LAMELLICOLIC ANHYDRIDE AND OTHER POLYKETIDES OF *VERTICILLIUM LAMELLICOLA* AND *ASPERGILLUS NIDULANS*.

A THESIS PRESENTED BY

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Summary.

A number of novel, phenolic 1,8-naphthalic anhydrides have been isolated from a strain of Verticillium lamellicola and the major metabolite, lamellicolic anhydride, shown to be 2,4,7-trihydroxy-5-methyl-1,8-naphthalic anhydride. Amongst a number of unusual and characteristic reactions exhibited by this compound was a remarkably facile aminolysis of ether groups in its O,O,O-trimethyl derivative with the formation of amino derivatives. Routes from lamellicolic anhydride have been studied in order to provide an entry into the dimeric series of compounds typified by duclauxin.

Minor metabolites of V. lamellicola include 2,7-dihydroxy-5-methyl-1,4-naphthaquinone - the in vitro synthesis of this compound from lamellicolic anhydride has been accomplished - and the monomethyl ester of 3-chlorolamellicolic acid. 4-O-Carbomethoxylamellicolic anhydride - the first natural product reported which contains a methyl carbonate group - co-occurs with lamellicolic anhydride and has been prepared from it. The biosynthesis of these compounds and the results of some preliminary feeding experiments are discussed.

Two phenolic, yellow pigments - arugoxanthones A and B - have been isolated from the spores of the yellow mutant of Aspergillus nidulans and identified as the first reported prenylated fungal xanthones. Model studies have confirmed the feasibility of the unusual chroman-4-ol unit found in arugoxanthone A being derived biogenetically via a novel

intramolecular cyclisation of an O-3,3-dimethylallyl unit and a formyl group.

Arugoxanthones A and B have also been isolated from mycelial extracts of the yellow mutant along with the closely related compounds isoarugoxanthone A and arugoxanthones C and D. The two latter compounds contain a substituent previously unrecorded in a natural product - namely the O-3,3-dimethyl-3-hydroxy-1-propyl ether group. The known mould metabolite arugosin co-occurs with these xanthones and routes to the latter compounds from it have been explored. In particular, the acid catalysed rearrangement of arugosin has been studied and the products of a novel intramolecular cyclisation reaction identified. The close relationship between arugosin and the xanthones has been demonstrated by the conversion of arugoxanthone B into anhydroarugosin and deisopentenyl-anhydroarugosin, reported as transformation products of arugosin.

The methyl ester of isocanadensic acid - a metabolite of Penicillium canadense and possible biogenetic precursor of the antibiotic canadensolide - has been synthesised by a five step route.

I should like to express my sincere thanks and appreciation to my supervisor, Dr. N.J. McCorkindale, for his constant guidance and advice during the course of this work and also in the presentation of this thesis.

I should also like to thank the Science Research Council for maintenance during the last three years and Professor R.A. Raphael, F.R.S., for the opportunity to carry out this research.

I am also indebted to Mrs. M. Tait, Miss M. McCartney and staff of the Mycology Department for technical assistance in the preparation and separation of fungal extracts. Thanks are also due to Miss F. Cowan (micro-analyses), to Mrs. F. Lawrie (infra-red spectra) and to Messrs J. Gall and A. Haetzman (n.m.r. spectra).

Finally, I should like to thank everyone who has assisted me in the preparation of this thesis.

Contents.

INTRODUCTION.

The Fungi.

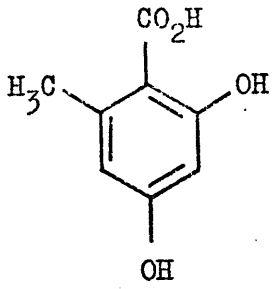
Fungi[1] and other organisms which possess neither leaves, roots nor stems, such as bacteria and algae, have been classified as belonging to that section of the plant kingdom called the Thallophyta. They absorb nutrients through the surface of their vegetative body or mycelium, which consists of an interwoven network of small tubes called hyphae containing a multi nucleate protoplasm. Depending on whether or not transverse walls divide the hyphae the mycelium is called septate or aseptate respectively. Some parts of the mycelium have the specialised function of producing the spores which ensure the continuation of the species and these may be sexual (formed by the coalescence of cells from two different hyphae) or asexual (formed by the coalescence of cells from the same hypha).

Fungi are often classified[2] according to the nature of their mycelium and to the type of spores produced. The Phycomycetes are distinguished by their having aseptate mycelium and are simple, often unicellular organisms- typified by Mucor.Rhizopus nigricans, a fungus which grows on bread. The Ascomycetes, e.g.truffles, and the Basidiomycetes, such as the puff-balls, have septate mycelium, the former group bearing their sexual spores in a sac-like vessel called an ascus and the latter bearing them on basidia. The Fungi Imperfecti- exemplified by the numerous species of Penicillia and Aspergilli produce only sexual spores and can be readily grown under laboratory conditions thus allowing the

metabolites which they produce to be investigated.

The same primary metabolic pathways are found in fungi as in other organisms. The breakdown of glucose by these routes provides the organism with the energy necessary for its existence and also leads to the synthesis of essential macromolecules such as proteins and nucleic acids. In fungi, however, as in bacteria and in higher plants secondary metabolic pathways exist resulting in the production of a wide range of structurally diverse molecules which have no obvious role to play in the survival of the organism and the distribution of which is species, and indeed often strain, dependent. The realisation that these secondary metabolites can be derived by a few basic pathways from a small number of key primary metabolites and the elucidation of these pathways is one of the most impressive achievements of modern organic chemistry.

This thesis deals with the isolation and identification of a number of polyketide derived secondary metabolites produced by a Verticillium species and an Aspergillus species.



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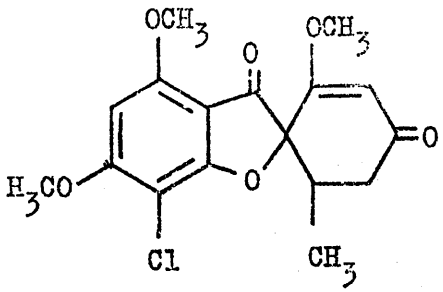

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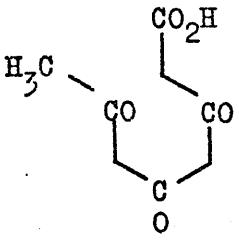

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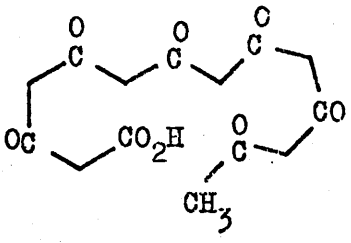

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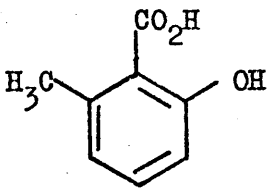

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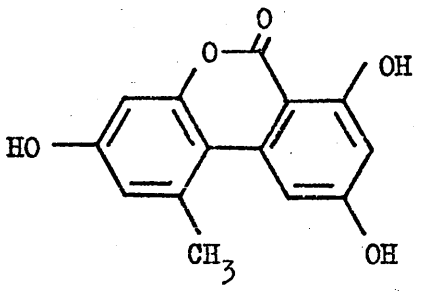

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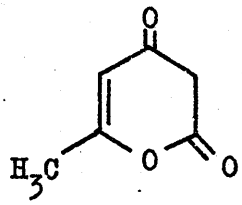
The Biosynthesis of Polyketides.

The suggestion that the structures of a large number of phenolic natural products were consistent with their being formed by the cyclisation (via Aldol or Claisen condensations) of β-polyketone chains, derived by the linear combination of acetate units, was first made by Birch[3], in 1953. The mould metabolites orsellinic acid (1) and griseofulvin (2), for example, could be derived from a tetramer (3) and a heptamer (4) respectively. The first experimental support for this hypothesis came from the observation[4] that ^{14}C labelled acetate fed to Penicillium griseofulvum was incorporated into 6-methylsalicylic acid (5) and subsequently many other plant and mould metabolites have been shown to be polyketide derived[2].

The enzymes involved in this pathway have not been isolated and purified- although cell-free extracts capable of synthesising polyketides such as 6-methylsalicylic acid[5] and alternariol[6] (6) have been obtained- and the mechanistic details of polyketide biosynthesis remain largely speculative. Radiotracer experiments[7], however, have shown that both acetate and malonate are involved and since 6-methylsalicylic acid and fatty acids coproduced in the presence of [1-^{14}C, 2-^{3}H] acetate have a distribution of radioactivity consistent with their formation by the same condensation processes[8] it is believed that there is a close relationship between the mechanism of polyketide biosynthesis and that of fatty acid biosynthesis. The details of the latter pathway have been



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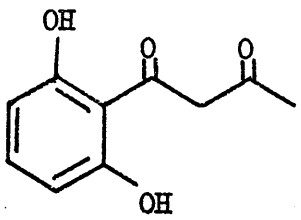

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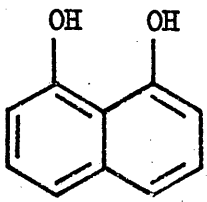

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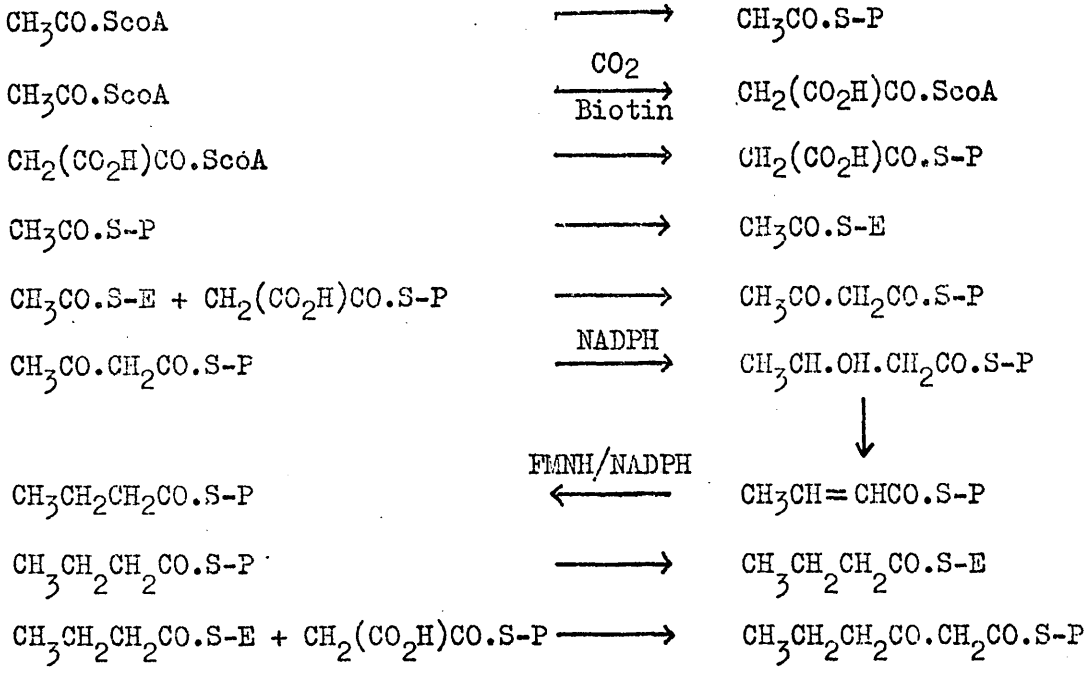
Figure 1. Fatty Acid Biosynthesis.



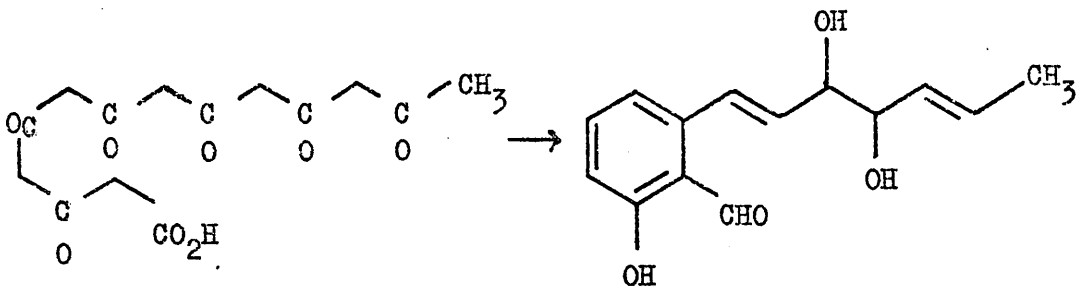
P = carrier protein.

E = enzyme.

elucidated[9] using purified enzyme systems and are as shown in figure 1. An acetyl group is transferred from coenzyme A via a carrier protein to a thiol group of an enzyme and then condensed with a protein-bound malonyl unit - formed by the carboxylation of acetyl coenzyme A. The acetoacetyl derivative formed is then converted, still bound to the protein, by a series of reduction and dehydration steps to the butyryl derivative which is then recycled and condensed with another malonyl unit. Continuation of this process leads to the long chain fatty acids which are only released from the protein when the desired chain length has been reached. Condensation of an acetyl starter group with malonyl units without the subsequent reduction and dehydration steps would lead to the production of β-polyketone chains. Indeed in the absence of NADPH, required for the reduction steps, fatty acid synthetase produces triacetic acid lactone[10] (7) - a stabilised polyketide. In accord with this theory experiments with cell-free preparations[5,7] have demonstrated that labelled acetate is more efficiently incorporated into the first two carbon atoms (i.e. those derived from the acetyl starter unit) than into the remainder of a polyketide chain.

The growing polyketide chain remains bound to the protein until it has reached its final length and is not released until it has been stabilised by cyclisation and aromatisation. The absence of free partially cyclised intermediates, e.g. the dione (8), is indicated by their not being incorporated into the final cyclised product, in this case the naphthalene (9)[11]. The mode of cyclisation of the chain will be

Figure 2. The Modes of Cyclisation of a Heptaketide Chain.



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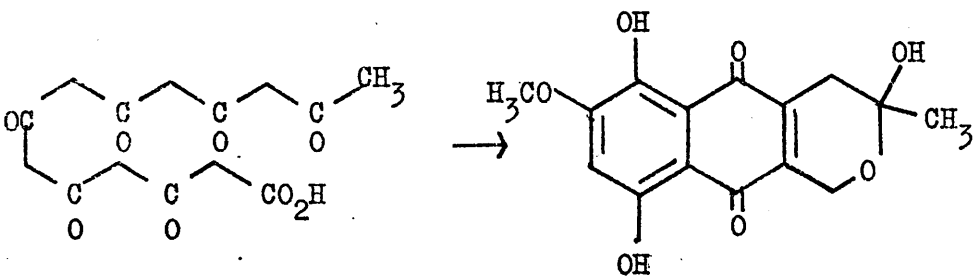

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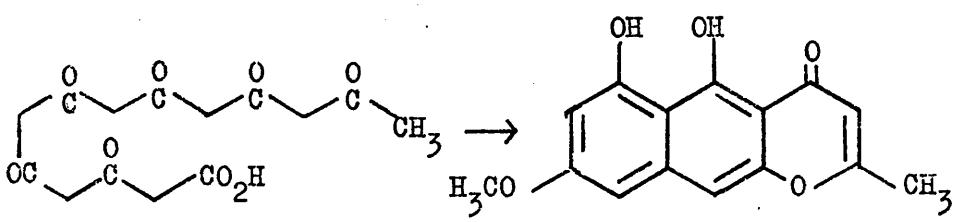

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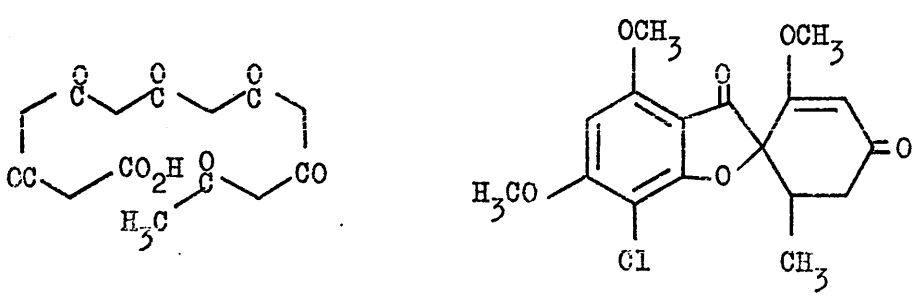

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Figure 3. Cyclisation of a Polyketide via an Electrocyclic Rearrangement.

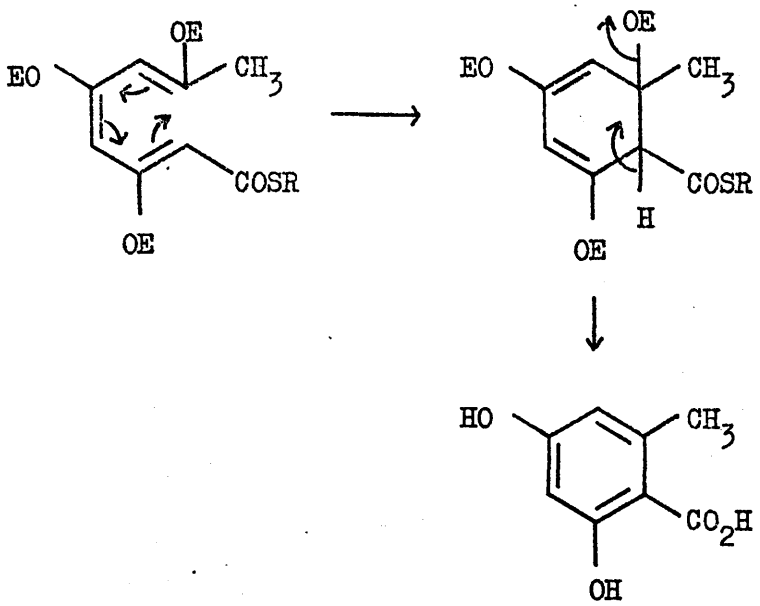
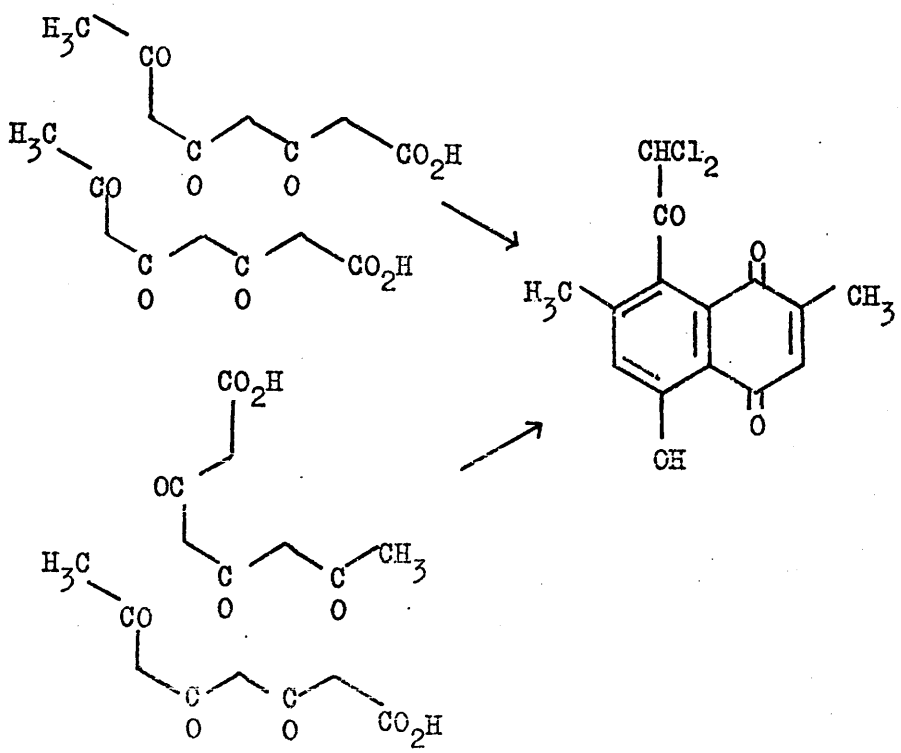
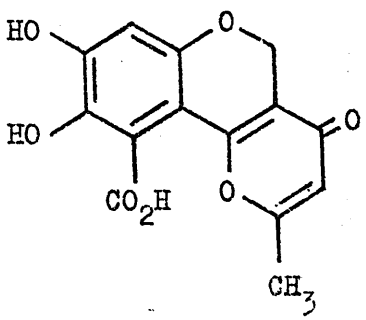
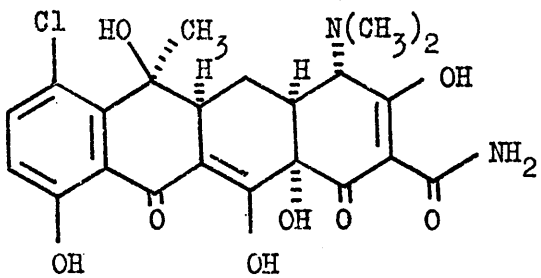


Figure 4. Alternative Biosyntheses of Mollisin.

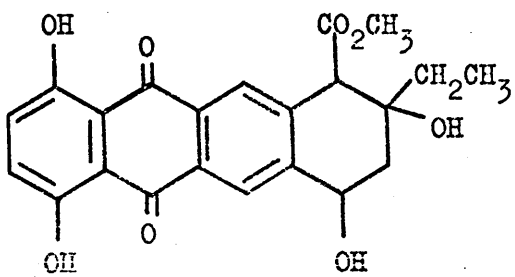



HO
HO
O
O
CO_2H
O
CH_3

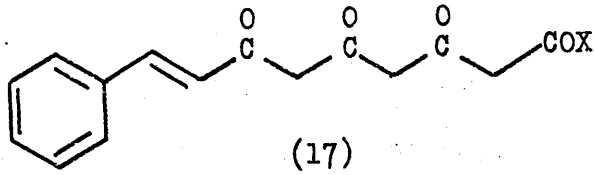
(14)


Cl
HO
CH_3
H
H
$N(CH_3)_2$
OH
NH_2
O
OH
O
O
OH
OH

(15)


OH
O
CO_2CH_3
CH_2CH_3
OH
O
OH
OH

(16)



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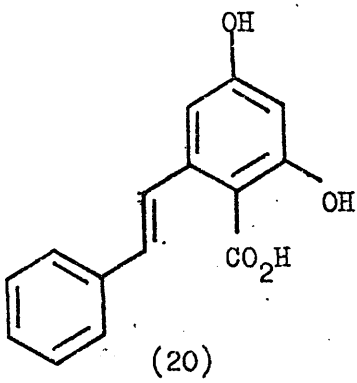

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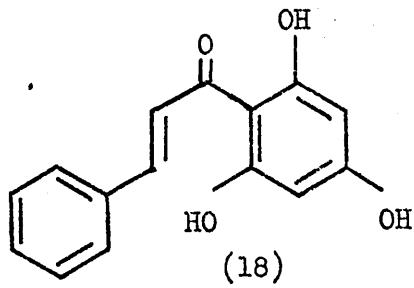

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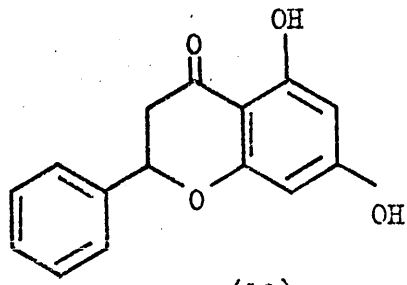
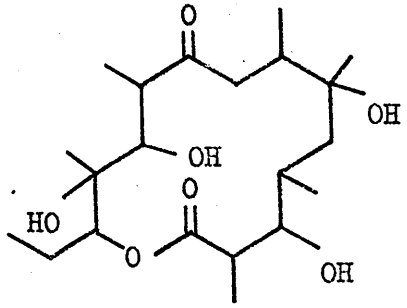


(19)

controlled by what Bu'Lock has called[12] 'the matrix of specific topography' associated with the protein in question and indeed the different modes of cyclisation of a chain of any one length can lead to a great variety of apparently structurally unrelated compounds. A heptaketide chain, for example, may be folded so as to produce such diverse mould metabolites as pyriculol[13] (10), fusarubin[14] (11), rubrofusarin[15] (12) and griseofulvin[16] (2) (figure 2). It has been suggested[17] that the highly reactive polyketide chains - stabilised on the proteins in the form of poly-β-enolates - may cyclise not via condensation reactions but by electrocyclic rearrangements followed by elimination reactions (figure 3).

Mollisin[18] (13) and a few other compounds arise by the condensation of two separate polyketide chains[19] (figure 4) and the detection of two acetate starter units has in some cases, e.g. citromycetin[20,21] (14), been taken as evidence of a two chain biosynthesis.

On occasion acyl coenzyme A derivatives other than acetyl coenzyme A can act as starter units - malonamate[22] in the case of some tetracyclines, e.g. 7-chlorotetracycline (15), and propionate in the case of the bacterial product ϵ-pyrromycinone[23] (16) can be instanced. In higher plants addition of three malonyl units to a shikimic acid derived aromatic starter unit[24] gives rise to a polyketide (17) which can yield chalcone (18) and compounds of the flavanoid series, e.g. flavanone (19), by Claisen condensation and compounds of the stilbene series, e.g. pinosylvic acid (20), by Aldol condensation. The structure of the



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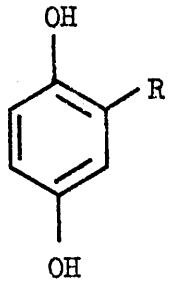
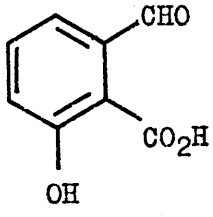


(22) R = CH_2OH

(23) R = CHO

(24) R = CO_2H



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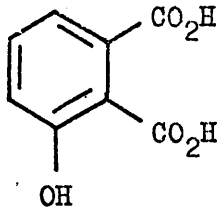

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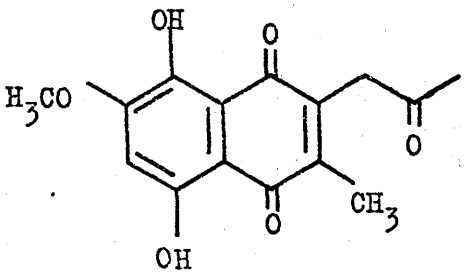

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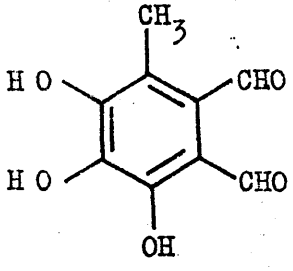

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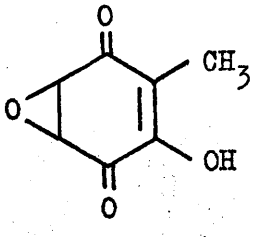

(29)

macrocyclic aglycone erythronolide[22] (21) exemplifies the fact that bacteria - unlike fungi - can also utilise propionate, in the form of methylmalonate, as a chain extender.

The range of polyketide structures isolated from natural sources is greatly increased by the large number of secondary transformations which can occur to the basic skeletons before or after cyclisation. These include processes involving oxidation, reduction, the insertion or removal of hetero-atoms, dehydration and electrophilic substitution.

The interconvertibility of acids, aldehydes and primary alcohols is demonstrated by the co-occurrence of groups of compounds such as gentisyl alcohol[25] (22), gentisyl aldehyde (23) and gentisic acid (24). On occasion methyl groups may be oxidised to carbonyl groups and to carboxyl groups, as in 6-formylsalicylic acid[26] (25) and 3-hydroxyphthalic acid[26] (26) respectively, and in a few cases carboxyl groups can be reduced to methyl groups, e.g. in javanicin[27] (27).

Mixed function oxygenases can introduce extra oxygen atoms into molecules, as in flavipin[28] (28) and terreic acid[29] (29). These enzymes are relatively non specific and this has allowed hydroxylated steroids- unobtainable by normal synthetic means- to be prepared by feeding suitable steroidal precursors to moulds containing such an enzyme[30].

Although polyketide derived, 6-methylsalicylic acid possesses one oxygen atom less than the number which would arise from cyclisation of the tetraketide chain (30). From the difficulties associated with the hydrogenolysis of a phenolic hydroxyl group it has been concluded that

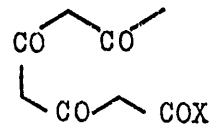

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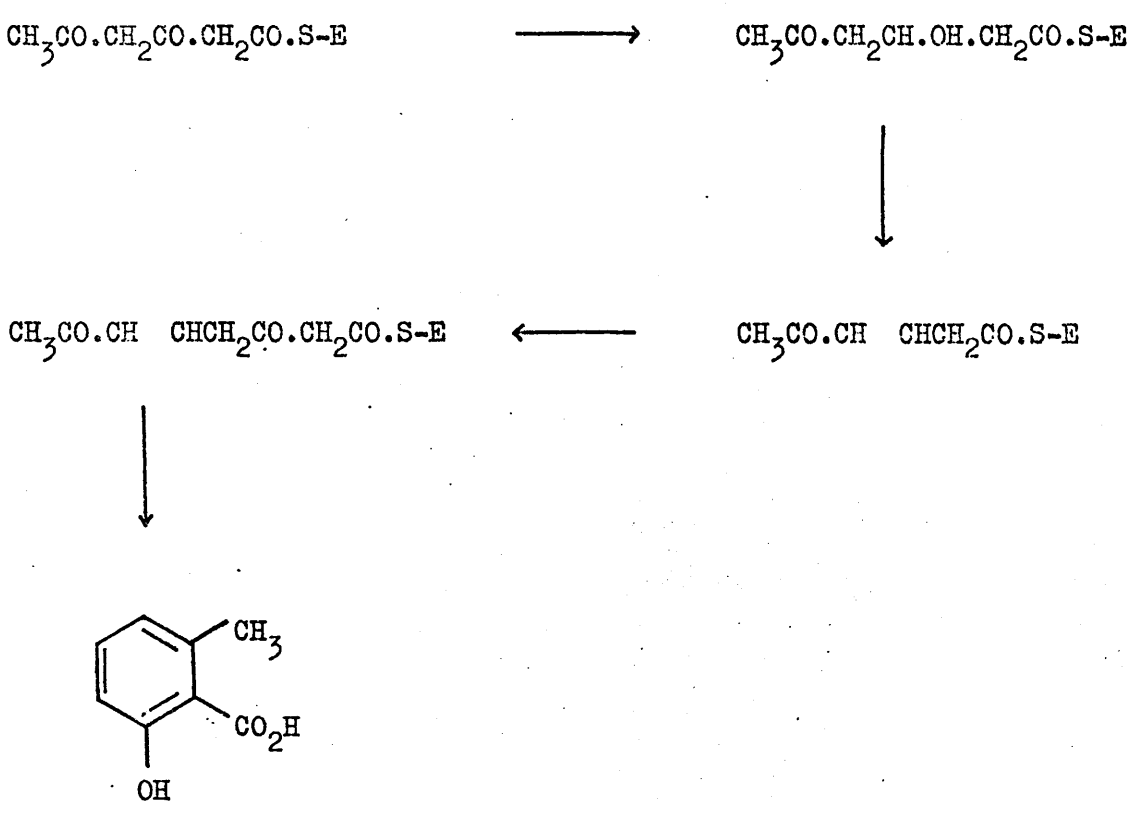
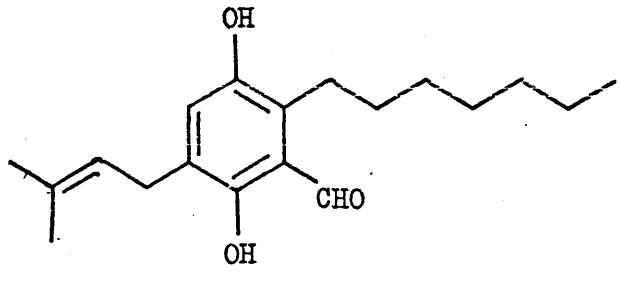
Figure 5. Biosynthesis of 6-Methylsalicylic Acid.





