THE SYNTHESIS OF POTENTIAL ANTITUMOUR COMPOUNDS.

by

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I would like to dedicate this thesis to my mother and father for their encouragement and understanding throughout the years.

Summary

The work carried out in this thesis concerned the synthesis of the fungal metabolite, duclauxin, and other potential antitumour compounds.

The synthetic approach to duclauxin was to synthesise a pyrano[1,8-*c*]naphthalen-1(3*H*)-one derivative and then dimerise it to a derivative of duclauxin. The key step in the synthesis was treatment of the ethylene acetal of acetoacetyl chloride with the anion of methyl 3,5-dimethoxyphenylacetate to give the 5-ethylene acetal of methyl 2-(3,5-dimethoxyphenyl)-3,5-dioxohexanoate. Treatment of this acetal with acid gave methyl 2-hydroxy-5,7-dimethoxy-4-methyl naphthalene-1-carboxylate as the only product. This naphthalene was methylated to give methyl 2,5,7-trimethoxy-4-methyl naphthalene-1-carboxylate which when treated with methoxyacetyl chloride and tin(IV)chloride gave the desired lactone, 4,6,9-trimethoxy-7-methyl pyrano[1,8-*c*]naphthalen-1(3*H*)-one. Boron tribromide and aluminium chloride were used to cleave one of the methoxy ethers in this lactone.

A series of potential antitumour, phosphorus heterocycles were prepared. Their activities will be tested and compared. The most important step in their synthesis was treatment of the appropriate ester with the anion of ethyl phosphonic acid bis(dimethylamide) and then treating this β-ketophosphonamide with boron tribromide to give the corresponding cyclic phosphonate.

The synthesis of the fungal metabolite, differanisole A, another potential antitumour compound, was completed. The main difficulty in this synthesis was the first step which was to cleave the ethyl ester group of ethyl 3,5-dichloro-2-hydroxy-4-methoxy-6-propylbenzoate. This was achieved by treatment with cold concentrated sulphuric acid at $0^{o}C$ for six days to give 3,5-dichloro-2-hydroxy-4-methoxy-6-propylbenzoic acid, differanisole A.

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CHAPTER 1.

1.1 Introduction to the Biosynthesis of Natural Products.

For many centuries people have been isolating and using natural products for various purposes.

Primitive[1] as well as civilised societies have extensively used particular plants and plant preparations to relieve pain, kill infections and to poison their enemies. It was found that these natural compounds were different from metal salts and rock materials in their properties and elemental compositions. This led to the classification of organic and inorganic chemistry in the early nineteenth century.

Natural products were indeed the sole basis of organic chemistry up until the middle of the nineteenth century. The synthesis of organic compounds was developing but the main stimulus was from the challenge of natural compounds. Indeed even in modern day society natural products and their analogues have a very important role where drug design[2] has been helped by the increasing understanding of biochemical metabolism and biosynthesis.

Living organisms produce both primary and secondary metabolites, primary metabolites[3] being the fundamental building blocks of living matter (i.e. polysaccharides, proteins, fats and nucleic acids) whereas secondary metabolites are not essential for the existence of the individual (e.g. terpenes, alkaloids

and some pigments). It is not clearly understood[4] why secondary metabolites are produced but one theory is that they are detoxification products of poisonous or overabundant metabolites. Another idea is that these metabolites are used as a store of energy and food in plants which can be used in times of need. However a lot is known about their chemistry and how they are formed.

Fungi[4] produce many secondary metabolites and many hundreds [illegible] now [illegible] is very convenient for the chemist since fungi can be grown on artificial media in the laboratory then extracted to give secondary metabolites. Fungi are members of part of the plant kingdom called Thallophyta, which comprises of organisms with no roots, stems or leaves. They cannot utilise carbon dioxide and water in photosynthesis to give them energy sources so instead they use food obtained from dead or living plants, animals or micro-organisms.

There are four classes of fungi which are:

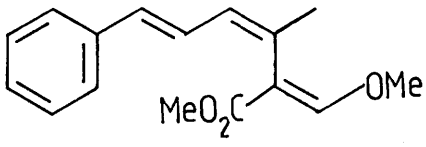
(a) The Phycomycetes which are the most primitive class of fungi, and are often unicellular e.g. *Rhizopus* (which forms on bread).

(b) The Ascomycetes range from unicellular yeasts to species with large fruiting bodies. Many are parasitic on plants and some yeasts are even responsible for causing human disease e.g. *Claviceps purpurea.*

(c) The Basidiomycetes are the highest class of fungi and they bear their species on basidia e.g. mushrooms, bracket fungi, puff-balls.

(d) Fungi Imperfecti include organisms for which no sexual stage has been observed (the Ascomycetes and the Basidiomycetes assign an organism depending upon the nature of its sexual spores). These form asexual spores borne on conidiophores. Examples include *Penicillia* and *Aspergilli*, where these cause the grey-green growth on "mouldy" food. There also appears to be certain species[4] of this class which are conidic forms of the Ascomycetes e.g. *Fusarium moniliforme* is the conidic form of *Gibberella fujikuroi*.

Antifungal products[5] are also isolated from plants. They arise in plants, either as pre-formed secondary metabolites or from pathogen-induced metabolism. An example of one of these natural fungicides is strobilurin A (1)[6] which is found in the basidiomycete fungi *Oudemansiella mucida* and *Strobilurus tenacellus*.

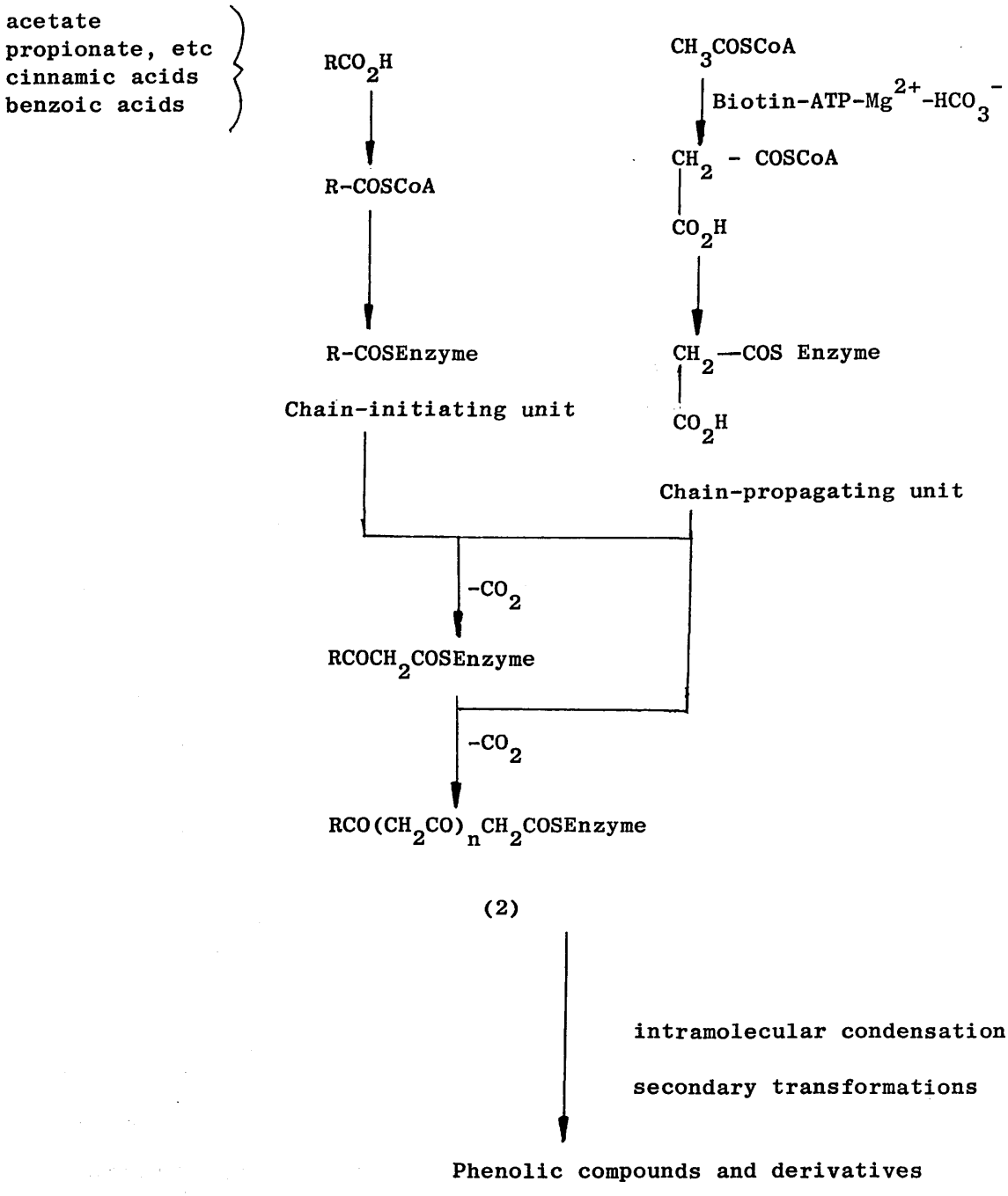


(1)

The polyketide biosynthetic route leads almost exclusively to fungal metabolites. However even amongst the fungi it is only characteristic to secondary metabolites of the Fungi Imperfecti and the Ascomycetes. This route is very rarely observed in the Basidiomycetes.

A paper published by J.N. Collie in 1907 put forward some interesting and innovative ideas about the biosynthesis of certain compounds deriving from the condensation of acetate units.[7] He also postulated that some well known laboratory reactions such as hydration, dehydration, oxidation, reduction, c-acylation and methylation may in fact take place biologically. It wasn't until 1955 that some conclusive evidence appeared.[8] Feeding experiments by A.J. Birch showed isotopically labelled sodium acetate was incorporated into 2-hydroxy-6-methylbenzoic acid (a fungal metabolite isolated from *Penicillium griseofulvum*). This supported his hypothesis, put forward two years previously, that the biosynthesis of many phenolic compounds may occur by the head-to-tail linkage of acetic acid units.[9]

The acyl-polymalonate biosynthetic route[10] to naturally occurring phenolic compounds involves condensation of an enzyme-bound carboxylic acid derivative with a variable number of malonate units to produce intermediate enzyme-bound β-polyketo-thiol esters (2). This is shown in Scheme 1.

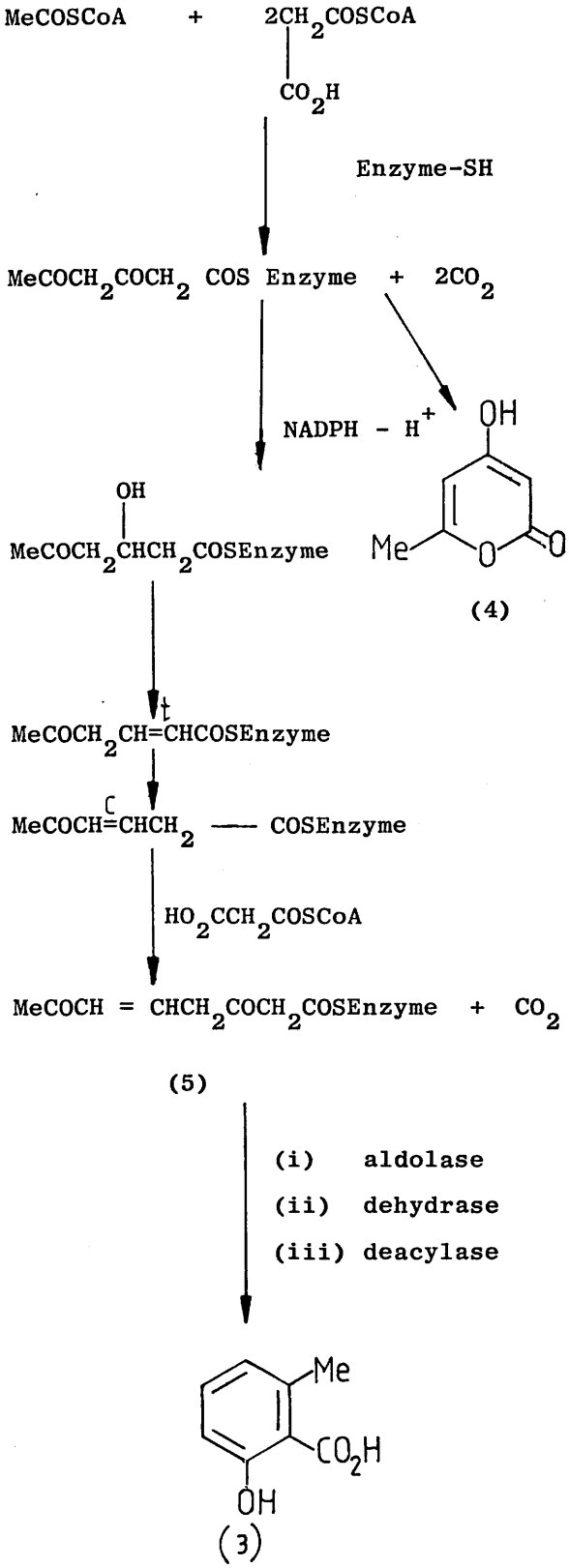

acetate
propionate, etc
cinnamic acids
benzoic acids
RCO2H
R-COSCoA
R-COSEnzyme
Chain-initiating unit
CH3COSCoA
Biotin-ATP-Mg2+-HCO3-
CH2 - COSCoA
CO2H
CH2—COS Enzyme
CO2H
Chain-propagating unit
-CO2
RCOCH2COSEnzyme
-CO2
RCO(CH2CO)nCH2COSEnzyme
(2)
intramolecular condensation
secondary transformations
Phenolic compounds and derivatives

SCHEME 1.

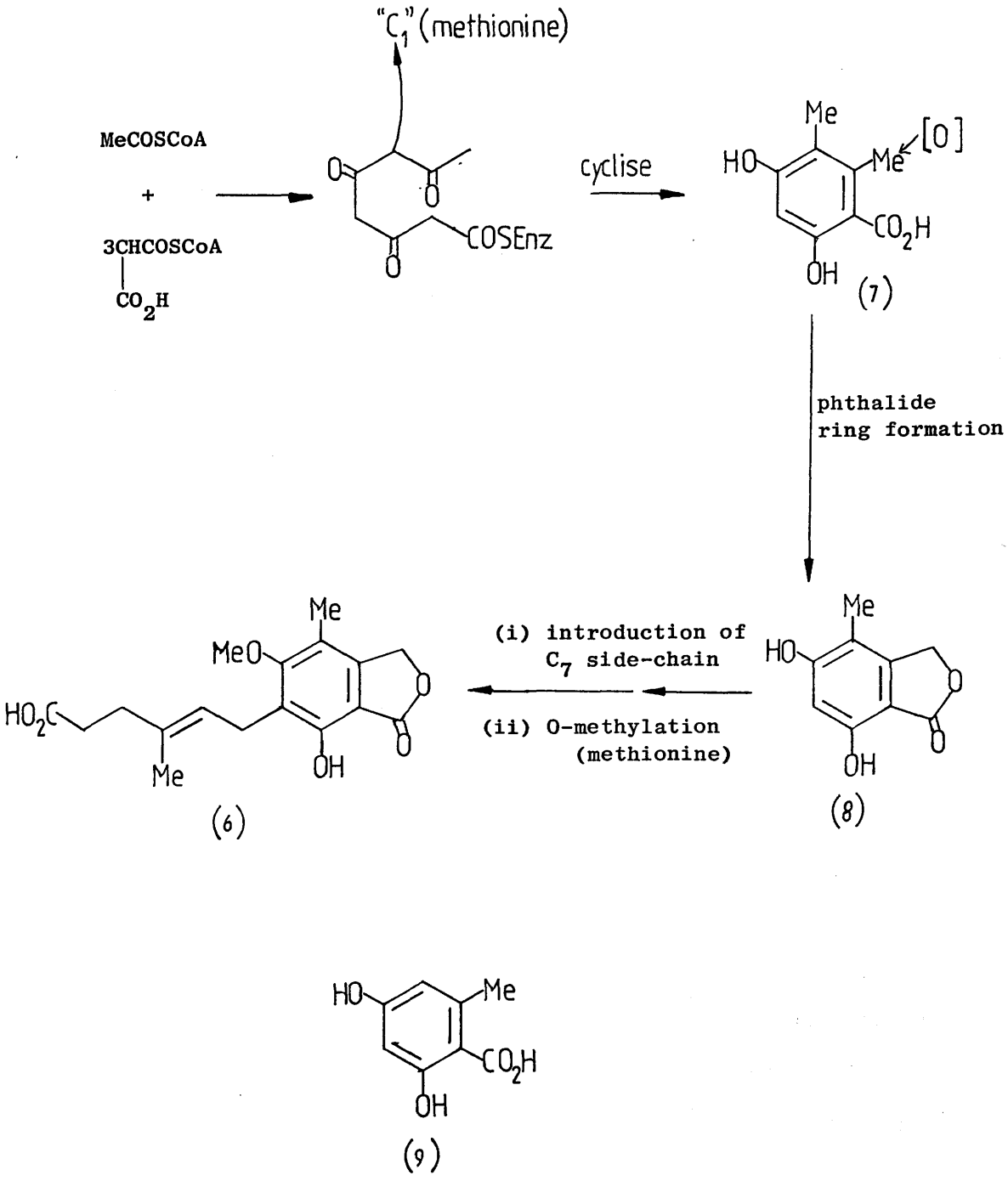
6-Methylsalicylic acid (6-MSA) (3) is a metabolite of *P. patulum*[11] and was one of the first extensively studied compounds in the enzymology of biological systems. It has been reported that an ammonium sulphate protein fraction from *P. patulum* catalyses the synthesis of 6-MSA, TAL (triacetic acid lactone) (4), and fatty acids in the presence of malonyl-coenzyme A and NADPH. It was shown that 6-MSA was derived from one acetate and three malonate units,[12] and also both 6-MSA and fatty acid synthetase activity were inhibited by sulphydryl blocking agents. Previous work had shown that purified 6-MSA synthetase system contains two sulphydryl sites. An inhibition of 6-MSA synthetase activity by acetylenic thioesters e.g. 3-hexynoyl-*N*-acetylcysteamine gives indirect evidence for the presence of an unsaturated enzyme-bound thiol ester intermediate (5). This supports the biosynthetic scheme outlined in Scheme 2.

Mycophenolic acid (6), a metabolite of *Penicillium brevicompactum*, is an example of mixed polyketide isoprenoid origin. This has been proven by incorporations of methylorsellinic acid (7) and phthalide (8).[13,14] Orsellinic acid (9) was not incorporated, which suggests that introduction of the extra methyl group occurs at the acyclic polyketide stage. The side chain of mycophenolic acid (6) is derived from farnesol. The biosynthetic scheme is shown in Scheme 3.

Methylorsellinic acid (7) has also been found to be a normal metabolite of the fungus, a fact which


MeCOSCoA + 2CH2COSCoA
CO2H
Enzyme-SH
MeCOCH2COCH2 COS Enzyme + 2CO2
NADPH - H+
OH
Me
O
O
(4)
OH
MeCOCH2CHCH2COSEnzyme
MeCOCH2CH=CHCOSEnzyme
MeCOCH=CHCH2 —— COSEnzyme
HO2CCH2COSCoA
MeCOCH = CHCH2COCH2COSEnzyme + CO2
(5)
(i) aldolase
(ii) dehydrase
(iii) deacylase
Me
CO2H
OH
(3)

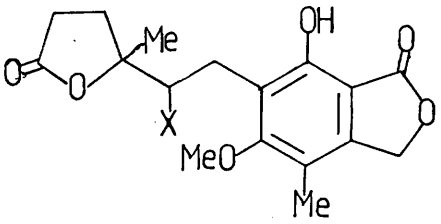
SCHEME 2.


"C1" (methionine)
MeCOSCoA
+
3CHCOSCoA
CO2H
COSEnz
cyclise
Me
HO
Me
[O]
CO2H
OH
(7)
phthalide
ring formation
Me
HO
OH
(8)
(i) introduction of
C7 side-chain
(ii) O-methylation
(methionine)
Me
MeO
HO2C
Me
OH
(6)
HO
Me
CO2H
OH
(9)

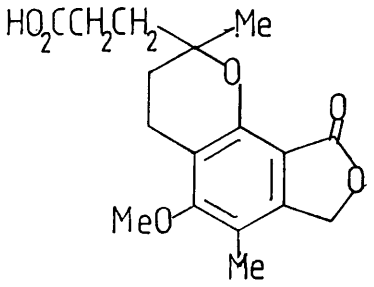
SCHEME 3

supports its role as a natural intermediate in Scheme 3.

Mycophenolic acid has antifungal, antibacterial and more importantly anticancer[15] properties. Many derivatives and analogues have been prepared, both chemically and microbiologically, but none is as active as the natural product. Reduction of the double bond, cyclisation to compounds of types (10) and (11), modification of the phenolic group (for example by methylation), or of the aromatic methyl or phthalide methylene groups, lead to destruction of activity.

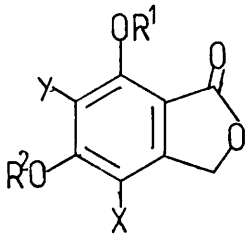


(10)



(11)

P. brevi-compactum is able to transform non-natural precursors[16] such as 5,7-dihydroxyphthalide (12) into (13) and 6-geranyl-5,7-dihydroxy 4-methylphthalide (14) into (6). The biosynthesis of the aromatic part is slower than the biosynthesis of the terpenoid portion of the molecule. Halogenated analogues of mycophenolic acid (6)[17] were produced when cultures of *P. brevi-compactum* were fed respectively with 4-bromo-5,7-dihydroxyphthalide (15) and 4-chloro-5,7-dihydroxyphthalide (16).

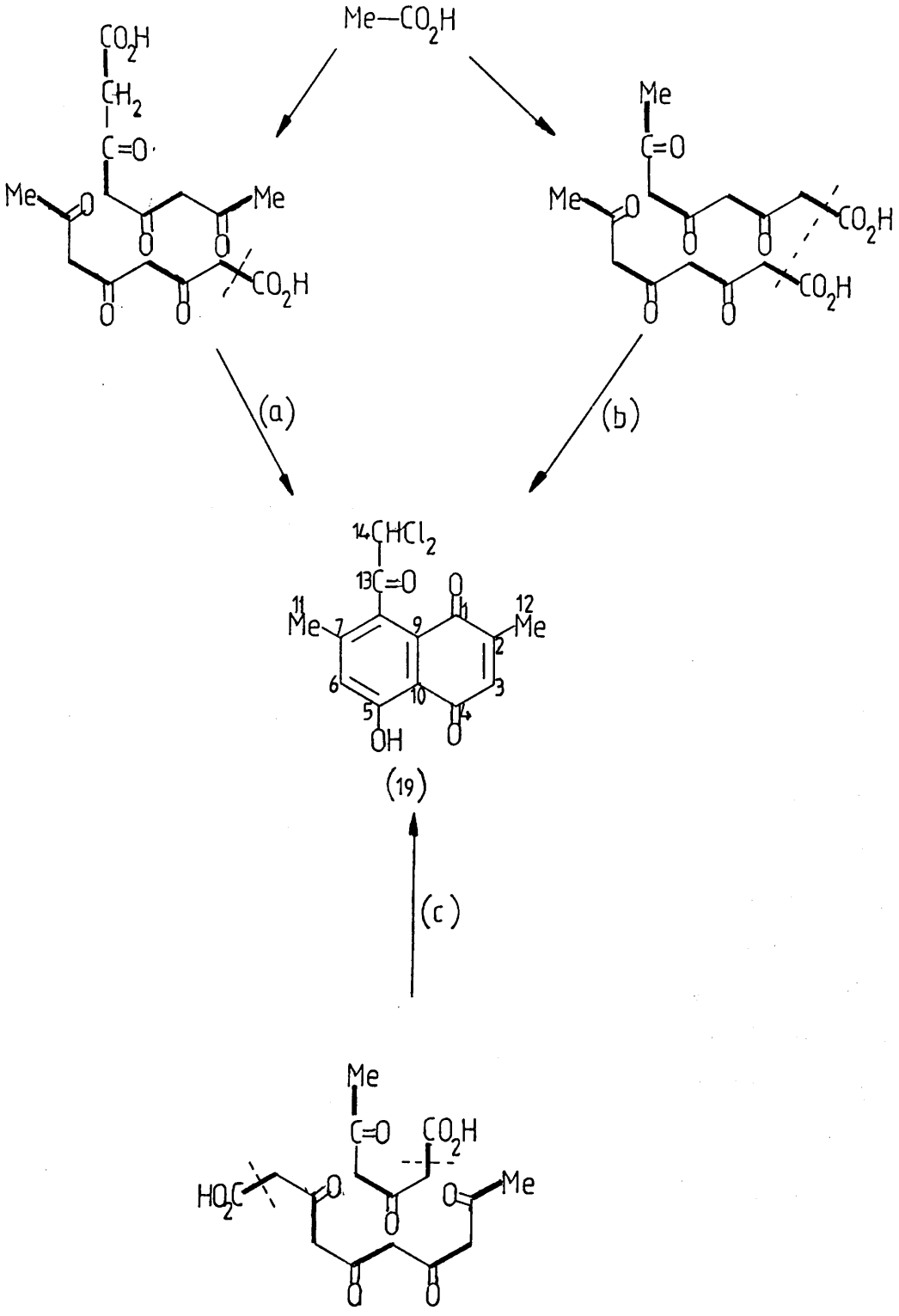


(6) R^1 = H; R^2 = Me; X = Me; Y = $HO_2C[CH_2]_2$ C(Me) = $CHCH_2$-
(12) R^1 = R^2 = H; X = Y = H
(13) R^1 = R^2 = H; X = H; Y = $HO_2C[CH_2]_2$ C(Me) = $CHCH_2$-
(14) R^1 = R^2 = H; X = Me; Y = geranyl
(15) R^1 = R^2 = H; X = Br; Y = H
(16) R^1 = R^2 = H; X = Cl; Y = H
(17) R^1 = H; R^2 = Me; X = Br; Y = $HO_2C[CH_2]_2$ C(Me) = $CHCH_2$-
(18) R^1 = H; R^2 = Me; X = Cl; Y = $HO_2C[CH_2]_2$ C(Me) = $CHCH_2$-

When the bromophthalide (15) is fed to the culture a 1:1 mixture of (6) and its bromo-analogue (17) is found. Likewise when the chlorophthalide (16) is added to the culture a mixture of (6) and the chloro-analogue (18) is formed. These mixtures are separated to give (17) and (18). However it was found that when (15) and (16) were added to the culture their transformation into (17) and (18) was slower than that of the natural precursors of (6).

Fourier-transform (FT) nmr is extremely useful in the elucidation of many biosynthetic pathways. The polyketide biosynthetic route to mollisin (19),[18] a metabolite of *Mollisia caesia*, was one of the first examples to use ^{13}C-^{13}C coupling.

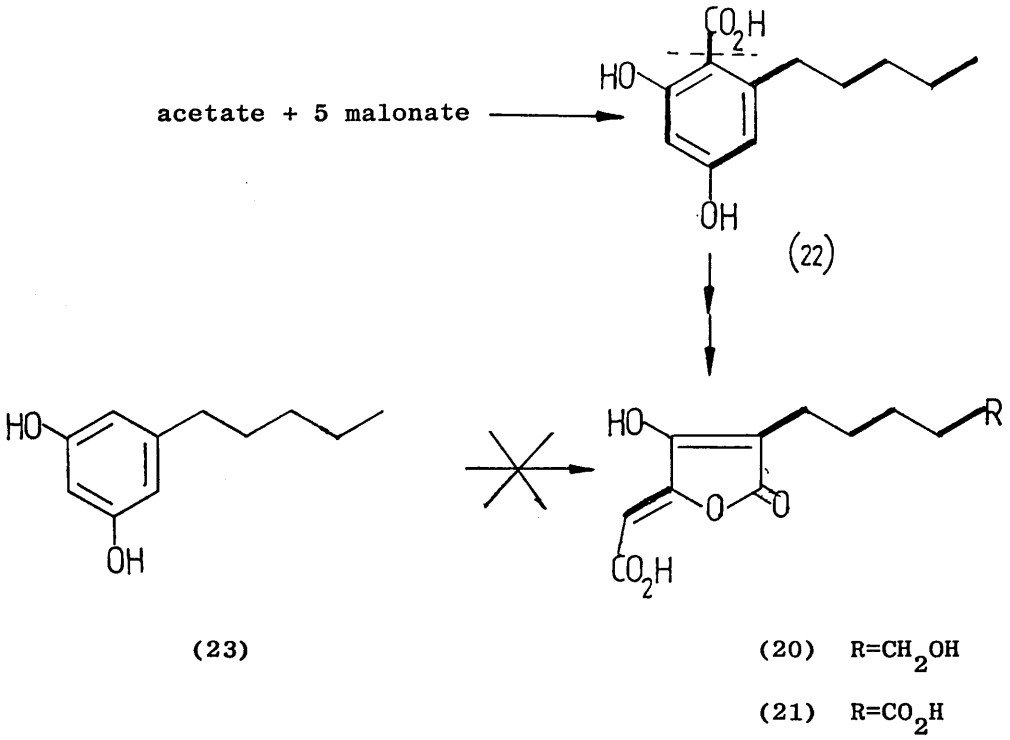
Two biosynthetic routes (a) and (b) for mollisin (19) have been suggested.[19] However another route (c)


Me—CO2H
CO2H
CH2
C=O
Me
Me
CO2H
Me
C=O
Me
CO2H
CO2H
(a)
(b)
14CHCl2
13C=O
11Me
12Me
(19)
(c)
Me
C=O
CO2H
HO2C
Me

SCHEME 4.

was shown by using ^{13}C doubly labelled acetate ($^{13}CH_3{}^{13}CO_2Na$, 90% enriched) to be the true pathway (Scheme 4). The results showed that ^{13}C-^{13}C coupling is observed with C-3, C-6, C-12 and C-14 but not with C-11. However low enrichment level and low mollisin yield resulted in a poor signal to noise ratio obscuring the ^{13}C-^{13}C coupling of C-2, C-4, C-7 and C-13. Therefore there are pairs of carbons at C-2 and C-12, C-3 and C-4, C-6 and C-7, and C-13 and C-14 (and probably C-5 and C-10, and C-8 and C-9) which are derived from the same molecule of acetic acid.

