TOWARDS THE BIOSYNTHESIS OF MOLLISIN

by

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A special gratitude is owed to my parents and this thesis is dedicated to them.

CONTENTS

SUMMARY

Mollisin, a unique dichloroacetylnapthoquinone metabolite of the fungus Mollisia caesia is of polyketide origin. The biosynthesis of mollisin is proposed to proceed either through the condensation of two polyketide precursors or through the condensation of a single octaketide chain by means of a phenanthrenoid intermediate. M.caesia was incubated with sodium [2-$^{2}H_{3}$,1-^{13}C] acetate. Subsequent isolation of mollisin and examination by NMR spectroscopy has shown that acetate was incorporated. Close examination of ^{13}C and ^{2}H spectra provided evidence that biosynthesis may proceed via the octaketide route.

A postulated biosynthetic intermediate is 1,5,7,10-tetrahydroxy-3-methylphenanthrene. A synthetic route to its 1,5,7-trimethyl ether has been developed which utilises the condensation of a 3,5-dimethoxyphenylacetonitrile anion with a benzoate ester. The enol acetate of the resulting β-ketonitrile undergoes photocyclisation to give a 10-acetoxy-9-cyanophenanthrene. Photolysis of the corresponding methyl enol ether failed to give cyclised product. It was found that the condensation product of the phenylacetonitrile with methyl 2-methoxy-4-methylbenzoate gave a mixture of 5,7-dimethoxy- and 1,5,7-trimethoxy-phenanthrenes upon photoylsis of the enol acetate. These result from non-oxidative and oxidative reaction pathways respectively, the former involving loss of methanol. In contrast the enol acetate of the condensation product of a 2,6-dimethoxy-4-methylbenzoate upon photolysis gave the 10-acetoxy-9-cyano-1,5,7-trimethoxyphenanthrene as the sole product -

the oxidative mechanism being precluded. Hydrolysis of the nitrile functionality of this material proved impossible even under forced conditions. This proved similarly so for the corresponding 10-hydroxy- and 10-methoxy-phenanthrenes prepared from this compound. On attempting to prepare the aldehyde by reaction of the nitrile with Raney nickel in formic acid, a novel reductive decyanation was observed to furnish the hydroxytrimethoxyphenanthrene. Mechanistically, this degradation is proposed to proceed either via a Birch-type process or via a series of hydrogenation and elimination steps. Complete demethylation of 10-hydroxy-1,5,7-trimethoxy-3-methylphenanthrene using standard techniques was not found to be possible.

Attempts to adapt this synthetic route to provide 4-substituted phenanthrenes were unsuccessful. Condensation of 3,5-dimethoxyphenylacetonitrile with a 2,5-dimethoxybenzoate ester followed by photolysis of the enol acetate of the resulting ketone gave exclusively the 2-methoxyphenanthrene through non-oxidative methanol loss. No 1,4-dimethoxyphenanthrene was obtained. A series of methyl 2,3,6-trimethoxybenzoates was prepared. However, condensation with the phenylacetonitrile was not possible because of steric hindrance of the ester moeity.

Bromination of 10-acetoxy-9-cyano-1,5,7-trimethoxy-3-methylphenanthrene has been found to be reversible and temperature dependant. The 2-bromo, 6-bromo, 2,6-dibromo, and 4,6-dibromophenanthrenes can be formed depending upon the reaction conditions. On prolonged reaction, a thermodynamic equilibrium

mixture is formed. The structure of these bromophenanthrenes was elucidated by 200 MHz proton NMR spectroscopy.

CHAPTER 1

INTRODUCTION

For more than two centuries the elucidation of the chemistry of a natural product - its molecular structure, chemical properties, synthesis and biosynthesis - has been a dominant theme of organic chemistry[1]. Ever since 1769-85 when Carl Wilhelm Scheele first isolated tartaric acid in pure form from grapes, citric acid from lemons, malic acid from apples and gallic acid from gall nuts the chemist has tried to decipher and elucidate the ways and means of nature.

Since this time, much has been discovered to aid our understanding of natural systems and it is now clear that living organisms synthesise and degrade chemical compounds by means of a series of enzyme-mediated chemical reactions, collectively known as metabolism. All organisms have some metabolic pathways in common that are essential for their survival, for example those through which amino acids, sugars, common fatty acids and nucleotides are constructed. In addition to such primary metabolites, a considerably greater body of natural substances - alkaloids, terpenes, polyenes, pigments, phenols, mycotoxins and so forth - occur throughout nature. Such secondary metabolites are products biosynthesised from primary metabolites and are often the result of long series of complex functionalisation requiring a large number of specific enzymes. Generally they are species, and often strain, specific.

The question of the role of secondary metabolism has been the subject of much speculation but the ideas of Bu'Lock[2] have perhaps been most interesting. Bu'Lock proposed that secondary metabolism serves to maintain basic, ie. primary, metabolism at periods when through nutritional imbalances cell replication cannot take place. So it is the process of secondary metabolism, rather than the metabolites so produced, which is seen as advantageous. A particularly fertile source of secondary metabolites are the fungi[3] and to date many hundreds of novel compounds have been isolated from fungal sources. It is with the secondary metabolites of fungi that this thesis will be concerned.

Together with the algae and the bacteria, fungi have traditionally been classified as members of the Thallophyte, a division of the plant kingdom which is comprised by organisms with no true roots, stems or leaves. The individual reproductive bodies of fungi are the spores which are borne by sporophores. Under favourable conditions, spores become detached from the sporophores and, if they reach a suitable environment, they grow to produce the vegetative phase of the fungus. This is known as the mycelium and consists of a network of fine branches, termed hypha.

All fungi lack photosynthetic pigments and are therefore unable to manufacture organic compounds from carbon dioxide and water; they therefore require oxidisable organic compounds as energy sources. In a natural environment these can be obtained from dead or living plants, animals, or from other micro-organisms; in culture

they are generally grown on sugars although they can often be induced to use many organic compounds.

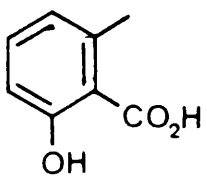
One of the most important biosynthetic routes in the secondary metabolism of fungi is the acylpolymalonate or polyketide pathway. The idea that naturally occurring, polyoxygenated compounds may arise from the condensation of acetate units was first suggested by J.N.Collie in 1893[4,5]. However, this inspired speculation was largely ignored until it was restated by Birch over fifty years later. Birch supported the proposition with the experimental evidence that ^{14}C-labelled acetate was incorporated into 6-methylsalicyclic acid (6-MSA) (1), a metabolite of Penicillium griseofulvum and other fungi[6,7]. The basic concepts which underline the acetate hypothesis can be summarised thus:

(a) Acetic acid units are joined by the formal elimination of water in head to tail linkages with each other or with other naturally occurring carboxylic acids to form β-polyketomethylene chains.

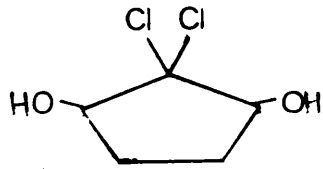
(b) The β-ketomethylene chains may undergo secondary changes: notably cyclisations of the Claisen or Aldol type to form aromatic rings.

(c) The carbon skeleton so formed may be modified by the introduction of alkyl groups.

(d) Secondary processes of reduction and of oxidation may occur either before or after cyclisation.



(1)



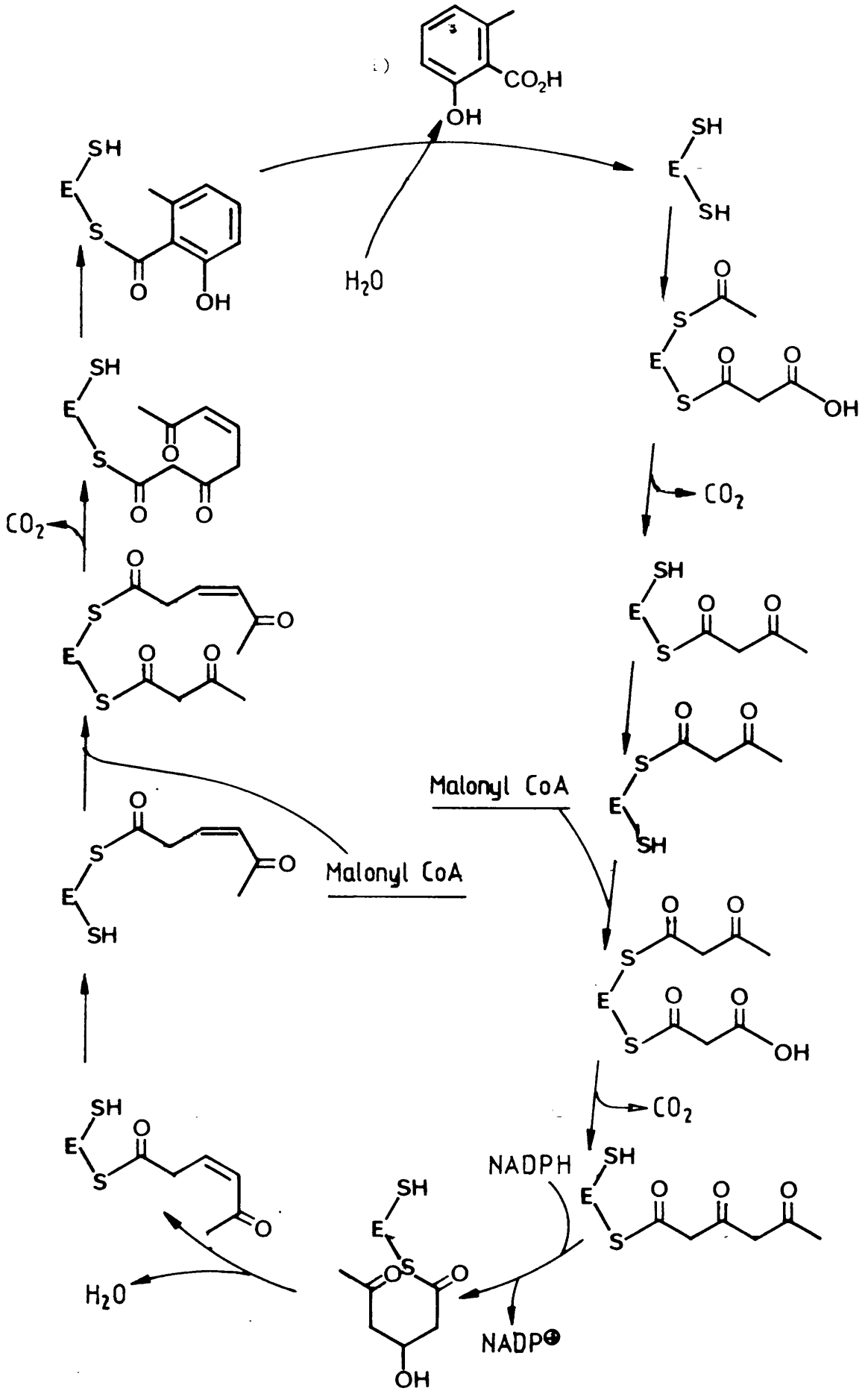
(2)

6-Methylsalicylic acid(1) can be regarded as a typical polyketide-derived aromatic metabolite. Bu'Lock showed that 6-MSA (1) was formed from one acetate unit and three malonate units[8], and on this basis Lynen proposed a mechanism for the biosynthesis of 6-MSA(1) by analogy with his hypothesis for the biosynthesis of fatty acids[9,10]. This mechanism involves acetyl-CoA, malonyl-CoA and NADPH in a multienzyme complex (figure 1). This complex (6-MSA synthetase) has been purified by several groups and studied in some detail and, in accordance with Lynen's scheme, has been shown to contain two sulphydryl group in its active site[11,12].

Of the many known in vivo modifications of polketides and other classes of metabolite, chlorination is frequently encountered. Well over 100 chlorine containing secondary metabolites have been isolated from fungal sources alone[3] and bacteria and higher plants are also prodigious producers. This area has been reviewed by Miller and Fleminson[13] and by Engrild[14].

The mechanism for biological chlorination is far from clear. However, chlorination does not generally occur at a late stage in biosynthesis and more usually occurs in intermediates with active methylene groups, such as β-dicarbonyl compounds, and certain phenols. The biosynthesis of caldariomycin (2), a chlorine containing metabolite of Caldariomyces fumago has been studied by Hager et al[15]. Growing cultures of C.fumago produce caldariomycin (2) as shown in figure 2. The chlorination of β-ketoadipate can be represented thus:

Figure 1. After Lynen

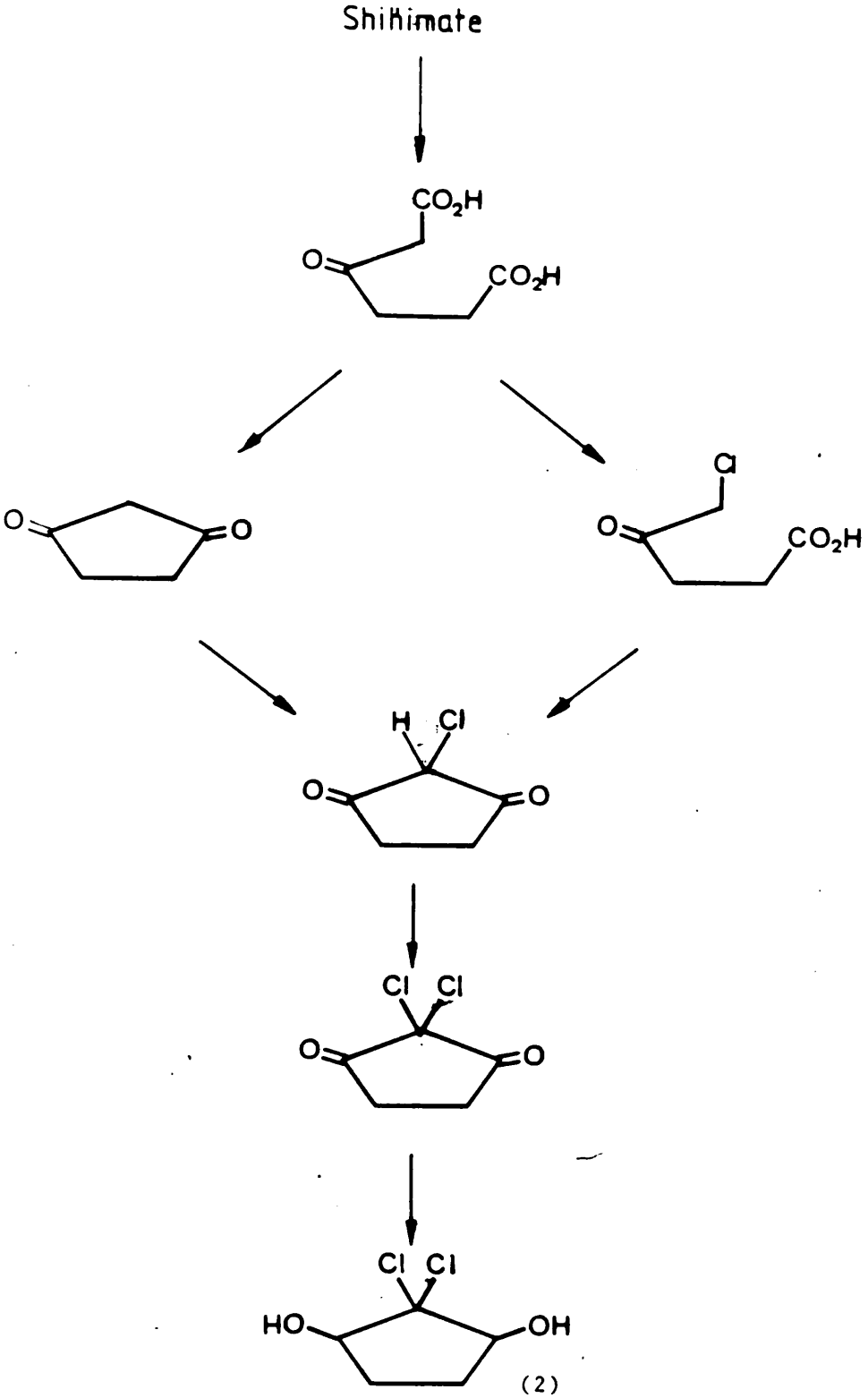


$$A\text{-}H + Cl^- + H_2O_2 \longrightarrow A - Cl + H_2O + OH^-$$

The reaction has been found to be catalysed by a chloroperoxidase enzyme crystallised from C.fumago extracts. The enzyme is a glycoprotein which contains one mole of ferriprotoporphyrin IX as prosthetic group and has a molecular weight of 42,000 of which 25-30% is carbohydrate. Detailed knowledge of the structure of the active site is lacking and in particular the nature of the process by which the halogen is transferred to the substrate is unclear. It is assumed that the oxidation of chloride to hypochlorite anions or their equivalent is involved. The chloroperoxidase is relatively non-specific and will catalyse chlorination of cyclic or acyclic 1,3-dicarbonyl compounds. β-Ketoadipic acid is converted into δ-chlorolevulinic acid with simultaneous decarboxylation. It is suggested that 1,3-cyclopentandione is converted in seperate steps firstly into the 2-chloro- and then the 2,2-dichloro- derivative which is subsequently converted to caldariomycin (2) by C. fumago[16,17]. In the absence of chlorine both bromine and iodine will act as alternative substrates for the chloroperoxidase (figure 2).

Chlorine is one of the most abundant elements in nature and it is universally present in plants and fungi. However, it was not until 1954 when Broyer[18] showed that it has an essential role. Only in 1959 was the first chlorine containing metabolite isolated from a higher plant: the pyrrolizidine alkaloid jaconine (3) from Senecia jacobanea (Compositae)[19]. Chlorine occurs with greater frequency in the secondary products of fungi and lichens and it is

Figure 2. The Biosynthesis of Caldariomycin (2).



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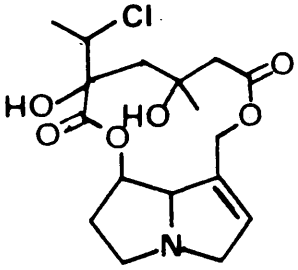

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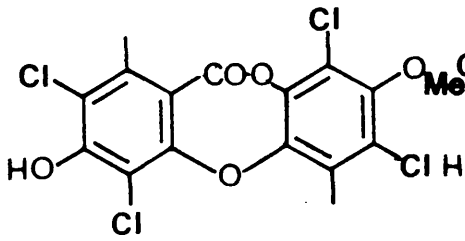

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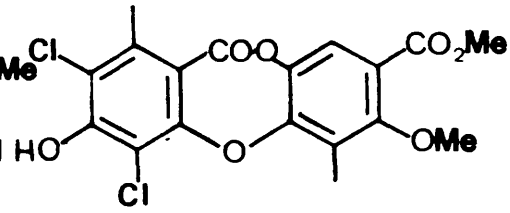

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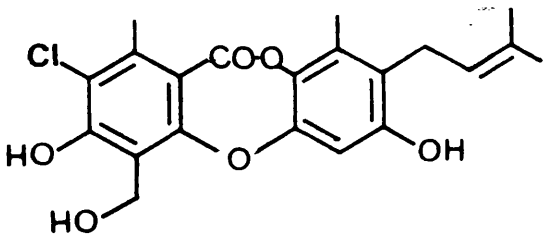

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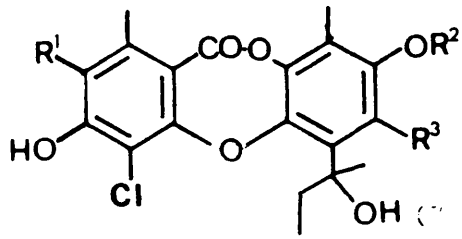
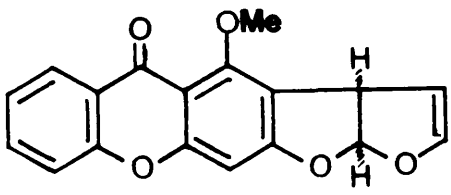


(7)

$R^1 = R^3 = H$
$R^2 \neq Me$
$R^1 = R^3 = Cl$
$R^2 = H$

(8)



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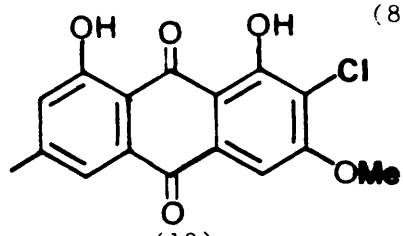

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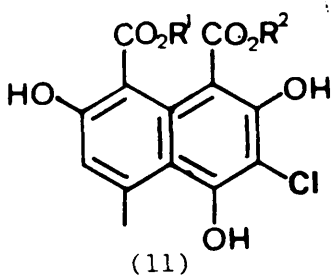


(11)

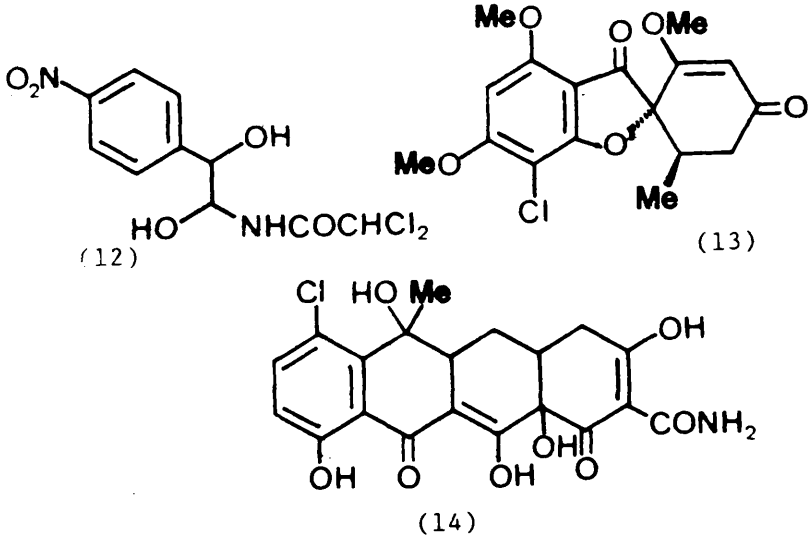
R^1 or R^2 = Me

thought that the chloro-metabolites produced by lichens are in fact produced by their fungal partners[20]. Zopf in 1904[21] was first to isolate a chlorometabolite from a lichen: the depsidone diploicin (4) from Diploicia canascens. Interestingly no deshalo analogue of diploicin (4) has yet been reported.

Depsidones and depsides are a particularly rich class of chlorine containing fungal product. Among many known examples are gangeloidin (5) (Leconara gangeloides)[22], mollicelin D (6) (Chaetominium mollicellum)[23], rubinin (7) and shirin (8) (Aspergillus ungins)[24]. Chlorinated examples of almost all classes of polyketide (and many non-polyketide) metabolite have been found: the xanthone austocystin A (9) (A.ustus)[25]; the anthraquinone fragilin (10) (Caloplacca spp)[26]; and the phelanone derived Verticullinum lamellicola chloronapthalic ester (11) (V.lamellicola)[27].

Many chlorine containing metabolites have antibiotic properties and among those used clinically are chloramphenicol (12), griseofulvin (13) and chlorotetracycline (14) produced from strains of Streptomyces venezuelae, Penicillium urticae, and S.aureofaciens respectively. These three antibiotics can be obtained from their fungal sources in various deshalo-, chloro- and bromo- forms depending upon the culture conditions employed.

The biosynthesis of griseofulvin (13) has been studied by Harris[29] and has been shown to proceed via cyclocondensation of a



(12) (13) (14)

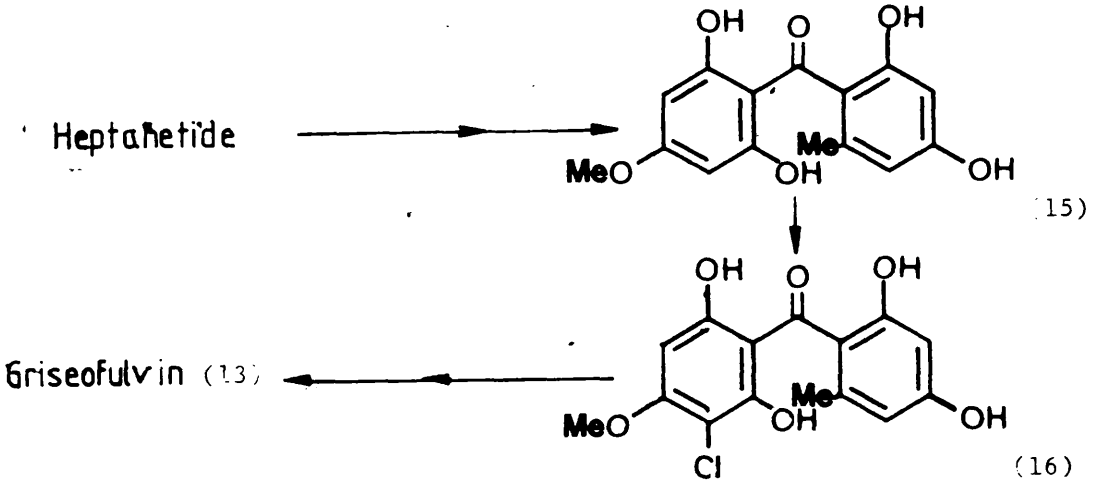
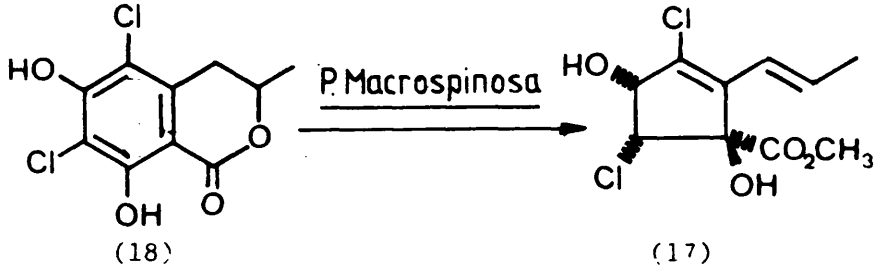


Figure 3. The Biosynthesis of Griseofulvin (13)



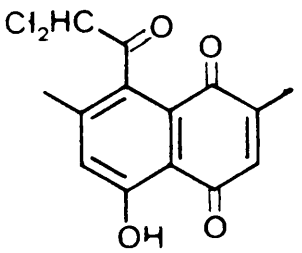
(18) (17)

heptaketide to produce an intermediate benzophenone, griseophenone C (15). Aromatic chlorination gives a second benzophenone, griseophenone B (16) which is subsequently converted into griseofulvin (13) by P.urticae (figure 3).

It will have been noted that most polyketide derived chloro-compounds mentioned above have had an aromatic bonded chlorine atom. This is generally but not always true and there are many cases of non-aromatic chloro-fungal metabolites. Howeve often the chloro-metabolite has been found to be derived from an aromatic precursor which may then undergo ring degradation. For example cryptosporiopsinol (17) which is derived from the chloroisocoumarin (18) in Periconia macrospinosa[30].

The major route to organo-halogen compounds in nature is regarded to be the halo-peroxidase mediated system previously described. However, Harper[31] has suggested that the formation of chloromethane by the wood-rotting fungus Phellinus pomaceus occurs via a quite distinct mechanism. Chloromethane production was found to mirror loss of chloride from the medium and available chloride was methylated with >90% efficiency. Harper states that this involves the enzymatic incorporation of halide ion directly into a one carbon compound.

A structually unique fungal metabolite is the napthoquinone pigment mollisin (19) isolated from Mollisia caesia and M.fallens[32,33]. Mollisin (19) has been shown to be of polyketide origin and is the only known polyketide natural product that contains



(19)

an aromatic dichloroacetyl moeity. Dichloroacetyl groups are not unknown in secondary metabolites but they are extremely rare from fungal sources. One of the few examples is chloramphenicol (12) which contains a dichloroacetamide grouping[28]. The origin of this dichloroacetyl group is unclear but it does not appear to arise either from the chlorination of an acetamide or via the formation of the amide from dichloroacetic acid in S. veneznelae. It has been suggested that biosynthesis proceeds through the fixation of carbon dioxide into a three carbon unit followed by chlorination of the resultant three carbon compound or its derivative.

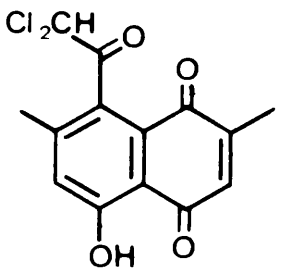
Marine algae are a more abundant source of dichloroacetyl moeities and indeed of halo-compounds generally[34]. Chlorocarbons are thought to play an important role in the regulation of atmospheric ozone density and marine plants have been suggested as the source of such air-borne compounds[35].

Volatile components of the oil of the Hawaiian sea-weed Aspargopsin armata have been examined by GC-MS techniques[36,37,38]. Among the many identified halogenated compounds are dibromoacetaldehyde, hexachloroacetone, 1,4-dibromo-1,4-dichlorobutenone, chloro-iodoacetamide, dichloroacetic acid and dibromoacetic acid. Curiously when a different source of A.armata was investigated (Mexican or Spannish), different halogenated compounds were observed[39]. Thus a series of halogenated acetones were identified including 1-bromo-3,3-dichloro-, 1,3,3-trichloro-1,1-dibromo-, 3,3-dichloro-, 1-bromo-1-, 3,3-trichloro-, and 1,1,3,3-tetra-chloroacetone (Table 1).

Table 1 Dihaloacetyl metabolites of A.armata

Br_2CHCHO	$BrCH_2COCHCl_2$
$Cl_3CCOCCl_3$	$ClCH_2COCHCl_2$
$BrClC{=}CHCOCHBrCl$	$BrClCHCOCHBrCl$
$ClIHCCONH_2$	$BrClCHCOCHCl_2$
Cl_2CHCO_2H	$Cl_2CHCOCHCl_2$
Br_2CHCO_2H	

Mollisin (19) was the first natural product to be discovered to contain a dichloroacetyl group directly attached to carbon. It remains the only known natural product to contain a dichloroacetyl group directly attached to an aromatic carbon atom, and so is of biosynthetic interest. An investigation into the biosynthesis of mollisin (19) by M.caesia is the subject of this dissertation.



(19)

CHAPTER 2

BIOSYNTHETIC STUDIES ON MOLLISIA CAESIA

Fungi of the genus Mollisia have been the subject of research for a number of years now, in particular the species M.fallens and M.caesia have been closely studied because of the characteristic yellow pigment produced by them. This was first described by Gremmen[40] in 1956 and the pigment, termed "mollisin", was later identified by Overeem and Van der Kerk[32] in 1964 as 8-dichloroacetyl-5-hydroxy-2,7-dimethyl-1,4-napthoquinone (19). This structure is biosynthetically unique in that it is the only known fungal metabolite to contain an aryl dichloracetyl moeity and so mollisin (19) has naturally been the focus of a significant amount of attention since its discovery.

The molecular structure of mollisin consists of a relatively simple carbon framework and is highly oxygenated therefore implying a biosynthetic origin from the acylpolymalonate pathway. This origin was first proposed by Overeem and Van der Kerk[31,32] and was confirmed by Bentley and Gatenbeck[41] through labelling experiments using [1-^{14}C] acetate and [1,3-^{14}C] malonate. This study demonstrated the likelihood that carbons 2 and 7 are derived from C-1 of acetate. On feeding [$^{14}CH_3$] methionine, no label was found at either C-11 or C-12 and so by implication these carbons are derived from C-2 of acetate. The authors therefore concluded that "if the nucleus and the dichloroacetyl side chain was derived from a single [polymalonate] chain, at least one methyl group would have to be added by a C_1 addition " and therefore " more than one

[poly-malonate] chains are involved"(sic). On the basis that the known chloroperoxidase enzyme system catalyses the chlorination of β-diketones and β-ketoacids rather than methyl ketones, the proposed biosynthetic scheme involved condensation of two tetraketide units followed by subsequent modification to give mollisin (19) (Figure 4).

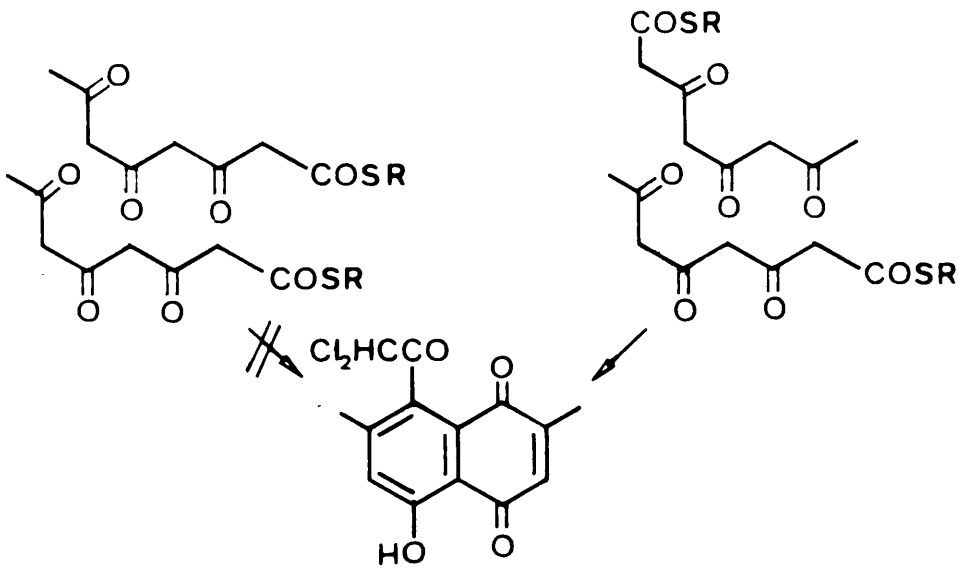
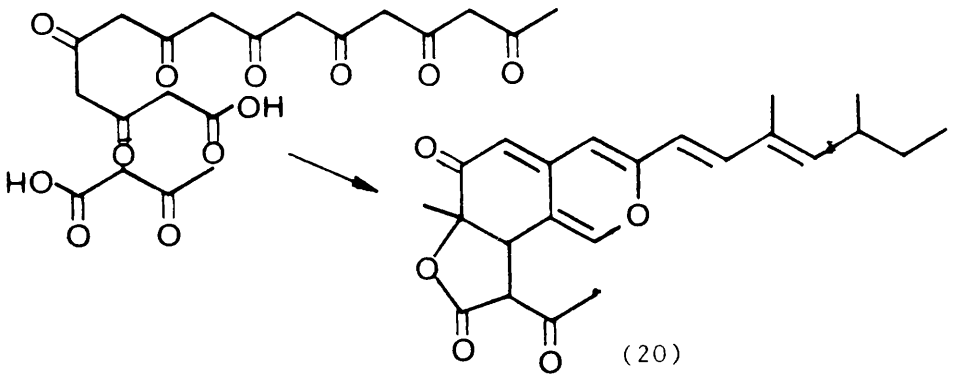


Figure 4. (After Bentley and Gatenbeck[41])

In a subsequent study by Tanabe and Seto[42] in 1970 using [2-^{13}C] acetate feedings it was determined that C-3, -6, -11, -12 and -14 were derived from C-2 of acetate. Whereas the previous studies were hampered by poor levels of incorporation of precursors and by the degradative techniques then required, this later study benefitted from the rapidly developing magnetic resonance (nmr) techniques which greatly increased the amount of information available from a feeding experiment while making that information much more readily accessible.



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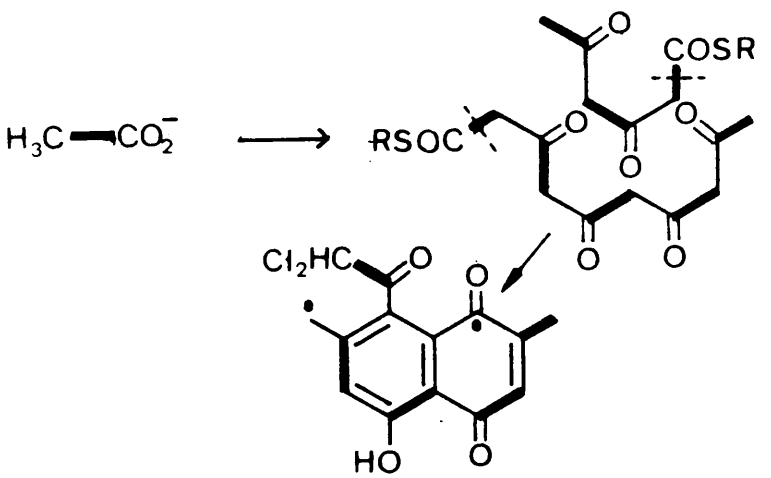


Figure 5. (After Seto et al[44])

Tanabe and Seto[42] found that, within experimental error, the above carbon atoms were equally labelled and so concluded that these results supported the two chain hypothesis and that the route proposed by Bentley and Gatenbeck[41] (figure 4) was correct. This conclusion is slightly surprising as in their discussion they state that in all known cases of condensation products derived from two polyacetate chains, the chains are unequally labelled after incorporation of labelled precursor (for example on feeding of labelled acetate to Penicillium multicolor, the acetoacetate unit of the resulting ocrephilone (20) exhibits a higher level of incorporation than the remainder of the molecule[43]).

In 1973, the complementary [1,2-^{13}C] acetate feeding experiments were conducted by Seto et al.[44] who found through observation of ^{13}C-^{13}C coupling in the nmr spectrum of mollisin (19) that neither of the previously proposed biosynthetic routes could account for the experimental results. The results demonstrated that the pairs of carbon atoms C-2 and C-12, C-3 and C-4, C-5 and C-10, C-6 and C-7, C-8 and C-9, and C-13 and C-14 arise through the intact incorporation of acetate units. To account for this a third biosynthetic route via condensation of two polyacetate chains was proposed (figure 5).

In 1975 McInnes and Wright[45] proposed that the ^{13}C labelling patterns observed in these experiments could be accounted for through a biosynthetic route utilising only a single octaketide chain. According to this scheme the octaketide would condense initially to produce a phenanthroid intermediate which may then

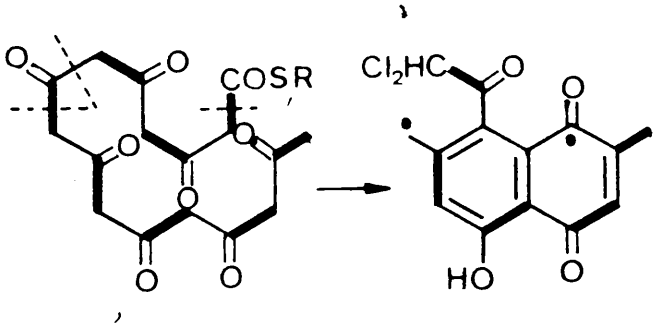


Figure 6. (After McInnes and Wright[45])

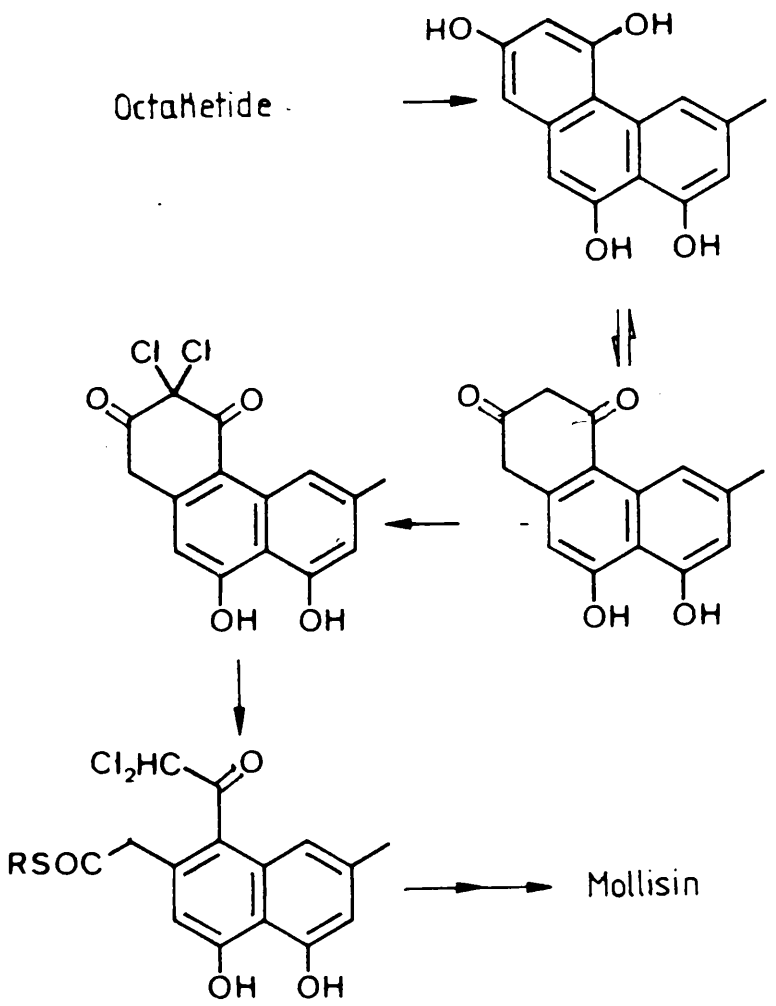


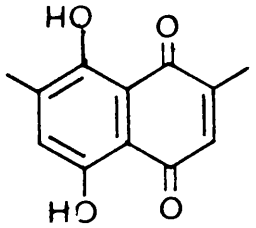
Figure 7.

undergo ring degradation to give mollisin (19) after further modification (figure 6). The envisaged mechanism of ring degradation would be through an enzyme mediated tautomerism of the phenanthrene to a 5,7-dioxo-intermediate. Dichlorination at the resultant methylene group followed by basic attack (enzymic) on the C-7 carbonyl would result in cleavage of the C6-C7 bond giving the proposed intermediate (figure 7).