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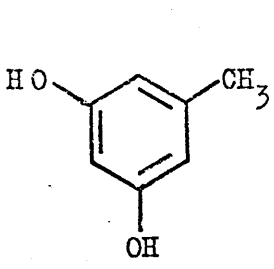

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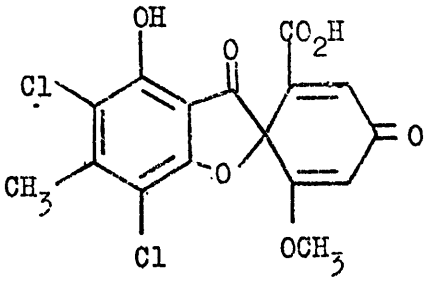

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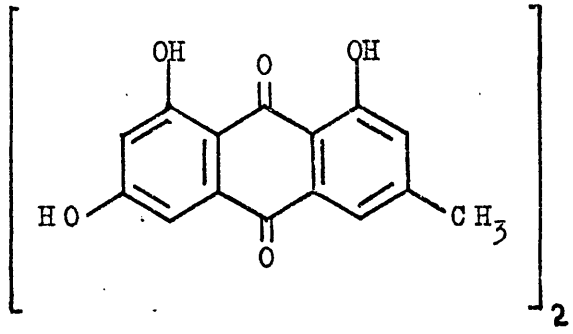


(34)

Figure 6. Biosynthesis of Patulin from 6-Methylsalicylic Acid.

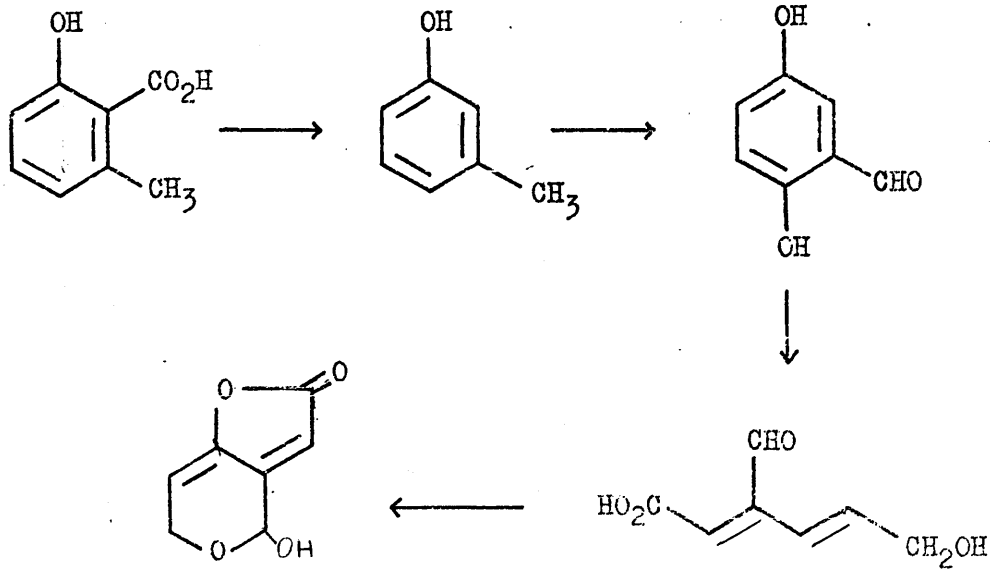
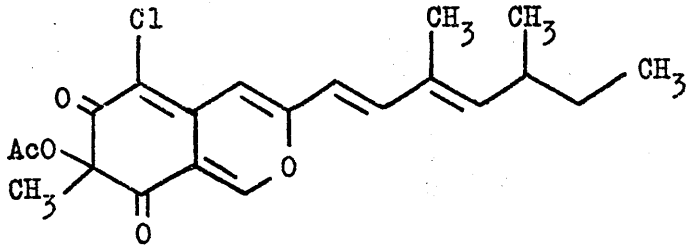
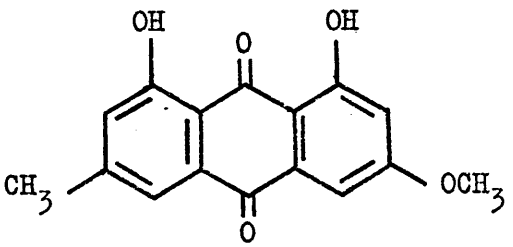


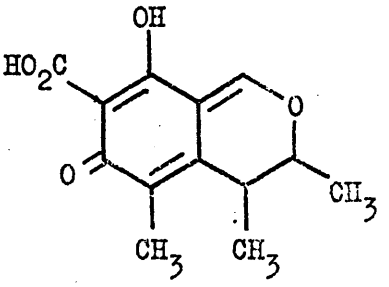
Figure 7.



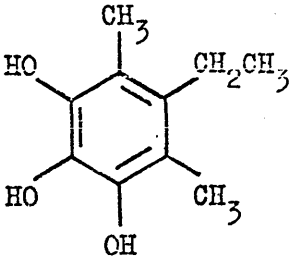
(36)



(37)



(38)



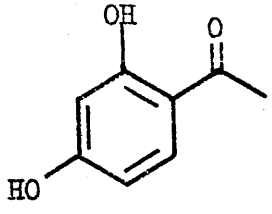
(39)

this oxygen atom is lost by sequential reduction and dehydration prior to aromatisation. Experiments with a purified 6-methylsalicylic acid synthetase[31] isolated from P. patulum have suggested that the sequence of events involved is as shown in figure 5. Further reduction of the double bonds produced by reduction and dehydration produces, in some compounds e.g. flavoglaucin[32] (31), long alkyl chains.

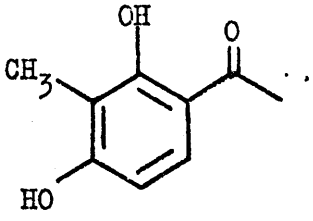
Orcinol[33] (32) exemplifies the fact that carbon atoms can be lost from some polyketide chains by decarboxylation. Clearly a combination of the oxidation of a methyl group to a carboxyl group and subsequent decarboxylation would result in the loss of that methyl group.

Another oxidative process which is found in polyketide metabolism is that of phenolic oxidative coupling, either of an intramolecular nature - as in erdin[34] (33) - or of an intermolecular nature - as in the dimeric anthraquinone skyrin[35] (34). More drastic oxidations can occur producing an apparently unrelated and non polyketide derived skeleton. The formation of patulin (35) from 6-methylsalicylic acid is an example of such a process and the mechanism shown in figure 6 has been proposed for this transformation[36].

Polyketides are frequently encountered which possess extra methyl groups i.e. ones which do not arise from a polyketide chain. These groups are transferred to the natural product from the tertiary sulphonium salt methionine by an S_N2 mechanism (figure 7) - it has been shown[37], using CD_3-methionine, that in the biosynthesis of sclerotiorin (36) the methyl groups are incorporated with retention of all three hydrogen atoms. The



(40)



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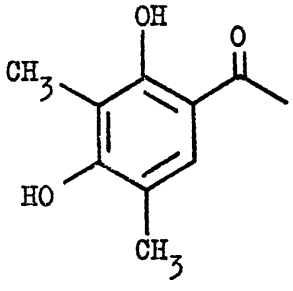

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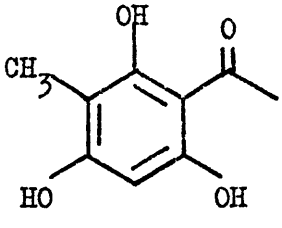

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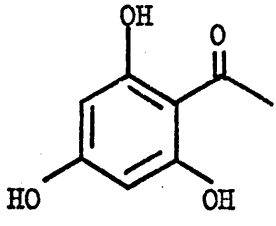

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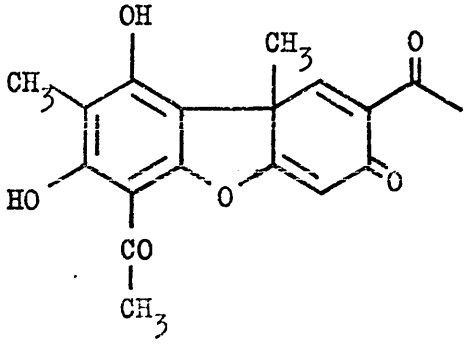

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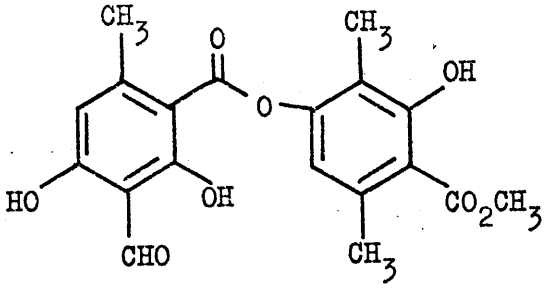
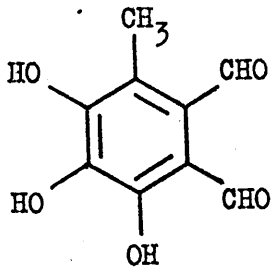


(46)



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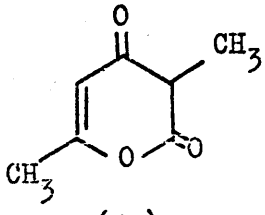


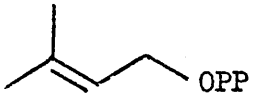
(48)

nucleophile involved in methylation may be an oxygen atom, as in the anthraquinone physcion[38] (37), or a carbon atom - almost invariably the carbon atom of one of the methylene groups of a polyketide chain - as in citrinin[39] (38). The only exception to this rule is barnol[40] (39) where a methyl group has been introduced onto the terminal methyl group of a polyketide chain to form an ethyl group - perhaps via an intermediate quinone-methide.

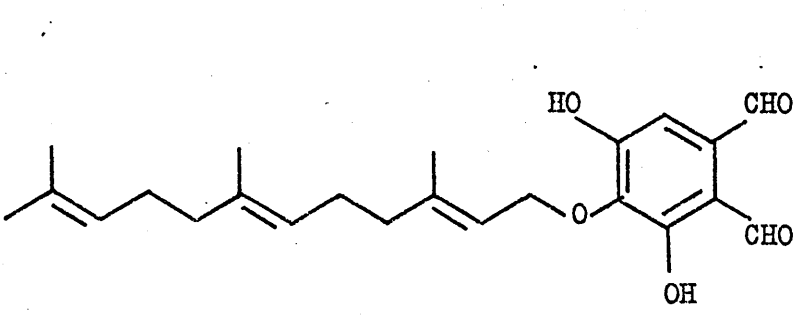
The fact that the ketones (40) and (41) are not incorporated into clavatol (42) has been taken[41] as evidence that in this case methylation occurs before aromatisation. Evidence from other sources, vis. the fact that the methylphloracetophenone (43) is incorporated[42] into usnic acid (44) whereas phloracetophenone (45) is not, and that 3-methylorsellinic acid but not orsellinic acid is incorporated into both atranorin[43] (46) and mycophenolic acid[44] (47), suggests that this may be generally true.

There is little evidence to determine whether methylation occurs on a growing or on a completed chain. A cell-free preparation from an Aspergillus species which produces flavipin (28) has been shown[45] to catalyse the methylation of what is thought to be a tetra-acetic acid suggesting that in this case methylation occurs onto a completed chain. In contrast, methyltriacetic acid lactone (48) has been isolated[46,47] from P. stipitatum - a tropolone producer - suggesting the occurrence of methylation onto a growing chain.

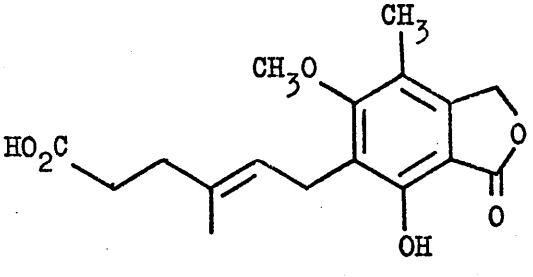
In flavoglaucin (31) a mevalonate derived C-5 unit has been incorporated into the heptaketide nucleus by an electrophilic substitution


OPP

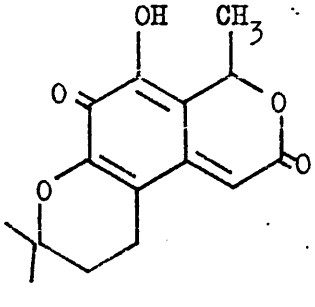
(49)


HO
CHO
CHO
O
OH

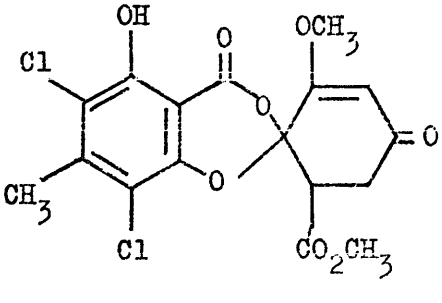
(50)


CH3
CH3O
HO2C
O
O
OH

(47)


OH
CH3
O
O
O
O

(51)


OH
O
OCH3
Cl
O
O
CH3
Cl
CO2CH3

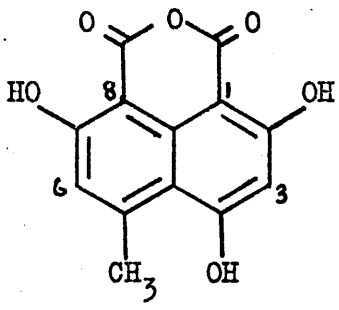
(52)

reaction. In this case the electrophile producing agent is dimethylallyl pyrophosphate (49) although it is possible that a more reactive enzyme-bound species is involved in the actual reaction. In a similar manner other prenyl units may be incorporated into polyketides, e.g. the farnesyl group seen in asperugin[48] (50), and further modifications can occur to them. In mycophenolic acid (47), for example, a farnesyl side-chain has been oxidised[49], while in fuscin[50] (51) cyclisation of a 3,3-dimethylallyl unit has occurred. Little is known about the timing of the incorporation of these units although in mycophenolic acid it occurs at a post-aromatic stage and it seems likely that this is generally the case.

A crystalline chloroperoxidase capable of carrying out biological halogenations has been isolated[51] from Caldariomyces fumago and a considerable number of polyketides, e.g. geodoxin[52] (52), contain chlorine atoms. It is believed that the incorporation of this moeity - which is generally not the last process to occur in the biosynthesis of a particular compound - involves a reaction between the electrophile Cl^+ and a nucleophilic site on the polyketide chain.

Although a considerable amount of effort has been expended in order to unravel the complexities of polyketide metabolism much remains to be done. It may be that modern instrumental techniques, such as ^{13}C n.m.r. spectroscopy, will provide the answers to many of the problems concerning the assembly of the basic polyketide skeletons but the isolation and identification of late-stage intermediates in the biosynthetic pathways will still require the traditional skills of the organic chemist.

CHAPTER 1.



(53)

Lamellicolic Anhydride.

In 1951 a white fungus, found growing on bracken collected from moorland north of Glasgow, was isolated and provisionally named Cephalosporium cretatum. Recently, however, it has been identified as a strain of Verticillium lamellicola[53]. It was noticed that extracts of its culture filtrate possessed antibacterial activity[54] and the initial objective of the present investigation was to ascertain whether or not the active constituent was produced in reasonable yield and if so to identify it.

The mould was grown on surface culture and the culture filtrate extracted with methylene chloride. This solvent was chosen since it produced an active solid extract. In contrast, extraction with ethyl acetate or methanol gave less active black tars. Monitoring of the growth of the mould by microbiological assay indicated that the activity reached a peak after 28 days and cropping off was carried out after that time.

Preliminary fractionation of the extract was achieved using column chromatography. In a number of the relatively polar fractions (eluted from the column by 5-25% ethyl acetate-chloroform mixtures) an enhancement of activity over that of the crude extract was observed and these fractions were combined to give the crude 'active fraction'.

The major component of this mixture, later given the trivial name lamellicolic anhydride and assigned structure (53), was isolated by fractional crystallisation as an inactive yellow solid ($C_{13}H_8O_6$, parent

Figure 8. Ultraviolet Spectrum of Lamellicolic Anhydride.

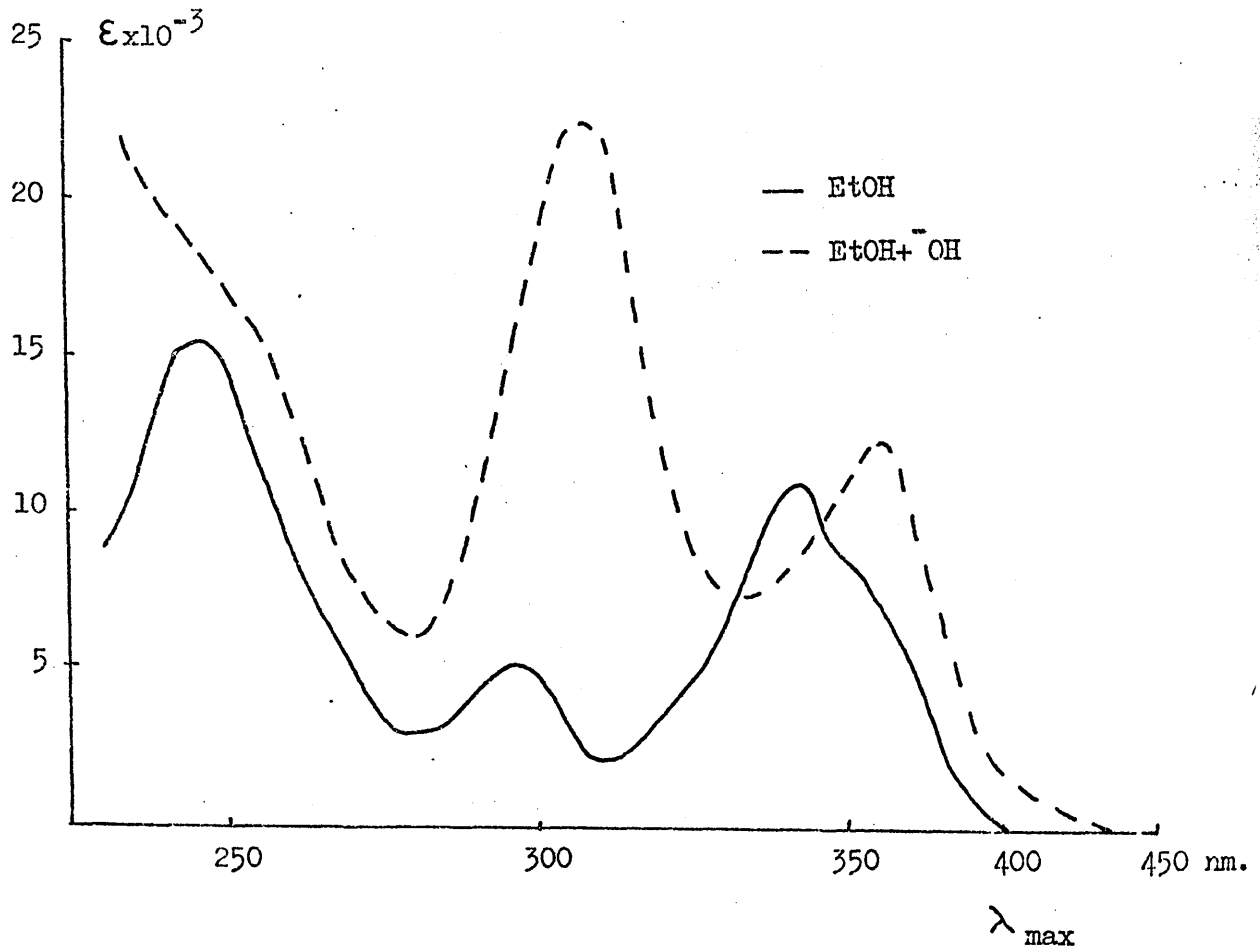


Table 1. N.M.R. Spectrum of Lamellicolic Anhydride.

a) in D_6-DMSO

3.20	1H	singlet
3.65	1H	singlet
7.26	3H	singlet

b) in D_6-acetone

3.25	1H	singlet
3.55	1H	singlet
7.20	3H	singlet

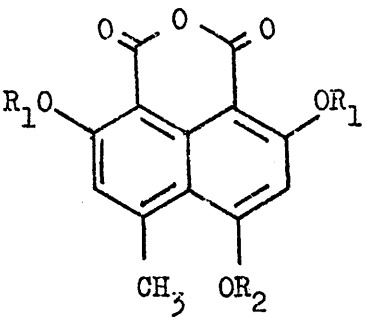
ion at m/e = 260) only sparingly soluble in chloroform but soluble in methanol. Its polarity on T.L.C. (Rf 0.35, 10% methanol-chloroform) and solubility in aqueous sodium hydrogen carbonate indicated an acidic or phenolic character and the brick red colour formed with methanolic ferric chloride solution supported the presence of one or more phenolic groups.

The ultraviolet spectrum of the compound (figure 8) underwent complex changes on basification - reversible on reacidification - including an apparent bathochromic shift of the long wavelength band. This suggested that if the compound were polyphenolic a resorcinol or catechol structure was more likely than a hydroquinone since the latter gives rise to hypsochromic shifts on basification[55]. In view of the complex nature of the spectrum, however, the appearance of an intense band at 314 nm. could be interpreted ambiguously and did not allow a hydroquinone structure to be entirely eliminated.

Two low field 1H singlets in the n.m.r. spectrum (table 1) were assigned to isolated aromatic protons while a 3H singlet at ca. 7.2τ was assigned to an aromatic methyl group. Sharpening of the signal at 3.2τ on irradiation at 7.2τ suggested that the aryl methyl group was situated ortho to an unsubstituted position.

The infra-red spectrum could not be immediately interpreted, strong absorption appearing in the carbonyl region (ν_{max} 1700, 1650 cm^{-1}) together with bands in the aromatic (C=C) stretching region (ν_{max} 1615, 1595 cm^{-1}).

The metabolite (53) failed to give any product in reasonable yield



(54) $R_1 = R_2 = CH_3$

(55) $R_1 = H$, $R_2 = CH_3$

(56) $R_1 = R_2 = CO.CH_3$

Table 2. N.M.R. Spectrum of the Trimethyl Ether (54).

a) in trifluoroacetic acid			b) in $CDCl_3$		
2.73	1H	singlet	3.12	1H	singlet
3.14	1H	singlet	3.51	1H	singlet
5.71	6H	singlet	5.87	6H	singlet
5.73	3H	singlet	5.90	3H	singlet
6.94	3H	singlet	7.14	3H	singlet

on reaction with diazomethane but treatment with dimethyl sulphate and potassium carbonate afforded a trimethyl ether (54, $C_{16}H_{14}O_6$) in which there were no unreacted hydroxyl groups (no absorption visible in the i.r. above 3100 cm^{-1}). The upward shift of around 50 cm^{-1} in the carbonyl absorption ($\nu_{C=O}$ 1750, 1715 cm^{-1}) suggested that hydrogen bonding, between the carbonyl system and one or more phenolic groups, within the molecule (53) had been removed by methylation.

Resonances ca. 0.3 p.p.m. lower than normal were observed for all 3 methoxyl groups (table 2). In the case of two of them this was reasonably explained by their location peri to carbonyl groups and in keeping with this they underwent cleavage on reaction of (54) with magnesium iodide-etherate[56] to give a monomethyl ether (55, $C_{14}H_{10}O_6$, 3H singlet at 5.8τ) which showed carbonyl absorption ($\nu_{C=O}$ 1710, 1670 cm^{-1}) at approximately the same frequencies as the natural product. The fact that this compound was less polar (Rf 0.48, 2% methanol-chloroform) on T.L.C. than the trimethyl ether (Rf 0.27, 5% methanol-chloroform) was in accord with the masking of the full polarity of the phenolic groups and the carbonyl groups by hydrogen bonding.

With acetic anhydride and pyridine the natural product (53) formed a triacetate (56, $C_{19}H_{14}O_9$, parent ion at m/e = 386 and ions corresponding to three successive losses of 42 mass units from the parent, 9H singlet at 7.51τ). As in the case of the trimethyl ether, the removal of hydrogen bonding was reflected in higher carbonyl frequencies, in this case 1750 and ca. 1775 cm^{-1}, although the upper band was partly obscured by the

Table 3. N.M.R. Spectrum of the Triacetate (56).

a) in $CDCl_3$

7.18	3H	singlet
7.51	9H	singlet

(A signal at 2.75τ was partly obscured by the $CHCl_3$ signal.)

b) in D_6-DMSO

2.38	1H	singlet
2.42	1H	singlet
7.16	3H	singlet

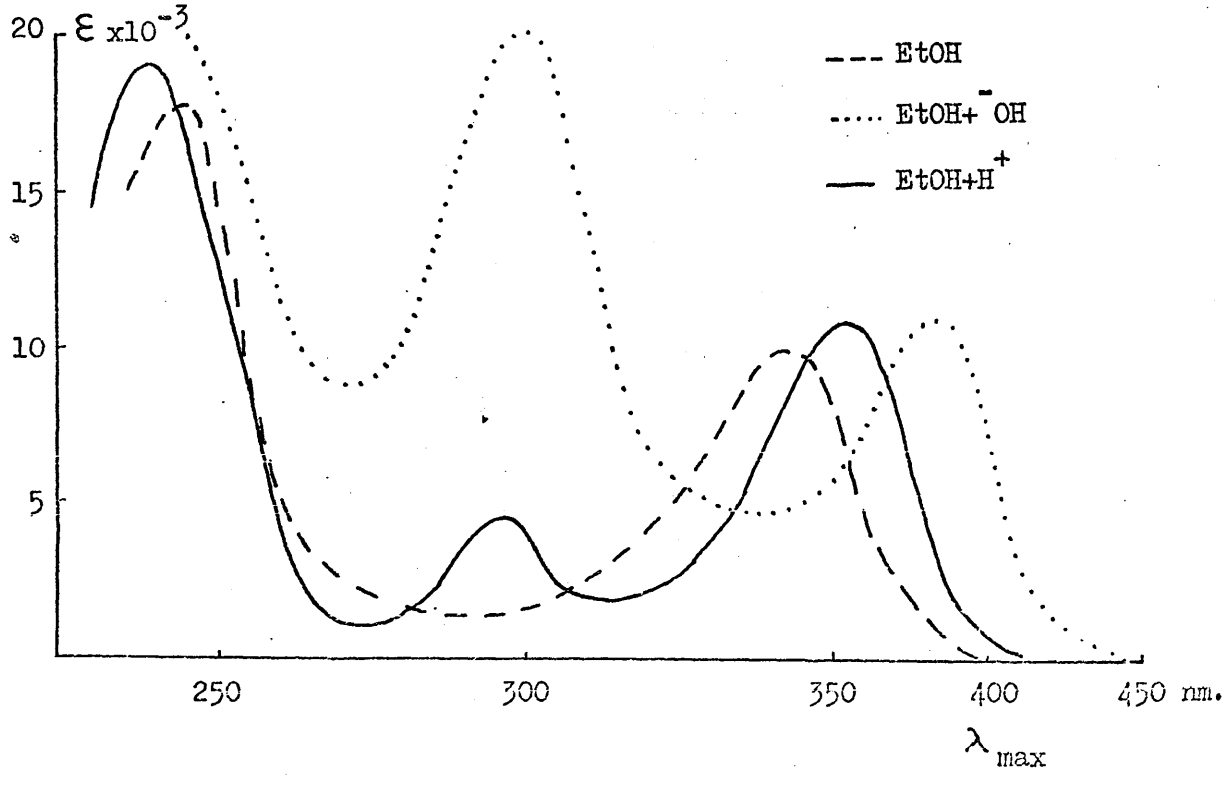
(A signal at 7.6τ was partly obscured by the DMSO signal.)

Table 4. Ultraviolet Spectra of (53) and (56).

(53): λ_{max}(EtOH): 250(15000), 292(5700), 352(10000), 368(7800) nm.

(56): λ_{max}(EtOH): 248(17000), 341(6000) nm.

Figure 9. Ultraviolet Spectrum of the Triacetate (56).

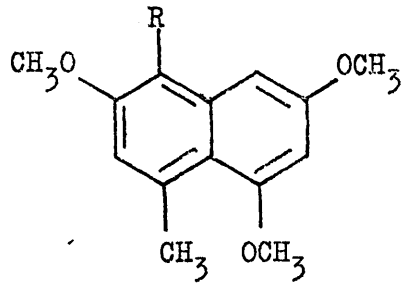


carbonyl absorption of the acetate group (ν_{max} 1780 cm^{-1}).

The chemical shifts (ca. 3.2 and 3.5τ) of the aromatic protons in the metabolite (53) were consistent with their being situated ortho to one and two hydroxyl groups respectively. On acetylation the shielding effect of these substituents was removed resulting in a downfield shift in the resonances of the aromatic protons. The values observed (table 3), however, for these protons in the triacetate were consistent with their being deshielded by another group in the molecule.

The ultraviolet spectrum of the triacetate reflected the lowering of the electron density of the aromatic ring system, by the acetate groups, compared with (53) (table 4) - the bands being shifted to lower wavelength. On basification and reacidification, however, the spectrum obtained was identical with that of (53) (figure 9) suggesting that hydrolysis of the ester groups had occurred. The instability exhibited by the triacetate during chromatography on silica supported the possibility of facile deacetylation.

Treatment of the metabolite (53) with sodium hydroxide, under nitrogen, and subsequent reaction of the products with diazomethane gave a trimethoxy methyl ester (57, $C_{16}H_{18}O_5$, parent ion at m/e = 290, $\nu_{C=O}$ 1720 cm^{-1}, 3H singlets at 5.96 and 6.05τ and a 6H singlet at 6.10τ). Two doublets in the n.m.r. at 3.40 and 3.62τ (J=2Hz) suggested the presence of a pair of aromatic protons situated meta to each other. The detection of long range coupling between the protons appearing as singlets at 7.14 and 3.18τ (3H and 1H respectively) indicated that the



(57) R = CO_2CH_3

(58) R = H

Figure 10. Ultraviolet Spectra of Substituted Naphthalenes.

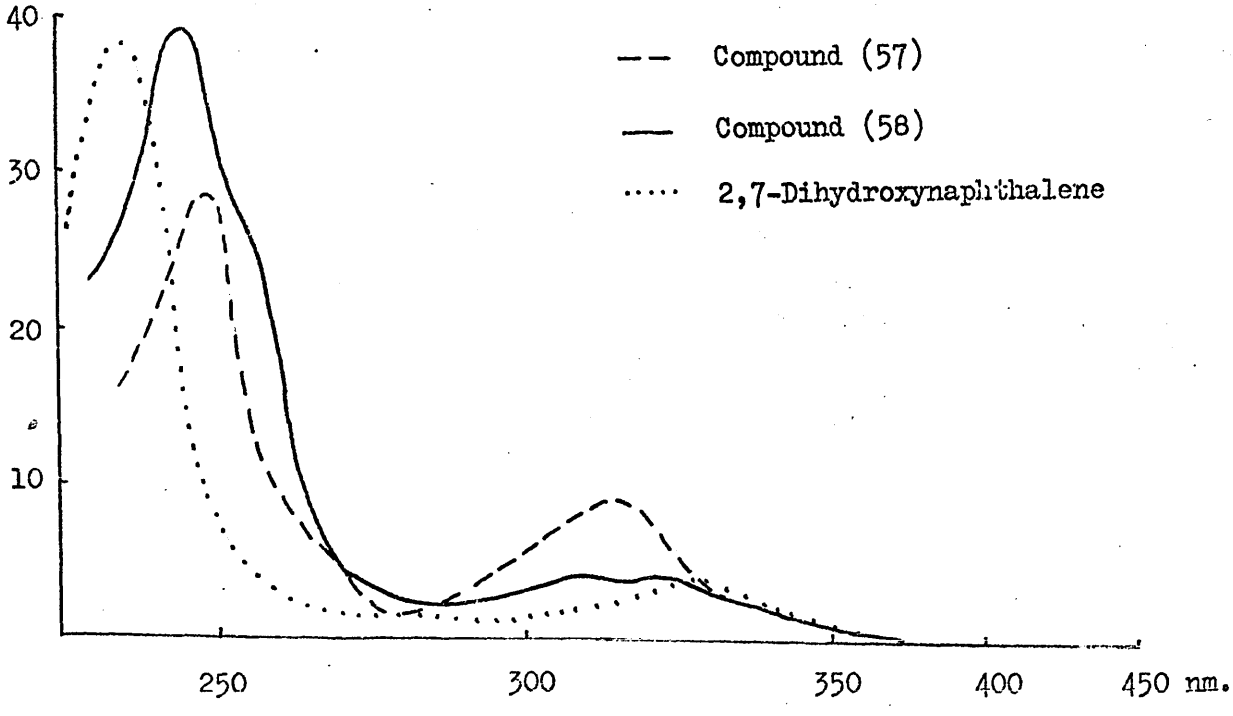


Table 5. Carbonyl Absorption of 1,8-Naphthalic Anhydrides.