Another case where the use of singly and doubly labelled ^{13}C-acetate was extremely valuable was in the elucidation of the biosynthesis and the structures of multicolic and multicolosic acids (20), (21) from *Penicillium multicolor*.[20] It was shown that the biosynthesis of multicolic acid (20) and multicolosic acid (21) involves the intermediate formation of 6-pentylresorcylic acid (22), followed by cleavage of the C(4)-C(5) bond. The ^{13}C-^{13}C couplings in the ^{13}C nmr spectrum of multicolic acid derived from doubly labelled acetate showed that intact C_2 units were arranged as shown in (20), thus excluding the possibility of cleavage at C(1)-C(2) in (22) and the intermediacy of a symmetrical intermediate such as 5-pentylresorcinol (23) (Scheme 5).

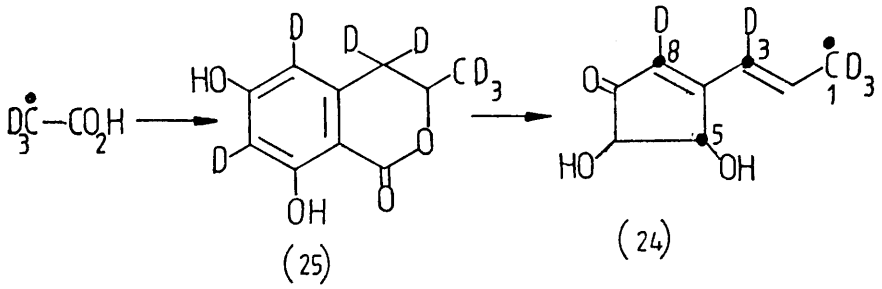


SCHEME 5.

The use of ^{2}H-labelling in the elucidation of polyketide biosynthesis developed during the late seventies. The ^{2}H-label can be detected either directly by ^{2}H nmr or indirectly through its coupling to ^{13}C in the ^{13}C nmr spectra of metabolites enriched from doubly labelled [^{2}H,^{13}C]-precursors. Staunton[21] showed that the detection of ^{2}H through its coupling to ^{13}C in the ^{13}C nmr spectra of metabolites derived from doubly labelled [^{2}H,^{13}C]-precursors offers the possibility of establishing the integrity of C-H bonds during the course of a biosynthetic pathway. In his experiments [2-^{13}C,2-$^{2}H_3$]acetate was used to investigate the retention of hydrogen from the methyl group of acetate in the biosynthesis of the polyketide, terrein (24).

[2-$^{2}H_3$,2-^{13}C]acetate was incorporated into cultures of *Aspergillus terreus*. The dihydroiso-

coumarin (25) is an established intermediate which undergoes an interesting ring contraction to give (24) (Scheme 6).

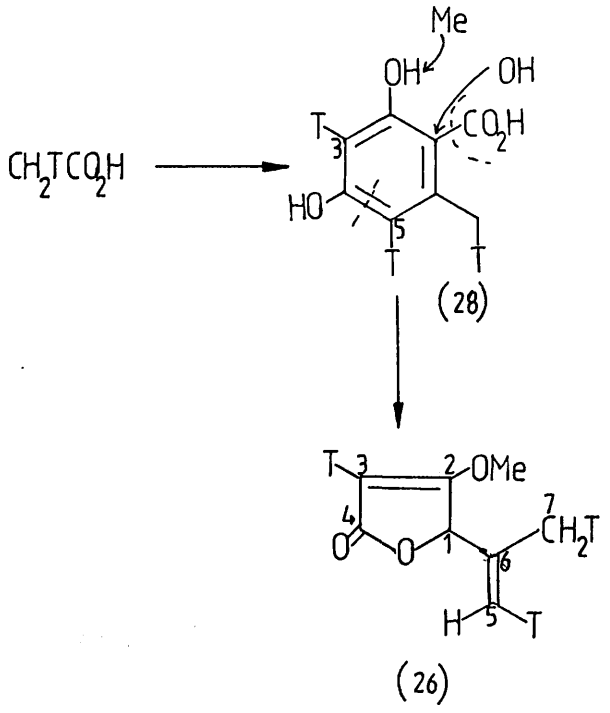


SCHEME 6.

The ^{13}C nmr spectrum of terrein (24) enriched with [2-^{13}C,2-$^{2}H_3$]acetate showed intense signals for C-1, C-3, C-5 and C-8, indicating that ^{13}C had been incorporated at these positions. The presence of deuterium on C-1, C-3 and C-8 was shown by comparing their lowered intensity to C-5 (which cannot retain deuterium). The deuterium-decoupled ^{13}C nmr spectrum confirmed that C-1 is a chain starter unit.[22] The spectrum showed a singlet at 17.95ppm (0.81ppm upfield of the normal chemical shift value for C-1) which was assigned to molecules trisubstituted with deuterium at this position (the normal chemical shift difference for isotopic substitution is ca. 0.3ppm for each deuterium). There is also a doublet (J_{C-H} 123 Hz) at 18.22ppm (0.55ppm upfield from normal) which indicates molecules labelled with CHD_2. The presence of an enriched CH_3 singlet (in the ^{1}H noise decoupled spectrum) shows that there is considerable exchange of hydrogen from the methyl group during biosynthesis. The ^{2}H noise

decoupled spectrum also showed singlets at 125.3ppm and 124.8ppm for C-3 and C-8 each carrying one ^{2}H only.

Another method for detecting hydrogen isotopes is by using tritium nmr. This technique became available at about the same time as ^{2}H nmr. One of the first reported biosynthetic applications of ^{3}H nmr was a study of the incorporation of [^{3}H]acetate into penicillic acid (26),[23] a metabolite of *Penicillum cyclopium*. The results are as shown in Scheme 7, where tritium was found to be present at the 3-, 5- and 7- positions, consistent with the overall mode of biosynthesis.

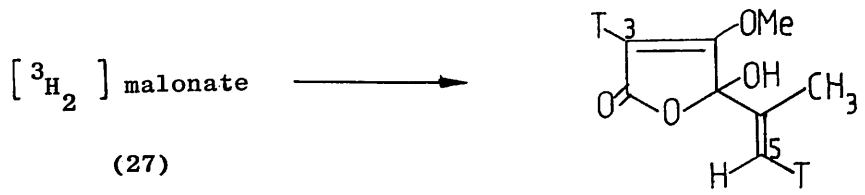


SCHEME 7.

The 7- position showed less exchange of tritium

label than the 3- and 5- positions. This is due to the 7- position being derived from a chain-starter methyl group rather than an activated chain-building methylene position.

Both [$^3H_2$]malonate (27) and [3,5-$^3H_2$]orsellinic acid (28) were also incorporated into the fungus. When [$^3H_2$]malonate is incorporated only the 3- and 5- positions are labelled (Scheme 8).



(27)

(26)

SCHEME 8.

There was a very high exchange at C-3, whereas C-5 was selectively labelled. This gives some clues to the nature of some of the complex biosynthetic steps which could not have been obtained from studies using carbon isotopes.

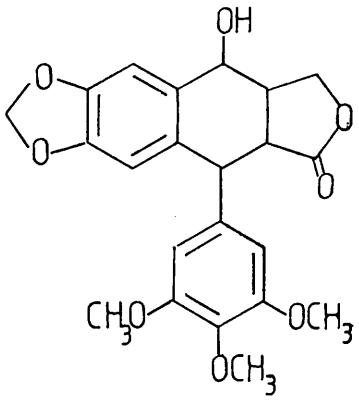
As shown in Scheme 7 [3,5-$^3H_2$]orsellinic acid (28) has been found to be an advanced precursor by incorporation of tritium into (26) with the same pattern of distribution of labels between the 5α- and 5β- positions as the established precursors.

There are definite advantages of ^3H nmr spectroscopy over more conventional methods and indeed

it can be even more useful when used in conjunction with other methods. However high incorporation ratios are required and the high level of radioactivity may be hazardous.

1.2 Introduction to Anticancer Agents.

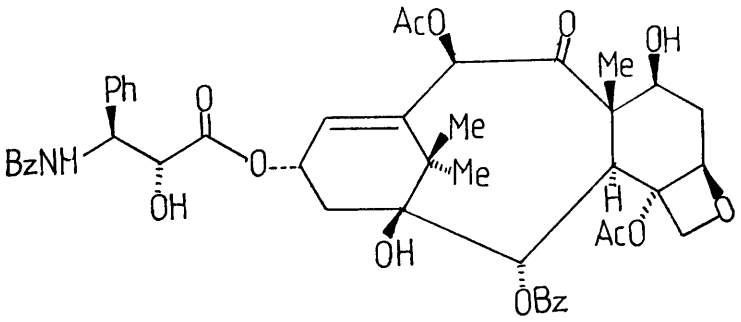
The search for natural products as potential anticancer agents dates back at least to the Ebers papyrus in 1550 B.C.[24] Hartwell's investigations on the application of podophyllotoxin (29) and its derivatives as anticancer agents started the actual scientific research in the early 1950's.[25,26]



(29)

Many hundreds of active compounds have so far been isolated from plant, marine and microbial sources.[27] Although relatively few of these lead compounds have survived the rigorous testing required before introduction into normal clinical use, those that have done so have proved enormously beneficial, and cancer treatment would be greatly impoverished without such drugs as vincristine, vinblastine, adriamycin, arthramycin and other natural products. Not only are natural products important in cancer treatment in their isolated form, but formation of analogues with improved activity sometimes is also extremely important.

The novel diterpenoid taxol (30) has become one of the most important lead compounds to emerge from the screening of natural products in recent years. Wall and his coworkers first isolated taxol (30) from the stem bark of the western yew, *Taxus brevifolia* in 1971.[28] Taxol has potent antileukaemia and tumour inhibitory properties and is the first compound possessing the taxane ring which has been demonstrated to have such activity.

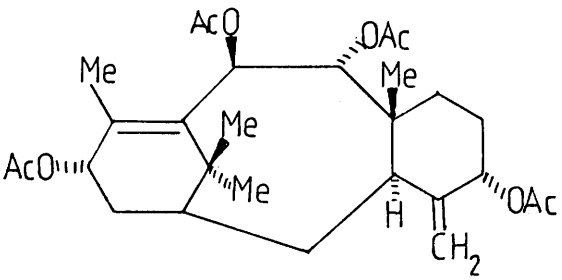


(30)

It has shown significant activity against various leukaemias, the Walker 256 carcinosarcoma, Sarcoma 180, and the Lewis lung tumour. The only difficulty that held up the development of this compound as a drug was actually isolating it, and its lipophilicity made formulation difficult. Clinical trials on this compound have been shown to be extremely encouraging. It was shown that taxol has excellent activity against ovarian cancer.

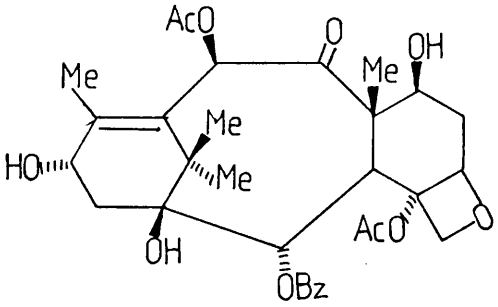
The total synthesis of taxol has yet to be attained but a simpler derivative has been synthesised

by Holton and his co-workers.[29] The compound which was actually synthesised was taxusin (31).



(31)

The partial synthesis of taxol from the simpler diterpenoid baccatin (32) has been reported. This approach thus offers, in principle, a source of taxol from the renewable resource of yew leaves, as opposed to yew bark, since in order to obtain sufficient amounts of taxol a large part of the pacific yew and its habitat, the virgin rainforest, is threatened.



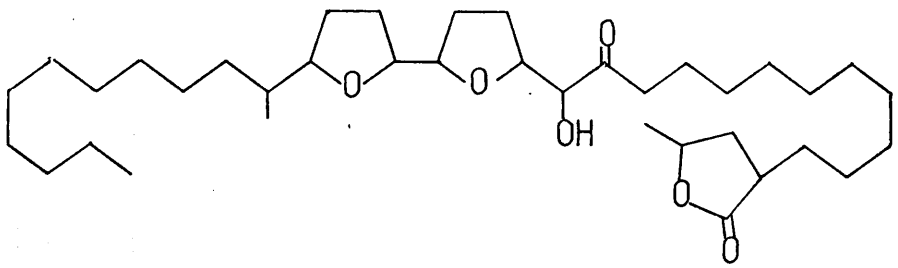
(32)

Although chemotherapy for cancers with a high growth fraction has achieved important advances, little impact has been made on the solid human cancers such as lung, colon, breast, ovarian, prostate, pancreas and brain.[30] New directions will however have to be taken

in the approach to discovery of drugs for these diseases. This has proceeded recently with an aim to uncovering non-toxic agents which might prevent the development of cancer.

Recent studies on tumour cell cytotoxic agents from plants will be discussed.

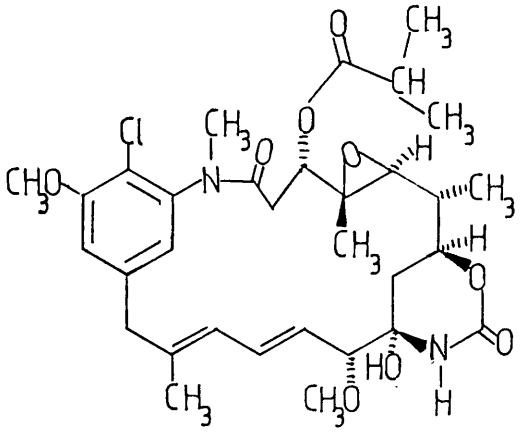
1.Constituents of the Annonaceae: Several novel cytotoxic polyketides have been extracted from the stem bark of *Annona densicoma*.[31,32] Annonacin was the first member of the C_{35} polyketide (acetogenin) series to be discovered. This series was found to possess a single tetrahydrofuran ring in contrast to the more common C_{37} polyketide (acetogenin) series, which has two adjacent tetrahydrofuran groups. One such acetogenin is rollenone (33) which also bears a γ-lactone and is isolated from *Rollinia papilionella*.[33] It demonstrated cytotoxicity against the P-388 lympholytic leukaemia *in vitro* and activity *in vivo* against the P-388 lympholytic leukaemia in mice.



(33)

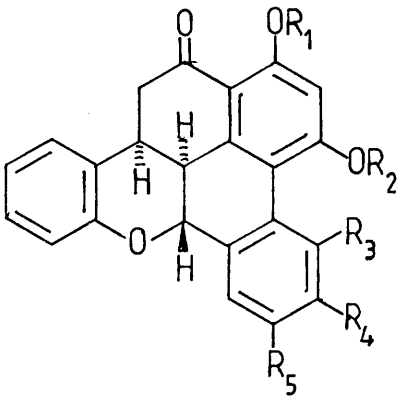
Isoannonacin and isoannonacinone are the first reported members of the iso-series of C_{35} polyketides. These compounds have demonstrated very interesting selective cytotoxicity in human tumour cell lines.

2. Constituents of Mosses (Thuidiaceae): Antitumour activity was discovered in bryophytes in 1980. *Claopodium crispifolium* showed significant antitumour activity. There was a minor cytotoxic constituent isolated which is known as ansamitocin P-3 (34).



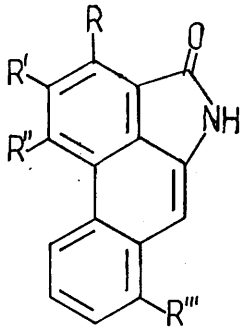
(34)

3. Studies on Polytrichum ohioense (Polytrichaceae):
A series of novel natural products named ohioensins A (35), B(36), C(37), D(38), and E(39). The structure of isomer ohioensin A was established by X-ray analysis to be a novel polycyclic benzo[*c*]naphthoxanthenone system that apparently results from coupling of *o*-hydroxycinnamate and hydroxylated bibenzyl precursors.[34]



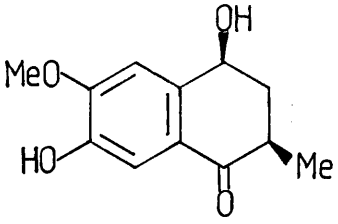
(35) $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = OH$ Ohioensin A
(36) $R_1 = R_2 = R_4 = R_5 = H$, $R_3 = OH$ Ohioensin B
(37) $R_1 = R_4 = R_5 = H$, $R_2 = Me$, $R_3 = OH$ Ohioensin C
(38) $R_1 = R_5 = H$, $R_2 = Me$, $R_3 = R_4 = OH$ Ohioensin D
(39) $R_1 = R_5 = H$, $R_2 = Me$, $R_3 = OH$, $R_4 = OMe$ Ohioensin E

4. Studies on Pararistolochia flos-avis: Two new aristolactams, FI (40) and FII (41) were isolated from active extracts of *Pararistolochia flos-avis*.[35] Aristolactam-I (42) and -AII (43) were also isolated from this plant. Aristolactam-AII showed cytotoxicity against PS and KB cells in culture. Aristolactam AII showed approximately tenfold selective cytotoxicity against human small cell lung and colon tumours.



(40) R = R^{111} = H, R^{1} = OMe, R^{11} = OH aristolactam FI
(41) R = R^{11} = OMe, R^{1} = OH, R^{111} = H aristolactam FII
(42) R = H; R^{1}, R^{111} = O-CH_2-O; R^{111} = OMe aristolactam I
(43) R = R^{111} = H, R^{1} = OH, R^{11} = OMe aristolactam AII

A new tetralone derivative and a new lignan named flossonol (44) and (-)-phillygenin (45) respectively have been isolated from the active part of the same plant. Flossonol (44) was found to be cytotoxic to murine leukaemia cells in culture.[36]



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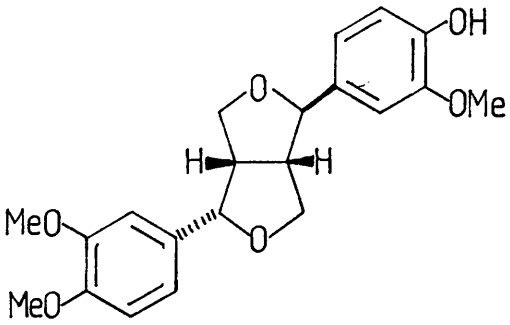
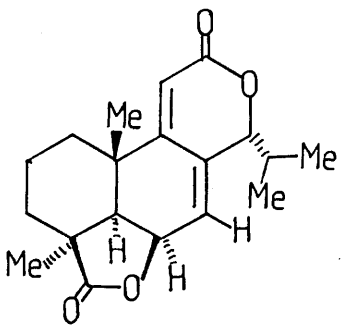


(45)

5. Studies on Podocarpus: This genus is widely distributed in tropical and subtropical areas of eastern Asia and the southern hemisphere and is a well known rich source of terpenic substances.[37] A number of nor- and bisnor-diterpene dilactones have been isolated. This group of compounds shows antitumour activity, plant growth regulatory activity, termiticidal activity and toxicity toward insect larvae. The cytotoxic nor-diterpene dilactones, nagilactone F (46) and its new congener nagilactone G (47) were isolated from the stem bark of *Podocarpus sellowii* and *Podocarpus milanjianus*.[38]



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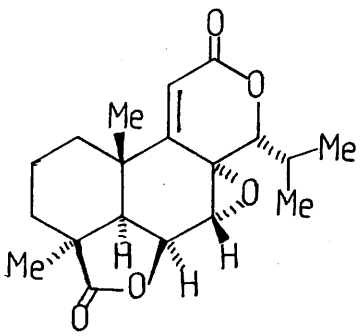
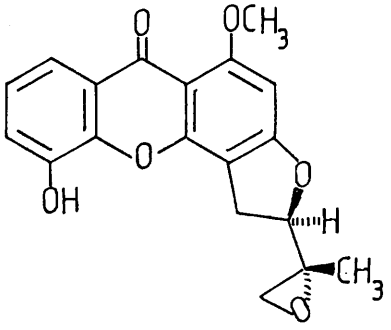


(47)

6. Constituents of Psorospermum febrifugum: Detailed studies have been carried out on the furanoxanthone psorospermin (48) due to its significant cytotoxicity and antitumour activity.[39] Psorospermin (48) was originally isolated by Kupchan and co-workers. The absolute stereochemistry was assigned[40] and a series of novel bioactive analogues was discovered. These analogues include 3′,4′-deoxypsorospermin; 3′,4′-deoxypsorospermin-3′,4′-diol; 3′,4′-deoxy-4-chloropsorospermin-3′-ol; and O^5-methyl-3′,4′-deoxypsorospermin-3′-ol. It was found after examining some analogues of psorospermin, e.g. psorospermin-diol (49) and 3′-O-acetylpsorospermin-diol (50), that the most potent cytotoxic agents in the series possess an alkylating group at the 3′,4′ position. Compounds lacking this

group show only borderline cytotoxicity.



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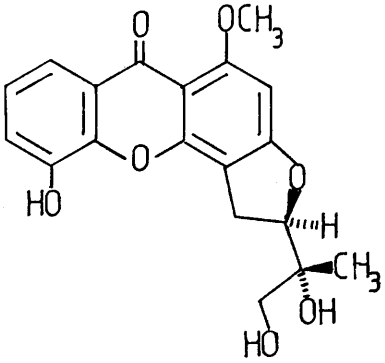

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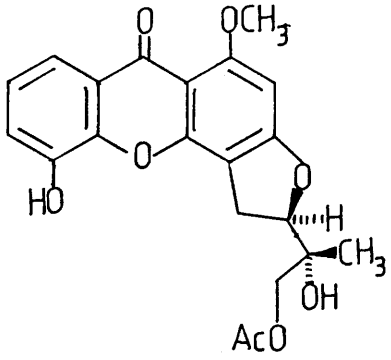


(50)

1.3 Background to Cancer

Simply, cancer is described as the continual, uncontrolled production of cells that are of no benefit to the body.[41] In most cases, as the cells proliferate, they form a swelling or "tumour". Tumours have been recognised ever since the time of the ancient Greeks. The word "cancer" originated from the Latin for crab, which is used to describe the appearance where a central area has channels spreading out, like arms, as the rapidly dividing cells, which are already squashed closely together, start to invade healthy tissue.

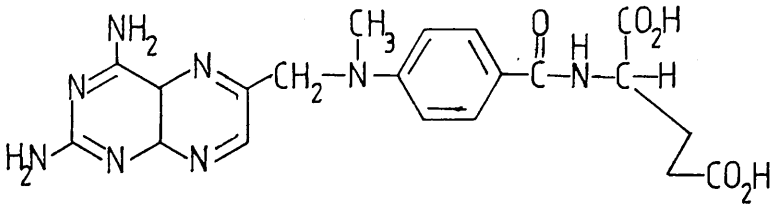
The reason why cancer cells are so harmful is that once the cells start to grow out of control their specific function is lost. So instead of the cells doing a job useful to the organism as a whole, they become independent entities, seeking nutrition and support wherever they can, often at the expense of normal healthy tissue. Cancer is also detrimental in that it can spread to other parts of the body. This is termed secondary or metastatic growth and the cancers which spread in this way are called malignant. Tumours which are localised and grow at a relatively slow rate are termed benign.

Once cancer has been diagnosed, the type of treatment to be given will be dependent upon the type of cancer, the location of the cancer and the actual person. The types of treatment include surgery, radiotherapy and chemotherapy. Usually treatment

involves a combination of all three or at least two of the methods. Surgery is also invaluable in the actual diagnosis of cancer as well as the treatment. With radiotherapy, each individual will be given a unique dosage depending upon the size of the tumour and their response to it. Drug treatment is usually used once cancer has spread widely, so that it affects many systems within the body. The drugs may either be hormones that mimic the body's natural chemical messengers which control the growth of certain kinds of cells, or drugs (called cytotoxins), that kill cancer cells. Breast cancer for example can be controlled by hormonal agents. Although cytotoxins are frequently effective, they have serious side-effects. There is however a fundamental distinction between the way hormones affect cell division and the action of other classes of antitumour drugs.[42] Hormones interact with receptors to form complexes that normally modulate cell division and maturation, and thus endocrine manipulation is essentially the application of natural mechanisms. Cytotoxins work by inhibiting normal pathways, either destroying of the biomolecules or causing them to malfunction.

The first drug to be successfully used against human cancer was in fact a by-product of research into chemical warfare.[41] As part of a development programme during the First World War, compounds that were irritating to the skin and eyes, were tested on volunteers. It was found that there was a very rapid fall in the number of white blood cells. One compound in particular had a pronounced effect on the white blood

cell count. This substance was mustard gas, which contained nitrogen mustard (51). In the early 1940's nitrogen mustard was used to treat lymphosarcoma with a good initial response. Soon afterwards a drug which inhibited the creation of the building blocks of DNA was synthesised. This compound, methotrexate (52), was found useful in treating leukaemia and is still used in chemotherapy treatment to this day.



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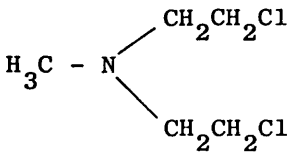
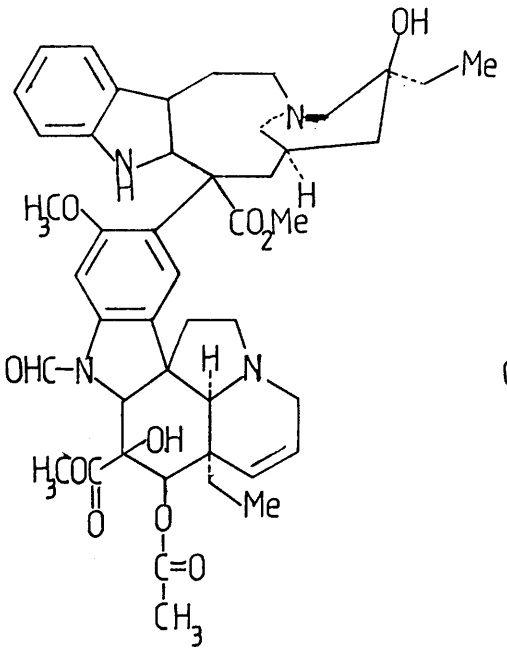


(51)

The rate of discovery and application of new anticancer drugs escalated during the late 1960's, but has since slowed considerably.[41] A trial of drug therapy in 1965 by the National Cancer Institute in Washington was set up in which patients with lymphatic cancer were treated simultaneously with four drugs. These four drugs - nitrogen mustard, vincristine (53), procarbazine (54) and prednisone (55) - had previously been found to be of some help individually. The results were extremely encouraging and this breakthrough led to interest in new approaches of combining different

compounds to treat a whole range of different cancer types. The main emphasis in chemotherapy today is in fact on drug combinations with a rational basis.



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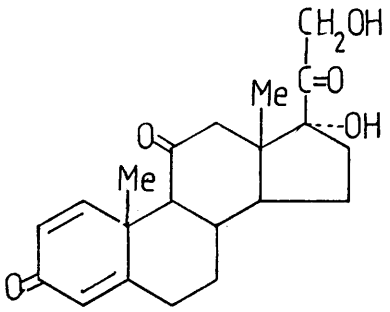

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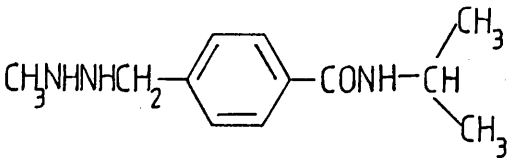

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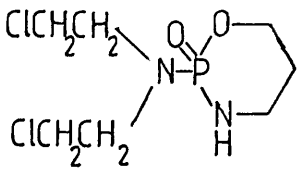


(56)

Many anticancer compounds have been discovered by accident. Some have been designed by pharmaceutical chemists to have a specific anticancer action, and others have been found by routine testing of a wide range of existing compounds or have been isolated from naturally occurring plant materials. There is a great chemical diversity in these classes of compounds.[43] This is an advantage in that a variety of approaches can be made for selecting optimal compounds. Chemopreventive agents can be placed into two broad categories. The first category includes compounds that are effective against complete carcinogens. The second includes compounds effective against tumour promoters. Some compounds fall into both categories.

1.4 Introduction to Alkylating Agents

Alkylating agents provide almost half of today's clinically useful products.[44] They were the first group of chemicals systematically investigated in order to find effective inhibitors against neoplastic cells. The definition of a biological alkylating agent[45] is a compound which can replace a hydrogen atom by an alkyl group under physiological conditions (pH 7.0-7.4, 37°C, aqueous solution).

There have been at least two main trends in the early development of alkylating agents.[44] The first one being the search for new cytotoxic alkylating functions which led to the first generation of clinically useful agents e.g. nitrogen mustard (51). The other trend is the search for structures with enhanced selectivity towards malignant cells, by exploiting, in the design of the new alkylating agents, some theoretical principles e.g. cyclophosphonamide (56). As well as these agents being able to inhibit tumour cell growth, they can often act as potent carcinogens. Their actual antitumour effect is their ability to induce lethal mutations and to inhibit DNA synthesis. True monofunctional alkylating agents (e.g. methyl-methane sulphonate) are carcinogenic since they generate mutations associated with miscoding which leads to transformed cells. In general it is only bifunctional alkylating agents that are effective antitumour compounds. A larger number of alkylating moieties accumulated in the same molecule does not lead to a proportional increase in the activity.[46]

The reaction between an alkylating agent and a substrate takes place according to the following equation[44],

$$R\text{-}H + alkyl\text{-}X \longrightarrow R\text{-}alkyl + H^{+} + X^{-}$$

Biological alkylating agents react following the equation at pH 7.0-7.4, 37°C in aqueous media. In aqueous media they are transformed into electrophilic species which interact directly through a bimolecular process, with target macromolecules.

The following factors are important in defining and evaluating the reactivity of the direct or the "ultimate" alkylators (i.e. those that are responsible for DNA cross-linking directly and are released by other alkylating agents):

1. the mechanism of the reaction with the nucleophiles or DNA nucleophilic sites;
2. the electrophilicity of the alkylating species;
3. the softness of the alkylating moieties;
4. the stereochemistry of the electophilic species;
5. the nature, reactivity and stereochemistry of the DNA nucleophilic site.