By invoking Occam's razor the proposal of McInnes and Wright was declared a null hypothesis by Casey et al.[46] on repeating the ^{13}C acetate experiments, but as was commented by Simpson in 1977[47] "the biosynthesis of mollison by cleavage of a single octaketide would appear to be at least as likely as the two-chain pathway proposal".

To date, no further work has been published on the biosynthesis of mollisin (19) and the nature of the polyketide precursors is still open to debate.

As the overall purpose of a biosynthetic study is, by definition, the elucidation of the biological precursors of a natural product, one obvious course would be to isolate co-metabolites which may then be identified (or rejected) as precursors. To this end, large scale batches of Mollisia caesia were grown and the whole culture extracted with chloroform, ethyl acetate and methanol. The extract was then resolved by chromatography into its component parts.



(21)

Surprisingly few minor metabolites of M.caesia were observed and with the exception of a red oil which ran just ahead of mollisin (19) on silica, none were present in sufficient amounts to permit characterisation. This was true even from the combined extracts of up to 50 l of culture medium. The red oil, which crystallised on standing, has previously been reported by Tanabe and Seto[42] and was identified by them as 2,7-dimethylnapthzarin (21). The napthazarin (21) is proposed to arise through a biological degradation of mollisin (19) and is not thought to be a biosynthetic precursor. Unfortunately, then, no new metabolites of M.caesia could be identified through this present study.

A second approach to the study of biosynthesis is through the feeding to an organism of an isotopically labelled postulated precursor followed by subsequent isolation of the metabolite of interest which is monitorred for incorporation of the isotopic label. At its simplest level this approach means the feeding of such fundamental precursors as acetate or malonate, or on a more advanced level the synthesis of complex molecules is often required. As has been described above, feeding experiments with M.caesia using C-1, C-2 and C-1,2 labelled acetate have been performed but have been unable to distinguish between the possible biosynthetic routes to mollisin (19). However, a feeding experiment involving deuterium labelled acetate would potentially provide enough information to prove one or other biosynthetic pathway.

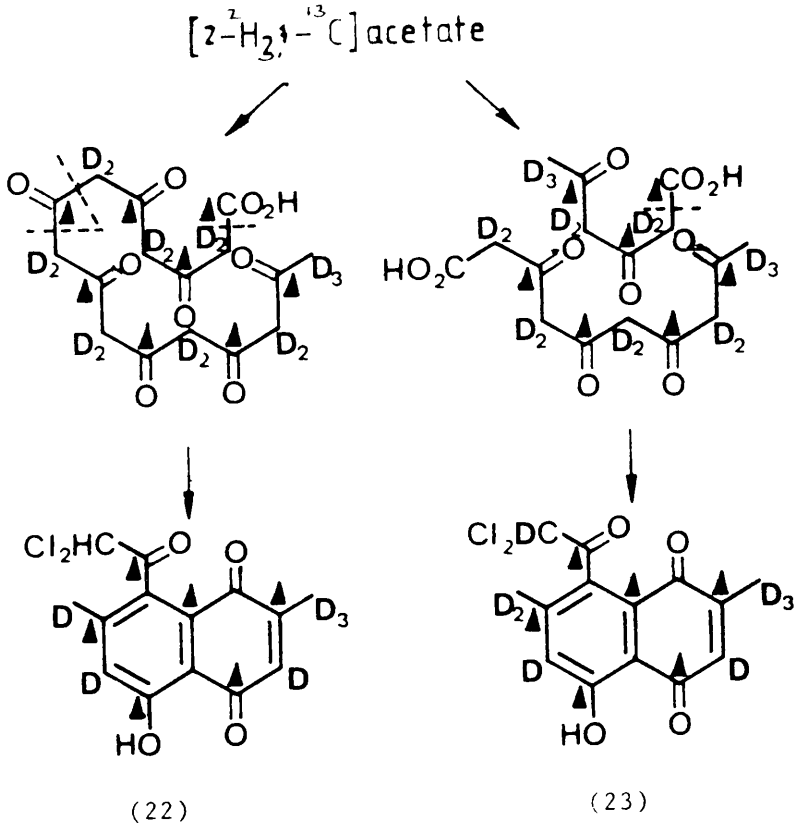
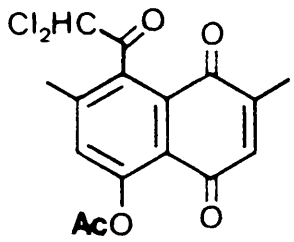


Figure 8

The fate of deuterium in biosynthesis may be monitorred either directly by ^{2}H nmr spectroscopy or indirectly by ^{13}C nmr spectroscopy[48,49]. If the deuteron is directly attached to a ^{13}C atom then the ^{13}C nmr signal for that carbon is shifted, normally upfield, and shows deuterium coupling. This technique is disadvantaged by the signal to noise ratio of the shifted signal being reduced by poor relaxation, signal mutliplicity and loss of nuclear Overhauser effects (NOE). If the deuteron is placed β to the ^{13}C nucleus, then again an upfield shift in the ^{13}C nmr spectrum results but the ^{2}H-^{13}C coupling over two bonds is negligible, the shifted signal is therefore a singlet and the level of enrichment can more easily be determined as the problems with relaxation and NOE are avoided.

If an experiment was conducted on M.caesia using [2-$^{2}H_{3}$,1-^{13}C]-acetate then, assuming equal rates of $^{1}H/^{2}H$ exchange at all positions, the resulting mollisin (19) would have a particular labelling pattern dependent upon its biosynthetic origin (22) or (23). Of course $^{1}H/^{2}H$ exchange is unlikely to be equivalent at all sites but (22) and (23) do represent the maximum possible extent of deuterium labelling of mollisin (19) (assuming that one of the two postulated biosynthesis does operate) and so the two routes may be distinguished by appropriate means (figure 8).

^{13}C NMR spectroscopy would be expected to demonstrate the location of those carbon atoms derived from C-1 of the labelled acetate, as has previously been shown for [1,2-$^{13}C_{2}$] acetate feedings by Casey et al[46]. However, because the fed acetate is



(24)

also deuterium labelled on the β-carbon then when this deuterium is incorporated into a metabolite the resulting ^{13}C nmr spectrum would show a geminal isotope shift of *circa* 0.1 ppm per deuteron to the ^{13}C nucleus. The magnitude of this shifted resonance would be proportional to the level of deuteriation achieved. The level of deuterium incorporation could therefore be measured directly from the ^{13}C nmr spectrum.

Thus to a culture of *M.casia* were added aqueous solutions of sodium [2-$^{2}H_3$, 1-^{13}C] acetate after 10, 13, 16 and 19 days growth. After 26 days growth the whole culture was extracted with ethyl acetate and the extract purified to provide a sample of mollisin (19). Because of the insolubility of mollisin (19) in organic solvents, it was carefully acetylated by the method of Casey *et al*.[46] to give mollisin acetate (24) which is appreciably more soluble. This was examined by ^{13}C and ^{2}H nmr.

The level of incorporation of [2-$^{2}H_3$,1-^{13}C] acetate was found to be very low. The levels of enrichment of ^{13}C in the sample of mollisin (19) were of the order of 0.2%. This compares with the natural abundance level of ^{13}C of 1.1% and so there was no clear indication of the alternative labelling pattern as found before by Casey *et al*[46].

It is unclear why such low levels of incorporation of [2-$^{2}H_3$, 1-^{13}C] acetate were found as previous studies have obtained incorporation levels of [1,2-$^{13}C_2$] acetate of up to 6.5%[46]. It may be that the deuterium isotope effect is a

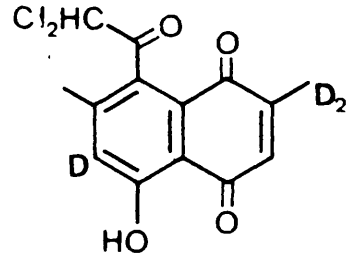


Figure 9. Observed deuterium incorporation of mollisin (19)

determining factor in this instance: the rate of cleavage of a carbon-hydrogen bond is generally about seven times faster than the corresponding carbon-deuterium bond. Hence, in the presence of an abundance of unlabelled acetate the uptake of deuteriated acetate will be much reduced.

No further information can be obtained about the ^{13}C labelling pattern from this experiment, but if it is assumed that, given higher levels of incorporation, results consistent with Casey et al.[46] would have been obtained then information may be abstracted from these results regarding deuterium labelling.

Thus, close examination of the ^{13}C nmr spectrum of the labelled mollisin acetate (24) reveals the presence of deuterium through geminally shifted resonances. The signal for C-7 shows a ^{2}H-^{13}C peak shifted upfield by 0.1 ppm to 143.7 ppm and the signal for C-2 shows resonances 0.1 ppm and 0.2 ppm upfield from the unlablled signal. No other geminal shifted deuterium signals could be detected.

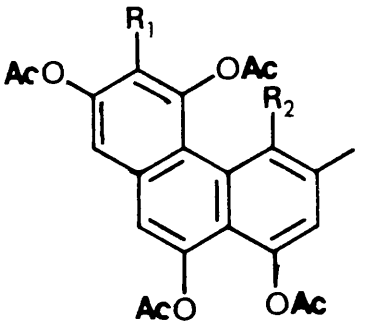
The low probability of two [2-$^{2}H_3$, 1-^{13}C]-acetate units joining together within an otherwise unlabelled metabolic acetate pool is such that these results indicate the presence of a single deuterio-label at C-6 and two deuterio-labels at C-12 (figure 9).

^{2}H-NMR of this sample confirmed these results, showing the presence of deuterium at positions -6 and -12. Again, no other positions showed evidence of deuterium incorporation.

Because of the low levels of incorporation achieved, no firm conclusion can be drawn from this study. It will be remembered that if the two chain hypothesis of mollisin (19) biosynthesis is correct, then evidence of up to two deuterons at C-11 could be predicted. That none were found at this position but were found at other positions predicted by both hypotheses would tend to suggest that a two polyketide chain biosynthesis of mollisin does not operate. Therefore the biosynthesis of mollisin (19) may proceed through cyclisation of a single polyketide chain.

It is re-iterated that the deuterium isotope effect at each position in a polyketide chain would differ such that the rates of ^{1}H/^{2}H would be non-equivalent. This may explain the varying levels of incorporation of the labelled acetate at each position in mollisin (19).

Similarly this may explain why no deuterium was found at sites where it would be expected on the basis of the biosynthetic hypotheses. For example, no deuterium incorporation was observed at C-3 which, assuming subsequent cyclised intermediates are more stable towards exchange, implies a rapid rate of ^{1}H/^{2}H exchange at this site in the polyketide. The washing out of deuterium label by exchange reactions is a well recognised limitation of such systems[48] and is difficult to overcome.



(25) $R_1 = R_2 = H$

(26) $R_1 = Cl$, $R_2 = H$

(27) $R_1 = Cl$, $R_2 = OAc$

(28) $R_1 = Cl$, $R_2 = CO_2H$

(29) $R_1 = H$, $R_2 = OAc$

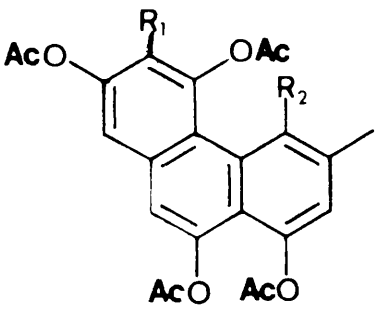
(30) $R_1 = H$, $R_2 = CO_2H$

Although it is realised that this is not the only possible interpretation of these results, it is the simplest and illustrates the need for further work to be done on this system.

In summary, these results indicate that the biosynthesis of mollisin (19) may proceed via a single polyketide chain according to that shown in figure 7. However, because of the poor levels of incorporation achieved this cannot be proven. As likely biosynthetic intermediates can be proposed on the basis of this scheme, one way of further investigating the biosynthesis would be to chemically synthesise isotoptically labelled postulated intermediates and then conduct further feeding experiments on M.caesia. If the hypothesis is correct then because these compounds are late intermediates, higher levels of incorporation into mollisin (19) are likely to be achieved.

Further assuming that this hypothesis is correct, then by synthesis of a series of isotopically labelled substituted phenanthrenes the sequence of the chlorination, decarboxylation and oxidation steps in mollisin biosynthesis may be established. The required compounds are 1,5,7,10-tetracetoxy-3-methylphenanthrenes with a 4-substituent of either hydrogen, carboxyl or acetoxyl, and a 6-substituent of either hydrogen or chlorine (25) (26) (27) (28) (29) and (30).

Although the postulated biosynthetic intermediates are free phenols, because of their polyphenolic nature these compounds are likely to be highly reactive and so require to be chemically protected. It is assumed that on feeding to M.caesia that these acetate moeities would be enzymically cleaved and so become able to enter the biosynthetic pathway.



(25) $R_1 = R_2 = H$
(26) $R_1 = Cl, R_2 = H$
(27) $R_1 = Cl, R_2 = OAc$
(28) $R_1 = Cl, R_2 = CO_2H$
(29) $R_1 = H, R_2 = OAc$
(30) $R_1 = H, R_2 = CO_2H$

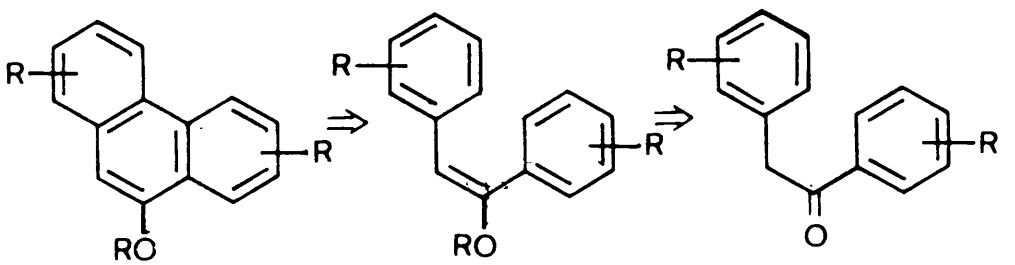


Figure 10. Retrosynthesis of 9-hydroxyphenanthrenes.

CHAPTER 3

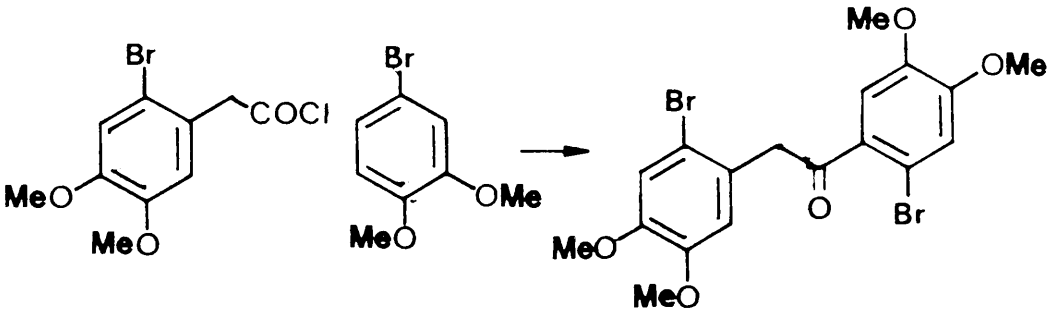
THE SYNTHESIS OF POSTULATED BIOSYNTHETIC INTERMEDIATES

As described in the previous chapter, the biosynthetic study of mollisin (19) requires the synthesis of deuterium labelled hydroxyphenanthrenes. For reasons of economy, isotopic labels are generally introduced as late in a synthetic route as possible. Incorporation of deuterium into active aromatic rings is well recognised and so it was proposed that deuteriation should occur after synthesis of the hydroxyphenanthrenes as the protected acetates (25), (26), (27), (28), (29) and (30).

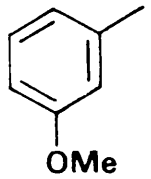
3.1. The Synthesis of 10-Hydroxy-1,5,7-trimethoxy-3-methylphenanthrene

3.1.1. Friedel-Crafts Acylation and Related Reactions

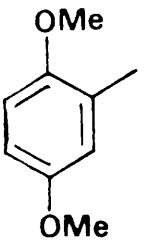
Generally the most direct route to the phenanthrene carbon skeleton involves the photocyclisation of the corresponding Z-stilbene[50]. In this case the target phenanthrene has a 10-hydroxy substituent and so the target stilbene is the tautomeric enol of a benzyl phenyl ketone (a desoxybenzoin). The principal synthetic step therefore is the photocyclisation of the enol ether or enol ester of a desoxybenzoin. This ketone is the first synthetic target (Figure 10).



(31)



(32)



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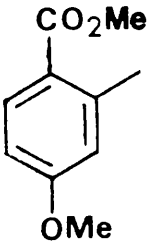

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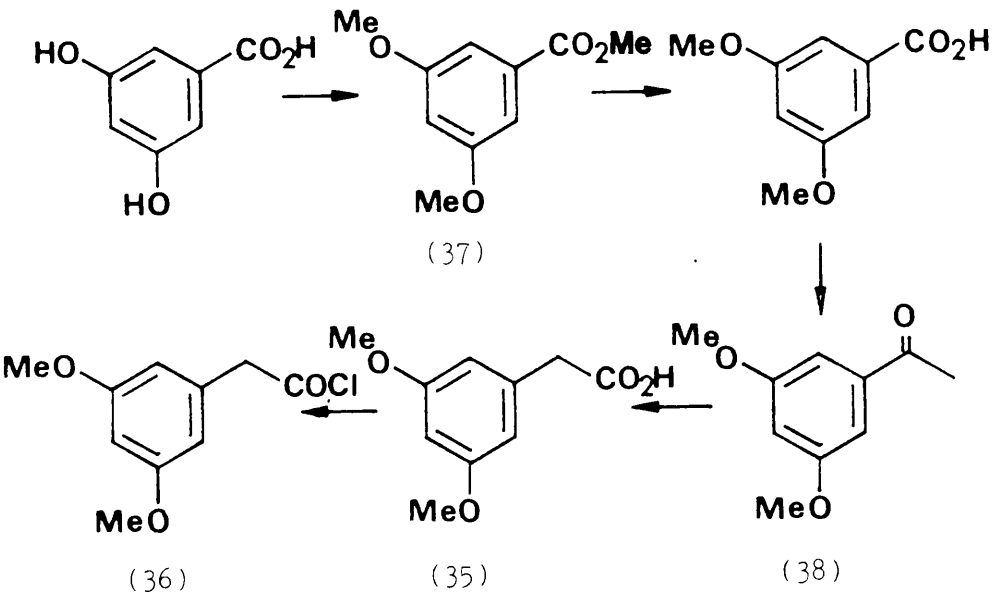


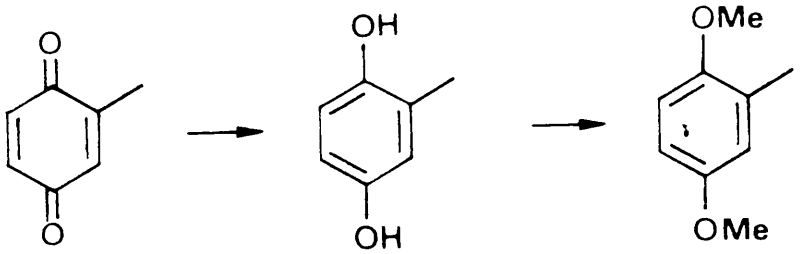
(37)

(36) (35) (38)

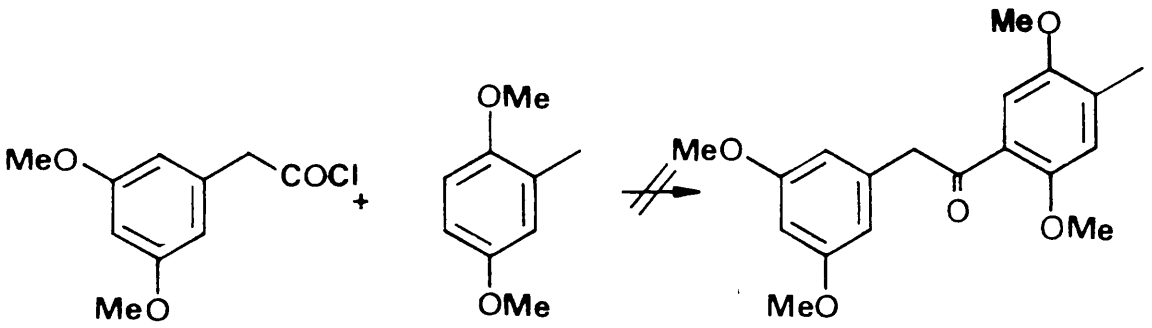
Scheme 1

Classically the favoured route to such aromatic ketones involves the Friedel-Crafts acylation of an aromatic substrate with a phenylacetic acid or acid chloride moeity. For example, the desoxybenzoin (31) has been synthesised by this method by Dominguez et al[51]. This is an attractive route because each of the target desoxybenzoins is potentially available by acylation at the para position to the methyl group of each desired substrate: 3-methoxytoluene (32), 2,5-dimethoxytoluene (33), and methyl 4-methoxy-2-methylbenzoate (34). This position of the toluenes (32), (33) and (34) is known to be the most active towards acylation. Also, 3,5-dimethoxyphenylacetic acid (35) and acid chloride (36) can be readily prepared.

Thus, 3,5-dihydroxybenzoic acid was methylated and the resulting dimethoxy methyl ester (37) hydrolysed and then treated with methyl lithium to furnish 3,5-dimethoxyacetophenone (38). Willgerodt-Kindler reaction[52] of this ketone gave the phenylacetic acid (35) in high yield which was identical to an authentic sample. The corresponding acid chloride (36) was obtained by reaction of the acid (35) with phosphorus trichloride (Scheme 1). The mechanism of the Willgerodt-Kindler reaction is unclear but reaction is known not to proceed via rearrangement of the carbon skeleton. Mayer[53] suggests that several independent mechanisms may operate and that "this complex and variable reaction cannot be described by a single mechanism".



(39) (33)



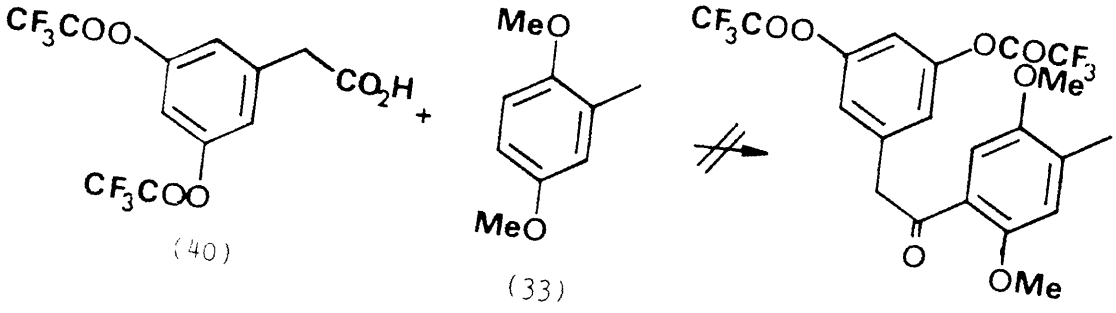
(36) (33)

The chosen substrate for the initial acylation reactions was 2,5-dimethoxytoluene (33) which was prepared by catalytic hydrogenation of methyl-p-benzoquinone and methylation of the resulting hydroquinone (39).

On attempting the Friedel-Crafts acylation of the toluene (33) with the phenylacetyl chloride (36) catalysed by aluminium chloride in carbon disulphide, none of the desired ketone could be isolated; instead a large amount of polymeric material was obtained of indeterminate nature. The nmr spectrum of this material suggested that the primary reaction occurring was that of self-condensation of the acid chloride: the spectrum of the acid chloride (36) shows aromatic resonances of a two proton doublet (J=2Hz) and a one proton triplet (J=2Hz) and it would be expected that the desired product would have a similar pattern in its nmr spectrum. However, the spectrum of the polymeric material shows a series of singlets in the aromatic region suggesting that the substitution pattern had been altered on the aromatic ring and that self-condensation had occurred.

This result is explained by the relative reactivities of the two aromatic reactants: because of the additive effects of the 1,3-dioxygenation pattern of the acid chloride (36), this aromatic ring is more active to acylation then the 1,4-dioxygenated toluene (33).

The overall usefulness of the Friedel-Crafts reaction[53,54] is partly due to the variety of conditions that can be employed, for example the catalyst, solvent and substrate can all be varied and



(40)

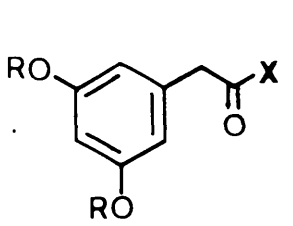
(33)

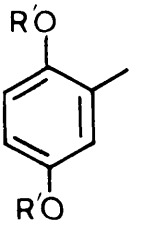
each variation may bring about a change in the course of reaction. A number of such variations were tried in our system but unfortunately without success. The parameters altered were the substitution of the phenylacetic acid (35) for the acid chloride (36) as acylating agent; substitution of polyphosphoric acid (PPA) or trifluoroacetic acid (TFA) in trifluoroacetic anhydride (TFAA) as catalyst; or variation of the substrate from dimethoxy- to dihydroxytoluene (see Table 2). In all cases no identifiable products other than starting materials could be isolated.

In an attempt to reverse the relative reactivities of the two aromatic components the methyl protecting groups of 3,5-dimethoxyphenylacetic acid (35) were replaced by trifluoroacetate groups. It was hoped that the electron withdrawing effects of this group would reduce the activity of the ring to a level below that of 2,5-dimethoxytoluene (33). However, when the trifluoroacetate (40) was generated in situ from the dihydroxy acid and subsequently reacted in TFA/TFAA with the toluene (33), no acylation was observed.

Although all combinations of these variables were not investigated it seemed indicative that an alternative approach to the desoxybenzoins should be sought.

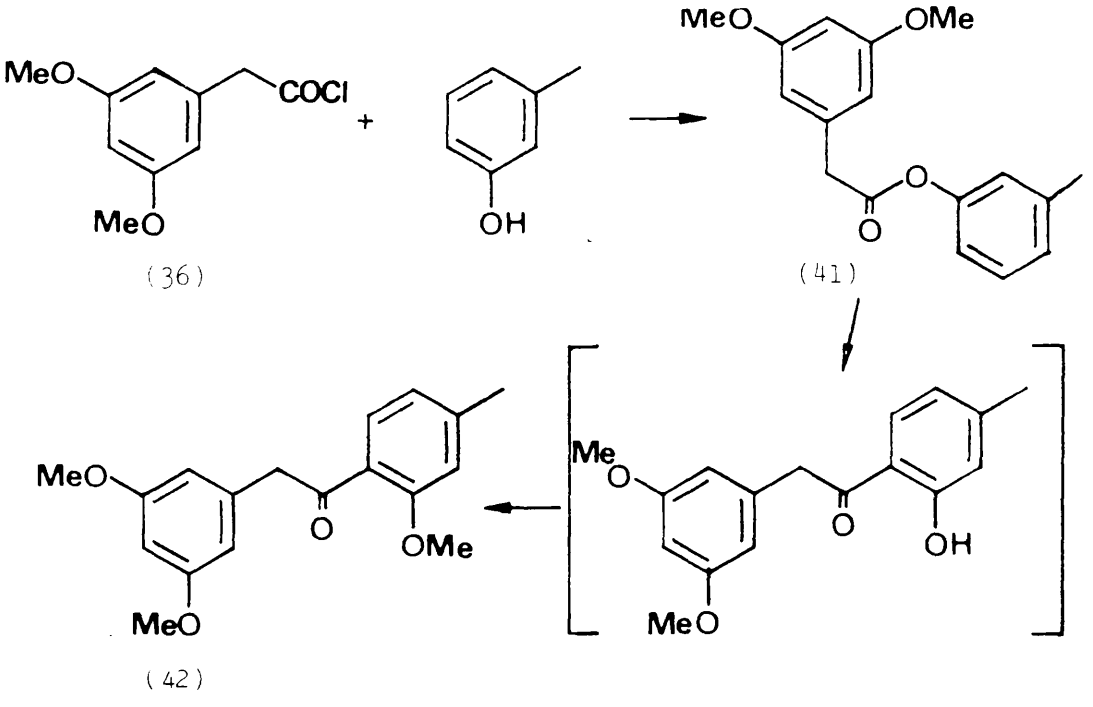
A related reaction to the Friedel-Crafts acylation is the Fries rearrangement of phenyl esters. One important difference between the two reactions is that the Fries rearrangement can operate through an intramolecular reaction mechanism and that by careful choice of reaction conditions the amount of intramolecular reaction versus intermolecular reaction can be altered.





Acylating Agent		Substrate	Catalyst
$R = CH_3$	$X = Cl$	$R' = CH_3$	$AlCl_3$
			PPA
			TFA/TFAA
		$R' = H$	$AlCl_3$
			TFA/TFAA
	$X = OH$	$R' = CH_3$	$AlCl_3$
			PPA
			TFA/TFAA
		$R' = H$	PPA
$R = H$	$X = OH$	$R' = CH_3$	TFA/TFAA

Table 2. Attempted Friedel Crafts Acylations.



(36) (41) (42)

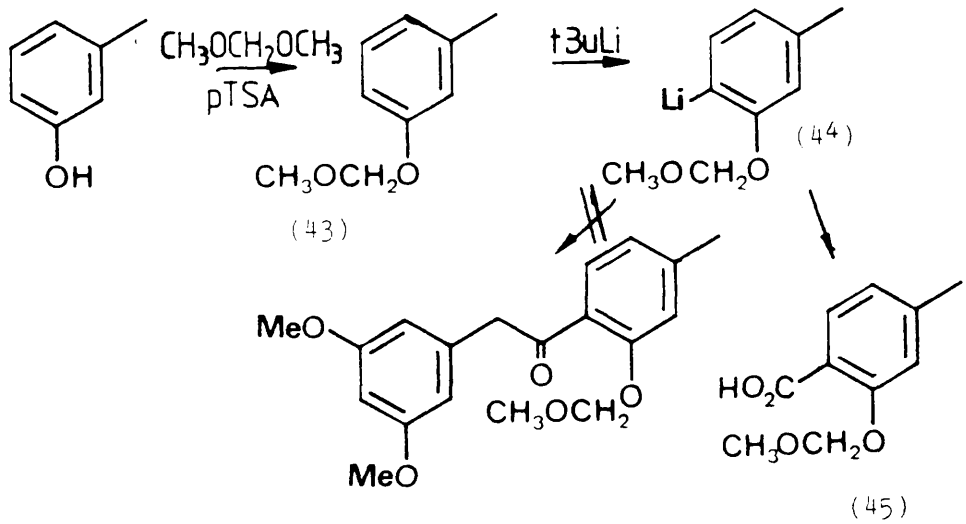
Scheme 2

Gerecs[55] states that *para* rearrangement occurs solely *via* an intermolecular process whereas *ortho* rearrangement of the phenyl ester may occur *via* a similar intermolecular process but also *via* an intramolecular process. The *ortho* rearrangement is reported to be favoured by high reaction temperatures and by use of excess catalyst.

The model compound used for this reaction was the *m*-cresyl ester of 3,5-dimethoxyphenylacetic acid (41). This was prepared by the method of Spassow[56] from the acid chloride (36) and *m*-cresol using magnesium metal as dessicant.

Reaction of this ester (41) with aluminium chloride in the absence of solvent at elevated temperature gave a complex mixture of mainly polymeric products. To facilitate purification, phenols were firstly base extracted and this extract was then methylated (Scheme 2). Chromatography of the products of this reaction showed a large number of compounds to be present, but from this mixture could be isolated the desired ketone (42) in <5% yield. The benzylic resonance in the nmr spectrum of ketone(42) occurs at δ4.20 ppm which compares with a value of δ4.21 ppm for the corresponding resonance of the parent desoxybenzoin[57].

On varying the reaction conditions (eg. shorter time, higher temperature) no improved yield could be obtained. It seems that in this system, under the utilised conditions, the intermolecular mechanism predominates and so the major products were similar to the polymeric materials obtained from the Friedel-Crafts reactions. Although the Fries rearrangement is a more successful route, it did


CH3OCH2OCH3
pTSA
t3uLi
OH
CH3OCH2O
(43)
Li
(44)
CH3OCH2O
MeO
O
CH3OCH2O
MeO
HO2C
CH3OCH2O
(45)

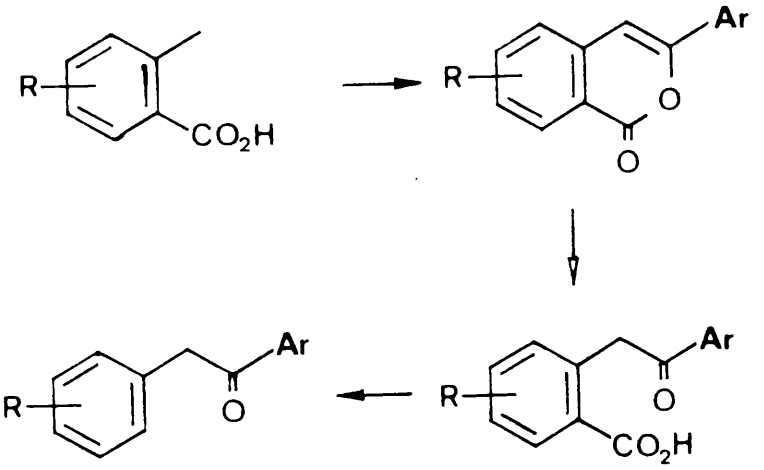
not furnish the desired product in synthetically useful amounts and so it too was abandoned.

3.1.2. Condensation of Benzoate Esters with Benzyl Anions

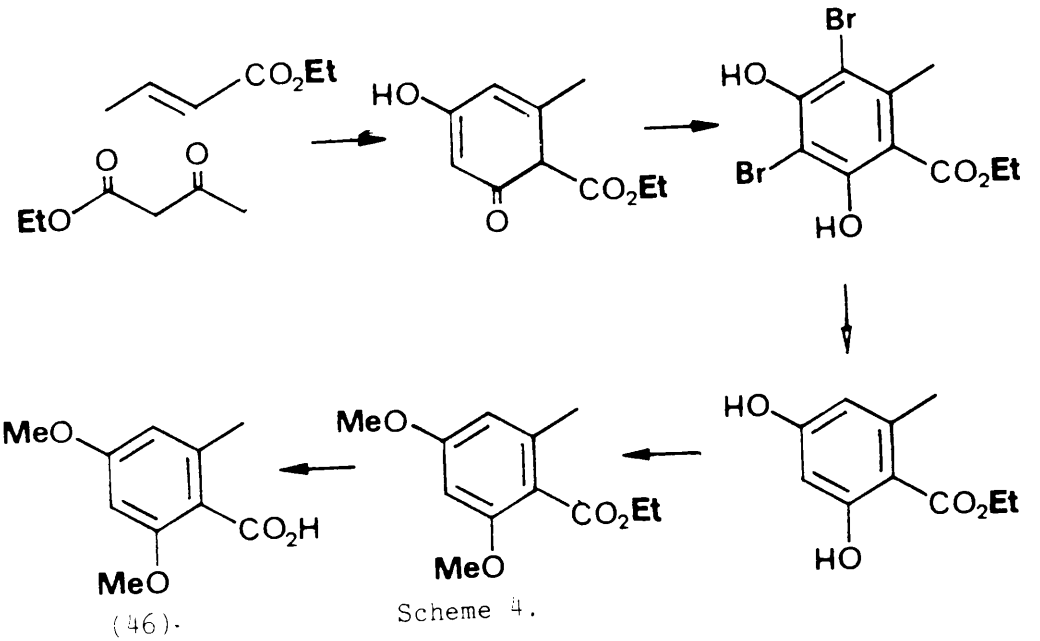
A more general approach to the synthesis of ketones is to utilise the condensation of an organometallic compound with an ester or acid chloride[58]. It has been reported[59] that when the methoxymethyl ether of m-cresol (43) is treated with t-butyl lithium the anion so formed (44) is exclusively at the 6-position because of complexation between the acetal oxygens and the metal cation. It was therefore proposed that the anion may condense with 3,5-dimethoxyphenylacetyl chloride (36) to give a desoxybenzoin. Such aryl anions are known to condense with esters and acid chlorides to give ketones[60].

Methoxymethyl-m-cresyl ether (43) was prepared by the acetal exchange method of Yardley and Fletcher[61] from m-cresol and dimethoxymethane. Treatment of this ether (43) with a slight excess of t-butyl lithium followed either by addition of or addition to 3,5-dimethoxyphenylacetyl chloride (36), however, gave only methoxymethyl-m-cresyl ether (43) and 3,5-dimethoxyphenylacetic acid (35), the latter being formed by hydrolysis of the acid chloride during work-up. No ketonic products could be isolated.

The formation of the aryl anion (44) was established by reaction with carbon dioxide which produced the corresponding acid (45) in ca. 75% yield. This suggests either that the anion (44) did

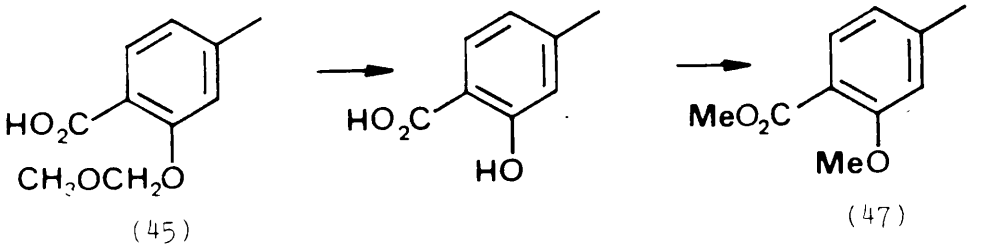


Scheme 3



(46).

Scheme 4.



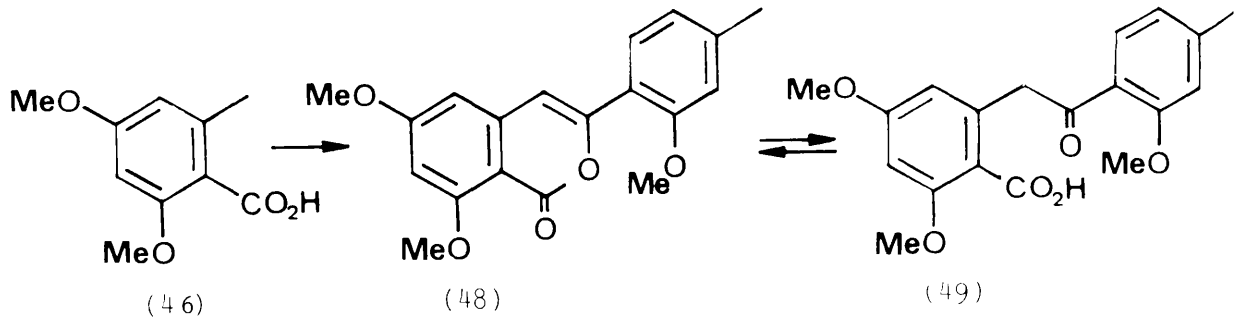
(45)

(47)

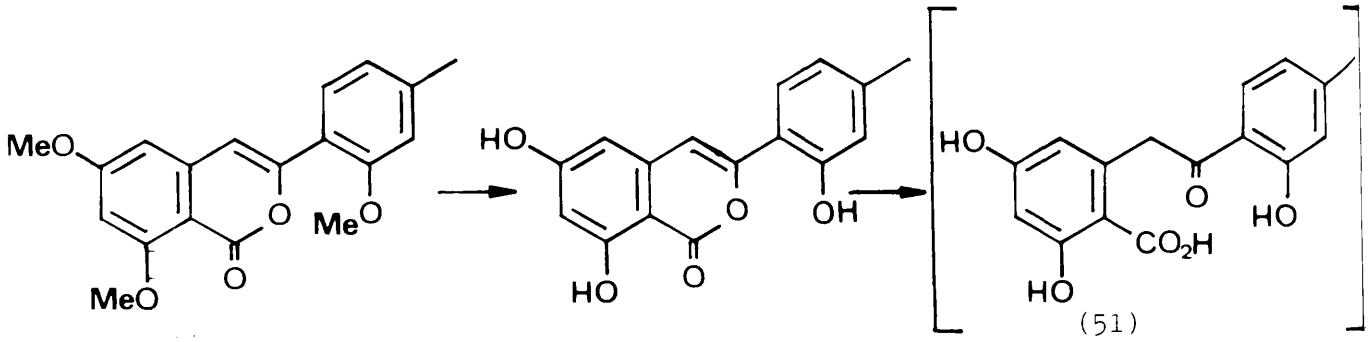
not react with the acid chloride (36) for steric or electronic reasons, or that the anion was quenched before reaction could occur with the active carbonyl. It seems likely that the latter is true and that the aryl anion (44) functioned as a base and abstracted the relatively acidic α-protons of the acid chloride (36).