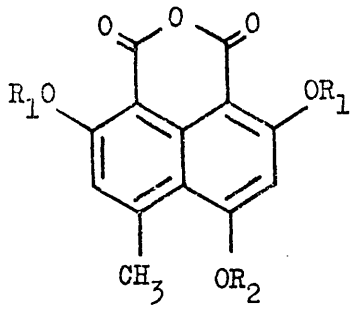
Compound.	$\nu_{C=O}$(KBr or nujol) (cm^{-1})
1,8-Naphthalic anhydride	1765, 1735
2,7-Dihydroxy-1,8-naphthalic anhydride	1720, 1685
Lamellicolic anhydride	1700, 1650
2,7-Dimethoxy-1,8-naphthalic anhydride	1750, 1720
Trimethyl ether (54)	1750, 1715
2,7-Diacetoxy-1,8-naphthalic anhydride	1760, 1720
Triacetate (56)	1775, 1730

position meta to the proton on the ring bearing the methyl group was still substituted.

A minor product of the reaction was a trimethoxy compound (58, $C_{14}H_{16}O_3$, parent ion at m/e = 232, 3H singlet at 6.10τ and 6H singlet at 6.20τ) which showed no carbonyl absorption in the ir. Two pairs of doublets (1H doublets, J=3Hz, at 3.10, 3.24, 3.31 and 3.61τ) were visible in the n.m.r. and double irradiation revealed that the position para to the aryl methyl group was now unsubstituted. The fact that both of these compounds showed uv. spectra characteristic of substituted naphthalene derivatives[55] (figure 10) suggested that the naphthalene nucleus was present in the metabolite (53).

The most likely explanation for the loss of the carbonyl system of the natural product under basic conditions was that a stepwise double decarboxylation had occurred. This was consistent with the presence in the metabolite of a potential o,o'-dihydroxydicarboxylic acid system[57,58]. The latter could be derived from a 1,8-naphthalic anhydride upon basic hydrolysis. Now 1,8-naphthalic anhydrides have strong and characteristic carbonyl absorption[59] and such a system with two peri phenolic groups would give rise to the carbonyl absorption[60] observed for the metabolite (53) and its derivatives (table 5). The metabolite could not have contained a 1,2 or a 2,3-naphthalic anhydride system since on decarboxylation these would have given rise to a pair of aromatic protons ortho to each other.

It was therefore concluded that the metabolite was a trihydroxy

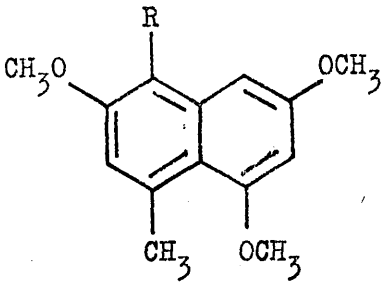


(53) $R_1 = R_2 = H$

(54) $R_1 = R_2 = CH_3$

(55) $R_1 = H$, $R_2 = CH_3$

(56) $R_1 = R_2 = CO.CH_3$

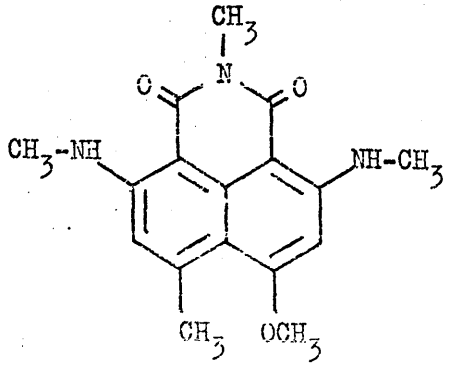


(57) $R = CO_2CH_3$

(58) $R = H$

Table 6. Nuclear Overhauser Effects Observed for the Trimethyl Ether (54)

Point of irradiation (τ)	Signal observed (τ)	% Enhancement
6.94	2.73	21
6.94	3.14	0
5.73	2.73	11
5.73	3.14	37

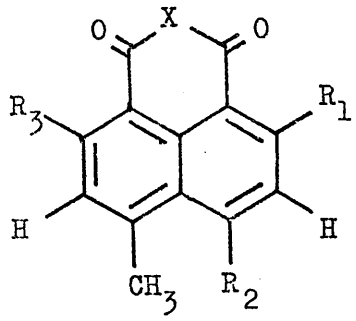


(59)

methyl-1,8-naphthalic anhydride in which two of the hydroxyl groups were peri to carbonyl groups, the methyl group was para to one carbonyl group and two aromatic protons - one of which was ortho to the methyl group - were each meta to a carbonyl group. These requirements can only be satisfied by assigning structure (53) to the metabolite. Structures (54), (55) and (56) follow for the trimethyl ether, the monomethyl ether and the triacetate respectively while (57) and (58) can be given to the products of base degredation. Such a formulation was also supported by the Nuclear Overhauser Effects (table 6) observed for the trimethyl ether.

Chemical confirmation of the presence of the anhydride system was obtained by reaction of (54) with aqueous methylamine[61] to give two yellow, crystalline products in good yield. The major product was the diaminoimide (59, parent ion at m/e = 313, $\nu_{C=O}$ 1635, 1605 cm^{-1}). Three N-methyl resonances were evident in the n.m.r., (3H singlet at 6.5τ and 6H doublet at 6.95τ), the singlet being assigned to an imido methyl group and the doublet to the methyl protons of a pair of aryl methylamino groups. Double irradiation confirmed the presence of coupling (J=4Hz) between the methyl groups and the N-H protons (2H broad multiplet at -1.5τ). One O-methyl resonance was also evident (3H singlet at 6.05τ). The amino functions were assigned to positions 2 and 7 on the basis of the low field N-H resonance and of the low imide carbonyl frequency (cf. 1,8-naphthalimide $\nu_{C=O}$ 1681 cm^{-1}) due to the presence of hydrogen bonding.

The minor product (parent ion at m/e = 314) was also an imide



(60) $R_1 = NH\text{-}CH_3$, $R_2 = R_3 = CH_3$

(61) $R_1 = R_2 = CH_3$, $R_3 = NH\text{-}CH_3$

Table 7. Chemical Shifts Observed for H-3 and H-6.

Compound	X	R_1	R_2	R_3	H-3 (τ)	H-6 (τ)	Solvent
(53)	O	OH	OH	OH	3.55	3.25	D_6-acetone
(54)	O	OCH_3	OCH_3	OCH_3	3.51	3.12	$CDCl_3$
(59)	$N\text{-}CH_3$	$NH.CH_3$	OCH_3	$NH.CH_3$	4.15	3.61	$CDCl_3$

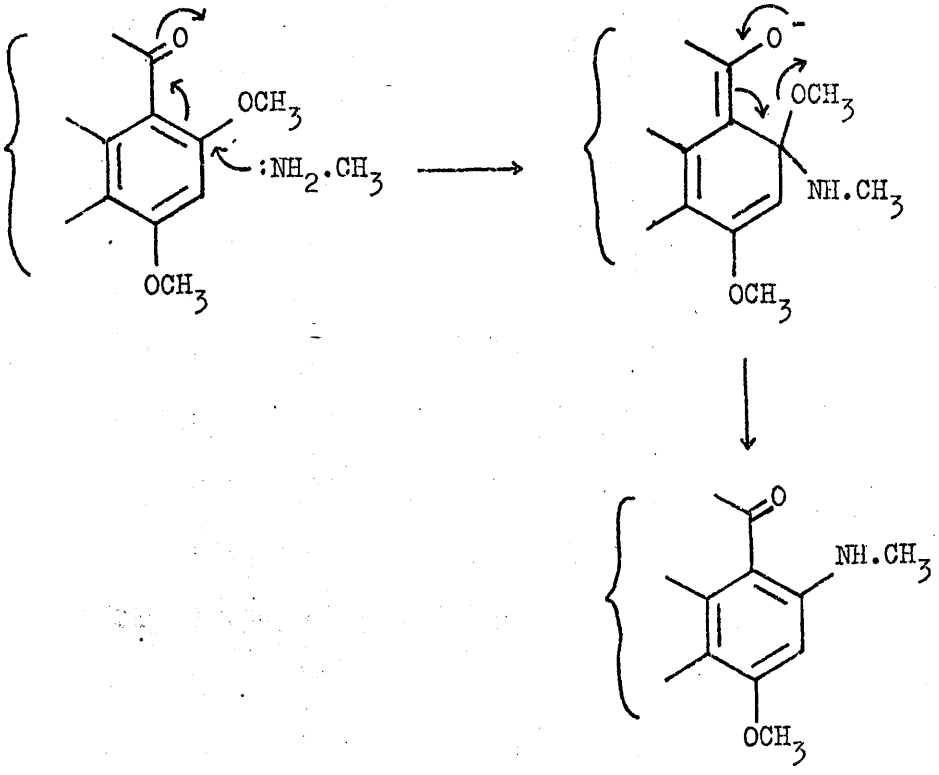
Table 8. Chemical Shifts Predicted for H-3 and H-6.

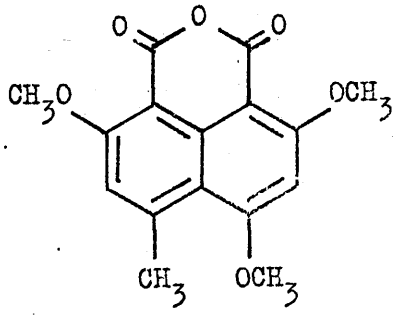
Compound	X	R_1	R_2	R_3	H-3 (τ)	H-6 (τ)
(60)	$N\text{-}CH_3$	$NH.CH_3$	OCH_3	OCH_3	4.15	3.12
(61)	$N\text{-}CH_3$	OCH_3	OCH_3	$NH.CH_3$	3.51	3.61

Table 9. Chemical Shifts Observed for H-3 and H-6.

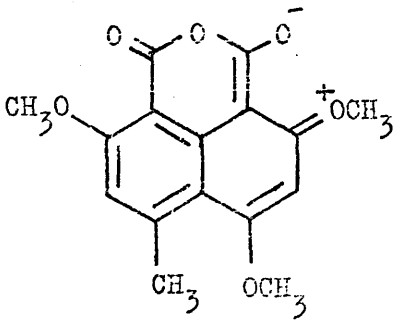
Compound	H-3 (τ)	H-6 (τ)	Solvent
Monoaminoimide	3.92	3.25	$CDCl_3$

Figure 11. Mechanism of Nucleophilic Substitution on (54).

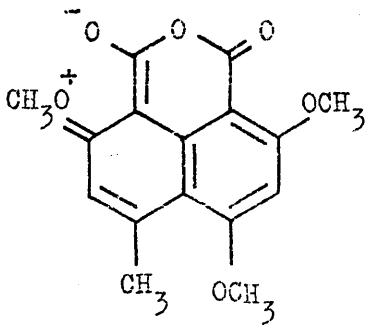




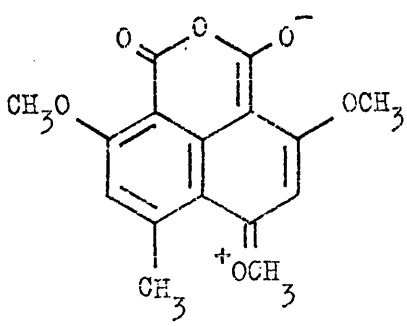
(54)



(63)



(62)



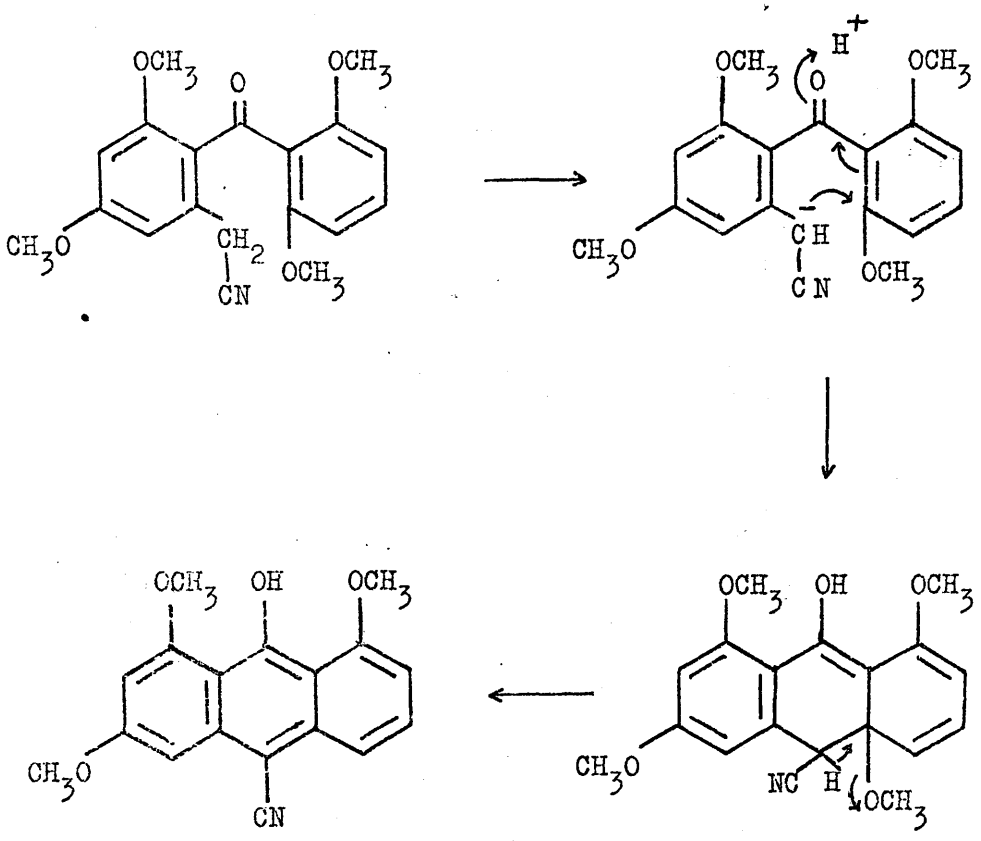
(64)

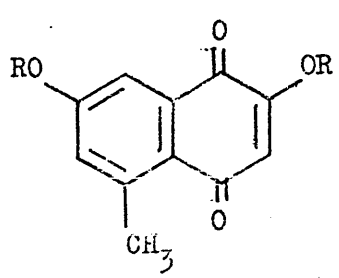
(3H singlet 6.52τ) but contained one methylamino group (3H doublet at 6.95τ) and two methoxyl groups (3H singlets at 5.92 and 6.05τ). That the methylamino group was peri to a carbonyl group was evident from the low field N-H resonance (-0.3τ) and from the lowering of the imide carbonyl frequency, relative to 1,8-naphthalimide, by hydrogen bonding ($\nu_{C=O}$ 1660, 1620 cm^{-1}) albeit to a lesser extent than in (59).

The chemical shifts observed for H-3 and H-6 in the derivatives described above (table 7) show the larger shielding effect of a methylamino group compared to a methoxyl group and it was possible to predict the approximate shifts expected for these protons in the two possible structures for the monoaminoimide (60) and (61) (table 8). Comparison with the values observed (table 9) led to structure (60) being preferred for this compound.

This unusually facile aromatic nucleophilic substitution reaction is presumably facilitated by the nearby carbonyl system and proceeds by the mechanism shown opposite (figure 11). The fact that nucleophilic substitution occurs on the ring which possesses the higher electron density is somewhat surprising. This result, however, may be rationalised by cosidering that the resonance forms (62), (63) and (64) contribute to the structure of the trimethyl ether (54). Therefore the ring bearing one methoxyl group will be likely to possess the greater degree of aromatic character. Now the initial, and presumably slower, step in the substitution reaction involves a loss of aromaticity in the ring on which substitution is occuring. Thus one would expect a more rapid reaction on

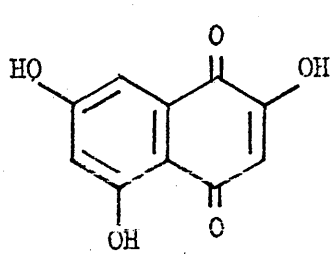
Figure 12. Elimination of Methanol From a Benzophenone.





(65) R = H

(67) R = CH_3

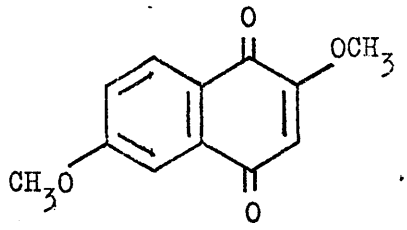


(66)

$\nu_{C=O}$(KBr): 1675, 1630 cm^{-1}

the ring possessing the lower degree of aromatic character i.e. on the ring bearing two methoxyl groups. It seems likely that steric effects will favour attack of the nucleophile at position 2 over attack at position 4. A comparable displacement of a methoxyl group by a nucleophile has been observed in the case of suitably functionalised benzophenones[62] (figure 12).

Further evidence as to the structure of lamellicolic anhydride was obtained from the isolation of a related metabolite from the fungal extracts. Chromatography of the material eluted from the column by 40% ethyl acetate-chloroform, i.e. more polar than the active fraction, afforded a pure sample of a brown-red, inactive, crystalline solid (65, $C_{11}H_8O_4$, parent ion at m/e = 204). The n.m.r. spectrum in D_4-methanol showed the presence of an aryl methyl group (3H singlet at 7.40τ) with unsubstituted positions ortho and para to it (1H doublets, J=2Hz, at 2.65 and 3.10τ). The solubility of the compound in aqueous sodium hydrogen carbonate and a strong carbonyl absorption ($\nu_{C=O}$ 1660 and 1620 cm^{-1}) was consistent with the presence of a 2-hydroxy-1,4-naphthaquinone system[38] (cf. flaviolin[63] (66)). Structure (65) which was suggested by this evidence was confirmed by the formation, on reaction with dimethyl sulphate and potassium carbonate, of a yellow dimethyl ether (67, parent ion at m/e = 232, 3H singlets at 6.05 and 6.10τ). Apart from signals (1H doublets at 2.42 and 3.00τ, J=3Hz) attributable to the protons para and ortho to the aryl methyl group a 1H singlet was seen at 3.92τ. This signal showed substantial enhancement on irradiation at the methoxyl signal and was assigned to H-3. This proton was not observed in the

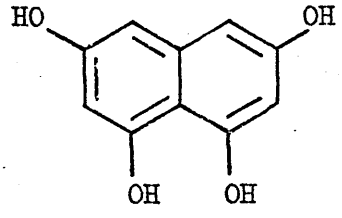


(68)

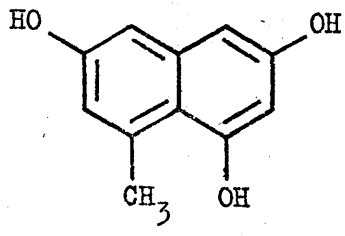
Table 8. Ultraviolet Spectra of (67) and (68)

(67) λ_{max}(EtOH): 263(18000), 291(10000), 343(1700), 384(1300) nm.

(68) λ_{max}(EtOH): 266(16000), 292(15000), 378(3000) nm.



(69)



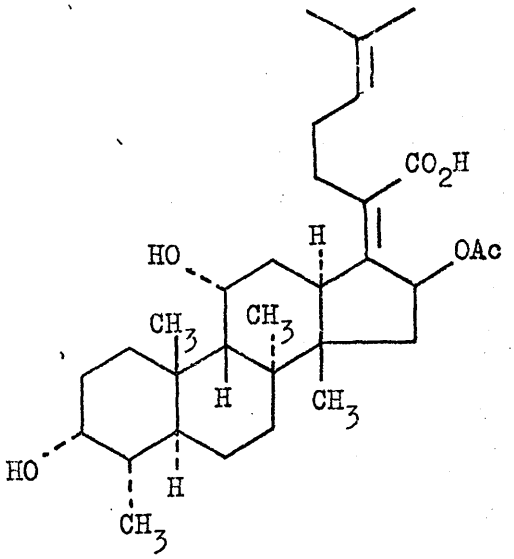
(70)

spectrum of the parent quinone since with D_4-methanol as solvent it would have been exchangeable with deuterium. The compound possessed a uv. spectrum very similar to that of the naphthaquinone[64] (68) (table 8).

The oxidation of the naphthalene (69) to flaviolin, under mildly basic conditions, has been reported[65] and it seemed possible that the quinone (65) might be prepared from lamellicolic anhydride by a similar oxidation after hydrolysis and decarboxylation. Attempts to effect this transformation in one step by reacting (53) with base in air were unsuccessful and produced only a mixture of very polar gums containing none of the desired product. When, however, the triphenol (70), formed by the reaction of lamellicolic anhydride with sodium hydroxide under nitrogen, was stirred in air with alkaline methanol the naphthaquinone was obtained in good yield.

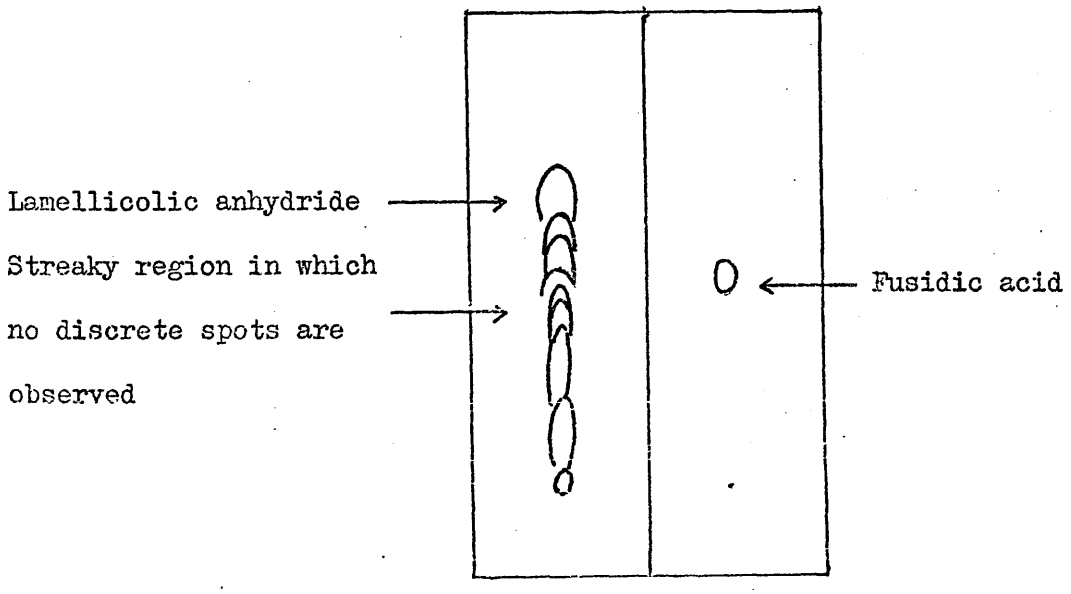
Whilst it is possible that the quinone is formed to some extent as an oxidative artefact during the work up proceedure it is also likely that it is formed during the fermentation process since it is detected by T.L.C. in fresh extracts, although whether or not an enzymic reaction is involved is not known.

All attempts to isolate the active constituent from the gummy residue of the active fraction after the removal of lamellicolic anhydride were unsuccessful and it was concluded that it was present, in surface culture extracts at least, in very small quantities. The antibiotic fusidic acid[66] (71) has been reported[67] as a metabolite of strains of V. lamellicola and T.L.C. evidence showed that it had a similar polarity

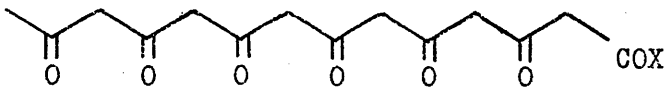


(71)

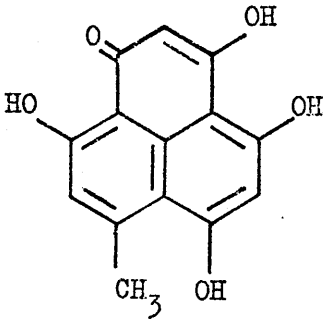
Figure 13. T.L.C. of the Active Fraction Residue and of a Pure Sample of Fusidic Acid.



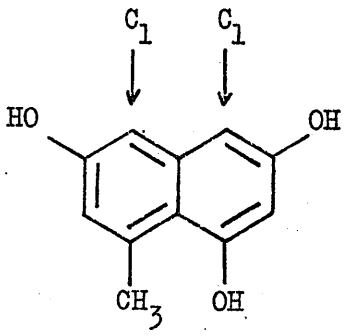
Eluting solvent: 5% methanol-chloroform



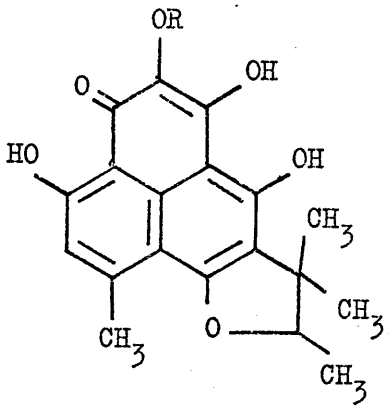
(72)



(73)

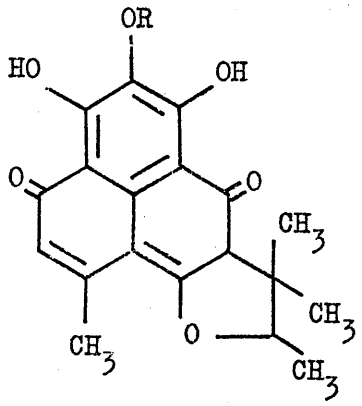


(74)



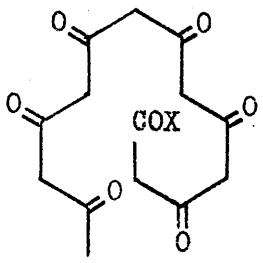
(75) R = H

(78) R = CH_3

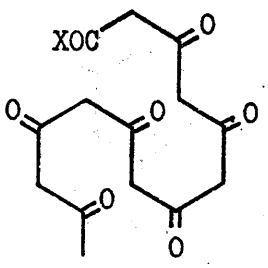


(76) R = CH_3

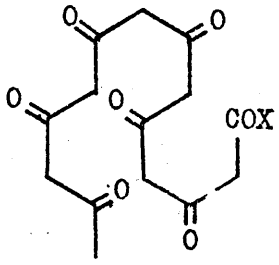
(77) R = H



(79)



(80)



(81)

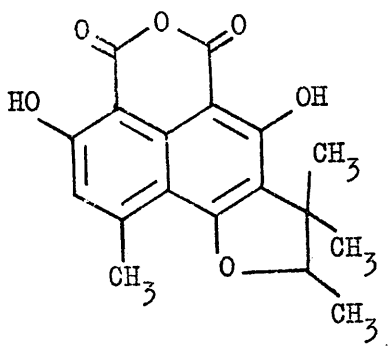
(figure 13) to the unknown active compound. No other evidence, however, has been obtained to support the view that they are one and the same thing.

Interest therefore shifted to a study of lamellicolic anhydride and other phenolic metabolites of the mould and since it was observed that they were produced in reasonable quantity after about 10 days growth the fungus was subsequently cropped off after that time.

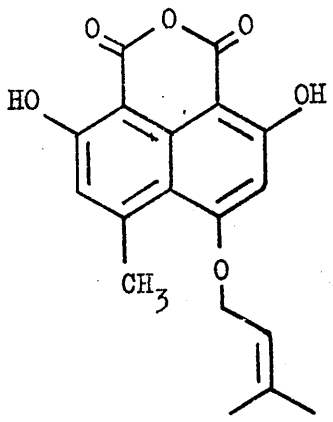
The orientation of the substituents around the aromatic nucleus suggests that lamellicolic anhydride is a polyketide, i.e. it is derived by the condensation of acetate and malonate units. The cyclisation of a protein-bound heptaketide chain (72) would lead to a phenalenone intermediate (73) which on oxidation, would give the anhydride (53). A less likely possibility is the intermediacy of a hexaketide derived species (74) followed by incorporation and oxidation of 2 C_1 units (see below).

A number of phenalenone heptaketide metabolites have been isolated from the moulds Penicillium atrovenetum and Penicillium herquei. The pigments atrovenetin (75), a metabolite of both species[68,69], and herqueinone[69] (76), norherqueinone[69] (77) and deoxyherqueinone[70] (78), produced only by the latter, arise by the condensation of the same polyketide derived phenalenone mentioned above with a mevalonate derived C_5 unit[71]. The three possible modes of cyclisation of the β-polyketone chain (79), (80) and (81) are indistinguishable by labelling experiments.

Also isolated from P. herquei has been the one other reported example

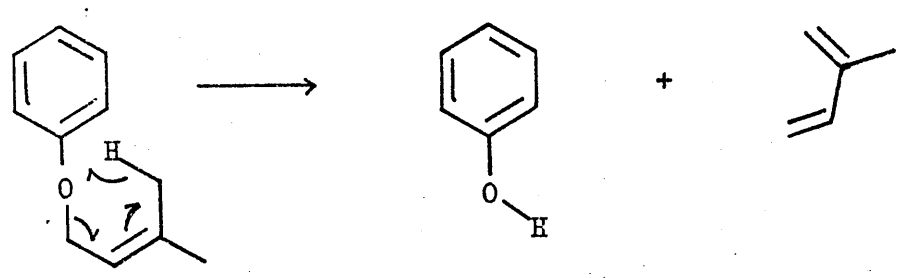


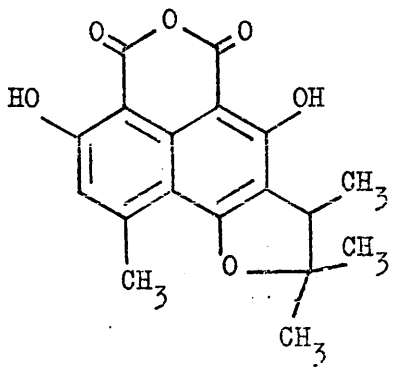
(82)



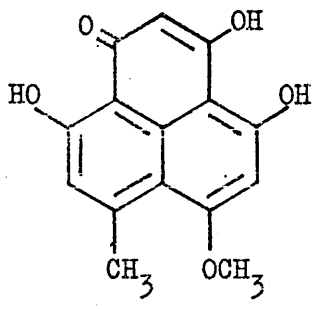
(83)

Figure 14. Mass Spectral Fragmentation of an O-3,3-Dimethylallyl Group.





(84)

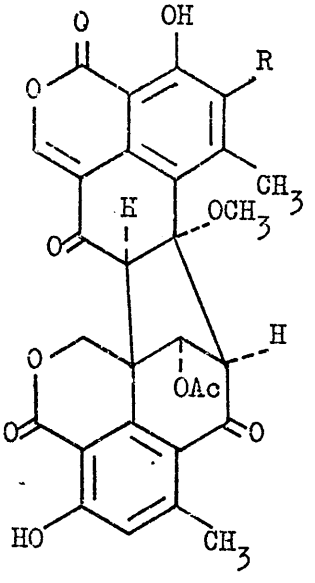


(85)

of a fungal 1,8-naphthalic anhydride[72] (82), and its preparation from lamellicolic anhydride provided further confirmation of the structure assigned to the latter. Treatment of (53) with 3,3-dimethylallyl bromide and potassium carbonate gave the 4-O-3,3-dimethylallyl ether (83), the n.m.r. spectrum of which showed signals attributable to an O-3,3-dimethylallyl group (1H triplet, J=6Hz, at 4.50τ, 2H doublet, J=6Hz, at 5.30τ and 3H singlets at 8.16 and 8.20τ). The mass spectrum showed a strong M^+-68 peak at m/e = 260 reflecting the characteristic fragmentation[73] of an O-3,3-dimethylallyl group (figure 14).