Alkylating agents can react in aqueous media by a unimolecular (S_N1), a bimolecular (S_N2) pathway (Scheme 9) or by enzymatic activation. Most of the agents alkylating by S_N1 or S_N2 mechanisms attack the nucleotides in order of their increasing basicity, guanine>adenosine>cytosine>tyrosine(uridine), whereas those alkylating by a carbenoid or radical mechanism

exhibit a reverse order of attack for guanine.

Knowing the reaction mechanisms for alkylating agents is very useful practically for their design.

$$\text{Alkyl} - \text{X} \xrightarrow{k_1} \text{Alkyl}^+ + \text{X}:^- \quad \text{(prior ionisation)}$$

$$\text{Alkyl}^+ + \text{Nu}:^- \xrightarrow{k_2} \text{Alkyl} - \text{Nu} \quad \text{for } k_1 << k_2 \qquad \Big\} \; S_N1$$

$$\text{Alkyl-X} + \text{Nu}:^- \xrightarrow{k_1} [\text{X---Alkyl---Nu}] \xrightarrow{k_2} \text{Alkyl-Nu} + \text{X}:^-$$

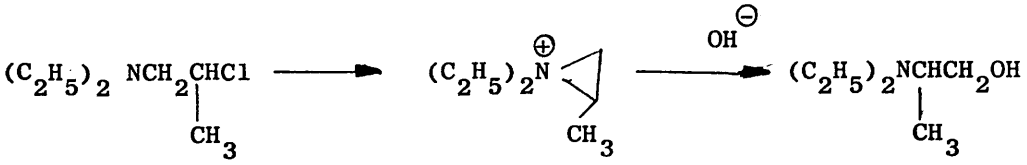
$$d[\text{Alkyl-X}]/_{dt} = -k_1[\text{Alkyl-X}][\text{Nu}:^-] \qquad \Big\} \; S_N2$$

SCHEME 9.

Those alkylating agents proceeding by an S_N1 mechanism rather than an S_N2 mechanism exhibit lower sensitivity to the substrate generally. This is due to the high reactivity of the carbenium ion intermediates.

The kinetics of the hydrolysis rate of biological alkylating agents were used as an early detector of reaction mechanisms. The structure and the concentration of the final hydrolysis products can give some evidence for the actual reaction mechanism. For example, the alkylating hydrolysis of 1-diethylamino-2-chloropropane (57), which occurs with rearrangement of the methyl group from the halogenated sidechain, is consistent with an S_N2 mechanism involving a cyclic

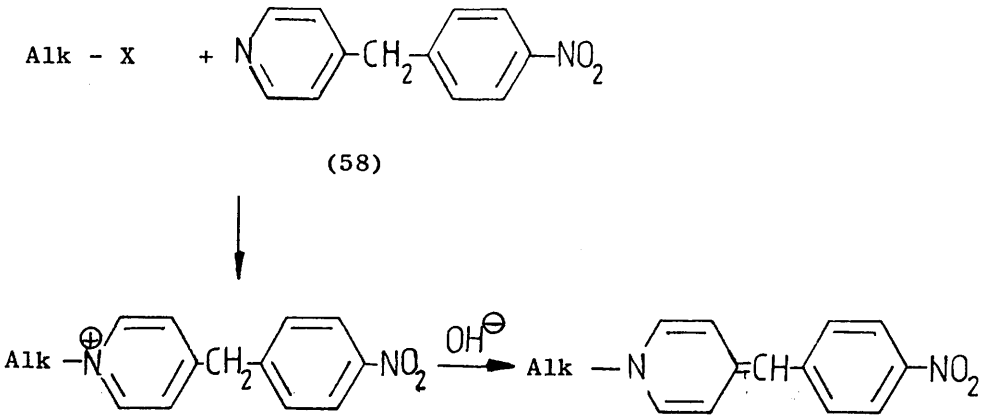
intermediate (Scheme 10).



(57)

SCHEME 10.

p-Nitrobenzylpyridine (NBP) (58) was used as another indicator of alkylating ability, as opposed to water that could better define the reactivity of alkylating agents. The reactivity of NBP (58) towards alkylating species results from the nucleophilicity of the unshared electron pair of the pyridine nitrogen. Using this reagent the reactivity of an alkylating agent is defined as the time-dependent alkylation of NBP in the presence of competing nucleophiles such as water or other solvent molecules (Scheme 11).[47,48]



(58)

SCHEME 11.

The concentration of alkylated NBP, determined under standard conditions, allows the calculation of the "alkylating activity" of the investigated derivative. Usually the reaction mechanism of NBP alkylation is of S_N2 type. However this parameter does not entirely reflect the behaviour of alkylating agents against target macromolecules.

The reaction between alkylating agents and DNA[44] yields addition products which either involve a single purine or pyrimidine nucleus (i.e. for methylating and ethylating agents) or link together two such halves (i.e. for bifunctional alkylating agents). The latter linkage is called crosslinking.

Bifunctional alkylating agents are able to induce several types of crosslinkings, i.e.

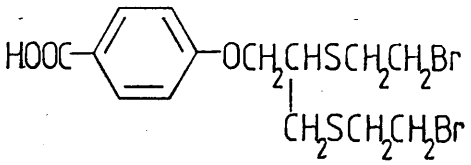
(a) intrastrand (both alkylated sites belonging to the same strand);

(b) interstrand;

(c) DNA-protein cross-links.

Interstrand cross-links are especially related to the cytotoxicity of alkylating agents.[49] The detrimental effects of these bifunctional alkylating agents could be due to the blocking of DNA replication by these crosslinks and also by a significantly lower efficiency of the removal of these adducts by the repair systems. Depurination, depyrimidination and alkali-labile DNA breaks are some effects that can also occur at DNA level.

The antitumour effectiveness of alkylating agents can be improved by increasing either their cytotoxicity or their selectivity towards malignant cells.[44] Cytotoxicity is easier to deal with because it is closely related to the chemical reactivity of the alkylating moiety and the bifunctional character of the alkylating agent. Very reactive and hence cytotoxic alkylating agents may be designed. One such example is (59).



(59)

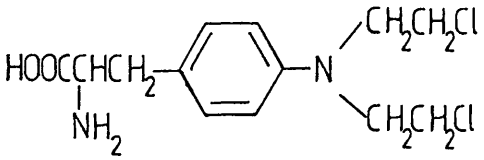
Three concepts have been developed to achieve better selectivity in the design of alkylating agents which are as follows:

(a) The search for new alkylating moieties e.g. quinone-methides or for unusual combinations of alkylating moieties grafted on the same molecule e.g. cyclophosphonamide (56) with methane-sulphonyl groups grafted on.[50,51]

(b) The latent activity concept where the idea is to use a highly reactive drug in a chemically inactive form

(prodrug) able to undergo metabolic activation.[52] This concept led to the design of latent phosphoramide nitrogen mustards, which finally led to a series of cyclic congeners with outstanding antitumour properties.

(c) The carrier-concept based on the properties of certain molecules to accumulate in cancer cells. This led to very active compounds being produced such as melphalan (*L*-PAM) (60).



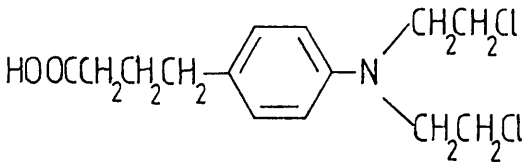
(60)

Both latent activity and carrier concepts are based on the possibility of modulating the chemical reactivity of the alkylating moieties.

Molecular structure and often stereochemistry are very important elements both for the cellular permeation of the alkylating agent and for its transport across the cytoplasm. There are two main types of carriers (the molecules to which the alkylating moieties are grafted) which are as follows:

(a) Xenobiotic structures e.g. Chlorambucil (61) which exhibit some preselected physio-chemical properties.

(b) Physiological structures whereby such carriers are believed to permeate cell membranes by specific transport processes, thus accumulating in tumour cells.

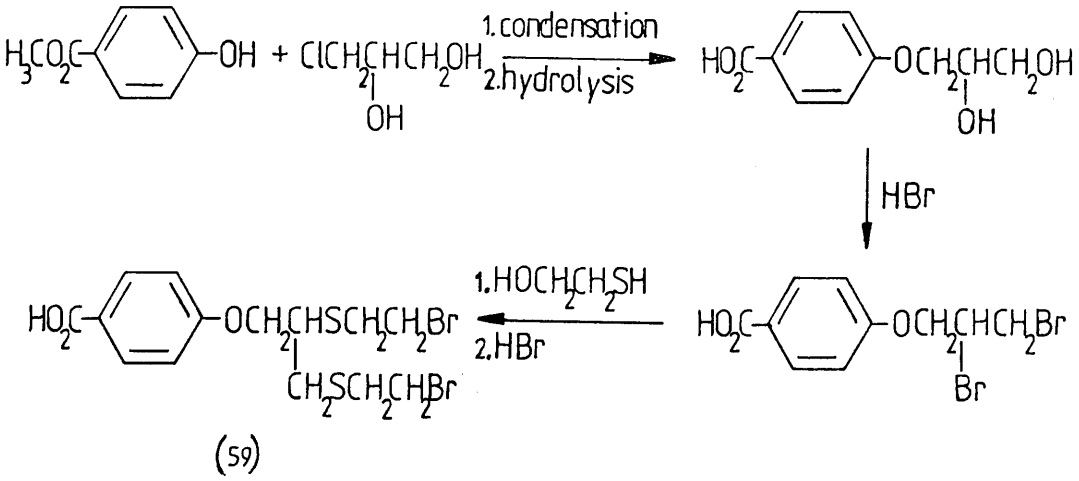


(61)

1.4 Preparative Chemistry of Alkylating Agents

(a) Sulphur mustards:

The very reactive sulphur mustard (59) used for intra-arterial or intratumoral infusions was synthesised as shown (Scheme 12)[53] using some relatively simple chemistry.



(59)

SCHEME 12.

(b) Nitrogen mustards:

One of the most widely used alkylating moieties is *N*,*N*-bis-(2-chloroethyl)amine, attached to xenobiotics or to physiological carriers, as such or in a latent form.

Aliphatic or aromatic nitrogen mustards are generally prepared as follows:

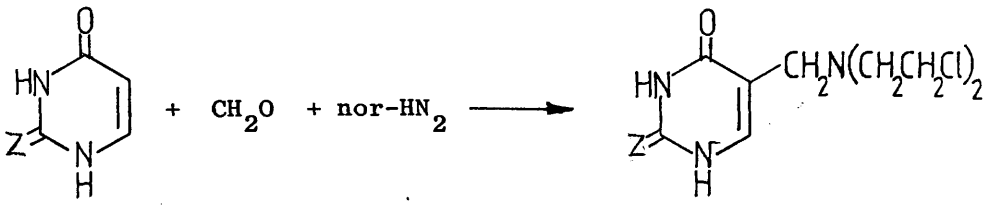
$$R\text{-}NH_2 \longrightarrow \underset{(62)}{RN(CH_2CH_2OH)_2} \longrightarrow \underset{(63)}{RN(CH_2CH_2X)_2}$$

The precursor (62) is prepared by treating the corresponding primary amine with 2-chloroethanol, or better with ethylene oxide in acetic acid medium. The product (63) is usually obtained by treating (62) with a number of reagents such as phosphorus trichloride or tribromide, phosphoryl chloride or bromide or thionyl chloride. Iodine is introduced by boiling the corresponding alkyl halides (63) (X = Cl, Br) with sodium iodide in dry acetone.[54]

Nitrogen mustard (51) was one of the first synthetic compounds used in cancer chemotherapy[55] and is still used clinically. A large number of nitrogen mustards with a wide range of chemical reactivities were synthesised by varying the halogens or their reactive sidechains (e.g. *n*-propyl, isopropyl).

The Mannich reaction proved to be extremely useful in the synthesis of nitrogen mustards linked by methylene bridges to the aromatic nucleus. Nitrogen mustards derived from uracil (64) and thiouracil (65)

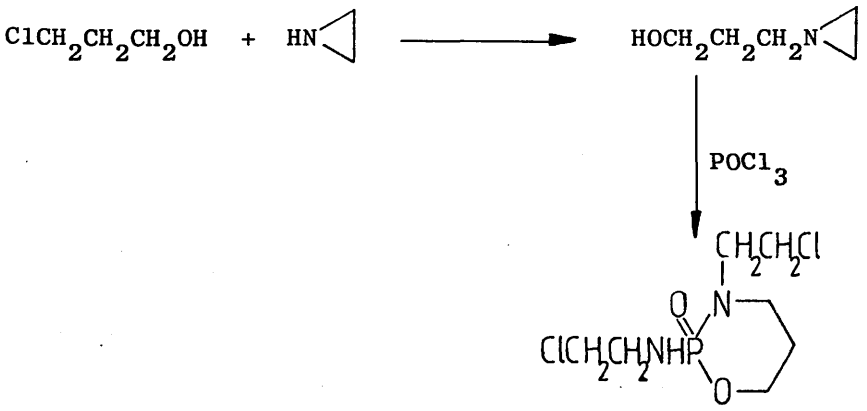
were prepared[56] (Scheme 13).



(64) Z = O
(65) Z = S

SCHEME 13.

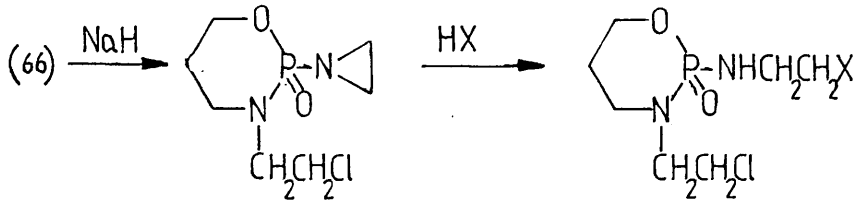
In order to obtain compounds with low chemical reactivity the nitrogen mustard moiety was modified. One such example is the synthesis of the cyclic phosphonamide, Iphosphamide (IF) (66) (Scheme 14).[57]



(66)

SCHEME 14.

The synthesis of new IF derivatives of the type (67) was recently reported (Scheme 15).[58] It was based on the idea that any modification of the parent molecule which does not affect the activation process, but does change the alkylating activity of the ultimate alkylator, should affect the IF antitumour activity. The derivative (67) (X-Br) appears to be effective compared to IF (66) on animal tests.

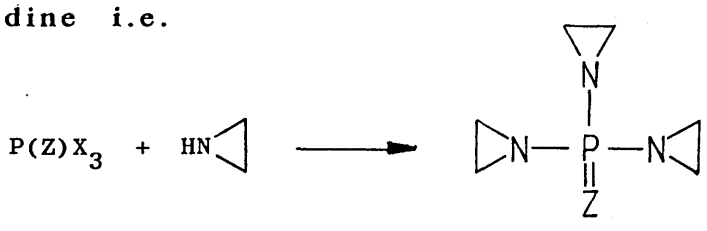


(67) (X = Br, $OSO_2C_6H_4CH_3$, etc)

SCHEME 15.

(c) Aziridines

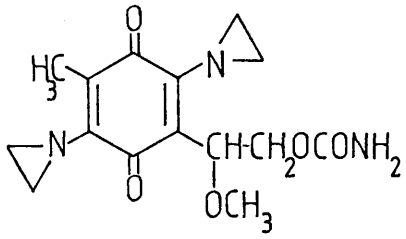
Phosphoramide derivatives (68) were first prepared[44] by Cyanamid Corporation by treating halogenated or thiohalogenated derivatives of phosphorus with aziridine i.e.



X = Cl, Br; Z = O,S

(68)

Aziridinylbenzoquinones were designed in order to find new antitumour agents for the central nervous system. This is because the compounds possess, beside a high cytotoxicity, several of the molecular characteristics necessary for CNS penetration i.e. suitable lipophilicity and a low degree of ionisation. One such compound, Esquinone (69), emerged as a clinically useful derivative.



(69)

(d) Methanesulphonates:

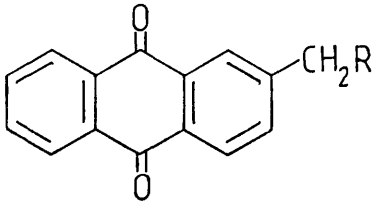
One of the methanesulphonates prepared, 1,4-dimethane-sulphonyloxybutane (70) (n = 4), exhibits a remarkable activity in chronic leukaemias. These compounds were first prepared in order to resemble nitrogen mustards.

$$CH_3SO_2O(CH_2)_nOSO_2CH_3$$

(70) (n = 1-9)

(e) Benzo- and naphthoquinones:

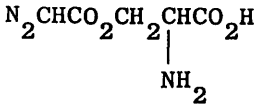
These are compounds activated by enzyme reduction. They require reduction by NADPH-dependent enzyme systems in order to exert their alkylating activity. Derivatives formed include anthraquinone derivatives (71).[59]



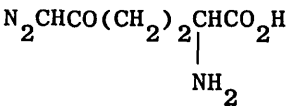
(71) (R=Cl, Br, CH_3CO_2, etc)

(f) Diazoalkanes:

These alkylating agents have an alternative type of antitumour activity because the alkylation occurs by a different mechanism, which possibly involves carbenoid or radical intermediates. Some examples include azaserine (72) and 6-diazo-5-oxo-norleucine (DON) (73) which have been extracted from *Streptomyces* cultures.



(72)

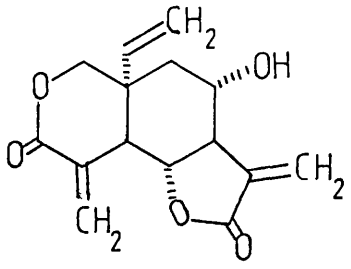


(73)

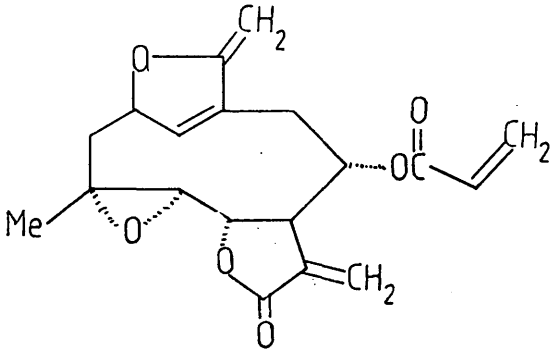
They probably act by a specific inhibition of the various *L*-glutamine-aminotransferases.[60] At high concentrations they also exhibit alkylating properties. However it is not certain that their antitumour properties are due to this effect, because only the *L*-enantiomer is active.

(g) α-Methylene-γ-lactones:

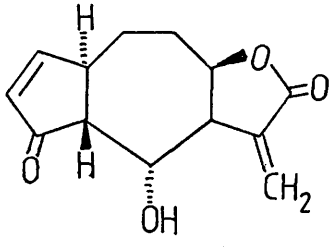
Sequiterpene lactones such as vernolepin (74), elephantopin (75), and helenalin (76) were found to possess significant *in vivo* antitumour activity.[61]



(74)



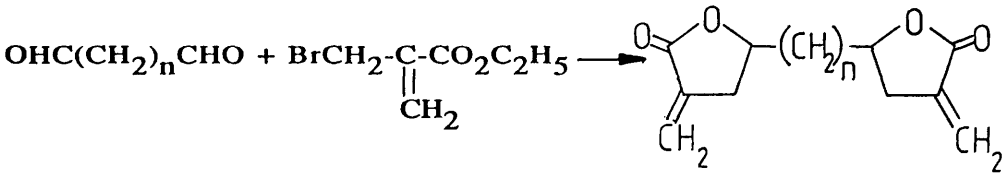
(75)



(76)

Their actual mode of action is that they act as alkylating agents by the conjugate addition of biological nucleophiles to the α-methylene-γ-lactone moiety. These compounds alkylate the thiolic functions of the enzymes as well as the substrates.

Bifunctional derivatives were synthesised to increase the cytotoxicity e.g. (77).



(n = 3-6) (77)

α-Methylene-γ-lactones possessing a leaving group linked to the α-methylene undergo Michael-type nucleophilic addition elimination reactions much more readily than the simple enones and are in this respect more effective alkylators.

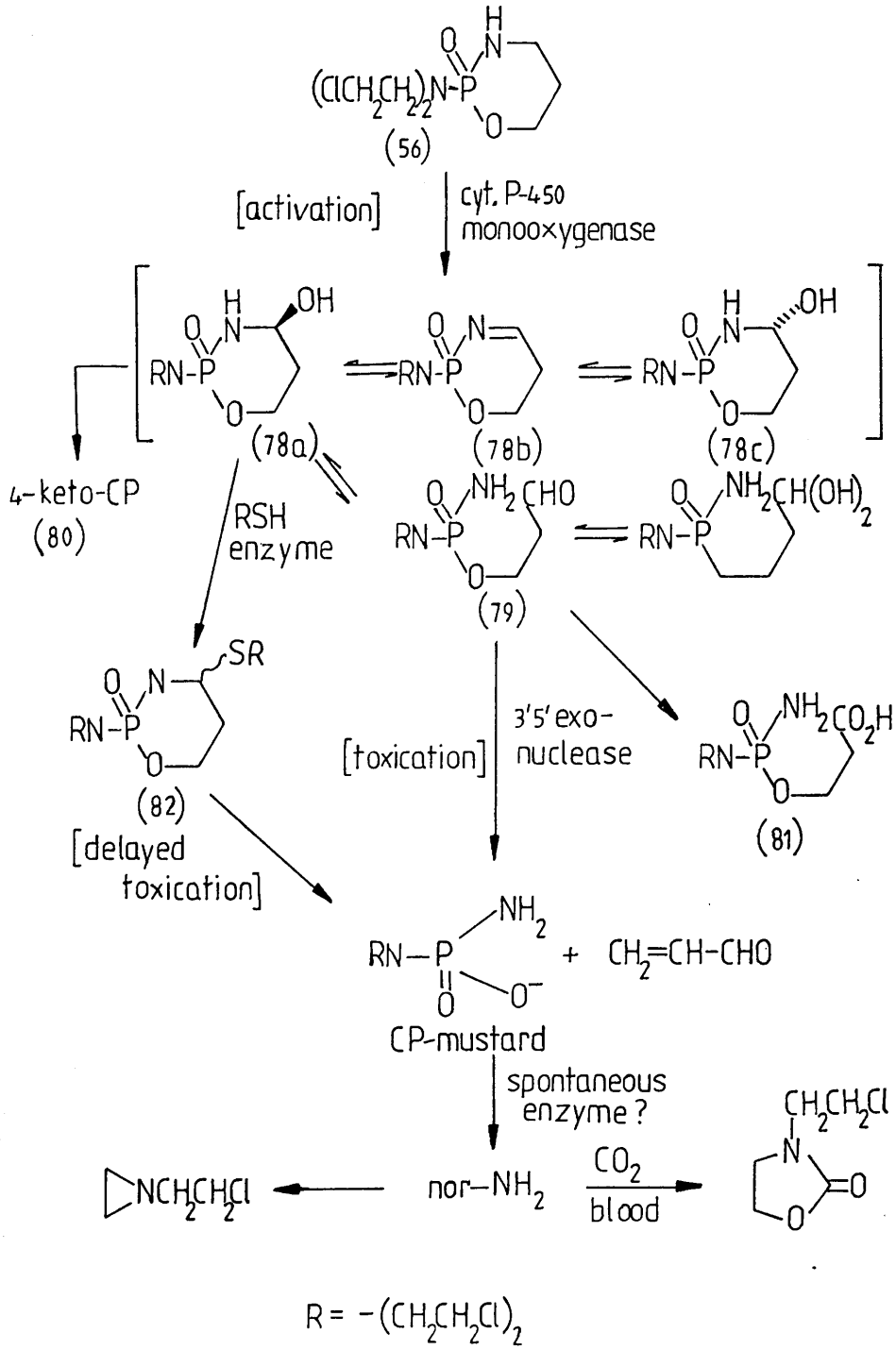
1.5 The Metabolism of Alkylating Agents.

For latent alkylating agents, the metabolism is of great importance in determining the cytotoxicity and especially the specificity of the compound.

The metabolism of cyclophosphamide (CP) (56) has been studied intensively in order to explain its specificity towards malignant cells. CP activation takes place mainly in the liver and requires O_2.[62] This process is due to cytochrome P-450 dependent monooxygenates which convert the basic compound to 4-HO-CP (78) at equilibrium with its tautomeric form, aldophosphamide (79). This hydroxylation is probably stereoselective, the 4-*cis*-HO-CP isomer being preferentially formed (78a).[63] However, *trans*-4-HO-CP (78c)is also detected, because of the proteolytic equilibrium existing between the two conformers. The metabolism of cyclophosphamide (56) is shown in Scheme 16.

4-HO-CP (78) is actually the key product of cyclophosphamide metabolism, being the most selective among the cyclophosphamide metabolites. The formation of the 4-keto-CP (80) and the acyclic acid (81) represent detoxication pathways catalysed by aldehyde oxidases. Another detoxication pathway consists of the 4-HO-CP conversion to the thioether (82) by reaction with glutathione or cysteine. This last intermediate may be recycled in the metabolic turnover by the enzymatic cleavage of the thioether bond (delayed toxication). This discovery has led to the design of a

new generation of compounds with better pharmacological properties.



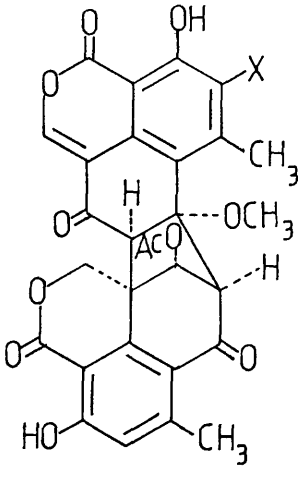
SCHEME 16.

CHAPTER 2.

2.1 Introduction to Duclauxin.

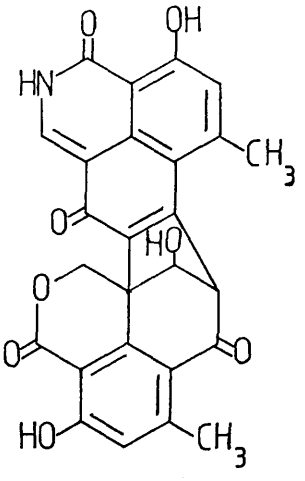
Duclauxin (83) is the main metabolite of *Penicillium duclauxi*.[64] It forms colourless crystals (from benzene or acetone-ethanol), $C_{29}H_{22}O_{11}$, m.p. 230°C, $[\alpha]_D$ + 272.5°. It dissolves in concentrated sulphuric acid, and gives a violet ferric reaction.

Chemical approaches to determine the structure of duclauxin (83) were unsuccessful, so an X-ray study on crystals of monobromoduclauxin (84) was carried out.[65].



(83) X=H

(84) X=Br



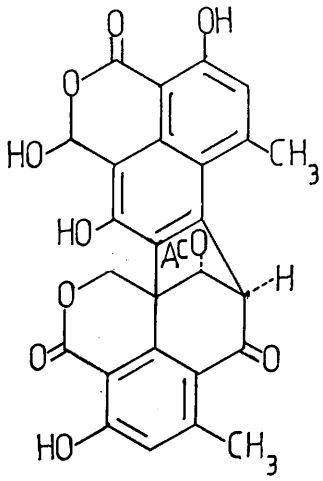
(85)

A characteristic of duclauxin (83) is the formation of orange coloured nitrogenous pigments, giving a positive magnesium acetate reaction with ammonia and some primary amines.[66] This type of compound is given the name duclauxamide (85). Duclauxin gives derivatives which decompose at their melting points : diacetate, m.p. 257°C; dimethyl ether,

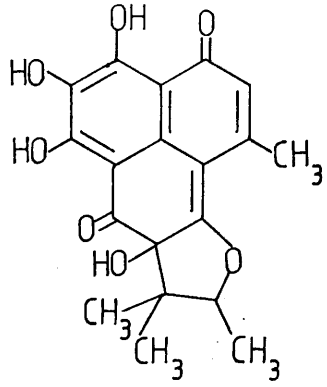
m.p. 180°C; dihydro derivative, m.p. 225°C; and the monobromoderivative (84) m.p. 260°C.

The nmr spectrum of monobromoduclauxin (84) showed the loss of one aromatic proton which had been replaced by one atom of bromine, and a downfield shift of the O*H* signal when compared with the original duclauxin.[67] This indicates that the bromine atom was introduced to a position adjacent to a hydroxyl group and no significant change arises in the skeletal structure by bromination.

Xenoclauxin (86) is another metabolite isolated from *Penicillium duclauxi*. In contrast to duclauxin, it gives no colour with ammonia, indicating the absence of the isocoumarin system.