The failure of this reaction to produce ketone suggested the possibility of the reverse synthesis - namely via the condensation of a benzyl anion with a benzoate or benzoyl chloride. The ideal anion would be that of 3,5-dimethoxytoluene but such a methyl anion is an unlikely species and would be inherently unstable. Creger[62] has shown that such aromatic methyl anions can be stabilised by an ortho carboxyl substituent and that the resulting dianion can condense with aromatic ketones to produce 3-aryl-dihydroisocoumarins. Thus it was proposed that the anion of a toluic acid would condense with a benzoate ester to give, on cyclisation, a 3-arylisocoumarin. Hydrolysis of this would give the corresponding keto-acid. If this could be induced to decarboxylate then the desired desoxybenzoin would result (Scheme 3).

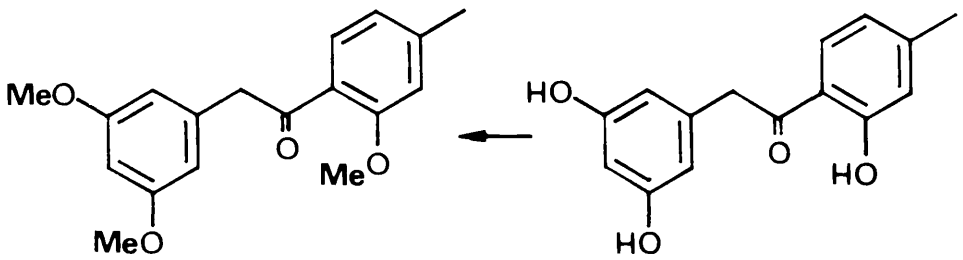
In this proposed scheme the required dianion is that of 2,4-dimethoxy-6-methylbenzoic acid (46). This acid is readily available via the route of Sargent et al[63] (Scheme 4). The required benzoate ester, methyl 2-methoxy-4-methylbenzoate (47) was prepared by quenching the anion of methoxymethyl ether (43) with carbon dioxide. The resulting acid acetal (45) was deprotected and then methylated to give the ester (47).



(46) (48) (49)



(47) (50) (51)

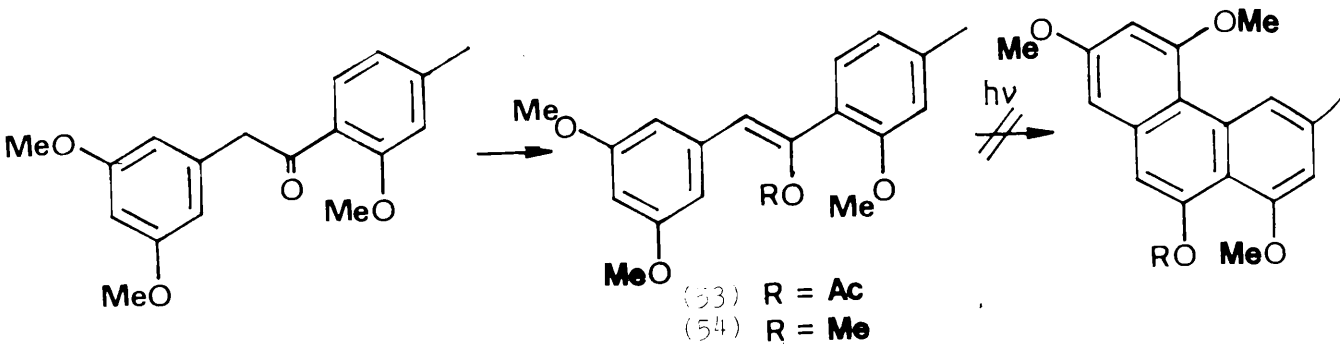


(42) (52)

Condensation was effected by treatment of the acid (46) with 2.2 equivalents of lithium diisopropylamide (LDA) followed by quenching of the dianion with the benzoate ester (47). Cyclisation occurred upon acid work-up to furnish the isocoumarin (48) in moderate yield. Base hydrolysis of (48) gave the keto-acid (49) which was stable under neutral or basic conditions. The nmr spectrum of the isocoumarin (48) shows a 1H singlet at δ7.19 due to the benzylic proton at C-4. On hydrolysis this resonance disappears and is replaced by a 2H singlet at δ4.42 due to the benzylic protons of the acid (49). However, attempted copper sulphate catalysed decarboxylation in quinoline at elevated temperature resulted only in dehydration of the keto-acid to give the isocoumarin (48) again.

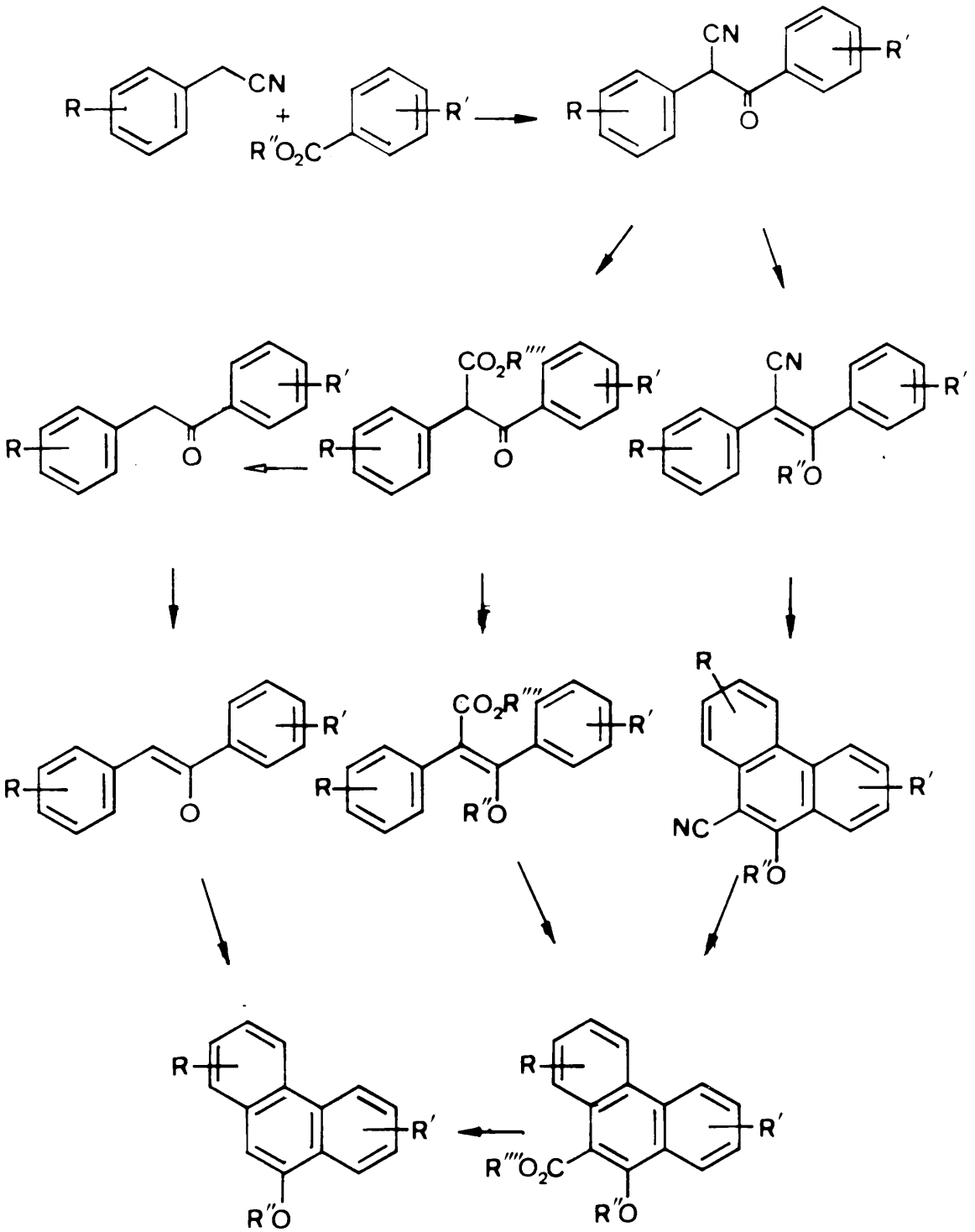
Decarboxylation could be achieved by prior demethylation of the trimethoxyisocoumarin (48). This demethylated smoothly upon treatment with boron tribromide and subsequent hydrolysis of the resulting trihydroxyisocoumarin (50) facilitated decarboxylation. Because the initial hydrolysis product is an ortho-hydroxy aromatic acid (51), extended hydrolysis results in loss of carbon dioxide to give the trihydroxyketone (52). Methylation of this gave the desired desoxybenzoin (42) in good yield, identical to that prepared by the Fries rearrangement route.

Formation of the enol acetate (53) of the desoxybenzoin (42) was achieved upon treatment with potassium acetate and acetic anhydride via the method of Barnes[64]. The methyl enol ether (54) was produced by reaction of the parent ketone (42) with trimethylorthoformate.

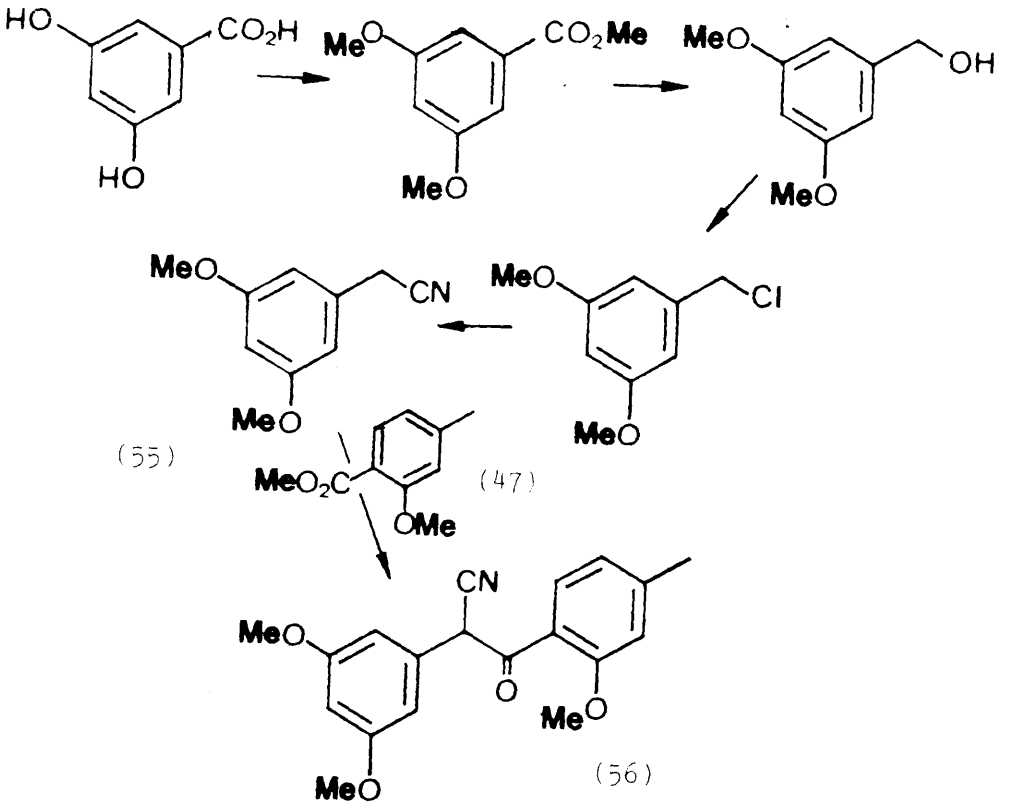

MeO
MeO
O
MeO
(52)
Me
O
MeO
RO
Me
O
MeO
(53) R = Ac
(54) R = Me
hν
Me
O
OMe
RO
MeO

When either protected enol, (53) or (54) was irradiated in cyclohexane with U.V. light unfortunately no cyclisation was observed. There is little literature precedent for the photocyclisation of α-oxy stilbenes but it is known that the methyl enol ether of desoxybenzoin does cyclise in 7-20% yield[65]. Also the corresponding enol acetate is known to cyclise to 9-acetoxyphenanthrene although the yield is not reported[66]. Scholz et al.[67] have attempted to explain the failure of certain stilbene photocyclisations by means of molecular orbital calculations and have implied that substituents can change the occurrence or otherwise of a photocyclisation. It seems likely that such substituent effects are operating in these systems.

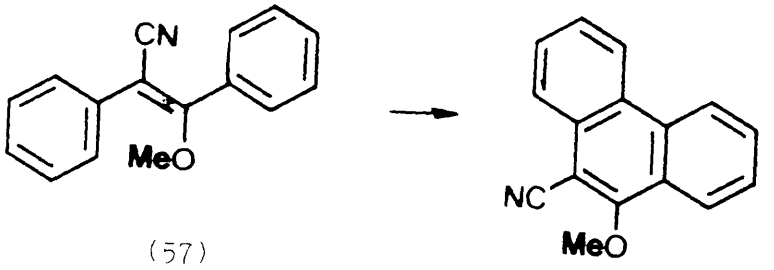
While this work was in progress it was realised that, because of the unpredictable nature of untried photocyclisations, it may be prudent to have a choice of substrate. To this end a second, more adaptable route was developed. This involved condensation of a phenylacetonitrile moeity with a benzoate ester (Scheme 5) to give, in the first instance, a β-ketonitrile. The advantage of this route is that potentially the nitrile group could easily be manipulated to produce the corresponding β-ketoester of the desoxybenzoin and that the enol ether or ester of any of these (the ketone, β-ketoester or, β-ketonitrile) may give cyclised product. It was envisaged that any 9-cyano- or 9-carboxy- 10-hydroxyphenanthrene so produced would be susceptible to hydrolysis and subsequent decarboxylation.



Scheme 5.


HO
CO2H
HO
Me
O
CO2Me
MeO
MeO
OH
MeO
MeO
CN
MeO
MeO
Cl
MeO
(55)
MeO2C
OMe
(47)
CN
MeO
O
MeO
MeO
(56)

Scheme 6.

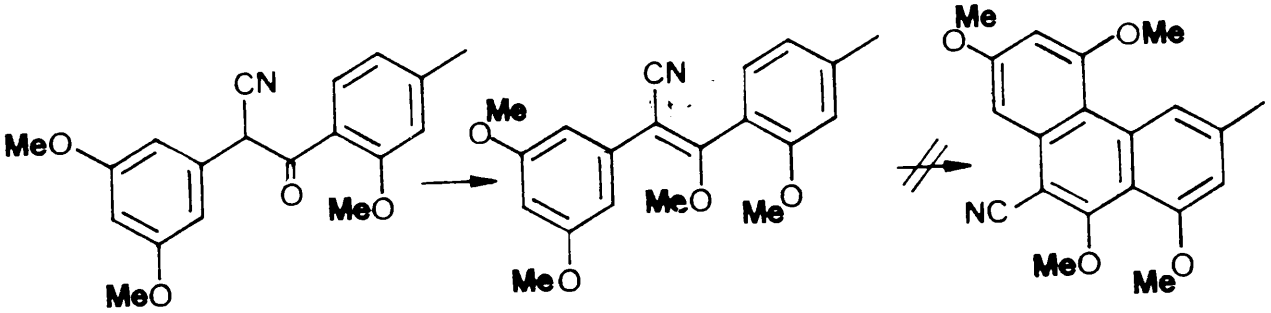

CN
MeO
NC
MeO

(57)

3,5-Dimethoxyphenylacetonitrile (55) was prepared by the route of Hill[68] (Scheme 6). In order to facilitate the condensation in good yield it is necessary to use two equivalents of base. If only one equivalent is used then because the benzylic proton of the first formed β-ketonitrile is more acidic than the benzylic protons of nitrile (55), this anion is quenched. Thus the maximum theoretical yield is 50%, but by using two equivalents of base this problem is overcome.

The dianion of nitrile (55) formed upon treatment with excess n-butyl lithium and was condensed with the ester (47) to give the β-ketonitrile (56) in good yield. This was shown spectroscopically to exist largely as its keto-tautomer: the nmr spectrum of (55) shows a 1H resonance at 5.77 ppm due to the benzylic proton and the IR spectrum shows a strong carbonyl stretch at 1683 cm^{-1} and only a relatively weak OH stretch (3250 cm^{-1}) due to the enolic tautomer.

By this time it was realised that the original target desoxybenzoin (42) did not photocyclise as its methyl enol ether (54) or enol acetate (53). The photochemistry of the protected enol of the β-ketonitrile (56) was therefore investigated. As α-cyano- α-methoxystilbene (57) is known to be photolytically labile[60], the methyl enol ether of the β-ketonitrile (58) was synthesised: treatment of β-ketonitile (55) with trimethylorthoformate afforded essentially one stereoisomeric enol ether (58) in almost quantitative yield. It is generally true[50] that E-stilbenes are thermodynamically more stable than the Z-isomer and also that the

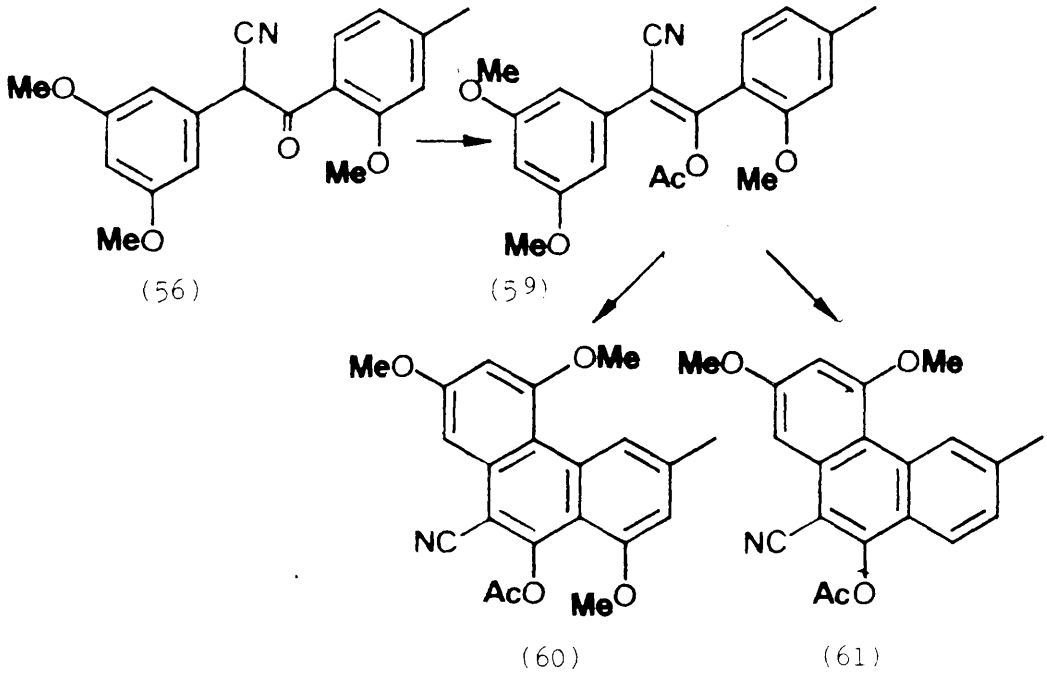


(56) (58)

former are generally crystalline whereas the latter are often oils. As the methyl ether obtained was the sole product and was a crystalline solid it was therefore proposed to be the E-isomer.

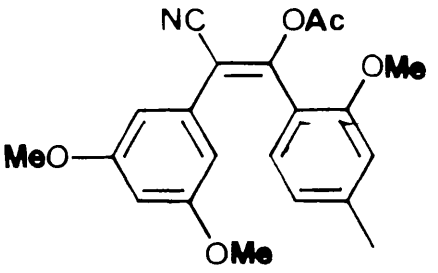
U.V. irradiation of an ethereal solution of (58) containing dissolved iodine however also failed to give cyclised product. It is interesting to note that the E-isomer was recovered largely unchanged even on prolonged reaction times. This implies either that the photolytic equilibrium lies strongly towards the E isomer or that the rate of E/Z photoisomerism is very slow. Thus it seems that the major problem is lack of isomerisation rather than the lack of cyclisation.

Remembering Scholz's statement about substituents affecting the course of a photocyclisation[67], the enol acetate of the β-ketonitrile (59) was the next chosen susbstrate. This was produced by acid catalysed acetylation of the nitrile (55). Unlike the methyl enol ether (58) the enol acetate (59) was produced as a stereoisomeric mixture. This was not resolvable by chromatography but, on the assumption that the major isomer had the E configuration, the ratio of E:Z was determined to be circa 3:2 by integration of the nmr spectrum. The nmr spectra of the isomers is in accordance with these assignments: the 6-H resonance of the major isomer (assigned E) occurs as a doublet (J=8Hz) at 7.12ppm whereas the corresponding resonance in the minor isomer (assigned Z) occurs as a similar doublet but is deshielded, presumably by the adjacent aryl group, to 7.45ppm.

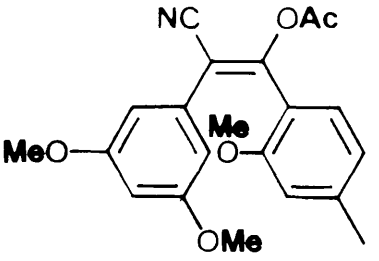

CN
MeO
O
MeO
MeO
(56)
CN
Me
O
Ac
O
Me
O
MeO
(59)
MeO
OMe
NC
AcO
Me
O
(60)
MeO
OMe
NC
AcO
(61)

The stereoisomeric mixture was irradiated in benzene containing iodine and to our delight gave cyclised product. Unfortunately on closer examination it was discovered that two phenanthrenes had been produced in approximately equal amounts. These were identified as the desired phenanthrene (60) and its deoxy analogue (61). The elimination of methanol during photocylisations of stilbenes with ortho-methoxy substituents is well recorded and the process by which it occurs is well understood[50]. The planar Z-isomer required for photocylisation can occur in two rotomeric forms (59a) and (59b) (Scheme 6). Reversible cycloaddition of these gives the 1-substituted dihydrophenanthrene (62) and the 4a-substituted dihydrophenanthrene (63) respectively. The photoreaction of (62) can proceed in the normal fashion by oxidative trapping by iodine or dissolved O_2 to give the desired product (60). However, the photoreaction of (63) proceeds by way of a non-oxidative elimination of methanol to give the phenanthrene (61).

One way of increasing the ratio of photolysis products (60):(61) would perhaps be to increase the amount of oxidant present to ensure more efficient trapping of the dihydro intermediate (62), but as the photocyclisation was carried out in an open reaction vessel it is debatable whether this would significantly alter the results. However, if a 6-methoxy substituent could be introduced into ring A of enol acetate (59) then, because of the resulting symmetry, any photolysis that occurred would be forced to occur by means of a non-oxidative elimination of methanol to give the desired phenanthrene (60). (The aromatic ring of the β-ketonitrile and its

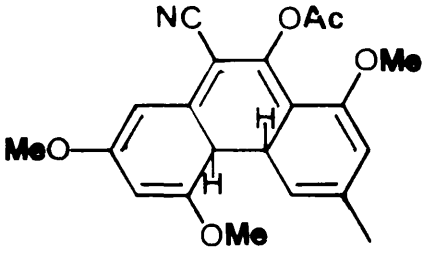


(59a)

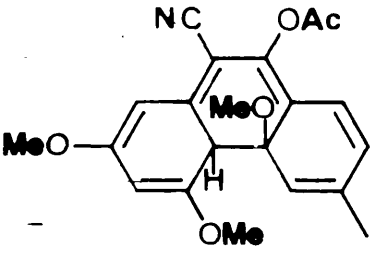


(59b)

hν hν

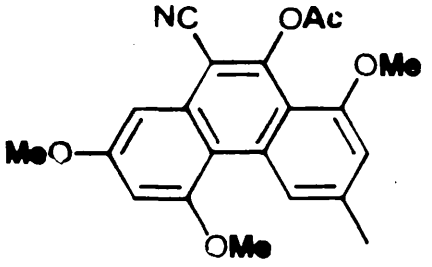


(62)

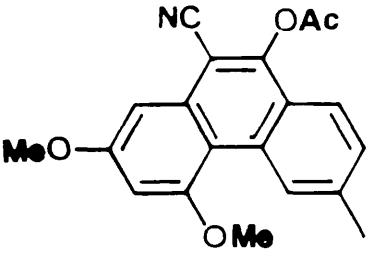


(63)

[O] -MeOH

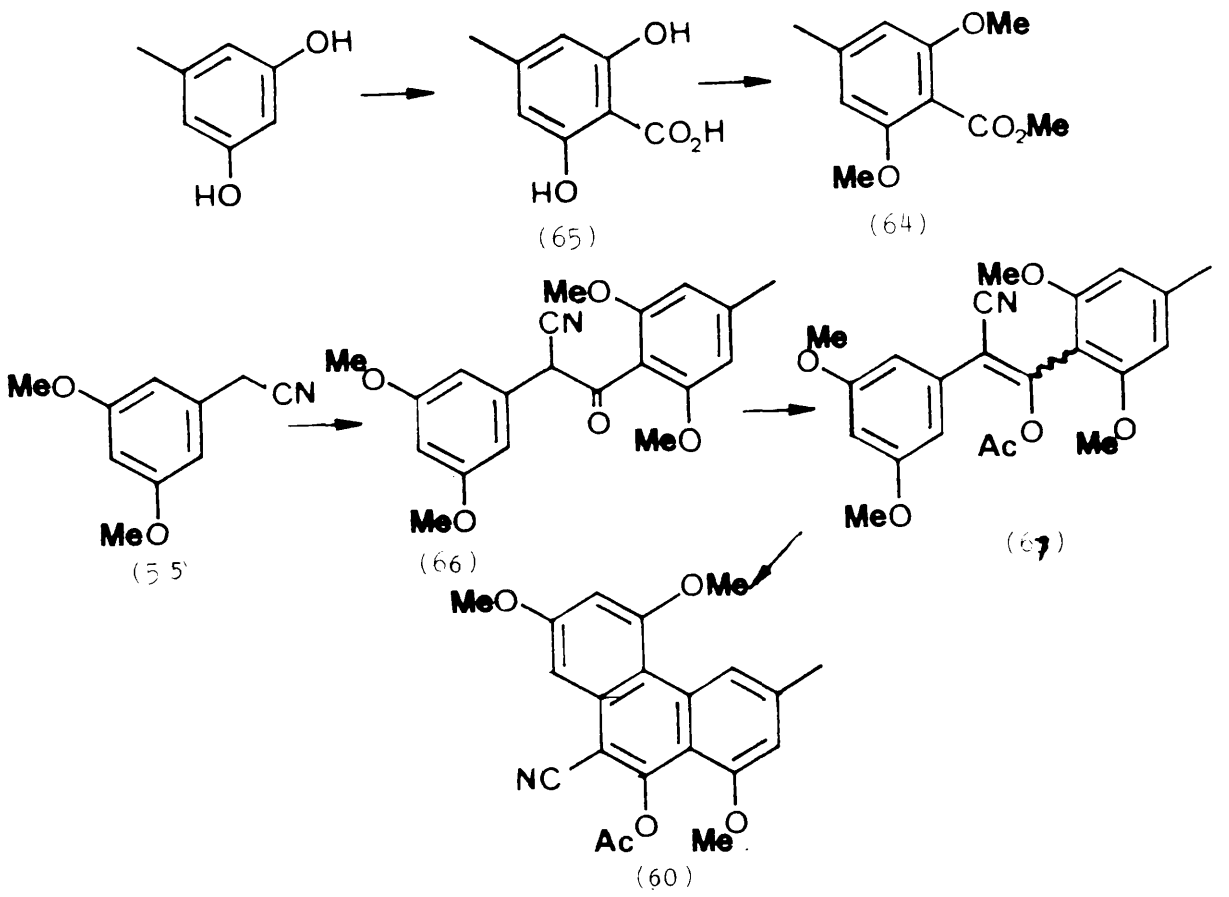


(60)



(61)

Scheme 6:


OH
HO
OH
CO2H
HO
(65)
OMe
CO2Me
MeO
(64)
MeO
CN
Me
O
MeO
(55)
MeO
CN
Me
O
O
MeO
MeO
(66)
MeO
CN
Me
O
Ac
O
Me
O
MeO
(67)
MeO
OMe
NC
Ac
O
Me
O
(60)

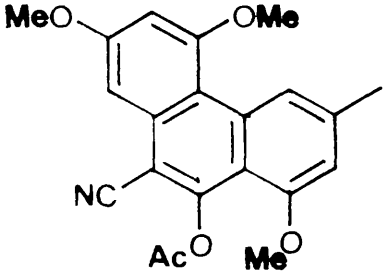
Scheme 7.

derivatives derived from the benzoate ester is here designated ring A; that derived from the phenylacetonitrile as ring B).

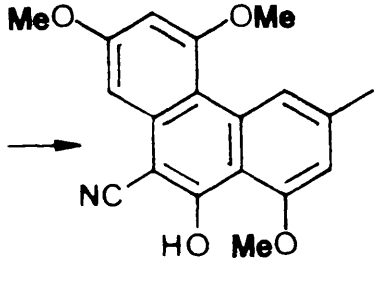
The general synthetic route was therefore adapted. Methyl 2,6-dimethoxy-4-methylbenzoate (64) was obtained by methylation of the dihydroxy acid (65), this being obtained by the Kolbe-Schmidt reaction of orcinol[70]. Condensation of the ester (64) with the phenylacetonitrile (55) was effected as before to give the β-ketonitrile (66) in good yield. Acetylation gave the enol acetate (67) again as a steroisomeric mixture; in this case the ratio of major:minor isomer was ca. 2:1 (Scheme 7).

In comparison with the 2-methoxy-β-ketonitrile (56) which exists largely as a keto-tautomer, the 2,6-dimethoxy-β-ketonitrile (66) was shown to exist almost exclusively as its enolic tautomer: whereas the IR spectrum of (56) exhibits a small OH stretch and a strong carbonyl stretch, the spectrum of (66) shows a strong OH stretch (3250 cm^{-1}) and the absence of a carbonyl stretch; also no benzylic resonance could be observed in the nmr spectrum of (66).

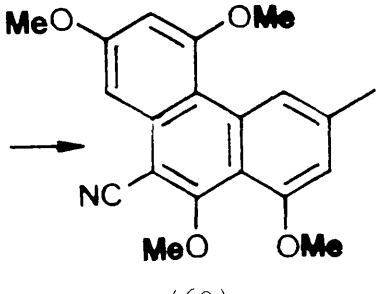
The stereoisomeric mixture of enol acetates (67) was irradiated under nitrogen in the absence of oxidant and gave the phenanthrene (60) as the sole cyclised product. On relatively short irradiation times (eg 16 hours) the yield of phenanthrene (60) was ca. 40%. From the reaction mixture could also be isolated in pure, crystalline form the major stereoisomeric enol acetate (67) and a smaller amount of gummy material which largely comprised of the minor



(60)



(68)



(69)

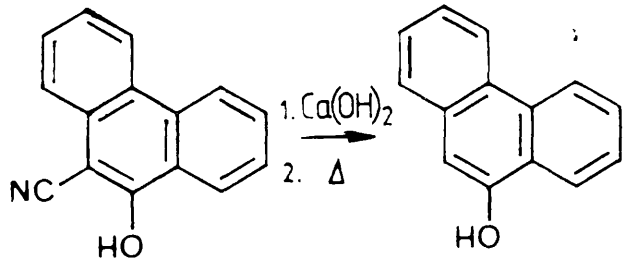
enol acetate (67). The major stereoisomer was therefore proposed to have the E-configuration and the minor the Z-configuration.

Interestingly the yield of phenanthrene (60) was not significantly increased on prolonged irradiation (eg up to 4 days) but by removing phenanthrene (60) from the system periodically, yields increased to ca.75%. The phenanthrene (60) absorbs strongly U.V. radiation in the region 200-350 nm whereas the lamp used in these experiments emits radiation in the range 200-400 nm. As the concentration of phenanthrene (60) increases through photocyclisation therefore the amount of radiation available to promote further cyclisation falls until eventually little or no cyclisation ensues. By removing phenanthrene (60) from solution further cyclisation can occur.

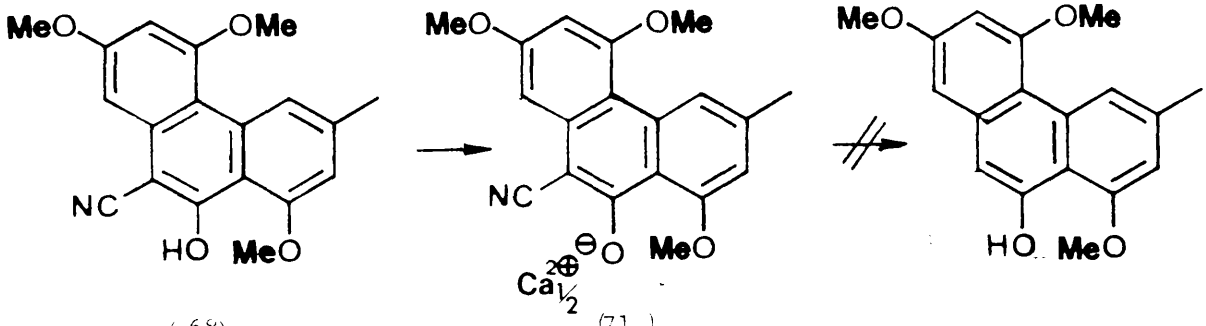
It will be remembered that hydrolysis and decarboxylation of the phenanthrene (60) was not anticipated to be troublesome. Ortho-substituted aromatic nitriles are known to be sometimes difficult to hydrolyse and some o-oxy substituted nitriles particularly so, but long reaction times usually ensure complete hydrolysis[71]. Unfortunately the nitrile of phenanthrene (60) proved completely resistant to hydrolysis under simple acid or basic conditions (Table 3) and only hydroxyphenanthrene (68) resulted. Similarly no reaction of the nitrile occurred during attempted hydrolysis of the 9-cyano-10-methoxyphenanthrene (69), obtained by methylation of (68).

Table 3. Reaction of Cyanophenanthrene

Substrate	Conditions
(60) 10-OAc	$KOH/H_2O/CH_3OH$ $48\%HBr$
(69) 10-OMe	$KOH/H_2O/CH_3OH$ H_2O_2/acetone
(68) 10-OH	NaOH/EtOH / sealed tube/190° PPA/200° $Ca(OH)_2$ / 150° Na / $NH_3(l)$ H_2S/Et_3N / py


NC
HO
1. Ca(OH)2
2. Δ
HO

(70)


MeO
OMe
NC
HO
MeO
MeO
OMe
NC
O
MeO
Ca
2⊕
½
MeO
OMe
HO
MeO

(68)

(71)

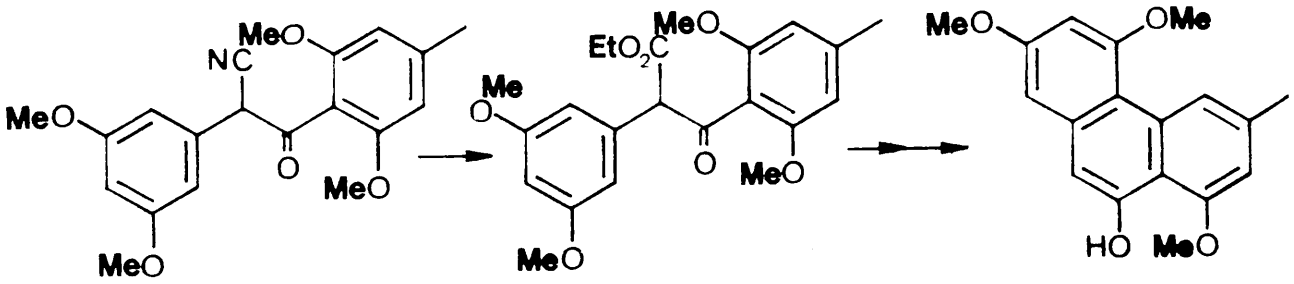
Sepiol and Mirck[71] report that decyanation of such compounds, presumably through hydrolysis (or alcoholysis) and decarboxylation, can generally be achieved by reaction of the aromatic nitrile with sodium hydroxide and ethanol at elevated temperature and pressure. In our hands, though, no reaction occurred with hydroxyphenanthrene (67) even at 200° in a sealed tube.

The parent phenanthrene, 9-cyano-10-hydroxyphenanthrene (70) is also known to be resistant to hydrolysis but has been decyanated through the pyrolysis of its calcium salt[72]. When the calcium salt of phenanthrene (71) was heated, only phenanthrene (68) could be recovered on work-up.

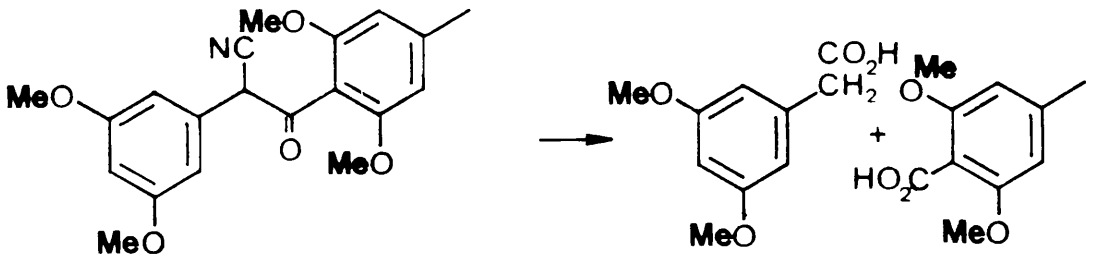
Decyanation of aromatic nitriles has been brought about by reaction with polyphosphoric acid[73]. Again however, no rection of the nitrile occurred under these conditions with hydroxy- or acetoxyphenanthrenes (68) and (60).

Complete removal of a nitrile moeity has also been achieved under dissolving metal reduction conditions[74]. Treatment of a solution of the hydroxyphenanthrene (68) in liquid ammonia with sodium metal however gave a large amount of polar material and starting material as the only identifiable product (see Table 3).

An alternative appoach to this problem is to firstly convert the nitrile group into some other functional group which may then be more susceptible to hydrolysis. For example nitriles can generally be converted into amides upon treatment with basic hydrogen


MeO
NC
MeO
O
MeO
MeO
(60
EtO2C
MeO
Me
O
O
MeO
MeO
(72)
MeO
OMe
HO
MeO

Scheme 8.

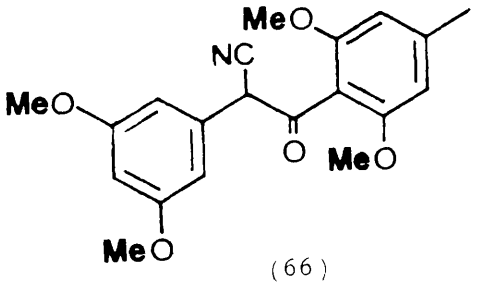

MeO
NC
MeO
O
MeO
MeO
(66)
CO2H
CH2
MeO
Me
O
+
HO2C
MeO
MeO
(35)
(73)

peroxide[75]. Again though, our system (as the methyl ether (69)) proved totally inert under these conditions. Similarly an attempt to produce the corresponding thiamide by treatment of the nitrile (60) with hydrogen sulphide was also a complete failure[76].

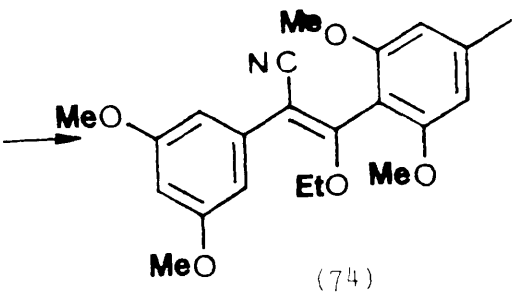
It will be remembered that the original synthetic route was designed to be adaptable in the light of the experimental failure of any particular step: ie if the target 10-acetoxyphenanthrene (25) is not available from the β-ketonitrile (66) then it may be obtainable from the corresponding β-ketoester (72). It was assumed that β-ketoester (72) would be available by alcoholysis of β-ketonitrile (66) (Scheme 8).