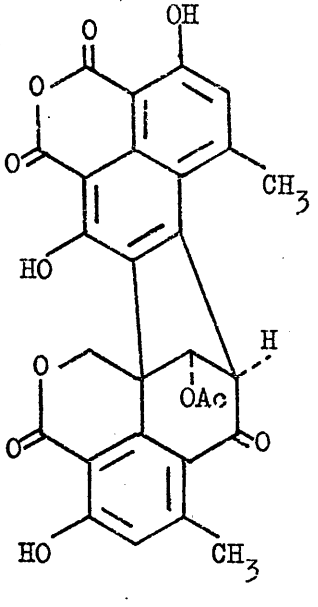
Pyrolysis of (83) in dry dimethylformamide at 120°C gave material identical to an authentic sample of the anhydride (82), prepared from herqueinone (76) by reduction to deoxyherqueinone (78) with zinc dust and subsequent oxidation with chromium trioxide[60,74]. In contrast, pyrolysis of the dimethylallyl ether (83) in a sublimation block at 160°C[75] gave material which although homogeneous by T.L.C. was seen by n.m.r. to be a mixture of (82) and the product of abnormal Claisen rearrangement[76] (84). The relative intensities of the signals attributed to the proton geminal to oxygen (quartet at 5.25τ) and to the benzilic proton (quartet at 6.62τ) revealed that (82) and (84) were present in the ratio of 1:2.

During the course of a projected synthesis of atrovenetin by a Nottingham group the phenalenone (85) was prepared[77]. Oxidation with potassium permanganate at 0°C gave the methoxy anhydride[78] (55) and subsequent demethylation with pyridine hydrochloride yielded the

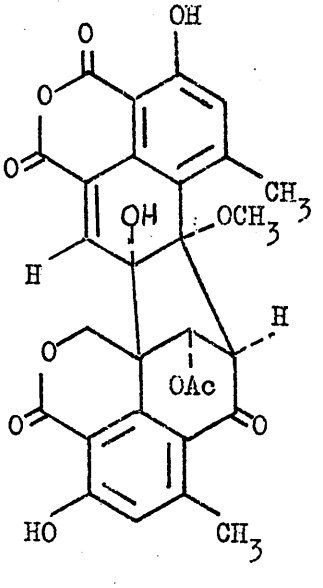


(86) R = H

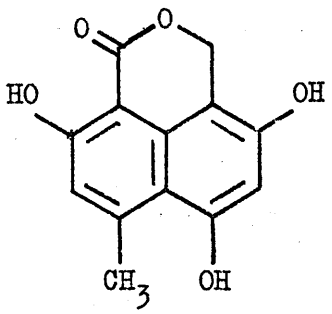
(87) R = Br



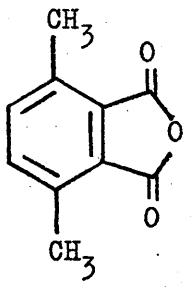
(88)



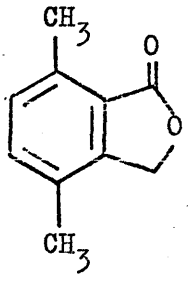
(89)



(90)



(91)



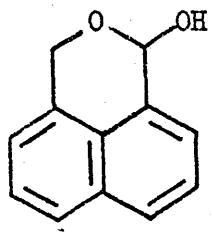
(92)

anhydride (53). Samples of these compounds supplied by Dr. B.W. Bycroft were found to be identical to 4-O-methyllamellicolic anhydride and lamellicolic anhydride respectively, confirming the structure assignments made previously.

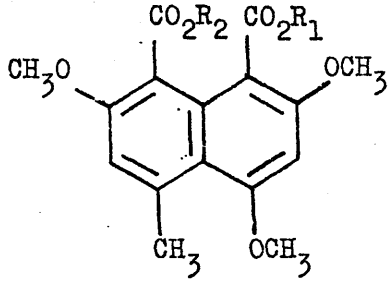
The dimeric compound duclauxin (86) has been obtained from Penicillium duclauxi[79] and its structure and absolute stereochemistry revealed by X-ray analysis of a monobromo derivative[80] (87). The involvement of the phenalenone (73) in the biosynthesis of duclauxin has been suggested by the results of radiotracer feeding experiments[81] which were consistent with a pathway involving the dimerisation by oxidative coupling and Aldol condensation of two heptaketide fragments. Nothing, however, is known about the level of oxidation of the monomers when dimerisation occurs. The closely related compounds xenoclauxin (88) and cryptoclauxin (89) have been isolated from the same source but no monomeric intermediates have been reported.

The isolation of lamellicolic anhydride has provided an opportunity to study the mechanism of the dimerisation reactions leading to the P. duclauxi metabolites by synthesising suitable monomers which can be used both for in vitro studies and also, in a labelled form, for radiotracer experiments when fed to the mould. The lactone (90) is likely to be involved in the biosynthesis of all three dimers and it was taken as an initial synthetic objective.

The reduction of the anhydride (91) to a lactone (92) with lithium aluminium hydride, has been reported[83]. Reaction, however, of O,O,O-tri-



(93)



(94) $R_1 = CH_3$, $R_2 = CH_2CH_3$

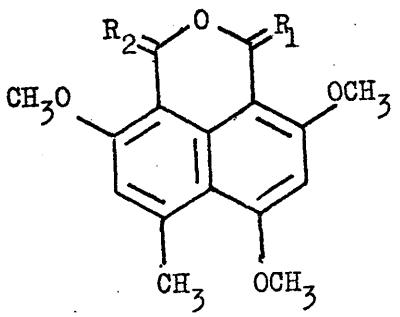
(95) $R_1 = CH_2CH_3$, $R_2 = CH_3$

(96) $R_1 = H$, $R_2 = CH_2CH_3$

(97) $R_1 = CH_2CH_3$, $R_2 = H$

methyllamellicolic anhydride with this reagent, in either diethyl ether or tetrahydrofuran, gave only mixtures of unstable non polar compounds whose ir. spectra showed no bands attributable to a lactone carbonyl absorption, suggesting that further reduction of the desired compounds had occurred. Reactions for a shorter time or at a lower temperature produced the same result.

Sodium borohydride has been used to prepare lactones from anhydrides[84]. When, however, the trimethyl ether (54) was treated with this reagent in tetrahydrofuran or dimethylformamide no products were obtained. Since it was observed that 1,8-naphthalic anhydride reacted with sodium borohydride in ethanol to give a good yield of the hemiacetal (93, parent ion at m/e = 186, ν_{OH} 3570 cm^{-1}) (54) was treated under these conditions. T.L.C. indicated that a single product, more polar than the starting material had been formed. It, however, was easily reconverted, during the work up, to starting material and the formation of a diester (94, parent ion at m/e = 362, $\nu_{C=O}$ 1720 cm^{-1}) the n.m.r. of which revealed the presence of both carboxymethyl (3H singlet at 6.15τ) and carboxyethyl groups (2H quartet at 5.70τ and 3H triplet at 8.65τ) on reaction with diazomethane suggested that the initial product was the acid ester (96). Since the trimethyl ether (54) was stable to solvolysis in ethanol alone, the reaction was attributed to the slow decomposition of the borohydride with the production of ethoxide ions. This was supported by the rapid formation of the acid ester (96) on reaction of (54) with sodium ethoxide in ethanol. Structure (96) was preferred to the alternative (97) on the



(98) $R_1 = H_2$, $R_2 = O$ or $R_1 = O$, $R_2 = H_2$

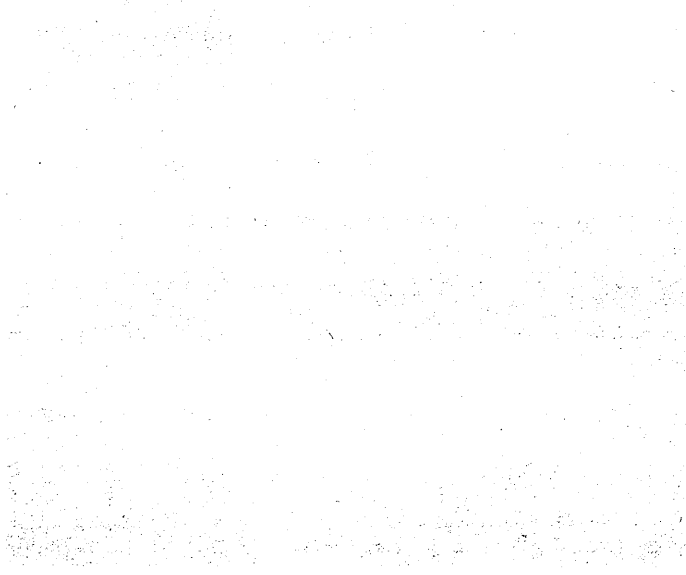
(99) $R_1 = O$, $R_2 = H_2$ or $R_1 = H_2$, $R_2 = O$

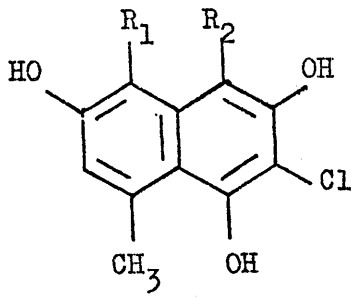
grounds that nucleophilic attack by ethoxide ought to occur on the more electrophilic carbonyl centre.

An attempt was made to exploit this unexpected solvolysis reaction by treating the acid ester (96) with lithium borohydride[85] - a reagent reputed to reduce esters but not acids. The only product, however, formed in reasonable yield was the trimethyl ether (54).

In view of the failure to achieve the desired transformation with hydride reducing agents attempts were made to effect the reduction by catalytic means. Hydrogenation of lamellicolic anhydride and of its trimethyl ether, in ethyl acetate, over 10% palladium-charcoal, however, failed to give any products. The use of Adams catalyst, in the same solvent, produced the same result when applied to the trimethyl ether. A major difficulty appeared to be the extreme insolubility of the substrates in most organic solvents. Trifluoroacetic acid has been used as a solvent for hydrogenation reactions[86] and since the ether (54) dissolved freely in it reaction in this solvent was attempted. With Adams catalyst a mixture of the isomeric lactones A and B (98) and (99) was obtained in low yield. Both products showed typical lactone carbonyl absorption ($\nu_{C=O}$ 1690 and 1700 cm^{-1} respectively) and possessed n.m.r. spectra consistent with the structure assigned in which a signal attributable to the benzilic methylene group was evident (2H singlet at 4.55τ). Although these compounds were found to be rather unstable and have been obtained, as yet, in only low yields it may be possible to convert them into the phenolic lactones required for the *in vitro* and *in vivo* conversion to dimeric compounds.

CHAPTER 2.

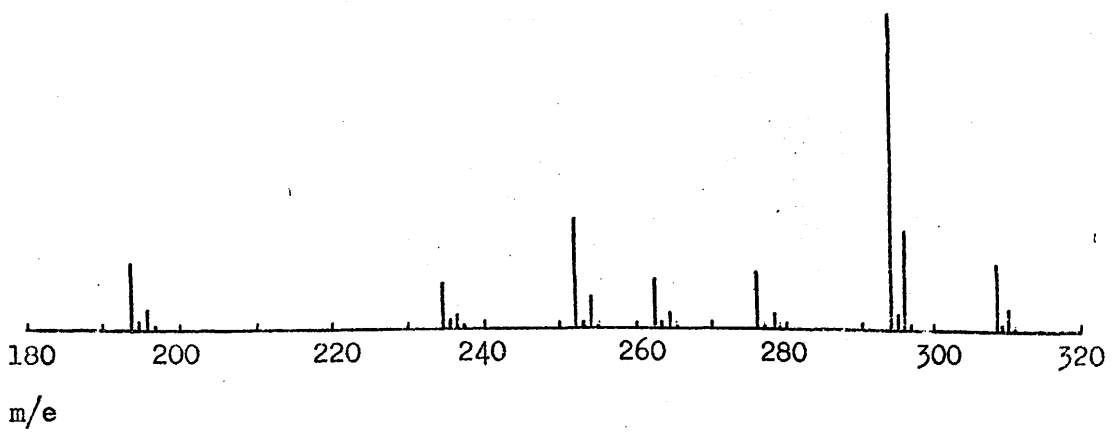




(100a) $R_1 = CO_2CH_3$, $R_2 = CO_2H$

(100b) $R_1 = CO_2H$, $R_2 = CO_2CH_3$

Figure 16. Mass Spectrum of the Chlorine-containing Metabolite.



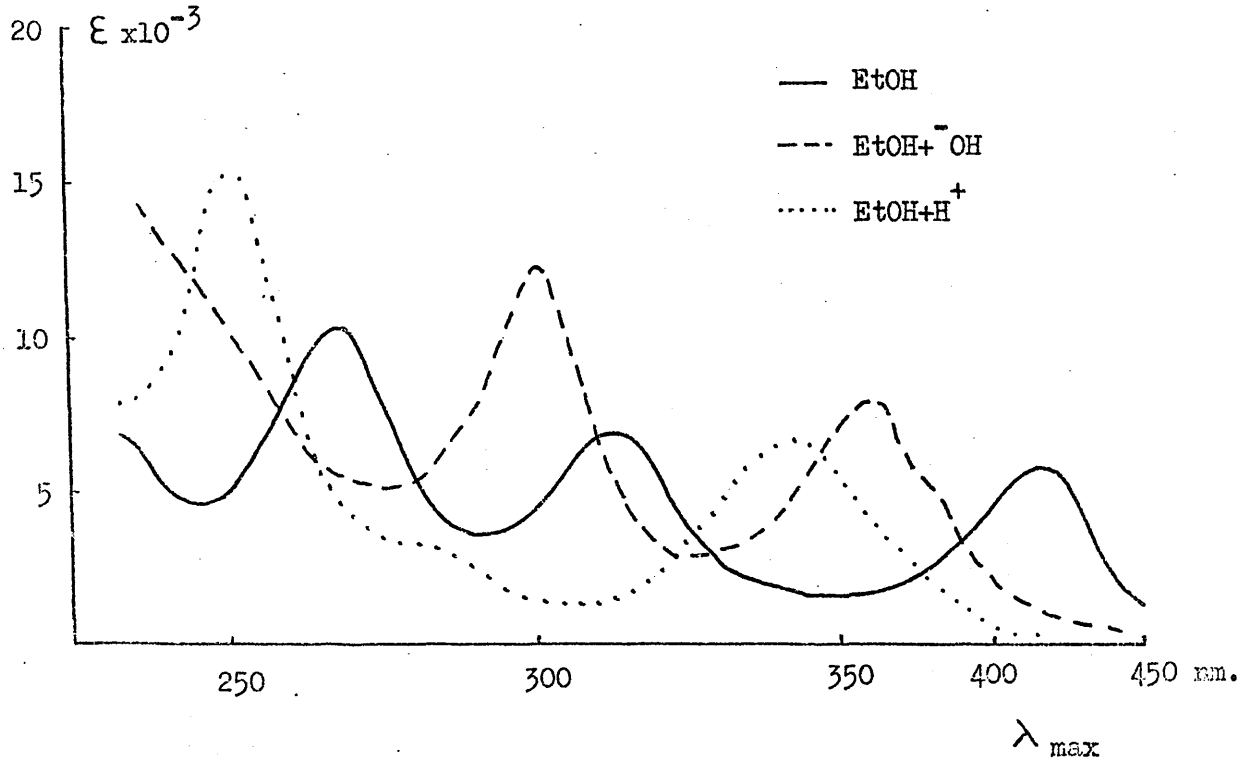
A Chlorine-containing Metabolite of V. Lamellicola.

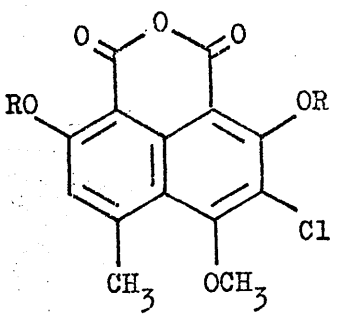
The solid, isolated by crystallisation from the crude active fraction of the broth extracts of V. lamellicola, consisted (T.L.C.) of two components. The major constituent, lamellicolic anhydride (53), was obtained pure by fractional crystallisation as previously described. Further chromatography of the residue afforded the minor component (100 a or b) as a yellow solid, which was recovered only with difficulty from preparative layer plates due to its insolubility in all solvents except methanol.

This compound was phenolic in character (red colour with methanolic ferric chloride) and contained a halogen atom (positive Beilstein test[87]). The presence of a chlorine atom was confirmed by the appearance in the mass spectrum (figure 16) of the doublets (in the ratio of 3:1) characteristic of the isotopes (mass numbers 35 and 37) of this element. The highest ion was observed at m/e = 308 and a high resolution mass spectrum suggested the molecular composition $C_{14}H_9ClO_6$. The base peak in the spectrum, however, was at m/e =294, i.e. 14 mass units lower than the highest ion. Since initial losses of 14 mass units are unusual it seemed possible that the peak at m/e = 308 was not a molecular ion.

The n.m.r. spectrum in D_6-dimethylsulphoxide revealed the presence of an aryl methyl group (3H singlet at 7.25τ), a methoxyl group (3H singlet at 6.30τ) and an uncoupled aromatic proton (1H singlet at 3.50τ). The four intense bands seen in the ir. between 1700 and 1580 cm^{-1} were

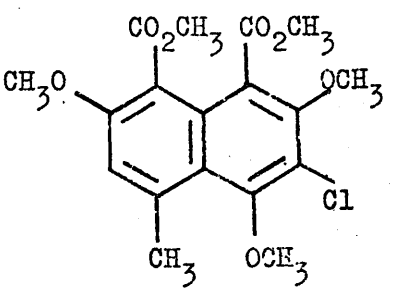
Figure 17. Ultraviolet Spectrum of the Chlorine-containing Metabolite.





(101) R = H

(103) R = CH_3



(102)

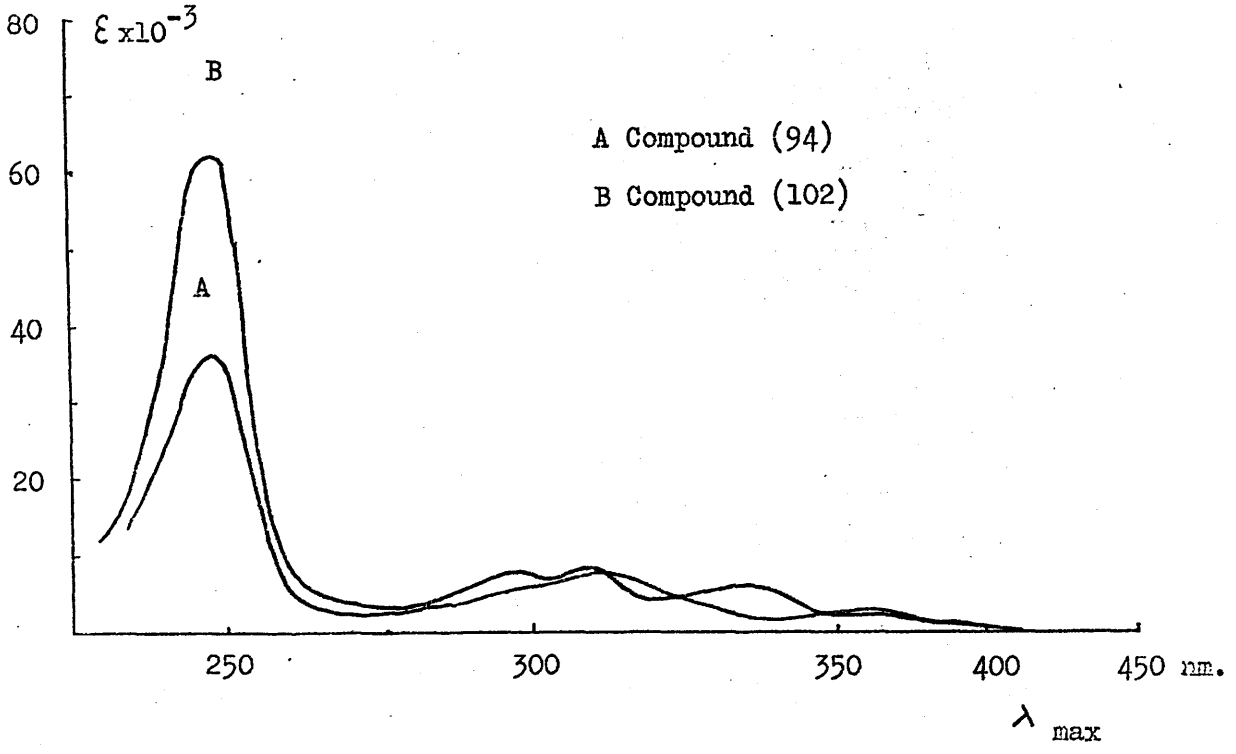
similar to those seen for lamellicolic anhydride although the upper two bands (1675, 1640 cm^{-1}) were at a lower frequency than the corresponding peaks produced by the latter compound. The complicated uv. spectrum (figure 17) underwent changes on basification, and on reacidification the yellow colour was destroyed producing a spectrum very similar to that of lamellicolic anhydride.

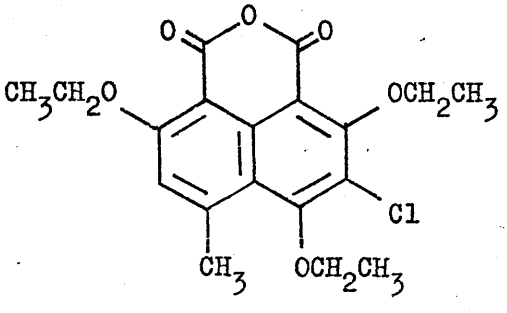
On the basis of this data structure (101), i.e. 3-chloro-4-O-methyl-lamellicolic anhydride, was at first tentatively assigned to this metabolite (100). This formulation, however, did not explain the acid lability of the compound, the apparent M^+-14 peak in the mass spectrum nor the carbonyl frequency being lower than in lamellicolic anhydride.

Reaction of the natural product (100) with diazomethane gave the trimethoxy dimethyl ester (102, 6H singlet at 6.05τ and 3H singlets at 6.10, 6.15 and 6.20τ, $\nu_{C=O}$ 1730 cm^{-1}). In keeping with this structure, double irradiation indicated that the aromatic proton (1H singlet at 3.05τ) was ortho to the aryl methyl group (3H singlet at 7.15τ), the uv. spectrum (figure 18) was characteristic of the substituted naphthalene nucleus, cf. the diester (94), and the mass spectrum showed twin molecular ions at m/e = 384 and 382. The formation of this ester (102) instead of the expected trimethyl ether (103) suggested that the assignment of structure (101) to the chlorine-containing metabolite had been erroneous.

In order to determine the position of the methoxyl group in the natural product it was treated with diethyl sulphate and potassium

Figure 18. Ultraviolet Spectra of Compounds (94) and (102).



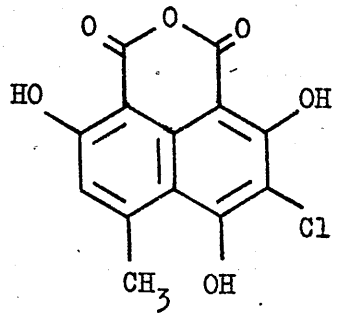


(104)

carbonate. The product obtained contained three O-ethyl groups (6H broad multiplet between 5.55 and 5.90τ, 3H triplet at 8.45τ and 6H triplet at 8.50τ), no methoxyl groups and showed the twin carbonyl absorption characteristic of a 1,8-naphthalic anhydride ($\nu_{C=O}$ 1760 and 1725 cm^{-1}). From the mass spectrum (molecular ion at m/e = 378) it was clear that the only tenable structure was (104), i.e. 3-chloro-O,O,O-triethyl-lamellicolic anhydride and this was confirmed by synthesis as indicated later.

In order to resolve the ambiguity in the structure of the natural product, an unambiguous synthesis of (101) from O,O,O-trimethyl-lamellicolic anhydride was carried out. Reaction of (54) with chlorine in carbon tetrachloride gave the monochloro trimethoxy anhydride (103, twin molecular ions in the ratio of 1:3 at m/e = 338 and 336, 3H singlet at 5.85τ and 6H singlet at 5.90τ, $\nu_{C=O}$ 1760, 1725 cm^{-1}). The detection of long range coupling between the aryl methyl group (3H singlet at 7.0τ) and the aromatic proton (1H singlet at 2.80τ) confirmed that substitution had occurred at C-3. Selective demethylation with magnesium iodide-etherate[56] gave the chloro methoxy anhydride (101, $\nu_{C=O}$ 1710, 1670 cm^{-1}, 3H singlet at 5.95τ), clearly different from the chlorine-containing metabolite.

From this information it was deduced that the metabolite was the half ester of 3-chlorolamellicolic acid (100 a or b). This formulation explained the uv. spectral data, the ready closure to an anhydride being expected to occur under basic conditions and was also in accord with the



(105)

Table 9.

	$\nu_{C=O}$ (cm^{-1})
Methyl salicylate	1675
Salicylic acid	1660

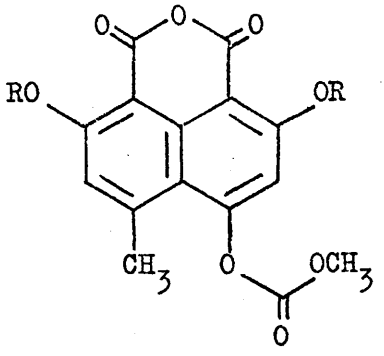
formation of a trimethoxy diester on methylation under neutral conditions but a triethoxy anhydride on ethylation in base. Facile losses of H_2O (18 mass units) and of CH_3OH (32 mass units) in the mass spectrometer[88] would result in the two highest ions observed, namely those at m/e = 308 and 294 (which differ by 14 mass units). Lastly, the carbonyl absorption expected for such a structure would be in agreement with that observed (cf. methyl salicylate and salicylic acid, table 9).

One way in which such an acid ester could arise would be by solvolysis of an anhydride either during the fermentation or during the work up proceedure and in order to determine how facile this process was the anhydride (105) was prepared from lamellicolic anhydride by reaction with chlorine in carbon tetrachloride. 3-Chlorolamellicolic anhydride (molecular ions at m/e = 296 and 294, $\nu_{C=O}$ 1710, 1660 cm^{-1}) was only sparingly soluble in methanol and possessed a uv. spectrum identical to that obtained from the half ester (100) on basification and reacidification. Its structure was confirmed by the formation of 3-chloro-O,O,O-trimethyl-lamellicolic anhydride on reaction with dimethyl sulphate and potassium carbonate. Prolonged treatment of (105) with methanol, however, failed to effect solvolysis suggesting that this process is unlikely to occur during the work up. In view of the sensitivity of the half ester to acid and of lamellicolic anhydride itself to base, the *in vitro* accomplishment of this solvolysis with sodium methoxide promised to present some difficulties. An attempt to carry out the solvolysis under milder conditions (sodium borohydride/methanol) was equally unsuccessful and no

product was obtained in reasonable yield.

Treatment of 3-chlorolamellicolic anhydride with diethyl sulphate and potassium carbonate yielded 3-chloro-O,O,O-triethyllamellicolic anhydride (104) identical (Rf, spectral data) to the material (104) prepared from the chlorine-containing metabolite thus providing further confirmation that this metabolite was the monomethyl ester of 3-chloro-lamellicolic acid (100 a or b). An alternative explanation for the biosynthetic formation of the half ester (100) is that it arises not by solvolysis of an anhydride but by methylation of a diacid. The fact that a diacid is a likely intermediate between a phenalenone and an anhydride supports this hypothesis.

CHAPTER 3.



(106) R = H

(107) R = CH_3

Table 10. N.M.R. Spectrum of 4-O-Carbomethoxylamellicolic Anhydride.

			τ
Ar-OH	1H	s	-1.60
Ar-OH	1H	s	-1.50
H-3	1H	s	3.02
H-6	1H	s	3.06
$-OCH_3$	3H	s	6.10
$Ar-CH_3$	3H	s	7.30

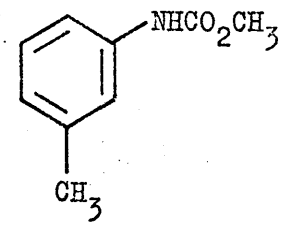
Table 11. Resonance of H-3 in the N.M.R. Spectra of (53) and (106).

	τ
(53)	3.55
(106)	3.02

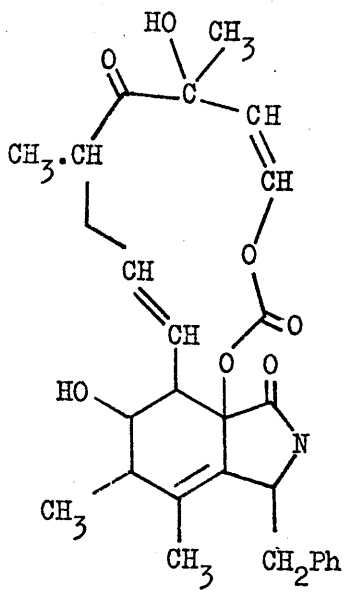
4-O-Carbomethoxylamellicolic Anhydride.

A minor metabolite which was present in column fractions eluted just before the main lamellicolic anhydride band was isolated by fractional crystallisation. This compound (106) was clearly closely related to lamellicolic anhydride since it gave the same characteristic brick red colour with methanolic ferric chloride and showed carbonyl absorption at 1725 and 1675 cm^{-1}. A third carbonyl absorption band, however, could be seen in the infra-red spectrum at 1765 cm^{-1}. The n.m.r. spectrum (table 10) of this compound was similar to that of lamellicolic anhydride, showing the presence of two chelated O-H groups and a pair of isolated aromatic protons with an aryl methyl group ortho to one of them. A 3H singlet was visible at 6.10τ and this was assigned to an O-methyl group. From this evidence structure (106), i.e. 4-O-carbomethoxy-lamellicolic anhydride, was assigned to this metabolite - a formulation which was supported by mass spectral data (parent ion at m/e = 318) and by the downfield shift in the resonance of H-3 compared to the value observed in lamellicolic anhydride (table 11), due to the removal of the shielding effect of a hydroxyl group situated at position 4.

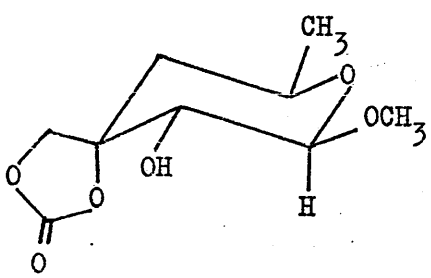
On treatment with dimethyl sulphate and potassium carbonate (106) gave a mixture of the dimethyl ether (107, parent ion at m/e = 346, $\nu_{C=O}$ 1760 and 1720 cm^{-1}, 6H singlet at 5.84τ and 3H singlet at 5.98τ) and O,O,O-trimethyllamellicolic anhydride (54). One would expect the carbonate group to be susceptible to base hydrolysis and this was further



(108)



(109)



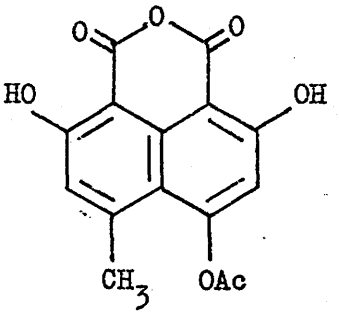
(110)

shown by the formation of triacetoxy lamellicolic anhydride on treatment of (106) with acetic anhydride and pyridine. An attempt to trap the carbonate unit, as the urethane (108), by refluxing (106) with m-toluidine was unsuccessful and the desired product could not be detected by T.L.C.

Final confirmation of structure (106) for the minor metabolite came from its preparation from lamellicolic anhydride on treating the latter with methyl chloroformate and potassium carbonate.