(86)



(87)

It has been shown that duclauxin and other metabolites of *Penicillium duclauxi* are dimers of

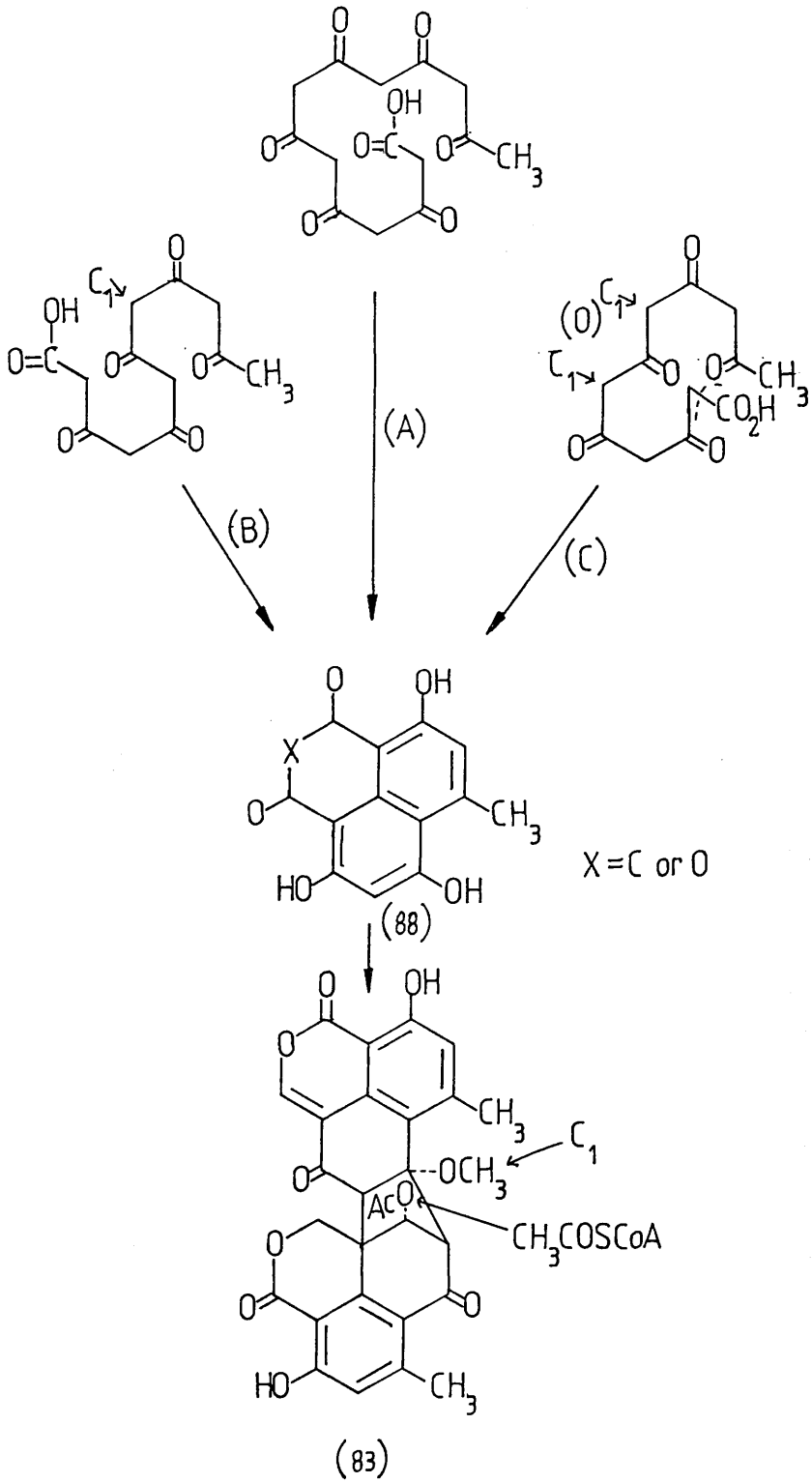
modified phenalenones. Only a few naturally occurring phenalenones are known, e.g. nor-herqueinone (87).[67]

Biochemical studies on duclauxin (83) have shown that it has a marked uncoupling effect on oxidative phosphorylation by rat liver mitochondria at a dose of 25 nmole/mg protein.[68] It was also suggested that duclauxin is bound to protein by electrostatic, hydrophobic and covalent interactions. It inhibits respiration in tumour cells and isolated rat liver mitochondria.[69]

The effect of 32 natural and synthetic derivatives of coumarin, dicoumarin, and 4,4/-epoxydicoumarin on the uptake of ^{14}C-labelled precursors in nucleic acid and protein synthesis was studied in Ehrlick's ascites carcinoma cells *in vitro*. It was found that duclauxin (83) was the most potent inhibitor of cell proliferation.[70]

In the biosynthesis of duclauxin (83), any of the following three pathways (A), (B) or (C) (Scheme 17), could be considered, leading to a hypothetical intermediate (88) which might be dimerised by phenol oxidative coupling and aldol condensation.[71]

In each pathway, an acetate-polymalonate condensation would form the fundamental carbon skeleton of the monomer (88), while in the schemes (B) and (C), C_1-unit incorporation would be required to complete the skeleton. In all cases, after dimerisation, another C_1 unit should be introduced to form a methoxy group,

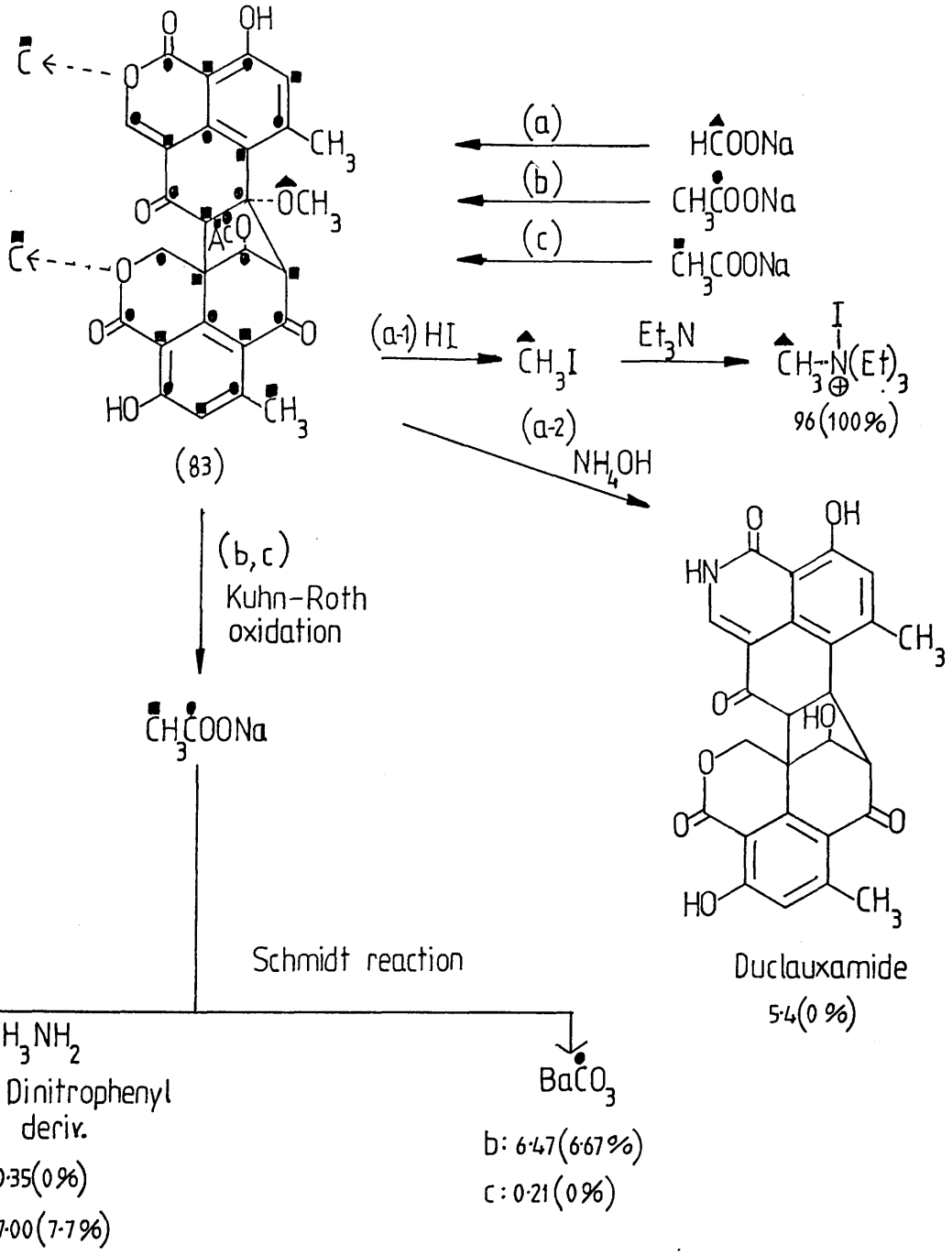


SCHEME 17

and an additional acetylation would provide the acetoxy group.

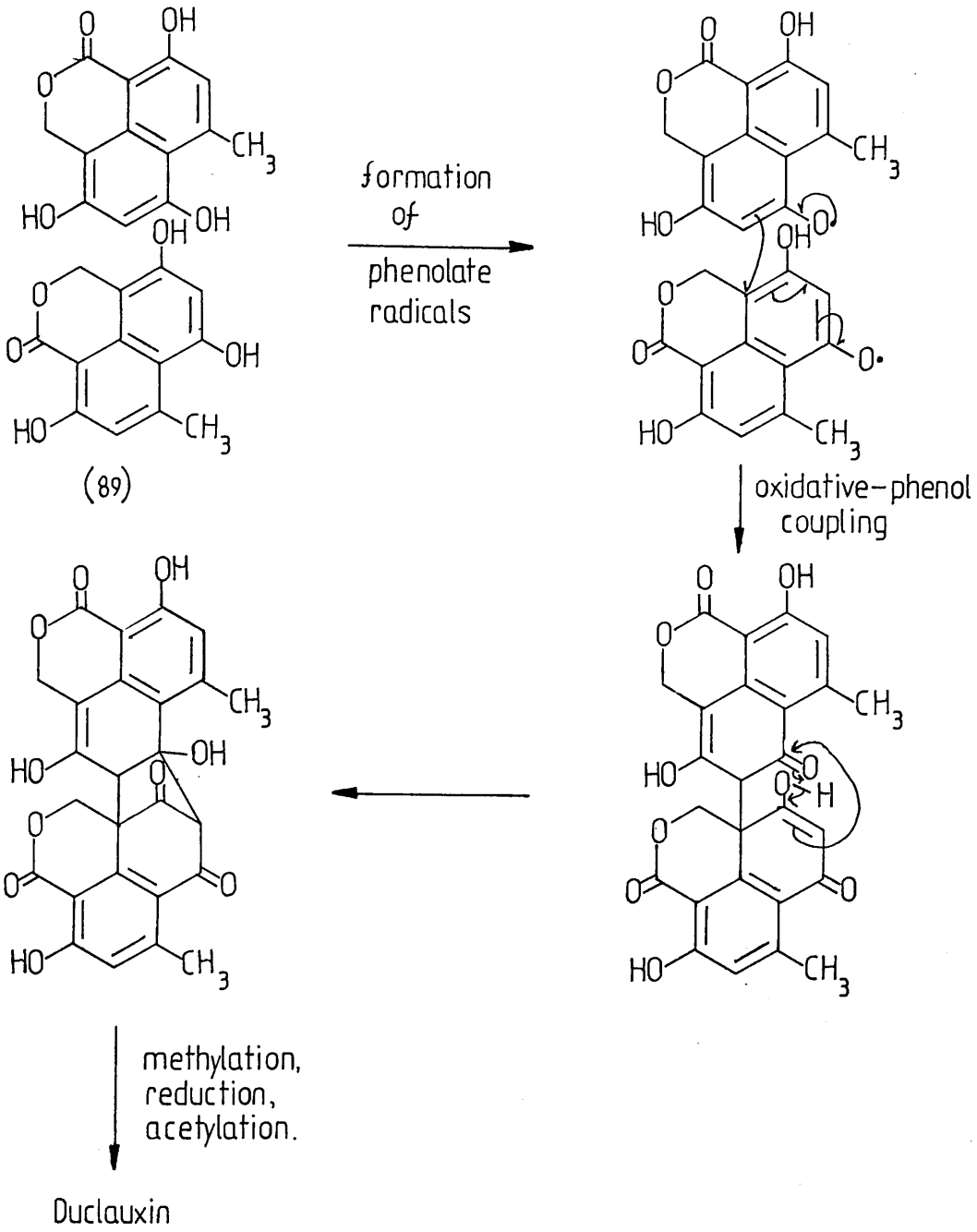
In feeding experiments[71], sodium formate-^{14}C, sodium acetate-1^{14}C, and sodium acetate-2^{14}C were incorporated into the fungus *Penicillium duclauxi*. The results are shown in Scheme 18 and they therefore indicate that pathway (A) is the most probable.

The monomer (89) could be dimerised by means of oxidative-phenol coupling.[72] Phenolate radicals are produced by removing an electron from a phenolate anion or by loss of a proton from a phenol. These radicals couple together in pairs to give stable products. The dimeric products are formed by abstracting one hydrogen atom from the starting phenol and coupling together these intermediates by C-C and C-O bonds, exclusively at *ortho* and *para* positions to the hydroxyl groups. A possible dimerisation route to duclauxin is shown in Scheme 19.



(The figures in the parentheses are the theoretical values of incorporation).

SCHEME 18.

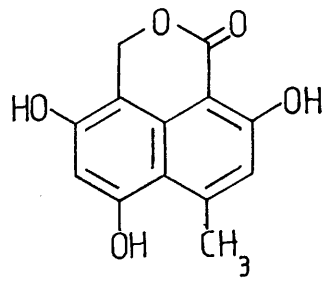

formation
of
phenolate
radicals
oxidative-phenol
coupling
methylation,
reduction,
acetylation.
Duclauxin

(89)

SCHEME 19

2.2 The Synthesis of Duclauxin

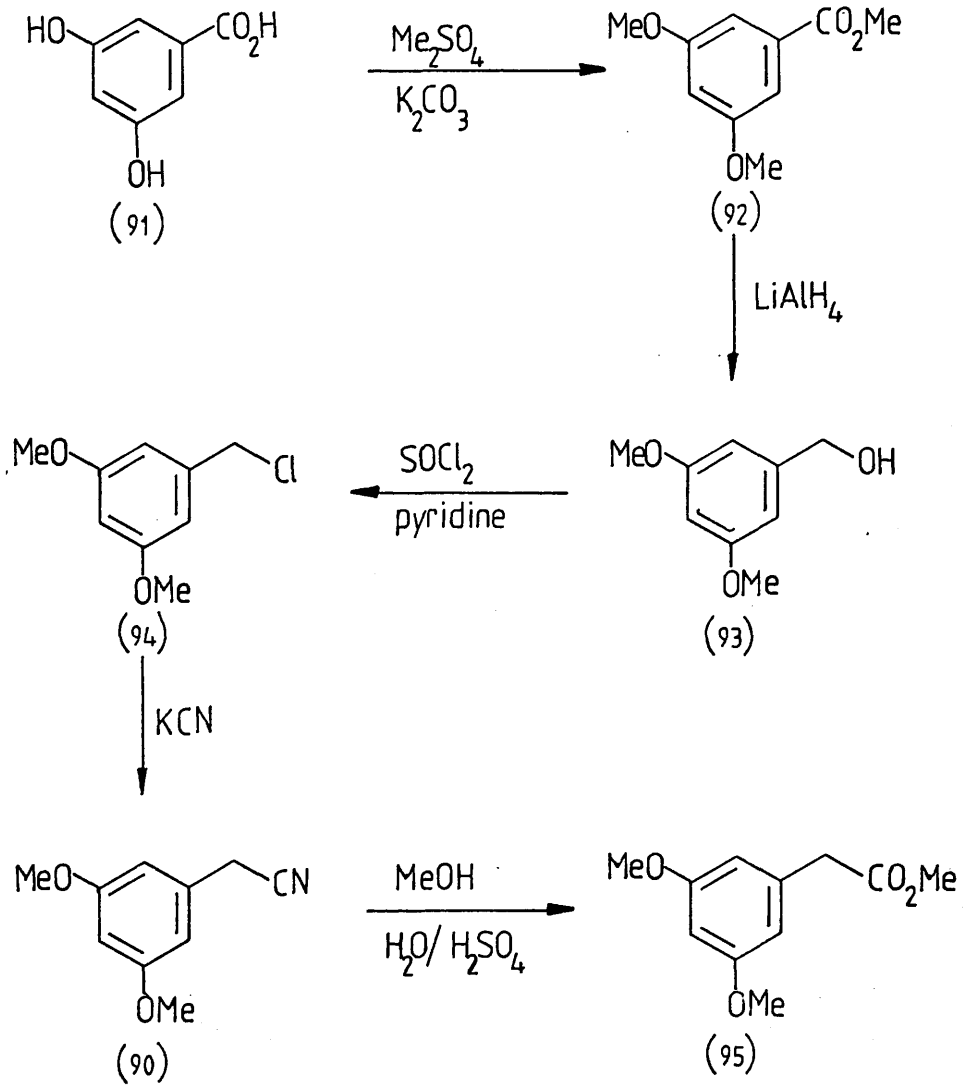
The main synthetic route to duclauxin (83) was to synthesise the monomer (89) and then form a derivative of duclauxin by some type of dimerisation reaction.



(89)

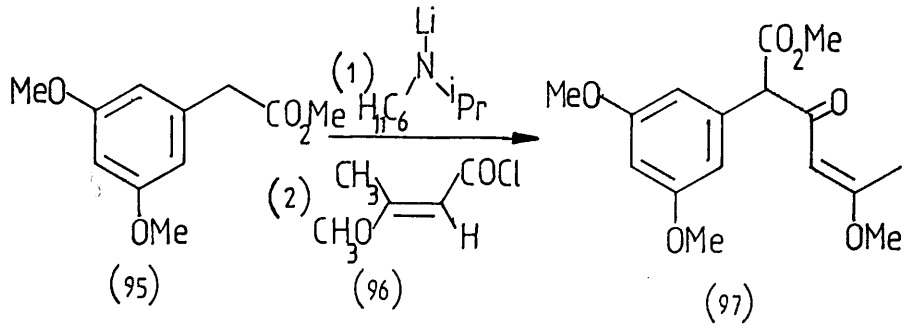
The synthesis involved production of 3,5-dimethoxybenzyl cyanide (90) from commercially available 3,5-dihydroxybenzoic acid (91) (Scheme 20). 3,5-Dihydroxybenzoic acid (91) was methylated using dimethyl sulphate and anhydrous potassium carbonate to give methyl 3,5-dimethoxybenzoate (92) which was then treated with the reducing agent, lithium aluminium hydride, to yield 3,5-dimethoxybenzyl alcohol (93). 3,5-Dimethoxybenzyl alcohol (93) was treated with thionyl chloride and pyridine to give 3,5-dimethoxybenzyl chloride (94) which was then treated with potassium cyanide to yield the nitrile, 3,5-dimethoxybenzyl cyanide (90). This synthetic route to the nitrile (90) was derived by Hinchliffe.[73] Methyl

3,5-dimethoxyphenylacetate (95) was prepared by methanolysis of the nitrile (90).



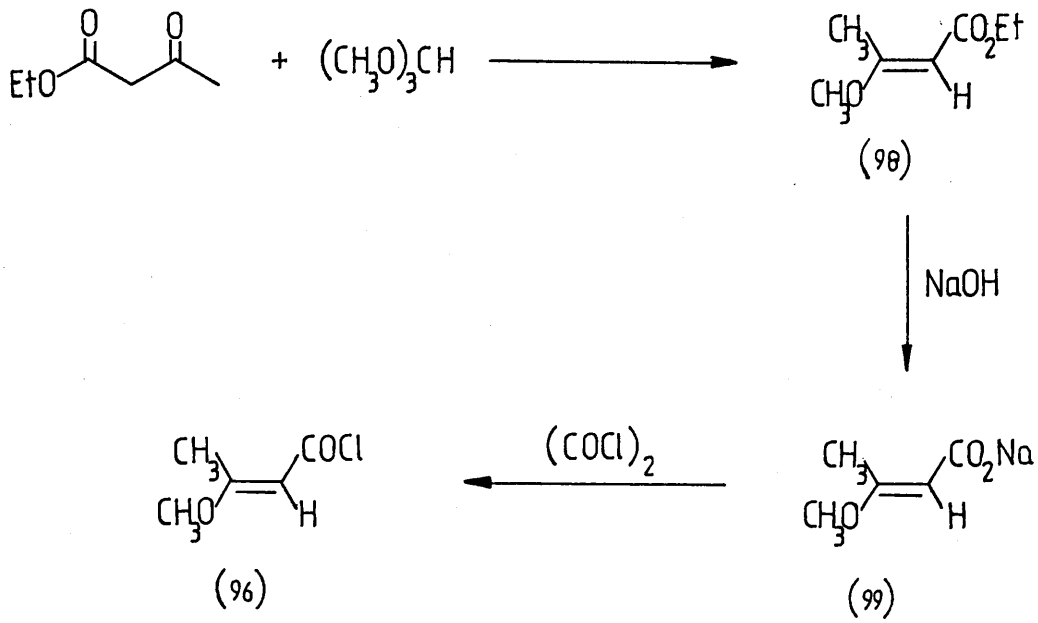
SCHEME 20

The next step in the synthesis involved treatment of methyl 3,5-dimethoxyphenylacetate (95) with lithium *N*-isopropylcyclohexylamide as the base, to form the anion of (95). The resulting lithium species was condensed with excess (*E*)-3-methoxy-2-butenoyl chloride (96) to give methyl 2-(3,5-dimethoxyphenyl)-5-methoxy-3-oxohex-4-enoate (97) (Scheme 21).

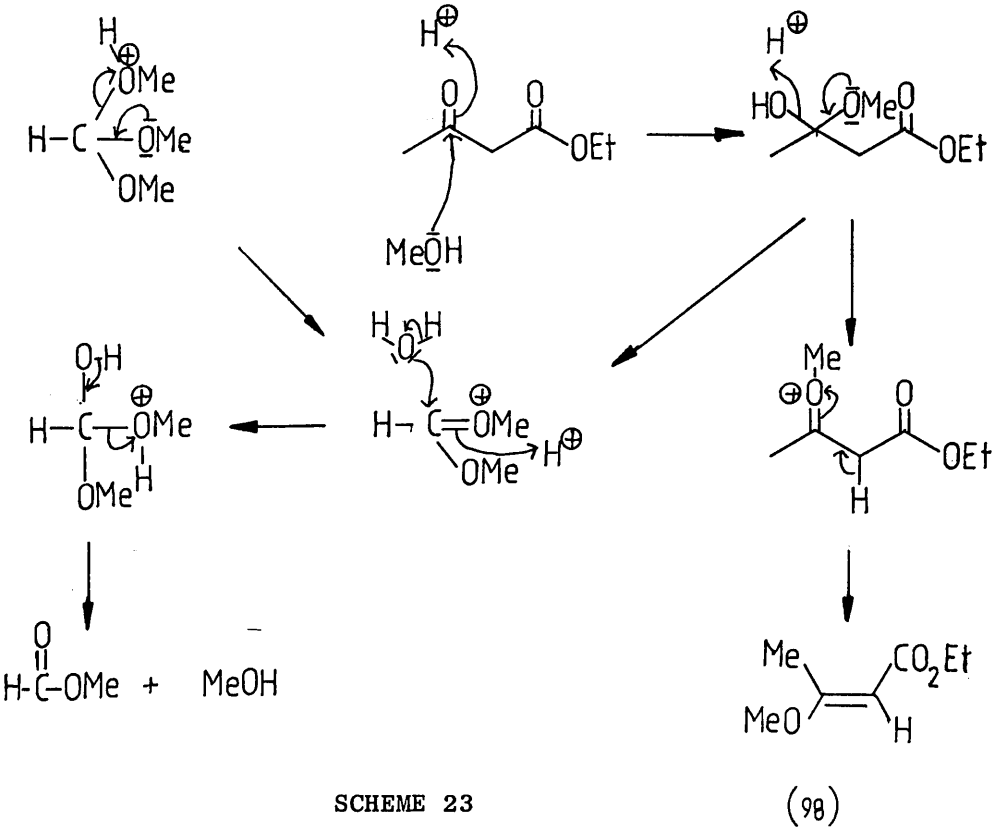


SCHEME 21

However, this reaction (Scheme 21) could not be carried out since attempts to prepare the highly unstable acid chloride, (*E*)-3-methoxy-2-butenoyl chloride (96), were unsuccessful (Scheme 22). The attempted preparation of the acid chloride (96) was carried out by the same procedure as Buchi and Leung.[74] Ethyl (*E*)-3-methoxy-2-butenoate (98) was prepared by treating a mixture of ethyl acetoacetate and trimethyl orthoformate with concentrated sulphuric acid (Scheme 23). The sodium salt of (*E*)-3-methoxy-2-butenoate (99) was then prepared by treating (98) with sodium hydroxide in an ester hydrolysis reaction. Finally this sodium salt (99) was treated with oxalyl chloride in an attempt to prepare (*E*)-3-methoxy-2-butenoyl



SCHEME 22

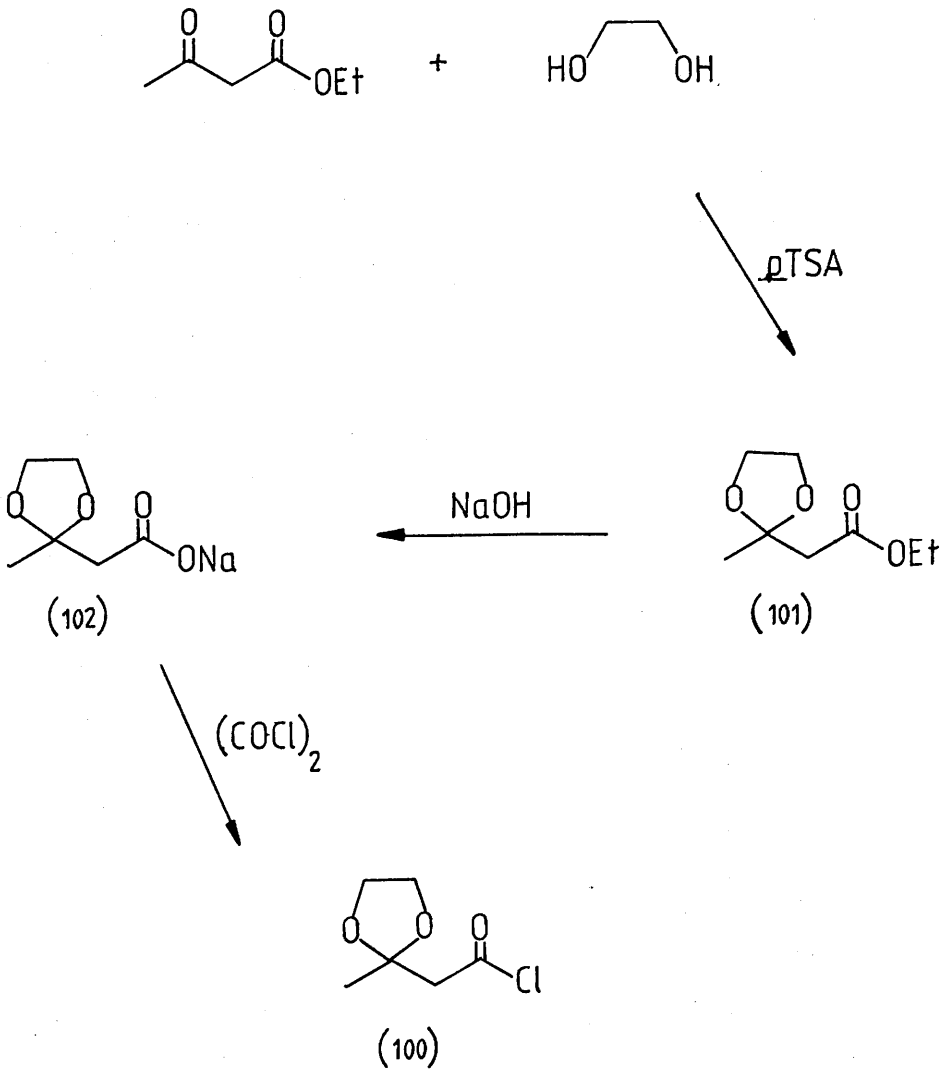


SCHEME 23

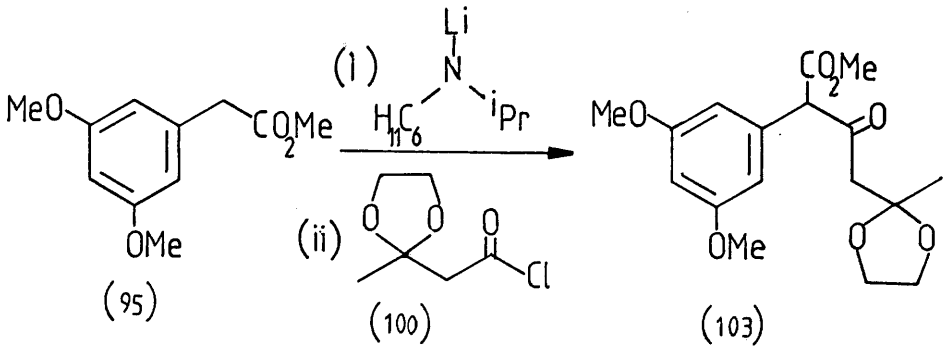
chloride (96). Many attempts to carry out this final step were unsuccessful. The acid chloride was so unstable in that it formed a brown-black viscous material before it could even be purified by distillation.

One way that overcame the formation of the unstable acid chloride (96) was to make an acid chloride with an acetal protecting group, i.e. the ethylene acetal of acetoacetyl chloride (100) (Scheme 24). The ethylene acetal of ethyl acetoacetate (101) was prepared by treating a mixture of ethyl acetoacetate and ethylene glycol with a catalytic amount of *p*-toluenesulphonic acid. This acetal (101) was then hydrolysed with base to give the corresponding sodium salt (102), which in turn was treated with oxalyl chloride to give the ethylene acetal of acetoacetyl chloride (100). This acid chloride was prepared just prior to use.

The ethylene acetal of acetoacetyl chloride (100) was reacted with the lithium species formed by treatment of methyl 3,5-dimethoxyphenylacetate (95) with lithium *N*-isopropylcyclohexylamide (Scheme 25).

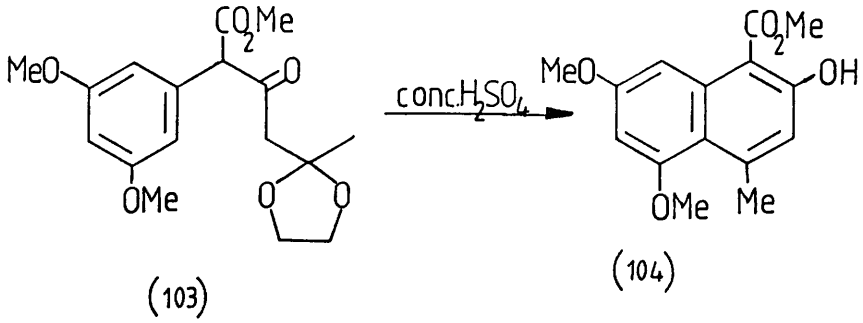


SCHEME 24



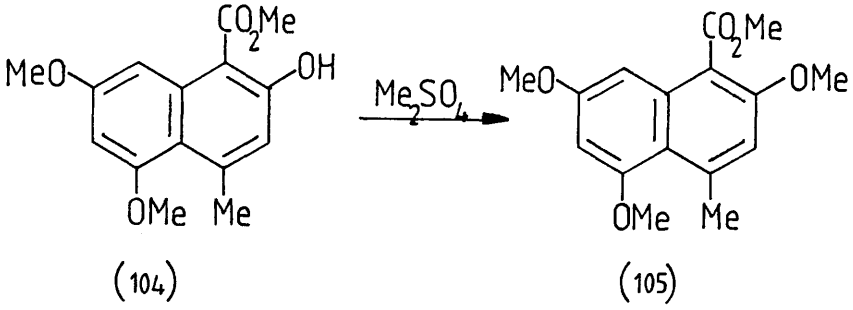
SCHEME 25.