The chemistry of such α-cyanodesoxybenzoins has long since been a matter of record. Bodroux[77] in 1910 discovered that base hydrolysis of the parent ketonitrile resulted in hydrolytic attack on the carbonyl rather than on the nitrile to give benzoic acid and phenylacetonitrile which is subsequently hydrolysed to phenylacetic acid. We found a similar cleavage of the α-cyanodesoxybenzoin (66) resulted upon treatment with base to give the corresponding phenylacetic and benzoic acids (35) and (73).

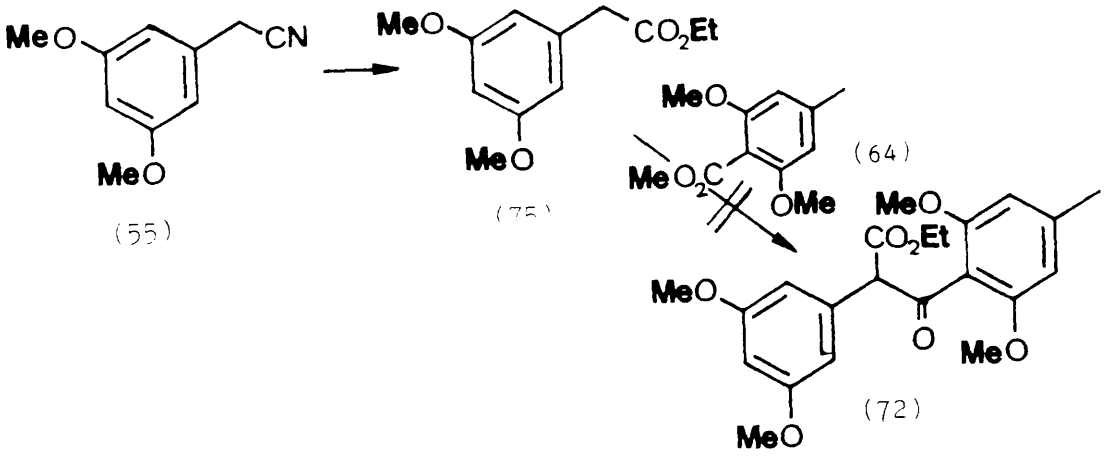
It is reported[78] that the ester can be obtained by treatment of the nitrile with hydrogen chloride saturated alcohol. When a solution of β-ketonitrile (66) in ethanol was saturated with hydrogen chloride and heated at 100° for 24 hours, a 50:50 mixture of starting material and a second compound was obtained. This


MeO
NC
MeO
MeO
O
MeO
MeO

(66)


MeO
NC
MeO
EtO
MeO
MeO

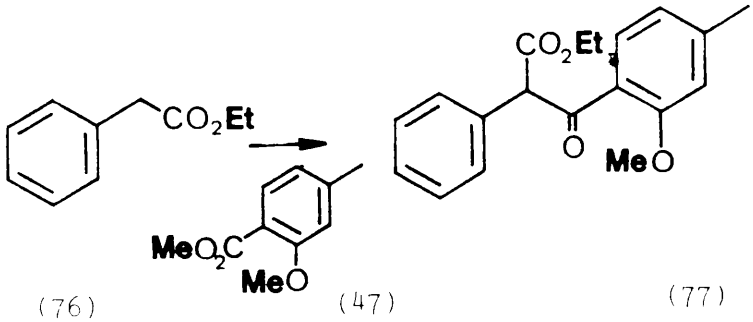
(74)


MeO
CN
MeO
(55)
MeO
CO2Et
MeO
(75)
MeO
MeO2C
OMe
(64)
MeO
CO2Et
MeO
O
MeO
MeO
(72)

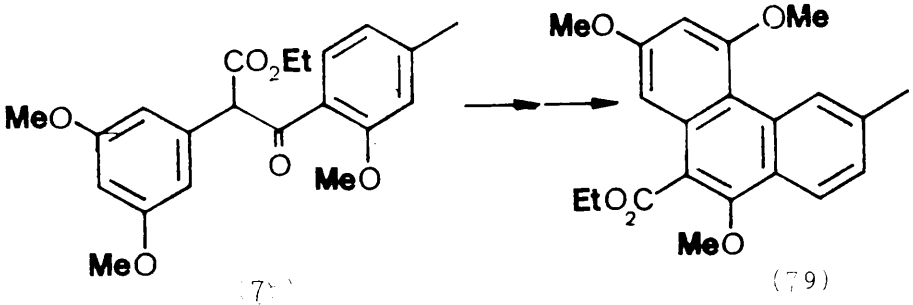
compound, however, was shown to be the ethyl enol ether of the β-ketonitrile (74) rather than the desired ester (72): the nmr spectrum of the product showed the presence of an ethoxy group and the IR spectrum showed the absence of hydroxyl groups (from the enolic tautomer of (65)) as well as the continued presence of a nitrile (ν_{max} = 2205 cm^{-1}). The identity of the enol ether (74) was confirmed by high resolution mass spectrometry. Even at 0° under these conditions no alcoholysis of the nitrile could be detected, the primary reaction again being enol ether formation (although the rate was consequently much slower). Attempted alcoholysis of the nitrile (66) was therefore abandoned in favour of a more direct route to the ester (72).

It was postulated that a condensation analogous to that used to form β-ketonitrile (66) would result in β-ketoester (72) if the anion used was that of the phenylacetic ester (75) rather than that of the phenylacetonitrile (55). The ethyl phenylacetate (75) was produced by ethanolysis of 3,5-dimethoxyphenylacetonitrile (55), but unfortunately and unexpectedly the anion of (75) repeatedly failed to condense with the dimethoxy ester (64). That the anion had been formed was shown by the incorporation of deuterium into the benzylic positions of the ester (75) upon quenching of the anion with deuterium oxide (D_2O).

The failure of the reaction can be explained on steric grounds. The ester functionality of methyl 2,6-dimethoxy-4-methylbenzoate (64) is sterically hindered by two ortho-methoxy groups, but not sufficiently hindered to prevent condensation with


CO2Et
MeO2C
MeO
CO2Et
O
MeO

(76) (47) (77)


CO2Et
MeO
O
MeO
MeO
MeO
OMe
EtO2C
MeO

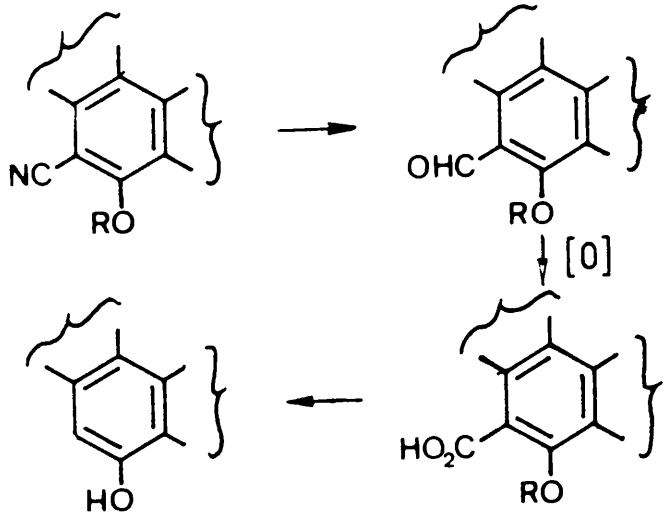
(78) (79)

the anion of a phenylacetonitrile. However, it seems that the greater bulk of the anion of an ethyl phenylacetate is sufficiently large to prevent condensation with the hindered ester moeity of (64). This postulation is supported by the observation that ethyl phenylacetate (76) does condense with the less hindered methyl 2-methoxy-4-methylbenzoate (47) to give β-ketoester (77).

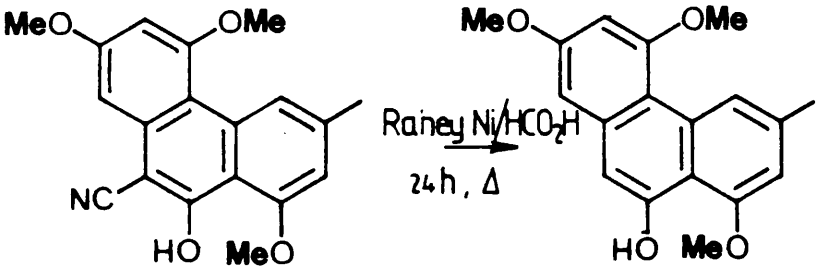
Although this latter result implies that the trimethoxy-β-ketoester (77) analogue (78) could be made, it was felt that if the protected enol of (78) proved photochemically labile then this route would lead to a large amount of the unwanted de-oxy phenanthrene (79) through a similar loss of methanol as described earlier. The 2,6-dimethoxy-β-ketoester (72) is the required substrate for this route but as it cannot be obtained by direct methods, this too was abandoned.

The situation can therefore be described as follows: the cyanophenanthrene (60) is obtainable *via* photocyclisation of a protected β-ketonitrile but the cyanophenanthrene (60) is completely resistant to hydrolysis; the β-ketoester (72) is unavailable either *via* alcoholysis of the β-ketonitrile (66) or *via* direct condensation of a phenylacetic ester with a benzoate ester; and the desoxybenoin (42) is available *via* the isocoumarin route but its protected enols are photochemically inert. The chemistry of the cyanophenanthrene (60) was therefore further investigated.

It was proposed that the nitrile functionality of (60) could be reduced to an aldehyde moeity which could then be reoxidised to a



Scheme 9,



(68) (80)

carboxylic acid; this acid could then undergo decarboxylation to give the desired 9-H-phenanthrene. Several methods are available for the reduction of aromatic nitriles to aldehydes, among them that of Staskun and Backeberg[79], which uses Raney nickel in aqueous formic acid to effect the reduction (Scheme 9).

Staskun and Backeberg's procedure[79] consists of a short (1-2 hour) open reflux of the reagents and is reported to furnish the aldehyde in high yield even from hindered nitriles. However, when either the acetoxy-, hydroxy- or methoxyphenanthrenes (60), (68) and (69) were reacted under these conditions, starting material was recovered largely unchanged. No aldehyde could be detected on analytical tlc by means of a 2,4-DNP spray.

Upon longer reaction time with hydroxy-phenanthrene (68) as substrate (eg 24 hours) a large amount of dark polymeric material resulted, but from this could be isolated a number of minor components. The nature of most of these components is unclear but the IR spectra of the majority showed the nitrile functionality to be intact. However, one component was shown by IR to lack a nitrile group and also, curiously, lack any carbonyl functionality. NMR suggested the presence of five aromatic protons in this product: the aromatic coupling pattern in the spectrum of this product was essentially similar to that of the starting material but with a fifth, uncoupled proton. This, in conjunction with the infra-red evidence implied that a reductive decyanation had occurred and that the product was the 10-hydroxyphenanthrene (80). The identity of (80) was confirmed by high resolution mass spectrometry.

The yield of hydroxyphenanthrene (80) was ca.10% but this could be improved to a more synthetically useful 42% by adapting the procedure such that reflux was carried out under a nitrogen filled balloon. Interestingly, when reaction was conducted under a gentle flow of nitrogen gas no improved yield was observed. That the decyanation is a function of the metal was demonstrated by the lack of reaction in the absence of catalyst.

It is interesting to note that this novel and serendipitious reaction was found not to be specific to Raney nickel catalysts. A number of other metals were investigated and the results are summarised in Table 4. When catalysed by 'Reformatsky grade' zinc, the reaction was notable for its clean product and almost all material could be accounted for between product (80) and recovered starting material (68). Although the zinc reaction gave up to 43% of decyanated material, long reaction times were required and the yield was very variable even when the preparation of the metal was thought to have been standardised.

Table 4. Reductive Decyanation of (68)

Catalyst	Reaction time	Yield (%)
Raney nickel	24h	42
zinc	5d	43
tin	48h	4
magnesium	4d	0

The mechanism of this interesting and wholly unexpected transformation is far from clear. One suggestion is that reaction may proceed *via* a process analogous to that of decyanation under Birch reduction conditions[74]. This would involve a series of one electron transfers to form the aryl free radical which would then be reduced to the carbanion, which on protonation by the solvent would give the decyanated aromatic product (Figure 11).

If this mechanism does operate in this system it would be expected that the yield would be increased on exclusion of air by a nitrogen filled balloon, as was observed. However, the yield would also be expected to similarly increase on flushing the system with nitrogen and no such effect resulted. It will also be remembered that under the classical Birch conditions of sodium dissolved in liquid ammonia, cyanohydroxyphenanthrene (68) exhibited no decyanation. This proposed mechanism is therefore unsupported.

A second mechanistic suggestion is that reaction may proceed *via* reduction of the 9,10-double bond of phenanthrene (68) to give an intermediate dihydrophenanthrene (81) which could then undergo elimination of hydrogen cyanide to furnish the observed product. However, the intermediate (81) could equally well eliminate water to give a 9-cyanophenanthrene (82) and both (80) and (82) could also undergo further reduction and elimination steps (Figure 12). The hydrogen source for the reduction would be the Raney nickel catalysed decomposition of formic acid to hydrogen gas and carbon dioxide. In support of this proposed mechanism is the increased yield of phenanthrene (80) when reaction is carried out under a balloon, which

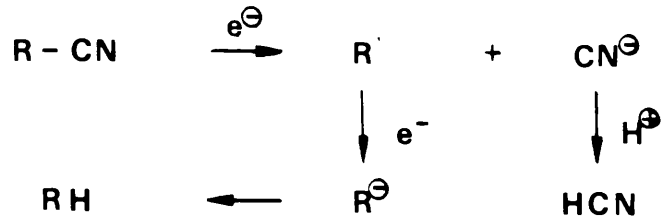


Figure 11. Birch reduction of nitriles.

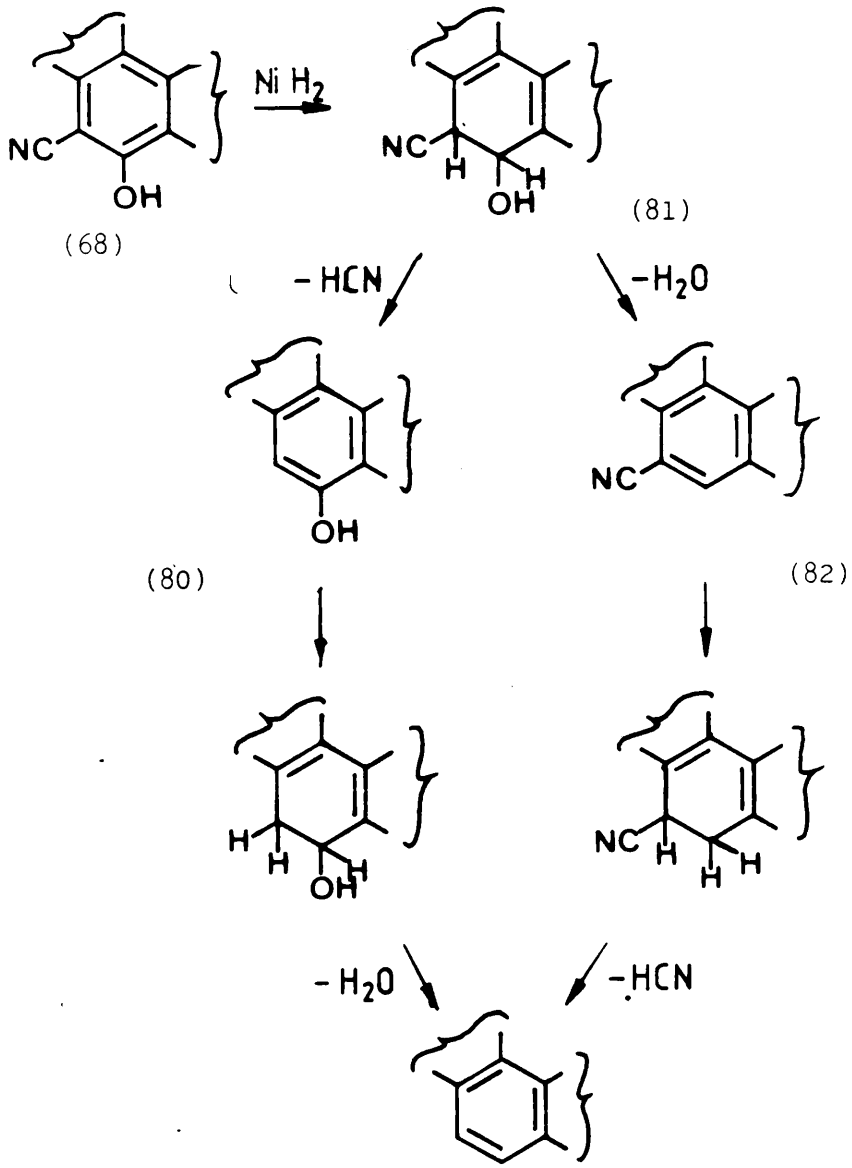
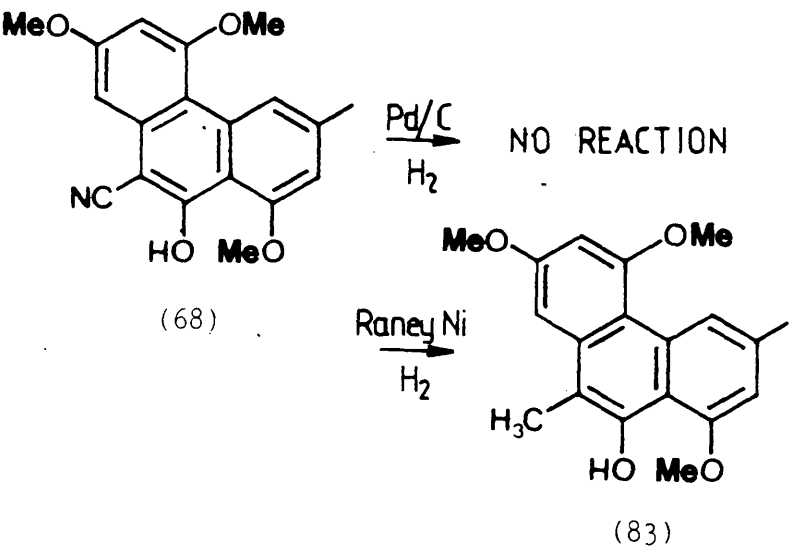


Figure 12. Proposed decyanation mechanism.


MeO
OMe
NC
HO
MeO
Pd/C
H2
NO REACTION
Raney Ni
H2
MeO
OMe
H3C
HO
MeO

(68)

(83)

would act to trap any evolved H_2 and so promote the initial reduction; in addition, sweeping the system with nitrogen would remove this hydrogen, as would an open condenser, hinder the reduction step and account for the observed results under these circumstances. Also the numerous products observed at first could be accounted for by this mechanism, but it does not explain why these products largely disappear when the reaction is carried out under a balloon.

Partly as an investigation of this proposed mechanism and partly as a search for a possible improved decyanation it was attempted to reduce the 9,10-double bond of phenanthrene (68) by other means and study the elimination reactions of the dihydrophenanthrene (81). Fu and Harvey[80] have shown that the 9,10-double bond of phenanthrenes can be selectively hydrogenated by using a palladium catalyst at medium pressure. However, the hydroxyphenanthrene (68) was totally resistant to hydrogenation over palladium even on prolonged exposure to hydrogen at high pressure and temperature.

The phenanthrene (68) was also resistant to hydrogenation over Raney nickel at atmospheric pressure, but at high pressure it was the nitrile moeity that was reduced rather than the 9,10-bond. The product of this reaction was the dimethylhydroxyphenanthrene (83) which arose from the step-wise addition of three moles of hydrogen.

This may seem to imply that the decyanation reaction does not proceed <u>via</u> the hydrogenation of the 9,10-double bond, but

because this hydrogenation was carried out in a neutral solvent (ethyl acetate) and the decyanation reaction in formic acid this would not be a valid conclusion. Unfortunately it is not practical to conduct a high pressure hydrogenation using Raney nickel catalyst in acidic solvents as the catalyst is quickly decomposed under such conditions. No conclusions can therefore be drawn about the decyanation mechanism from this.

In a further attempt to reduce the 9,10-double bond of the phenanthrenes, the methoxyphenanthrene (69) was reacted with lithium aluminium hydride (LAH). A complex mixture of products arose which could not be resolved by chromatography. IR analysis of the mixture showed the absence of starting material and also that the nitrile functionality had largely been reduced as only a weak, broad nitrile stretch (2250 cm^{-1}) was observed. It is likely that the major reaction in this case is reduction of the nitrile to the corresponding benzylamine; the phenanthrene 9,10-bond may then undergo further reduction and possibly elimination, thus accounting for the multiple products (see Table 5).

The reaction was repeated using lithium triethoxyaluminium hydride which was generated <u>in situ</u>. This reagent is reported[81] to reduce nitriles to aldehydes but no aldehyde could be detected among the complex mixture of products which was essentially similar to that produced by LAH.

Table 5. Reduction of cyanophenanthrenes.

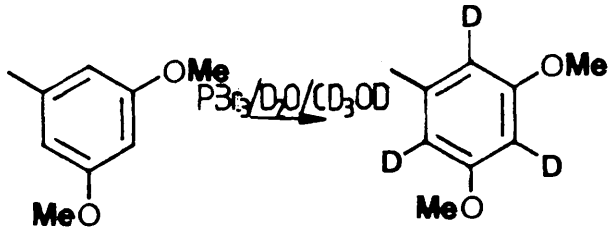
Substrate	Reagent	Reaction	Product
R = OH (68)	Pd C H_2	none	(68)
	Raney Ni H_2	CN reduc^n	(83)
R = OMe (69)	$LiAlH_4$	complex	multiple
	$LiAl(OEt)_3H$	"	"
	B_2H_6	"	"

When diborane was used as the reducing agent a green, polymeric substance was produced and no recognisable products could be isolated. Selective reduction of the 9,10-double bond was therefore abandoned as a means of improving the decyanation reaction and unfortunately no further information was provided about the reaction with Raney nickel and other metal catalysts. The mechanism of this transformation remains unknown but, on the basis of the precedent of dissolving metal reductions, it is perhaps most likely that some sort of radical mechanism may operate.

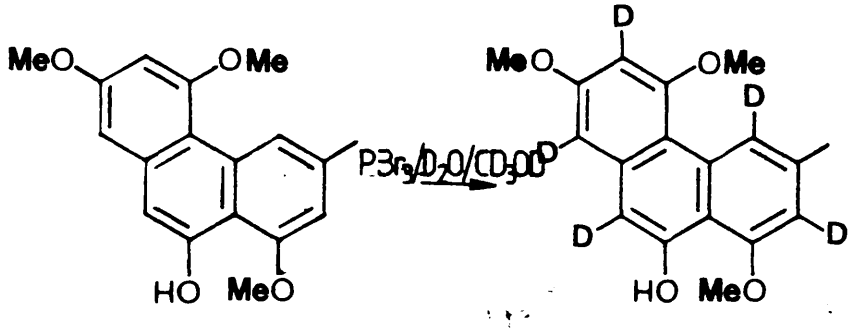
3.1.3. Reactions of 10-Hydroxy-1,5,7,trimethoxy-3-methylphenanthrene

It will be remembered that the ultimate synthetic target is a series of deuteriated phenanthrenes. One approach to the synthesis of deuteriated compounds is to employ a route whereby the label is carried along from a deuteriated starting material over a multi-step sequence into a deteriated product. A complementary approach is *via* synthesis of unlabelled material which is then made to undergo deuterium exchange within the finished molecular framework. Now that the phenanthrene skeleton had been synthesised, it seemed opportune to investigate deuterium exchange reactions of the phenanthrene (80).

Deuterium exchange of electron-rich aromatic compounds can be achieved under acid or base catalysis and the choice of conditions is often determined by the stability of the substrate. Wahala *et al.*[82] has shown that phenols undergo *ortho* and *para* exchange catalysed by deuterium bromide (DBr) generated *in situ* by reaction of D_2O and phosphorus tribromide. Yields are reported to be


OMe
MeO
$PBr_3/D_2O/CD_3OD$
D
OMe
D
D
MeO

(84)


MeO
OMe
HO
MeO
$PBr_3/D_2O/CD_3OD$
D
MeO
OMe
D
D
D
D
HO
MeO

(80)

improved on prior exchange of the acidic phenolic proton. To test the scope of this reaction 3,5-dimethoxytoluene (84) was heated at reflux with PBr_3 in D_2O. Deuterium exchange at the aromatic positions was seen to have occurred and the incorporation was ca. 85% by nmr integration. This figure was supported by mass spectrometry which suggested that the products were d_0, 1.6; d_1, 6.4; d_2, 42.2; d_3, 49.6%. Because the toluene (84) is insoluble in D_2O much charring occured and so chemical yields were moderate. However, by introducing methanol-d_4 as co-solvent quantitative recovery of material was obtained without affecting deuteriation yields. Satisfactory levels of incorporation are therefore obtainable under these conditions.

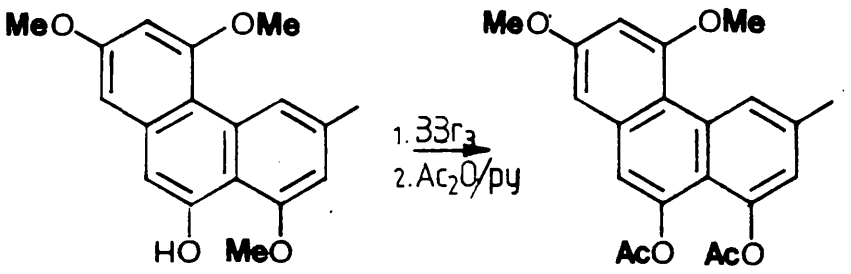
As all of the aromatic protons of phenanthrene (80) are ortho or para to either a phenolic or methoxyl substituent it was proposed that they would undergo DBr catalysed deuterium exchange. Thus the phenanthrene (80) after prior exchange of the phenolic proton was dissolved in D_2O/CD_3OD and treated with PBr_3 at reflux for 12 hours. NMR analysis of the product indicated that the overall level of incorporation of deuterium into the aromatic positions of (80) was around 40%. The level of incorporation at each position in detailed in table 6. These incorporations were worked out by integration of the nmr signals for the products on the assumption that deuterium exchange on the 3-methyl substituent was negligible. Thus the integral for Me-3 was assigned to three protons and the extent of deuteriation at other positions was determined accordingly.

In an attempt to increase the level of deuteriation the deuteriated phenanthrene was twice further reacted with $PBr_3/D_2O/CD_3OD$, each for 24 hours. The overall level of deuteriation was now found to be 77% by nmr integration. As before the individual incorporation at each site is shown in table 6. It is curious to note that the level of deuterium incorporation at position-9 does not increase on prolonged reaction and at position-8 the incorporation actually falls. This is not what would be expected under these circumstances and this observation is currently unexplained. Although not fully investigated, it is probable that the levels of incorporation of deuterium could be further increased by more prolonged treatment with PBr_3/D_2O.

time	position					overall
	2	4	6	8	9	
12 h	25	43	54	68	57%	40%
12+(2x24)h	78·5	89·5	72	51·5	57%	77%

Table 6. % Deuteriation of phenanthrene (80)

At this stage attention was turned towards the demethylation of the phenanthrene (80). There are a large number of reagents available for aromatic ether cleavage but the reagent of choice is often boron tribromide (BBr_3) which is generally clean and

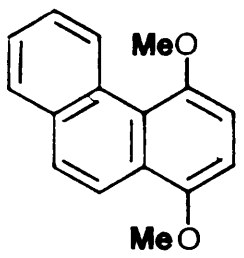


(80) (85)

effective[83]. Cleavage of the methyl ethers of phenanthene (80) was not anticipated to be a problem but because the product phenols would be expected to be readily oxidised in air they were immediately acetylated by reaction with acetic anhydride and pyridine.

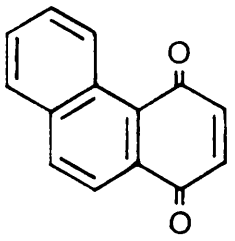
When phenanthrene (80) was reacted with a large molar excess of BBr_3, however, only partial demethylation resulted. The nmr spectrum of the major product (after acetylation) indicated the presence of two acetoxyl- and two methoxyl-substituents. It is difficult to conclusively prove the structure of this product phenanthrene by simple means, but it is chemically very likely that the previously free phenol has acetylated. This proposition is supported by the observation that the nmr signal for H-9 undergoes an upfield shift of 0.22 ppm from δ 6.98. NMR also indicated that the second acetoxyl group is at carbon-1: the H-4 signal is shifted upfield by 0.25 ppm from δ9.10 implying the substitution of a methoxyl group by an acetoxyl. This suggests that the product of this reaction is 1,10-diacetoxy-5,7-dimethoxy-3-methylphenanthrene (85). It is emphasised however that no rigorous proof is offered in support of this structure. This result was repeatable.

The failure of BBr_3 to fully demethylate phenanthrene (80) was somewhat surprising. The methoxyl at carbon-1 would be expected to be particularly labile towards BBr_3 because the peri-hydroxyl group could facilitate delivery of a borane moeity, and the methoxyl at C-7 is unhindered and free to rotate and so it is difficult to reason that it should be inert towards BBr_3. The methoxyl at C-5 is partially hindered due to its position in the aromatic bay region but would still be predicted to be reactive towards BBr_3.

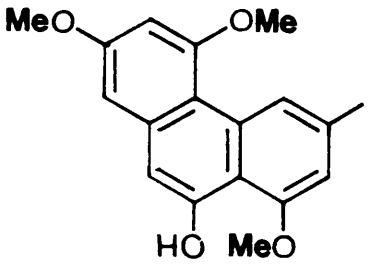


(86)

1. TMSI
2. [O]

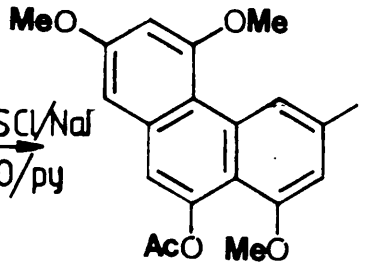


(87)



(80)

1. TMSCl/NaI
2. Ac_2O/py



(88)

One of the many alternative reagents available for the cleavage of aromatic methyl ethers is trimethylsilyl iodide (TMSI) which is reported[84] to give clean, quantitative demethylation for many systems and is often complementary to BBr_3. For example 1,4-dimethoxyphenanthrene (86) is fully demethylated upon treatment with TMSI to give the quinone (87) (upon oxidation); 1,4-dimethoxyphenanthrene (86) is resistant to demethylation by BBr_3[85].

A standard method for *in situ* preparation of TMSI is *via* transhalogenation of trimethylsilyl chloride with sodium iodide[86]. Thus, phenanthrene (80) was reacted with a large molar excess of TMSI generated as above in acetonitrile under nitrogen and the reaction products immediately acetylated as before. A significant amount of decomposition was observed but the major component isolable by chromatography was the acetylated starting phenanthrene (88). Apparently little demethylation had occurred under these reaction conditions.

TMSI can be used directly to effect ether cleavage without the need for *in situ* generation. The phenanthrene (80) was reacted with a large excess of TMSI in refluxing chloroform under nitrogen. Chromatography of the acetylated mixture separated the major product (*ca*.40%) of the reaction from a large amount of dark tar. NMR spectroscropy of this product, however, showed that only partial demethylation had occurred: resonances due to only two acetate groups (δ 2.38 and 2.44 ppm) were seen but interestingly and confusingly only one methoxyl resonance (δ3.91 ppm) was observed in addition to

an ethoxyl resonance (δ 1.42, 3H,t and δ 4.17, 2H, q ppm). This suggested that during reaction two methyl ether groups had been cleaved and that one had subsequently been ethylated. This was confirmed by high resolution M.S.

The identity of the ethylating agent in this reaction was not immediately apparent but it will be remembered that reaction was carried out in chloroform solvent which customarily contains 0.75% ethanol for stabilisation. A known reaction of aliphatic alcohols with TMSI is to produce the alkyl iodide[87] and so in this system a small amount of ethyl iodide is unavoidably produced. Although 35mg of phenanthrene (80) was initially dissolved in only 10 ml of chloroform, because the reaction entailed prolonged reflux under a flow of nitrogen gas, blown-off solvent had to be replaced periodically so that ca.60ml of chloroform was used in total. Simple calculation suggests that sufficient ethyl iodide would be produced to effect the observed ethylation.

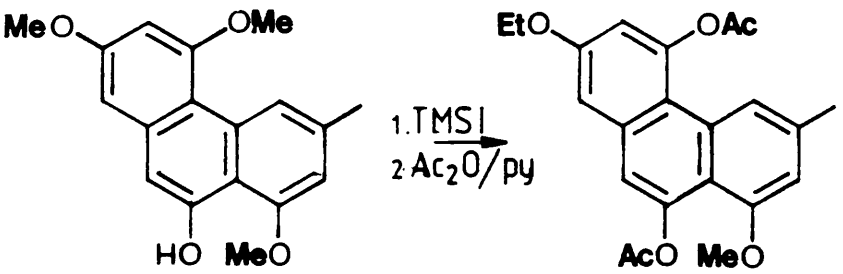
Although the nature of the ethylating agent is probably clear, the nature of the ethylation is not. It is curious to say the least that ether formation occurs under conditions designed to produce ether cleavage and no satisfactory explanation is forthcoming. Assuming that a formal demethylation/ethylation scheme operates, it is interesting to note that of the three phenolic groups present (as phenyl silyl ethers) only one is specifically ethylated to give the observed product.

The structure of this ethoxyphenanthrene (89) was not immediately evident from its spectral characteristics. However, a series of spin decoupling experiments on the nmr spectrum of (89) revealed the position of the remaining methoxy group. Irradiation of the methyl signal, δ 2.52, resulted in the aromatic multiplet due to H-4, δ8.49, collapsing to a doublet. More significantly the broad singlet at δ6.82 became resolved to a doublet. This signal therefore is due to H-2.

On irradiating the methoxyl resonance at δ3.92 the H-2 resonance sharpened appreciably and so H-2 must be adjacent to this methoxyl group. The methoxyl group is therefore at C-1. No other signal was affected in this experiment.

On irradiation of the ethoxyl methylene resonance (δ 4.15) only the ethyl CH_3 signal is affected, collasping into a singlet. The aromatic resonances are unchanged thus giving no information about the position of the ethoxyl moeity.

A related nmr technique is that of nuclear Overhauser enhancement (NOE) difference spectroscopy. For a heteronuclear two spin system (A_2) the signals observed in nmr due to radio-frequency (r.f.) induced transitions are proportional to the appropriate population differences in the systems in the absence or presence of any r.f. magnetic field. If a double resonance experiment is performed such that the A_1 signal is monitorred while the A_2 resonances are simultaneously irradiated strongly, then there will be decoupling. If there is complete saturation of the A_2



(80) (89)

magnetisation then there will be a resultant intensity change in the signal due to A_1.

In a NOE difference experiment the nmr spectrum of the A_2 system is recorded followed by the double resonance spectrum on irradiating A_1 (say). The spectra are then subtracted from each other and any NOE can be directly observed.

A series of NOE difference experiments was conducted on the ethoxyphenanthrene (89) where most signals were irradiated in turn. The results are shown in Table 7. Thus irradiation of the ethoxy methylene signal (δ4.15) produced a NOE enhancement in two aromatic signals indicating that it is situated at C-7. The two acetate groups must therefore be situated at the remaining C-5 and C-10 positions. This is proven by irradiation of the methoxy signal which resulted in enhancement of the signal at δ2.37, identifying this signal as being due to a C-10 acetate. Irradiation of the C-5 acetate, δ 2.48, produced an enhancement of the aromatic signal at δ6.88, identifying this signal as H-6. H-8 therefore appears at δ7.05 and H-9 at 7.15 ppm.

The structure of the ethoxyphenanthrene (89) is clearly elucidated and its proton nmr fully assigned by this technique.

Chemically it appears that demethylation occurs at both positions -5 and -7 (but not at postion -1) and because position -7 is least hindered it is preferentially ethylated under the reaction conditions to ultimately give phenanthrene (89).

Table 7. NOE difference experiment on phenanthrene (89).

irradiate	δ 2·37 10-OAc	3·92 1-OMe	6·82 2-H	2·53 3-CH_3	8·49 4-H	2·48 5-OAc	6·88 6-H	4·15 7-OCH_2—	1·47 —CH_3	7·05 8-H	7·15 9-H
δ 2·37	x	2·3									
3·92	3·3	x	21·1								
2·53			9·4	x	12·2						
8·49				5·2	x	4·8					
2·48					12·2	x	1·5				
4·15							4·5	x	9·3	7·8	
7·05								8·8		x	10·7

Figures are % enhancement upon irradiation.

In contrast to reaction with BBr_3 where OMe-1 is most reactive towards demethylation, in this case OMe-1 is resistant towards demethylation by TMSI. As has been said above, reaction of OMe-1 with BBr_3 may be facilitated by the C-10 *peri* hydroxy group but this group would not be predicted to reduce the activity of OMe-1 towards TMSI. Differences in reactivity of ethers towards BBr_3 and TMSI are well known but in general are unexplained.

In a final attempt to completely demethylate this system the phenanthrene (80) was reacted with hydrobromic acid. As would be expected for such a ring active molecule, the reaction product was an intractable black tar and no identifiable products could be isolated.

The synthesis was therefore abandoned at this stage. It is probable that by repeating the reaction of phenanthrene (80) with TMSI in the absence of alcohols a triacetoxy-1-methoxyphenanthrene would result. Subsequent reaction of this product with BBr_3 may result in cleavage of the final methyl ether but it is likely that the poor yields obtained in each step would limit the synthetic value of such a route. If the overall synthetic strategy is to be retained in future work, a more beneficial approach would perhaps be to repeat the synthesis using a more easily removed protecting group, eg. benzyl ethers. A likely complication of this scheme is that the course of each reaction may alter with the different reactants and a whole new chemistry may need to be devised. However, such unpredictability is part of the fundamental nature of chemical research.