4-O-Carbomethoxylamellicolic anhydride (106) is the first natural product reported which contains the methyl carbonate unit. Two compounds, however, - the macrocyclic mould metabolite cytochalasin E[89] (109) and aldgamycin, an antibiotic whose total structure is unknown but a degredation product of which - aldgarose[90] - has structure (110) - contain a cyclic carbonate group. The origin, therefore, of this unit is of considerable interest. Since 4-O-carbomethoxylamellicolic anhydride could be detected by T.L.C. in fresh methylene chloride extracts and none was obtained by refluxing a pure sample of lamellicolic anhydride in methylene chloride for a considerable time (ca. 10 days) it seemed likely that (106) was not an artefact formed from (53) during the purification proceedure.

If 4-O-carbomethoxylamellicolic anhydride was a genuine natural product formed during the fermentation process it seemed possible that one or indeed both of the carbon atoms of the carbonate unit might arise from the C_1 pool. Accordingly [^{14}C-methyl]-methionine was fed to V. lamellicola and the lamellicolic anhydride and 4-O-carbomethoxy-

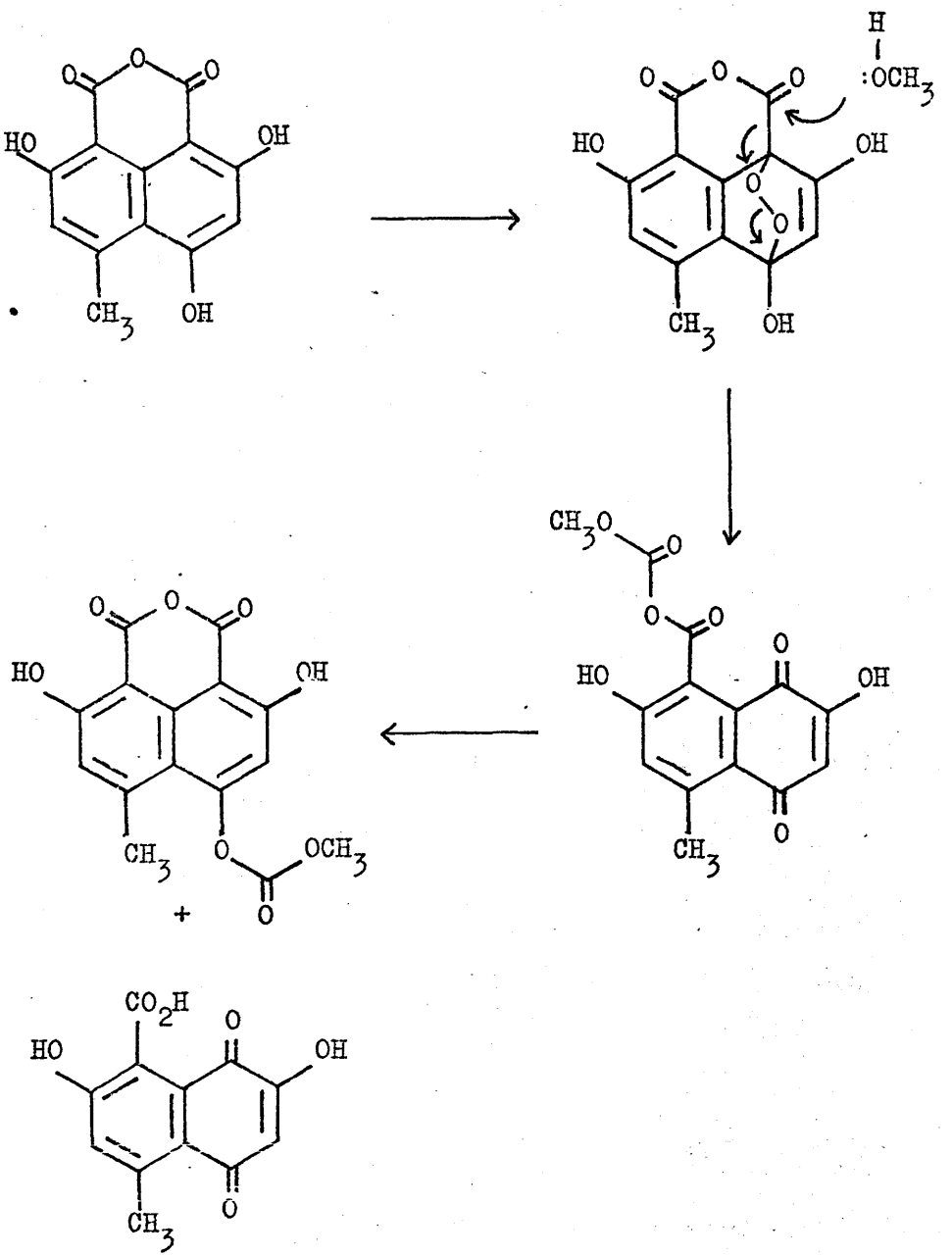


(111)

Table 12.

Compound	dpm./mM. ($\times 10^{-4}$) ^{14}C	dpm./mM. ($\times 10^{-4}$) ^{3}H	$^{3}H/^{14}C$ Ratio
4-O-Carbomethoxy-lamellicolic anhydride	5.64	35.3	6.25
lamellicolic anhydride	5.24	35.2	6.69

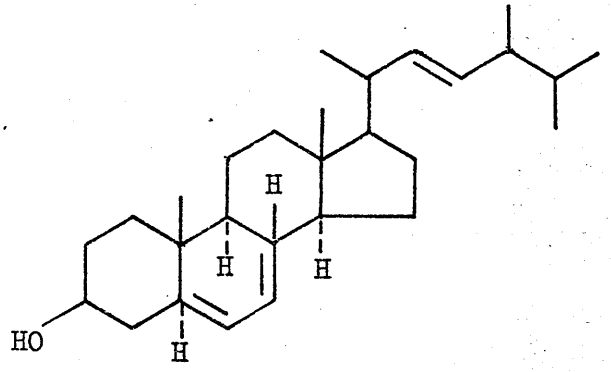
Figure 19. Possible Route to 4-O-Carbomethoxylamellicolic Anhydride.



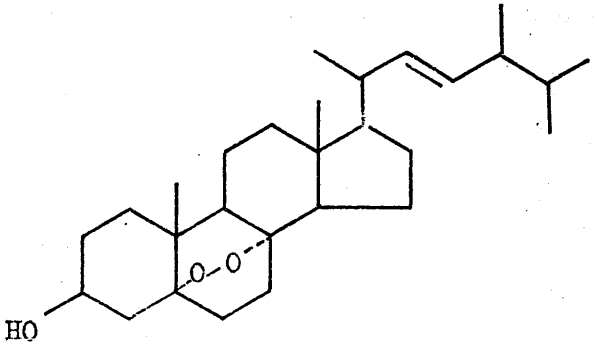
lamellicolic anhydride produced isolated. No radioactive label was incorporated into either of the compounds suggesting that, if this result is accepted, the C_1 pool is not involved in the biosynthesis of the carbonate unit. This result also eliminated the unlikely possibility of lamellicolic anhydride being derived from a cyclised hexaketide species and two C_1 units (see above).

It is conceiveable that the carbonate unit may arise from acetate via a Baeyer-Villager type oxidation of the monoacetate (111) and it was therefore decided to fed labelled acetate to the mould. Difficulties, however, were experienced at this point in the growth of the fungus and so far only a preliminary feeding experiment has been completed. [1-^{14}C, 2-^{3}H]-acetate was incorporated into both lamellicolic anhydride and 4-O-carbomethoxylamellicolic anhydride (table 12) but both compounds showed almost the same $^{3}H/C$ ratio suggesting that acetate was not incorporated into the carbonate unit of the latter. Lack of time prevented the carrying out of the more detailed feeding studies necessary to clarify this point.

Another possible route to the carbonate (106) is via an endoperoxide as shown in figure 19. Peroxides of this type can be formed *in vitro* from substituted naphthalenes[91] by the action of ultraviolet light in the presence of a photosensitizer and it may be that pigments present in the culture filtrate can act as photosensitizers and facilitate this reaction. In support of this hypothesis, ergosterol peroxide (112) has been isolated from fungal sources and it has been shown[92] that it is formed



(113)



(112)

from ergosterol (113) in the presence of photosensitizing pigments. Attempts to carry out a similar transformation of lamellicolic anhydride, in vitro, were unsuccessful. Under mild conditions (methylene blue as sensitizer, strong light) no reaction was obtained whilst extensive decomposition occurred under more forcing conditions (no sensitizer, ultraviolet light). If, however, the carbonate (106) is formed by this route then one of the carbonyl carbon atoms of lamellicolic anhydride will be incorporated into the carbonate unit, i.e. feeding of [1-^{14}C]-acetate ought to result in the incorporation of activity into the carbonate unit. The preliminary acetate feeding experiment described above did not eliminate this possibility and clearly further feeding experiments are necessary. It is particularly important that conditions be found which permit the selective removal of the carbonate unit in order to determine the amount of activity present in it. At present, then, it can be said that 4-O-carbomethoxylamellicolic anhydride is either a most unusual natural product or a most unusual artefact.

CHAPTER 4.

The Spore Pigments of the Mutants of Aspergillus Nidulans.

By irradiating an organism with ultraviolet light or by exposing it to a chemical mutagen it is possible to produce mutants of the organism which differ from the parent only in one gene. If the mutant thus does not possess a particular enzyme and is consequently unable to carry out a particular metabolic reaction a biosynthetic pathway will be disrupted. This technique allows information to be obtained about the biosynthetic pathway involved since intermediates, prior to the enzyme block, will accumulate and can be identified by chemical analysis. In addition, normal growth will be restored to the organism if the compound whose synthesis is blocked is fed to the mutant. Thus a picture of the sequence of reactions involved in a pathway can be built up by examination of a large number of mutants.

The enormous amount of work involved in the growth and examination of the required number of mutants has, so far, restricted the use of this technique in the elucidation of the secondary metabolism of micro-organisms to a few cases, e.g. the biosynthesis of the tetracyclines[93]. It is, however, conveniently applied to the study of pigment production since mutants which do not possess a particular pigment are readily recognisable.

The spores produced by the wild type strain of the mould Aspergillus nidulans are green. Mutants, however, have been isolated which produce white, fawn and yellow spores[94] and strains carrying more than one

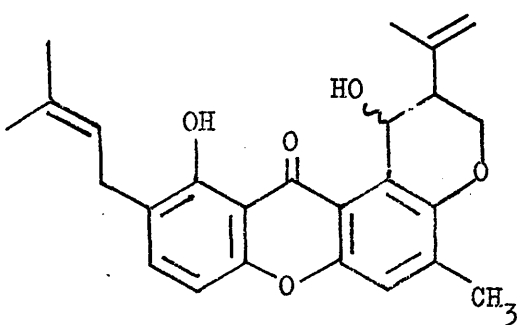
Table 13.

Mutant	Spore Colour
White mutant	White
White, fawn mutant	White
Fawn mutant	Fawn
Fawn, yellow mutant	Fawn
Yellow mutant	Yellow

Figure 20.

No pigment (white) → Fawn pigment → Yellow pigment → Green pigment

White mutant block Fawn mutant block Yellow mutant block



(114)

Figure 21. Ultraviolet Spectrum of Arugoxanthone A.

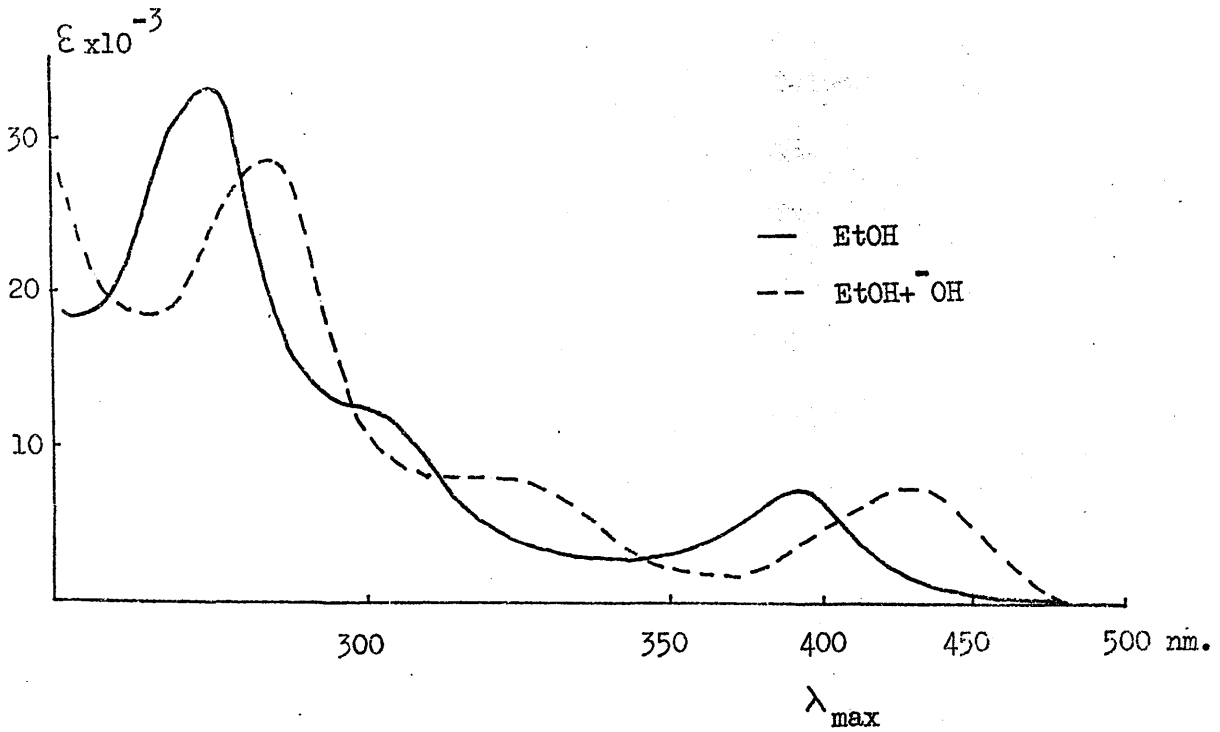
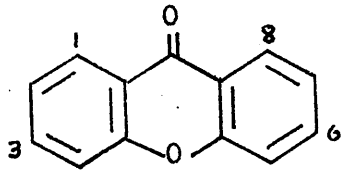
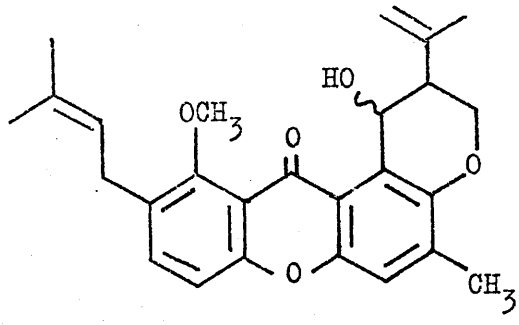


Table 14. Ultraviolet Spectra of Xanthones.

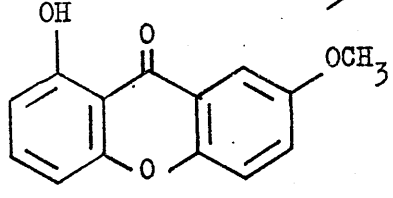
Arugoxanthone A	242,	257(inf.),	270(inf.),	276, 297,	394 nm.
1-Hydroxy-7-methoxy-xanthone[98] (117)	237,		260,	287,	385 nm.
Mangostin[99] (118)	243,	259,		318,	351 nm.
Calabaxanthone[100] (119)	240,		287,	292, 314,	384 nm.
1,6-Dihydroxyxanthone[101]	230,	248, 265,	288,	305,	353 nm.
1,5-Dihydroxyxanthone[101]	235,	248,		315,	370 nm.



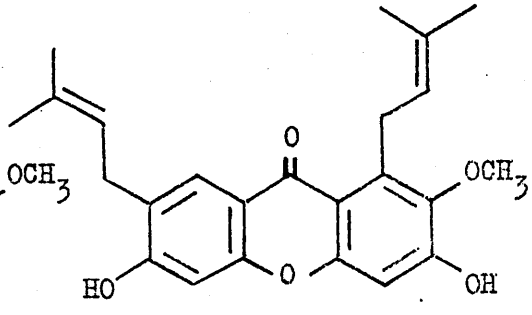
(115)



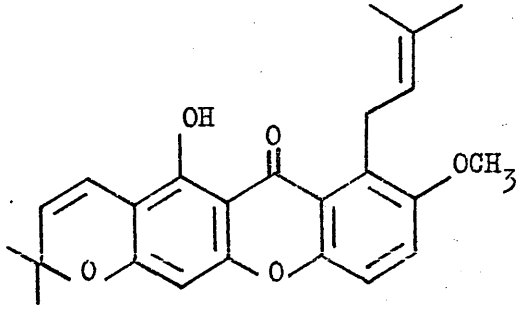
(116)



(117)



(118)



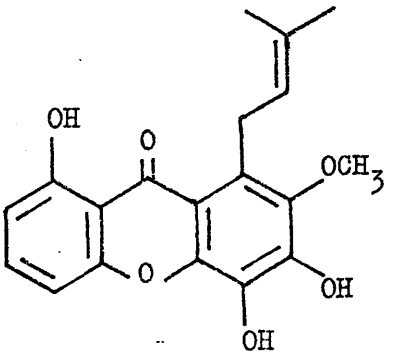
(119)

mutation behave as shown in table 13 suggesting that the differences in colour between the mutants are due to the blocking of pigment production at the stages shown in figure 20[95]. The present investigation was initiated in order to isolate and identify the pigments present in the various mutants with a view to understanding the enzymic basis for the differences in colour. Spores of the wild type strain contain a p-diphenol oxidase which is believed to be involved in the conversion of the yellow pigment into the green pigment and cell-free extracts containing this enzyme have been shown[96] to oxidise gallic acid, pyrogallol and 2,6-dimethoxyphenol. The isolation, therefore, of the yellow pigment was of particular interest since it would then be possible to test whether or not the enzyme can be induced by its substrate when presented externally.

Two yellow pigments, phenolic in character (blue colour with methanolic ferric chloride), were isolated by chromatography from light petroleum extracts of the spores of the yellow mutant. The ultraviolet spectrum (figure 21) of the less polar compound ($C_{25}H_{26}O_5$, parent ion at m/e = 406) - later given the trivial name arugoxanthone A and assigned structure (114) - showed the bathochromic shift on basification expected for a phenol and suggested that it was not a quinone[38] (no absorption > 400 nm.). Comparison, however, with the uv. (table 14) and infra-red (table 15) spectra of a number of xanthones[97] provided evidence for the presence of this chromophore (115), possibly with the same oxygenation pattern as seen in 1-hydroxy-7-methoxyxanthone.

Table 15. Carbonyl Absorption of Xanthones.

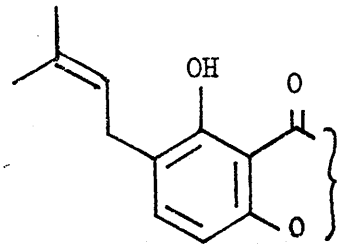
	$\nu_{C=O}$ (cm^{-1})
Arugoxanthone A	1635
Calabaxanthone	1645
1,5-Dihydroxyxanthone	1650



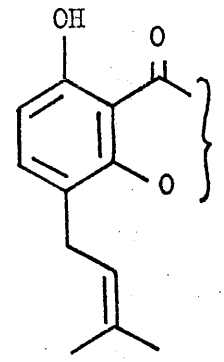
(120)

Table 16. Resonances of Aromatic Protons in (120)

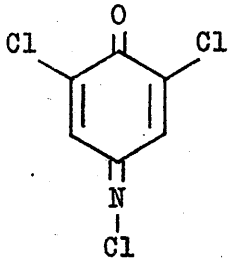
Compound	Proton	Chemical Shift (τ)
(120)	H-2	3.49
(120)	H-3	2.59
(120)	H-4	3.25



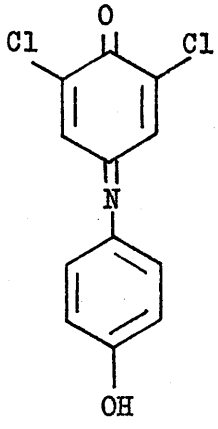
(121)



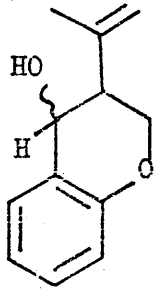
(122)



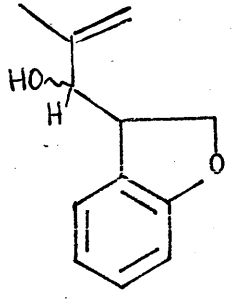
(123)



(124)



(125)



(126)

Arugoxanthone A (114) failed to react with diazomethane but on treatment with dimethyl sulphate and potassium carbonate it formed a monomethyl ether (116, $C_{26}H_{28}O_5$, 3H singlet at 6.00τ) suggesting the presence of a hydroxyl group in the 1-position. This was supported by a low field O-H resonance in the n.m.r. of (114) (1H exchangeable singlet at -2.55τ) and by the bathochromic shift observed in the ultraviolet spectrum on the addition of a trace of aluminium trichloride[100].

Of the three aromatic protons evident in the n.m.r. spectrum of arugoxanthone A, two (1H doublets, J=8Hz, at 2.55 and 3.27τ) possessed chemical shifts consistent with their being assigned to positions 3 and 4 respectively or 3 and 2 respectively on a 1-hydroxyxanthone nucleus, cf. celebixanthone[102] (120) (table 16). The presence of a 3,3-dimethylallyl group (1H broad triplet, J=6Hz, at 4.75τ, 2H doublet, J=6Hz, at 6.57τ and 3H singlets ca. 8.2τ) ortho to H-3 was deduced from the long range coupling detected between that proton and the methylene group of the isopentenyl unit. Accordingly the substitution pattern around ring A in arugoxanthone A was as shown in partial structure (121) or (122).

Phenols with unsubstituted positions para to the hydroxyl group have been recognised and differentiated from those with unsubstituted ortho positions by the characteristic green colour which the former give on mixing with Gibbs reagent[103] - 2,6-dichlorobenzoquinone-4-chloroimide (123) - through the formation of an indophenol (124). An intense absorption is seen between 500 and 700 nm. in the ultraviolet spectrum of an indophenol and this has allowed the Gibbs test to be put onto a

Figure 22. Gibbs Test on Arugoxanthone A.

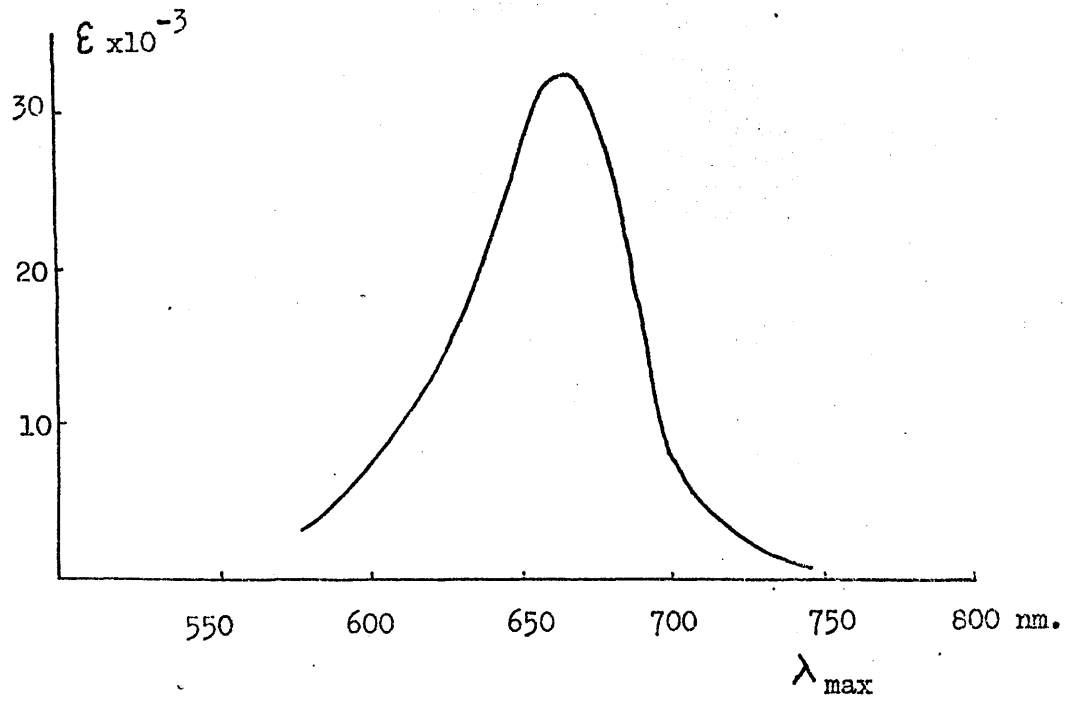


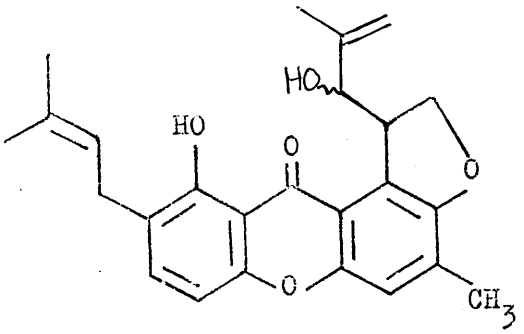
Table 17. Chemical Shifts of the Protons in Ring C of (117).

	τ
H-5	2.7
H-6	2.9
H-8	2.28

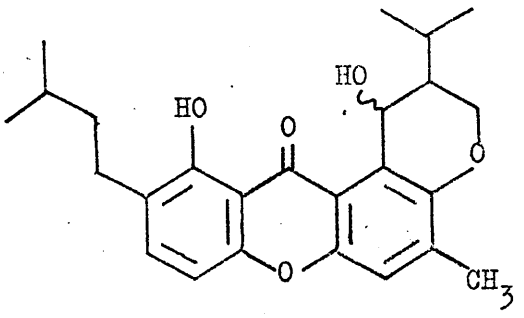
spectroscopic basis[104]. When the substituent ortho or para to the hydroxyl group is a halogen atom, a carboxyl group or an alkoxy group the validity of the test has been questioned[105,106]. Anomalous results, however, are not obtained when the substituent is an alkyl group and the test is carried out quantitatively[107]. With arugoxanthone A a positive test was obtained (figure 22) (λ_{max} 660 nm., ε = 30000) suggesting that position 4, i.e. para to the hydroxyl group was unsubstituted and that ring A possessed the substitution pattern shown in (121).

The chemical shift of the third aromatic proton in arugoxanthone A (1H singlet at 2.72τ) suggested that it was situated at position 5 on a 1,7-dioxygenated xanthone nucleus, cf. 1-hydroxy-7-methoxyxanthone (table 17). An aryl methyl group (3H singlet at 7.7τ) ortho to this proton (irradiation at 7.7τ produces sharpening of the signal at 2.7τ) could then be assigned to position 8.

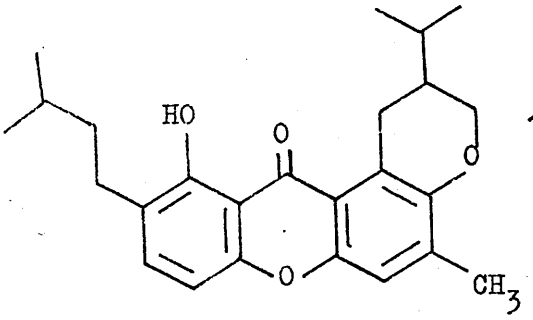
The remaining resonances were consistent with the presence in arugoxanthone A of the structural unit (125) or (126) and were assigned to: a hydroxyl group (1H exchangeable doublet, J=3Hz, at 5.02τ, ν_{OH} seen in the ir. at 3460 cm^{-1}) geminal to a benzilic or allylic proton (1H multiplet at 4.65τ); a vinyl methyl group (3H singlet at 8.21τ); a terminal methylene group (1H singlets at 5.26 and 5.47τ); a methylene group bonded to oxygen (2H doublet at 5.69τ); and an allylic or benzilic proton (1H multiplet at 7.35τ). Double irradiation revealed coupling between these protons consistent either with partial structure (125) or (126). On spectroscopic evidence, therefore, arugoxanthone A could be



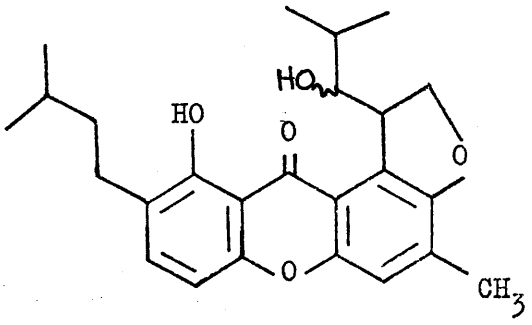
(127)



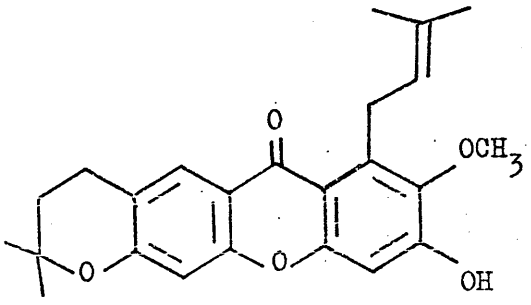
(128)



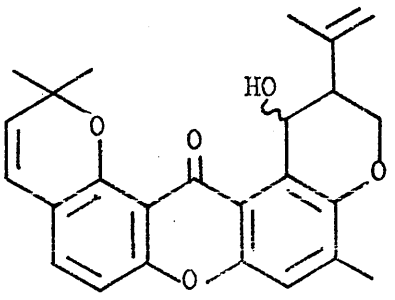
(130)



(129)



(131)



(132)

assigned either structure (114) or (127).

Hydrogenation of arugoxanthone A, in ethyl acetate, over 10% palladium-charcoal gave not the expected tetrahydro compound, (128) or (129), but the product of concomitant hydrogenolysis (parent ion at m/e = 394). The proton seen in the starting material at 7.35τ now resonated at ca. 8.3τ. This upfield shift was consistent with structure (114) being assigned to arugoxanthone A and structure (130) to the reduction product.

The allylic secondary hydroxyl group in (114) resisted oxidation with manganese dioxide or even with Jones reagent at 20°C. This was attributed partly to the group being sterically hindered and partly to it being hydrogen bonded to the xanthone carbonyl group.

Chemical confirmation of the presence of a 3,3-dimethylallyl group ortho to a hydroxyl group in mangostin (118) by cyclisation to the pyran (131) has been reported[108]. Attempts, however, to effect this transformation in arugoxanthone A have been unsuccessful, perhaps due to strong hydrogen bonding between the xanthone carbonyl group and the hydroxyl group. Treatment of (114) with p-toluenesulphonic acid at room temperature in benzene failed to produce any reaction while at elevated temperatures no stable product could be isolated. Reaction of (114) with 3% sulphuric acid in acetic acid gave a large number of unisolable polar compounds. An attempt to effect an oxidative cyclisation of arugoxanthone A to the chromene (132), using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone[109], was equally unsuccessful giving only an unstable product which could not be characterised.

Figure 23. Fragmentation Process Resulting in the Loss of 56 Mass Units.

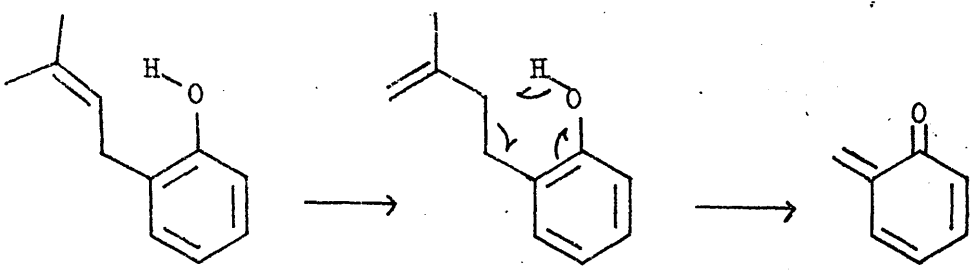


Figure 24. Mass Spectrum of Arugoxanthone A.