The 5-ethylene acetal of methyl 2-(3,5-dimethoxyphenyl)-3,5-dioxohexanoate (103) could not be isolated from the reaction mixture as a single compound. The ^1H nmr spectrum and t.l.c. showed the crude reaction mixture to be a complex mixture of products that could not be resolved by chromatography. The crude reaction mixture was used in the next step of the synthesis to give methyl-2-hydroxy-5,7-dimethoxy-4-methyl naphthalene-1-carboxylate (104) as the only product. This crude reaction mixture was treated with concentrated sulphuric acid in water and methanol to give methyl 2-hydroxy-5,7-dimethoxy-4-methyl naphthalene-1-carboxylate (104) as a fine, white, crystalline compound (Scheme 26).



SCHEME 26

Methyl 2-hydroxy-5,7-dimethoxy-4-methyl naphthalene-1-carboxylate (104) was then methylated using dimethyl sulphate to give methyl 2,5,7-trimethoxy-4-methyl naphthalene-1-carboxylate (105) (Scheme 27).

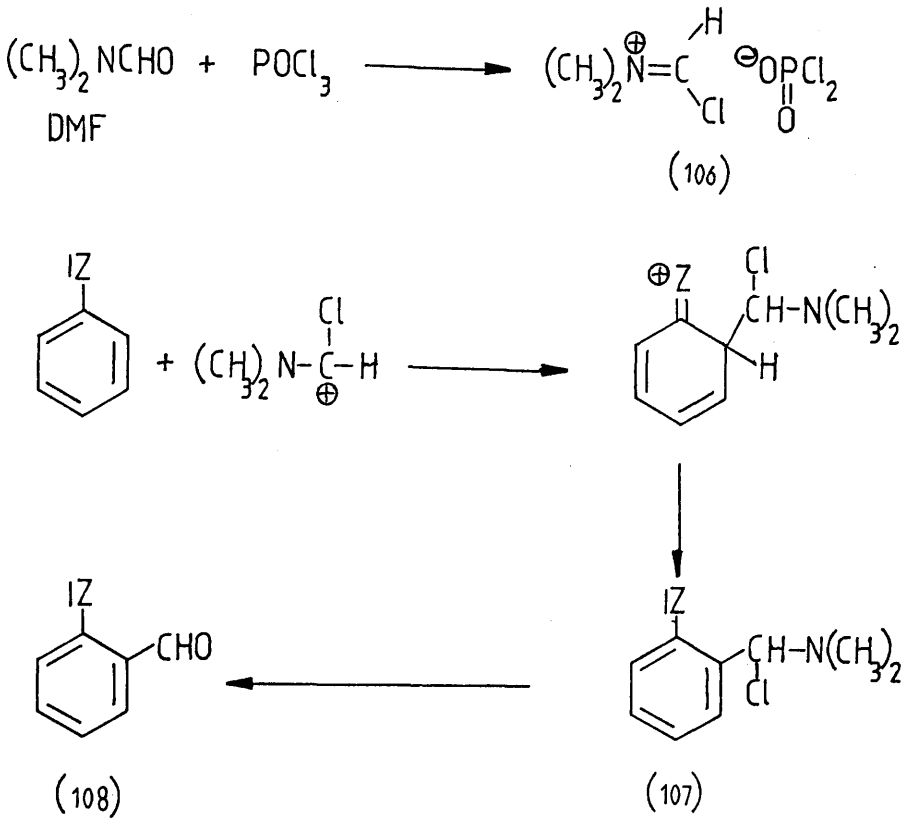


(104) (105)

SCHEME 27

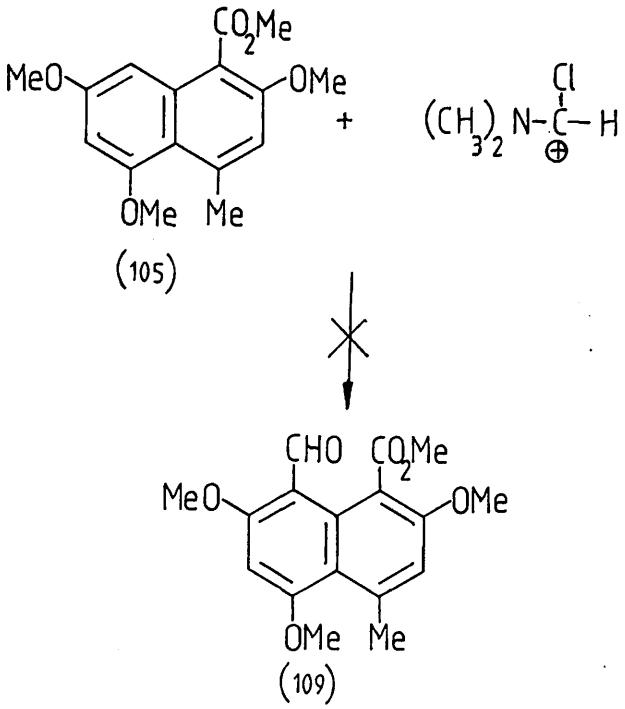
The next step in the synthetic route involved trying to formylate methyl 2,5,7-trimethoxy-4-methyl naphthalene-1-carboxylate (105) at position 8 in the ring system. This was attempted by means of the Vilsmeier reaction.

The Vilsmeier reaction is the most common method for the formylation of aromatic rings.[75] Aromatic hydrocarbons can only be formylated if they are much more active than benzene. Phosphorus oxychloride and dimethylformamide (DMF) react together to form the complex (106). The probable reaction mechanism is shown in Scheme 28.



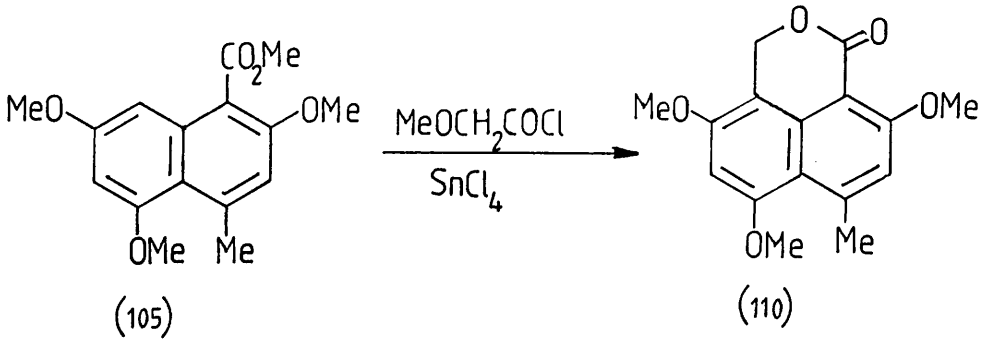
SCHEME 28

The chloro amine (107) is unstable and easily hydrolysed to the product (108). When methyl 2,5,7-trimethoxy-4-methyl naphthalene-1-carboxylate (105) was treated with the complex (106) no reaction took place and only starting material was recovered. The desired product from the reaction would have been methyl 8-formyl-2,5,7-trimethoxy naphthalene-1-carboxylate (109) (Scheme 29). Position 8 of (105) should be activated due to the *ortho*- and *para*-directing methoxyl group.



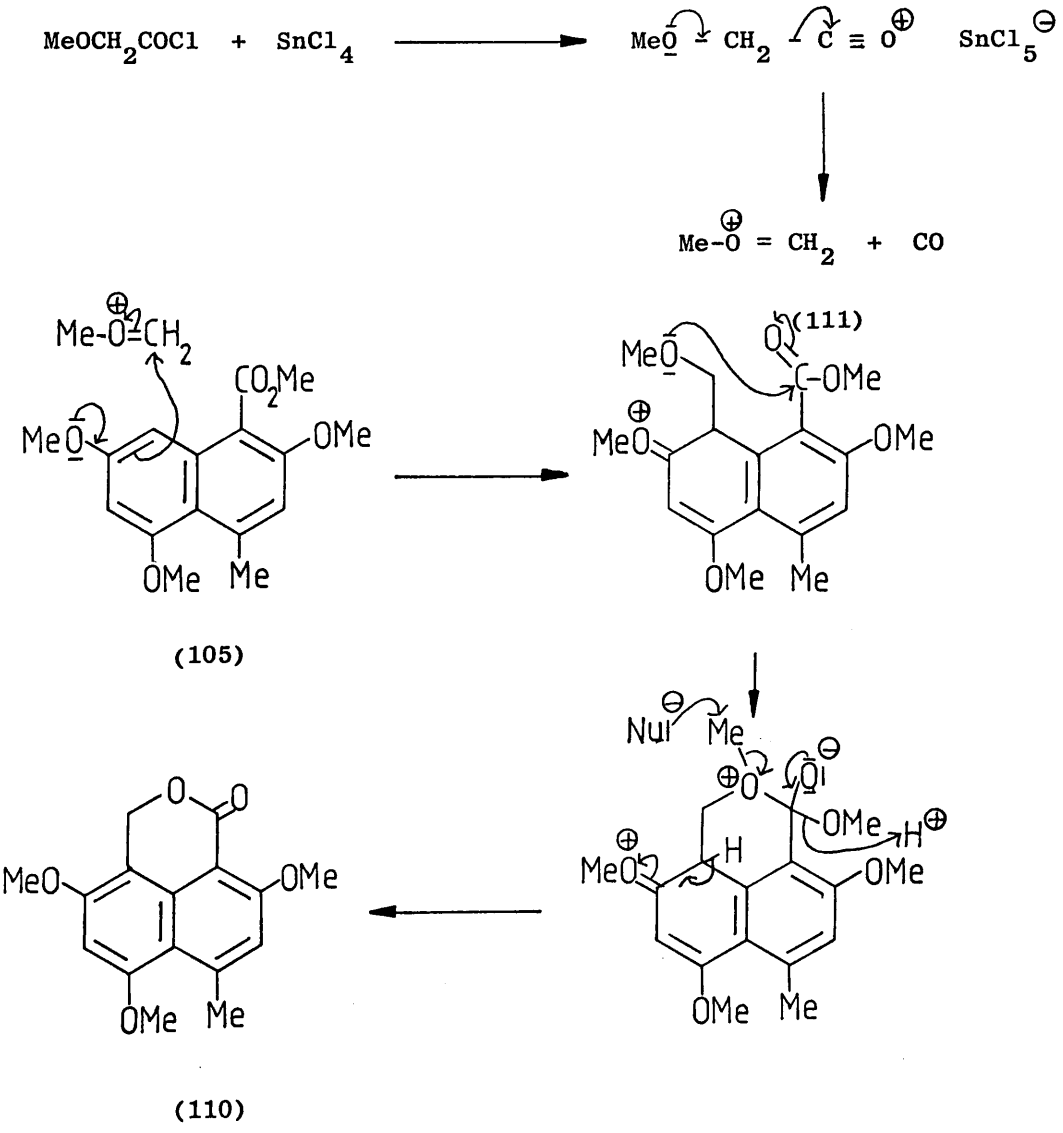
SCHEME 29

Fortunately the next reaction that was tried gave the desired product with the lactone ring attached, 4,6,9-trimethoxy-7-methyl pyrano[1,8-*c*]naphthalen-1(3*H*)-one (110). Methyl 2,5,7-trimethoxy-4-methyl naphthalene-1-carboxylate (105) was treated with methoxyacetyl chloride and tin(IV)chloride to give (110) (Scheme 30).



SCHEME 30

The mechanism for the chloromethylation of aromatic compounds with methoxyacetyl chloride and aluminium chloride developed by McKillop[76] where the methoxymethyl cation (111) is postulated as an intermediate is analogous to the one for the formation of the lactone ring in (110). The formation of the lactone ring results from Lewis acid induced cyclisation of the intermediate methoxymethyl derivative (Scheme 31).



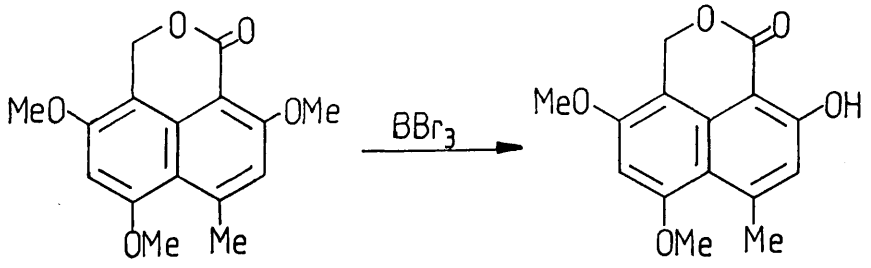
SCHEME 31

Various reagents have been used in an attempt to demethylate 4,6,9-trimethoxy-7-methyl pyrano[1,8,*c*] naphthalen-1(3*H*)-one (110). These are as follows:-

(a) Boron tribromide: This reagent has been used widely to cleave ether protecting groups under mild conditions without affecting a large number of other functional groups. Also the need for the use of strongly acidic or basic reaction conditions, or reducing environments can be avoided. These advantages would therefore be ideal for this reagent to be used to demethylate (110).

Boron tribromide was found to be a useful reagent by Benton and Dillon as early as 1942[77] but this was not recognised until 1963 when McOmie published papers on the usefulness of boron tribromide.[78]

Using boron tribromide to demethylate 4,6,9-trimethoxy-7-methyl pyrano[1,8,*c*]naphthalen-1(3H)-one (110) was somewhat disappointing in that it only cleaved one of the methoxyl groups. The product obtained was 9-hydroxy-4,6-dimethoxy-7-methyl pyrano[1,8,*c*]-napthalen-1(3*H*)-one (112) (Scheme 32).

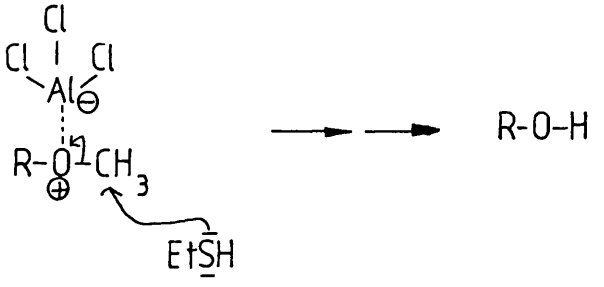


(110) (112)

SCHEME 32

(b) Hydrobromic acid: Aqueous hydrobromic acid, particularly with acetic acid as a co-solvent, has been extensively used to cleave ethers. Using this reagent to cleave 4,6,9-trimethoxy-7-methyl pyrano[1,8,*c*] napthalen-1(3*H*)-one (110) gave a complex mixture of products which could not be resolved.

(c) Aluminium chloride with ethanethiol: This system works on the principle of using a hard acid, aluminium chloride, with a soft nucleophile, ethanethiol.[79] Aluminium chloride is complexed to the oxygen atom of the ether group to be cleaved. Ethanethiol then attacks in a S_N2 reaction as follows:



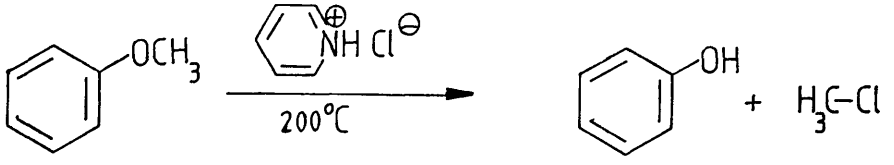
This system was however ineffective in the demethylation of 4,6,9-trimethoxy-7-methyl pyrano[1,8,*c*]naphthalen-1(3*H*)-one (110).

(d) Aluminium chloride: As with other Lewis acids, aluminium chloride forms addition compounds of the acid-base type with most ethers. It has also been used to selectively cleave methoxy groups adjacent to an aldehyde or a ketone function without affecting other

methoxy groups present in the molecule.

The product obtained from the treatment of 4,6,9-trimethoxy-7-methyl pyrano[1,8,*c*]napthalen-1(3*H*)-one (110) was 9-hydroxy-4,6-dimethoxy-7-methyl pyrano[1,8,*c*] naphthalen-1(3*H*)-one (112).

(e) Pyridine hydrochloride: The reaction involving the use of the salt, pyridine hydrochloride, is carried out at approximately 180-200°C without an additional solvent and using a large excess of the salt. For example (113) is completely demethylated to give (114):



(113) (114)

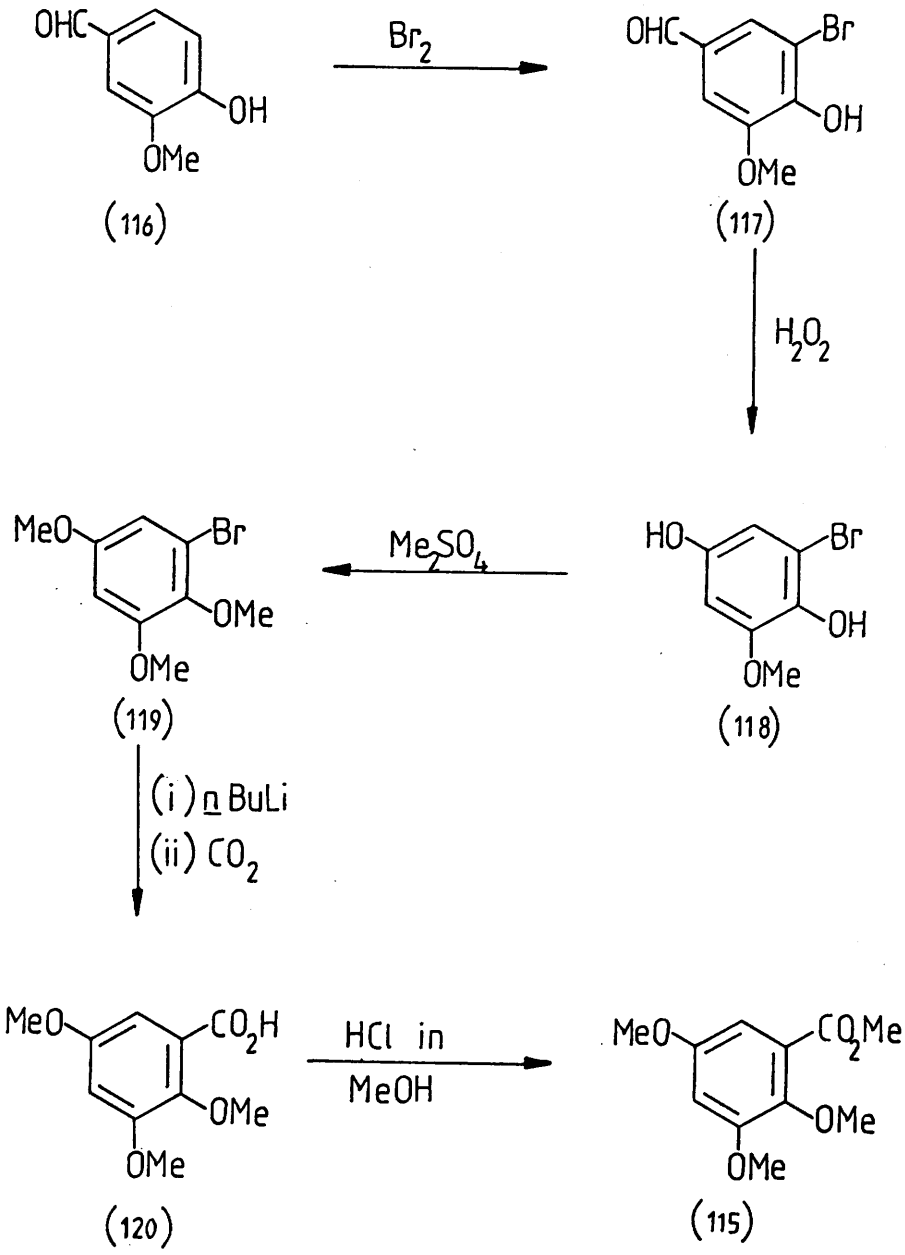
This reagent failed to demethylate 4,6,9-trimethoxy-7-methyl pyrano[1,8,*c*]naphthalen-1(3*H*)-one (110).

CHAPTER 3.

The Synthesis of Cyclic Phosphonates as Potential Antitumour Compounds.

3.1 The Synthesis of 2-Dimethylamino-6,8-dihydroxy-3-methyl-2*H*-1,2 λ^5-benzoxaphosphorin-2,4(3*H*)dione

The synthesis involved the production of methyl 2,3,5-trimethoxybenzoic acid (115) from vanillin (116) (Scheme 33). Vanillin (116) was brominated using bromine in glacial acetic acid to give 3-bromo-4-hydroxy-5-methoxybenzaldehyde (117). (117) was then oxidised in a Baeyer-Villiger oxidation reaction to yield 2,5-dihydroxy-3-methoxybromobenzene (118) which was then methylated using dimethyl sulphate to give 2,3,5-trimethoxybromobenzene (119).[80] The anion of 2,3,5-trimethoxybromobenzene (119) formed using *n*-butyllithium was then carboxylated to give 2,3,5-trimethoxybenzoic acid (120).[81] 2,3,5-Trimethoxybenzoic acid (120) was esterified by saturating the compound in methanol with hydrogen chloride to yield methyl 2,3,5-trimethoxy-benzoate (115).[82]


OHC
OH
OMe
(116)
Br2
OHC
Br
OH
OMe
(117)
H2O2
MeO
Br
OMe
OMe
(119)
Me2SO4
HO
Br
OH
OMe
(118)
(i) n BuLi
(ii) CO2
MeO
CO2H
OMe
OMe
(120)
HCl in
MeOH
MeO
CO2Me
OMe
OMe
(115)

SCHEME 33

Before the next step in the synthesis could be carried out, ethylphosphonic acid bis(dimethylamide) (121) had to be prepared. Ethylphosphonic dichloride (122) was firstly prepared by the preparation of J.P. Clay.[83] The method may be represented by the following reactions:

(1) $CH_3CH_2Br + PCl_3 + AlCl_3 \longrightarrow C_2H_5PX_3\ ^{+}AlX_4^{-}$

(insoluble complex)

X = Br or Cl

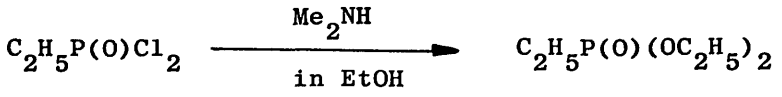
(2) $C_2H_5PX_3\ ^{+}AlX_4^{-} \xrightarrow{\text{conc. HCl}} C_2H_5P(O)Cl_2$

(122)

However, several factors are important in the preparation of (122) which include the order of mixing of the reagents, control of temperature, anhydrous condition of the reagents and apparatus, and molar ratio of concentrated hydrochloric acid to complex used in the hydrolysis.

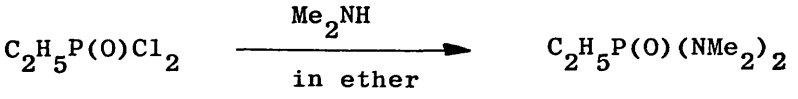
The preparation of ethylphosphonic acid bis(dimethylamide) (121) was attempted by reacting ethylphosphonic dichloride (122) with a solution of dimethylamine in ethanol. The product obtained was actually diethyl ethanephosphonate (123). The strong phosphorus-oxygen bond will preferentially form instead

of the phosphorus nitrogen bond.



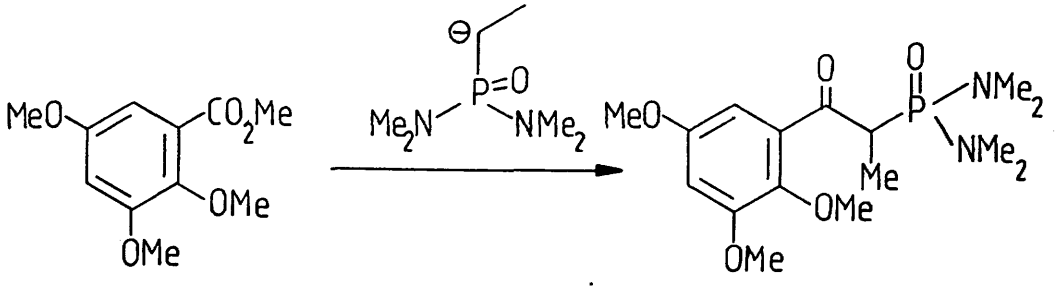
(122) (123)

Ethylphosphonic acid bis(dimethylamide) (121) was actually prepared by reacting (122) with a solution of dimethylamine in ether.



(122) (121)

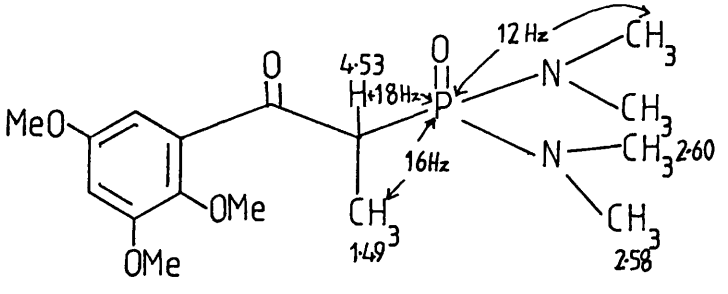
Methyl 2,3,5-trimethoxybenzoate (115) reacted successfully with the anion of ethyl phosphonic acid bis(dimethylamide) to give the β-ketophosphonamide (124) in high yield (Scheme 34).



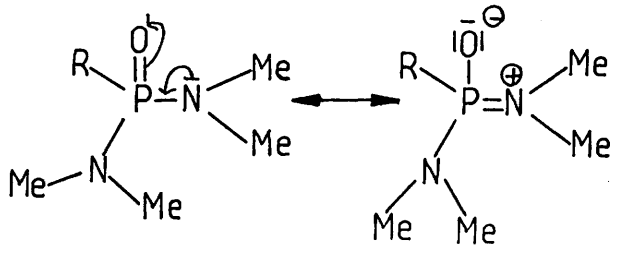
(115) (124)

SCHEME 34.

The proton nmr spectrum (run in d_1-chloroform) of the β-ketophosphonamide (124) was unusual in that there was coupling observed between the phosphorus atom ($I=^1/_2$) and the protons. This coupling was actually observed over three bond lengths. Due to this effect the nmr signal of the proton α- to the ketone carbonyl group is split into a doublet of quartets at 4.53 ppm (J_{PH} = 18Hz). The nmr signal of the methyl group α- to the ketone carbonyl is split into a doublet of doublets at 1.49 ppm (J_{PH} = 16Hz). The *N*-methyls are not equivalent and their nmr signal consists of two doublets at 2.58 ppm and 2.60 ppm. The non-equivalence of the methyls is due to contribution of the resonance structure (125) of the phosphonamide group and the phosphorus coupling ($^3J_{PH}$ = 12Hz) (see diagram).

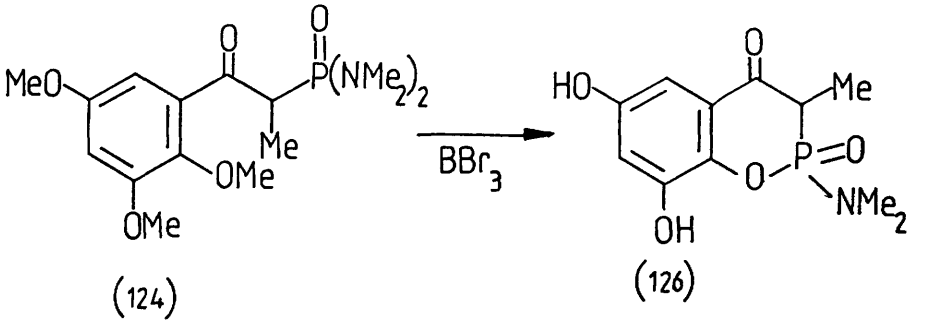


(124)

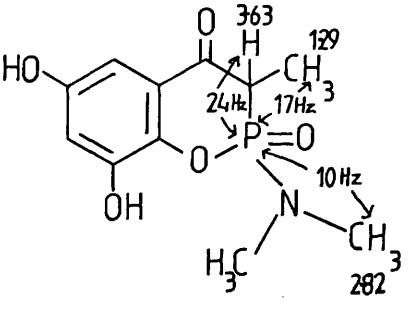


(125)

When the β-ketophosphonamide (124) is treated with boron tribromide an interesting phosphorus heterocyclic compound is formed. This cyclic phosphonate, 2-dimethylamino-6,8-dihydroxy-3-methyl-2*H*-1,2λ^5-benzoxaphosphorin-2,4(3*H*)-dione (126), also has an unusual nmr spectrum. The chemical shift (in d_6-acetone) of the proton α- to the ketone carbonyl moves upfield to 3.63 ppm and the shift of the protons of the methyl groups moves upfield to 1.29 ppm. The signal of the *N*-methyls appears downfield as a doublet at 2.82 ppm. The *N*-methyls are accidentally equivalent in this structure ($^3J_{PH}$ = 10Hz) (see diagram).