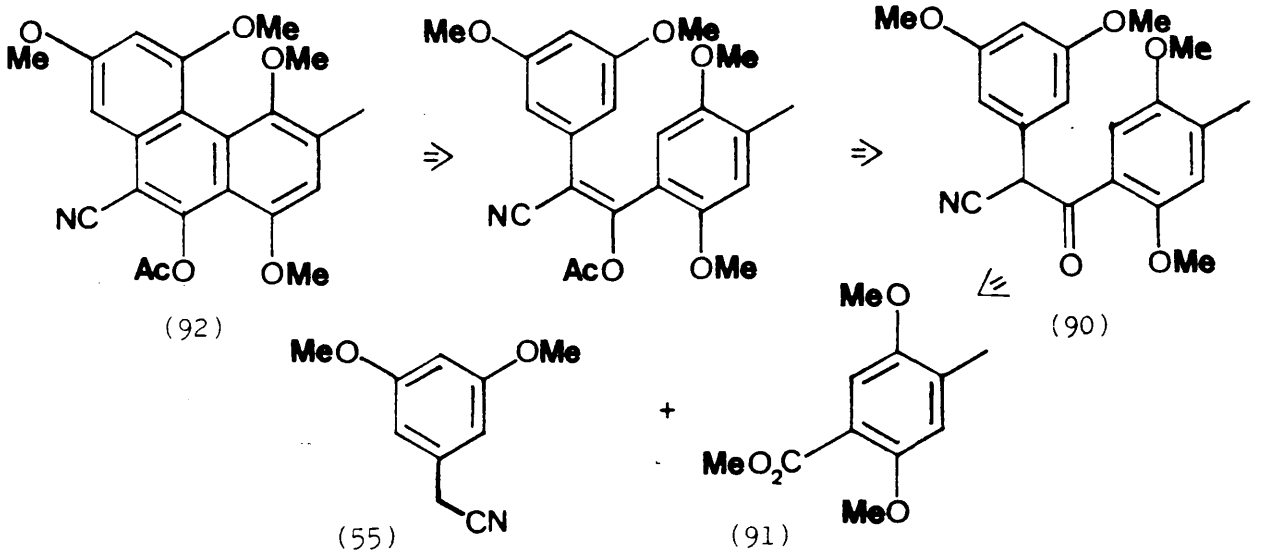
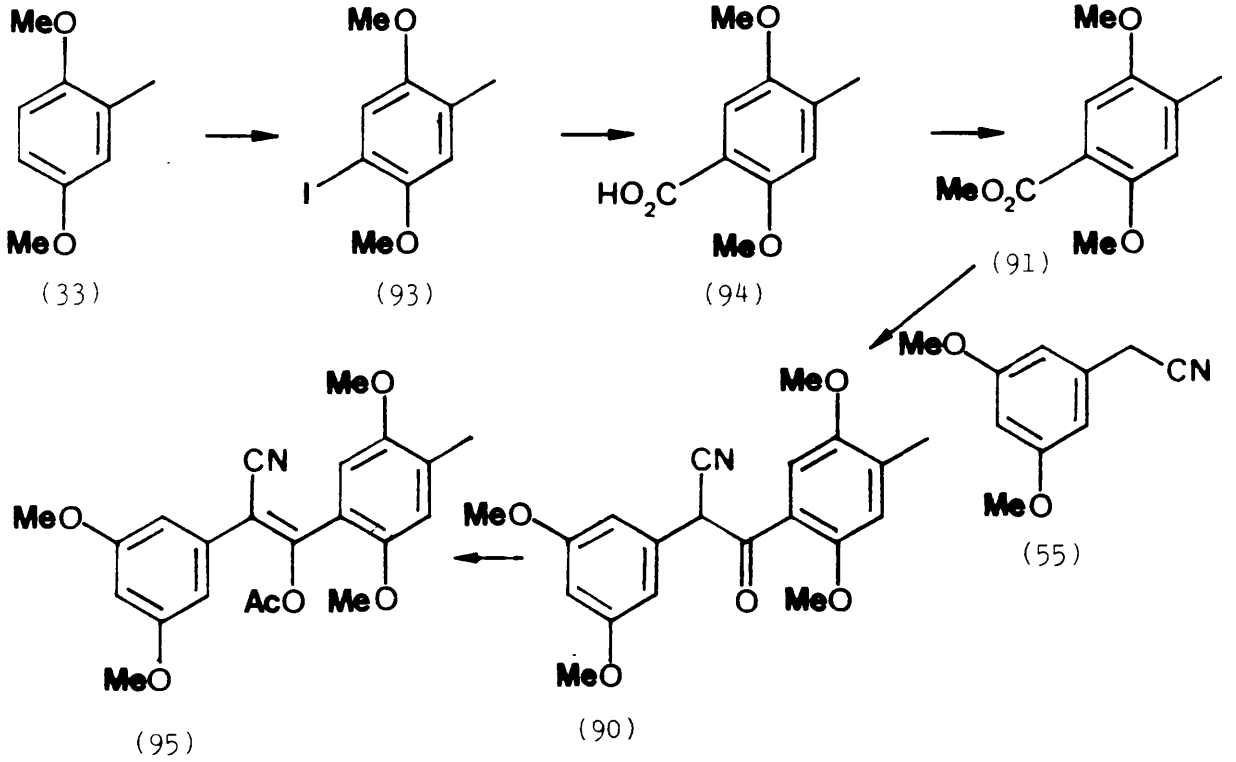


Figure 13



Scheme 10.

3.2. The Synthesis of 4-Substituted Phenanthrenes

3.2.1. Direct Methods

In the first instance it was postulated that the general route outlined in the previous section to 1,5,7,10-tetraoxygenated phenanthrenes could be extended to produce 4-hydroxy and 4-carboxy phenanthrenes. Thus it was proposed that the β-ketonitrile (90) resulting from condensation of 3,5-dimethoxyphenylacetonitrile (55) with methyl 2,5-dimethoxy-4-methylbenzoate (91) may photocyclise as its enol acetate to give the corresponding 4-methoxyphenanthrene (92) (figure 13).

Hence, 2,5-dimethoxytoluene (33) was iodinated using iodine and silver trifluoroacetate and the resulting aryl iodide (93) reacted with carbon dioxide according to the method of Gilman[88] to give 2,5-dimethoxy-4-methylbenzoic acid (94). Methylation gave the desired ester (91) which condensed with the phenylacetonitrile (55) in good yield. The resulting β-ketonitrile (90) was acetylated as before to furnish the enol acetate (95). The ratio of major:minor stereoisomeric enol acetates was ca. 2:1 and, as before, the major isomer was proposed to have the E-configuration (Scheme 10).

In comparison to the previously produced 2-methoxy- and 2,6-dimethoxy-β-ketonitriles (56) and (66), which exist largely as their ketonic and enolic tautomers respectively, the 2,5-dimethoxy-β-ketonitrile (90) exists largely as the ketonic tautomer

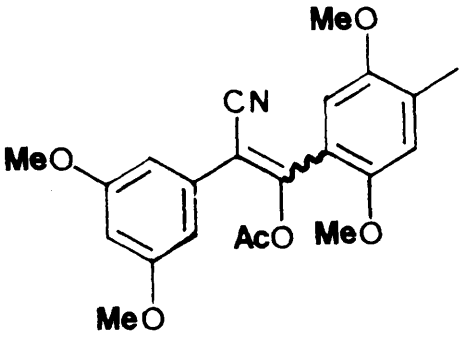
(see Table 8). The IR spectrum of (90) exhibits a strong carbonyl stretch and only a very weak OH stretch; similarly the nmr spectrum of (90) shows a 1-H singlet at δ5.95 ppm due to the benzylic proton.

Compound	Sub^n	$\nu_{C=O}(cm^{-1})$	$\nu_{OH}(cm^{-1})$	δ_{benzyl}(ppm)
(56)	2-OMe	1 683	-	5·77
(66)	2,6-OMe	-	3 250	-
(90)	2,5-OMe	1 675	-	5·95

Table 8. Spectroscopic properties of β-ketonitriles.

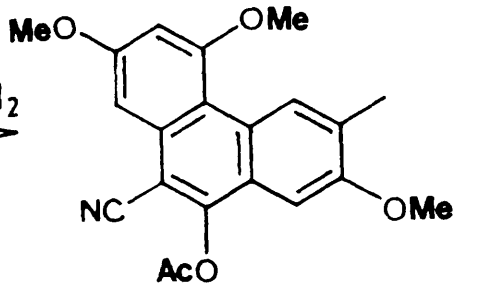
It would be predicted on electronic grounds that the 2,6-dimethoxy compound (66) would be least likely of these three β -ketonitriles to exist as an enol. For the keto-form to be stable the ketone functionality must be co-planar with the adjacent aromatic ring. However, this would bring the lone electron pairs of the ketonic oxygen into close contact with those of the ortho-methoxyl group. This crowding is relieved through tautomerism to the corresponding enol form. Such crowding can also be envisaged for both the 2-methoxy- and 2,5-dimethoxy- compounds (56) and (90) but in these cases the crowding can be relieved by rotation of the aromatic ring through 180°. Hence the ketonic tautomer is favoured by both (56) and (90).

Irradiation of the isomeric mixture of enol acetates (95) in benzene containing iodine gave a single product. However, nmr analysis of this product showed the presence of four aromatic



(95)

$h\nu/I_2$



(96)

protons, including a 1H singlet at δ9.25ppm indicating that the product was unsubstituted at the 4-position; also only three methoxyl resonances could be observed. This compares with the expected three aromatic protons and four methoxyl groups of the desired product. Thus the product was identified as 10-acetoxy-9-cyano-2,5,7,trimethoxy-3-methylphenanthrene (96).

This phenanthrene (96) arose by a similar non-oxidative cyclisation with loss of methanol as previously described. Unlike the photocyclisation of the 2-methoxy enol acetate (59) though, only the non-oxidative mechanism was seen to operate: before, both oxidative and non-oxidative mechanisms were seen. This result can be explained on steric grounds[50]. The photocyclisation of the Z-enol acetate (95a) gives rise to a dihydro-intermediate (97a) and the rotomeric Z-enol acetate (95b) gives rise to the intermediate (97b). If, as is likely, oxidative trapping of the intermediate (97a) is not 100% efficient then because of its highly strained nature the rate of thermal ring opening of (97a) to give (95a) is greatly enhanced relative to that of the less strained (97b) to give (95b). This is due to the relief of the destabilising methoxy-methoxy interaction that accompanies the conversion of (95a) to (97a). Thus it appears that the overall relative rates of phenanthrene production via the two competing mechanisms is so much faster through the less crowded non-oxidattive aromatisation that effectively no phenanthrene is produced by the oxidative mechanism (Figure 14).

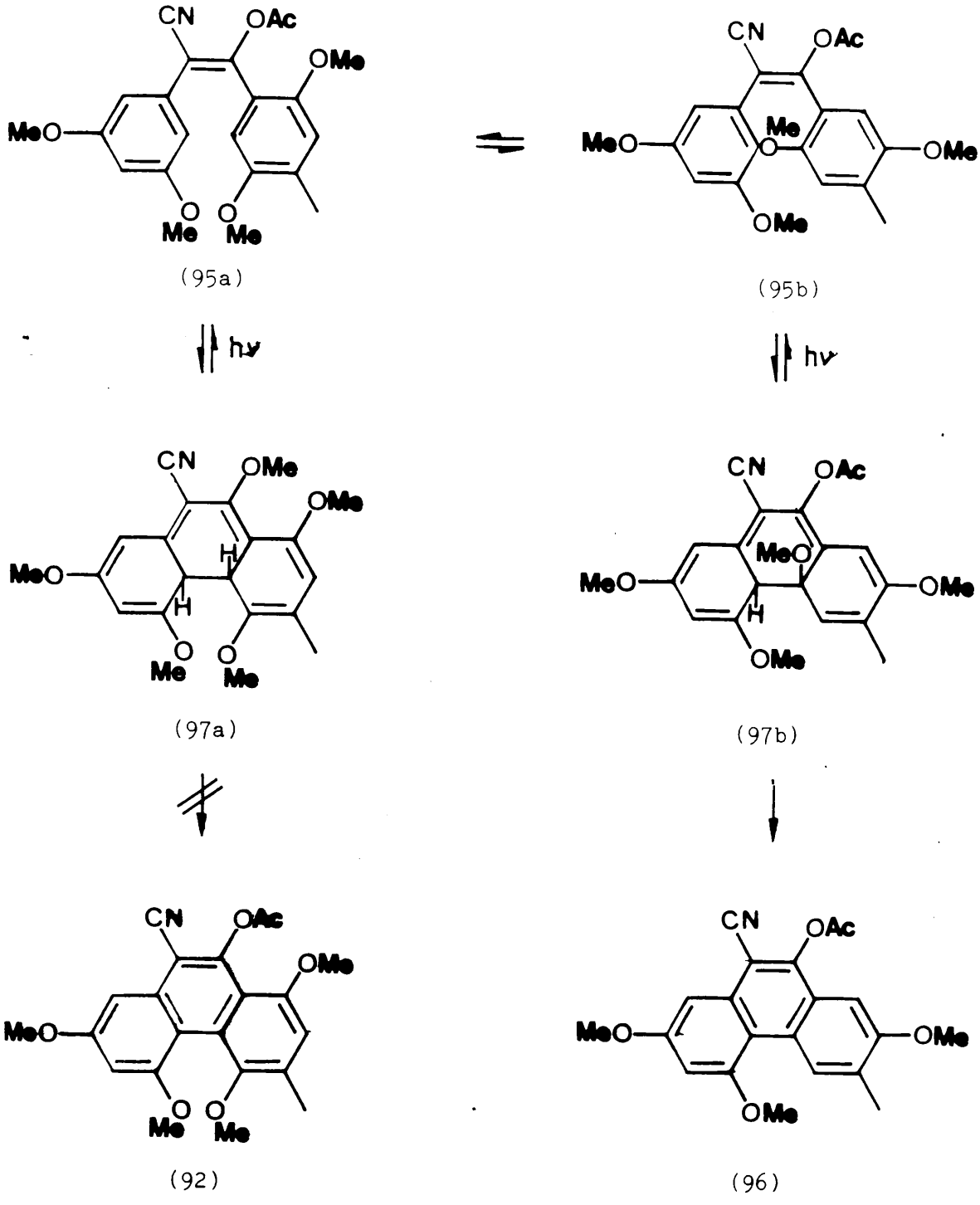
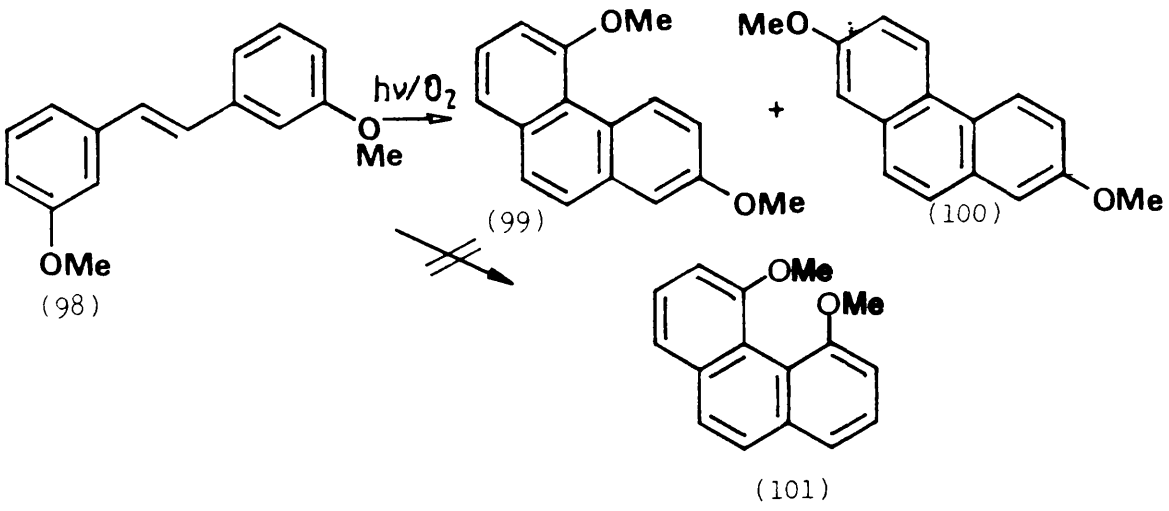


Figure 14. Photocyclisation of (95).


OMe
(98)
hν/O2
O
Me
OMe
OMe
(99)
MeO
+
OMe
(100)
OMe
OMe
(101)

It is notable that photocyclisation of m,m'-dimethoxy stilbene (98) with oxygen as oxidant gives 2,5- and 2,7-dimethoxy phenanthrene (99) and (100) and that, as above, none of the more crowded 4,5-dimethoxyphenanthrene (101) results [89].

If the desired 4-substituted phenanthrenes are to be synthesised via the β-ketonitrile route then these results dictate that a non-oxidative photocyclisation must be utilised: ie.3-substituted 2,6-dimethoxy-β-ketonitriles are required. However, because such β-ketonitriles are no longer symmetrical then again two possible products can arise upon photolysis of the enol acetate (102a and 102b): the desired 4-substituted phenanthrene (103) and the undesired 2-substituted phenanthrene (104). The product ratio would be determined by the relative rates of trapping of the respective dihydrointermediates (105) and (106) (assuming equal rates of formation of the two intermediates). But it would perhaps be predictable that the sterically crowded intermediate (105) would be more labile towards thermal ring opening than intermediate (106) and so the overall rate of formation of the 2-substituted phenanthrene (104) would be faster. That is phenanthrene (104) would be predicted to predominate over (103) as the major product of this reaction (Figure 15).

It was hoped that this anticipated difficulty could be overcome by the introduction of a substituent, Y, into the final unsubstituted position of ring A. This would give rise to a pair of rotomeric Z-enol acetates (107a) and (107b). The intermediate dihydrophenanthrenes (108) and (109) formed on irradiation of these

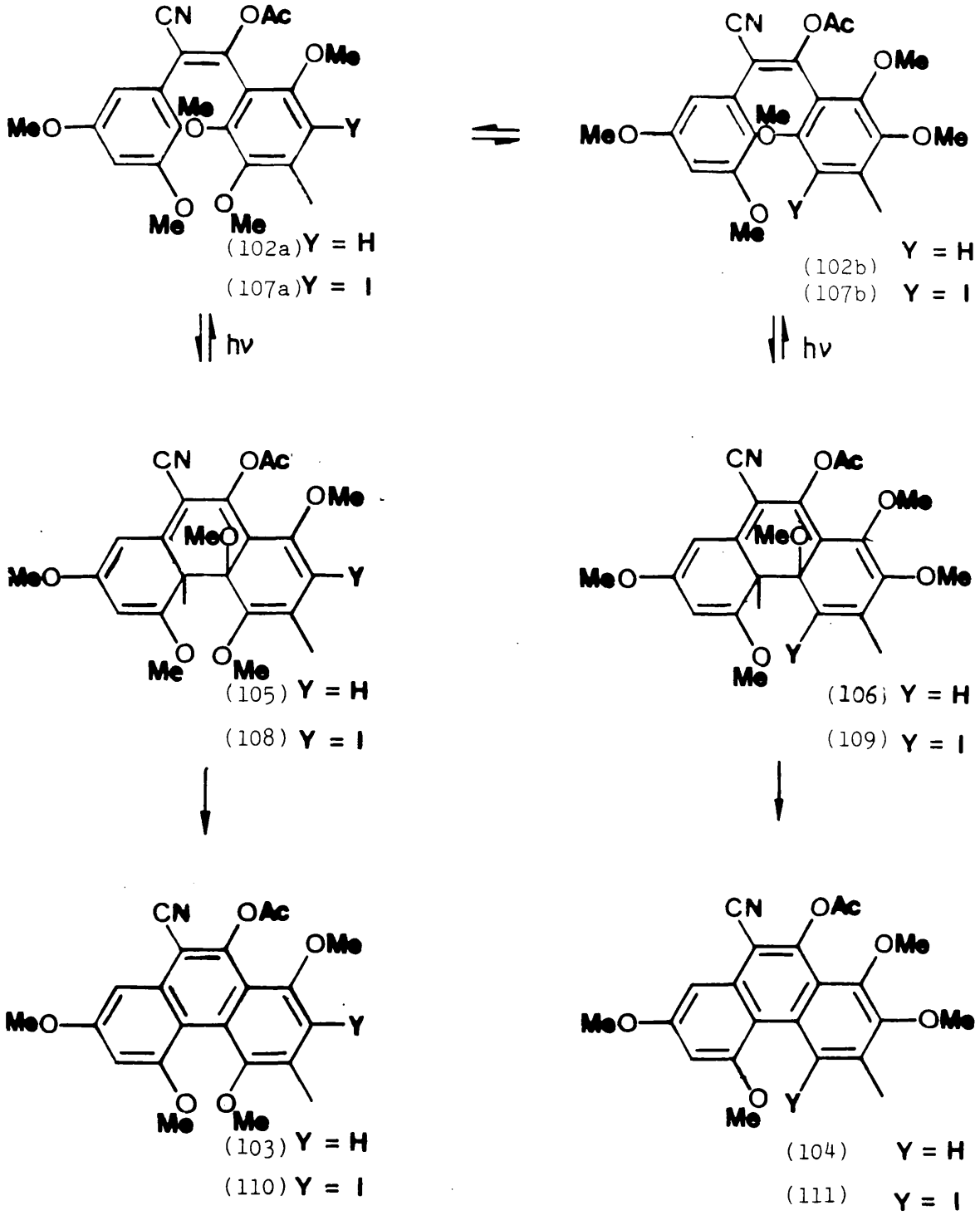
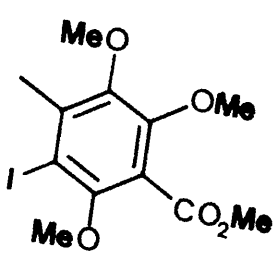
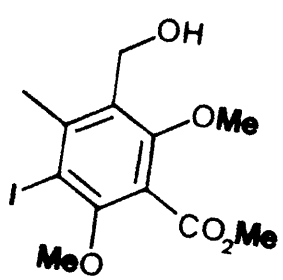

CN
OAc
OMe
Me
MeO
Y
O
Me
(102a) Y = H
(107a) Y = I
hν
(102b) Y = H
(107b) Y = I
(105) Y = H
(108) Y = I
(106) Y = H
(109) Y = I
(103) Y = H
(110) Y = I
(104) Y = H
(111) Y = I

Figure 15.



(112)

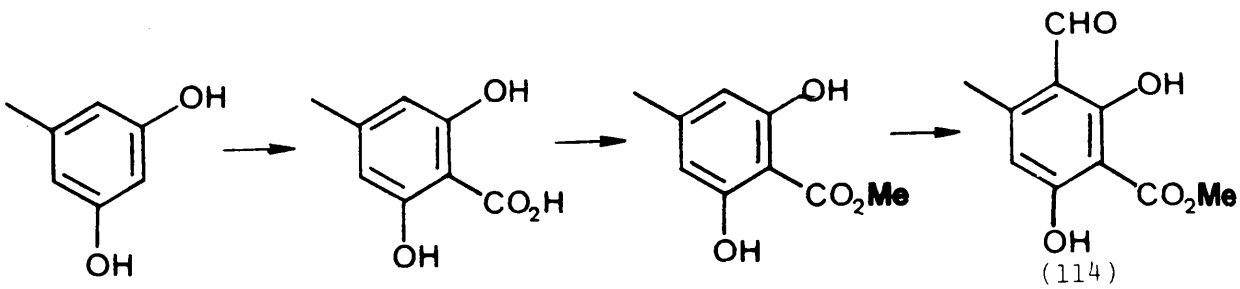


(113)

would then both be sterically hindered. It was hoped that the relative rates of thermal ring opening of these intermediates would be such that the ratio of desired: undesired photocylisation product (110):(111) would be improved.

For the purposes of the synthesis, such an additional substituent, Y, must be easily introduced at some stage in the synthesis, be inert to subsequent synthetic steps, be physically large enough to influence the photocyclisation, and be easily removed after cyclisation. It was decided that an iodide substituent would be suitable and that it should be introduced into the benzoate ester moeity prior to condensation.

Thus the iodobenzoate ester required to ultimately produce a 4-hydroxyphenanthrene would be methyl 5-iodo-2,3,6-trimethoxy-4-methylbenzoate (112). Similarly, the ester required to produce a 4-carboxyphenanthrene by this route is methyl 3-hydroxymethyl-5-iodo-2,6-dimethoxy-4-methylbenzoate (113), the alcohol functionality of which would be protected during the condensation step. The hydroxymethyl group of (113) is being used as a pro-carboxylic acid: a carboxylic acid cannot be used directly in this position as it would interfere with the phenylacetonitrile condensation; nor could an acid be protected as its ester as this would be susceptible to attack by the nitrile anion. A hydroxymethyl group, suitably protected, should be stable under the employed reaction conditions and be readily oxidised to the carboxylic acid at a later stage.



(114)

Scheme 11.

(I) p-tol-SO2-N(Na)Cl (115) + ArOH → p-tol-SO2-N(H)Cl + ArONa

(II) p-tol-SO2-N(H)Cl + NaI ⇌ p-tol-SO2-N(H)Na + ICl

(III) p-tol-SO2-N(Na)Cl + ICl ⇌ p-tol-SO2-N(I)Cl + NaCl

(117)

The two hexasubstituted esters (112) and (113) have not previously been reported in the literature and so a route to both was developed. Because of the obvious similarities of these molecules (each has five identical substituents) it seemed logical to derive them both from the same starting material. Methyl 3-formyl-2,6-dihydroxy-4-methylbenzoate (114) is readily available *via* the route of Barton *et al.*[90] (Scheme 11). It was reasoned that the aldehyde functionality of (114) could be reduced to provide the desired hydroxymethyl group or oxidised to provide, on methylation of the resultant phenol, the desired methoxy substituent. It was further proposed that the iodide group could be introduced at any suitable stage in the synthesis.

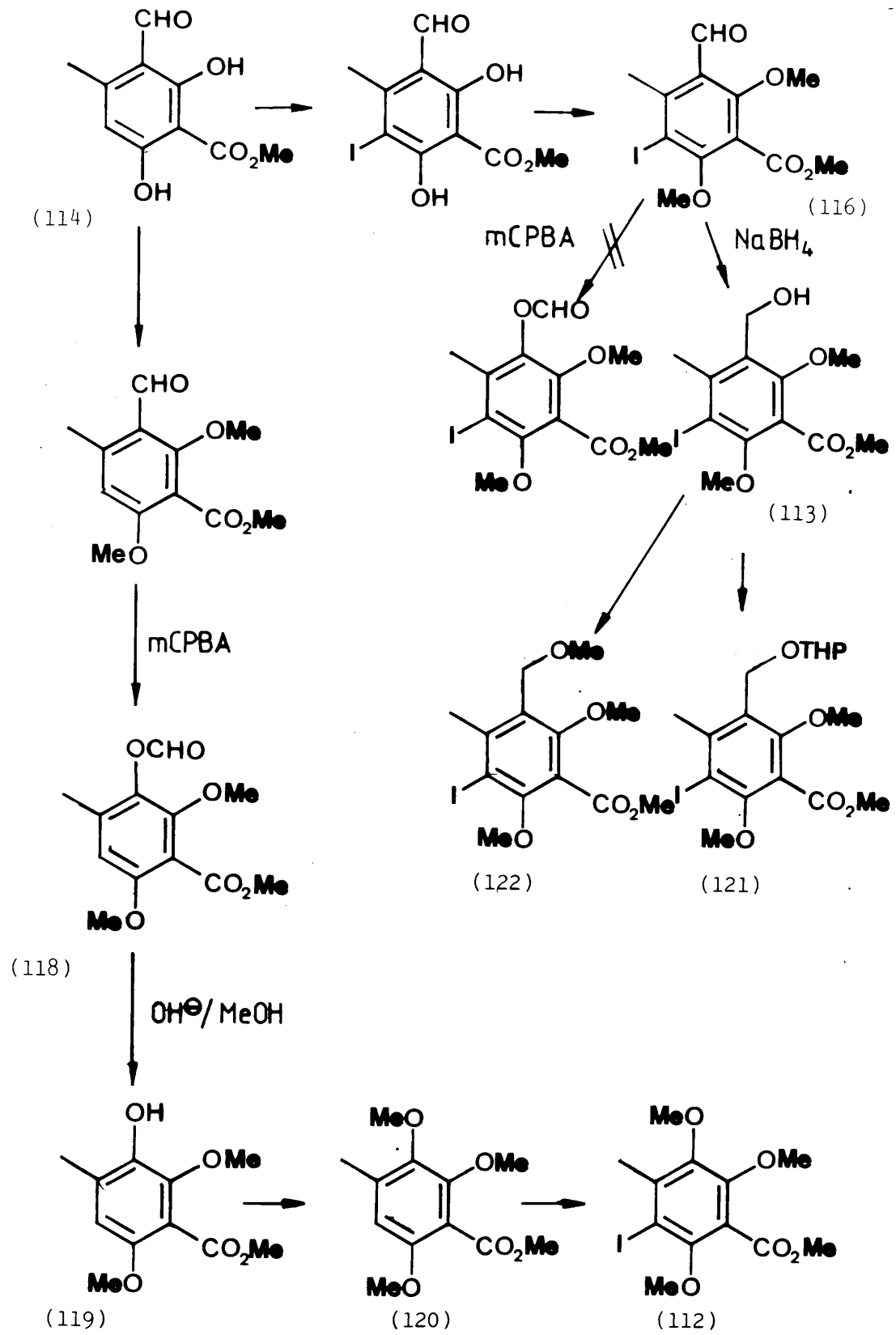
About this time Kometani *et al.*[91] reported a new method for the iodination of phenols using sodium iodide and chloramine T(115) in DMF. The dihydroxy ester (114) iodinated smoothly under these conditions in high yield and the product was methylated to give the iodo-dimethoxy ester (116). The aromatic ring of (116) was demonstrated to be hexasubstituted by nmr spectroscopy which showed the absence of any aromatic protons.

Kometani[91] does not comment upon the mechanism of this iodination reaction but it seems likely that reaction proceeds in an analogous fashion to chlorination of phenols with chloramine T: thus the actual iodinating agent is probably *N*-chloro-*N*-iodo -*p*-toluenesulphonamide (117) which could be generated from chloramine T and iodide ions (equations (I)(II) and (III)).

It was hoped that the aromatic aldehyde (116) would be susceptible to a Baeyer-Villiger type oxidation. Godfrey et al.[92] has shown that benzaldehydes undergo reaction with m-chloroperbenzoic acid (mCPBA) to furnish formate esters which can then be hydrolysed under mild conditions to give phenols. Godfrey[92] found that for more substituted (ie. more hindered) benzaldehydes generally longer reaction times are required for complete reaction. However, it was found that the hexasubstituted iodo-benzaldehyde (116) was completely resistant to oxidation, even on prolonged reaction times.

This problem could be overcome by reversing the order of the iodination step such that the oxidation substrate is a less hindered pentasubstituted benzaldehyde. Thus the formyl-dihydroxy ester (114) was methylated and then treated with mCPBA to furnish the corresponding formate ester (118) in almost quantitative yield. The formate ester functionality of (118) was found to be stable under anhydrous conditions but could be selectively hydrolysed by treatment with methanolic sodium hydroxide at room temperature. The resulting phenol (119) was methylated to give the trimethoxy ester (120). Iodination of this ester was achieved by reaction with elemental iodine with silver trifluoracetate as oxidant. The desired iodo-trimethoxy ester (112) was obtained in excellent overall yield (Scheme 12).

The desired hydroxymethyl compound (113) was prepared by reduction of the iodo-aldehyde (116) with sodium borohydride which left all other functionalities intact. It was decided that the alcohol functionality of (113) should be protected as its


CHO
OH
CO2Me
OH
(114)
CHO
OH
I
CO2Me
OH
CHO
OMe
I
CO2Me
MeO
(116)
mCPBA
NaBH4
OCHO
OMe
I
CO2Me
MeO
OH
OMe
I
CO2Me
MeO
(113)
CHO
OMe
CO2Me
MeO
mCPBA
OCHO
OMe
CO2Me
MeO
(118)
OMe
OMe
I
CO2Me
MeO
(122)
OTHP
OMe
I
CO2Me
MeO
(121)
OH⊖/MeOH
OH
OMe
CO2Me
MeO
(119)
MeO
OMe
CO2Me
MeO
(120)
MeO
OMe
I
CO2Me
MeO
(112)

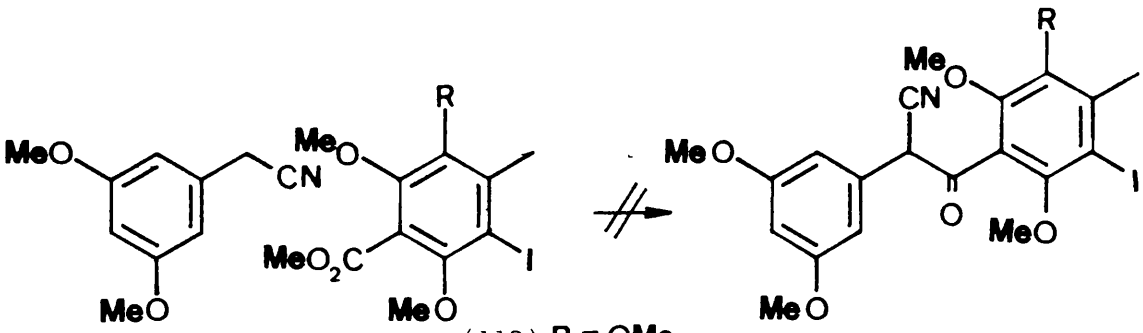
Scheme 12

tetrahydropyranyl (THP) ether: THP ethers are stable under neutral or basic conditions and to alkyl lithium reagents; furthermore they are easily made but can be cleaved under mildly acid conditions and so fulfil the criteria required of the chosen protecting group[93].

THP ether formation proceeded cleanly and gave the ether (121) quantitatively. The synthetic routes to the desired hexasubstituted esters are summarised in Scheme 12.

The nmr spectrum of the THP ether (121) exhibited an AB quartet (δ4.70 ppm) due to the benzylic protons as the large bulk of the THP moeity hinders free rotation about the benzylic C-O bond. The benzylic protons therefore cease to be magnetically equivalent and so appear as an AB quartet. In comparison, free rotation can occur about this bond in the parent alcohol (113) and the corresponding benzylic nmr signal is a sharp singlet (δ 4.66 ppm).

Attempts to condense the THP ether (121) with the anion of the phenylacetonitrile (55) were a failure: only starting material was recovered and no β-ketonitrile could be detected. The failure of this reaction was assumed to be due to steric hindrance of the ester functionality of the ether (121). Polysubstituted benzoate esters are well known to be hindered to nucleophilic attack because of the additive neighbouring group effects which result in the carbonyl of the ester being forced out of the plane of the aromatic ring. However, in the light of this result, it was hoped that by replacing the bulky THP ether by the physically smaller methyl ether protecting



(112) R = OMe

(121) R = CH_2OTHP

(122) R = CH_2OMe

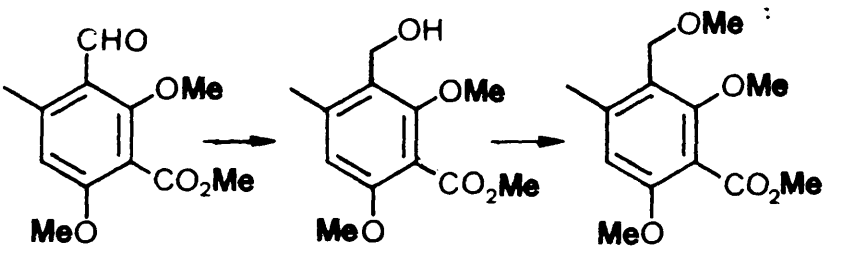
Scheme 13

group that steric hindrance would be reduced sufficiently so as to allow condensation (Scheme 13).

Thus the methylbenzyl ether (122) was formed from the hydroxymethyl ester (113). The benzylic protons of this ether (122) appear in its nmr spectrum as a sharp singlet (δ4.59 ppm) in contrast to the observed AB quartet of the corresponding resonance of THP ether (121). This implies at least a slight decrease in steric crowding about this molecule. Unfortunately, the ester functionality was found still to be too hindered for condensation with the phenylacetonitrile (55) to occur; as before only starting material could be recovered (Scheme 13).

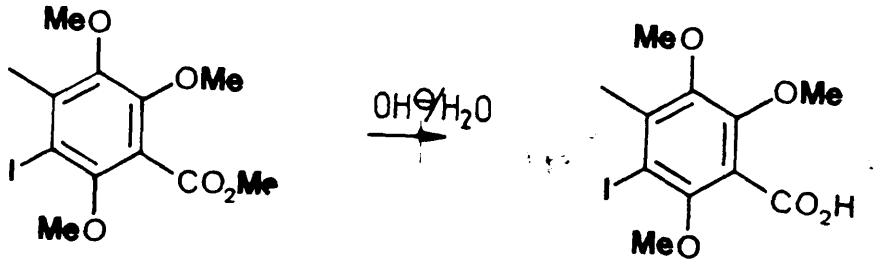
A similar result was obtained upon reaction of the trimethoxy ester (112) where, again, only starting materials could be recovered from the reaction mixture (Scheme 13).

It will be remembered that the reasoning behind the synthesis of these hexasubstituted esters was that the iodide substituent should influence the photocyclisation of the subsequent enol acetate to provide a more acceptable product ratio. Since these iodo-esters have been found not to condense with the phenylacetonitrile then the next logical step is to attempt reaction of the des-iodo esters (120) and (123). If these are found to condense with the nitrile (55) then the experimental ratio of 4-substituted: 2-substituted phenanthrenes resulting from subsequent photolysis could be determined.



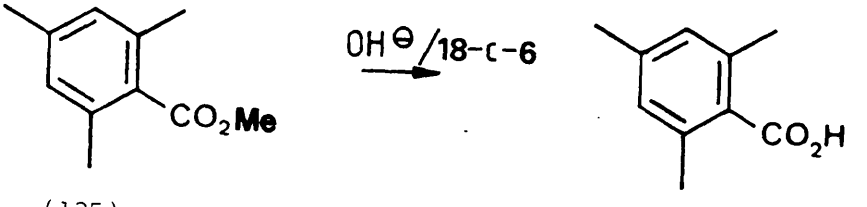
(123)

Scheme 14.



(112)

(124)



(125)

The preparation of the des-iodo trimethoxy ester (120) has been described above. The methoxymethyl ether (123) was prepared by omitting the iodination step from the route to the iodo ester (122) (Scheme 14).

To our dismay it was found that both esters (120) and (123) were still sufficiently crowded to prevent condensation with the phenylacetonitrile and that omission of the iodide did not alter the course of this reaction. It would appear that for condensation reactions of this series of ortho-substituted esters that both the degree of further substitution and the size of the attacking nucleophile are crucial in determining whether or not reaction occurs. It will be recalled that whereas the 2-methoxy ester (47) reacts smoothly with both a phenylacetonitrile and an ethyl phenylacetate anion, the corresponding 2,6-dimethoxy ester (64) is now hindered such that reaction with the large ester nucleophile is prevented. On further increasing the substitution to the pentasubstituted (120) and (123) or the hexasubstitued esters (112), (121) and (122) than the steric crowding about the ester functionality is increased such that even the nitrile anion is excluded. Notably however, reacton may still occur with small nucleophiles: the iodo-trimethoxy ester (112) is easily hydrolysed to the corresponding acid (124), and so these esters are less hindered than the tetrasubstituted methyl 2,4,6-trimethylbenzoate (125) for which crown ether is required for saponification[94].

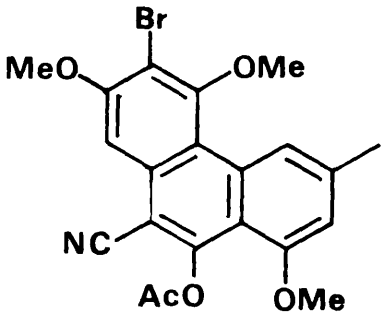
These results mean that the desired 4-substituted phenanthrenes are not available via synthesis of β-ketonitriles with

the appropriate substitution pattern. However, it may be possible to obtain the desired phenanthrenes by modification of the existing cyanophenanthrene (60) which, on decyanation, may give the required substitution pattern.

3.2.2. Introduction of a 4-Substituent

It is unlikely that a substituent could be directly introduced exclusively in the 4-position of phenanthrene (60). It would be predicted that the more electronically active position to electrophilic substitution would be position-6 or -8, with position-2 then being more active than position-4 for steric reasons. It was reasoned that if halogen substituents could be introduced into position-4 and all more active positions then, as the more active positions to halogenation should also be the more active towards lithium exchange of the halogen, the halogens could be removed sequentially to provide a 4-halo-phenanthrene. This, it was further reasoned, could then be converted to the desired 4-substituted compounds by standard techniques. The bromination reactions of the phenanthrene (60) were therefore investigated and were found to be surprisingly complex.

It should be noted at this point that the products of these reactions were identified by high field nmr spectroscopy and that the structure determination will be described in the next section. It should also be noted that as would be expected from the structural similarities of the bromo derivatives the products of these reactions were very difficult to separate chromatographically. Therefore once



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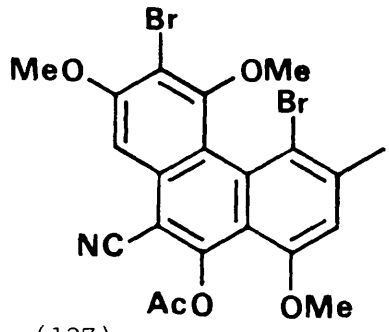

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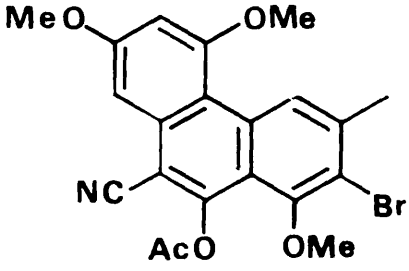

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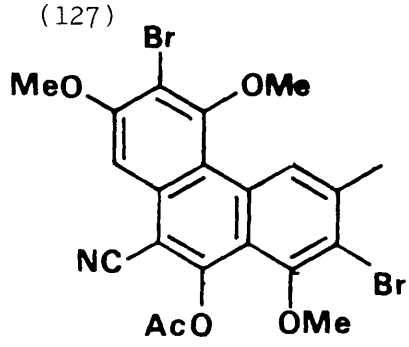


(129)

all products had been fully characterised and their nmr spectra assigned then product ratios were determined on the basis of the nmr spectra of crude product mixtures.

The phenanthrene (60) was thus reacted with 1.1 molar equivalents of bromine in acetic acid at room temperature but was found to be resistant to halogenation under these conditions. However, heating this reaction mixture to 70° gave a complex mixture of products. Resolution of this mixture by tlc proved extremely difficult but the major products could be obtained by multiple development techniques. These were identified by high field nmr as the 6-bromo derivative (126) and the 4,6-dibromo derivative (127) and were produced in 40% and 20% yields respectively. In addition to a small trace of starting material, also recovered were two as yet unidentified bromo derivatives accounting for 15% and 5% of the products. On repeating this reaction with 2.0 molar equivalents of bromine it was found that one of the primary products again was the 6-bromide (126) (35%), but the other main product (35%) was the major unidentified bromide from the previous reaction. This was purified and identified as the 2-bromo derivative (128). A trace of the 4,6-dibromide (127) was also observed and the previously unidentified trace product was found in 25% yield. This was identified as the 2,6-dibromo derivative (129).