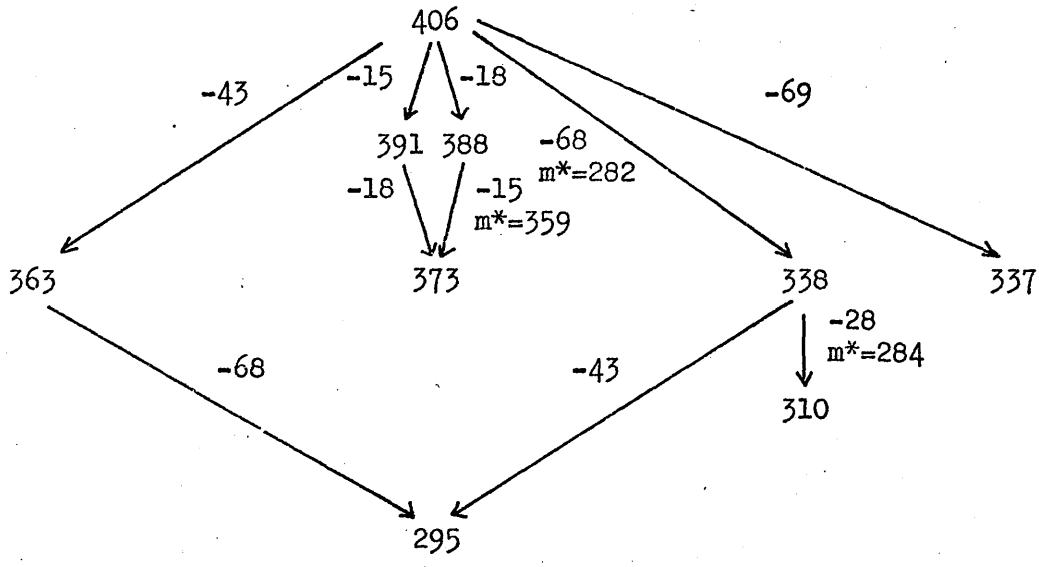


Figure 25. Fragmentation Process Resulting in a Loss of 43 Mass Units.

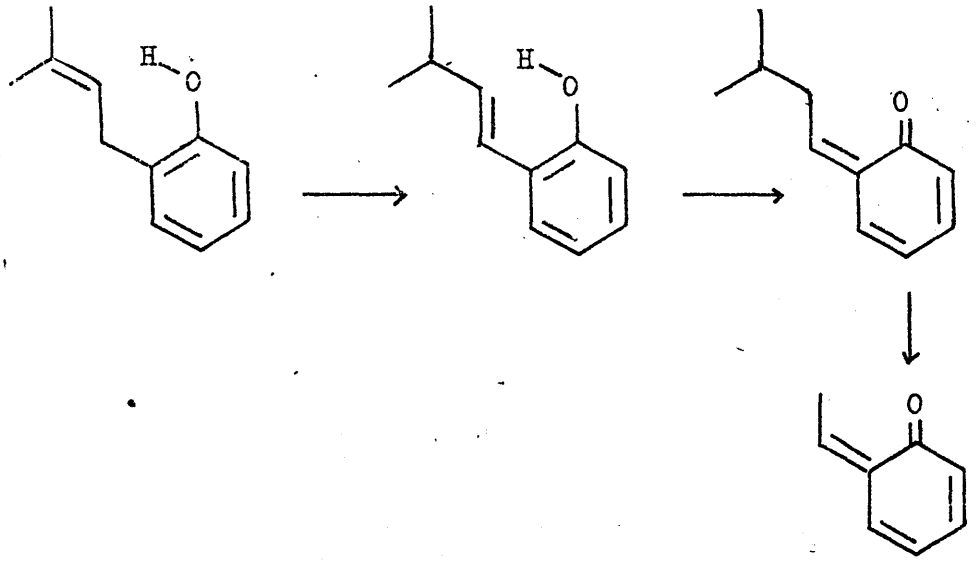


Figure 26. Fragmentation Processes Resulting in Losses of 68 and 69 Mass Units.

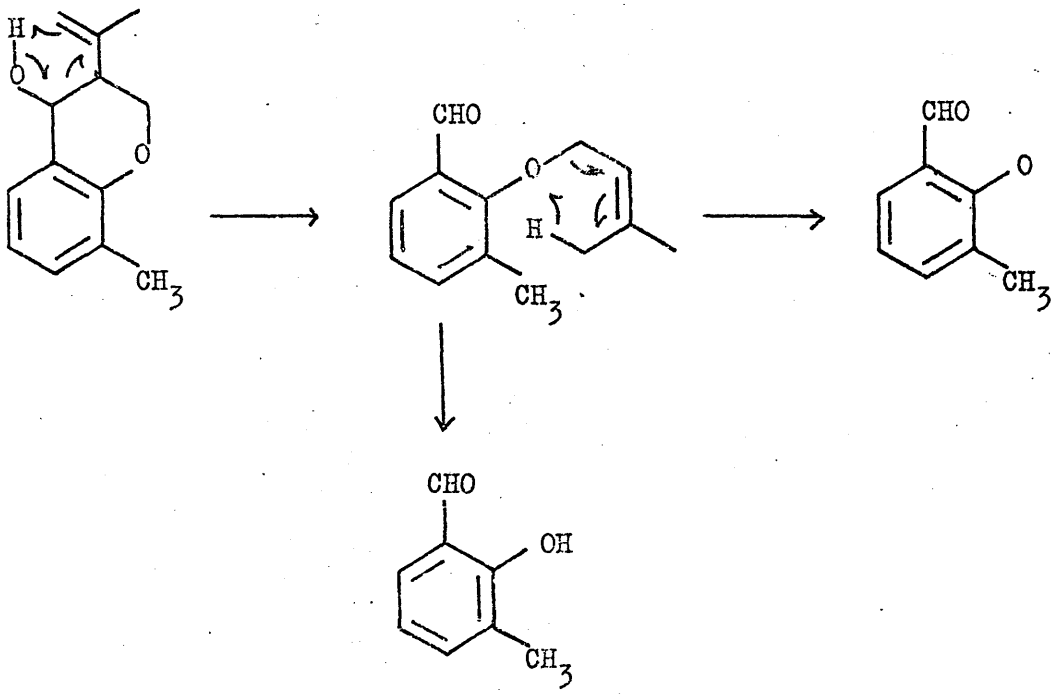


Figure 27. Alternative Fragmentation Process Leading to a Loss of 68 Mass Units.

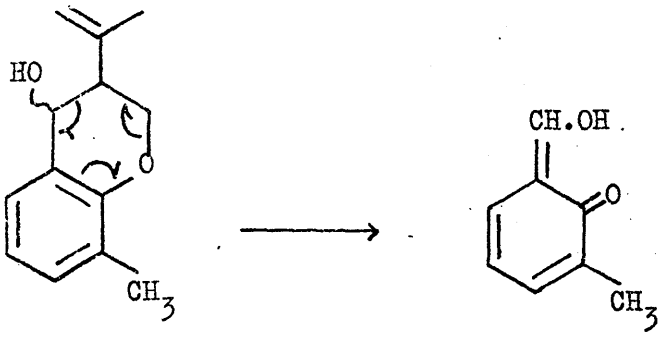
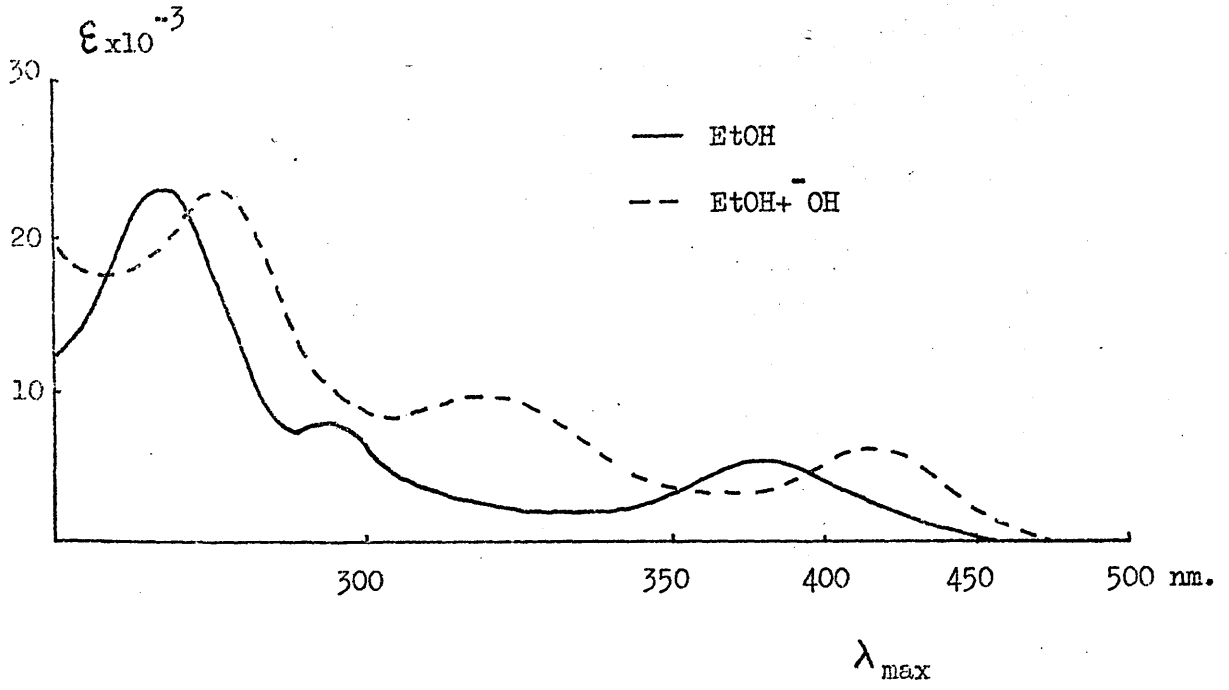
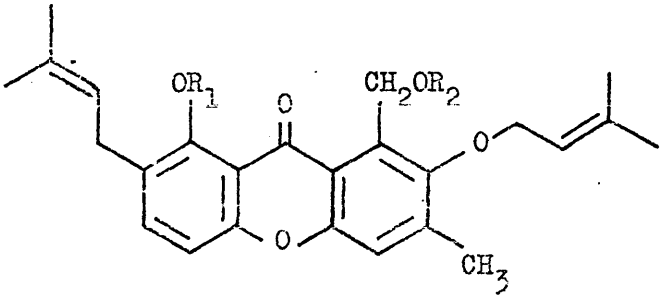


Figure 28. Ultraviolet Spectrum of Arugoxanthone B.





(133) $R_1 = R_2 = H$

(134) $R_1 = CH_3$, $R_2 = H$

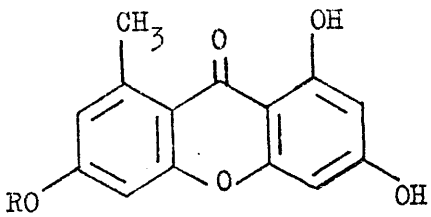
(135) $R_1 = H$, $R_2 = CO.CH_3$

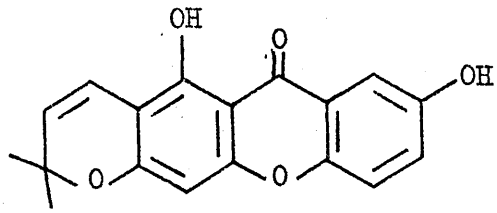
(136) $R_1 = R_2 = CO.CH_3$

It has been alleged[73] that 3,3-dimethylallyl units ortho to hydroxyl groups can be recognised by a characteristic fragmentation in the mass spectrum resulting in the loss of 56 mass units (figure 23). The mass spectrum of arugoxanthone A (figure 24) showed no evidence of the occurrence of such a fragmentation, the C-3,3-dimethylallyl group fragmenting with a loss of 43 mass units (figure 25) while the losses of 68 and 69 mass units from the parent ion could arise following rearrangement to the O-3,3-dimethylallyl system as shown (figure 26). The loss of 68 mass units could alternatively arise directly as shown in figure 27.

The ultraviolet (figure 28) and infra-red (e.g. $\nu_{C=O}$ 1640 cm^{-1}) absorption of the more polar pigment ($C_{25}H_{28}O_5$, parent ion at m/e = 408) suggested that it also was a xanthone and it was assigned the trivial name arugoxanthone B and structure (133). On treatment with dimethyl sulphate and potassium carbonate it gave a monomethyl ether (134, parent ion at m/e = 422, 3H singlet at 6.15τ). The hydroxyl group in the pigment (133) could be assigned to position 1 since it resonated in the n.m.r. at -1.5τ. A positive Gibbs test (λ_{max} 670 nm., ε = 28000) suggested that the 4-position was unsubstituted.

The n.m.r. spectrum of (133) showed the presence of several features occurring in arugoxanthone A: aromatic protons situated at positions 3 and 4 (1H doublets, J=8Hz, at 2.54τ and 3.25τ); a 3,3-dimethylallyl substituent at the 2-position (1H broad triplet, J=7Hz, at 4.67τ, 2H doublet, J=7Hz, at 6.52τ and 3H singlets ca. 8.20τ); an aryl methyl group

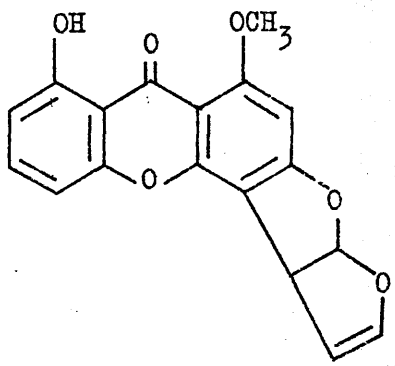




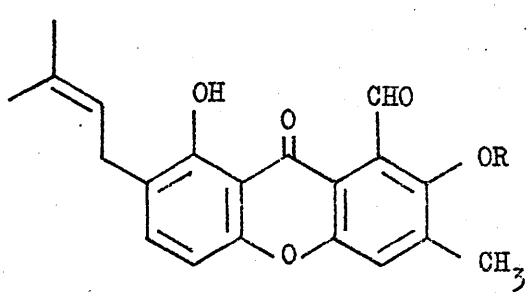
(137) R = H

(139) R = CH_3

(138)



(140)



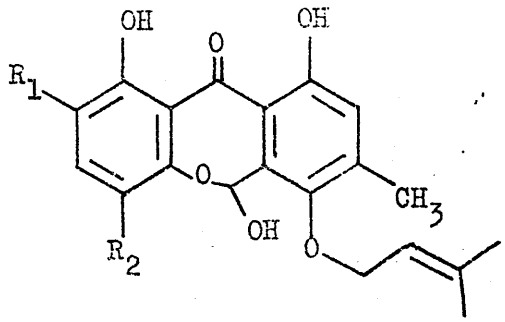
(141) R = $CH_2CH.C(CH_3)_2$

(142) R = H

(3H singlet at 7.54τ) ortho to a proton (1H singlet at 2.65τ) at position 5. New structural features were an O-3,3-dimethylallyl group (1H broad triplet, J=6Hz, at 4.38τ, 2H doublet, J=6Hz, at 5.55τ and 3H singlets ca. 8.20τ) and an aryl hydroxymethyl group (1H exchangeable multiplet at 5.55τ and a 2H doublet, J=7Hz, at 4.91τ which collapsed on shaking with D_2O to a 2H singlet). From this evidence structure (133) was assigned to arugoxanthone B.

Treatment of arugoxanthone B with acetic anhydride and pyridine gave a mixture of the monoacetate (135, $\nu_{C=O}$ 1730 (acetate), 1640 (xanthone) cm^{-1}, 3H singlet at 7.99τ, blue colour with $FeCl_3$) and the diacetate (136, $\nu_{C=O}$ 1760 (phenolic acetate), 1730 (alcoholic acetate), 1655 (xanthone) cm^{-1}, 3H singlets at 7.70 and 8.05τ, no colour with $FeCl_3$). 2H singlets at ca. 4.3τ in these acetates supported the presence in arugoxanthone B (133) of a hydroxymethyl group, the methylene protons of which would be subject to a downfield shift on acetylation.

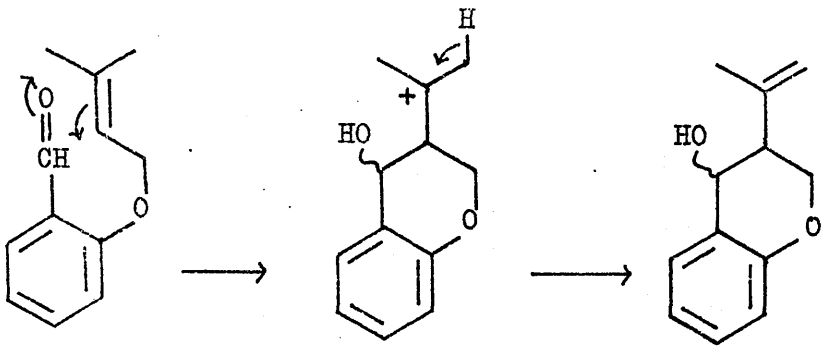
Xanthones are frequently found as metabolites of lichens, e.g. norlichexanthone[110] (137), and of higher plants, e.g. osajaxanthone[107] (138). Only a few, however, have been isolated from fungal sources - e.g. griseoxanthone C[111] (139) from P. patulum and sterigmatocystin[112] (140) from A. versicolor - and arugoxanthones A and B are the first prenylated fungal xanthones reported. The prenylated xanthones anhydroarugosin (141) and deisopentenylanhydroarugosin (142) have, however, been reported as in vitro transformation products of arugosin - a metabolite of the mould Aspergillus rugulosus - which has been shown[113]

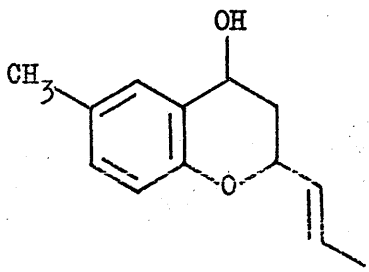


(143) $R_1 = CH_2CH.C(CH_3)_2$, $R_2 = H$

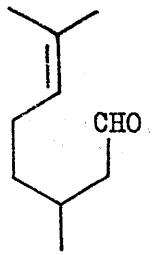
(144) $R_1 = H$, $R_2 = CH_2CH.C(CH_3)_2$

Figure 29.

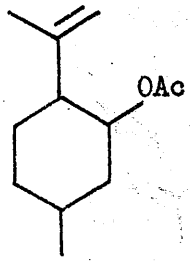




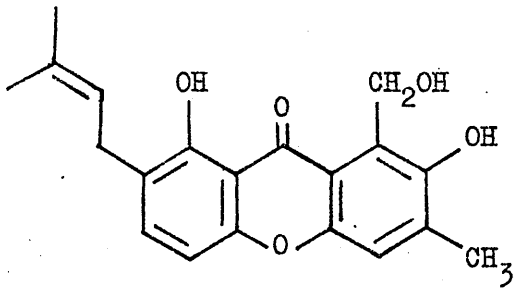
(145)



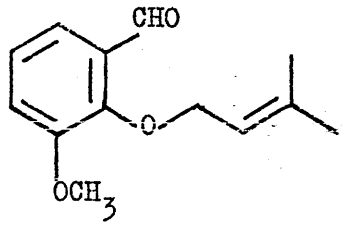
(146)



(147)



(148)



(149)

to be a mixture of the isomeric hemiacetals (143) and (144).

An unusual feature of the structure of arugoxanthone A is the chroman-4-ol unit which might arise biosynthetically from the aldehyde (141) by the cyclisation of the O-3,3-dimethylallyl group onto the formyl group situated *ortho* to it (figure 29). This has not hitherto been reported as an *in vivo* process - other chroman-4-ols found as natural products, e.g. myrochromanol[114] (145), are formed by different biosynthetic routes - nor as an *in vitro* reaction although the analogous cyclisation of citronellal (146) to (147) in acetic anhydride has been known for a considerable time[115]. Reduction of the formyl group in (141) - a reaction commonly observed[116] in polyketide biosynthesis - would yield arugoxanthone B. Thus (141) may be an intermediate on the route to both (114) and (133), in which case arugosin is also a precursor of these compounds.

Attempts to prepare (141) from arugoxanthone B by oxidation with manganese dioxide were unsuccessful - the starting material being recovered unchanged. Treatment of (133), however, with Jones reagent gave a product (141, parent ion at m/e = 406), in poor yield (ca. 27%), the presence in which of a formyl group was evident from the characteristic carbonyl absorption in the infra-red spectrum ($\nu_{C=O}$ 1705 cm^{-1}) and the non exchangeable low field resonance seen in the n.m.r. (1H singlet at -0.74τ). This compound (141) possessed melting point and spectral data identical to that reported for anhydroarugosin (141). Deisopentenylanhydroarugosin (142) was most conveniently prepared

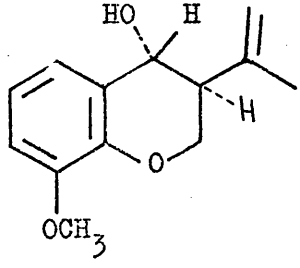
from arugoxanthone B by removing the O-3,3-dimethylallyl group under acid conditions to give the alcohol (148, parent ion at m/e = 340) followed by oxidation with manganese dioxide to give an aldehyde (142, parent ion at m/e = 338, $\nu_{C=O}$ 1640 cm^{-1}) which showed identical physical characteristics to those reported for deisopentenylanhydroarugosin, further confirming the structural assignment made to arugoxanthone B.

Due to the difficulty encountered in preparing a reasonable quantity of the aldehyde (141) a suitable model compound was synthesised in order to investigate the cyclisation reaction. Treatment of 2-hydroxy-3-methoxybenzaldehyde with 3,3-dimethylallyl bromide and potassium carbonate gave the ether (149, parent ion at m/e = 220, $\nu_{C=O}$ 1690 cm^{-1}) in good yield. The presence of an O-3,3-dimethylallyl group was evident from the n.m.r. (1H broad triplet, J=8Hz, at 4.45τ, 2H doublet, J=8Hz, at 5.35τ, and 3H singlets at 8.25 and 8.40τ) and from the mass spectrum (large M^+-68 ion observed). No reaction occurred on treatment of (149) with acetic anhydride and pyridine but on stirring in benzene with a trace of p-toluenesulphonic acid three products were obtained, one of which was 2-hydroxy-3-methoxybenzaldehyde.

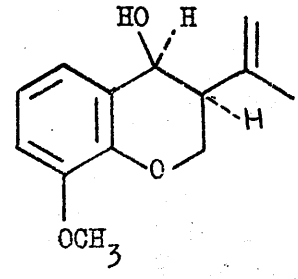
The other two compounds (150) and (151), were isomeric in structure (parent ion at m/e = 220) and had almost identical polarity on T.L.C. (Rf 0.21 and 0.20 in 100% chloroform respectively). Their ir. spectra differed only in the fingerprint region and provided evidence for the presence in both compounds of a hydroxyl group (ν_{max} ca. 3400 cm^{-1}) and a double bond (ν_{max} ca. 1640 cm^{-1}). The n.m.r. spectra of (150) and

Table 18. N.M.R. Spectra of Chroman-4-ol Units.

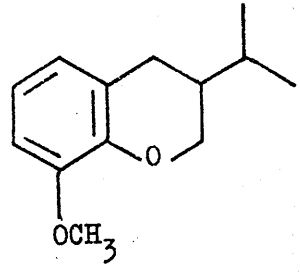
		τ (150)	τ (151)	τ arugoxanthone A.
Ar-H	3H	3.10	3.10	--
Olefinic H	1H	4.90	5.05	5.26
Olefinic H	1H	5.25	5.20	5.47
Ar-C-H	1H	5.25	5.23	4.65
O-CH_2	2H	5.65	5.65, 5.85	5.69
O-CH_3	3H	6.10	6.20	--
Allylic H	1H	7.35	7.45	7.35
OH	1H	8.05	8.05	5.02
Vinyl CH_3	3H	8.05	8.20	8.26



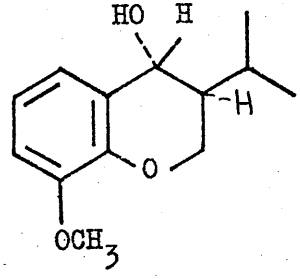
(150)



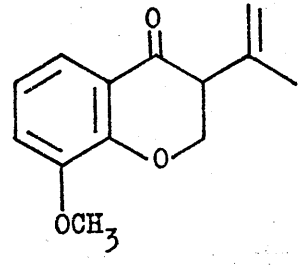
(151)



(152)



(153)



(154)

(151) (table 18) were consistent with the presence in these compounds of a chroman-4-ol unit and good agreement was seen between the chemical shifts of the protons of this unit and those of the equivalent protons in arugoxanthone A (114).

From the observation of a strong M^+-18 peak in the mass spectrum of the more polar compound (Rf 0.20) but not in that of the less polar isomer (Rf 0.21) structure (151) was assigned to the former and structure (150) to the latter since in (151) a facile loss of H_2O would be expected in the mass spectrometer.

Further confirmation of the assignment of structures (150) and (151) to the alcohols came from the hydrogenation of (150) over 5% palladium-charcoal giving a mixture of the hydrogenolysis product (152, parent ion at m/e = 206, no absorption in the ir. > 3100 cm^{-1}) and the dihydro compound (153, parent ion at m/e =222, ν_{OH} 3600 cm^{-1}). In the n.m.r. spectrum of the latter (153) the proton which had been allylic to the terminal methylene group in (150) now resonated at 8.5τ.

Treatment of either (150) or (151) with Jones reagent gave a ketone (154, parent ion at m/e = 2?8, $\nu_{C=O}$ 1695 cm^{-1}). In the n.m.r. spectrum of (154) the deshielding effect of the carbonyl group was seen in the downfield shift of the peri aromatic proton (1H double doublet at 2.59τ, J_{ortho}=7Hz, J_{meta}=3Hz) relative to the other aromatic protons (2H multiplet at 3.10τ). Reduction with sodium borohydride of a sample of (154) prepared from either (150) or (151) gave a mixture in which both of the isomeric alcohols could be detected by T.L.C.

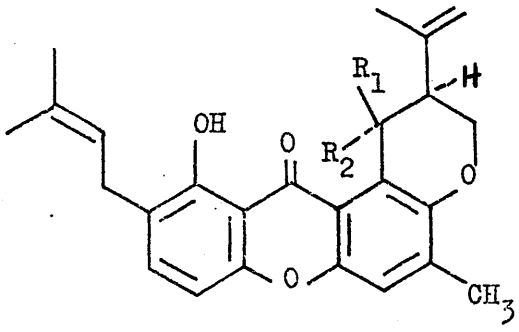
From this evidence it seems likely that arugoxanthone A (114) and arugoxanthone B (133) are derived from a common intermediate - the aldehyde (141).

Arugoxanthones A and B were detected by T.L.C. in extracts of the spores of the green mutant although they were present in smaller quantities relative to the other constituents than in yellow spore extracts. The green pigment has not so far been observed in any extracts. This may be due to its extreme polarity or to incomplete disruption of the spores resulting the pigment being trapped within them.

CHAPTER 5.

Table 19. U.V. Spectra of Arugoxanthone A and Isoarugoxanthone A.

Arugoxanthone A	λ_{max}:	242,	257(inf.),	270(inf.),	276,	297(inf.), 394 nm.
Isoarugoxanthone A	λ_{max}:	242,	256,	269(inf.),	275,	296, 394 nm.



(114a) R_1 = OH, R_2 = H

(114b) R_1 = H, R_2 = OH

Table 20. N.M.R. Spectra of Arugoxanthone A and Isoarugoxanthone A.

			Arugoxanthone A	Isoarugoxanthone A
Ar-OH	1H	s	-2.55	-2.50
Ar-H	1H	d, J=8Hz	2.55	2.60
Ar-H	1H	s	2.72	2.75
Ar-H	1H	d, J=8Hz	3.27	3.30
H geminal to O	1H	m	4.65	4.53
3,3-Dimethylallyl olefinic H	1H	t, J=6Hz	4.75	4.68
O-H	1H	m	5.02	5.45
Terminal methylene H	1H	s	5.26	4.95
Terminal methylene H	1H	s	5.47	5.20
$-OCH_2$	2H	m	5.69	5.60
$Ar-CH_2$	2H	d, J=6Hz	6.57	6.52
Allylic H	1H	m	7.35	7.50
$Ar-CH_3$	3H	s	7.72	7.65
Vinyl CH_3	3H	s	8.21	7.98
Vinyl CH_3	3H	s	8.30	8.20

The Mycelial Pigments of Aspergillus Nidulans.

Since only small amounts of arugoxanthones A and B, (114) and (133), were isolated from spore extracts the yellow mutant of Aspergillus nidulans was grown on surface culture in the hope that a more plentiful supply of the pigments might be obtained from this source. The mycelium was extracted with light petroleum and a preliminary fractionation of the resultant yellow oil carried out by column chromatography. P.L.C. of some of the initial fractions afforded a band which appeared, by T.L.C., to consist of arugoxanthone A. Subsequent crystallisation afforded material identical in all respects (melting point, Rf, spectral data) to the xanthone (114) obtained from spore extracts.

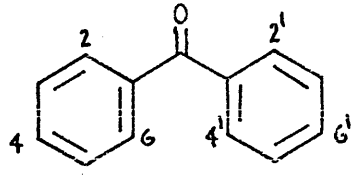
P.L.C., using multiple elution, of the residues from these crystallisations gave, apart from more arugoxanthone A, a yellow crystalline solid with an Rf (0.78, 100% chloroform) almost the same as that of arugoxanthone A (0.77, 100% chloroform). Mass spectral data (parent ion at m/e = 406) indicated that this compound was isomeric with arugoxanthone A and accordingly it was given the trivial name isoarugoxanthone A (114). The compound possessed ultraviolet (table 19) and infra-red ($\nu_{C=O}$ 1645 cm^{-1}) absorption consistent with it being a xanthone and an n.m.r. spectrum (table 20) very similar to that of arugoxanthone A, the main difference being that the vinyl methyl group and the terminal methylene protons resonated at lower field. From this evidence it appears that isoarugoxanthone A is a diastereoisomer of arugoxanthone A differing

Table 21. Ultraviolet Spectra of 2,2'-Dihydroxybenzophenones.

	λ_{max} (nm.)
Arugosin	280, 368
2,2',4'-Trihydroxy-4-methoxy-6,6'-dimethylbenzophenone[117]	292, 345
2,2',3-Trihydroxy-6'-methoxy-benzophenone[118]	270, 347

Table 22. Carbonyl Absorption of 2,2'-Dihydroxybenzophenones..

	ν_{max} (cm^{-1})
Arugosin	1625
2,2',4'-Trihydroxy-4-methoxy-6,6'-dimethylbenzophenone	1618
2,2',3-Trihydroxy-6'-methoxy-benzophenone	1620

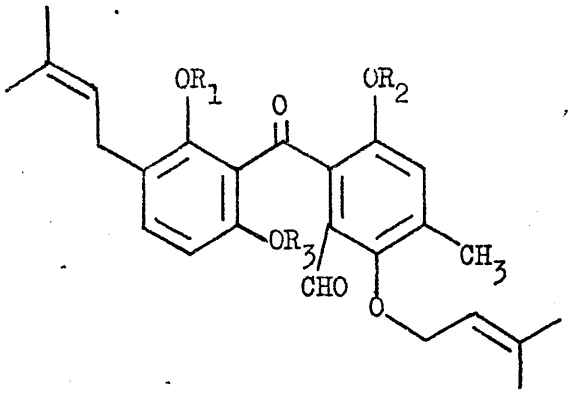


in the stereochemistry around the chroman-4-ol ring, i.e. arugoxanthone A and isoarugoxanthone A have structures (114a) or (114b). The relative stereochemistry in each case has not so far been elucidated.

P.L.C. of these initial fractions afforded two other yellow pigments. Crystallisation of the band containing the less polar of these compounds gave material identical (melting point, Rf, spectral data) to arugoxanthone B (133).