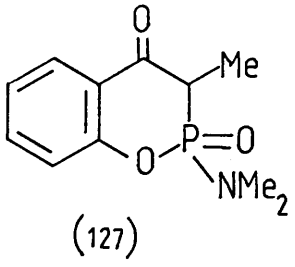


(124) (126)

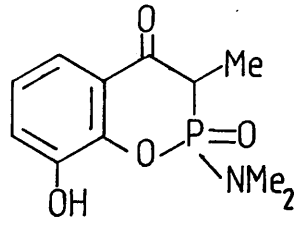


3.2 The Synthesis of Some Other Cyclic Phosphonates.

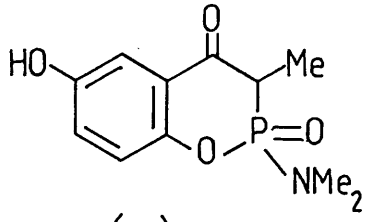
Several other cyclic phosphonate compounds were prepared. These are as follows:



(127)

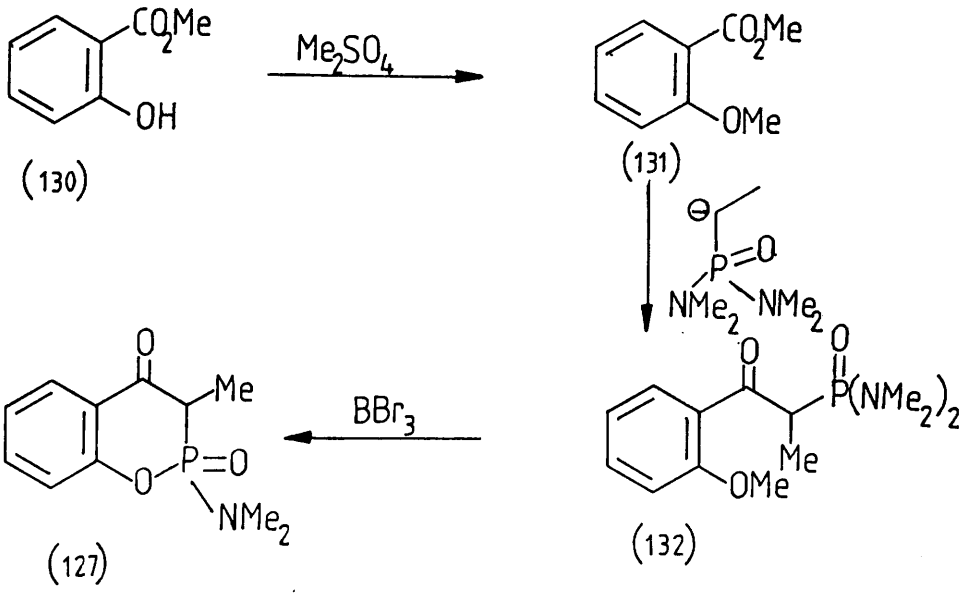


(128)



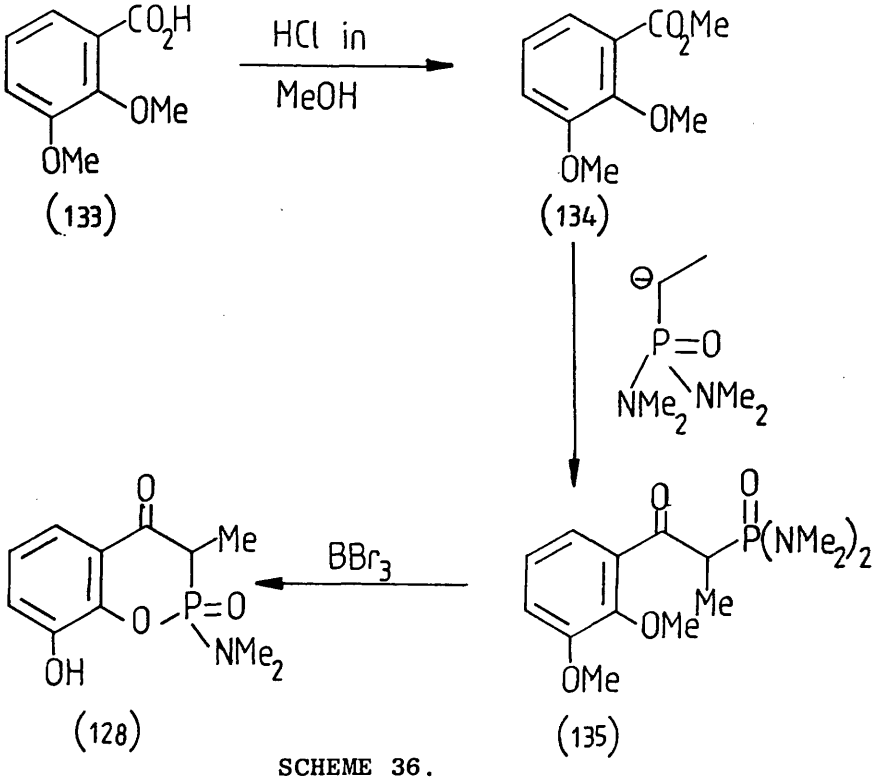
(129)

The cyclic phosphonate (127) was prepared using methyl 2-hydroxybenzoate (130) as the starting material (Scheme 35).



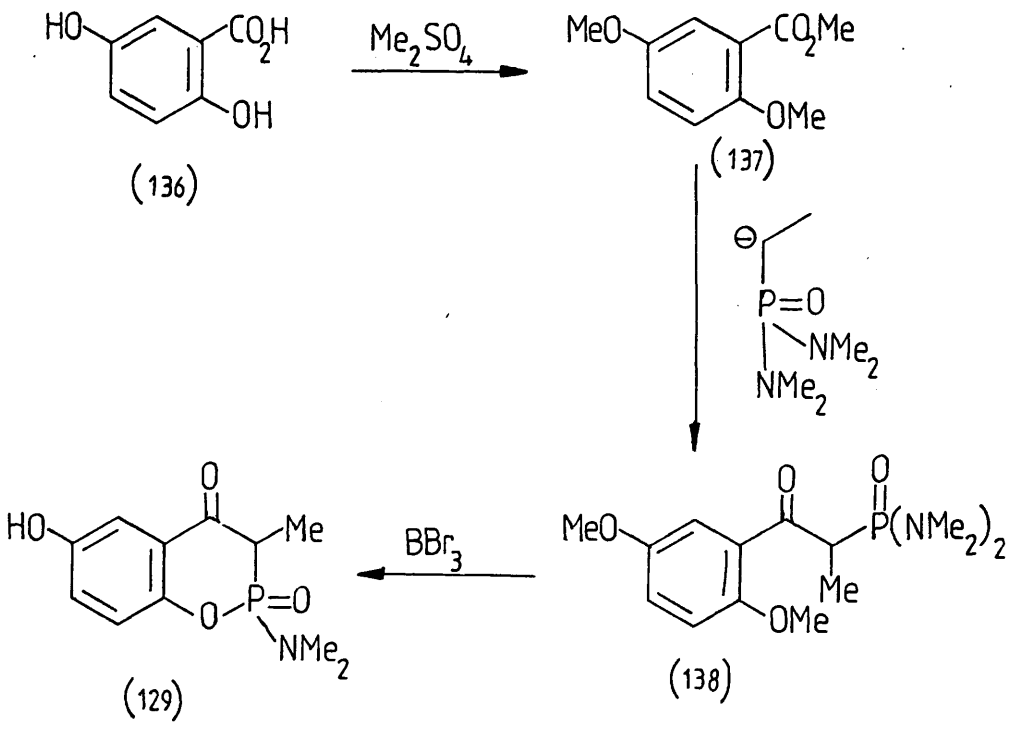
SCHEME 35

The cyclic phosphonate (128) was similarly prepared using 2,3-dimethoxybenzoic acid (133) as starting material. (133) was esterified by saturating the compound in methanol with hydrogen chloride to yield methyl 2,3-dimethoxybenzoate (134) (Scheme 36).



SCHEME 36.

Again the cyclic phosphonate (129) was prepared by methylating the starting material 2,5-dihydroxybenzoic acid (136) with dimethyl sulphate to give methyl 2,5-dimethoxybenzoate (137) (Scheme 37).



SCHEME 37.

These cyclic phosphonate compounds are potential antitumour compounds. They are soon to be sent away to assess their potential antitumour properties.

CHAPTER 4.

4.1 Introduction to Differanisole A

Differanisole A (137), a secondary metabolite, was first discovered in the cultural broth of a *Chaetemium* strain RB-001 from a soil sample.[84,85] It has been found to induce differentiation in mouse myeloid leukaemia M1 cells and mouse melanoma B16 cells as judged by the induction of lysozyme and tyrosinase activity respectively. However differentiation of human promyeloid leukaemia HL60 cells by differanisole A was not observed when lysozyme induction was used as the test. When neuroblastoma cells treated with differanisole A are transplanted to A/J mice apparently inhibiting the growth of tumours, it was found to decrease the turmogenicity of the cells. It is therefore apparent that differanisole A appears to exhibit both *in vivo* and *in vitro* antitumour effects. This is a very beneficial property since many substances are known to induce differentiation of tumour cells *in vitro*, but few have been reported which give a positive *in vivo* effect.

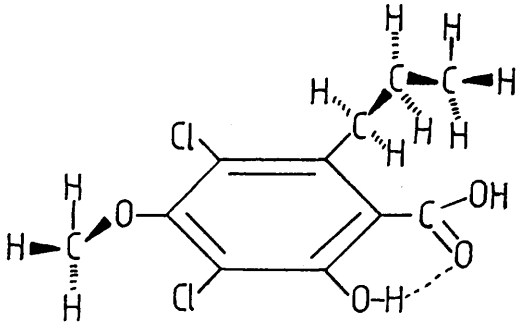
Differanisole A is an acidic compound, and mass spectroscopic analysis showed it to have molecular formula $C_{11}H_{12}O_4Cl_2$ (M.W. 279.1193). It shows no optical activity and has three ultraviolet absorption maxima in methanol at 318 nm (ε 2 700), 257 nm (ε 4 060), and 220 nm (ε 2 300). The infra-red spectrum (KBr disc) shows three major absorption bands. The large broad absorption at 3600-3300 cm^{-1} is typical of a hydrogen bonded hydroxyl group stretch. An absorption

band at 1640 cm^{-1} is typical of a carbonyl stretch, and an absorption band at 1575 cm^{-1} indicates an aromatic ring absorption frequency. The 100 MHz ^{1}H nmr spectrum (in d_6-acetone) shows resonances at δ 3.94(s), 3.15(m), 1.61(m) and 1.02 ppm (t). The ^{13}C nmr spectrum (in d_4-methanol) shows six types of aromatic carbons at δ 113.4, 115.0, 119.1, 142.7, 153.8 and 157.1 ppm.

Chemical tests on differanisole A have shown that it gives positive iodine and $KMnO_4$ reactions but negative Fehling and 2,4-dinitrophenylhydrazine reactions. It is soluble in H_2O, MeOH, acetone, DMF and DMSO but insoluble in *n*-hexane or petroleum ether.

The spectroscopic and chemical data therefore indicate that differanisole A is a highly substituted benzene ring with *n*-propyl, methoxy and carbonyl groups as the substituted groups on the ring.

The structure of differanisole A was confirmed by single crystal X-ray diffraction analysis.[86] It was found that there was an intramolecular hydrogen bond between a carboxylic oxygen and the adjacent oxygen group. The planar aromatic ring has a methoxy group attached at C-4 which lies below the ring and a propyl group at C-6 lying above the ring, in planes which are almost perpendicular to the ring. Finally there are two chlorine atoms at C-3 and C-5 with the carboxyl and hydroxyl groups, at C-1 and C-2 respectively, lying in the plane of the ring. The crystal structure of differanisole A is thought to consist of dimers linked by hydrogen bonds.



(139)

Differanisole A is probably biosynthesised via the polyketide pathway (Scheme 38).

(a) Activation 1.

$$CH_3CO_2H + CoASH \longrightarrow CH_3COSCoA$$

(b) Activation 2.

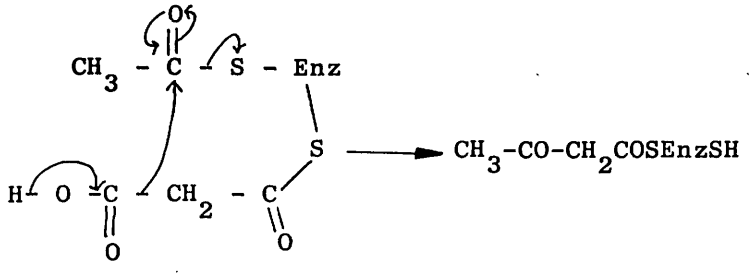
$$CH_3COSCoA + CO_2 \longrightarrow \begin{matrix} CH_2\text{-}COSCoA \\ | \\ CO_2H \end{matrix}$$

(c) Priming Reaction.

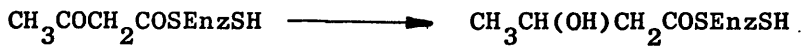
(i) $$CH_3COSCoA + Enz\begin{matrix} SH \\ SH \end{matrix} \longrightarrow CH_3COS\text{-}Enz\text{-}SH + CoASH$$

(ii) $$\begin{matrix} CH_2\text{-}COSCoA \\ | \\ CO_2H \end{matrix} + CH_3\text{-}COSEnzSH \longrightarrow CH_3COS\text{-}Enz\text{-}SCOCH_2\text{-}CO_2H + CoASH$$

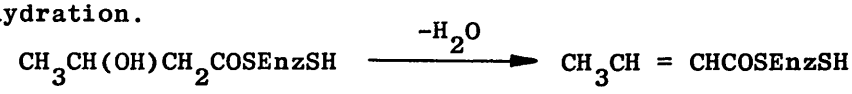
(d) Condensation.



(e) Reduction 1.

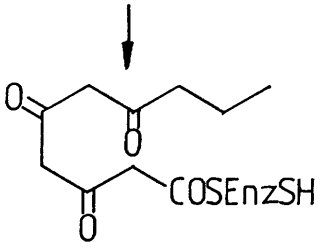


(f) Dehydration.

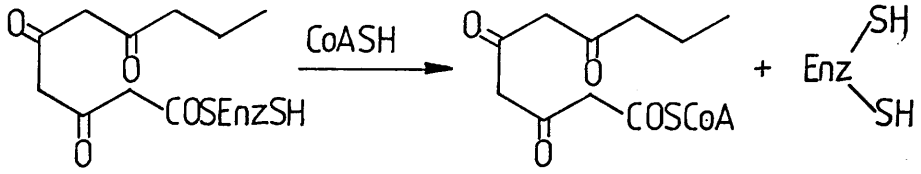


(g) Reduction 2.

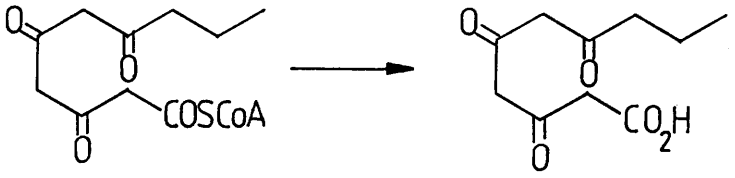
(h) Repeat steps (c)(ii) to (d) three times



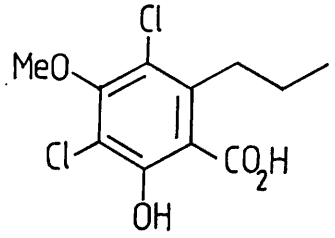
(i) Termination.



(j) Hydrolysis.



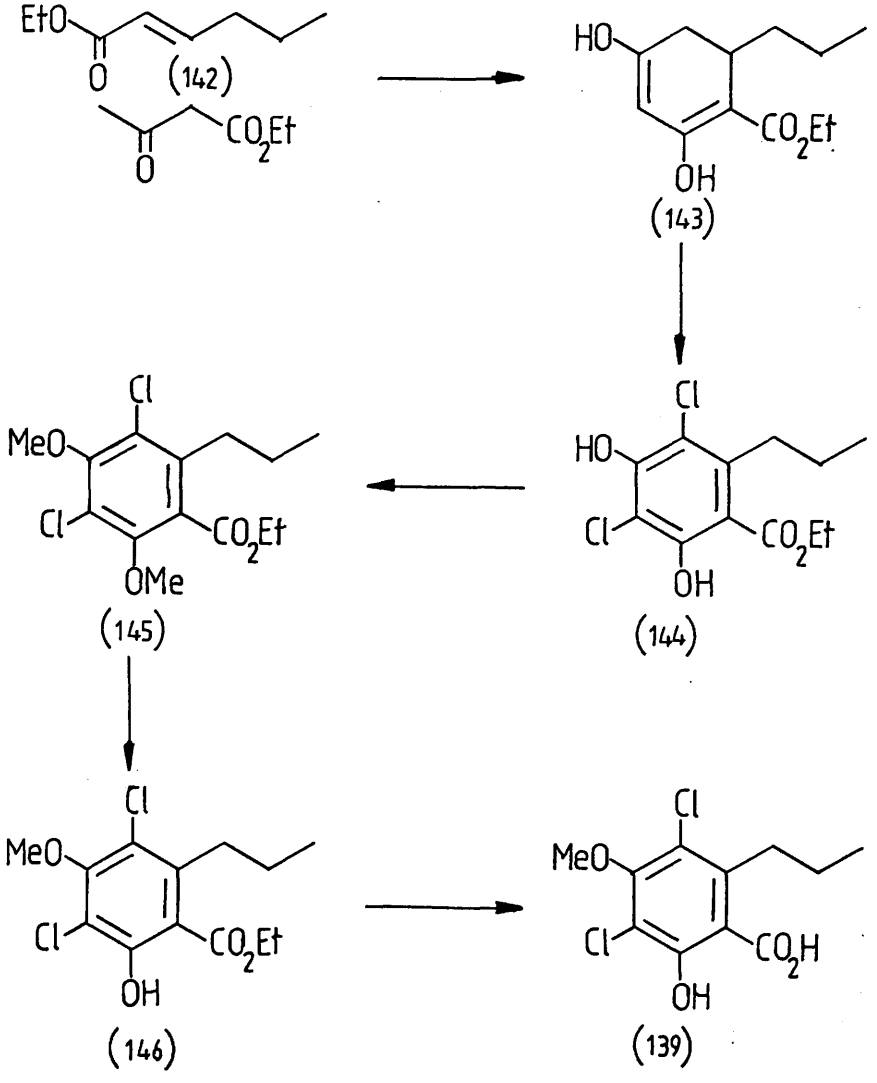
(k) (i) Aldol condensation
(ii) Enolisation
(iii) Chlorination
(iv) Methylation



SCHEME 38

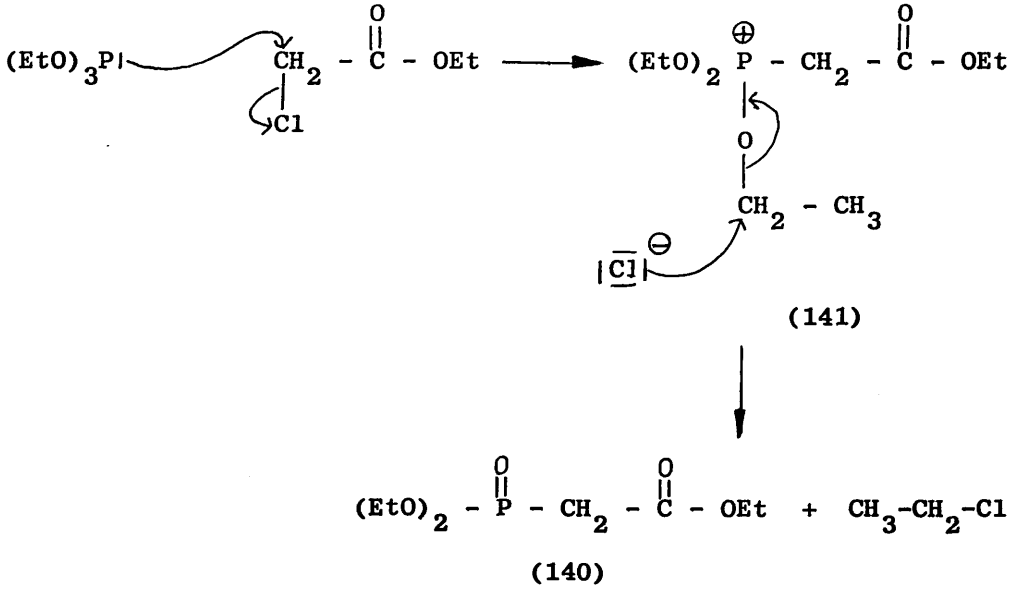
4.2 The Synthesis of Differanisole A.

The synthetic route to differanisole A is shown in Scheme 39.



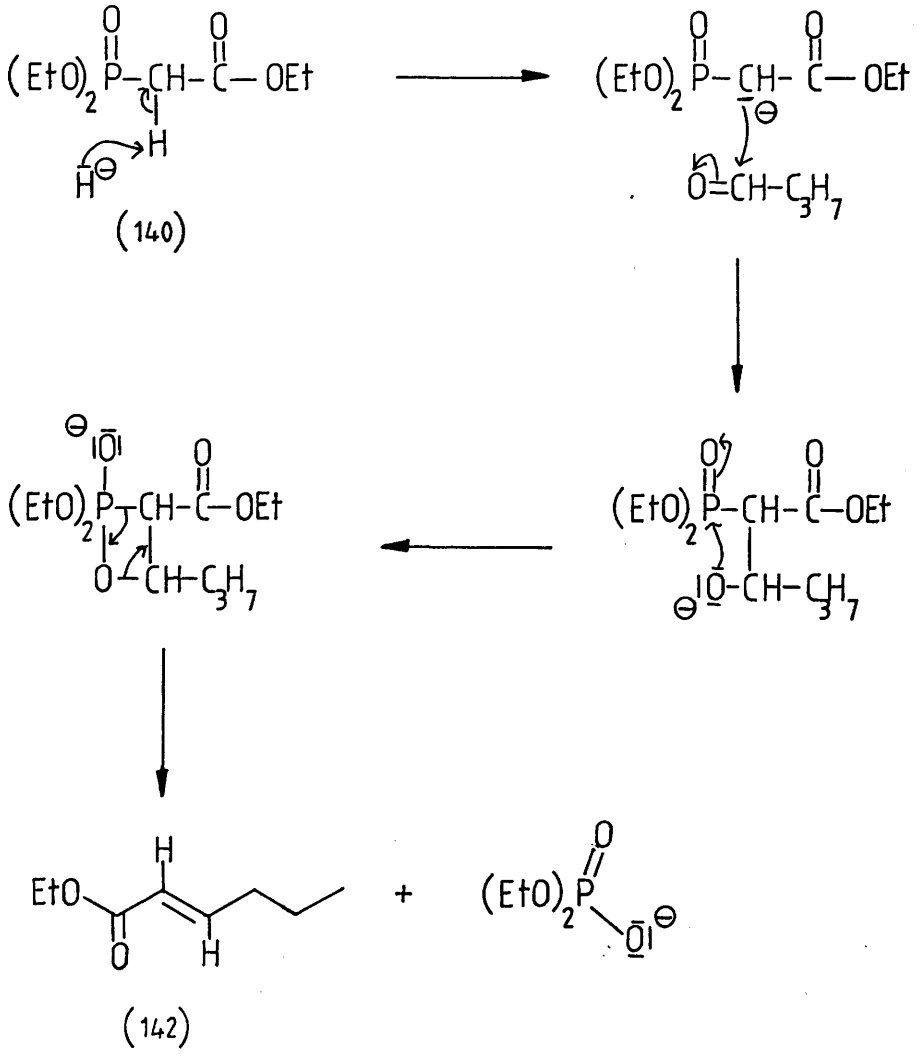
SCHEME 39

The first step in the synthesis involves reacting together ethylchloroacetate and triethylphosphite in a Michaelis-Arbusov type of reaction to produce the phosphonate ester, triethyl phosphonoacetate (140). The reaction proceeds by an initial quaternisation of the phosphite by nucleophilic attack on the halide to give a dioxyphosphonium salt (141) and the subsequent dealkylation of the phosphonium cation by the halide ion which has been displaced in the previous step.[87,88] Both steps are S_N2-type reactions. The driving force of this reaction is undoubtedly the formation of the extremely strong P=O bond (Scheme 40).



SCHEME 40.

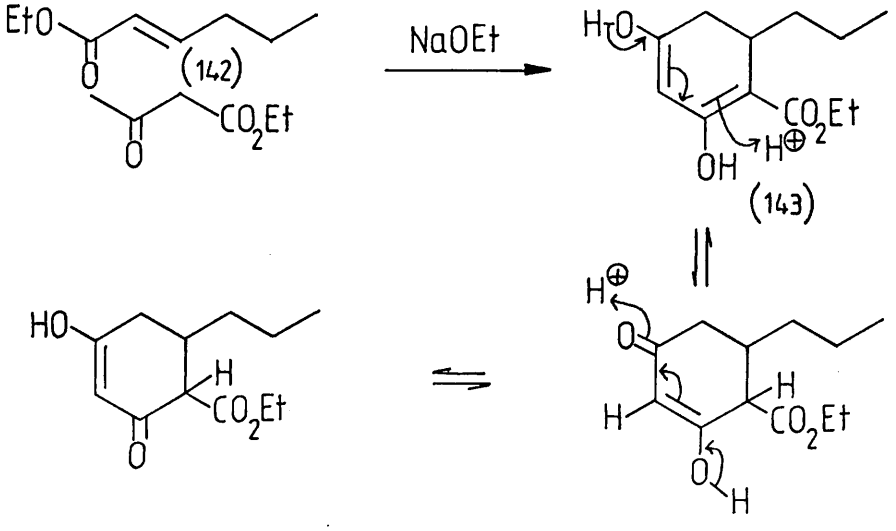
The phosphonate ester (140) was then deprotonated by sodium hydride and the reactive carbanion (an ylide) so formed attacked the aldehyde carbon of butyraldehyde. A four membered ring (a betaine) is formed when the oxygen from butyraldehyde forms a bond to the phosphorus. The phosphorus-carbon bond breaks as the phosphorus-oxygen bond is far stronger and, therefore, more favourable. This results in the formation of ethyl hex-2-enoate (142) (Scheme 41).



SCHEME 41

The Michaelis-Arbusov reaction has several advantages over the Wittig reaction. It is actually a modification of the Wittig reaction in that it utilises a phosphonate carbanion instead of a trialkyl phosphonium ion. Phosphonate carbanions are more reactive and hence the reaction time is reduced. Another reason for using the phosphonate anion is that greater selectivity is shown for the *trans*-olefin as opposed to using the normal Wittig reagent where a mixture of *cis*- and *trans*- isomers would be obtained.

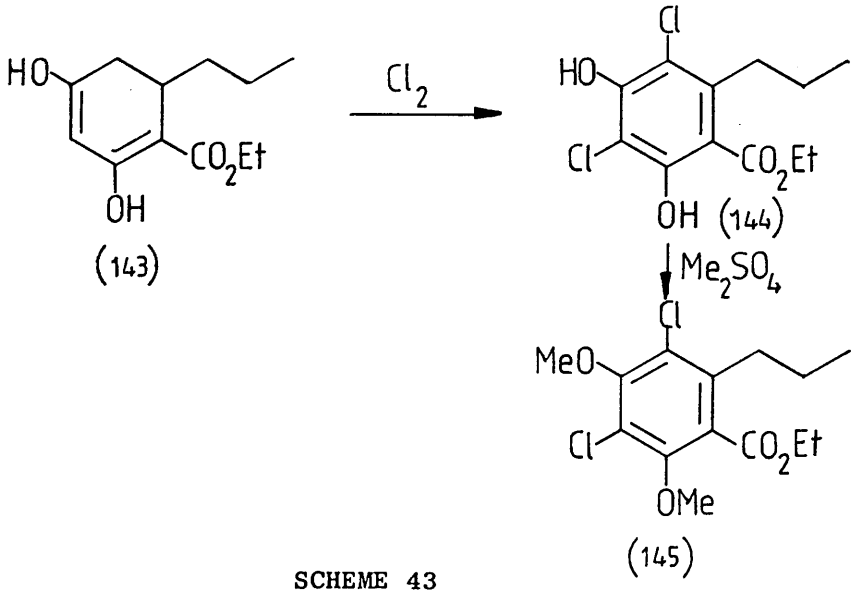
The next step in the synthesis involves heating ethyl hex-2-enoate (142) with ethyl acetoacetate in an ethanolic solution of sodium ethoxide to give ethyl 6-propyl-5,6-dihydro-β-resorcylate (143) as pale yellow crystals. The ethoxide ion forms a carbanion with ethyl acetoacetate which attacks the double bond of ethyl hex-2-enoate (142) in a Michael addition. The product, ethyl 6-propyl-5,6-dihydro-β-resorcylate (143) has the ability to undergo keto-enol tautomerism (Scheme 42).



SCHEME 42

The ^{1}H nmr spectrum of (143) is very complex due to the fact that it exists as a mixture of tautomers.

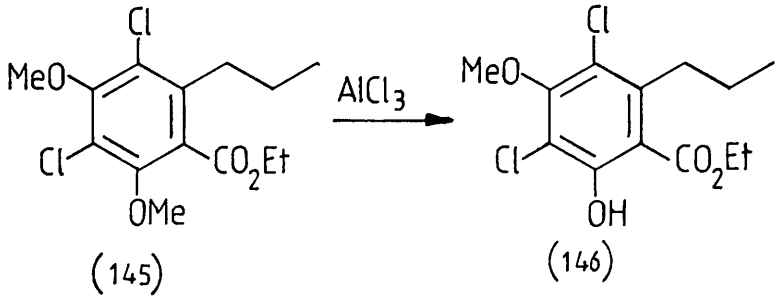
Ethyl 6-propyl-5,6-dihydro-β-resorcylate (143) was then chlorinated and aromatised using chlorine. This was carried out by preparing a solution of chlorine in acetic acid which was refluxed with (143) to give ethyl 3,5-dichloro-6-propyl-β-resorcylate (144). (144) was then methylated with dimethyl sulphate to yield ethyl 3,5-dichloro-2,4-dimethoxy-6-propylbenzoate (145) as an oil (Scheme 43).



SCHEME 43

Specific demethylation of the methyl ether at C-2 in ethyl 3,5-dichloro-2,4-dimethoxy-6-propylbenzoate (145) was carried out by heating with aluminium chloride in nitrobenzene to give ethyl 3,5-dichloro-2-hydroxy-4-methoxy-6-propylbenzoate (146), the ethyl ester of differanisole A, as white crystals. Aluminium chloride acts as a Lewis acid and a complex is formed with the lone pair of electrons of the methoxyl oxygen

at C-2 and the carbonyl of the ester group.