Furthermore, when reaction was repeated at 40° with 1.1 equivalent of bromine the observed products were the 2-bromide (128) (53%), the 6-bromide (126) (37%) and the 4,6-dibromide (127) (10%). At 120° (reflux), reaction with 1.1 equivalent of bromine produced

the 2,6-dibromide (129) as the major product (c.90% on correction for recovered starting material) with only trace amounts of the other halo-compounds being seen. Similarly with two equivalents of bromine at reflux, the dibromide (129) was the major product (90%) with only trace amounts of the other bromides. On reaction with excess bromine no further bromination occurred even at reflux and a similar product distribution was observed as for reaction with two equivalents. It should be recorded that these results were repeatable (see Table 9).

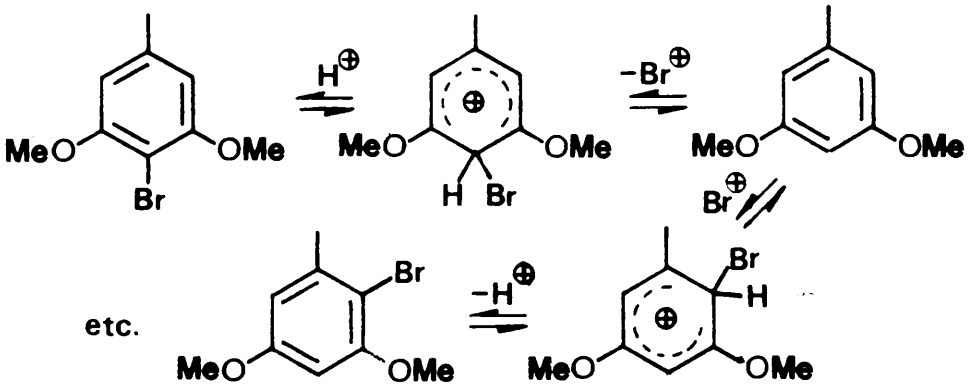
T(°C)	eq.Br_2	Products(%) 2-Br (128)	6-Br(126)	2,6-Br (129)	4,6-Br(127)
40	1.1	53	37	0	10
70	1.1	15	40	5	20
70	2.0	35	35	25	5
120	1.1	t	t	90	t
120	2.0	t	t	90	t
120	2.0	t	t	90	t

t=trace only

Table 9. Bromination of phenanthrene(60)

The bromination reactions of the phenanthrene (60) are seen to be surprisingly temperature dependant and intriguingly complex. At low temperature (40°) the 2-position of the phenanthrene (60) is seen to be more active towards electrophilic substitution than the 6-position such that the ratio of (128):(126) is ca.3:2. However, on moderately increasing the temperature (to 70°) this situation is apparently reversed such that 6-bromination predominates over 2-bromination. Furthermore, on increasing the temperature further (to 120°) it seems that the reactivity of the first formed monobromide is such that the rate of dibromination is faster than that of bromination of starting material (60), a phenomenon that occurred only to a small extent at lower temperatures. It is assumed that the small amounts of 4,6-dibromide (127) arise via 4-bromination of the 6-bromide (126) rather than vice versa as no 4-bromophenanthrene could be detected.

These results are not easily explained. Unlike aromatic iodination which is known to be reversible, bromination in common with most other electrophilic substitutions is generally assumed to be irreversible under normal conditions and the products formed are kinetically controlled. Rearrangements and disproportionations of aromatic bromides are known but generally only under the influence of Friedel-Craft catalysts. For example treatment of o-bromotoluene with aluminium chloride results in a mixture of toluene and o-, m-, and p- bromotoluenes[96]. However, O'Bara et al.[96] have shown that certain p-bromophenols, produced by bromination of the parent phenol, isomerise and disproportionate under the influence of the hydrogen bromide produced in the course of the bromination.



Scheme 15. (After Cannon [97])

Also Cannon et al.[97] have described the disproportionation of bromodimethoxytoluenes under similar circumstances to give isomeric bromides. These observations are explained on the following mechanistic grounds[96,97]. The rate determining step during bromination is thought to be reaction of the arene with bromine to form an intermediate σ-complex. Therefore the rate determining step during debromination must be the cleavage of the carbon-bromine bond and formation of molecular bromine. At equilibrium during reversible bromination the concentration of each product is dependant on the rate of formation and the rate of debromination of that product: the most rapidly formed bromide, A, (the kinetic product) will also be debrominated most rapidly; the more slowly formed bromide, B, (the thermodynamic product) will have a slower rate of debromination. Therefore the ratio of the products A:B will fall with time until a thermodynamic equilibrium is reached (Scheme 15).

Although not widely reported, it seems probable that other active aromatic systems may also be subject to this process. This would largely explain the observed behaviour of phenanthrene (60). On the assumption that temperature determines the absolute rate of the bromination/debromination reactions but does not alter the relative rates of competing brominations and debrominations then at low temperature the kinetic product will predominate. The concentration of other components will be determined by their relative rates of formation. The 2-bromide (128) is the kinetic product and is formed more rapidly than the competing 6-bromide. However, on increasing the temperature (equivalent to a longer reaction time), because the kinetic product (128) is also less stable

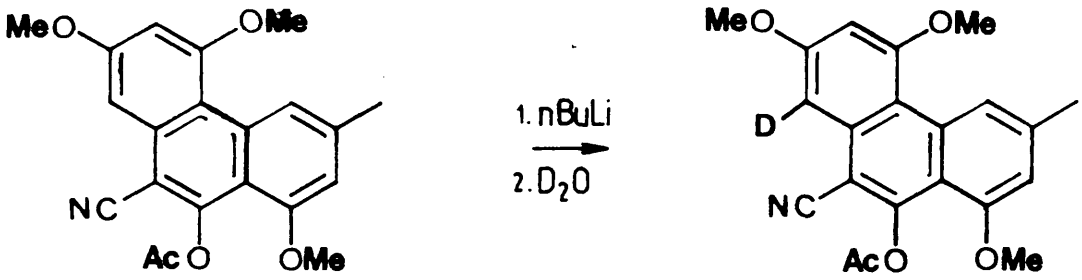
Starting mixture (%)		Product mixture (%)	
(128) 2-Bromo	44	(60) desbromo	17
(126) 6-Bromo	56	(128) 2-Bromo	17
		(126) 6-Bromo	17
		(129) 2,6-Dibromo	49

Table 10. Disproportionation of bromophenanthrenes.

to debromination than the 6-bromide (126), the ratio of (128):(126) falls such that at 70° (126) predominates. It will be remembered that a small amount of the 2,6-dibromide (129) is produced at this temperature. On further increasing the temperature it appears that this 2,6-dibromide (129) is the true thermodynamic product and is formed by debromination-rebromination of the more rapidly formed bromides (126), (127) and (128).

If this theory is correct then it would be predicted that a mixture of the 2-bromide (128) and the 6-bromide (126) on heating to 120° in HBr/acetic acid would result in disproportionation to give the 2,6-dibromide (129), the des-bromo-phenanthrene (60) and traces of (126), (127) and (128). Thus heating a 44:56 mixture of (128) and (126) under these conditions for 1 hour gave the 2,6-dibromide (129) (35%) as the major product with approximately equal amounts of the mono-bromides (126) and (128) and the phenanthrene (60). The excess of dibromide (129) over phenanthrene (60) can be accounted for by further bromination by elemental bromine present in the HBr solution. That the des-bromophenathrene (60) is at all present proves that disproportionation has occurred. It is notable that no disproportionation occurred on heating the above mixture in acetic acid alone, implying that reaction is a function of the HBr present. These results are therefore in excellent accordance with the theory (Table 10).

It is not altogether clear why the 2,6-dibromide (129) is the thermodynamically most stable product. However, remembering that the rate determing step in bromination is formation of the



(60)

intermediate o-complex, then the stability of this complex will be increased by the presence of an electron withdrawing bromide substituent which can further delocalise the positive charge (although the rate of formation will decrease). Therefore provided that the reaction is reversible and that the rate of bromination of the monobromide is greater than the rate of debromination of the dibromide, then a long reaction time or high temperature will favour dibromination. The 2,6-dibromide (129) is presumably favoured over other possible dibromides for steric and electronic reasons.

An interesting aspect of these reactions is that no 8-bromination was seen. It would be predicted that the 8-position would be sterically less hindered than the 6-position. Treatment of the phenanthrene (60) with n-butyl lithium followed by quenching with D_2O results in deuterium exchange only at the 8-position. These results appear to be anomalous and are currently unexplained.

It will be recalled that the rationale behind bromination was to introduce a 4-substituent into the phenanthrene (60). Unfortunately, 4-brominated phenanthrenes were not obtained in synthetically useful amounts and so this route was abandoned. In view of these results it is not easy to see how 4-substituted phenanthrenes may be made by extension of the chemistry described in this thesis. It may be that a new synthetic strategy will be required.

3.2.3. N.M.R. Analysis of Bromophenanthrenes

The bromination reactions described in the previous section gave rise to a series of four brominated products which, on the basis of mass spectrometry and elemental analysis were identified as two mono-brominated and two dibrominated phenanthrenes. The substitution patterns of these products could not be unambigiously determined by low field nmr spectroscopy but by examining the high field (200 MHz) spectra the bromides could be identified.

Before assigning the nmr spectra of the bromo-derivatives it is first necessary to fully assign that of the parent phenanthrene (60). 90 MHz nmr shows H-2 and H-4 as singlets (δ 6.79 and 8.99 ppm respectively). These signals are broadened due to meta coupling unresolved at this low field strength. H-6 and H-8 appear as meta coupled doublets (J 2.5 Hz) at δ 6.70 and 7.12 ppm but these resonances cannot be unambiguously assigned. However, at 200 MHz these resonances appear as a doublet of quartets (J 2.5 and 0.4 Hz) and a broad doublet (J 2.5 Hz) respectively. Moreover the previously unresolved meta coupling between protons-2 and -4 can be seen with H-4 appearing as a doublet of quartets (J 1.4 and 0.8 Hz) due to further coupling with the 3-methyl substituent (which is seen as a doublet of doublets, J 0.81 and 0.45 Hz). H-2 is now seen as a complex multiplet. Furthermore the methoxyl resonances are now seen as two doublets (J 0.22 and 0.32 Hz) and a broadened singlet. Schaeffer[98] has shown that the $^{5}J_{o}$ coupling between aromatic methoxyl protons and adjacent ortho protons can be observed at high

magnetic field and that the value of $^{5}J_{o}$ (H, CH_3) is dependant on the spatial orientation of the methoxyl group.

The observed coupling constants suggest that the broad doublet at δ7.12 is coupled to the broadened methoxyl signal and that the doublet of quartets at δ6.70 is coupled to the methoxyl doublet with J 0.32 Hz. The other methoxyl doublet (with J 0.22 Hz) is therefore coupled with H-2. Because OMe-5 is situated in the bay region of the phenanthrene it would be expected to adopt a cis orientation towards H-6 in order to relieve steric crowding. According to Schaeffer[98] this situation would result in a $^{5}J_{o}$ (H, CH_3) coupling of ca.0.28 Hz, and so the observed resonance at δ6.70 is assigned to H-6. The resonance at δ7.13 is therefore assigned to H-8. OMe-7 is unhindered and so free rotation can occur; this accounts for the reduced coupling between it and H-8. These assignments are detailed in table 11.

Returning to the bromination products, consider firstly the monobromide (126). The 90 MHz nmr spectrum of (126) is essentially similar to that of phenanthrene (60), differing only in the resonances of H-6 and H-8 such that a 1H singlet is seen (δ 7.38). This implies that substitution has occurred at either the 6- or 8-position. At 200 MHz, this 1H singlet is seen to be in fact a quartet (J 0.2 Hz). Also the methoxyl resonances are seen to be two doublets (J 0.22 and 0.26 Hz) and a sharp singlet. H-2 as before is seen as a multiplet. If 8-substitution had occurred then it would be expected that rotation of OMe-7 would be partially restricted by the adjacent bromide and that this would manifest itself as an increased

	– (60)	2-BROMO (128)	6-BROMO (126)	4,6-DIBROMO (127)	2,6-DIBROMO (129)
δ H-2 δ(ppm) multiplicity J(Hz)	6·786 m	–	6·893 dq 1·4 &0·4	6·912 m	–
H-4	8·976 dq 1·4&0·8	9·300 q 0·8	9·035 dq 1·4 &0·4	–	9·314 q 0·8
H-6	6·696 dq 2·5 &0·4	6·757 dq 2·4 &0·35	–	–	–
H-8	7·127 br d 2·5	7·186 dq 2·4 0·2	7·379 q 0·3	7·250 (obscured)	7·377 q 04
OMe-1	3·913 d 0·22	3·903 s	3·968 d 0·26	3·972 d 0·24	3·926 s
OMe-5	4·012 d 0·32	4·040 d 0·34	3·806 s	3·276 s	3·812 s
OMe-7	3·948 br s	3·971 d	4·082 d 0·23	4·091 d 0·28	4·080 d 0·27
OMe-3	2·529 dd 0·81 &0·45	2·616 d 0·12	2·573 dd 0·8 &0·48	2·636 d 0·48	2·642 d 0·85
Ac-10	2·459 s	2·515 s	2·481 s	2·459 s	2·519 s

Table 11.

coupling between OMe-7 and H-8. Thus one would expect to see three methoxyl doublets. That two doublets and a sharp singlet are observed is indicative that 6-substitution has occurred and that compound (126) is therefore the 6-bromide. The methoxyl resonances of monobromide (126) can be fully assigned on the basis of the observed coupling constants as before (see table 11).

The structure of the dibromoderivative (127) can also be determined by these means. A resonance in the nmr spectrum of a phenanthrene with $\delta 9.0 \pm 0.5$ ppm is indicative of an unsubstituted 4-position. The lack of any resonance in the nmr spectrum of the dibromide (127) with $\delta > 7.26$ shows that 4-substitution has occurred. That 6-bromination has also taken place was shown in a similar way for the monobromide (126). The methoxyl resonances appear as two doublets (J 0.28 and 0.24 Hz) and a sharp singlet and so 6-bromination must also have occurred. Interestingly the signal due to OMe-5 of the bromide (127) is shifted downfield to $\delta 3.28$; because OMe-5 is sandwiched between two bromine atoms it is forced out of the plane of the aromatic system and into the shielding zone in order to reduce steric crowding.

The 90 MHz nmr spectrum of the monobromide (128) suggested that 2-bromination had taken place. A 4-proton was observed ($\delta 9.30$) shifted slightly upfield because of the meta bromo substituent and the resonances due to protons-6 and -8 were essentially identical to those of phenanthrene (60). The structure was confirmed as the 2-bromide (128) by high field nmr which showed the C-3 methyl substituent as a clean doublet, coupled only with H-4 (J 0.8 Hz).

Curiously whereas the coupling between H-8 and OMe-7 in phenanthrene (60) is too small to be measured (producing only signal broadening), in the 2-bromide (128) this coupling is between 0.1 and 0.2 Hz. It is unclear why these two systems should differ in this respect.

Finally, the structure of the dibromide (129) was deduced to be the 2,6-dibromide. The two observed aromatic resonances are sharp at 90 MHz implying that both meta coupled systems have been disrupted. A H-4 signal is seen (δ 9.31) demonstrating that 2-substitution has taken place. As before, the 200 MHz spectrum confirms this by virtue of the observed 3-methyl doublet (J 0.85 Hz). The methoxyl resonances, two singlets and one doublet (J 0.27 Hz) demonstrate that 6-bromination has also occurred. The methoxyl resonance seen as a doublet is therefore assigned to OMe-7. The methoxyl singlets were assigned on the basis of their chemical shifts: the OMe-5 resonance of the 6-bromide (126) occurs at δ 3.81 and the OMe-1 resonance of the 2-bromide (128) is seen at δ3.90. Therefore the observed resonance of the 2,6-dibromide (129) at δ3.81 is assigned to OMe-5 and that at δ3.93 to OMe-1.

The full assignment of the spectra of these bromo-compounds and subsequent structure elucidation illustrates the utility of Schaeffer's technique[98].

CHAPTER 4

EXPERIMENTAL

General Procedures

Melting-points were determined on a Kofler hot-stage apparatus and are uncorrected. Ultra-violet spectra were measured on a Pye Unicam SP8-100 spectrometer. Infra-red spectra were recorded for potassium bromide discs (unless otherwise stated) on a Perkin-Elmer S80 spectrometer. The following abbreviations are used: s-strong, m-medium, w-weak and br-broad

Proton nuclear magnetic resonance spectra were determined on a Perkin-Elmer RB 32 spectrometer using deuteriochloroform as solvent (unless otherwise stated) and using tetramethylsilane (TMS) as internal standard. The following abbreviations are used: s-singlet, d-doublet, t-triplet, q-quartet, m-multiplet, br-broad. Carbon nuclear magnetic resonance spectra were determined on a Bruker WP200 SY spectrometer in the pulsed F.T. mode using deuteriochloroform as solvent and TMS as internal standard.

Mass spectra were obtained with a MS 12 or MS 902 mass spectrometer.

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

Preparative layer chromatography was carried out using Merck Kieselgel GF_{254}. Analytical thin layer chromatography was carried out on commercially prepared plates with a 0.25 mm layer of the same silica gel.

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

Culture and Harvesting of Mollisia caesia

M.caesia (CBS 220.56) was obtained from Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. Large scale cultures of M.caesia were grown as surface cultures in Roux bottles on a Blakeslee malt extract agar prepared from glucose (20 g), Oxoid malt extract (20 g), Bacto peptone (1 g), and sodium chloride (0.5 g) in di-ionised water (1 l) containing 2% agar. The medium was innoculated by smearing the surface with a small piece of mycelium and allowed to grow at 20-24°C for up to 24 days.

Isolation of Mollisin (19)

The contents of the Roux bottles were mixed with water and blended. The resulting mixture was constantly extracted with ethyl acetate for 48 hours. The solution was evaporated to leave a red-brown gum. The extract was first passed through a short column of silica (2.5 x 5 cm) under suction using ethyl acetate as eluent to remove highly polar resinous material and then subjected to PLC using

ether:hexane (2:3) as eluent. Mollison (R_f 0.5) was isolated as orange needles (typical yield: 40 mg/l of medium). Mollisin was recrystallised from methanol as felty needles, m.p. 203-204° (lit[41]., 203-204°) (found: C, 53.73; H, 3.30; Cl, 22.5. Calc. for $C_{14}H_{10}Cl_2O_4$, C, 53.71; H, 3.23; Cl, 22.64%); ν_{max} 1 720 s 1 649 s, and 1 618 s cm^{-1}; δ_H 7.20 (1H, s, H-6), 6.83 (1H, q, J 1.5 Hz, H-3), 6.33 (1H, s, H-14), 2.40 (3H, s, Me-12), and 2.15 (3H, d, J 1.5 Hz, Me-12); δ_C 191.90 (C-13), 189.12 (C-3), 130.08 (C-8, C-9), 126.07 (C-6), 112.82 (C-10), 70.91 (C-14), 20.55 (C-11), and 16.41 (C-12); m/e 229 ($M-CHCl_2$).

Isolation of 2,7-Dimethylnapthazarin (21)

From the foregoing extract could be isolated by PLC, using ether:hexane (2:3) as eluent, the napthazarin as a red oil (R_f 0.6). This was crystallised from petroleum ether (60-80°) as long black needles, m.p. 134-139° (lit[41]., 125-6°); ν_{max} 1 740 s and 1 655 s cm^{-1}, δ_H 6.95 (2H, s, ArH), and 2.25 (6H, s, $ArCH_3$); m/e 218 (M).

Mollisin Acetate[46] (24)

Mollisin (0.643 g) was suspended in acetic anhydide (6.2 ml) and pyridine (4.9 ml) was added with stirring. When dissolution occurred the mixture was allowed to stand for 5 minutes and then poured onto 2M hydrochloric acid (90 ml). The precipitate was collected, dried over P_2O_5 and sublimed (150-200°, 0.1 mm) to give the acetate as pale yellow needles (0.566 g, 78%), m.p.

213-214.5° (lit[46]., 211-213°) (found: C, 54.21; H, 3.28; Cl, 19.89. Calc. for $C_{16}H_{12}Cl_2O_5$, C, 54.10; H, 3.41; Cl, 19.96%); ν_{max} 3 440 m br, 1 779 s, 1 719 s, and 1 652 cm^{-1}; δ_H 7.28 (1H, s, H-6), 6.70 (1H, q, J 1.5 Hz, H-3), 6.37 (1H, s, H-14), 2.46 (3H, s, Me-11), 2.40 (3H, s, Ac-16), and 2.10 (3H, d, J 1.5 Hz, Me-12); δ_C 191.79 (C-13), 186.23 (C-1) 182.46 (C-4), 169.05 (C-15), 150.37 (C-5), 146.15 (C-2), 144.70 (C-7), 137.50 (C-3), 135.26 (C-8), 132.27 (C-9), 131.97 (C-6), 121.51 (C-10), 70.74 (C-14), 21.03 (C-16), 20.35 (C-11), and 15.95 (C-12); m/e 271 ($M-CHCl_2$), 229 ($M-CHCl_2O$).

Methyl 3,5-Dimethoxybenzoate

3,5-Dihydroxybenzoic acid (10 g) dissolved in dry acetone (500 ml) with anhydrous potassium carbonate (50 g) and dimethyl sulphate (20 ml) was heated at reflux for 7h with stirring. After cooling the solution was filtered and the residue washed with acetone (2 x 50 ml). The combined acetone solutions were evaporated and the golden brown residue dissolved in ether (150 ml), washed with concentrated ammonia solution (2 x 50 ml) and water (2 x 50 ml) and evaporated. The residue solidified on cooling and was recystallised from dry ether as colourless needles (10.2 g, 78%), m.p. 41-42° (lit[68]., 42°); ν_{max} ($CHCl_3$) 1 725 s and 1 595 s cm^{-1}; δ_H 7.18 (2H, d, J 3.5 Hz, ArH), 6.62 (1H, t, J 3.5 Hz, ArH), 3.88 (3H, s, CO_2Me), and 3.78 (6H, s, OMe); m/e 196 (M).

3,5-Dimethoxybenzoic acid (37)

Methyl 3,5-dimethoxybenzoate (4.4 g) was suspended in 10% sodium hydroxide solution (250 ml) and heated at reflux for 1h. After cooling, the mixture was acidified with concentrated hydrochloric acid. The precipitate was collected, washed with cold water and recrystallised from methanol as colourless needles (4.3 g, 93%), m.p. 186° (lit[99]., 185-186°); ν_{max} 2 920br s, 2 600 br s, 1 690 s, and 1 610 s cm^{-1}; δ_H ($(CD_3)_2CO$) 7.18 (2H, d, J 2 Hz, ArH), 6.69 (1H, t, J 2 Hz, ArH), and 3.82 (6H, s, OMe); m/e 182 (M).

3,5-Dimethoxyacetophenone (38)

Lithium metal (4.7 g) in the form of strips was added to anhydrous ether (400 ml) under a nitrogen atmosphere. To this was added drop-wise and with stirring methyl iodide (18.7 ml) in ether (50 ml) at such a rate that a gentle reflux ensued. Stirring was continued for 1 h after which time 3,5-dimethoxybenzoic acid (18.2 g) suspended in anhydrous ether (200 ml) was added over 10 minutes. The mixture was stirred under nitrogen for a further 30 minutes and then water (250 ml) was added to hydrolyse the lithium salt. The aqueous layer was separated and extracted with ether (2 x 75ml) and the combined organic solutions were washed with half of their volume of water, dried and evaporated to yield a pale oil which was crystallised from petroleum ether (40-60°) (14.0g, 78%), m.p. 41° (lit[100]; 42-43°); ν_{max} 1 690 cm^{-1}; δ_H 7.08 (2H, d, J 2 Hz, ArH), 6.62 (1H, t, J 2Hz, ArH), 3.82 (6H, s, OMe), and 2.53 (3H, s, CH_3CO); m/e 180 (M).

3,5-Dimethoxyphenylacetic acid (35)

3,5-Dimethoxyacetophenone (3.1 g) was brought slowly to the boil with sulphur (0.82 g) and morpholine (2.2 g) and heated at reflux for 14 hours. The crude thiomorpholide was hydrolysed with 10% potassium hydroxide solution (100 ml) by heating at reflux for 12 hours. After cooling, the mixture was acidified with concentrated hydrochloric acid and the precipitate was collected and washed with cold water. Recrystallisation from water gave colourless needles (2.3 g, 69%), m.p. 103-104° (lit[101]., 100.5-101°); ν_{max} (Nujol)3 000 br m and 1 610 s cm^{-1}; δ_H 6.38 (3H, m, ArH), 3.74 (6H, s, OMe), and 3.55 (2H, s, $ArCH_2$); m/e 196 (M).

2,5-Dihydroxytoluene (39)

Methyl-p-benzoquinone (1.01 g) was dissolved in methanol (25 ml) and glacial acetic acid (0.02 ml) and hydrogenated over 10% palladium on charcoal (0.10 g) at atmospheric pressure for 1/2 hour. The mixture was filtered and then the solvents evaporated to give a solid. Sublimation (100°, 0.2 mm) gave colourless needles (1.00 g, 98%), m.p. 126-128° (lit[102]., 126-127°); ν_{max} 3 310 s br and 1 505s cm^{-1}; δ_H ($(CD_3)_2CO$) 6.61 (3H, m, ArH) and 2.13 (3H, s, $ArCH_3$); m/e 124 (M).

2,5-Dimethoxytoluene (33)

2,5-Dihydroxytoluene (8.9 g) was dissolved in dry acetone (500 ml) containing anhydrous potassium carbonate (20 g) and dimethyl

sulphate (20 ml) and heated at reflux for 5 hours. On cooling, the mixture was filtered, the residue washed with acetone (150 ml) and the combined acetone solutions evaporated. The brown residue was taken up in ether (150 ml) and washed with concentrated ammonia solution (2 x 50 ml) and water (2 x 50 ml), dried and evaporated. The resulting gum distilled as a colourless viscous liquid (9.4 g, 85%), b.p.$_{2.0}$ 91° (lit[103]., 214-218°); ν_{max} ($CHCl_3$) 2950 m and 1 505 s cm^{-1}; δ_H 6.72 (3H, s, ArH), 3.80 (3H, s, OMe), 3.79 (3H, s, OMe), and 2.21 (3H, s, $ArCH_3$); m/e 152 (M).

3,5-Dimethoxyphenylacetyl Chloride (36)

3,5-Dimethoxyphenylacetic acid (2.05 g) was dissolved in carbon tetrachloride (25 ml) and phosphorus trichloride (0.55 ml) was added. The solution was heated at reflux for 16h, then cooled, filtered and evaporated to give 3,5-dimethoxyphenylacetyl chloride (2.19 g, 98%) which was used without further purification; δ_H 6.39 (3H, m, ArH), 4.03 (2H, s, CH_3), and 3.75 (6H, s, OMe).

3,5-Dihydroxyphenylacetic acid

To a solution of 3,5-dimethoxyphenylacetic acid (1.62 g) in chlorobenzene (30 ml) was added aluminium chloride (3.29 g) with mechanical stirring. The stirred mixture was heated at reflux for 5h, cooled and treated with ice-cold 2N hydrochloric acid. The mixture was extracted with ethyl acetate (3 x 50 ml) and this extract further extracted with saturated sodium carbonate solution (4 x 50 ml). The basic solution was acidified with concentrated hydrochloric

acid, extracted with ethyl acetate and this extract dried and evaporated to give 3,5-dihydroxyphenylacetic acid (1.40 g, 96%). A portion was recrystallised from ethyl acetate-benzene as needles, m.p. 126-8° (lit[101]., 128°); m/e 168 (M).

Attempted Desoxybenzoin Formation through Friedel-Crafts Acylation

(i) Catalysed by Aluminium Chloride:

(a) To a solution of 2,5-dimethoxytoluene (1.05 g) in carbon disulphide (10 ml) containing aluminium chloride (0.93 g) was added drop-wise a solution of 3,5-dimethoxyphenylacetyl chloride (1.05 g) in carbon disulphide (10 ml). The mixture was heated at reflux for 3h, cooled, and the solvent decanted. The residue was treated with ice-cold 3N hydrochloric acid (75 ml) and the resulting mixture extracted with ethyl acetate (2 x 75 ml) and ether (2 x 75 ml). The combined extracts were dried and evaporated to yield a dark brown gum (2.10 g) which was non-resolvable by chromatography on silica.

(b) The foregoing procedure was repeated using 2,5-dimethoxytoluene (1.41 g), aluminium chloride (1.80 g) and 3,5-dimethoxyphenylacetic acid (2.00 g) to give a dark brown intractable tar (2.36 g) which was non-resolvable by chromatography on silica.

(c) The foregoing procedure was repeated using 2,5-dihydroxytoluene (1.24 g), aluminium chloride (1.80 g) and

3,5-dimethoxyphenphenylacetyl chloride (2.15 g) yielding a dark brown gum which was non-resolvable by chromatography on silica.

(ii) Catalysed by Polyphosphoric Acid

(a) 3,5-Dimethoxyphenylacetyl chloride (2.15 g) and 2,5-dimethoxytoluene (1.52 g) were dissolved in polyphosphoric acid (35 ml) and the solution heated at 90-100° for 3h. The mixture was poured onto water (150 ml) and extracted with ethyl acetate (2 x 75ml) and ether (2 x 75 ml). The combined extracts were dried and evaporated to give a brown oil (3.15 g) which proved non-resolvable by chromatography on silica.

(b) The foregoing procedure was repeated using 3,5-dimethoxyphenylacetic acid (0.96 g), 2,5-dimethoxytoluene (1.52 g) and polyphosphoric acid (35 ml) to give a dark brown gum. No identifiable products were obtained by chromatography on silica.

(c) The foregoing procedure was repeated using 3,5-dimethoxyphenylacetyl chloride (2.15 g), 2,5-dihydroxytoluene (1.24 g) and polyphorsphoric acid (35 ml). The resultant dark brown gum was non-resolvable by chromatography on silica.

(iii) Catalysed by Trifluoroacetic Acid

(a) A solution of 3,5-dimethoxyphenylacetic acid (1.60 g) and 2,5-dimethoxytoluene (1.25 g) in trifluoracetic acid (10 ml) and

trifluoroacetic anhydride (2.4 ml) was heated at reflux for 8h. The mixture was cooled and poured slowly onto saturated sodium bicarbonate solution (100 ml). The aqueous mixture was extracted with ethyl acetate (3 x 70 ml) and the extract dried and evaporated. Chromatography on silica (chloroform as eluent) of the residue gave a dark brown polar material (1.20 g) and 2,5-dimethoxytoluene (0.42 g). The bicarbonate solution was acidified with concentrated hydrochloric acid and the precipitate of 3,5-dimethoxyphenylacetic acid (0.81 g) recovered by filtration.

(b) The foregoing procedure was repeated using 3,5-dihydroxyphenylacetic acid (0.84 g) and 2,5-dimethoxytoluene (0.76 g) in trifluoroacetic acid (15 ml) and trifluoroacetic anhydride (2.80 ml) and the intermediate 3,5-di(trifluoroacetoxy)phenylacetic acid was reacted as before to give, on work-up, starting materials 3,5-dihydroxyphenylacetic acid (0.51 g) and 2,5-dimethoxyltoluene (0.21 g) after chormotography on silica (chloroform as eluent).

3-Methylphenyl 3,5-Dimethoxyphenylacetate (41)

A solution of 3,5-dimethoxyphenylacetyl chloride (2.18 g) and m-cresol (1 ml) in benzene (40 ml) containing magnesium filings (0.11 g) was heated at reflux for 16h. After cooling the mixture was filtered and the magnesium washed with ether (40 ml). The combined organic solutions were washed with 0.1N sodium hydroxide solution (2 x 30 ml) and water (2 x 30 ml), dried and evaporated to give the ester which was distilled as a clear oil (1.63 g, 62%), b.p.$_{0.05}$ 95° (Found: M^+ 286.119. $C_{17}H_{18}O_4$ requires 286.120); ν_{max}

($CHCl_3$) 1 750 s and 1 610 s cm^{-1}; δ_H 7.20 (1H, m, ArH), 6.35 (3H, m, ArH), 6.55 (2H, m, ArH), 6.40 (1H, m, ArH), 3.78 (8H, br s, OMe and CH_2), and 2.30 (3H, s, CH_3).

3-Methyoxy-4-(3,5-dimethoxyphenylacetyl)toluene (42)

A solution of the ester (41) (1.63 g) in carbon disulphide (15 ml) was added drop-wise to a mechanically stirred suspension of aluminium chloride (0.76 g) in carbon disulphide (15 ml). The solvent was evaporated and the dark red residue was heated at 130-145° for 2h. After hydrolysis with iced dilute hydrochloric acid the mixture was extracted with ethyl acetate (3 x 50 ml) and this was then extracted with 10% sodium hydroxide solution (3 x 50ml). The basic extract was acidified with concentrated hydrochloric acid and then re-extracted with ethyl acetate (3 x 50 ml) and washed with saturated sodium bicarbonate solution (2 x 50 ml) and water (2 x 50 ml), dried and evaporated to give a brown gum (0.25 g). The gum was dissolved in dry acetone (40 ml) and heated at reflux for 16h with dimethyl sulphate (1 ml) and dry potassium carbonate (2 g). After cooling the mixture was filtered and the acetone solution evaporated, the residue was dissolved in ether (40 ml) and washed with concentrated ammonia solution (3 x 15 ml) and water (2 x 15 ml), dried and evaporated to give a red oil. This was purified by tlc on silica using ethyl acetate as eluent and the fraction (R_f 0.85-1.0) was further chromatographed using chloroform as eluent to give the trimethoxyketone (31) as needles (48 mg, 3%), m.p. 199-200° (from chloroform-hexane) (Found: C, 71.8; H, 6.8%;

M', 300.135. $C_{18}H_{20}O_4$ requires C, 71.9; H, 6.7%; M, 300.136); ν_{max} ($CHCl_3$) 1 680 s and 1 610 s cm^{-1}; δ_H 7.60 (1H, d, J 9 Hz, ArH), 6.77 (2H, m, ArH), 6.35 (3H, m, ArH), 4.20 (2H, s, CH_2), 3.89 (3H, s, OMe), 3.75 (6H, s, OMe) and 2.33 (3H, s, $ArCH_3$).

Methoxymethyl-m-cresyl Ether (43)[61]

m-Cresol (86.2 g) was dissolved in methylene chloride (1.5 l) and dimethoxymethane (320 ml) was added with p-toluenesulphonic acid (0.8 g). The mixture was heated at reflux for 90h below a soxhlet apparatus containing type 3A molecular sieve (90 g) which were replaced every 12h. On cooling, triethylamine (6.4 ml) was added and the solution washed with 1N sodium hydroxide solution (2 x 250 ml), dried and evaporated to give a pale green liquid (87.6 g, 72%). Distillation gave a free colourless liquid, b.p.$_{12}$ 96° (lit[61]., 91-92° at 12 mm);δ_H 7.10 (1H, m, ArH), 6.85 (3H, m, ArH), 5.10 (2H, s, CH_2), 3.45 (3H, s, OCH_3), and 2.28 (3H, s, $ArCH_3$); m/e 152 (M).

Attempted Condensation of Methoxymethyl-m-cresyl Ether (43) with the Phenylacetyl Chloride (36)

To a solution of the ether (0.5 g) in dry petroleum ether (40-60°) (15 ml) was added t-butyl lithium (2.6 ml, 1.28M in pentane) at 0° and with stirring under nitrogen. After 1h a solution of 3,5-dimethoxyphenylacetyl chloride (0.8 g) in dry ether (10 ml) was added drop-wise. After stirring for a further 1h water

(50 ml) followed by benzene (50 ml) was added. The organic solutions were separated, washed with water (30 ml), dried and evaporated to leave a pale oil. TLC (chloroform eluent), gave methoxymethyl-m-cresyl ether (0.41 g) and 3,5-dimethoxyphenylacetic acid (0.63 g) as the sole identifiable products.

2-Hydroxy-4-methylbenzoic acid

To a solution of methoxymethyl-m-cresyl ether (3.0 g) in dry petroleum ether (40-60°) (25 ml) was added t-butyl lithium (17.2 ml, 1.28M in pentane) at 0° with stirring under nitrogen. After 1h, the mixture was poured onto crushed dry ice (200 g) and after 1h was acidified with dilute hydrochloric acid (100 ml) and the aqueous mixture refluxed for 15 minutes. On cooling the crystalline precipitate was collected and recrystallised from water as needles (2.03 g, 67%) m.p. 173° (lit[104]., 177°); ν_{max} 3 010 br, 1650 s, and 1 625 s cm^{-1}; δ_H ($(CD_3)_2CO$) 7.70 (1H, d, J 9 Hz, ArH), 6.71 (2H, m, ArH), and 2.28 (3H, s, $ArCH_3$); m/e 152 (M).

Methyl 2-Methoxy-4-methylbenzoate (47)

2-Hydroxy-4-methylbenzoic acid (2.2 g) was dissolved in dry acetone (55 ml) containing dimethyl sulphate (3.5 ml) and dry potassium carbonate (12.5 g) and the mixture stirred at reflux for 3h. After cooling the mixture was filtered and the residue washed with acetone (2 x 20 ml). The combined acetone solutions were evaporated and the residue taken up in ether (50 ml), washed with

concentrated ammonia solution (2 x 30 ml) and water (2 x 30 ml), dried and evaporated to give a pale yellow oil. Distillation gave the ester as a free flowing colourless liquid (2.33 g, 91%), b.p.$_{12}$ 136-7° (lit[105]., 137-139° at 14 mm); ν_{max} (neat) 1 730 s and 1 615 s cm^{-1}; δ_H 7.70 (1H, d, J 9H z, ArH), 6.75 (2H, m, ArH), 3.85 (3H, s, CO_2CH_3), 3.84 (3H, s, OMe), and 2.35 (3H, s, $ArCH_3$); m/e 180 (M).

3-(2-Methoxy-4-methylphenyl) isocoumarin

n-Butyl lithium (15.2 ml, 1.55M in hexane) was added to a solution of di-isopropylamine (3.3 ml) in tetrahyrofuran (THF) (25 ml) at 0° and with stirring under nitrogen. After 10 minutes the solution was cooled to -60° and a solution of o-toluic acid (0.4 g) in THF (20 ml) was added drop-wise to form a raspberry coloured solution of the dianion. The cooling bath was removed and after 1h a solution of methyl 2-methoxy-4-methyl-benzoate (1.0 g) in THF (20 ml) was added drop-wise at 0°. After 2h, water (60 ml) was added and the organic solvents evaporated. The yellow residue was washed twice with ether, acidified with 2N hydrochloric acid and then extracted with ethyl acetate (3 x 40 ml), dried and evaporated to give a pale yellow solid. Recrystalised from ethanol as needles (0.30g, 37%); ν_{max} 1 719 s and 1 628 s cm^{-1}; δ_H 8.25 (1H, m, ArH), 7.80 (1H, d, J 9 Hz, ArH), 7.50 (3H, m, ArH), 7.30 (1H, s, vinylic), 6.85 (2H, m, ArH), 3.95 (3H, s, OMe) and 2.40 (3H, s, $ArCH_3$); m/e 266 (M).

Ethyl 1,2-Dihydro-o-orsellinate[106]

To a solution of sodium (23 g) in ethanol (300 ml) was added ethyl acetoacetate (126 g) and ethyl crotonate (102 g). The mixture was heated at reflux for 2h by which time a slurry of the enolate had appeared and then stirred at room temperature overnight. The mixture was acidifed with 5% sulphuric acid and then filtered. The filtrate was diluted with water (200 ml) and then extracted with chloroform, dried and evaporated to leave an oily product which was chilled until crystallisation began. This was then diluted with petroleum ether (40-60°) and left standing at 0° overnight. The product was collected by filtration and recrystallised from benzene-light petroleum (40-60°) as needles (90g, 46%), m.p. 85° (lit[107]., 89-90°); ν_{max}3 200 br and 1 730 s cm^{-1}; δ_H5.50 (1H, s,)H-3), 4.22 (2H, q, J 7 Hz, $OC\underline{H}_2CH_3$), 3.05 (1H, d, J 10 Hz, H-1), 2.0 -2.80 (3H, m. H-5 and H-6), 1.25 (3H, t, J 7 Hz, $OCH_2C\underline{H}_3$), and 1.05 (3H, d, J 6 Hz, CH_3); m/e 196 (M).