The ultraviolet spectrum (table 21) and carbonyl absorption in the ir. (table 22) of the more polar pigment, which was the major constituent of the extract, suggested that this compound - obtained as a viscous yellow oil ($C_{20}H_{18}O_5$, parent ion at m/e = 424) - was not a xanthone but a 2,2'-dihydroxybenzophenone (155). That it was in fact arugosin (143 and 144) was indicated by its possessing an n.m.r. spectrum identical to that previously reported[113] for this pigment when it was isolated from Aspergillus rugulosus. That arugosin is a mixture of the isomeric hemiacetals (143) and (144) is evident from the four exchangeable singlets (-3.37,$\frac{2}{5}$H; -2.78,$\frac{1}{5}$H; -1.09,$\frac{1}{5}$H; -0.87,$\frac{2}{5}$H) visible at low field which integrate in pairs, i.e. the first and second and the third and fourth, for one proton. The absence of any carbonyl absorption characteristic of a formyl group or of a low field non exchangeable singlet in the n.m.r. suggested that arugosin does not exist to any observable extent in the tautomeric form (156).

On treatment with either methyl iodide and potassium carbonate or dimethyl sulphate and potassium carbonate arugosin gave, not as previously reported/



(156) $R_1 = R_2 = R_3 = H$

(157) $R_1 = R_2 = R_3 = CH_3$

(158) $R_1 = H$, $R_2 = R_3 = CH_3$

Figure 30. Mass Spectrum of the Dimethyl Ether (158).

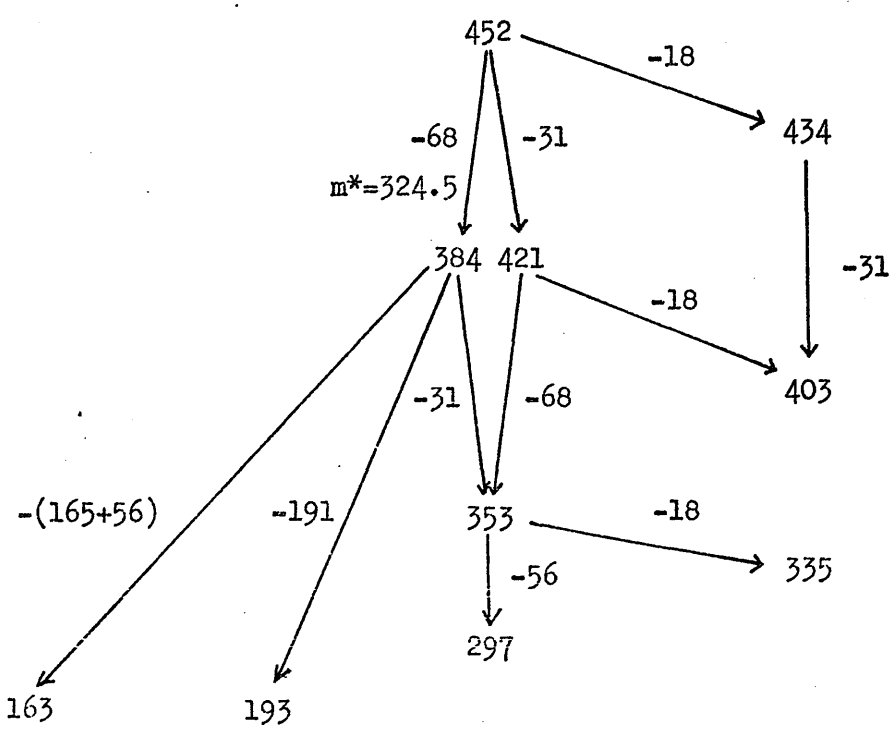


Figure 31. Mass Spectral Fragmentation of Compound (158).

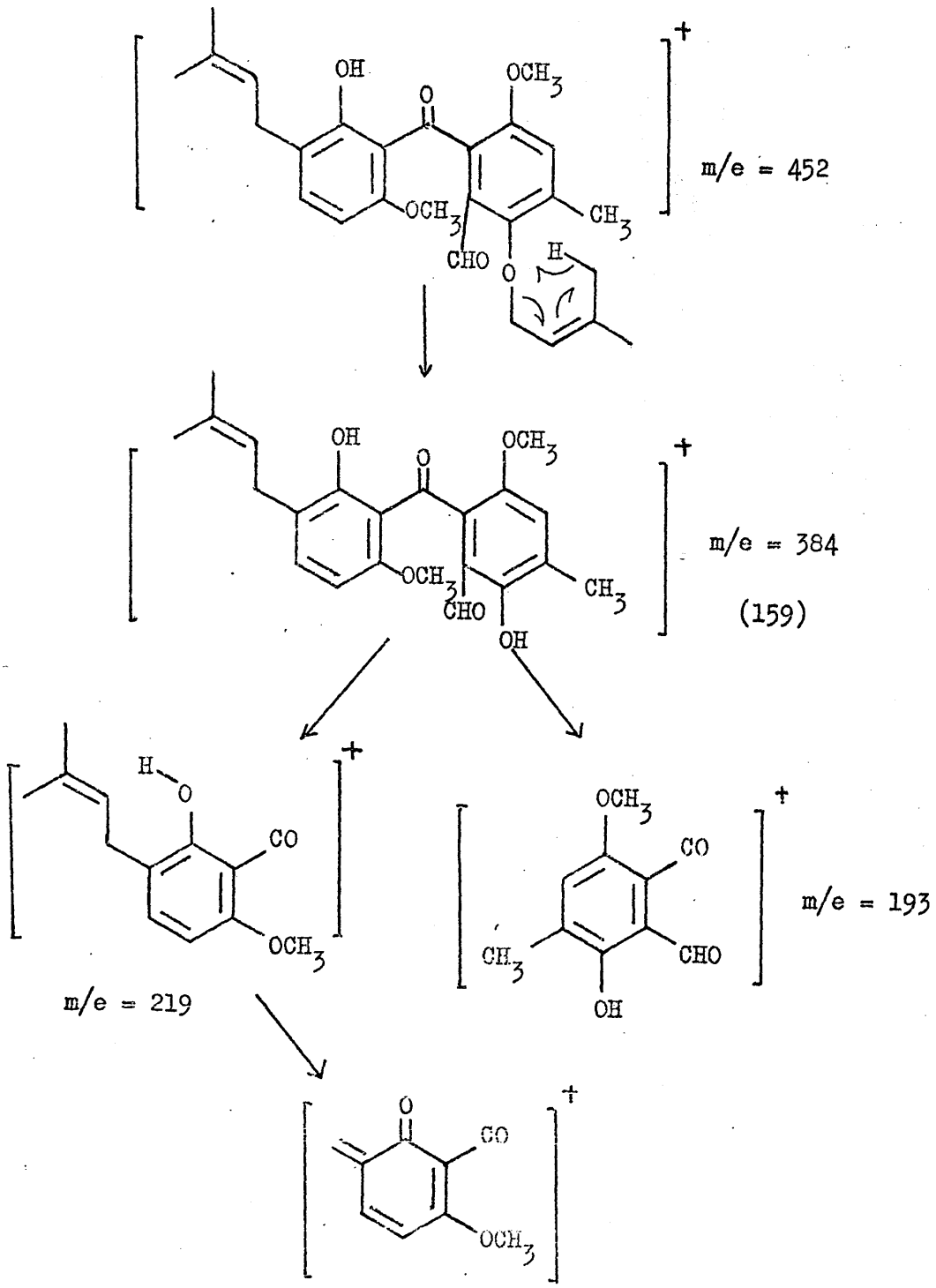
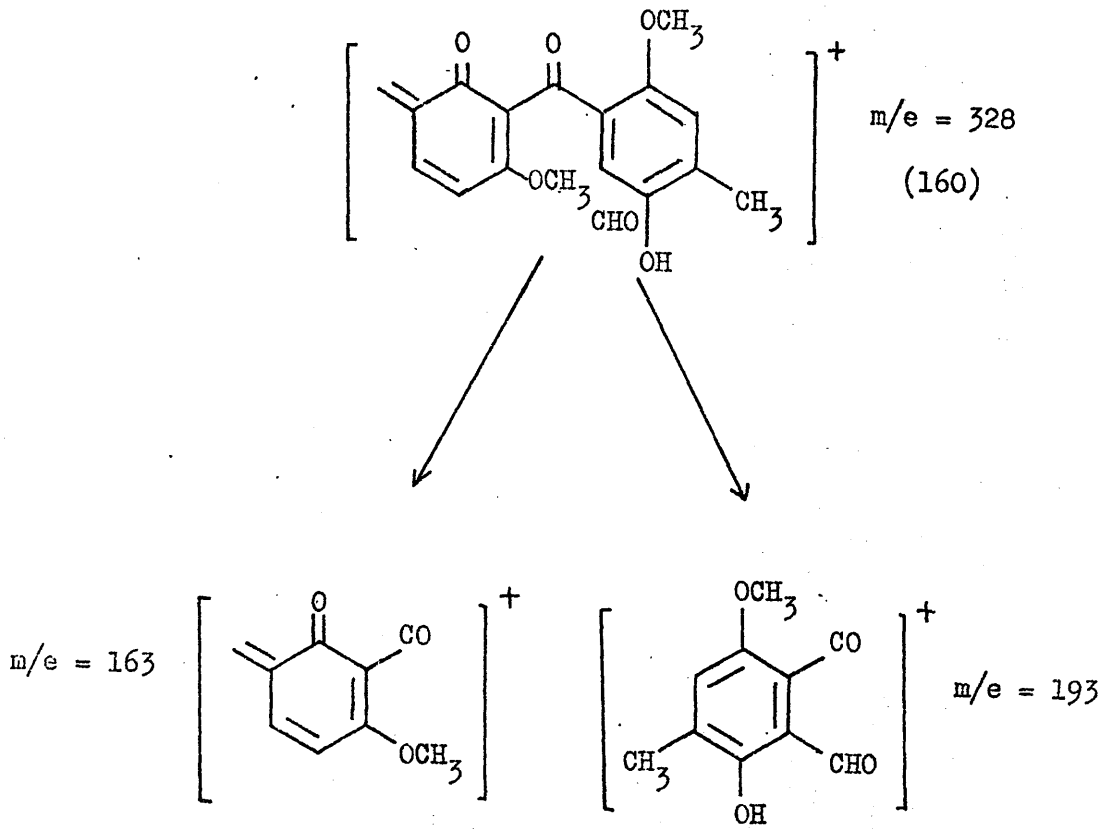
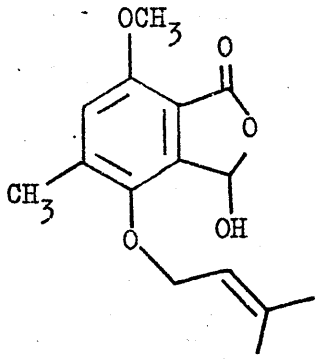


Figure 32.



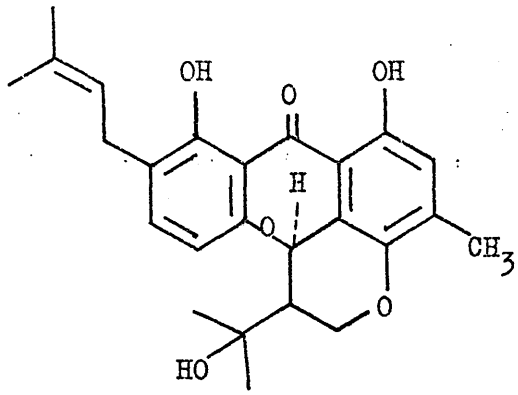
the trimethyl ether (157) but a dimethoxy benzophenone (158, parent ion at m/e = 452, $\nu_{C=O}$ 1610 cm^{-1}, 3H singlets at 6.36 and 6.74τ) which contained a formyl group ($\nu_{C=O}$ 1690 cm^{-1}, 1H non exchangeable singlet at -0.05τ). The unusually high values for the resonances of the methoxyl groups could be attributed to the shielding effect of the nearby aromatic rings of the benzophenone system. The mass spectrum of this compound (figure 30) showed losses consistent with the occurrence of the fragmentation processes shown in figure 31. An initial loss of 68 mass units would give the benzophenone (159) which would fragment to yield acylium ions[111], seen at m/e = 193 and 163. The observation of the ion at m/e = 193 is consistent with the presence in the dimethyl ether of a methoxyl group situated on the ring bearing the formyl group. The appearance of the ion at m/e = 163 could be explained by a fragmentation of (159) to yield an acylium ion at m/e = 219 followed by a facile loss of 56 mass units via the rearrangement process shown in figure 31 with the result that no significant peak was seen at m/e = 219. It may be that the rearrangement process occurs prior to the fragmentation (figure 32) resulting in the intermediacy of the benzophenone (160). Since, however, significant ions were not observed at m/e = 219 or 328 it was not possible to decide which fragmentation pathway was occurring. The rearrangement process, however, suggests the situation in the dimethyl ether of an aryl 3,3-dimethylallyl group ortho to a phenolic group. Consequently structure (158) was assigned to this compound and this was supported by a positive Gibbs test (λ_{max} 630 nm., ε = 30000).



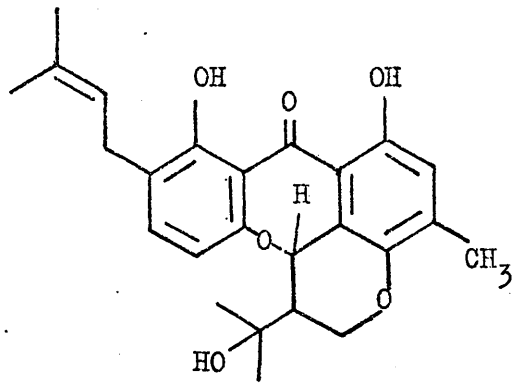
(161)

As mentioned above arugosin is a likely precursor of arugoxanthones A and B, (114) and (133), and therefore it ought to be possible to form these xanthones from arugosin in vitro. Since methoxy phenolic benzophenones are known to eliminate methanol under basic conditions[111] to give xanthones an attempt was made to exploit the unexpected formation of the dimethyl ether (158) in this way. On treatment, however, of (158) with 2% ethanolic potassium hydroxide the only product formed in good yield was a pseudo-acid (161, ν_{OH} 3380 cm^{-1}, $\nu_{C=O}$ 1750 cm^{-1}). The proton geminal to two oxygen atoms was seen in the n.m.r. as a singlet at 3.40τ which sharpened on shaking with D_2O. The formation of (161) was in accord with the structure assigned to the dimethyl ether (158). An attempt to form a xanthone from (158) under acid conditions - stirring in benzene with p-toluenesulphonic acid - was also unsuccessful, an intractable black gum being formed.

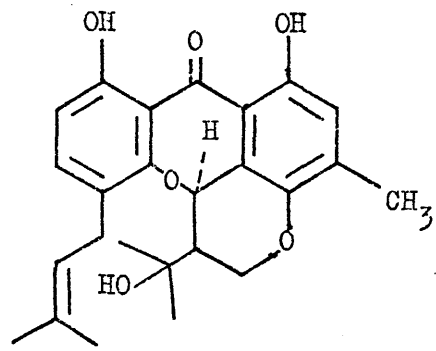
It has been reported[113] that arugosin can be converted into the xanthones anhydroarugosin (141) and deisopentenylanhydroarugosin (142) by passing through a column of activated silica. Attempts, however, to reproduce this result have been unsuccessful, the material being recovered unchanged. Arugosin has also been found to be stable to repeated P.L.C. on silica and to being refluxed in benzene with silica. The compound is, however, unstable to acid and attempts were made to find conditions under which (141) and (142) would be formed. Treatment of arugosin with p-toluenesulphonic acid in benzene gave a slow conversion to a large number of products while no reaction was observed



(162)



(163)



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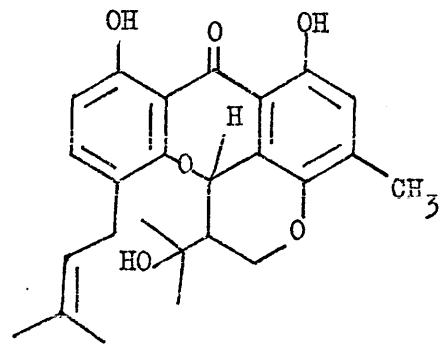
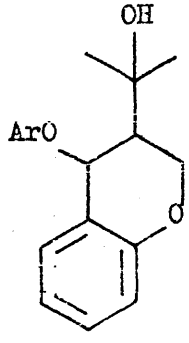


(166)

Table 23. N.M.R. Spectra of Compounds (162) and (163).

			τ (162)	τ (163)
Ar-OH	1H	s	-4.00	-3.75
Ar-OH	1H	s	-0.65	-0.60
Ar-H	1H	d, J=8Hz	2.75	2.75
Ar-H	1H	s	3.20	3.20
Ar-H	1H	d, J=8Hz	3.65	3.62
3,3-Dimethylallyl olefinic H	1H	t, J=6Hz	4.75	4.70
Ar-CH-O	1H	d, J=4Hz	4.95	4.95
Ar-O-CH_2	2H	m	5.40-5.90	5.60-5.80
Ar-CH_2	2H	d, J=6Hz	6.70	6.70
O-H	1H	s	7.30	8.35
Ar-O-C-C-H	1H	m	7.95	7.60
Ar-CH_3	3H	s	7.75	7.75
Vinyl CH_3	3H	s	8.23	8.22
Vinyl CH_3	3H	s	8.26	8.25
CH_3 geminal to O	3H	s	8.60	8.66
CH_3 geminal to O	3H	s	8.65	8.72

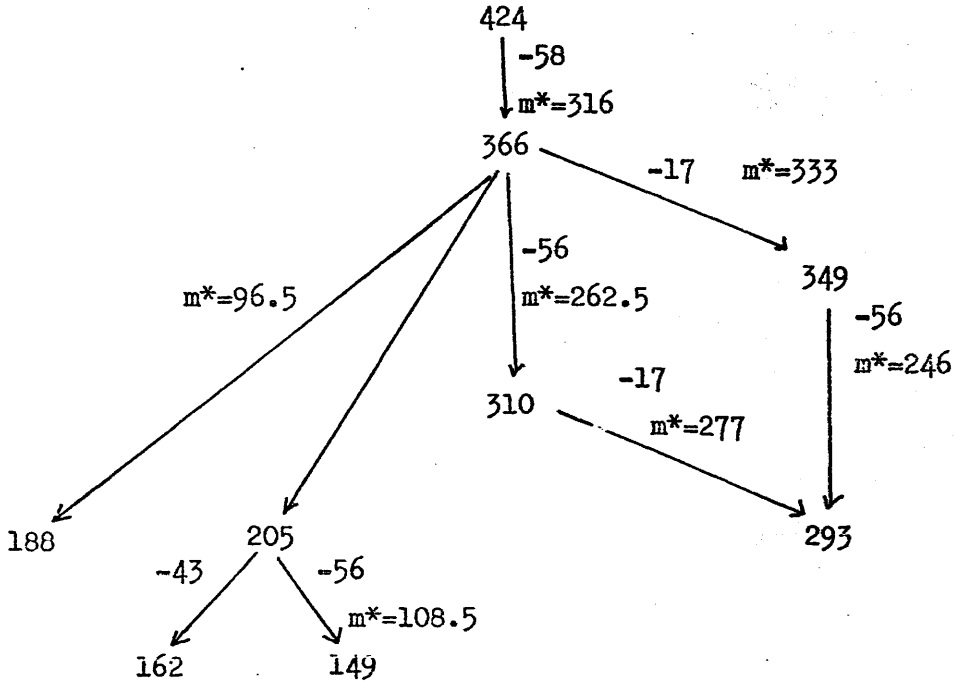


(164)

Table 24. Gibbs Test on (162) and (163).

	λ_{max} (nm.)	ε
(162)	632	31000
(163)	635	32000

Figure 33. Mass Spectrum of (162) or (163).



when it was stirred for 48 hours in ethereal sulphuric acid. When, however, arugosin was treated for a short time with a 3% solution of sulphuric acid in acetic acid three main products, isolable by P.L.C., were formed.

Two of these compounds, (162) and (163), were isomeric with arugosin (both showed parent ion at m/e = 424) and possessed almost identical polarity on T.L.C. (Rf 0.58 and 0.50 respectively, in 100% chloroform). Their infra-red spectra were very similar apart from the fingerprint regions and showed the presence in both of an alcoholic OH group (ν_{OH} ca. 3600 cm^{-1}) and a 2,2'-dihydroxybenzophenone group ($\nu_{C=O}$ 1610 cm^{-1}). Their n.m.r. spectra (table 23) showed the presence in each of: two chelated hydroxyl groups; a pair of aromatic protons situated ortho to each other at positions 3 and 4 or 4 and 5; a C-3,3-dimethylallyl group situated at position 3 or position 5; an aromatic proton at position 3'; and an aryl methyl group at position 4'. In addition there were signals evident which suggested the presence of a unit of structure (164), namely: a hydroxyl group; two methyl groups geminal to oxygen; a methylene group bonded to oxygen; and a proton geminal to the methylene group. Double irradiation revealed coupling between these protons appropriate to structure (164). From this evidence any of the isomeric structures (162), (163), (165), and (166) could be assigned to these compounds. Positive Gibbs tests (table 24), however, were observed for both of the isomers and so structures (162) and (163) were to be preferred.

The mass spectra (figure 33) of (162) and (163) were identical and

Figure 34. Mass Spectral Fragmentation of (162) or (163).

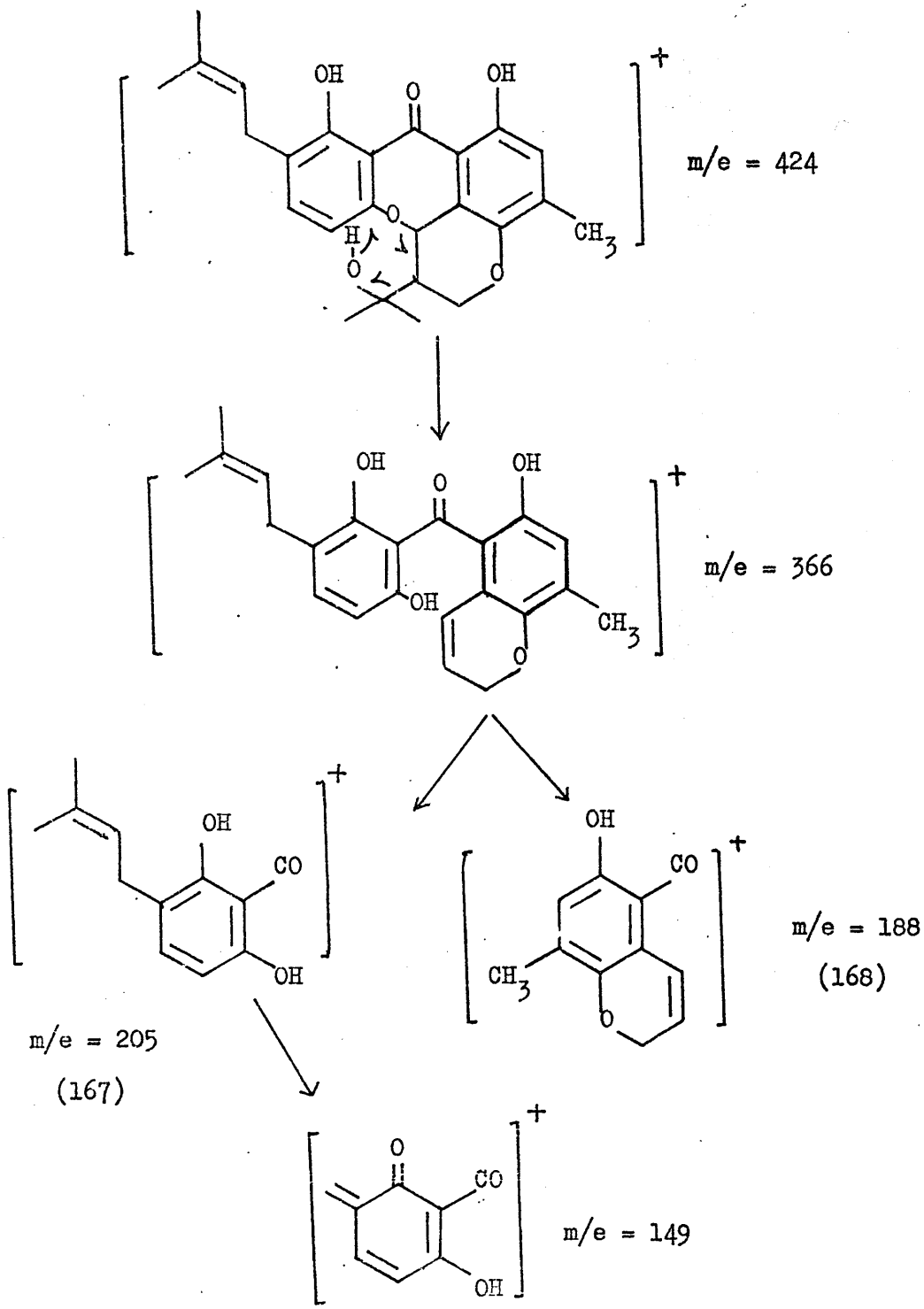
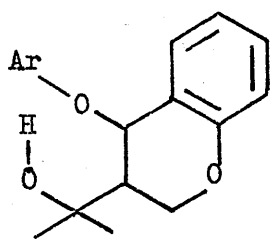
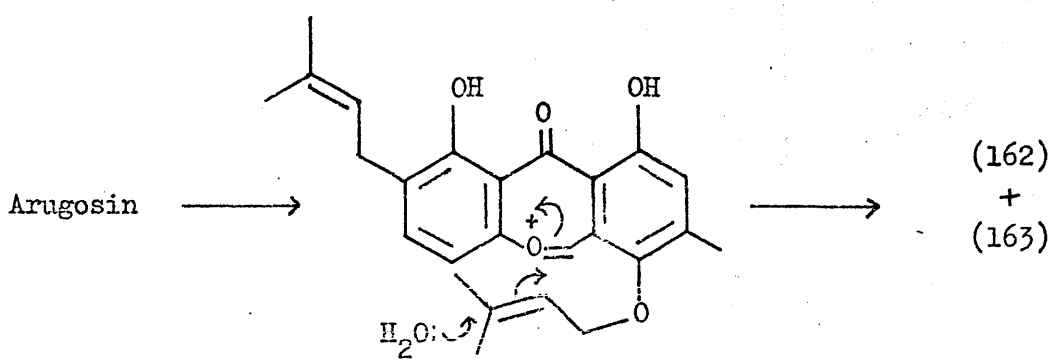


Table 25. Hydroxyl Absorption in (162) and (163).

	ν_{OH} (cm^{-1})
(162)	3615(w), 3580(m)
(163)	3620(m)

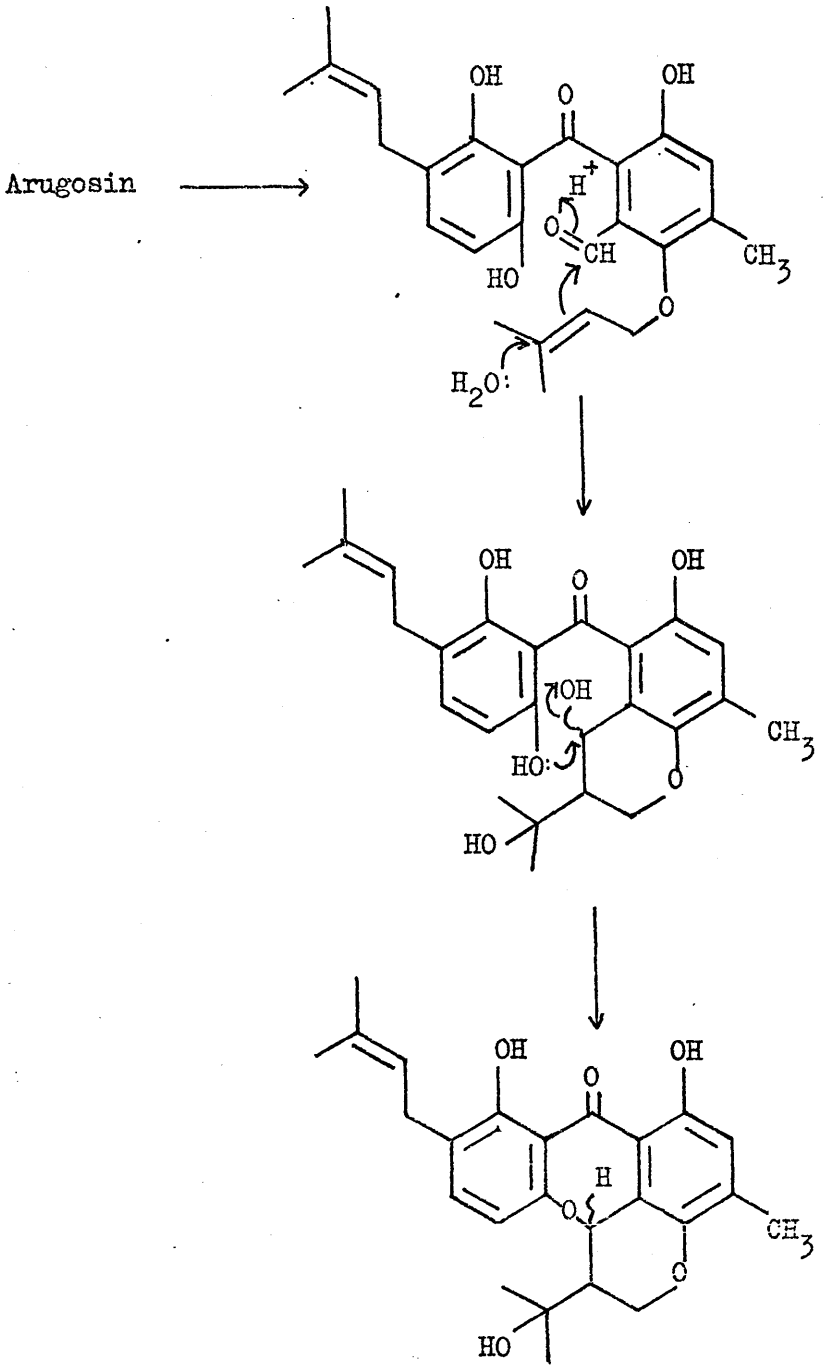
Figure 35.