(145) (146)

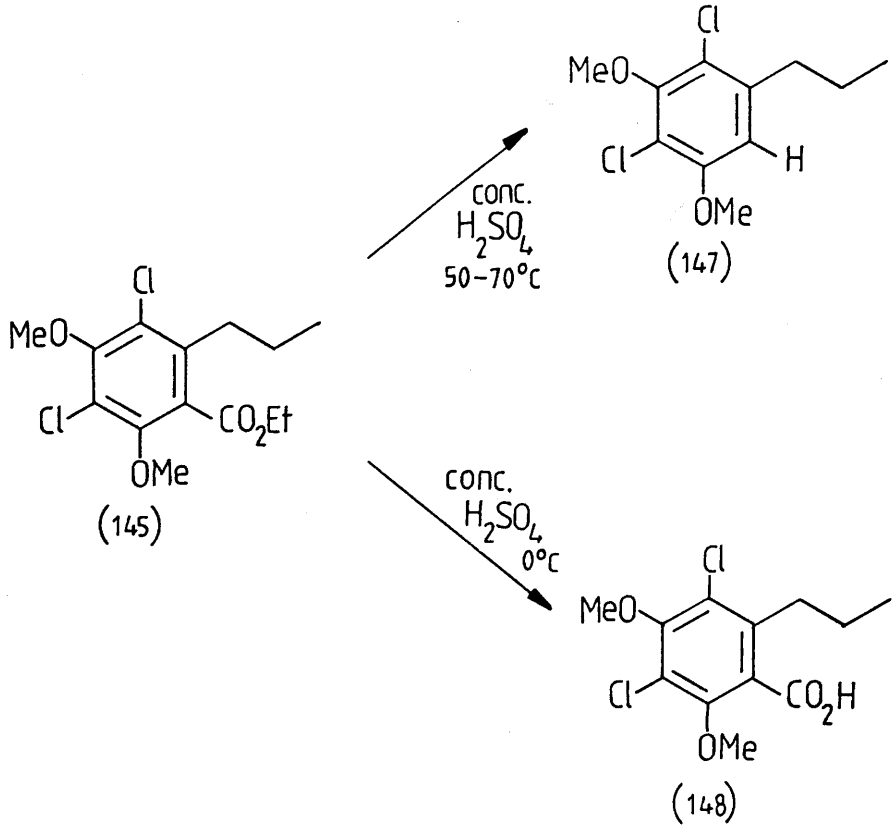
The final step in the synthesis was in fact to cleave the sterically hindered ethyl ester of (146) to give differanisole A. It is known that esters of di-*ortho*- substituted benzoic acids are difficult to hydrolyse and that they give rise to a special effect known as the *ortho*-effect which is not necessarily just a steric effect.[89] The *ortho*-effect operates mainly in benzoic acids. Benzoic acid is a resonance hybrid, and so the carbonyl group is coplanar with the ring. An *ortho*- substituent tends to prevent this coplanarity, thus, resonance is diminished or even prevented. The oxygen atom of the hydroxyl group has a greater positive charge resulting in increased acid strength. Hence the greater the steric inhibition of resonance, the stronger the acid.

Several ester cleavage reactions were carried out on ethyl 3,5-dichloro-2,4-dimethoxy-6-propylbenzoate (145). These are as follows:-

(a) Sodium hydroxide: No reaction took place using sodium hydroxide. Steric crowding by the *ortho* substituents prevents the hydroxide ion from attacking

the ester.

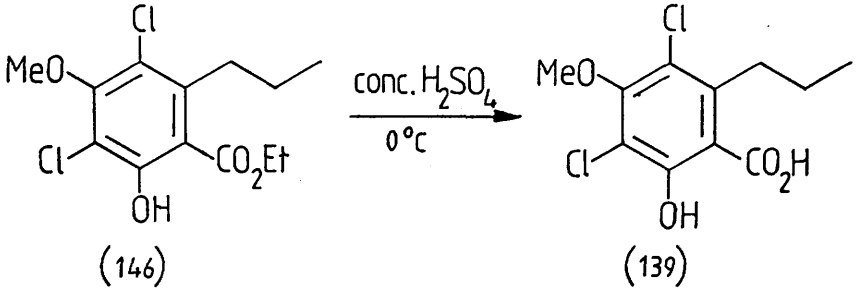
(b) Concentrated sulphuric acid: Heating (145) at 50-70°C resulted in complete decarboxylation. The substituents on the ring must therefore be affecting the hydrolysis and thus promoting decarboxylation to give (147). Treatment of (145) with cold concentrated sulphuric acid gave the desired acid (148).



(c) Trimethylsilyliodide: Treatment of (145) with trimethylsilyliodide in carbon tetrachloride was unsuccessful, giving only starting material.

Ethyl 3,5-dichloro-2-hydroxy-4-methoxy-6-propyl-benzoate (146) was treated with cold concentrated sulphuric acid at O°C for six days in the final step of

the synthesis. This resulted in the formation of 3,5-dichloro-2-hydroxy-4-methoxy-6-propylbenzoic acid, differanisole A (139).



(146) (139)

The analysis of differanisole A (139) was shown to be consistent with that which was reported [84,85].

CHAPTER 5.

General Experimental Procedures

Melting points (m.p) were determined on a Kofler hot-stage apparatus and are uncorrected. Infra-red spectra were recorded on a Perkin-Elmer 983 spectrophotometer. The following abbreviations are used: s-strong, m-medium, w-weak and br-broad. Routine ^1H nmr spectra were determined on a Perkin-Elmer R32 (90MHz) spectrometer using tetramethylsilane as internal standard. ^1H nmr spectra were also recorded at 200 MHz on a Bruker WP 200 SY instrument, employing a deuterium lock system, setting chloroform ($CHCl_3$) in $CDCl_3$ at δ 7.25 ppm as internal standard. Routine mass spectra were determined using a VG/Kratos MS 12 spectrometer.

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

Column chromatography was performed using Fluka Kieselgel HF_{254}. Preparative thin layer chromatography (t.l.c) was performed using 20 x 20cm glass plates coated with 1mm of Fluka Kieselgel GF_{254}.

Solvents and reagents were dried and purified prior to use as follows: tetrahydrofuran was distilled from sodium/benzophenone and used immediately; ether was dried using sodium wire; methanol and ethanol were dried using magnesium activated with iodine and stored over 3A molecular sieves; pyridine and *N*-isopropylcyclohexylamine were refluxed over sodium hydroxide pellets,

distilled under nitrogen and stored over potassium hydroxide; thionyl chloride was distilled from iron; quinoline was dried using sodium sulphate and was distilled from zinc dust; dichloromethane was distilled from phosphorus pentoxide and stored over 4A molecular sieves; chlorobenzene was washed with concentrated sulphuric acid, then aqueous sodium bicarbonate and water, followed by drying using calcium chloride then with phosphorus pentoxide and distilled; butyraldehyde was dried using calcium chloride then fractionally distilled; nitrobenzene was extracted with aqueous 2M sodium hydroxide solution, then water, dilute hydrochloric acid, and water, dried using calcium chloride and fractionally distilled under reduced pressure.

Methyl 3,5-dimethoxybenzoate (92)[90]

3,5-Dihydroxybenzoic acid (91) (30.0 g) dissolved in Analar acetone (300 ml) with anhydrous potassium carbonate (130 g) and dimethyl sulphate (60 ml) was heated at reflux for 7 h with stirring. After cooling, the solution was filtered and the residue washed with acetone (200 ml). The combined acetone solutions were evaporated and the golden brown residue dissolved in ether (200 ml), washed with ammonia liquor (3 x 100 ml), 10% sodium hydroxide solution (2 x 100 ml), and water (100 ml), then dried and evaporated. The residue solidified on cooling and was recrystallised from ether as white needles (30.6 g, 78%), m.p. 41-42°C (lit.[90]

42°C); δ($CDCl_3$) 7.18 (2H,d, *J* 2Hz, aromatic), 6.63 (1H, t, *J* 2Hz, aromatic), 3.92 (3H, s, $COOCH_3$), 3.82 (6H, s, OCH_3); ν_{max} (KBr) 1720 s and 1600 s cm^{-1}.

3,5-Dimethoxybenzyl alcohol (93)[90]

Methyl 3,5-dimethoxybenzoate (92) (25.0 g) in dry tetrahydrofuran (200 ml) was added slowly to lithium aluminium hydride (6.0 g) in THF (100 ml) and the mixture was stirred at reflux for 8 h. After cooling, water (6.0 ml) was added cautiously, followed by 15% sodium hydroxide solution (6.0 ml) and more water (18.0 ml) with stirring. The granular aluminium hydroxide was filtered and washed with ether (300 ml). The organic solutions were evaporated to dryness to give a white solid which was recrystallised from diisopropyl ether as needles (17.1 g, 80%), m.p. 46-47°C (lit.[90] 47°C); δ($CDCl_3$) 6.47 (2H, d, *J* 2Hz, aromatic), 6.34 (1H, t, *J* 2Hz, aromatic), 4.53 (2H, s, $ArCH_2OH$), 3.73 (6H, s, OCH_3), 3.20 (1H, br s, *OH*); ν_{max} (KBr) 3400 br m and 1600 s cm^{-1}.

3,5-Dimethoxybenzyl chloride (94)[90]

Thionyl chloride (10 ml) and pyridine (1 ml) in dry ether (150 ml) were added over 1 h to 3,5-dimethoxybenzyl alcohol (93) (15.0 g) in ether (100 ml) with stirring. More thionyl chloride (5 ml) was added

in one portion and the reaction mixture gently heated until complete dissolution ocurred. After a further 2 h at room temperature the excess thionyl chloride was destroyed with water (100 ml), and the ether layer washed with water (100 ml), 10% sodium hydroxide solution (100 ml) and more water (100 ml). The ether solution was dried and evaporated to afford a fawn coloured solid which was recrystallised from ether as fine white needles (14.5 g, 87%), m.p. 47-48°C (lit.[90] 46°C); $\delta(CDCl_3)$ 6.53 (2H, d, *J* 2Hz, aromatic), 6.41 (1H, t, *J* 2Hz, aromatic), 4.51 (2H, s, CH_2), 3.80 (6H, s, OCH_3); ν_{max} (KBr) 1600 s cm^{-1}.

3,5-Dimethoxybenzyl cyanide (90)[90]

Potassium cyanide (13.5 g) and 3,5-dimethoxybenzyl chloride (94) (12.5 g) in ethanol (200 ml) and water (60 ml) were stirred at reflux for 4 h then poured onto ice. The cream precipitate was allowed to stand for 2 h then filtered, washed thoroughly with cold water and dried over phosphorus pentoxide in a vacuum desiccator. Recrystallisaion from methanol yielded fine white needles (8.1 g, 63%), m.p. 54°C (lit.[90] 53°C); $\delta(CDCl_3)$ 6.44 (3H, m, aromatic), 3.78 (6H, s, OCH_3), 3.66 (2H, s, CH_2); ν_{max} (KBr) 2240 w and 1610 s cm^{-1}.

Methyl 3,5-dimethoxyphenylacetate (95)[91]

Concentrated sulphuric acid (40 ml) was carefully added to 3,5-dimethoxybenzyl cyanide (90) (2.0 g) in methanol (170 ml) and water (10 ml) and the solution heated at reflux for 16 h. Upon cooling, the methanol was removed under reduced pressure and the acidic solution extracted with ether (3 x 100 ml). The combined organic extracts were washed with saturated aqueous sodium bicarbonate (2 x 100 ml) followed by brine (100 ml) and then dried. Evaporation of the solvent afforded a brown oil which was distilled *in vacuo* to give the ester (95) as a colourless liquid (1.40 g, 59%), b.p. 125°C/0.06 mm Hg (lit., [91] 94°C/0.04 mm Hg); $\delta(CDCl_3)$ 6.40 (3H, m, aromatic), 3.74 (6H, s, OCH_3), 3.65 (3H, s, CO_2CH_3), 3.52 (2H, s, CH_2); ν_{max} ($CHCl_3$) 3020 m, 1730 s and 1600 s cm^{-1}; *m/z* 210 (M^+) and 151 (M^+-CO_2CH_3).

Ethyl (*E*)-3-methoxy-2-butenoate (98)[92]

Concentrated sulphuric acid (1 ml) was added to ethyl acetoacetate (52.0 g) and trimethyl orthoformate (43.0 g) and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was neutralised with quinoline (2.5 ml). Distillation of the dark mixture *in vacuo* afforded ethyl (*E*)-3-methoxy-2-butenoate (98) (48.6 g, 85%), b.p. 66-68°C/12 mm Hg (lit. [93] 188-193°/760 mm Hg); $\delta(CDCl_3)$ 4.93 (1H, s,

olefinic), 4.11 (2H, q, *J* 3Hz, $CO_2CH_2CH_3$), 3.60 (3H, s, CH_3), 2.25 (3H, s, OCH_3), 1.23 (3H, t, *J* 4Hz, $CO_2CH_2CH_3$) ν_{max} (thin film) 1705 s, 1620 s and 1143 s cm^{-1}.

Attempted preparation of (*E*)-3-methoxy-2-butenoyl chloride (96)[74]

A mixture of ethyl (*E*)-3-methoxy-2-butenoate (98) (38.6 g), sodium hydroxide pellets (11.9 g), water (160 ml) and methanol (175 ml) was refluxed for 16 h. The solvent was evaporated under reduced pressure, with the last traces of water being removed by azeotropic distillation with benzene. The remaining white solid was dried thoroughly to give sodium (*E*)-3-methoxy-2-butenoate (99) in quantitative yield, which was finely powdered for the next step, m.p. 205-208°C; ν_{max} (KBr) 1580 s, 1400 s, and 1210 m cm^{-1}.

To a stirred mixture of the sodium salt (99) (5.0 g) thus obtained in anhydrous ether (100 ml) at -5°C was added dropwise oxalyl chloride (10.0 ml) under nitrogen. Stirring was continued for 2 h at room temperature and excess oxalyl chloride and solvent were then distilled under atmospheric pressure. Vacuum distillation afforded (*E*)-3-methoxy-2-butenoyl chloride (96)[74] as a pale yellow oil (12.2 g, 50%), b.p. 64°C/4 mm Hg; ν_{max} (thin film) 1750, 1570, 1432, 1385, 1268, 1074 and 1050 cm^{-1}. This acid chloride is unstable and was stored at -78°C prior to use.

However when this preparation was attempted, no product was obtained. A brown/black viscous material was formed which could not be characterised.

Attempted preparation of methyl 2-(3,5-dimethoxyphenyl)-5-methoxy-3-oxohex-4-enoate (97)

To a stirred solution of *N*-isopropylcyclohexylamine (0.589 g) in THF (10 ml) at -5°C was added dropwise *n*-butyllithium (1.60 ml, 2.61M in hexane). After the mixture was stirred for 0.5 h and cooled to -78°C, a solution of methyl 3,5-dimethoxyphenylacetate (95) (0.40 g) in THF (10 ml) was added dropwise. Stirring was continued for 0.5 h at -78°C, and then ethyl (*E*)-3-methoxy-2-butenoate (98) (0.36 g) in THF (10 ml) was added dropwise. The reaction mixture was allowed to warm up to room temperature slowly, and then quenched with cold water, poured into saturated aqueous ammonium chloride, and extracted with ethyl acetate (2 x 100 ml). Following removal of the solvent *in vacuo*, the residue was dissolved in ether (150 ml), washed with saturated aqueous sodium bicarbonate (2 x 100 ml) and dried. Evaporation of the solvent left a brown oil. The nmr spectrum revealed the product to be a mixture of the starting materials.

The ethylene acetal of ethyl acetoacetate (101)[94]

A mixture of ethyl acetoacetate (30 g), ethylene glycol (14.3 g) and a small crystal of *p*-toluenesulphonic acid in benzene (100 ml) was refluxed for 16 h using a Dean-Stark apparatus. The amount of water collected was approximately 5 ml (4.2 ml theoretical value). The benzene layer was washed with saturated aqueous sodium bicarbonate (2 x 150 ml), then water (150 ml), dried and evaporated. Vacuum distillation affored the ethylene acetal of ethyl acetoacetate (101) as a colourles oil (20.3 g, 51%), b.p. 88-92°C/14 mm Hg (lit. [94] 43-44°C/0.1 mm Hg); $\delta(CDCl_3)$ 4.17 (2H, q, *J* 3Hz, OCH_2CH_3), 4.00 (4H, s, OCH_2CH_2), 2.67 (2H, s, CH_2), 1.50 (3H, s, CH_3), 1.27 (3H, t, *J* 3Hz, OCH_2CH_3); ν_{max} (thin film) 1738 s, 1188 s, and 1114 s cm^{-1}.

The ethylene acetal of acetoacetyl chloride (100)

A mixture of the ethylene acetal of ethyl acetoacetate (101) (12.0 g), sodium hydroxide pellets (2.76 g), methanol (220 ml) and water (180 ml) was refluxed for 16 h. The solvent was evaporated under reduced pressure, with the last traces of water being removed by azeotropic distillation with benzene. The remaining white solid was dried thoroughly to give the sodium salt (102) (9.2 g, 79%), m.p. 208-214°C; δ (100 MHz, D_2O), 4.26 (4H, s, OCH_2CH_2), 2.77 (2H, s, CH_2), 1.68 (3H, s, CH_3); ν_{max} (KBr) 1594 br s, and

1400 br m cm^{-1}.

To a stirred mixture of the sodium salt (102) (5.0 g) thus obtained in dry ether (150 ml) at -5°C was added dropwise oxalyl chloride (7.61 ml) under nitrogen. Stirring was continued for 1 h at room temperature, then the reaction mixture was refluxed for 45 minutes. Solvent and excess oxalyl chloride were evaporated. Vacuum distillation afforded the acid chloride (100) as a colourless oil (2.8 g, 57%), b.p. 65-70°C/0.35 mm Hg; $\delta(CDCl_3)$ 4.03 (4H, s, OCH_2CH_2), 3.27 (2H, s, CH_2) 1.50 (3H, s, CH_3); ν_{max} (thin film) 1810 br s, 752 s, and 647 s cm^{-1}.

The 5-ethylene acetal of methyl 2-(3,5-dimethoxyphenyl)-3,5-dioxohexanoate (103)

To a stirred solution of *N*-isopropylcyclohexylamine (1.18 g) in THF (10 ml) at -5°C was added dropwise *n*-butyllithium (3.20 ml, 2.61M in hexane). After the mixture was stirred for 0.5 h and cooled to -78°C a solution of methyl 3,5-dimethoxyphenylacetate (95) (0.80 g) in THF (15 ml) was added dropwise. Stirring was continued for 0.5 h at -78°C, then the temperature was slowly brought up to 0°C. A solution of the acid chloride (100) (0.81 g) in THF (15 ml) was added dropwise and the reaction mixture was then stirred at room temperature for 0.5 h. The mixture was quenched with cold water, poured into saturated aqueous ammonium chloride, and extracted with ethyl acetate (2 x 100 ml).

Following removal of the solvent *in vacuo*, the residue was dissolved in ether (150 ml), washed with saturated aqueous sodium bicarbonate (2 x 100 ml), and dried to give an amber oil (1.24 g, 96%).

The ^{1}H nmr spectrum and t.l.c showed the crude reaction mixture to be a complex mixture of products which could not be resolved by chromatography. The crude reaction mixture was used in the next step to give methyl 2-hydroxy-5,7-dimethoxy-4-methyl naphthalene-1-carboxylate (104) as the only product.

Methyl 2-hydroxy-5,7-dimethoxy-4-methyl naphthalene-1-carboxylate (104)

The crude reaction mixture obtained from the preparation of the 5-ethylene acetal of methyl 2-(3,5-dimethoxyphenyl)-3,5-dioxohexanoate (103) (7.87 g) was mixed with methanol (160 ml), water (5 ml), and concentrated sulphuric acid (4 ml) and heated at 50°C for 16 h. Half of the methanol was then evaporated under reduced pressure, resulting in the precipitation of fine grey crystals. These were filtered, washed with cold methanol and recrystallised from methanol to give fine white crystals (2.18 g, 34%), m.p. 130-131°C; $\delta(CDCl_3)$ 11.89 (1H, s, O*H*), 7.86 (1H, d, *J*1Hz, aromatic) 6.76 (1H, s, aromatic), 6.44 (1H, d, *J*1Hz, aromatic), 4.09 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 2.80 (3H, s, CH_3); ν_{max} (KBr) 1640 s, 1615 s, 1597 s, and 1575 s cm^{-1}; *m/z* 276 (M^+) 244, 216, 173.

(Found: C, 65.07; H, 5.70. $C_{15}H_{16}O_5$ requires C, 65.21; H, 5.84%).

Methyl 2,5,7-trimethoxy-4-methyl naphthalene-1-carboxylate (105)

A mixture of the naphthalene carbonate (104) (0.16 g), dimethyl sulphate (0.14 ml) and anhydrous potassium carbonate (0.64 g) in Analar acetone (60 ml) was refluxed for 16 h. After cooling, the solution was filtered and the residue washed with acetone (100 ml). The combined acetone solutions were evaporated and the beige solid obtained was dissolved in ethyl acetate (100 ml), washed with ammonia liquor (2 x 100 ml), 10% sodium hydroxide solution (2 x 100 ml) and brine (2 x 100 ml), then dried and evaporated to give a cream coloured solid (0.15 g, 88%), m.p. 126-127°C; δ(200 MHz, $CDCl_3$) 6.78 (1H, d, *J* 0.8Hz, aromatic), 6.54 (1H, d, *J* 2.3Hz, aromatic), 6.33 (1H, d, *J* 2.3Hz, aromatic), 3.99 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 2.83 (3H, d, *J* 0.9Hz, CH_3); ν_{max} (KBr) 1720 s, 1620 s, and 1590 s cm^{-1}; *m/z* 290 (M^+), 259 ($M^+ - OCH_3$), 231 ($M^+ - OCOCH_3$).
(Found: C, 66.28; H, 6.21. $C_{15}H_{18}O_5$ requires C, 66.19; H, 6.25%).

Attempted preparation of methyl 8-formyl-2,5,7-trimethoxy naphthalene-1-carboxylate (109)

Phosphorus oxychloride (0.05 ml) was added dropwise to dimethyl formamide (0.05 ml) at $0^{o}C$. The naphthalene carboxylate (105) (0.10 g) in dimethyl formamide (2 ml) was then added dropwise to the reaction mixture. The reaction mixture was then stirred at room temperature for 24 h. Water (50 ml) was added to destroy the excess phosphorus oxychloride, then the solution was extracted with ethyl acetate (3 x 50 ml), dried, and evaporated to give a cream coloured solid (0.84 g).

The ^{1}H nmr spectrum and t.l.c. analysis showed the product to be solely starting material.

Methoxyacetyl chloride[95]

Methoxyacetic acid (50 g) was dropped with stirring onto thionyl chloride (80 g), the flask being cooled in an ice bath. The mixture was warmed on a water bath to 50 - $80^{o}C$ until the evolution of sulphur dioxide gas and hydrogen chloride gas ceased. The resulting reaction mixture was distilled *in vacuo* to give methoxyacetyl chloride as a colourless liquid (51 g, 87%), b.p. 38-$40^{o}C$/15 mm Hg (lit.[96] 112-$113^{o}C$/760 mm Hg); $\delta(CDCl_3)$ 4.37 (2H, s, CH_2), 3.50 (3H, s, OCH_3).

4,6,9-Trimethoxy-7-methyl pyrano[1,8-*c*]naphthalen-1(3*H*)-one (110)

To a stirred solution of the naphthalene carboxylate (105) (0.10 g) and methoxyacetyl chloride (0.10 ml) in dichloromethane (8 ml) at -5°C was added dropwise tin(*IV*)chloride (0.24 ml) under nitrogen. The reaction mixture was stirred at -5°C for 0.5 h then heated at 30-40°C for 2 h. It was then poured into a mixture of ice and 10% aqueous hydrochloric acid (20 ml) and extracted with dichloromethane (4 x 50 ml), washed with saturated aqueous sodium bicarbonate (2 x 100 ml), dried, and evaporated to give a yellow coloured solid (93 mg, 94%), m.p. 125-126°C; δ(200 MHz, $CDCl_3$), 6.83 (1H, d, *J* 0.83Hz, aromatic), 6.42 (1H, s, aromatic), 5.45 (2H, s, CH_2), 4.07 (3H, s, OCH_3), 3.93 (6H, s, OCH_3), 2.83 (3H, d, *J* 0.90 Hz, CH_3); ν_{max} (KBr) 1700 s, 1620 s, and 1600 s cm^{-1}; *m/z* 288 (M^+), 259, 243. (Found: C, 66.72; H, 5.78. $C_{16}H_{16}O_5$ requires C, 66.66; H, 5.60%).

Attempted demethylation reactions of 4,6,9-trimethoxy-7-methyl pyrano[1,8-*c*]naphthalen-1(3*H*)-one (110)

(a) Using boron tribromide as a reagent

To a solution of (110) (300 mg) in dichloromethane (20 ml) was added boron tribromide (0.5 ml) at -70°C under nitrogen. The reaction mixture was kept at this temperature for 3 h and then kept at room temperature for 16 h. Ether was added, then water cautiously and the organic layer was dried and evaporated to give a brown solid. Purification by silica gel chromatography using ethyl acetate as eluant yielded 9-hydroxy-4,6-dimethoxy-7-methyl pyrano[1,8-*c*]naphthalen-1(3*H*)-one (112) as a yellow solid which was recrystallised from ether/hexane as fine, bright yellow needles (71 mg, 24%), m.p. 217-220°C; δ(200 MHz, DMSO) 6.74 (2H, s, aromatic), 5.59 (2H, s, CH_2), 3.96 (6H, s, OCH_3), 2.75 (3H, s, CH_3); *m/z* 274 (M^+), 273, 245, 230. (Found: C, 65.85; H, 5.31. $C_{15}H_{14}O_5$ requires C, 65.69; H, 5.14%).

(b) Using hydrobromic acid as a reagent

A solution of (110) (100 mg) in hydrobromic acid (3 ml, 48%) and glacial acetic acid (3 ml) was heated at 100-120°C for 3 h. The hydrobromic acid and acetic acid were evaporated to yield a brown/black solid which could not be resolved and thus identified.

(c) Using aluminium chloride with ethanethiol

To a stirred solution of aluminium chloride (0.28 g) in ethanethiol (2.5 ml) at $O^{o}C$ was added (110) (100 mg) and stirring was continued for 0.5 h at $O^{o}C$ and 3 h at room temperature. The reaction mixture was poured into water, acidified with dilute aqueous hydrochloric acid, extracted with dichloromethane, washed with brine, dried using sodium sulphate, and evaporated to give a yellow solid.

The ^{1}H nmr spectrum showed the product to be starting material.

(d) Using aluminium chloride

A solution of (110) (100 mg) and aluminium chloride (139 mg) in dry chlorobenzene (40 ml) was refluxed for 3-5 h. The reaction mixture was then poured into water and extracted with ether (3 x 50 ml). The ether extracts were dried and evaporated to give a yellow/brown solid. Purification by silica gel chromatography using ethyl acetate as eluant yielded 9-hydroxy-4,6-dimethoxy-7-methyl pyrano[1,8-*c*]naphthalen-1(3*H*)-one (112) as a yellow solid.

(e) Using pyridine hydrochloride

A mixture of (110) (100 mg) and pyridine hydrochloride (3.20 g) was heated at $220^{o}C$ under

nitrogen for 10 minutes after the solid had melted. Dilute sulphuric acid was added which resulted in a black homogeneous solution being formed. This solution was extracted with ether (4 x 100 ml), dried, and evaporated to give a yellow/brown solid.

The ^{1}H nmr spectrum showed the product to be starting material.

3-bromo-4-hydroxy-5-methoxybenzaldehyde (117)[80]

Vanillin (75 g) was dissolved in glacial acetic acid (150 ml) and cooled to $0^{o}C$. Bromine (27.5 ml) in glacial acetic acid (100 ml) was added rapidly with stirring. Water (1000 ml) was added, the precipitate filtered and washed with water (200 ml). The product was recrystallised from aqueous ethanol as cubes (98 g, 84%), m.p. 163-164^{o}C (lit.[80] 163-164^{o}C); $\delta(CDCl_3)$ 9.55 (1H, s, C*H*O), 7.63 (1H, d, *J* 2Hz, aromatic), 7.36 (1H, d, *J* 2Hz, aromatic), 6.50 (1H, br s, O*H*), 4.00 (3H, s, OCH_3); ν_{max} ($CHCl_3$) 3500 br s, 1690 s, and 1600 m cm^{-1}.

2,5-dihydroxy-3-methoxybromobenzene (118)[80]

A solution of 3-bromo-4-hydroxy-5-methoxybenzaldehyde (50 g) in *N* potassium hydroxide solution (200 ml) was cooled to $0^{o}C$, and 100 vol. hydrogen peroxide (53.5 ml) in water (324 ml) was added dropwise over 3 h with cooling in an ice bath and stirring. The mixture was stirred for 1 h further, and the product filtered, washed with water, and dried. A small portion was recrystallised from water as pink needles (29.3 g, 63%), m.p. 140-141^{o}C (lit.[80] 141^{o}C); $\delta(CDCl_3)$ 6.58 (1H, d, *J* 3Hz, aromatic), 6.40 (1H, d, *J* 3Hz, aromatic), 5.50 (2H, br s, O*H*), 3.78 (3H, s, OCH_3); ν_{max} ($CHCl_3$) 3180 br s, 1620 m, and 1590 m cm^{-1}.

2,3,5-trimethoxybromobenzene (119)[80]

A solution of 2,5-dihydroxy-3-methoxybromobenzene (54 g) in Analar acetone (600 ml) with dimethyl sulphate (55 ml) and anhydrous potassium carbonate (138 g) was heated at reflux for 4 h with stirring. The reaction mixture was allowed to cool, filtered, and the potassium carbonate washed with acetone (100 ml). The combined acetone solutions were evaporated, and the residue was dissolved in ether. The ether solution was washed with ammonia liquor (4 x 150 ml), 10% sodium hydroxide solution (3 x 150 ml) and water (2 x 150 ml), then dried and evaporated. The resulting product was distilled

in vacuo to give 2,3,5-trimethoxybromobenzene as a colourless liquid which slowly crystallised (34.8 g, 59%), m.p. 37-38°C (lit.[80] 37-38°C); δ($CDCl_3$) 6.62 (1H, d, *J* 3Hz, aromatic), 6.43 (1H, d, *J* 3Hz, aromatic), 3.79 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 3.70 (3H, s, OCH_3); ν_{max} ($CHCl_3$) 1600 s, and 1570 s cm^{-1}.