Ethyl o-orsellinate

A solution of bromine (71 g) in glacial acetic acid (30 ml) was added to a solution of ethyl 1,2-dihydro-o-orsellinate (25 g), in glacial acetic acid (150 ml) with stirring at such a rate that the temperature of the reaction mixture remained above 60°. The reaction mixture was stirred overnight and then poured onto ice (1 l); a white precipitate of ethyl 3,5-dibromo-o-orsellinate separated and was collected by filtration, washed with water and air dried.

Ethyl 3,5-dibromo-o-orsellinate (20 g) was dissolved in 2N sodium hydroxide solution (240 ml) and Raney nickel alloy was added portion-wise with stirring at 0°. After complete addition of the alloy the mixture was stirred at 0° for a further 1h, filtered under suction and the filtrate poured onto ice-cold concentrated hydrochloric acid (150 ml). The acid solution was extracted with ether and the ether dried and evaporated to give the product which was crystallised from aq. acetic acid as needles (7 g, 67%), m.p. 131-132° (lit[108]., 131-133°); ν_{max} 3 360 br, 1 640s, and 1585 m cm^{-1}; δ_H ($(CD_3)_2CO$) 6.25 (2H, s, ArH), 4.38 (2H, q, J 8Hz, OCH_2CH_3), 2.45 (3H, s, $ArCH_3$), and 1.37 (3H, t, J 8 Hz, OCH_2CH_3); m/e 195 (M).

2,4-Dimethyoxy-6-methylbenzoic acid (46)

To a solution of ethyl o-orsellinate (7 g) in dry acetone (150 ml) was added dry potassium carbonate (15 g) and dimethyl sulphate (10.5 ml) and the mixture was heated at reflux for 8h. On cooling, the mixture was filtered and evaporated to leave a dark, oily residue which was taken up in ether (100 ml) and washed with concentrated ammonia solution (2 x 50 ml) and water (100 ml). The ethereal solution was evaporated and the resulting oil suspended in 10% sodium hydroxide solution (150 ml) and heated at reflux overnight. On cooling, the basic solution was washed with ether (2 x 30 ml) and acidified. The resulting precipitate was collected, washed with cold water and dried over P_2O_5. Recrystallisation from methylene chloride-hexane gave the acid as needles (5.4 g, 78%), m.p. 142° (lit[109]., 140°); ν_{max} 2 950 br m, 2 600 br m,

1 685 s and 1 600 s cm^{-1}; δ_H ($(CD_3)_2CO$) 6.42 (2H, s, ArH), 3.80 (6H, s, OMe), and 2.28 (3H, s, $ArCH_3$); m/e 196 (M).

6,8-Dimethyoxy-3-(2-methoxy-4-methylphenyl)isocoumarin(48)

A solution of n-butyl lithium (7.6 ml, 1.55M in hexane) was added to a solution of di-isopropylamine (1.7 ml) in THF (20 ml) at 0° under nitrogen with stirring. After 10 minutes the solution was cooled to -60° and a solution of 2,4-dimethyoxy-6-methylbenzoic acid (0.58g) in THF (20 ml) was added drop-wise. The cooling bath was removed and after 1h methyl 2-methoxy-4-methy-benzoate (1.06g) in THF (20 ml) was added drop-wise and the mixture stirred for 16 h. Water (30 ml) was added and the organic solvents were evaporated. The residue was washed with ether, acidified with dilute hydrochloric acid and then extracted with ethyl acetate (3 x 30 ml) which was dried and evaporated to give a yellow oil. The oil was dissolved in methanol (30 ml) and concentrated hydrochloric acid (5 ml) and the mixture was heated at reflux for 1h. After dilution with water the methanol was evaporated until crystallisation occurred and the crystals were collected to give the isocoumarin as cubes (0.60g, 62%), m.p. 166-168° (from ethanol) (found: C, 69.7; H, 5.6%; M+ 326.114. $C_{19}H_{18}O_5$ requires C, 69.9; H, 5.5%; M, 326.115); ν_{max}1720 s and 1610 s cm^{-1}; δ_H 7.85 (1H, d, J 8 Hz, ArH), 7.19 (1H, s, 4-H), 6.82 (2H, m, ArH), 6.42 (2H, s, ArH), 3.97 (3H, s, OMe), 3.93 (3H, s, OMe), 3.90 (3H, s, OMe), and 2.38 (3H, s, $ArCH_3$).

4-(2-Hydroxycarbonyl-3,5-dimethoxyphenylacetyl)-3-methoxytoluene (49)

A suspension of 6,8-dimethoxy-3-(2-methoxy-4-methyl-phenyl) isocoumarin (1.4 g) in 10% sodium hydroxide solution (75 ml) was heated at reflux for 3 hours. On cooling, the mixture was acidified by the slow addition for iced concentrated hydrochloric acid. The acid solution was extracted with ethyl acetate (3 x 50 ml) and the extract washed with water (75 ml), dried and evaporated to give the acid as a pale oil (1.3 g, 88%) which was used without further purification; δ_H 7.65 (1H, d, J 8 Hz, ArH), 6.75 (2H, m, ArH), 6.42 (2H, s, ArH), 4.56 (2H, s, CH_2), 3.88 (6H, s, OMe), 3.78 (3H, s, OMe), and 2.30 (3H, s, $ArCH_3$).

Attempted Decarboxylation of 4-(2-Hydroxycarbonyl-3,5-dimethoxyphenylacetyl)-3-methoxytoluene (49)

The crude acid (0.96) was dissolved in quinoline (12 ml) containing copper (II) sulphate (7.5 mg) and stirred at 200-220° for 4h. After cooling, the mixture was diluted with benzene (80 ml) and then washed with 1N hydrochloric acid (2 x 40 ml), 1N potassium carbonate solution (2 x 40 ml) and water (50 ml), dried and evaporated to give a yellow oil. Chromatography on silica (chloroform as eluent) gave the isocoumarin (0.55 g, 61%), identical to that previously produced.

6,8-dihydroxy-3-(2-hydroxy-4-methylphenyl)isocoumarin (50)

Boron tribromide (0.5 ml) was added to a solution of 6,8-dimethoxy-3-(2-methoxy-4-methylphenyl)isocoumarin (85 mg) in methylene chloride (25 ml) under nitrogen at -78°. The solution was kept at this temperature for 6h and then allowed to rise to room temperature over 16h. Ether (20 ml) was added followed by water (10 ml). The organic layer was separated, washed with water (15 ml), dried and evaporated to give the trihydroxyisocoumarin (49) as needles (70 mg, 90%), m.p. 202-204° (from ethanol) (Found: C, 67.5; H, 4.35%; M+, 284.070. $C_{16}H_{12}O_5$ requires C, 67.6; H, 4.25%; M, 284.068); ν_{max} 3 200 br s, 1660 s, and 1 610 s cm^{-1}; δ_H ($(CD_3)_2CO$) 7.75 (1H, d, J 9 Hz, ArH), 7.40 (1H, s, 4-H), 6.88 (1H, s, ArH), 6.80 (1H, d, J 9 Hz, ArH), 6.48 (1H, d, J 1 Hz, ArH), 6.40 (1H, d, J 1 Hz, ArH), and 2.27 (3H, s, $ArCH_3$).

3-Hydroxy-(3,5-dihydroxyphenylacetyl)toluene (52)

The trihydroxyisocoumarin (50) (0.33 g) was dissolved in 10% potassium hydroxide solution (50 ml) and heated at reflux for 6h. After cooling, the solution was acidifed with concentrated hydrochloric acid and extracted with ethyl acetate (2 x 50 ml). The extract was dried and evaporated to give the trihydroxyketone as cubes (0.25 g, 90%), m.p. 89° (from chloroform-hexane) (Found: C, 69.6, H, 5.55%; M+, 258.088. $C_{15}H_{14}O_4$ requires C, 69.75; H, 5.45%; M, 258.089); ν_{max} 3 250 br s, 1 620 s, and 1 610 s cm^{-1}; δ_H ($(CD_3)_2CO$)

7.90 (1H, d, J 9 Hz, ArH), 6.30 (3H, m, ArH), 4.18 (2H, s, CH_2), and 2.30 (3H, s, $ArCH_3$).

3-Methoxy-(3,5-dimethoxyphenylacetyl) toluene (42)

The trihydroxyketone (52) (0.25 g) was dissolved in dry acetone (50 ml) and treated at reflux for 16h with anhydrous potassium carbonate (2.5 g) and dimethyl sulphate (0.4 ml). After cooling, the mixture was filtered and the solvents evaporated. The residue was dissolved in ether (50 ml) and washed with concentrated ammonia solution (2 x 30 ml) and water (20 x 30 ml), dried and evaporated to give the trimethoxyketone as needles (0.26 g, 90%) which was identical in all respects to that obtained earlier.

3-Methoxy-4-(1-methoxy-2-(3,5-dimethoxyphenyl)ethenyl)toluene (54)

The trimethoxyketone (42) (90 mg) was dissolved in trimethylorthoformate (2 ml) and the mixture distilled through a short vigreux column to remove volatiles with b.p.<86°. The remainder was heated at reflux for 1h and then evaporated to dryness. The residue was crystallised from methylene chloride-hexane to give the methyl enol ether as cubes (87 mg, 92%), m.p. 138° (Found: C, 72.55; H, 6.8%; M^+, 314.150. $C_{19}H_{22}O_4$ requires C, 72.6; H, 7.05%; M, 314.51); ν_{max} 2 950 m and 1 610 s cm^{-1}; δ_H 7.30 (1H, m, ArH), 7.12 (2H, m, ArH), 6.72 (3H, m, ArH), 6.67 (1H, s, olefinic H), 6.40 (1H, m, ArH), 3.91 (3H, s, OMe), 3.85 (6H, s, OMe), 3.64 (3H, s, OMe), and 2.41 (3H, s, $ArCH_3$).

4-(1-Acetoxy-2 (3,5-dimethoxyphenyl)ethenyl)-3-methoxytoluene (53)

The trimethoxyketone (42) (0.22 g) was dissolved in acetic anhydride (30 ml) and heated at reflux with anhydrous potassium acetate (0.5 g) for 1.5h. After cooling, the dark mixture was poured onto iced water, stirred for 20 minutes and then extracted with ethyl acetate (3 x 40 ml). The extract was washed with saturated sodium carbonate solution (3 x 50 ml) and water (50 ml), dried and evaporated to give a yellow oil. The product was purified by tlc on silica using chloroform as eluent to give the enol acetate as an oil (0.13 g, 52%) (Found: M', 342.145 $C_{20}H_{22}O_5$ requires M, 342.146); ν_{max} ($CHCl_3$) 1 770 s and 1 600 s cm^{-1}; δ_H 7.30 (1H, m, ArH), 7.05 (1H, m, ArH), 6.70 (3H, m, ArH), 6.65 (1H, s, olefinic H), 6.40 (1H, m, ArH), 3.85 (3H, s, OMe), 3.78 (6H, s, OMe), 2.35 (3H, s, $ArCH_3$), and 2.20 (3H, s, Ac).

Irradiation of the Methyl Enol Ether (54)

A solution of the methyl enol ether (54) (0.10 g) in cyclohexane (80 ml) containing iodine (10 mg) was irradiated with a Hanovia 125W medium pressure lamp for 16h. After which time the solution was decanted, the apparatus rinsed with ethyl acetate (30 ml) and the combined solutions washed with 5% sodium thiosulphate solution (40 ml) and water (40 ml), dried and evaporated to give a brown oil. This oil was purified by tlC on silica to furnish only the starting methyl enol ether.

Irradiation of the Enol Acetate (53)

The foregoing procedure was repeated using the enol acetate (53) (0.13 g) dissolved in cychohexane (80 ml) containing iodine (10 mg). Chromatography of the products (chloroform eluent) gave only starting enol acetate.

3,5-Dimethoxybenzyl alcohol[68]

Methyl 3,5-dimethoxybenzoate (5 g), was treated with lithium aluminium hydide (1.2 g) in dry tetrahydrofuran (175 ml) and heated at reflux for 5 hours. After cooling, water (1.2 ml) was added cautiously followed by 15% sodium hydroxide solution (1.2 ml) and more water (3.6 ml) with stirring. The granular aluminium hydroxide was filtered and washed with ether (200 ml). The organic solutions were evaporated to give a colourless solid which was recrystallised from di-isopropyl ether as needles (3.5 g, 75%), m.p. 46-47° (lit[68]., 47°); ν_{max} ($CHCl_3$) 3400 br s and 1 610 s cm^{-1}; δ_H 6.51 (2H, d, J 2 Hz, ArH), 6.38 (1H, t, J 2 Hz, ArH), 4.60 (2H, s, $ArCH_2$), 3.76 (6H, s, OMe), and 2.05 (1H, s, OH); m/e 168 (m).

3,5-Dimethoxybenzyl chloride[68]

Thionyl chloride (2 ml) and pyridine (0.2 ml) in dry ether (50 ml) were added to 3,5-dimethoxybenzyl alcohol (2.5 g) in dry ether (35 ml) with stirring. After standing for 1 hour at room temperature the excess thionyl chloride was destroyed with water (10 ml) and the ether layer was separated, washed with water (20 ml), 10% sodium hydroxide

solution (2 x 30 ml) at water (2 x 30 ml). The ethereal solution was dried and evaporated to give a colourless solid which was recrystalised from dry ether as needles (2.5 g, 90%), m.p. 44-45° (lit[68]., 46°); ν_{max} ($CHCl_3$) 1 620 s and 1 600 s cm^{-1}; δ_H 6.51 (2H, d, J 2 Hz, ArH), 6.40 (1H, t, J 2 Hz, ArH), 4.49 (2H, s, $ArCH_2$), and 3.77 (6H, s, OMe); m/e 186, 188 (M).

3,5-Dimethoxyphenylacetonitrile[68](55)

Potassium cyanide (5.0g) and 3,5-dimethoxybenzyl chloride(2.7 g) in ethanol (50 ml) and water (15 ml) was heated at reflux for 3 hours and then poured onto ice with stirring. The cream coloured precipitate was allowed to stand for 2 hours and then filtered, washed with cold water and dried over P_2O_5. The product was recrystallised from petroleum ether (40-60°) as colourless needles (1.7 g, 55%), m.p. 52-53° (lit[68]., 53°); ν_{max} ($CHCl_3$) 2 240 w and 1 610 s cm^{-1}; δ_H 6.44 (3H, m, ArH), 3.71 (6H, s, OMe), 3.60 (2H, s, $ArCH_2$); m/e 177 (M).

4-(2-Cyano-2-(3,5-dimethoxyphenyl)acetyl)-3-methoxytoluene (56)

n-Butyl lithium (10.2 ml, 1.55M in hexane) was added to dry THF (10 ml) at 0° under nitrogen with stirring. 3,5-Dimethoxy-phenylacetonitrile (1.28g) in dry THF (10 ml) was added drop-wise during 5 minutes. After 1h, methyl 2-methoxy-4-methylbenzoate (1.3 g) in dry THF (10 ml) was added drop-wise and the mixture was stirred at reflux for 3h. After cooling, 3M hydrochloric acid (30 ml) was added and the organic solvents were evaporated. The residue was extracted

with ethyl acetate (2 x 40 ml) and the extract was dried and evaporated to give the cyanoketone as cubes (1.75 g, 76%), m.p. 144-145° (from acetone-hexane) (Found: C, 70.25; H, 5.95; N, 4.35%; M', 325.131. $C_{19}H_{19}NO_4$ requires C, 70.15; H, 5.9; N, 4.3%; M, 325.131); ν_{max} 2 250 m , 1 685 s, and 1 610 cm^{-1}; δ_H 7.55 (1H, d J 8 Hz, ArH), 6.78 (2H, m, ArH), 6.40 (2H, m, ArH), 6.33 (1H, m, ArH), 5.77 (1H, s, CH), 3.87 (3H, s, OMe), 3.68 (6H, s, OMe), and 2.33 (3H, s, $ArCH_3$).

3-Methyoxy-4-(1-methoxy-2-cyano-(3,5-dimethoxyphenyl)ethenyl)toluene(58)

The β-ketonitrile (56) (0.31 g) was dissolved in trimethylorthoformate (5 ml) and the mixture distilled through a short vigreux column to remove volatiles with b.p.<86°. The remainder was heated at reflux for 1h and then evaporated to give a red solid (0.31 g, 91%). The enol ether was recrystallised as cubes, m.p. 146° (from methanol) (Found: C, 70.65; H, 6.3; N, 4.1%. $C_{20}H_{21}NO_4$ requires C, 70.7; H, 6.2; N, 4.1%); ν_{max} 2 215 m and 1 610 s cm^{-1}; δ_H 7.38 (1H, d, J 8 Hz, ArH), 6.90 (2H, m, ArH), 6.55 (2H, ABq, J 18 Hz, ArH), 6.40 (1H, s, ArH), 3.90 (3H, s, OMe), 3.85 (6H, s, OMe), 3.65 (3H, s, OMe), and 2.40 (3H, s, $ArCH_3$).

Irradiation of the Methyl Enol Ether (58)

The methyl enol ether (58) (0.10 g) was irradiated as described for the enol ether (54). Chromatography (chloroform eluent) of the products gave only starting enol ether.

4-(1-Acetoxy-2-cyano-2-(3,5-dimethoxyphenyl)ethenyl)-3-methoxytoluene (59)

A solution of the β-ketonitrile (56) (0.26 g) in acetic anhydride (15 ml) containing concentrated sulphuric acid (0.01 ml) was heated at reflux for 1.5h. After cooling the mixture ws poured onto iced water (100 ml), stirred for 20 minutes and then extracted with ethyl acetate (2 x 60 ml). This extract was washed with saturated sodium bicarbonate solution (3 x 60 ml) and water, dried and evaporated to give a brown gum. Chromatography using chloroform as eluent gave the enol acetate as an oil (E:Z = 6:5) (0.21 g, 72%) (Found: M$^+$, 367.142. $C_{21}H_{21}NO_5$ requires M, 367.141); ν_{max} (neat) 2 220 s, 1 774 s, and 1 600 s cm^{-1}; δ_H (E isomer) 7.12 (1H, d, J 8 Hz, ArH), 6.75 (2H, m, ArH), 6.62 (1H, s, ArH), 6.62 (2H, s, ArH), 3.60 (6H, s, OMe), 3.59 (3H, s, OMe), 2.30 (3H, s, $ArCH_3$), and 2.25 (3H, s, Ac); δ_H(Z isomer) 7.45 (1H, d, J 8Hz, ArH), 6.80 (4H, m, ArH), 6.50 (1H, m, ArH), 3.92 (3H, s, OMe), 3.82 (6H, s, OMe), 2.40 (3H, s, $ArCH_3$), and 2.10 (3H, s, Ac).

Irradiation of 4-(1-Acetoxy-2-cyano-2-(3,5-dimethoxyphenyl)ethenyl)-3-methoxytoluene (59)

A solution of the enol acetate (59) (0.19 g) in benzene (80 ml) containing iodine (25 mg) was irradiated for 24h as described for the enol ether (54). The products were separated by tlc on silica using chloroform as eluent to give:

10-acetoxy-9-cyano-5,7-dimethoxy-3-methylphenanthrene (61) (56 mg, 32%) m.p. 256-257° (from methanol) (Found: C, 71.45; H, 5.2; N, 4.35%; M⁺, 335.116. $C_{20}H_{17}NO_4$ requires C, 71.6; H, 5.1; N, 4.2%; M, 335.115); ν_{max} 2 219 m, 1 760 s, and 1 615 s cm^{-1}; δ_H 9.31 (1H, s, 4-H), 7.80 (1H, d, J 8 Hz, ArH), 6.79 (1H, d, J 2 Hz, ArH), 6.40 (1H, d, J 8 Hz, ArH), 6.22 (1H, d, J 2 Hz, ArH), 4.08 (3H, s, OMe), 3.98 (3H, s, OMe), 2.58 (3H, s, $ArCH_3$) and 2.54 (3H, s, Ac).

10-acetoxy-9-cyano-1,5,7-trimethoxy-3-methylphenanthrene (60)(41 mg, 22%) m.p. 244-245° (from methanol) (Found: C, 69.15; H, 5.35; N, 3.6%; M⁺, 365.122. $C_{21}H_{19}NO_5$ requires C, 69.05; H, 5.25; N, 3.85%; M, 365.125); ν_{max} 2 221m, 1 770s, and 1 610s cm^{-1}; δ_H 8.99 (1H, s, H), 7.13 (1H, d, J 2.5 Hz, H-8), 6.79 (1H, s, H-2), 6.70 (1H, d, J 2.5 Hz, H-6), 4.01 (3H, s, OMe), 3.95 (3H, s, OMe), 3.91 (3H, s, OMe), 2.53 (3H, s, $ArCH_3$), and 2.46 (3H, s, Ac); δ_H(200 MHz) see table 11.

2,6-Dihydroxy-4-methylbenzoic acid[70] (65)

A mixture of orcinol (60 g) and potassium bicarbonate (120 g) in glycerol (200 ml) was heated at 120° under an atmosphere of carbon dioxide for 8h with manual mixing every 15 minutes. After cooling, the mixture was added to water (1 l) and acidified with concentrated hydrochloric acid. The precipitate was collected and recrystallised from water as needles (51 g, 63%), m.p. 164-165.5° (lit[70]; 172°); ν_{max} 3 500 br m, 3 000 br m, and 1670 s cm^{-1}; $\delta_H((CD_3)_2CO)$ 6.30 (2H, s, ArH), and 2.21 (3H, s, $ArCH_3$); m/e 168 (M).

Methyl 2,6-Dimethyoxy-4-methylbenzoate (64)

A solution of 2,6-dihydroxy-4-methylbenzoic acid (5.0 g) in dry acetone (200 ml) was heated at reflux with anhydrous potassium carbonate (30 g) and dimethyl sulphate (9.5 ml) for 5h. After cooling, the mixture was filtered, the filtrate washed with acetone (200 ml) and the combined acetone solutions evaporated. The golden residue was taken up in ether (100 ml), washed with concentrated ammonia solution (2 x 50 ml) and water (2 x 50 ml), dried and evaporated to give a white solid. Recrystallisation from ether gave the ester as hexagonal plates (4.2 g, 67%), m.p. 86.5° (lit[70]., 86°); ν_{max} 3000 m, 2 945 m, 1 735 s, and 1 610 s cm^{-1}; δ_H 6.36 (2H, s, ArH), 3.86 (3H, s, CO_2Me), 3.78 (6H, s, OMe), and 2.31 (3H, s, $ArCH_3$); m/e 210 (M).

4-(2-Cyano-2-(3,5-dimethoxyphenyl)acetyl)-3,5-dimethoxytoluene (66)

Prepared in a manner similar to that for the β-ketonitrile (56) above using 3,5-dimethoxyphenylacetonitrile (1.77 g) and methyl 2,6-dimethoxy-4-methylbenzoate (2.31 g) to furnish the β-ketonitrile (65) as plates (2.3 g, 65%) m.p. 164-165° (from acetone-hexane) (Found: C, 67.6; H, 5.8; N, 3.7%; $C_{20}H_{21}NO_5$ requires C, 67.6; H, 5.95; N, 395%); ν_{max} 3 250 m, 2 219 m 1 638 s, and 1 610 s cm^{-1}; δ_H 7.10 (2H, d, J 2 Hz, ArH), 6.43 (2H, s, ArH), 6.35 (1H, m, ArH), 3.83 (6H, s, OMe), 3.81 (6H, s, OMe), and 2.35 (3H, s, $ArCH_3$).

4-(1-Acetoxy-2-cyano-2-(3,5-dimethoxyphenyl)ethenyl-3,5-dimethoxytoluene (67)

Prepared in a manner similar to that for the enol acetate (59) above using the β-ketonitrile (66) (1.5 g) to give the enol acetate as a pale oil (1.4 g, 84%) after flash chromatography on silica using chloroform as eluent (E:Z = 3:1) (Found M$^+$, 397.154. $C_{22}H_{23}NO_6$ requires 397.179); $\nu_{max}(CHCl_3)$ 2 940 m, 2 212 m, 1 750 s, and 1 610 s cm^{-1}; δ_H (E isomer) 6.77 (2H, d, J 2 Hz, ArH), 6.41 (1H, m, ArH), 6.40 (2H, s, ArH), 3.83 (6H, s, OMe), 3.79 (6H, s, OMe), 2.32 (3H, s, $ArCH_3$), and 2.05 (3H, s, Ac); δ_H (Z isomer) 6.77 (2H, d, J 2 Hz, ArH), 6.31 (1H, m, ArH), 6.27 (2H, s, ArH), 3.58 (12H, s, OMe), 2.28 (3H, s, $ArCH_3$), and 2.20 (3H, s, Ac).

Irradiation of 4-(1-Acetoxy-2-cyano-2-(3,5-dimethoxyphenyl)ethenyl) -3,5-dimethoxytoluene (67)

The enol acetate (67) (1.4 g) was dissolved in deoxygenated benzene (800 ml) and irradiated under a nitrogen atmosphere for 20h. The solvents were evaporated and the residue crystallised from methanol. The crystals were collected and the mother liquors evaporated. This residue was redissolved in benzene and re-irradiated. This cycle was repeated twice to furnish 10-acetoxy-9-cyano-2,5,7-trimethoxy-3-methylphenanthrene (60) (1.06 g, 76%) as the sole product. It was identical to that produced by the previous route.

9-Cyano-10-hydroxy-1,5,7-trimethoxy-3-methylphenanthrene (68)

(i) A solution of 10-acetoxy-9-cyano-1,5,7-trimethoxy-3-methyl phenanthrene (0.61 g) in methanol (50 ml) containing water (5 ml) and potassium hydroxide (3 g) was heated at reflux for 2h. After cooling, the mixture was poured onto iced water (100 ml) and acidified with concentrated hydrochloric acid. The precipitate was collected and air dried. Recrystallisation gave needles (0.52 g, 96%), m.p. 192-193° (from ethanol) (Found: C, 70.6; H, 5.0; N, 4.2%; M', 323.117. $C_{19}H_{17}NO_4$ requires C, 70.6; H, 5.3; N, 4.3%; M, 323.115); ν_{max} 3 305 br s, 2 218 m, and 1 625 s cm^{-1}; δ_H 8.87 (1H, s, H-4), 7.00 (1H, d, J 2 Hz, ArH), 6.62 (1H, s, ArH), 6.51 (1H, d, J 2 Hz, ArH), 4.05 (3H, s, OMe), 4.00 (3H, s, OMe), 3.92 (3H, s, OMe), and 2.49 (3H, s, $ArCH_3$).

(ii) To a suspension of the acetoxyphenanthrene (60) (0.5g) in methanol (25 ml) was added 48% hydrobromic acid (15 ml) and the mixture heated at reflux for 12 hours. After cooling, the dark mixture was poured onto water (60 ml) and extracted with ethyl acetate (2 x 50 ml). This extract was washed with saturated sodium bicarbonate solution (2 x 30 ml) and water (50 ml), dried and evaporated to give a brown gum. Crystallisation from ethanol gave the hydroxyphenanthrene (68) (0.3g, 68%), identical to that previously obtained.

9-Cyano-1,5,7,10-tetramethoxy-3-methylphenanthrene (69)

A solution of the hydroxyphenanthrene (68) (96 mg) in dry acetone (50 ml) containing dimethyl sulphate (0.8 ml) and anhydrous

potassium carbonate (0.75 g) was stirred at reflux for 7h. After cooling, the mixture was filtered and the potassium carbonate washed with acetone (50 ml). The combined acetone solutions were evaporated and the residue taken up in ether (50 ml) and washed with concentrated ammonia solution (2 x 25 ml) and water (2 x 25 ml). The ethereal solution was dried and evaporated to give the phenanthrene as needles (99 mg, 98%), m.p. 179-180° (from methylene chloride-hexane) (Found: C, 71.35, H, 4.7; N, 4.15%; M$^+$, 337.131. $C_{20}H_{19}NO_4$ requires C, 71.2; H, 5.7; N, 4.15%; M,337.131); ν_{max} 2 935 m, 2 208 m and 1 622 s cm^{-1}; δ_H 8.98 (1H, S, ArH), 7.18 (1H, d, J 2 Hz, ArH), 6.88 (1H, s, ArH), 6.19 (1H, d, J 2 Hz, ArH), 4.04 (6H, s, OMe), 4.01 (3H, s, OMe), 3.95 (3H, s, OMe), and 2.55 (3H, s, $ArCH_3$).

Attempted Hydroylsis of
9-Cyano-1,5,7,10-tetramethoxy-3-methylphenanthrene (69)

(i) The tetramethoxyphenanthrene (69) (0.5 g) was dissolved in methanol (15 ml) and water (15 ml) and potassium hydroxide (2 g) was added. The mixture was heated at reflux for 48h. Only starting phenanthrene could be detected upon normal work-up.

(ii) Hydrogen peroxide solution (0.5 ml, 100 vol.) was added to the tetramethoxyphenanthrene (69) (99 mg) and acetone (3 ml) was added to effect solubility. To this was further added 10% sodium carbonate solution (2.5 ml) and the solution stirred at room temperature. After 3 days, the mixture was poured onto water (150 ml) and the precipitate collected by filtration and dried over P_2O_5 to furnish only starting phenanthrene (45 mg).

Attempted Hydrolysis of 9-Cyano-10-hydroxy-1,5,7-trimethoxy-3-methylphenanthrene (68)

(i) The hydroxyphenanthrene (68) (108 mg) was suspended in ethanol (8 ml) containing sodium hydroxide (0.2 g). The mixture was heated in a sealed tube at 190° for 20h. After cooling, the mixture was poured onto water (50 ml), acidified and extracted with ethyl acetate. The extract was dried and evaporated to yield a yellow solid which on tlc (chloroform eluent) furnished only starting material (60 mg).

(ii) The hydroxyphenanthrene (68) (70 mg) was dissolved in hot polyphosphoric acid (7 ml) and the solution heated at 200° for 1h. After cooling, iced water was added and the mixture extracted with ethyl acetate (2 x 50 ml). The extract was dried and evaporated to give a polymeric red oil of indeterminate nature.

(iii) The hydroxyphenanthrene (68) (130 mg) was dissolved in 0.05% calcium hydroxide solution (200 ml) at 95° and the solution allowed to cool to room temperature. The precipitate of the calcium salt was collected and heated at 150° for 9h. After cooling, water (20 ml) was added and the mixture acidified with concentrated hydrochloric acid and extracted with ethyl acetate. This extract was dried and evaporated to give the starting phenanthrene (47 mg) as the sole component.

Birch Reduction of the Hydroxyphenanthrene (68)

To a solution of the hydroxyphenanthrene (68) (123 mg) in liquid ammonia (15 ml) at -60° was added sodium metal (100 mg) and the mixture stirred for 1h. The reaction was quenched by the addition of methanol (10 ml). The ammonia was evaporated followed by evaporation of the methanol. The residue was purified by tlc (chloroform as eluent) to furnish starting phenanthrene (10 mg) and a large amount of highly polar material.

Attempted Thioamide formation

To a solution of the hydroxyphenanthrene (68) (92 mg) in dry pyridine (20 ml) was added triethylamine (2.5 ml). Hydrogen sulphide gas was passed through this solution with stirring at 50-60° for 5.5h. After cooling, the mixture was evaporated and the residue crystallised from methanol to give starting phenanthrene as the sole product.

Hydrolysis of 4-(2-Cyanao-2-(3,5-dimethoxyphenyl)acteyl)-3,5-dimethoxytoluene (66)

A solution of the β-ketonitrile (66) (0.10 g) in ethanol (5 ml) and 40% sodium hydroxide solution (5 ml) was heated at reflux for 20h. After cooling, the mixture was poured onto water (100 ml), acidified with concentrated hydrochloric acid and extracted with ethyl acetate (3 x 30 ml). This extract was dried and evaporated to give a clear oil (0.11 g). Chromatography on silica using 10% methanol-hexane

as eluent gave 3,5-dimethoxyphenylacetic acid (43 mg) and 2,6-dimethoxy-4-methylbenzoic acid (45 mg), identical to samples previously prepared.

Ethanolysis of 4-(2-Cyano-2(3,5-dimethoxyphenyl)acetyl)-3,5-dimethoxytoluene (66)

A solution of the β-ketonitrile (66) (57 mg) in absolute ethanol (25 ml) was satured with dry hydrogen chloride gas at 0°. The mixture was heated on a steam bath for 24h and then poured onto iced water (100 ml). The aqueous mixture was extracted with ethyl acetate (2 x 50 ml) and this extract was dried and evaporated to give a brown oil. Chromatography on silica with chloroform as eluent gave 3-methoxy-4-(1-ethoxy-2-cyano-(3,5-dimethoxyphenyl)ethenyl)toluene as an oil (Found: M', 383.173; $C_{22}H_{25}NO_5$ requires M, 383.173); ν_{max} ($CHCl_3$) 3 005 m, 2 205 m, and 1 600 s cm^{-1}; δ_H 7.10 (2H, d, J 2 Hz, ArH), 6.35 (3H, m, ArH), 3.82 (2H, q, J 5 Hz, OCH_2CH_3), 3.81 (6H, s, OMe), 3.75 (6H, s, OMe), 2.31 (3H, s, $ArCH_3$), and 1.30 (3H, t, J 5 Hz, OCH_2CH_3).

Ethyl 3,5-Dimethoxyphenylacetate (75)

A solution of 3,5-dimethoxyphenylacetonitrile (0.72 g) in ethanol (50 ml) and concentrated sulphuric acid (25 ml) was heated at reflux for 16h. After cooling, the mixture was poured onto iced water (150 ml) and extracted with ethyl acetate (2 x 60 ml). This extract was dried and evaporated to give a brown oil. Flash chromatography on silica using chloroform as eluent gave the ester as a colourless oil (0.68 g, 75%), b.p.$_{1.0}$ 145-150° (lit.,[110] 128-132° at 0.1 mm);

δ_H 6.40 (3H, m, ArH), 4.15 (2H, q, J 7.5 Hz, OCH_2CH_3), 3.75 (6H, s, OMe), 3.52 (2H, s, CH_2) and 1.21 (3H, t, J 7.5 Hz, OCH_2CH_3); m/e 224 (M).

4-(2-Ethoxycarbonyl-2-phenyl)acetyl-3-methoxytoluene (77)

n-Butyl lithium (6.6 ml, 1.6M in hexane) was added to a solution of di-isopropylamine (1.5 ml) in THF (15 ml) with stirring at 0° under nitrogen. After 10 minutes the mixture was cooled to -60° and ethyl phenylacetate (0.82 g) in THF (15 ml) was added drop-wise over 5 minutes. After 15 minutes the cooling bath was removed, methyl 2-methoxy-4-methylbenzoate (1.0 g) in THF (15 ml) was added drop-wise and the stirred mixture heated at reflux for 2h. After cooling, the mixture was acidified with dilute hydrochloric acid and the organic solvents evaporated. The residue was extracted with ethyl acetate (3 x 40 ml), dried and evaporated to give an oil. Purification by flash chromatography using ether-petroleum ether (40-60°) as eluent gave the β-ketoester (77) (0.48 g, 31%), m.p. 64-65° (Found: M^+, 312.136. $C_{19}H_{20}O_4$ requires M, 312.136); ν_{max} ($CHCl_3$)3 080 br m, 1 732 s, 1 680 s, and 1 605 s cm^{-1}; δ_H 7.66 (1H, d, J 9z, ArH), 7.25 (5H, s, ArH), 6.70 (2H, m, ArH), 5.61 (1H, s, CH), 4.15 (2H, q, J 8 Hz, OCH_2CH_3), 3.79 (3H, s, OMe), 2.75 (3H, s, $ArCH_3$), and 1.15 (3H, t, J 8 Hz, OCH_2CH_3).

Attempted condensation of Ethyl 3,5-Dimethoxyphenylacetate (75) with the Dimethoxy-Benzoate Ester (64)

The foregoing procedure was repeated using ethyl 3,5-dimethoxyphenylacetate (0.60 g) and methyl 2,6-dimethoxy-4-methyl benzoate (0.62 g). Only starting materials could be identified on similar work-up.

Activation of Raney Nickel catalyst

Following the procedure of Staskun and Backeberg[79], nickel-aluminium alloy (4.5 g) was stirred magnetically with 2N sodium hydroxide sodium (90 ml) for 45 minutes, the temperature being allowed to rise. The alkaline solution was then decanted and the catalyst washed with water (2 x 100 ml). Most of the water was decanted and the catalyst was generally used as an aqueous suspension.

If required dry, the catalyst was further washed with methanol (3 x 60 ml) and ether (2 x 10 ml) and then dried in a vacuum dessicator.

10-Hydroxy-1,5,7-trimethoxy-3-methylphenanthrene (80)

Raney nickel alloy (4.5 g) was added as a water slurry to a suspension of 9-cyano-10-hydroxy-1,5,7-trimethoxy-3-methylphenanthrene (0.46 g) in 75% formic acid solution (50 ml) and the mixture was stirred under nitrogen and heated at reflux for 12h. A further portion of Raney nickel (4.5 g) was added and heating continued for a

further 12h. After cooling, the mixture was filtered and the nickel was washed with ethyl acetate (150 ml). The combined solutions were washed with water (50 ml), saturated sodium bicarbonated solution (4 x 60 ml) and water (50 ml). The organic solution was dried and evaporated to give a dark brown gum. Polar material was removed by passing through a short column of silica using chloroform as eluent. Further purification by tlc on silica using chloroform as eluent gave starting material (42 mg, 9%) and the hydroxyphenanthrene (80) (177 mg, 42%) as plates m.p. 177-177.5° (from methylene chloride-hexane) (Found: C, 72.45; H, 6.2%; $\underline{M}^+$, 298.120. $C_{18}H_{18}O_4$ requires C, 72.45; H, 6.1%; $\underline{M}$, 298.120); ν_{max} 3 300 br s, 1 650 s, and 1 610 s cm^{-1}; δ_H 9.60 (1H, s, OH), 9.00 (1H, s, ArH), 6.86 (1H, s, ArH), 6.70 (1H, s, ArH), 6.60 (1H, d, $\underline{J}$ 2 Hz, ArH), 6.46 (1H, d, $\underline{J}$ 2 Hz, ArH), 3.96 (3H, s, OMe), 3.92 (3H, s, OMe), 3.82 (3H, s, OMe), and 2.46 (3H, s, $ArCH_3$).

Metal catalysed Decyanation of the Cyanophenanthrene (68)

(i) Zinc: To a solution of 9-cyano-10-hydroxy-1,5,7-trimethoxy-3-methylphenanthrene (68) (109 mg) in glacial acetic acid (25 ml) was added Reformatsky grade zinc dust (3 g) and the mixture stirred at reflux for 8 days with fresh portions of zinc (3 g) being added after 13h, 24h, 48h and 5 days. After cooling, the mixture was filtered and the zinc washed with ethyl acetate (100 ml). The combined solutions were washed with water (100 ml), saturated sodium bicarbonate solution (3 x 60 ml) and water (60 ml), dried and evaporated to give a dark green oil. Chromatography on silica using methylene chloride as eluent gave starting material (47 mg, 43%) and the hydroxyphenanthrene (80) (33 mg, 33%) identical to that previously produced.

(ii) Tin: The foregoing procedure was repeated using the cyanophenanthrene (68) (105 mg) and powdered tin metal (2 g) in 75% formic acid solution (25 ml). After 48h the mixture was worked-up as before to give, after chromatography on silica using chloroform as eluent, starting material (40 mg, 38%) and the hydroxyphenathrene (80)(4 mg, 4%).

(iii) Magnesium: The foregoing procedure was repeated using the cyanophenanthrene (68) (80 mg) and magnesium filings (3.0 g) in isopropanol (25 ml) and acetic acid (5 ml). After 4 days the mixture was worked-up as before to give starting material (60 mg) as the sole product after chromatography on silica (methylene chloride as eluent).

Attempted Catalytic Hydrogenation of Cyanophenanthrene (68)

A solution of 9-cyano-10-hydroxy-1,5,7-trimethoxy-3-methylphenanthrene (68) (65 mg) in ethyl acetate (80 ml) was stirred over 10% palladium on charcoal catalyst (7 mg) under an atmosphere of hydrogen gas at atmospheric pressure. After 5 days the mixture was filtered, the catalyst washed with ethyl acetate (100 ml) and the combined solutions evaporated. Chromatography on silica (methylene chloride as eluent) gave starting material (55 mg) as the sole product.

