4033.

125. D.C. Aldridge and W.B. Turner, J. Chem. Soc. C., 1970, 2598.

126. W.A. Hawksworth, P.W. Jeffs, B.K. Tidd and T.P. Toube, J. Chem. Soc., 1965, 1991.

127. S. Kobayashi, H. Ishikawa, E. Sasakawa, M. Kihara, T. Shingu and A. Kato, Chem. Pharm. Bull., 1980, 28, 1827.

128. M. Taneyama, S. Yoshida, M. Kobayashi and M. Hasegawa, Phytochemistry, 1983, 22, 1053.

129. T. Yoshida, T. Okuda, T. Koga and N. Toh, Chem. Pharm. Bull., 1982, 30, 2655.

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

131. E.J. Corey and J.W. Suggs, Tetrahedron Lett., 1975, 2647.

132. J.N. Chatterjea, B.K. Banerjee, C. Bhakta and J. Mukerji, J. Ind. Chem. Soc., 1972, 49, 797.

133. J.M. Brittain, P.B.D. De la Mare, N.S. Isaacs and P.D. McIntyre, J. Chem. Soc., Perkin Trans. 2, 1979, 933.

134. K. Auwers, Ber., 1911, 44, 3692.

135. Vogel's Textbook of Practical Organic Chemistry, fifth edition, Longman Scientific and Technical, 706.

136. M. Stiles, J. Am. Chem. Soc., 1959, 81, 2598.

137. D.R. Nadkarni and R.N. Usgaonkar, Ind. J. Chem., 1977, 15B, 185.

138. J.N. Chatterjea, S.K. Mukherjee and C. Bhakta, Ind. J. Chem., 1980, 20B, 359.

139. M. Lampilas and R. Lett, Tetrahedron Lett., 1992, 773.

140. M. Lampilas, Ph.D. Thesis, Université Pierre et Marie Curie, 1991.

141. H. Kumagai, T. Masuda, M. Ohsono and S. Hattori, J. Antibiotics, 1990, 43, 1505.