2,3,5-trimethoxybenzoic acid (120)[81]

Ether (100 ml) containing 2,3,5-trimethoxybromobenzene (10 g) was cooled to -70°C, and *n*-butyllithium (29.5 ml, 1.60M in hexane) was added with stirring. Stirring was continued for 1 h, then the mixture was poured onto crushed dry ice (300 g) and allowed to warm up to room temperature. Water (100 ml) was added and the ether layer separated and extracted with water (2 x 50 ml). The aqueous extracts were acidified and cooled. The precipitate was collected after standing at 0°C for 2 h, washed with water and dried. The product was recrystallised from hexane as fine prisms (5.5 g, 64%), m.p. 99-100°C (lit.[81] 99.5-100.5°C); δ($CDCl_3$) 10.35 (1H, br s, COO*H*), 6.81 (1H, d, *J* 3Hz, aromatic), 6.70 (1H, d, *J* 3Hz, aromatic), 3.82 (6H, s, OCH_3), 3.73 (3H, s, OCH_3); ν_{max} ($CHCl_3$) 3450 br s, 2740 s, 1730 s, and 1600 s cm^{-1}.

Methyl 2,3,5-trimethoxybenzoate (115)[82]

A solution of 2,3,5-trimethoxybenzoic acid (3 g) in dry methanol (50 ml) was saturated with hydrogen chloride and heated under reflux for 4 h. The methanol was removed *in vacuo* and the residue dissolved in ether. The ether solution was washed with saturated aqueous sodium bicarbonate and water, dried, and evaporated to give methyl 2,3,5-trimethoxybenzoate as an oil (2.58 g, 81%); δ ($CDCl_3$) 6.78 (1H, d, *J* 3Hz, aromatic), 6.61 (1H, d, *J* 3Hz, aromatic), 3.88 (6H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.76 (3H, s, OCH_3); ν_{max} ($CHCl_3$) 2850 m, 1750 s, 1600 m, 1490 s, and 1055 s cm^{-1}.

Ethylphosphonic dichloride (122)[83]

Firstly, anhydrous aluminium chloride (13.3 g) was weighed into a glass stoppered conical flask, followed by phosphorus trichloride (13.7 g). To this mixture was added ethyl bromide (32.7 g), and the flask was stoppered and clamped on a shaking machine. (All reagents and the reaction flask were cooled to about 4°C just prior to weighing).

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

continued for 1 h. The reaction flask was then placed in a refrigerator at 4°C for 24 h. The supernatant liquid was poured off, leaving 19 g of dry crystalline material which was thought to be the complex $C_2H_5PX_3^+AlX_4^-$, where X = Br or Cl. This crystalline complex was dissolved in methylene chloride (200 ml), transferred to a three-necked flask (500 ml), cooled to 0°C, and hydrolysed by adding dropwise over 20 mins cold concentrated hydrochloric acid (25.3 ml, 36%). The mixture was stirred for 2 h. The mixture was kept below 7°C throughout the hydrolysis. The cold mixture was filtered and the filtrate was distilled to remove excess solvent, followed by vacuum-distillation of the residue to give a colourless liquid (8.2 g, 57%), b.p. 60°C/15 mm Hg (lit.[83] b.p.174.5°C/760mm Hg); $\delta(CDCl_3)$ 2.61 (2H, dq, J_{HH} 7.5 Hz, and $^2J_{PH}$ 16Hz, $CH_3CH_2P(O)Cl_2$), 1.39(3H,dt, J_{HH} 7.5Hz and $^3J_{PH}$ 31Hz, $CH_3CH_2P(O)Cl_2$).

Ethylphosphonic acid bis(dimethylamide)(121)[97]

To a stirred solution of dimethylamine (18 g) in ether (250 ml) was added, while at 0°C and under nitrogen, ethylphosphonic dichloride (11 g). The resulting solution was stirred at 0°C for 1 h and at 25°C for 3 h. The precipitate of dimethylamine hydrochloride was removed by filtration and the filtrate evaporated under vacuum. Distillation of the residue afforded a colourless oil (11.2 g, 91%), b.p. 75°C/ 1.0 mm Hg (lit.[97] b.p. 142°C/31 mm Hg); $\delta(CDCl_3)$ 2.61

(12H, d, $^3J_{PH}$ 9Hz, RP(O)(N*Me*$_2$)$_2$), 1.77 (2H, dq, J_{HH} 5Hz and $^2J_{PH}$ 14 Hz, $CH_3CH_2P(O)(NR_2)_2$), 1.09 (3H, dt, J_{HH} 7.5 Hz and $^3J_{PH}$ 18.5 Hz, $CH_3CH_2P(O)(NR_2)_2$).

1-Oxo-1-(2,3,5-trimethoxyphenyl)propan-2-ylphosphonic Bis(dimethylamide)(124)[82]

A solution of *n*-butyllithium (3.9 ml, 1.6M in hexane) was added to a stirred solution of ethylphosphonic acid bis(dimethylamide)(121) (1 g) in dry tetrahydrofuran (15 ml) at - 78°C under nitrogen. The mixture was stirred at -50°C for 3 h after which it was cooled to -78°C; a solution of methyl 2,3,5-trimethoxybenzoate (115) (0.68 g) in dry tetrahydrofuran (10 ml) was then added. Stirring was continued for 2 h at -78°C after which the solution was allowed to warm to room temperature. Water (10 ml) was added and the organic solvents were evaporated. The aqueous solution was extracted with ethyl acetate and the combined organic extracts were washed thoroughly with aqueous sodium chloride (1M) to remove the unchanged ethylphosphonic acid bis(dimethylamide). The organic solution was dried and evaporated to give the β-oxophosphonamide (124) as an oil (0.97 g, 90%); δ($CDCl_3$) 6.60 (2H, s, aromatic), 4.53 (1H, dq, J_{HH} 7 Hz and J_{PH} 18 Hz, C*H*Me), 3.86 (3H, s, OC*H*$_3$), 3.82 (3H, s, OC*H*$_3$) 3.79 (3H, s, OC*H*$_3$), 2.60 (6H, d, J_{PH} 10 Hz, NC*H*$_3$), 2.58 (6H, d, J_{PH} 10 Hz, NC*H*$_3$), 1.49 (3H, dd, J_{HH} 7 Hz and J_{PH} 16 Hz, CHC*H*$_3$).

2-Dimethylamino-6,8-dihydroxy-3-methyl-2*H*-1,2λ^5-benzoxaphosphorin-2,4(3*H*)-dione (126)[82]

The β-oxophosphonamide (124) (0.23 g) was dissolved in dichloromethane (20 ml) at -78°C under nitrogen and boron tribromide (0.5 ml) was added. After 15 mins, the cooling bath was removed and the reaction left at room temperature for 16 h. Ether and then water were added and the aqueous layer was saturated with sodium chloride and extracted with ethyl acetate. The combined organic extracts were dried and evaporated. The residue was purified by chromatography on silica gel using ethyl acetate as eluant to give the cyclic phosphonate (126) (0.14 g, 82%); δ ($(CD_3)_2CO$) 8.55 (2H, s, O*H*), 6.76 (1H, d, *J* 3Hz, aromatic), 6.64 (1H, d, *J* 3Hz, aromatic), 3.63 (1H, dq, J_{HH} 7 Hz and J_{PH} 23.5 Hz, C*H*CH_3), 2.82 (6H, d, J_{PH} 10 Hz, NC*H*$_3$), 1.29 (3H,dd, J_{HH} 7Hz and J_{PH} 17 Hz, CHC*H*$_3$).

Methyl 2-methoxybenzoate (131)[98]

A mixture of methyl 2-hydroxybenzoate (10 g), dimethyl sulphate (15.5 ml), and anhydrous potassium carbonate (72.7 g) in Analar acetone (150 ml) was refluxed for 5 h. The solution was cooled, filtered, washed with Analar acetone (150 ml) and evaporated *in vacuo* to give a colourless oil. This residue was then dissolved in ether (200 ml), washed with ammonia liquor (3 x 100 ml), 10% sodium hydroxide solution (2 x 100 ml), water (2 x 100 ml), and then dried and evaporated to give a colourless oil (7.28 g, 67%), b.p. 146-147°C/15 mm Hg (lit.[98] 127°C/11 mm Hg); $\delta(CDCl_3)$ 7.79 (1H, m, aromatic), 7.48 (1H, m, aromatic), 7.00 (2H, m, aromatic), 3.88 (6H, s, OCH_3); ν_{max} ($CHCl_3$) 3020 m, 1720 s, and 1260 s cm^{-1}.

1-Oxo-1-(2-methoxyphenyl)propan-2-ylphosphonic Bis(dimethylamide) (132)

The method of preparation of the β-oxophosphonamide (124) was followed using methyl 2-methoxybenzoate (131) (1 g) in THF (10 ml), a solution of *n*-butyllithium (7.63 ml, 1.60M in hexane), and ethylphosphonic acid bis(dimethylamide)(121) (2.05 g) in THF (15 ml). An amber oil was formed (0.89 g, 49%); δ ($CDCl_3$) 7.56 (2H, m, aromatic), 7.01 (2H, t, J 4Hz, aromatic), 4.53 (1H, dq, J_{HH} 6Hz and J_{PH} 17 Hz, C*H*Me), 3.87 (3H, s,OCH_3), 2.65 (6H, d, J_{PH} 9Hz, NCH_3), 2.50

(6H, d, J_{PH} 9Hz, NCH_3), 1.51 (3H, dd, J_{HH} 6Hz and J_{PH} 16Hz, CHCH_3)

(Found: C, 56.31; H, 7.60; N, 9.45; P, 10.51. $C_{14}H_{23}N_2O_3P$ requires C, 56.36; H, 7.77; N,9.39; P, 10.38%).

2-Dimethylamino-3-methyl-2*H*-1,2 λ^5benzoxaphosphorin-2,4-(3*H*)-dione (127)

The method of preparation of the cyclic phosphonate (126) was followed using the β-oxophosphonamide (132) (0.30 g). The reaction yielded a dark brown coloured oil (0.20 g, 83%); δ ($CDCl_3$) 7.85 (1H, m, aromatic), 7.47 (1H, m, aromatic), 7.13 (2H, t, *J* 7Hz, aromatic), 3.21 (1H, dq, J_{HH} 7 Hz and J_{PH} 23 Hz, C*H*Me), 2.84 (6H,d, J_{PH} 10 Hz, NCH_3), 1.32 (3H,dd, J_{HH} 7 Hz and J_{PH} 16 Hz, CHCH_3). (Found : C, 55.15; H, 5.70; N, 5.95; P, 12.90. $C_{11}H_{14}NO_3P$ requires C, 55.23; H, 5.90; N, 5.86; P, 12.95%).

Methyl 2,3-dimethoxybenzoate (134)[99]

A solution of 2,3-dimethoxybenzoic acid (4.5 g) in dry methanol (50 ml) was saturated with hydrogen chloride, and refluxed for 4 h. The methanol was evaporated *in vacuo* and the residue dissolved in ether. The ether solution was washed with saturated aqueous sodium bicarbonate and then water, dried, and evaporated to give a white crystalline solid (3.22 g, 67%), m.p. 45-46°C (lit.[99] 46-48°C); δ ($CDCl_3$) 7.33 (1H, t, *J* 3Hz, aromatic), 7.07 (2H, d, *J* 3Hz, aromatic), 3.89 (9H,s, OCH_3); ν_{max} (KBr) 2950 m, 1700 s, 1480 s, 1260 s, and 1060 s cm^{-1}.

1-Oxo-1-(2,3-dimethoxyphenyl)propan-2-ylphosphonic-Bis(dimethylamide) (135)

The method of preparation of the β-oxo-phosphonamide (124) was followed using methyl 2,3-dimethoxybenzoate (134) (1.00 g) in THF (10 ml), a solution of *n*-butyllithium (6.47 ml, 1.60M in hexane), and ethylphosphonic acid bis(dimethylamide) (121) (1.73 g) in THF (15ml). The reaction yielded the β-oxophosphonamide (135) (1.18 g, 71%); δ ($CDCl_3$) 7.12 (3H, m, aromatic), 4.51 (1H, dq, J_{HH} 6 Hz and J_{PH} 17 Hz, C*H*Me), 3.89 (6H,s, OCH_3), 2.65 (6H, d, J_{PH} 9Hz, NCH_3), 2.54 (6H, d, J_{PH} 9Hz, NCH_3), 1.55 (3H, dd, J_{HH} 7 Hz and J_{PH} 16 Hz, $CHCH_3$)
(Found : C, 54.72; H, 7.81; N, 8.65; P, 9.40.

$C_{15}H_{25}N_2O_4P$ requires C, 54.87; H, 7.67; N, 8.53; P, 9.43%).

2-Dimethylamino-8-hydroxy-3-methyl-2*H* 1,2 λ^5-benzoxaphosphorin-2,4(3*H*)-dione (128)

The method of preparation of the cyclic phosphonate (126) was followed using the β-oxophosphonamide (135) (0.30 g) in dichloromethane (20 ml), and boron tribromide (0.5 ml). The reaction yielded the cyclic phosphonate (128) (0.22 g, 96%); δ [$(CD_3)_2CO$] 8.43 (1H,s, O*H*), 7.12 (3H, m, aromatic), 3.61 (1H, dq, J_{HH} 7 Hz and J_{PH} 18 Hz C*H*Me), 2.82 (6H, d, J_{PH}, 9 Hz, NCH_3), 1.22 (3H, dd, J_{HH} 7 Hz and J_{PH} 16 Hz, CHCH_3). (Found : C, 51.70; H, 5.63; N, 5.42; P, 12.10. $C_{11}H_{14}NO_4P$ requires C, 51.77; H, 5.53; N, 5.49; P, 12.14%).

Methyl 2,5-dimethoxybenzoate (137)[100]

A mixture of 2,5-dimethoxybenzoic acid (10 g), dimethyl sulphate (20 ml), and anhydrous potassium carbonate (50 g) in Analar acetone (500 ml) was refluxed for 5 h. The solution was cooled, filtered, washed with Analar acetone (150 ml), and evaporated. The residue was dissolved in ether (200 ml), washed with ammonia liquor (3 x 100 ml), 10% sodium hydroxide solution (2 x 100 ml), water (2 x 100 ml), and then

dried and evaporated to give a pale yellow coloured oil (9.1 g, 72%), (lit.[100] b.p. 95-98°C/1 mm Hg); δ ($CDCl_3$) 7.47 (1H, d, *J* 3 Hz, aromatic), 7.01 (2H, m, aromatic), 3.89 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.78 (3H, s, OCH_3); ν_{max} ($CHCl_3$) 3020 m, 1720 s, and 1500 s cm^{-1}.

1-Oxo-1-(2,5-dimethoxyphenyl)propan-2-ylphosphonic-Bis(dimethylamide) (138)

The method of preparation of the β-oxophosphonamide (124) was followed using methyl 2,5-dimethoxybenzoate (137) (1.00 g) in THF (10 ml), a solution of *n*-butyllithium (6.47 ml, 1.60M in hexane) and ethylphosphonic acid bis(dimethylamide) (121) (1.73 g) in THF (15 ml). The reaction yielded the β-oxophosphonamide (138) (1.16 g, 69%); δ ($CDCl_3$) 7.13 (1H, m, aromatic), 6.97 (2H, m, aromatic), 4.64 (1H, dq, J_{HH} 6 Hz and J_{PH} 16 Hz, C*H*Me), 3.87 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 2.66 (6H, d, J_{PH} 9 Hz, NCH_3), 2.56 (6H, d, J_{PH} 9 Hz, NCH_3), 1.52 (3H, dd, J_{HH} 7 Hz and J_{PH} 16 Hz, $CHCH_3$).

(Found : C, 54.82; H, 7.53; N, 8.37; P, 9.40. $C_{15}H_{25}N_2O_4P$ requires C, 54.87; H, 7.67; N, 8.53; P, 9.43%).

2-Dimethylamino-6-hydroxy-3-methyl-2*H*-1,2 λ^5-benzoxaphosphorin-2,4(3*H*)-dione (129)

The method of preparation of the cyclic phosphonamide (126) was followed using the β-oxophosphonamide (138) (0.35 g) in dichloromethane (20 ml), and boron tribromide (0.5 ml). The reaction yielded the cyclic phosphonate (129) (0.25 g, 93%); δ [$(CO_3)_2CO$] 8.51 (1H, s, O*H*), 7.22 (1H, d, *J* 3 Hz, aromatic), 6.98 (2H, t, *J* 3 Hz, aromatic), 3.12 (1H, dq, J_{HH} 7 Hz and J_{PH} 23 Hz, C*H*Me), 2.64 (6H, d, J_{PH} 10 Hz, NCH_3), 1.33 (3H, dd, J_{HH} 7 Hz and J_{PH} 16 Hz, CHCH_3).
(Found : C, 51.65; H, 5.70; N, 5.35; P, 12.20.
$C_{11}H_{14}NO_4P$ requires C, 51.77; H, 5.53; N, 5.49; P, 12.14%).

Triethyl phosphonoacetate (140)[101]

Ethyl chloroacetate (65 g) and triethyl phosphite (88 g) were thoroughly mixed in a round bottomed flask (250 ml) with a condenser and placed in an oil bath. The reaction mixture was heated and stirred and the temperature slowly brought to 125°C, then the external heat was discontinued for 30 mins. As the reaction proceeded a vigorous but controlled evolution of ethyl chloride occurred. The temperature was then brought up to 160°C over a 75 min period and held there for 8 h, after which time ethyl chloride evolution had stopped. The liquid was allowed to cool overnight then distilled *in vacuo* to yield triethyl phosphonoacetate (140) as a colourless oil (89.1 g, 75%), b.p. 86-90°C/0.4 mm Hg

(lit.[101] 109°C/0.8 mm Hg); δ ($CDCl_3$) 4.20 (4H, dq, J_{HH} 2 Hz and J_{PH} 7 Hz PO_2CH_2), 4.13 (2H, q, *J* 6 Hz, $CO_2CH_2CH_3$), 2.95 (2H, d, J_{PH} 20 Hz, PCH_2), 1.32 (6H, t, *J* 6 Hz, $POCH_2CH_3$), 1.27 (3H, t, *J* 6 Hz, $CO_2CH_2CH_3$); ν_{max} (thin film) 1740 s, 1270 s, and 1030 s cm^{-1}.

Ethyl hex-2-enoate (142)[102]

Triethyl phosphonoacetate (50 g) was added dropwise for a period of 1 h to sodium hydride (9 g, 60% mineral oil dispersion) in dry diethyl ether (300 ml) at 0°C. The mixture was allowed to warm up to room temperature and then heated to reflux for 1 h after which the solution was cooled to -10°C in an ice-salt bath and freshly distilled butyraldehyde (16 g) was added to the vigorously stirred solution during a period of 45 mins. As the reaction proceeded the solution became increasingly viscous. The reaction mixture was stirred for another hour and then brought slowly to reflux. On cooling a thick gummy precipitate of sodium diethyl phosphate was observed. The remaining liquid was decanted off and the gummy precipitate washed with ether. The combined ether solutions were washed with saturated aqueous sodium hydrogen carbonate, and then water. The ether extract was dried and evaporated *in vacuo* to give ethyl hex-2-enoate (142) (27.7 g, 89%); δ ($CDCl_3$) 6.94 (1H, dt, *J* 6 Hz and 14 Hz, 3-*H*), 5.81 (1H, dt, *J* 1.5 Hz and 14 Hz, 2-*H*), 4.18 (2H, q, *J* 6 Hz, OCH_2), 2.17 (2H, q, *J* 6 Hz, 4-H_2), 1.50 (2H, m, 5-H_2), 1.27 (3H, t, *J* 6 Hz, OCH_2CH_3), 0.91 (3H, t, *J* 6 Hz, 6-H_3); ν_{max} (thin film) 1725 s, 1655 s, 1270 s, and 1180 s cm^{-1}.

Ethyl 6-propyl-5,6-dihydro-β-resorcylate(143)[102]

Ethyl acetoacetate (18.3 g) was added dropwise to a stirred solution of sodium (3.65 g) in dry ethanol (100 ml) at a rate which maintained a gentle reflux. The solution was heated at reflux for a further 30 mins, then ethyl hex-2-enoate (20 g) was added dropwise during 15 mins. The mixture was heated at reflux for 6 h and then stirred at room temperature for a further 16 h. The sodium salt of the enolate produced was filtered, washed with dry ether and air dried. The dried salt was dissolved in water, washed with ether, acidified with dilute hydrochloric acid, and the aqueous solution extracted with ether. The extract was dried and evaporated to give ethyl 6-propyl-5,6-dihydro-β-resorcylate (143) as an oil, which slowly crystallised to give pale yellow crystals (11.7 g, 37%), m.p. 78-80°C (lit.[102] 80°C); δ ($CDCl_3$) 5.54 (1H, s, exchangeable with D_2O, O*H*), 4.67 (1H, s, exchangeable with D_2O, O*H*), 4.21 (2H, q, *J* 7 Hz, OCH_2), 3.55 (2H, m, 5-H_2), 3.20 (1H, d, *J* 10 Hz, 6-*H*), 2.53 (3H, m, $CHCH_2$), 1.30 (2H, m, $CH_2CH_2CH_3$), 1.25 (3H, t, *J* 7 Hz, OCH_2CH_3), 0.92 (3H, m, $CH_2CH_2CH_3$); ν_{max} (KBr) 1735 s, 1610 s, and 1510 s cm^{-1}.

Ethyl 3,5-dichloro-6-propyl-β-resorcylate (144)[102]

Concentrated hydrochloric acid was dropped onto potassium permanganate crystals in order to prepare a solution of chlorine. The gas was purified by bubbling it through a potassium permanganate solution and then water. It was collected in 400 ml of acetic acid so that the weight of the flask increased by 35.5 g.

Acetic acid (24.8 ml) containing chlorine (2.2 g) was added to a stirred solution of ethyl 6-propyl-5,6-dihydro-β-resorcylate (143) (3.3 g) in glacial acetic acid (20 ml) at $0^{o}C$. The mixture was stirred at this temperature for 30 mins, then at room temperature for 30 mins and finally at $60^{o}C$ for 4 h. Nitrogen was then passed through the solution to remove any hydrogen chloride gas which had evolved. A further quantity of chlorine (1.5 g) in acetic acid (16.9 ml) was added to the stirred mixture at approximately $0^{o}C$, assuring that the solution did not freeze. This mixture was then stirred for 30 mins and poured onto an ice-water mixture from which the product precipitated from solution. Ethyl 3,5-dichloro-6-propyl-β-resorcylate (144) was recrystallised from ether and hexane (2.56 g, 60%), m.p. 85-87^{o}C (lit.[102] 86-88^{o}C); δ ($CDCl_3$) 6.48 (2H, br s, exchangeable with D_2O, O*H*), 4.50 (2H, q, *J* 7 Hz, OCH_2CH_3), 3.05 (2H, m, $CH_2CH_2CH_3$), 1.53 (2H, m, $CH_2CH_2CH_3$), 1.46 (3H, t, *J* 7Hz, OCH_2CH_3), 1.03 (3H, t, *J* 7 Hz, $CH_2CH_2CH_3$); ν_{max} (KBr) 3400 s, 1640 s, and 1585 cm^{-1}.

Ethyl 3,5-dichloro-2,4-dimethoxy-6-propyl-benzoate (145)[102]

A solution of ethyl 3,5-dichloro-6-propyl-β-resorcylate (144) (2 g) in Analar acetone (20 ml) with dimethyl sulphate (1.28 g) and anhydrous potassium carbonate (3.76 g) was heated at reflux for 4 h. The solution was allowed to cool, then filtered, and washed with acetone. The acetone solutions were evaporated and the residue dissolved in ethyl acetate then washed with ammonia liquor (3 x 100 ml) and water (2 x 100 ml) respectively, dried and evaporated to give a brown oil. This residue was distilled *in vacuo* to yield a colourless oil (1.26 g, 58%), b.p. 220°C/0.60 mm Hg; δ ($CDCl_3$) 4.15 (2H, q, *J* 7 Hz, OCH_2), 3.92 (6H, s, OCH_3), 2.70 (2H, m, $CH_2CH_2CH_3$), 1.60 (2H, m, $CH_2CH_2CH_3$), 1.27 (3H, t, *J* 7 Hz, OCH_2CH_3), 1.00 (3H, t, *J* 7 Hz, $CH_2CH_2CH_3$); ν_{max} (thin film) 1734 s, and 1565 s cm^{-1}.

Ethyl 3,5-dichloro-2-hydroxy-4-methoxy-6-propyl-benzoate (146)

Aluminium trichloride (600 mg) was added slowly to a solution of ethyl 3,5-dichloro-2,4-dimethoxy-6-propyl-benzoate (200 mg) in nitrobenzene (15 ml). The mixture was stirred for 5 h at a temperature between 50-60°C and then poured onto an ice-water mixture and acidified with dilute hydrochloric acid. The acidic solution was extracted with ether (3 x 40 ml) and the combined extracts were extracted with 10% sodium hydroxide solution (2 x 40 ml). The basic solution was then washed with ether (2 x 40 ml), acidified with dilute hydrochloric acid, and again extracted with ether (3 x 50 ml). The final extracts were dried and evaporated to give a brown oil which slowly crystallised to give ethyl 3,5-dichloro-2-hydroxy-4-methoxy-6-propylbenzoate (146) (50 mg, 26%); δ ($CDCl_3$) 4.48 (2H, q, *J* 7 Hz, OCH_2CH_3) 3.95 (3H, s, OCH_3), 3.05 (2H, m, $CH_2CH_2CH_3$), 1.62 (2H, m, $CH_2CH_2CH_3$), 1.45 (3H, t, *J* 7 Hz, OCH_2CH_3), 1.02 (3H, t, *J* 8 Hz, $CH_2CH_2CH_3$); ν_{max} (KBr) 3430 br, 1650 s, and 1588 s cm^{-1}; *m/z* 298/296/294 (M^+).
(Found: C, 47.95; H, 5.30; Cl, 23.50.
$C_{12}H_{16}O_4Cl_2$ requires C, 48.83; H, 5.46; Cl, 24.02%).

Attempted preparation of 3,5-dichloro-2,4-dimethoxy-6-propylbenzoic acid (148) using sodium hydroxide

Ethyl 3,5-dichloro-2,4-dimethoxy-6-propylbenzoate (145) (100 mg) was dissolved in a 25% sodium hydroxide solution (3 ml) and allowed to stand at room temperature for 7 days. The mixture was then diluted with water, acidified with dilute hydrochloric acid and extracted with ether. The extract was dried and evaporated to give a brown oil which was identified as starting material.

Attempted preparation of 3,5-dichloro-2,4-dimethoxy-6-propylbenzoic acid (148) using trimethylsilyliodide

Ethyl 3,5-dichloro-2,4-dimethoxy-6-propylbenzoate (148) (110 mg) was dissolved in carbon tetrachloride (20 ml) which had been previously dried by passing it through a column of alumina. Trimethylsilyliodide (0.8 ml) was then added to the mixture which was heated at a temperature of 50^{o}C for a period of 48 h. Water was then added to the solution and the carbon tetrachloride layer separated and washed with a solution of sodium thiosulphate, dried and evaporated. The residue was dissolved in ether, dried and evaporated to give a clear oil which was then identified as starting material.

2,4-Dichloro-1,3-dimethoxy-5-propylbenzene (147)

Ethyl 3,5-dichloro-2,4-dimethoxy-6-propylbenzoate (145) (100 mg) in concentrated sulphuric acid (2.5 ml) was kept at 50-70°C for 2 h then poured onto ice water, extracted with ether, dried and evaporated to give 2,4-dichloro-1,3-dimethoxy-5-propyl benzene (147) as an oil; δ_H ($CDCl_3$) 6.58 (1H, s, aromatic), 3.88 (6H, s, OCH_3) 2.67 (2H, m, $CH_2CH_2CH_3$), 1.62 (2H, m, $CH_2CH_2CH_3$), 0.95 (3H, t, *J* 7 Hz, $CH_2CH_2CH_3$); ν_{max} (thin film) 2960 m, 2865 m, and 1575 s cm^{-1}; *m/z* 252/250/248 (M^+)
(Found : C, 52.88; H, 5.35; Cl, 28.27.
$C_{11}H_{14}O_2Cl_2$ requires C, 53.03; H, 5.66; Cl, 28.46%).

3,5-Dichloro-2,4-dimethoxy-6-propylbenzoic acid (148)

Ethyl 3,5-dichloro-2,4-dimethoxy-6-propylbenzoate (145) (100 mg) was dissolved in an ice-cold solution of concentrated sulphuric acid (5 ml) and allowed to stand at O°C for 6 days. The mixture was then poured onto ice-water and extracted with ether. The ether extract was dried and evaporated to give a brown oil (148) (90 mg, 98%); δ ($CDCl_3$), 6.06 (1H, br s, CO_2H), 3.98 (6H, s, OCH_3), 2.80 (2H, m, $CH_2CH_2CH_3$), 1.68 (2H, m, $CH_2CH_2CH_3$), 1.02 (3H, t, *J* 7 Hz, $CH_2CH_2CH_3$); ν_{max} (thin film) 1730 s, and 1570 s cm^{-1}; *m/z* 296/294/292 (M^+).
(Found : C, 49.03; H, 4.59; Cl, 24.31.
$C_{12}H_{14}O_4Cl_2$ requires C, 49.16; H, 4.81; Cl, 24.19%).

3,5-Dichloro-2-hydroxy-4-methoxy-6-propylbenzoic acid (139) (Differanisole A).

Ethyl 3,5-dichloro-2-hydroxy-4-methoxy-6-propylbenzoate (146) (40 mg) was dissolved in an ice cold solution of concentrated sulphuric acid (10 ml) and allowed to stand at $0^{o}C$ for 6 days. The mixture was then poured onto ice-water and extracted with ether. The ether extract was then dried and evaporated to give (139) as a brown solid (30 mg, 84%); δ [$(CD_3)_2CO$] 7.12 (1H, br s, CO_2H), 3.80 (3H, s, OCH_3), 3.17 (2H, m, $CH_2CH_2CH_3$), 1.57 (2H, m, $CH_2CH_2CH_3$), 0.98 (3H, t, *J* 7 Hz, $CH_2CH_2CH_3$); ν_{max} (KBr) 3400 m and 1630 s cm^{-1}; *m/z* 282/280/278 (M^+).

(Found : C, 47.23, H, 4.21; Cl, 25.37.

$C_{11}H_{12}O_4Cl_2$ requires C, 47.33; H, 4.34; Cl, 25.40%).

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