10-Hydroxy-1,5,7-trimethoxy-3,9-dimethylphenanthrene (83)

A solution of 9-cyano-10-hydroxy-1,5,7,-trimethoxy-3-methylphenanthrene (45 mg) in ethyl acetate (35 ml) was hydrogenated over freshly prepared and dried Raney nickel catalyst (2 g) at 130 p.s.i. for 4 days. After this time, the mixture was filtered, the

catalyst washed with ethyl acetate (50 ml) and the combined solutions evaporated. The residue was purified by tlc on silica using chloroform as eluent to give the phenanthrene (83) (42 mg, 97%) as the sole product, m.p. 165-166.5° (Found: C, 73.1; H, 6.5%; M+, 312.138. $C_{19}H_{20}O_4$ requires C, 73.0; H, 6.5%; M, 312.136); ν_{max} ($CHCl_3$)3 380 br s, 3 010 m, 2 945 m, and 1 610 s cm^{-1}; δ_H 10.30 (1H, s, OH), 9.10 (1H, s, ArH), 6.95 (1H, d, J 2 Hz, ArH), 6.86 (1H, s, ArH), 6.63 (1H, d, J 2 Hz, ArH), 4.06 (3H, s, OMe), 4.05 (3H, s, OMe), 3.96 (3H, s, OMe), 2.51 (3H, s, $ArCH_3$), and 2.49 (3H, s, $ArCH_3$).

Hydride Reduction of the Cyanotetramethoxyphenanthrene (69)

(i) To a stirred suspension of lithium aluminium hydride (3 mg) in THF (10 ml) was added 9-cyano-1,5,7,10-tetramethoxy-3-methylphenanthene (50 mg) in THF (15 ml) and the mixture stirred at reflux for 3h. After cooling, 2N sodium hydroxide solution (2 ml) was added cautiously and the mixture filtered through Celite. The solvents were evaporated to give a yellow gum (43 mg) which by chromatography on silica was shown to have numerous unidentifiable components.

(ii) Lithium triethoxyaluminium hydride was prepared *in situ* by the addition by absolute ethanol (70 ul) to lithium aluminium hydride (15 mg) in THF (10 ml) at 0° under argon. After 10 minutes the tetramethoxyphenanthrene (69) (105 mg) in THF (10 ml) was added drop-wise and the mixture heated at reflux for 1h. After cooling 2N sodium hydroxide solution (2 ml) was added, the mixture filtered through Celite and the solvents evaporated to give a yellow gum. Analytical tlc indicated a similar composition to that previously obtained.

(iii) Diborane was generated *in situ* by the drop-wise addition of boron trifluoride-etherate (48% BF_3, 0.022 ml) in anhydrous diglyme (5 ml) to a solution of the tetramethoxyphenanthrene (69) (50 mg) in anhydrous diglyme (5 ml) containing sodium borohydride (7 mg). The lime green solution was stired for 2h and then glacial acetic acid (15 ml) was added and the mixture heated at reflux for a further 2h. After cooling, the mixture was poured onto water (75 ml) and extracted with ethyl acetate. This extract was washed repeatedly with saturated sodium bicarbonate solution and water, dried and evaporated to give a dark green oil (25 mg). Chromatography on silica using chloroform as eluent gave no recognisable product.

Deuteriation of 3,5-Dimethoxytoluene (84)

To a solution of 3,5-dimethoxytoluene (250 mg) in methanol-d_4 (1 ml) and deuterium oxide (2 ml) was added phosphorus tribromide (0.3 ml) and the mixture stirred at reflux for 14h. After cooling, the mixture was extracted with ether (3 x 3 ml). This extract was washed with water (3 x 3 ml), dried and evaporated to give the deuteriated toluene (0.22 g, 88%); δ_H 6.30 (0.15H, m, ArH), 3.75 (6H, s, OMe), and 2.26 (3H, s, $ArCH_3$); m/e 152 (3%), 153 (12.2), 154 (79.8), 155 (100).

Deuteriation of 10-Hydroxy-1,5,7-trimethoxy-3-methylphenanthrene (80)

The hydroxyphenanthrene (80) (26.5 mg) was dissolved in deuteriochloroform (0.25 ml) and deuterium oxide (D_2O) (0.75 ml) was added with stirring. After 5 minutes the solvents were evaporated and the residue was re-dissolved in methanol-d_4 (1 ml) and D_2O (2 ml) and

phosphorus tribromide (0.1 ml) was added. The mixture was heated at reflux for 12h. After cooling, the mixture was extracted with ether (2 x 5 ml) and the extract was washed with D_2O (3 x 2 ml), dried and evaporated. The residue was re-dissolved in methanol-d_4 and the cycle repeated a further twice, to furnish the deuterio-hydroxyphenanthrene (21 mg, 80%); δ_H 9.65 (1H, s, OH), 9.08 (0.10 H, s, H-4), 6.92 (0.43H, s, H-9), 6.81 (0.22H, s, H-2), 6.70 (0.48H, s, H-8), 6.65 (0.28H, s, ArH), 4.07 (3H, s, OMe), 4.05 (3H, s, OMe), 3.90 (3H, s, OMe), and 2.52 (3H, s, OMe); m/e 298 (3.6%), 299 (11.6), 300 (39.0), 301 (47.7), 302 (25.5), 303 (14.9).

Attempted Demethylation of the Hydroxyphenanthrene (80)

(i) via BBr_3 To a solution of 10-hydroxy-1,5,7-trimethoxy-3-methylphenanthrene (71 mg) in methylene chloride (20 ml) under nitrogen at -78° was added boron tribromide (1.5 ml). The solution was kept at this temperature for 6h and then allowed to rise to room temperature over 16h. Ether (30 ml) was added followed by water (10 ml). The organic layer was separated, washed with water (15 ml), dried and evaporated. The residue was immediately redissolved in pyridine (10 ml) and acetic anhydride (10 ml) and the solution stirred for 2h. The solution was then poured onto iced water (50 ml), stirred for 20 minutes and then extracted with ethyl acetate (2 x 30 ml). This extract was washed with 0.1N hydrochloric acid (3 x 40 ml), saturated sodium bicarbonate solution (3 x 40 ml) and water (40 ml), saturated sodium bicarbonate solution (3 x 40 ml) and water (40 ml), dried and evaporated to give a brown gum. Chromatography on silica using chloroform as eluent gave a diacetoxydimethoxyphenanthrene (85) (45 mg, 51%) as needles, m.p. 160-161° (from ether) (Found: M^+, 368.127. $C_{21}H_{20}O_6$ requires 368.125); ν_{max} 1 770 s and 1 610 s cm^{-1};

δ_H 9.31 (1H, s, ArH), 7.20 (1H, s ArH), 7.00 (1H, d, J 1 Hz, ArH), 6.70 (2H, m, ArH), 6.70 (2H, m, ArH), 4.02 (3H, s, OMe), 3.78 (3H, s, OMe), 2.55 (3H, s, $ArCH_3$), 2.35 (6H, s, Ac).

(ii) via in situ generation of trimethylsilyliodide: to a stirred suspension of dry sodium iodide (403 mg) in a solution of the hydroxyphenanthrene (80) (50 mg) in dry acetonitrile (10 ml) under a nitrogen atmosphere at room temperature was added drop-wise trimethylsilyl chloride (0.43 ml) in dry acetonitrile (8 ml). The stirred mixture was heated at reflux for 48h. On cooling, water (10 ml) was added and the mixture extracted with ether (2 x 40 ml) and the extract washed with 5% aqueous sodium thiosulphate solution (2 x 30 ml) and water (30 ml), dried and evaporated. The residue was immediately reacted with acetic anhydride (10 ml) and pyridine (10 ml) in accordance with the foregoing procedure. Chromatography in silica using chloroform as eluent gave 10-acetoxy-1,5,7-trimethoxy-3-methylphenanthrene (88) (35 mg), 68%) as a golden gum (Found: M^+, 340.135. $C_{20}H_{20}O_5$ requires: M, 340.1305); ν_{MAX} ($CHCl_3$) 1 770 s and 1 630 s cm^{-1}; δ_H 9.15 (1H, s, ArH), 6.90 (1H, s, ArH), 6.73 (1H, d, J 2 Hz, ArH), 6.66 (1H, d, J 2 Hz, ArH), 6.61 (1H, s, ArH), 4.01 (3H, s, OMe), 3.92 (3H, s, OMe), 3.85 (3H, s, OMe), 2.48 (3H, s, $ArCH_3$), and 2.40 (3H, s, Ac).

(iii) via trimethylsilyl iodide To a stirred solution of the hydroxyphenanthrene (80) (35mg) in Analytical Grade chloroform (10 ml) under a slight flow of nitrogen gas was added trimethylsilyl iodide (0.50 ml). The mixture was heated at reflux for 3 days during which time the solvent lost by evaporation was periodically replaced. On cooling, methanol (10 ml) was added and the mixture evaporated to dryness. The

residue was taken up in ether (15 ml) and washed with 5% aqueous sodium thiosulphate solution (2 x 20 ml) and water (10 ml), dried and evaporated. The residue was immediately reacted with acetic anhydide (6 ml) and pyridine (6 ml) in accordance with the foregoing procedure. Chromatography on silica using methylene chloride as eluent furnished 5,10-diacetoxy-7-ethoxy-1-methoxy-3-methylphenanthrene (89) (18 mg, 46%) as needles m.p. 179-80° (from acetone-hexane) (Found: M', 382.141. $C_{22}H_{22}O_6$ requires: M, 382.141) ν_{max} ($CHCl_3$) 3 010 m, 1 745 s, 1 660 s, and 1 630 s cm^{-1}; δ_H 8.53 (1H, s, ArH), 7.20 (1H, s, ArH), 7.09 (1H, d, J 2.5 Hz, ArH), 6.9 (1H, d, J 2.5 Hz, ArH), 6.86 (1H, s, ArH), 4.17 (2H, q, J 7 Hz, OCH_2CH_3), 3.91 (3H, s, OMe), 2.51 (3H, s, $ArCH_3$), 2.44 (3H, s, Ac), 2.38 (3H, s, Ac), and 1.42 (3H, t, J 7 Hz, OCH_2CH_3); for δ_H (200 MHz) see table 12.

(iv) via hydrogen bromide: A solution of the hydroxphenanthrene (80) (50 mg) in glacial acetic acid (5 ml) and 48% hydrobromic acid (7 ml) was stirred at 140° (bath temperature) for 20h. On cooling, water (20 ml) was added and the mixture extracted with ether (2 x 40 ml). This extract was washed with saturated sodium bicarbonate solution (3 x 20 ml) and brine (2 x 20 ml), dried and evaporated. The residue was immediately reacted with acetic anhydride (10 ml) and pyridine (10 ml) in accordance with the foregoing proceduring to give a dark, intractable tar (9 mg).

4-Iodo-2,5-dimethoxytoluene (93)

To a suspension of 2,5-dimethoxytoluene (6.4 g) and silver triflruoacetate (9.4 g) in chloroform was added iodine (10.8 g) in chloroform (150 ml) drop-wise and with stirring. After complete addition,

stirring was continued for 1h. The mixture was filtered, the filtrate washed with chloroform (100 ml) and the combined solutions were dried and evaporated to give the iodotoluene which was recrystallised as needles (8.3 g, 71%) (from methanol), m.p. 85° (lit[88].,85°); ν_{max} 2 960 m, 2 845 m, and 1 490 s cm^{-1}; δ_H 7.18 (1H, s, ArH), 6.68 (1H, s, ArH), 3.80 (3H, s, OMe), 3.75 (3H, s, OMe), and 2.15 (3H, s, $ArCH_3$); m/e 278 (M).

2,5-Dimethoxy-4-methylbenzoic acid[103] (94)

n-Butyl lithium (20 ml, 1.6M in hexane) was added to a solution of 4-iodo-2,5-dimethoxytoluene (8.1 g) in petroleum ether (60-80°) (250 ml) with stirring at 0° under nitrogen. After 1h the mixture was poured onto crushed dry ice (600 g) under argon. After 2h the mixture was acidified with 3N hydrochloric acid and extracted with ether (3 x 150 ml). The ethereal solution was re-extracted with 15% sodium carbonate solution (3 x 150 ml) which was then acidified with concentrated hydrochloric acid. The white precipitate was collected and recrystallised to give the acid as needles (3.2 g, 55%), m.p. 126-217° (lit.,[103] 125-126°) (from water); ν_{max} 2 960 br m, 2 600 br m, 1 735 s, and 1 695 s cm^{-1}; δ_H ($(CD_3)_2CO$) 7.41 (1H, s, ArH), 7.09 (1H, s, ArH), 4.00 (3H, s, OMe), 3.82 (3H, s, OMe), and 2.22 (3H, s, ArH); m/e 169 (M).

Methyl 2,5-Dimethoxy-4-methylbenzoate (91)

A solution of 2,5-dimethoxy-4-methylbenzoic acid (3.0 g) in dry methanol (150 ml) was saturated with dry hydrogen chloride and the mixture heated at reflux for 16h. After cooling, the solvent was evaporated and the residue crystallised from ethyl acetate-petroleum ether (60-80°) as

cubes; δ_H 7.38 (1H, s, ArH), 6.79 (1H, s, ArH), 3.77 (3H, s, OMe), 3.75 (3H, s, OMe), 3.70 (3H, s, CO_2Me), and 2.22 (3H, s, $ArCH_3$); m/e 210 (M).

4-(2-Cyano-2-(3,5-dimethoxyphenyl)acetyl-2,5-dimethoxytoluene (90)

Prepared in a manner similar to that for the β-ketonitrile (56) above using 3,5-dimethoxyphenylacetonitrile (1.77 g) and methyl 2,5-dimethoxy-4-methylbenzoate (2.31 g) to furnish the β-ketonitrile (90) as plates (1.7 g, 48%) m.p. 120-121° (from acetone-hexane) (Found: C, 67.7; H, 6.0; N, 3.9; M', 355.144. $C_{20}H_{21}NO_5$ requires C, 67.6; H, 5.9; N, 3.9; M, 355.142); ν_{max} 2 842 m, 1 675 s, and 2 615 s cm^{-1}; δ_H 7.20 (1H, s, ArH), 6.80 (1H, s, ArH), 6.51 (2H, d, J 2 Hz, ArH), 6.40 (1H, t, J 2 Hz, ArH), 5.95 (1H, s, CH), 3.95 (3H, s, OMe), 3.80 (3H, s, OMe), 3.78 (6H, s, OMe), and 2.26 (3H, s, $ArCH_3$).

4-(1-Acetoxy-2-cyano-2-(3,5-dimethoxyphenyl)ethenyl)-2,5-dimethoxytoluene(95)

Prepared in a similar manner to that for the enol acetate (59) above using the β-ketonitrile (90) (1.1 g) to give the enol acetate (95) as a pale oil (0.97 g, 79%) after flash chromatography on silica using chloroform as eluent (E:Z= 3:2) (Found: M^+, 397.152. $C_{22}H_{23}NO_6$ requires 397.152) ν_{max} ($CHCl_3$) 2 920 m, 2 221 m, 1 778 s, and 1 600 s cm^{-1}; δ_H (E isomer) 7.00 (1H, s, ArH), 6.65 (1H, s, ArH), 6.70 (2H, d, J 2 Hz, ArH), 6.45 (1H, t, J 2 Hz, ArH), 3.85 (3H, s, OMe), 3.83 (3H, s, OMe), 3.79 (6H, s, OMe), 2.32 (3H, s, $ArCH_3$) and 2.05 (3H, s, Ac); δ_H (Z isomer) 7.18 (1H, s, ArH), 6.79 (1H, s, ArH), 6.35 (3H, m, ArH), 3.60 (9H, s, OMe), 3.55 (3H, s, OMe), 2.35 (3H, s, $ArCH_3$), and 2.17 (3H, s, Ac).

Irradiation of 4-(1-Acetoxy-2-cyano-2-(3,5-dimethoxyphenyl)ethenyl)-2,5-dimethoxytoluene (95)

The enol acetate (95) (0.91 g) in benzene (800 ml) containing iodine (650 mg) was irradiated as described for the enol ether (58). The crude product residue was crystallised from methanol to give 10-acetoxy-9-cyano-2,5,7-trimethoxy-3-methylphenanthrene (96) (0.45 g, 50%), m.p. 261-262° (from chloroform-hexane) (Found: C, 69.2; H, 5.2; N, 3.7%; M', 365.128. $C_{21}H_{19}NO_5$ requires C, 69.0; H, 5.2; N, 3.8%; M, 365.126); ν_{max} 2 945 m, 2 220 m, 1 770 s, and 1 620 s cm^{-1}; δ_H 9.25 (1H, s, ArH), 7.18 (1H, m, ArH), 7.05 (1H, s, ArH), 6.70 (1H, d, J 2 Hz, ArH), 4.01 (3H, s, OMe), 3.90 (3H, s, OMe), 3.88 (3H, s, OMe), 2.50 (3H, s, $ArCH_3$), and 2.38 (3H, s, Ac).

Methyl 2,6-Dihydroxy-4-methylbenzoate (90)

A solution of 2,6-dihydroxy-4-methylbenzoic acid (40 g) in dry acetone (200 ml) was treated with anhydrous sodium bicarbonate (27 g) and dimethyl sulphate (28 ml). The mixture was stirred at reflux for 16h. After cooling, the mixture was filtered and the filtrate concentrated to ca.25 ml and treated with methanol (60 ml). Large needles of the ester separated (32.3 g, 76%), m.p. 95-97° (lit.,[90] 98-99°); ν_{max} 3 425 s and 1 668 s cm^{-1}; δ_H ($(CD_3)_2CO$) 6.22 (2H, s, ArH), 4.05 (3H, s, CO_2Me), and 2.23 (3H, s, $ArCH_3$); m/e 182 (M).

Methyl 3-Formyl-2,6-dihydroxy-4-methylbenzoate[39](114)

Aluminium chloride (8 g) in anhydrous ether (50 ml) was added with mechanical stirring to a solution of methyl 2,6-dihydroxy-4-methylbenzoate (3.8 g) in ether (75 ml) containing zinc cyanide (7.5 g) at 0°. The mixture was saturated with hydrogen chloride at 0°, and after 3h the crystalline complex had separated and was filtered off, washed with ether, dissolved in water (500 ml) and left overnight. The aldehyde was produced as pale yellow needles (3.1 g, 71%), m.p. 148-149° (lit[39]., 147°) (from methanol); ν_{max} l 667s and l 640 cm^{-1}; δ_H 13.97 (1H, s, OH), 12.11 (1H, s, OH), 10.18 (1H, s, CHO), 6.48 (1H, s, ArH), 3.96 (3H, s, CO_2Me), and 2.58 (3H, s, $ArCH_3$); m/e 210 (M).

Methyl 3-Formyl-2,6-dihydroxy-5-iodo-4-methylbenzoate

To a solution of methyl 3-formyl-2,6-dihydroxy-4-methylbenzoate (4.2 g) in dimethylformamide (150 ml) containing sodium iodide (3.3 g) was added chloramine T (196 mg) with stirring. After 1.5h the mixture was poured onto water (300 ml), acidified with 2N hydrochloric acid and extracted with ethyl acetate (3 x 150 ml). This extract was washed with 5% sodium thiosulphate solution (2 x 300 ml) and brine (300 ml), dried and evaporated. The residue was crystallised from methanol to give the iodoaldehyde (5.15 g, 77%). A small portion of this was further recrystallised from chloroform-hexane to give needles, m.p. 183.5-184.5° (Found: C, 35.7, H, 2.6; I, 37.6%; $\underline{M}^+$, 335.950. $C_{10}H_9IO_5$ requires C, 35.7, H, 2.7 I, 37.8%; $\underline{M}$, 335.949); ν_{max} 2 750 br m, l 650 s, and l 618 cm^{-1}; δ_H ($(CD_3)_2CO$) 10.17 (1H, s, CHO), 4.00 (3H, s, CO_2Me), and 2.70 (3H, s, $ArCH_3$).

Methyl 3-Formyl-5-iodo-2,6-dimethoxy-4-methylbenzoate (116)

A solution of methyl 3-formyl-2,6-dihydroxy-5-iodo-4-methylbenzoate (3.7 g) in dry acetone (350 ml) containing anhydrous potassium carbonate (18 g) and dimethyl sulphate (2.5 ml) was stirred at reflux for 7h. After cooling the mixture was filtered and the potassium carbonate washed with acetone (150 ml). The combined acetone solutions were evaporated and the residue taken up in ether (150 ml). This ethereal solution was washed with concentrated ammonia solution (2 x 70 ml) and water (70 ml), dried and evaporated. The residue was purified by flash chromatography on silica using methylene chloride as eluent to give the dimethoxyaldehyde as a crystalline solid (2.9 g, 55%), m.p. 94-95° (from chloroform-hexane) (Found: C, 39.5; H, 3.4; I, 34.8%; $\underline{M}^+$, 363.981. $C_{12}H_{12}IO_5$ requires C, 39.6; H, 3.6; I, 34.85%; $\underline{M}$, 363.980); ν_{max} 2 975 m, 1 735 s, 1 692 s, 1 680 s, and 1 610 s cm^{-1}; δ_H 10.20 (1H, s, CHO), 3.95 (3H, s, CO_2Me), 3.88 (6H, s, OMe), and 2.72 (3H, s, $ArCH_3$).

Methyl 3-Hydroxymethyl-5-iodo-2,6-dimethoxy-4-methylbenzoate (113)

Sodium borohydride (2.5 g) was added portion-wise over 1h to a solution of methyl 3-formyl-5-iodo-2,6-dimethoxy-4-methylbenzoate (2.0 g) in methanol (70 ml) at 0° with stirring. The solution was poured onto 2N dilute hydrochloric acid (200 ml) and extracted with ether. This extract was washed with water (50 ml), dried and evaporated to give an oil which was distilled to give the hydroxymethylaldehyde (1.7 g, 85%) as a colourless

oil, b.p. 185° at 0.1 mm (Found: M', 365.996. $C_{12}H_{13}IO_5$ requires M, 365.996); ν_{max} (neat) 2 940 m, 1 742 s, and 1 580 s cm^{-1}; δ_H 4.66 (2H, s, CH_2), 4.15 (1H, br s, OH), 3.85 (3H, s, CO_2Me), 3.77 (3H, s, OMe), 3.75 (3H, s, OMe), and 2.55 (3H, s, $ArCH_3$).

Methyl 5-Iodo-2,6-dimethoxy-4-methyl-3-tetrahydropyranyloxymethylbenzoate (121)

To a solution of methyl 3-hydroxymethyl-5-iodo-2,6-dimethoxy-4-methylbenzoate (1.2 g) in methylene chloride (20 ml) containing dihydropyran (1.5 ml) at 0° was added p-toluenesculphonic acid (6 mg) with stirring. After 10 minutes the cooling bath was removed and stirring continued for 1.5h, after which time the mixture was partitioned between ether (50 ml) and a solution made from saturated brine solution (10 ml), saturated sodium bicarbonate solution (10 ml) and water (20 ml). The ether layer was washed with brine (2 x 35 ml), dried and evaporated to give a pale yellow oil. Flash chromatography on silica using methylene chloride as eluent gave the THP ether (121) as a colourless oil (1.4 g, 98%), b.p. 180° at 0.2 mm (decomp.) (Found: M^+, 450.055. $C_{17}H_{23}IO_6$ requires M. 450.053); ν_{max} ($CHCl_3$) 3 000 m, 2 945 m, and 1 740 s cm^{-1}; δ_H 4.72 (1H, m, CH), 4.70 (2H, ABq, J 48 Hz, CH_2), 3.92 (3H, s, CO_2Me), 3.83 (6H, s, OMe), 3.70-3.40 (2H, m, CH_2O), 2.59 (3H, s, $ArCH_3$), and 1.80-1.35 (6H, m, aliphatic H).

Methyl 5-Iodo-2,6-dimethoxy-3-methoxymethyl-4-methylbenzoate (123)

A solution of methyl 3-hydroxymethyl-5-iodo-2,6-dimethoxy-4-methylbenzoate (0.75 g) in dry DMF (15 ml) was added drop-wise to a stirred solution of sodium hydride (0.1 g, 50% dispersion in oil) and methyl iodide (1.2 ml) in DMF (15 ml). After 2.5h, water (70 ml) was added and the mixture extracted with ether (3 x 70 ml). This extract was dried and evaporated to give the pale oil which was distilled to give the methyl ether (123) (0.71 g, 90%) as a viscous oil, b.p. 180° at 3mm (Found: $\underline{M}^+$, 380.012. $C_{13}H_{17}IO_5$ requires $\underline{M}$, 380.012); ν_{max} (neat) 2 940 m, 1 786 s, and 1 580 s cm^{-1}; δ_H 4.59 (2H, s, CH_2), 3.92 (3H, s, CO_2Me), 3.84 (6H, s, OMe), 3.41 (3H, s, OMe), and 2.58 (3H, s, $ArCH_3$).

Methyl 3-Formyl-2,6-dimethoxy-4-methylbenzoate

Methyl 3-formyl-2,6-dihydroxy-4-methylbenzoate (1.82 g) was methylated in a similar manner to the iodo-ester (116) by treatment with dimethyl sulphate (2.0 ml) and anhydrous potassium carbonate (5 g) to give the dimethoxy-ester 1.99 g, 97%) as cubes, m.p. 95.5-96° (from ether) (Found: C, 60.5; H, 5.7%; $\underline{M}^+$, 238.085. $C_{12}H_{14}O_5$ requires C, 60.5; H, 5.9%; $\underline{M}$, 238.084); ν_{max} 2 955 m, 1 740 s, 1 680 s, and 1 602 s cm^{-1}; δ_H 10.32 (1H, s, CHO), 6.52 (1H, s, ArH), 3.90 (6H, s, CO_2Me and OMe), 3.88 (3H, s, OMe), and 2.59 (3H, s, $ArCH_3$).

Methyl 3-Formyloxy-2,6-dimethoxy-4-methylbenzoate (118)

A solution of methyl 3-formyloxy-2,6-dimethoxy-4-methylbenzoate (1.68 g) in methylene chloride (60 ml) containing m-chloroperbenzoic acid (2.15 g, 88%) was heated at reflux for seven hours after which time the solvents were evaporated. The residue was taken up in ether (100 ml) and washed with saturated sodium bicarbonate solution (2 x 40 ml) and brine (40 ml), dried and evaporated to give the formate ester as a pale oil (1.70 g, 98%) (Found: M^+, 254.079. $C_{12}H_{14}O_6$ requires M, 254.079); ν_{max} ($CHCl_3$) 2 975 m, 1 735 br s, and 1 610 s cm^{-1}; δ_H 8.22 (1H, s, CHO), 6.55 (1H, s, ArH), 3.91 (3H, s, CO_2Me), 3.80 (6H, s, OMe), and 2.20 (3H, s, $ArCH_3$).

Methyl 3-Hydroxy-2,6-dimethoxy-4-methylbenzoate (119)

A solution of methyl 3-formyloxy-2,6-dimethoxy-4-methylbenzoic acid (1.7 0g) in methanol (35 ml) containing 10% potassium hydroxide solution (8 ml) was stirred for 1h, then poured onto 3N hydrochloric acid (75 ml) and extracted with ether (3 x 30 ml). This extract was washed with brine (50 ml), dried and evaporated to give the phenol as a crystalline mass (1.34 g, 89%). Recrystallised as needles, m.p. 108° (from petroleum ether (60-80°)) (Found: M^+ 226.084. $C_{11}H_{14}O_5$ requires M, 226.084); ν_{max} 3 540 br m, 1 775 s, and 640 s cm^{-1}; δ_H 6.48 (1H, s, ArH), 3.91 (3H, s, CO_2Me), 3.82(3H, s, OMe), 3.76(3H, s, OMe), and 2.25 (3H, s, ArH).

Methy 2,3,6-Trimethoxy-4-methylbenzoate (120)

Prepared by methylation of methyl 3-hydroxy-2,6-dimethoxy-4-methylbenzoate (1.24 g) in a similar manner to the iodo-ester (116) by treatment with dimethyl sulphate (1 ml) and anhydrous potassium carbonate (3 g) to give the trimethoxy-ester (1.29 g, 98%) as a clear oil after flash chromatography on silica using chloroform as eluent, b.p. 186° at 0.1 mm (Found: $\underline{M}^+$, 240.100. $C_{12}H_{16}O_5$ requires 240.099); ν_{max} (neat) 2940 m and 1 745 s cm^{-1}; δ_H 6.44 (1H, s, ArH), 3.88 (6H, s, CO_2Me and OMe), 3.75 (6H, s, OMe), and 2.24 (3H, s, $ArCH_3$).

Methyl 5-Iodo-2,3,6-trimethoxy-4-methybenzoate (112)

To a solution of methyl 2,3,6-trimethoxy-4-methylbenzoate (1.28 g) in chloroform (10 ml) containing silver trifluoroacetate (1.11g) was added iodine (1.28 g) in chloroform (20 ml) drop-wise and with stirring over 2h. Stirring was continued for a further 1h and then the mixture was filtered, washed with 5% sodium thiosulphate solution (2 x 30 ml) and brine (2 x 30 ml), dried and evaporated to give a pale oil. Distillation gave the iodo-ester (1.46 g, 86%) as a clear liquid, b.p. 146° at 0.1 mm (Found $\underline{M}^+$, 365.996. $C_{12}H_{15}IO_5$ requires $\underline{M}$, 365.996); ν_{max} (NaCl)2 950 m and 1 765 s cm^{-1}; δ_H 3.91 (3H, s, CO_2Me), 3.88 (3H, s, OMe), 3.80(3H, s, Ome), 3.78(3H, s, OMe), and 2.40 (3H, s, $ArCH_3$).

Methyl 2,6-Dimethoxy-3-methoxymethyl-4-methylbenzoate (123)

Sodium borohydride (2.0 g) was added portion-wise over 30 minutes to a solution of methyl 3-formyl-2,6-dimethoxy-4-methylbenzoate in methanol (50 ml) at 0° with stirring. After a further 1h the mixture was poured onto 3N hydrochloric acid (150 ml) and extracted with ether (3 x 50 ml). This extract was dried and evaporated to give a pale oil. The oil was taken up in dry DMF (10 ml) and added drop-wise to a solution of sodium hydride (0.13 g, 50% dispersion in oil) and methyl iodide in DMF (15 ml). After 1h, this was poured onto water (100 ml) and extracted with ether (2 x 50 ml). This extract was dried and evaporated to give the methyl ether as a crystalline mass (0.98 g, 91%). Recrystallised from petroleum ether (60-80°) as plates, m.p. 68-69° (Found: C, 61.4; H, 7.2%; $\underline{M}^+$, 254.116. $C_{13}H_{18}O_5$ requires C, 61.4; H, 7.15%; $\underline{M}$, 254.115); ν_{max} 1 745 s and 1 738 s cm^{-1}; δ_H 6.55 (1H, s, ArH), 4.40 (2H, s, CH_2), 3.89 (3H, s, CO_2Me), 3.81 (3H, s, OMe), 3.79 (3H, s, OMe), 3.48 (3H, s, OMe) and 2.39 (3H, s, $ArCH_3$).

Attempted condensation of 3,5-Dimethoxylphenylacetonitrile (55) with Polysubstituted Benzoate Esters (121), (122), (112), (123) and (120).

(i) n-Butyl lithium (3.66 ml, 1.6M in hexane) was added to THF (10 ml) at 0° under nitrogen with stirring. 3,5-Dimethoxyphenylacetonitrile (0.49 g) in THF (10 ml) was added drop-wise during 5 minutes. After 1h methyl 5-iodo-2,6-dimethoxy-4-methyl-3-tetrahydropyranyloxymethylbenzoate (126) (1.38 g) in THF (10 ml) was added drop-wise and the mixture stirred at reflux for 3h. After cooling, 3M hydrochloric acid (25 ml) was added and the organic solvents were evaporated. The residue was extracted with

ethyl acetate (2 x 40 ml) and the extract was dried and evaporated to give a brown oil. Column chromatography on silica (ether as eluent) gave an amount of polymeric material, nitrile starting material and ester starting material only.

(ii) The foregoing procedure was repeated using 3,5-dimethoxyphenylacetonitrile (0.27 g) and methyl 5-iodo-2,6-dimethoxy-3-methoxymethyl-4-methylbenzoate (122) (0.86 g). The mixture was worked-up as before to give a large amount of polymeric material, nitrile starting material (85 mg) and ester starting material (480 mg) only.

(iii) The foregoing procedure was repeated using 3,5-dimethoxyphenylacetonitrile (0.44 g) and methyl 5-iodo-2,3,6-trimethoxy-4-methylbenzoate (112) (1.36 g). The mixture was worked-up as before to give polymeric material, nitrile starting material (0.11 g) and ester starting material (0.90 g) only.

(iv) The foregoing procedure was repeated using 3,5-dimethoxyphenylacetonitrile (0.57 g) and methyl 2,6-dimethoxy-3-methoxymethyl-4-methylbenzoate (123) (1.00 g). The mixture was worked-up as before to give a large amount of polymeric material and ester starting material (0.62 g) only.

(v) The foregoing procedure was repeated using 3,5-dimethoxyphenylacetonitrile (2.36 g) and methyl 2,3,6-trimethoxy-4-methylbenzoate (120) (3.52 g). The mixture was worked-up as before to give a large amount of polymeric material, nitrile starting material (0.52 g), and ester starting material (2.1 g) only.

5-Iodo-2,3,6-trimethoxy-4-methylbenzoic acid (124)

Methyl 5-iodo-2,3,6-trimethoxy-4-methylbenzoate (114 mg) was dissolved in methanol (3 ml) and water (2 ml) containing sodium hydroxide (1 g) and the mixture heated at reflux for 1.5h. After cooling, water (10 ml) was added and the mixture acidified with concentrated hydrochloric acid. This mixture was extracted with ethyl acetate (2 x 15 ml) and the extract washed with water (15 ml), dried and evaporated to give an oil (104 mg, 95%). The acid was purified by sublimation to give needles, m.p. 152-153° (Found, $\underline{M}^+$, 352. $C_{11}H_{13}IO_5$ requires $\underline{M}$, 352); ν_{max} 3 000 br s and 1 705 s cm^{-1}; δ_H 3.95 (3H, s, OMe), 3.90(3H, s, OMe), 3.80(3H, s, OMe), and 2.42(3H, s, $ArCH_3$).

Bromination of 10-Acetoxy-9-cyano-1,5,7-trimethoxy-4-methylphenanthrene (60):

At 40°: To a solution of the acetoxyphenanthrene (60)(150 mg, 1 mol.) in glacial acetic acid (15 ml) at 40° was added bromine (2.45 ml, 1% in glacial acetic acid, 1.1 mol.) and the mixture stirred for 2h and then poured onto water (75 ml). This was extracted with ethyl acetate (2 x 50 ml) and the extract washed with saturated sodium bicarbonate solution (3 x 60 ml) and water (2 x 60 ml), dried and evaporated to give a yellow solid. Purification by tlc on silica (using benzene-chloroform, 3:2 as eluent) gave:

10-acetoxy-2-bromo-9-cyano-1,5,7-trimethoxy-3-methylphenanthrene (128)

(97 mg, 53%), m.p. 231-233° (from methylene chloride-cyclohexane) (Found: C,56.5; H, 4.0; N, 3.2; Br, 18.2%; M$^+$ 443.036 and 445.035. $C_{21}H_{18}NO_5Br$ requires C, 56.8; H, 4.1; N, 3.15; Br, 18.0%; M, 443.036 and 445.034); ν_{max} 3 350 br m, 2 225 m, 1 760 s and 1 620 s cm^{-1}; δ_H 9.30 (1H, s, 4-H), 7.19(1H, d, J 2.5 Hz, 8-H), 6.76 (1H, d, J 2.5 Hz, H-6), 4.04 (3H, s, OMe), 3.97(3H, s, OMe), 3.90 (3H, s, OMe), 2.62 (3H, s, $ArCH_3$), and 2.52 (3H, s, Ac); for δ_H(200 MHz) see table 11.

10-acetoxy-6-bromo-9-cyano-1,5,7-trimethoxy-3-methylphenanthrene (126)

(67 mg, 37%) m.p. 249-251° (from methylene chloride-hexane) (Found:C,56.7; H,4.0; N,3.3; Br,18.2%; M$^+$ 443.036 and 445.038. $C_{21}H_{18}NO_5Br$ requires C,56.8; H, 4.1; N,3.15; Br, 18.0%; M, 443.036 and 445.034); ν_{max} 3 420 br m, 2 220 m, 1 770 s, and 1 602 s cm^{-1}; δ_H 9.04(1H, s, 4-H), 7.38 (1H, s, 8-H), 6.89 (1H, s, 2-H), 4.08 (3H, s, OMe), 3.97 (3H, s, OMe), 3.81 (3H, s, OMe), 2.57 (3H, s, $ArCH_3$), and 2.48 (3H, s, Ac); for δ_H(200 MHz) see table 11.

10-acetoxy-4,6-dibromo-9-cyano-1,5,7-trimethoxy-3-methylphenanthrene (127)

(21 mg,10%) m.p. 243-244° (from methylene chloride-hexane) (Found:C, 48.3; H, 3.4; N, 2.7; Br, 30.6%; M$^+$ 520.947, 522.945 and 524.944. $C_{21}H_{17}NO_5Br_2$ requires C, 48.2; H, 3.3; N, 2.7; Br, 30.55%; M, 520.947, 522.945 and 524.943); ν_{max} 3 420 br m, 2 225 m, 1 775 s, and 1 595 s cm^{-1}; δ_H 7.25 (1H, s, 8-H), 6.91 (1H, s, 2-H), 4.09 (3H, s, OMe),

3.97 (3H, s, OMe), 3.28 (3H, s, OMe), 2.64 (3H, s, $ArCH_3$), and 2.46 (3H, s, Ac); for δ_H(200 MHz) see table 11.

At 70°: (i) The foregoing procedure was repeated at 70° using the acetoxyphenanthrene (60) (101 mg, 1 mol.) and bromine (1.6 ml, 1% in glacial acetic acid, 1.1 mol.). The mixture was worked-up as before to give the 2-bromophenanthrene (128) (18 mg, 15%), the 6-bromophenanthrene (126) (49 mg, 40%), the 4,6-dibromophenanthrene (127) (29 mg, 20%), and 10-acetoxy-2,6-dibromo-9-cyano-1,5,7-trimethoxy-3-methylphenanthrene (129) (7 mg, 5%) m.p. 205-207° (Found: C, 48.25; H,3.3;, N,2.7; Br, 30.7%; M', 520.948, 522.945 and 524.945. $C_{21}H_{17}NO_5Br_2$ requires C, 48.2; H, 3.3; N, 2.7; Br, 30.55%; M, 520.947, 522.945 and 524.943); ν_{max} 3 420 br m, 2 215 m, 1 780 s, and 1 595 s cm^{-1}; δ_H 9.31 (1H, s, 4-H), 7.38 (1H, s, H-8), 4.08 (3H, s, OMe), 3.93 (3H, s, OMe), 3.81 (3H, s, OMe), 2.64 (3H, s, $ArCH_3$), and 2.52 (3H, s, Ac).

(ii) The foregoing procedure was repeated using the acetoxyphenanthrene (60) (100 mg, 1 mol.) and bromine (3 ml, 1% in glacial acetic acid, 2.2 mol.). The mixture was worked-up as before to give the 2-bromophenanthrene (128) (36 mg, 30%), the 6-bromophenanthrene (126) (36 mg, 30%), the 2,6-dibromophenanthrene (129) (31 mg, 22%) and the 2,6-dibromophenanthrene (127) (7 mg, 5%), identical to samples previously produced.

At 120° (i) The foregoing procedure was repeated as 120° using the acetoxyphenanthrene (60) (99 mg, 1 mol.). The mixture was worked up as before to give the 2,6-dibromophenanthrene (129) (57 mg, 43%), the acetoxyphenanthrene (60) (43 mg, 43%), and trace amounts of the

2-bromophenanthrene (128) the 6-bromophenanthrene (124) and the 4,6-dibromophenanthrene (127) by nmr analysis.

(ii) The foregoing procedure was repeated using the acetoxyphenanthrene (60) (100 mg, 1 mol.) and bromine (3.0 ml, 1% in glacial acetic acid, 1 mol.). The mixture was worked up as before to give the 2,6-dibromophenathrene (129) (57 mg, 43%), the acetoxyphenanthrene (50) (43 mg, 43%), and trace amounts of the 2-bromophenanthrene (128) the 6-bromophenanthrene (124) and the 4,6-dibromophenanthrene (127).

Disproportionation of the Bromphenanthrenes (128) and (126)

A solution of 10-acetoxy-2-bromo-9-cyano-1,5,7-trimethoxy-3-methylphenanthrene (22 mg) and 10-acetoxy-6-bromo-9-cyano-1,6,7-trimethoxy-3-methylphenanthrene (28 mg) in 20% (w/v) hydrogen bromide solution in glacial acetic acid (5 ml) was heated at reflux for 1.5h. After cooling the mixture was poured onto water (25 ml) and the precipitate extracted with ethyl acetate (2 x 20 ml). This extract was washed with saturated sodium bicarbonate solution (2 x 30 ml) and water (30 ml), dried and evaporated to give a brown oil which solidified on standing. This was shown by nmr to consist of the acetoxyphenanthrene (60) (17%), the 2-bromide (128) (17%), the 6-bromide (126) (17%) and the 2,6-dibromide (129) (49%).

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