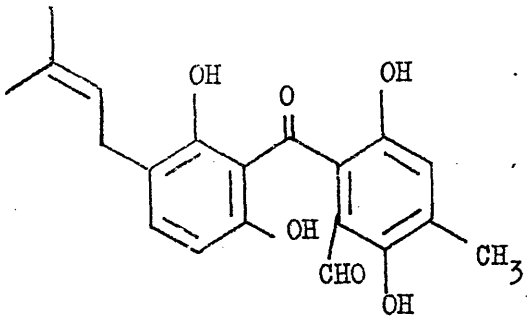




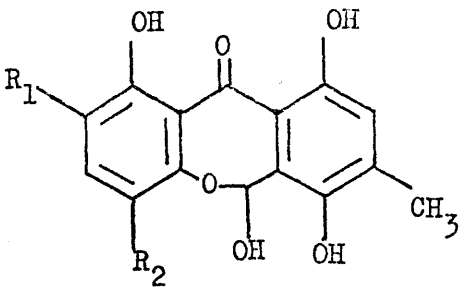
(169)

Figure 36. Formation of the Cyclic Products (162) and (163)



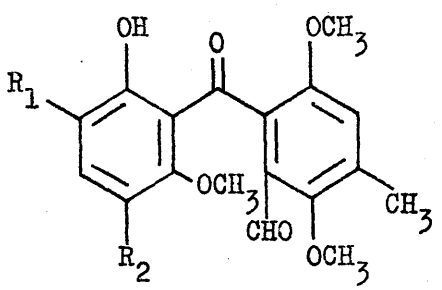


(170)



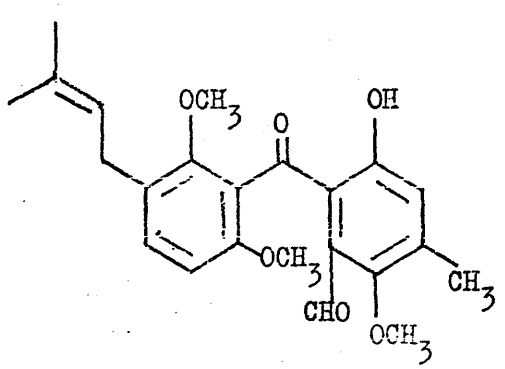
(171) $R_1 = CH_2CH.C(CH_3)_2$, $R_2 = H$

(172) $R_1 = H$, $R_2 = CH_2CH.C(CH_3)_2$



(173) $R_1 = CH_2CH.C(CH_3)_2$, $R_2 = H$

(174) $R_1 = H$, $R_2 = CH_2CH.C(CH_3)_2$



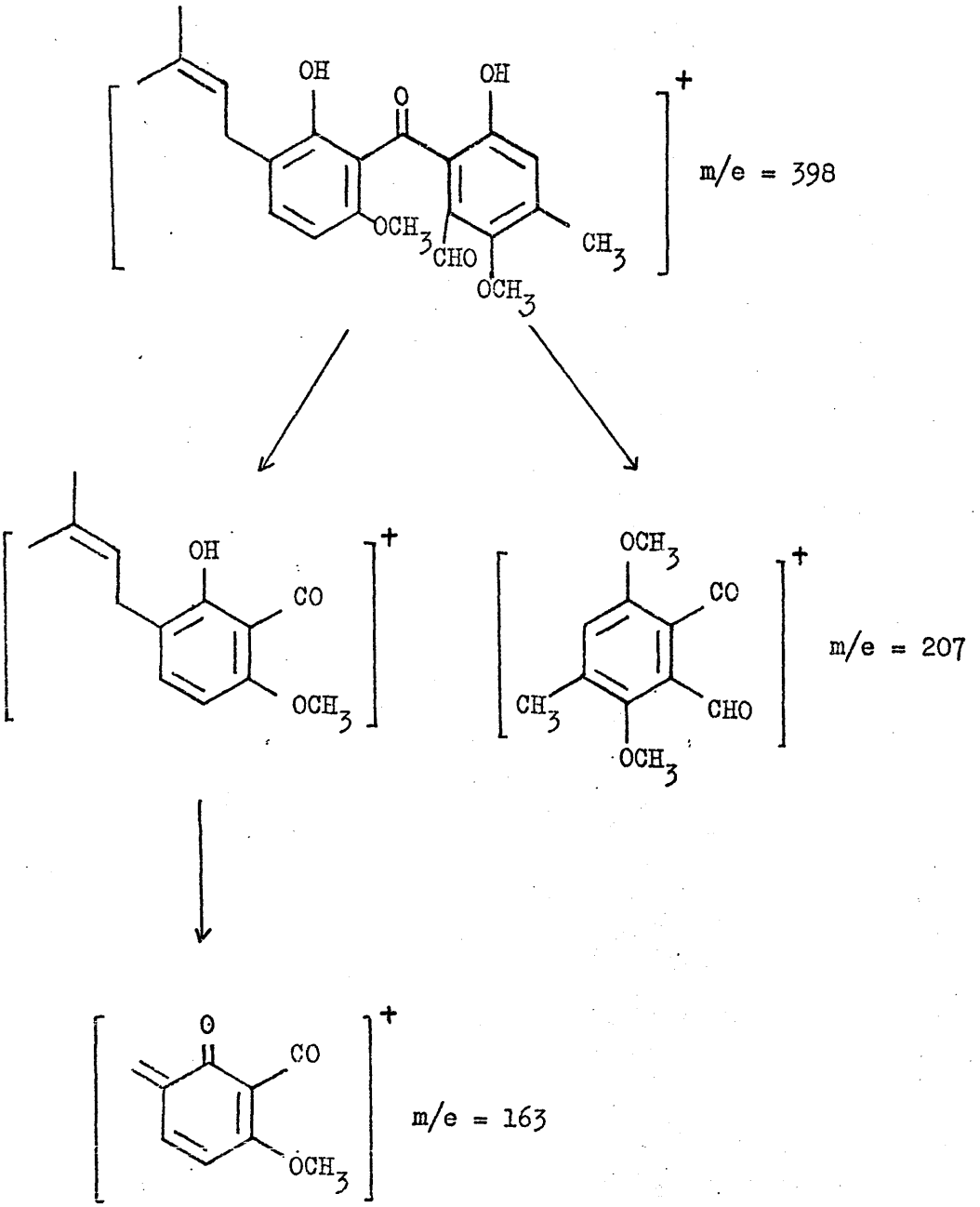
showed fragmentations, in accord with the formulations described above, corresponding to an initial loss of acetone (figure 34) to yield a benzophenone (m/e = 366) which fragments to give the acylium ions (167, m/e = 205) and (168, m/e = 188). The loss of a fragment of 56 mass units from the ions at m/e = 366 and 205 was characteristic of a 3,3-dimethyl-allyl group ortho to a hydroxyl group.

Intramolecular hydrogen bonding[119] of the alcoholic hydroxyl group to the ether oxygen atom (figure 35) was observed in the less polar isomer and not in the more polar compound (table 25). This hydrogen bonding was consistent with the trans configuration, i.e. structure (162), being given to the former and the cis configuration, i.e. structure (163), being given to the latter. These compounds presumably arise from arugosin by the intramolecular cyclisation process shown in figure 36. An alternative mechanism, however, involving cyclisation of the intermediate (169) cannot be excluded.

The major, and most polar, product of the reaction of arugosin with 3% sulphuric acid in acetic acid was deisopentenylarugosin (parent ion at m/e = 356). The n.m.r. spectrum showed low field signals consistent with this compound existing to the extent of about 60% in the tautomer (170) and about 40% in the hemiacetal forms (171) and (172). This was supported by the substantial carbonyl absorption seen at 1650 cm^{-1} in the infra-red spectrum due to the formyl group of (170).

On treatment with dimethyl sulphate and potassium carbonate deisopentenylarugosin formed a trimethyl ether (173, parent ion at m/e = 398, 3H singlets at 6.25, 6.35 and 6.65τ). Structure (173) was preferred

Figure 37. Mass Spectral Fragmentation of the Trimethyl Ether (173).

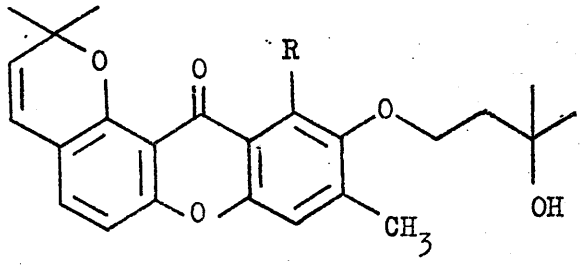


to the alternatives (174) and (175) on the grounds of the fragmentations observed in the mass spectrum of this compound (figure 37) and this was supported by the observation of a positive Gibbs test (λ_{max} 630 nm., ε = 23000).

An attempt to obtain deisopentenylanhydroarugosin (142) from deisopentenylarugosin (170) by refluxing the latter in dimethylformamide was unsuccessful and the starting material was recovered unchanged. Exposure of (170) to basic conditions (refluxing in acetone with potassium carbonate) resulted only in decomposition and (142) could not be detected in the reaction mixture by T.L.C.

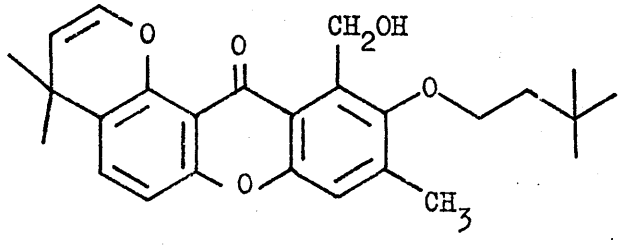
Table 26. Ultraviolet Spectrum of Arugoxanthone C (176)

λ_{max} (nm.): 238, 281, 319(inf.), 380.



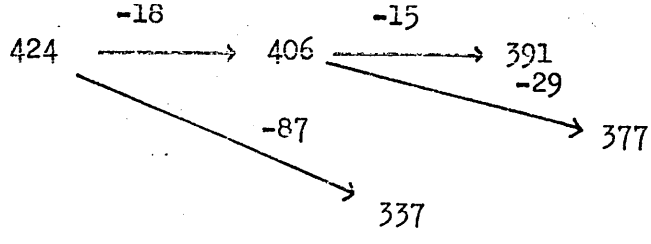
(176) R = CH_2OH

(178) R = CHO



(177)

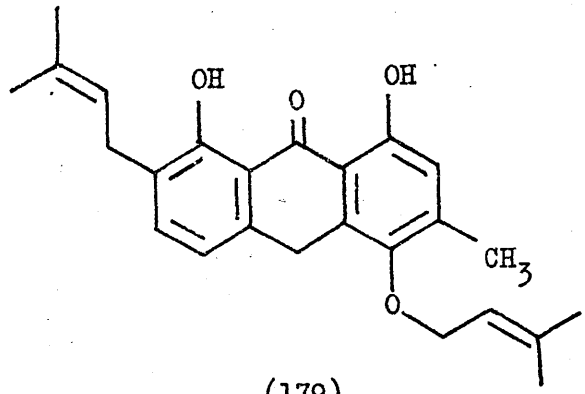
Figure 38. Mass Spectrum of Arugoxanthone C (176).



After the removal of arugoxanthones A and B, isoarugoxanthone A and arugosin from a column of the light petroleum extract continued elution produced material which consisted largely (T.L.C.) of two new pigments and these were purified by further chromatography and crystallisation.

The ultraviolet (table 26) and infra-red absorption ($\nu_{C=O}$ 1645 cm^{-1}) of the more polar compound - a crystalline yellow solid ($C_{25}H_{28}O_6$, parent ion at m/e = 424) - provided evidence that it was a xanthone and it was given the name arugoxanthone C (176). A negative ferric chloride test suggested that it was non phenolic in character and this was confirmed by the absence of any exchangeable low field signals in the n.m.r. and of a bathochromic shift in the uv. spectrum on addition of base. The following features were, however, evident in the n.m.r.: aromatic protons situated at positions 3 (1H doublet, J=8Hz, at 2.75τ), 4 (1H doublet, J=8Hz, at 3.20τ) and 5 (1H singlet at 2.85τ); an aryl methyl group at position 6 (3H singlet at 7.60τ); a hydroxymethyl group (3H broad singlet at 5.05τ which collapses on shaking with D_2O to a 2H singlet); and an O-3,3-dimethyl-3-hydroxy-1-propyl ether group (2H triplets, J=6Hz, at 5.90 and 7.95τ, 1H exchangeable multiplet at 7.30τ and 6H singlet ca. 8.5τ). The remaining resonances were consistent with the presence in arugoxanthone C of a dimethyl chromene unit (1H doublets, J=10Hz, at 3.70 and 4.40τ and 6H singlet ca. 8.5τ)[120]. From this evidence structure (176) was assigned to the metabolite - the isomeric structure (177) being discounted on biogenetic grounds. The mass spectrum (figure 38) of (176) showed losses of 18, (H_2O), and 87, ($C_5H_{11}O$), mass units from the

Figure 39. A Possible Biosynthetic Pathway to the Xanthones Isolated from Aspergillus Nidulans.



(179)

Arugosin

Anhydroarugosin

Arugoxanthone A

Arugoxanthone B

Arugoxanthone C

Arugoxanthone D

molecular ion consistent with the presence of the O-3,3-dimethyl-3-hydroxy-1-propyl ether group. Although many modifications of the dimethylallyl unit have been observed in natural products to our knowledge this group appears to be unique and may arise by reduction of an epoxide.

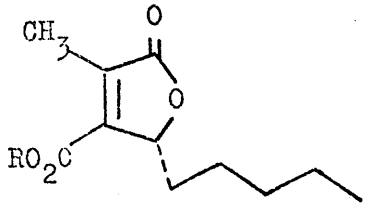
The less polar pigment (178) was obtained as a yellow oil (parent ion at m/e = 422), the n.m.r. spectrum of which was identical to arugoxanthone C except that no hydroxymethyl group was in evidence. A non exchangeable 1H singlet could be seen at -0.77τ suggesting the presence of a formyl group at position 8 and this was supported by the characteristic carbonyl absorption ($\nu_{C=O}$ 1700 cm^{-1}) seen in the ir. Accordingly this compound, which possessed infra-red spectral data consistent with it being a xanthone ($\nu_{C=O}$ 1650 cm^{-1}) was assigned structure (178) and called arugoxanthone D.

The co-occurrence of two pigments, (176) and (178), containing a dimethyl chromene unit with arugoxanthones A and B provides strong circumstantial evidence in favour of the structures (114) and (133) assigned to the latter pair of compounds in which the isopentenyl group is situated ortho to the hydroxyl group.

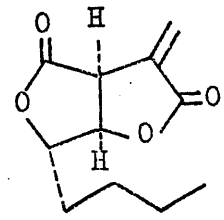
The xanthones isolated from this mould are all transformation products of the octaketide derived metabolite arugosin and a possible biosynthetic pathway to these compounds is shown in figure 39. It seems likely that arugosin itself is formed oxidatively from an anthrone, such as (179), by a process analogous to that proposed for the biosynthesis

of the ergochromones by Franck[121]. It is interesting to note that in the case of arugoxanthones B and C, (133) and (176), reduction has occurred at a late stage in their biosynthesis - in the latter case at two different sites - following oxidative steps.

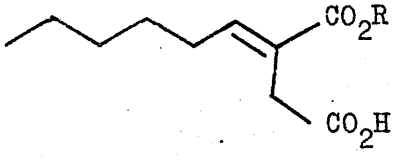
CHAPTER 6.



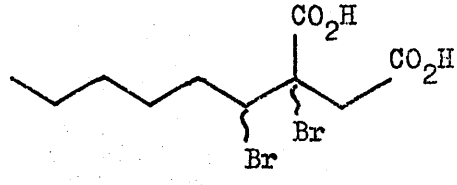
(180) R = H
(182) R = CH_3



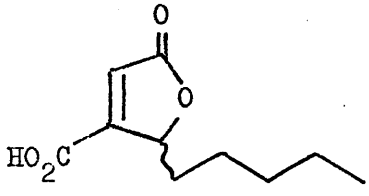
(181)



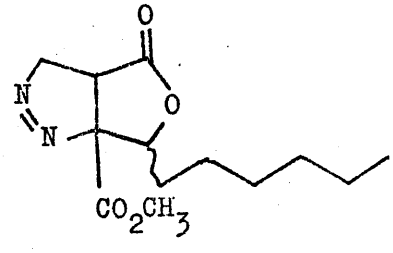
(183) R = CH_2CH_3
(184) R = H



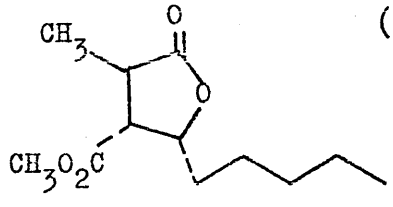
(185)



(186)



(187)



(188)

A Synthesis of Methyl Isocanadensate.

Isocanadensic acid (180), a possible biosynthetic precursor of the antibiotic canadensolide (181) - produced by the mould Penicillium canadense[122] -, has been isolated, in small quantities, from the same source[123] and purified and characterised as its methyl ester (182). A synthesis of (182) was undertaken in order to confirm the structural assignment made.

Condensation of diethyl succinate with n-hexanal in dry tertiary-butanol, using potassium tertiary-butoxide as base, gave the half ester (183) as a red oil. This was not purified but hydrolysed with 1N sodium hydroxide to yield 3-carboxy-3-nonenoic acid[124] (184) (ν_{OH} 3400-2500 cm^{-1}, $\nu_{C=O}$ 1720, 1700 cm^{-1}). Irradiation of (184) and bromine in carbon tetrachloride with a wide spectrum ultraviolet lamp gave the dibromo compound[125] (185) (ν_{OH} 3400-2500 cm^{-1}, $\nu_{C=O}$ 1730 cm^{-1}) and this on treatment with 1N sodium hydroxide afforded 5-n-pentylaconic acid (186) (ν_{OH} 3400-2600 cm^{-1}, $\nu_{C=O}$ 1740, 1710 cm^{-1}, $\nu_{C=C}$ 1630 cm^{-1}). Allylic coupling between H-3 (1H doublet, J=2Hz, at 3.2τ) and H-5 (1H multiplet at 4.7τ) was evident in the n.m.r.

Reaction of (186) with a large excess of ethereal diazomethane gave the pyrazoline[126] (187) which, on pyrolysis at 90°C, yielded methyl isocanadensate (182) identical in all respects save optical rotation with material obtained from natural sources.

Methyl dihydroisocanadensate (188) has also been obtained from

Penicillium canadense[123]. An attempt, however, to prepare it from (182) by reaction of the latter with zinc and acetic acid[127] was unsuccessful and the starting material was recovered unchanged.

EXPERIMENTAL.

Instrumentation.

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Ultraviolet spectra were obtained on a Unicam SP 800 recording spectrophotometer. Infra-red spectra were measured with Perkin-Elmer SP 220 and SP 257 spectrometers. Nuclear magnetic resonance spectra were recorded with Varian T-60 and HA-100 MHz spectrometers using tetramethylsilane as an internal standard. Routine mass spectra were obtained with an A.E.I. MS-12 mass spectrometer and high resolution mass spectra on an A.E.I. MS-9 mass spectrometer.

Thin Layer Chromatography (T.L.C.).

Rf values were determined from elution on 0.25 mm. layers of Kieselgel GF_{254}, the compounds being located under ultraviolet light and by spraying with a 5% solution of ferric chloride in methanol and with a 1% solution of ceric ammonium nitrate in 5N sulphuric acid and heating. The colours developed by these sprays are recorded thus:
T.L.C. Rf 0.45 (100% chloroform); $FeCl_3$: blue; Ce^{4+}: brown.
Numerical values quoted are indications of relative polarity and are not quantitative reproducible results. Preparative layer chromatography (P.L.C.) was carried out on 1 mm. layers of Kieselgel HF_{254}, bands being located by irradiation with ultraviolet light (λ = 254 nm.).

Counting of Radioactive Materials.

Radioactive assays were carried out using a Phillips liquid

scintillation counter. In experiments with doubly labelled precursors the external channel ratio method was used.

Solid samples were weighed on metal foil, transferred to Packard scintillation vials and dissolved in toluene scintillation solution (15 ml.): 2,5-diphenyloxazole (6g.); 1,4-bis-2-(4-methyl-5-phenyl-oxazalyl)-benzene (0.1g.); toluene (1 litre). Aqueous solutions were counted in a mixture of toluene scintillator solution (13.5 ml.), biosolv. BBS-2 solution (1 ml.) and distilled water (0.5 ml.). Samples were counted for sufficient time to achieve a counting error of less than $\pm 3\%$.

General.

Diazomethane was prepared by the method of Moore and Reed from bis-(N-methyl-N-nitroso)-terephthalimide.[128] Jones reagent refers to an aqueous solution containing chromium trioxide (266 mg./ml.) and concentrated sulphuric acid (405 mg./ml.). Active manganese dioxide was prepared by the method of Attenburrow et al.[129] Hydrogenation experiments were carried out at 15°C and under 1 atmosphere of hydrogen. All organic extracts were routinely washed with a saturated brine solution and dried over anhydrous magnesium sulphate. Solvents were removed using a rotary film evaporator. Light petroleum refers to a light petroleum fraction b.pt. 40-60°C.

The following abbreviations are used in reporting spectral data: in N.M.R. spectral data: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; d.d, double doublet.

in I.R. spectral data: s, strong; m, medium; w, weak; br, broad.

in U.V. spectral data: inf., point of inflection.

In the following typical description of N.M.R. spectral data: 4.8(1H,d,J=4Hz,irr.7.6→s,H-2);, irradiation at 7.6τ has resulted in the collapse of the 1H doublet at 4.8τ to a singlet.

EXPERIMENTAL.

CHAPTER 1.

Culture Medium for Verticillium Lamellicola.

Glucose	50g.
Sodium nitrate	2g.
Potassium chloride	1g.
Magnesium sulphate	1g.
Potassium hydrogen phosphate	0.5g.
Ferrous sulphate	0.01g.
Distilled water	1 litre.

The resulting fluid medium had ph = 6.6.

Growth and Extraction of the Mould.

The mould was maintained on 2% malt agar slants and seed grown on the same medium in Roux bottles (15x9 cm.). A spore suspension could be prepared by adding distilled water (10 ml.) to one of these bottles and gently scraping the surface of the agar. The suspension from 12 bottles was decanted off, made up to 2l. with distilled water and used to inoculate 100 Roux bottles which had been previously sterilised and contained 200 ml. each of the medium described opposite. The mould was grown at 75% humidity and 25°C and cropped off after 28 or 10 days.

The broth was extracted with methylene chloride in a continuous liquid-liquid extraction apparatus for 48 hours and the solvent evaporated to give the crude broth extract (0.6g.).

Antibacterial Assays.

The 7-21 days old surface growth of a 5 ml. agar slant culture of Bacillus subtilis, in a test tube, was scraped into 5 ml. of sterile distilled water. The suspension was then shaken and added to 250 ml. of Bouillon agar which had been melted and cooled to approximately 40°C. The inoculum was throughly mixed with the agar, and 20 ml. aliquots pipetted into 10 cm. flat-bottomed Petri dishes and the agar allowed to set. 5 µl. of a solution of the substance under test (1 mg./ml.) was pipetted onto a circle of filter paper (diameter 1.3 cm.) and the solvent allowed to evaporate. The disc was then placed on the surface of the agar and the plates incubated for 18 hours at 30°C. The diameter of the inhibition zone produced was then measured.

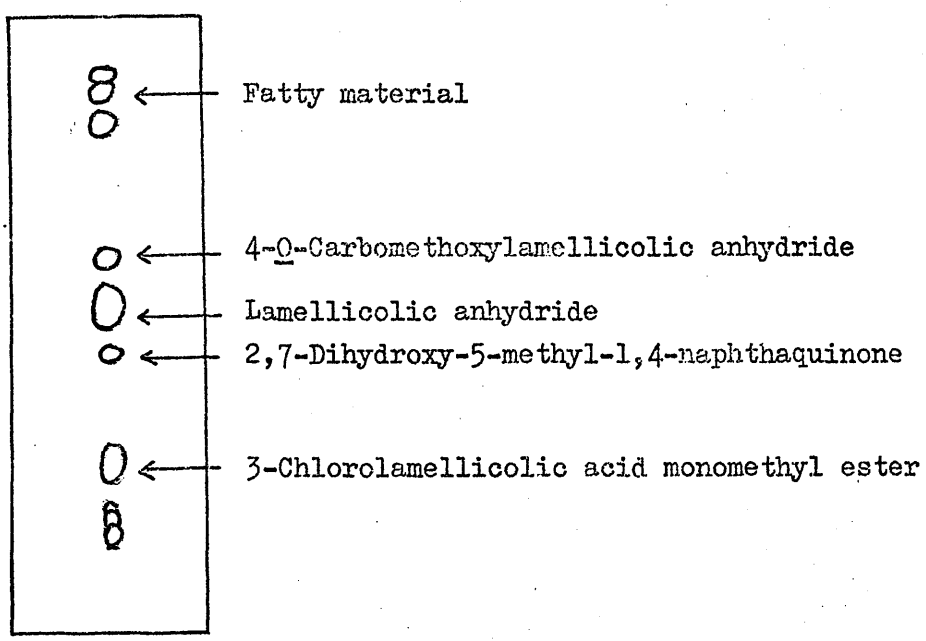
Summary of Chromatographic Separation of the V. Lamellicola Metabolites.

Fractions	Eluting solvent	Constituents.	Diameter of inhibition zone. (cm.)
1-6	chloroform and 1% ethyl acetate - chloroform.	non polar oils	0
7-9	2% ethyl acetate chloroform	4-O-carbomethoxy-lamellicolic anhydride; lamellicolic anhydride.	4.9
10-19	5-25% ethyl acetate-chloroform.	lamellicolic anhydride; 3-chlorolamellicolic acid monomethyl ester; unidentified minor metabolites.	7.8
20-22	40% ethyl acetate-	2,7-dihydroxy - 5-methyl- 1,4-naphthaquinone.	3.4
23-30	50-100% ethyl-acetate.	polar oils	2.9

Diameter of inhibition zone of crude extract = 5.6 cm.

T.L.C. of Crude Methylene Chloride Extract of V. Lamellicola.

Eluting solvent: 10% methanol-chloroform



Chromatographic Fractionation of the Metabolites of Verticillium Lamellicola.

Crude dry methylene chloride extract (6g.) of the broth in chloroform (50 ml.) was adsorbed on silica (25g.), evaporated under reduced pressure, finely powdered, and introduced onto a column of silica (600g., 120x5 cm.) made up in chloroform (600 ml.). Separation was achieved by elution with chloroform, 1, 5, 10, 25, 40, 60% ethyl acetate-chloroform mixtures and finally ethyl acetate using one column volume of each successively. Fractions (200 ml.) were collected and monitered by T.L.C.

Fractions 1-6 were combined to give a light green oil which was inactive. Since the i.r. spectrum indicated that this material was fatty in character (ν_{max} 2960, 1740, 1170 cm^{-1}) it was set aside and no further work carried out on it.

An enhancement of activity over that of the crude broth extract was evident in the material obtained by bulking fractions 10-19. Crystallisation from methanol-chloroform gave a solid which was shown by T.L.C. to consist of two components. Further crystallisation gave a pure sample of the major component- lamellicolic anhydride (600 mg.). P.L.C. of the residue from these crystallisations using ethyl acetate as eluting solvent afforded the minor component- 3-chlorolamellicolic acid monomethyl ester (10 mg.). After the removal of these two compounds the total residue consisted of a gummy red oil from which no other compound could be isolated in a pure state. T.L.C. of this oil using a variety of

different eluting solvents failed to show the formation of any discrete spots.

P.L.C. (2% methanol-chloroform) of material obtained from fractions 7-9 allowed 4-O-carbomethoxylamellicolic anhydride to be obtained in a pure state (50 mg.).

When fractions 20-22 were combined and separated by further chromatography (P.L.C., ethyl acetate) 2,7-dihydroxy-5-methyl-1,4-naphthaquinone was obtained (40 mg.).

The four phenolic metabolites mentioned above could be rapidly separated from the fatty material by, prior to fractionation, extracting the crude material with aqueous sodium hydrogen carbonate. Acidification of the alkaline extract at $0^{\circ}C$, followed by extraction with chloroform and evaporation gave a solid which by T.L.C. was a mixture of phenolic compounds. The acid lability, however, of 4-O-carbomethoxylamellicolic anhydride and of the chloro-compound prevented this technique being used as a means for the rapid separation of the phenolic compounds from the fats.

Lamellicolic Anhydride (53).

Lamellicolic anhydride (4-methyl-2,5,7-trihydroxy-1,8-naphthalic anhydride) was isolated as described above and crystallised from methanol-chloroform as pale yellow needles which slowly decomposed above 300°C. The compound dissolved freely in dilute aqueous sodium hydrogen carbonate and could be recovered by reacidification.

T.L.C. Rf 0.35 (10% methanol-chloroform); U.V.: blue-green; $FeCl_3$: brick red; Ce^{4+}: brown.

I.R. ν_{max}(KBr): 3260(br.w), 3070(w), 2920(w), 2850(w), 1700(s), 1650(s), 1615(s), 1595(s), 1465(m), 1420(m), 1345(m), 1320(m), 1230(m), 1175(s), 1030(s), 880(m), 850(m), 810(m), 780(m), 755(m) cm^{-1}.

U.V. λ_{max}(EtOH): 250(15000), 292(5700), 352(10000), 368(inf.)(7800) n.m.

λ_{max}(EtOH+$^-$OH): 251(inf.)(15000), 314(23000), 372(11000) n.m.: reverting to EtOH spectrum on acidification.

N.M.R. (100 MHz) τ (d_6DMSO): 3.20(1H,s,irr.7.26→-sharpening,H-6); 3.65(1H,s,H-3); 7.26(3H,s,Ar-CH_3).

(60MHz) τ ($d_6$acetone): 3.25(1H,s,H-6); 3.55(1H,s,H-3); 7.20 (3H,s,Ar-CH_3).

M.S. m/e(rel.abundance): 260(M^+)(100), 242(8), 216(38), 214(8), 188(8), 160(29), 131(2), 128(8), 103(8), 102(8).

HIGH RESOLUTION M.S. molecular ion at m/e = 260.0323; $C_{13}H_8O_6$ requires molecular ion at m/e = 260.0320.

ANALYSIS. Found: C, 58.49; H, 3.57%; $C_{13}H_8O_6$ requires C, 60.01; H, 3.10%.

Methylation of Lamellicolic Anhydride.

1) Reaction with diazomethane.

Lamellicolic anhydride (140 mg.), in methanol (10 ml.), was allowed to stand overnight with a large excess of ethereal diazomethane (5.14g. nitrosan). Ether (50 ml.) was added, the mixture filtered through glass paper and evaporated to give a mixture of a large number of components, none of which could be obtained in a pure state.

2) Reaction with dimethyl sulphate.

Lamellicolic anhydride (100 mg.), in dry acetone (10 ml.), was refluxed and stirred under nitrogen with anhydrous potassium carbonate (100 mg.) and excess dimethyl sulphate for 48 hours. Chloroform (100 ml.) was added, the mixture washed with dilute hydrochloric acid (2x50 ml.) and water (2x50 ml.) and evaporated. P.L.C. (2% methanol-chloroform) yielded two fractions of which the more polar crystallised from methanol-chloroform giving the trimethyl ether (54) as colourless needles which melted slowly above 290°C (81 mg., 69%).

T.L.C. Rf 0.27 (5% methanol-chloroform); U.V.: light blue; $FeCl_3$: negative; Ce^{4+}: brown.

I.R. ν_{max}(KBr): 3010(w), 2930(w), 2850(w), 1750(s), 1715(s), 1595(s), 1562(s), 1465(m), 1450(m), 1358(s), 1295(s), 1245(m), 1218(s), 1172(m), 1064(s), 1042(s), 1010(s), 966(m), 817(m), 743(m), 730(w) cm^{-1}.

ν_{max}($CHCl_3$): 1750(s), 1715(s), 1595(s), 1562(s), 1460(m), 1355(m), 1295(s), 1065(m), 1040(s), 1010(s) cm^{-1}.

U.V. λ_{max}(EtOH): 227(5900), 252(13000), 261(inf.)(12000), 346(5800),

363(4900), 380(3800) n.m.

λ_{max}(EtOH+$^{-}$OH): 255(15000) n.m.: reverting to EtOH spectrum on acidification.

N.M.R. (100 MHz) τ (TFA): 2.73(1H,s,H-6); 3.14(1H,s,H-3); 5.71(6H,s,O-CH_3); 5.73(3H,s,O-CH_3); 6.94(3H,s,Ar-CH_3).

(100 MHz) τ ($CDCl_3$): 3.12(1H,s,H-6); 3.51(1H,s,H-3); 5.87(6H,s, O-CH_3); 5.90(3H,s,O-CH_3); 7.14(3H,s,Ar-CH_3).

Nuclear Overhauser Effect. Irradiation at 6.94τ produced 11% and 37% enhancements of the signals at 2.73τ and 3.14τ.

M.S. m/e(rel.abundance): 302(M^+)(100), 287(13), 258(27), 229(22), 228(24), 195(10).

High Resolution M.S. molecular ion at m/e = 302.0803; $C_{16}H_{14}O_6$ requires molecular ion at m/e = 302.0790.

Analysis. Found: C, 63.88; H, 4.73%; $C_{16}H_{14}O_6$ requires C, 63.57; H, 4.67%.

The less polar fraction, consisting of the monomethyl ether (55), crystallised from chloroform as colourless needles which melted slowly above 260°C (12 mg., 10%). This material was identical in all respects (Rf, spectral data, mixed melting point) with a synthetic sample supplied by Dr. B.W. Bycroft.

T.L.C. Rf 0.48 (2% methanol-chloroform); U.V.: green-blue; $FeCl_3$: blue; Ce^{4+}: grey.

I.R. ν_{max}($CHCl_3$): 3120-2800(v.br.), 2990(w), 2920(w), 2850(w), 1710(s), 1670(s), 1620(s), 1605(s), 1300(s), 1040(s), 990(m) c.m.$^{-1}$

ν_{max}(KBr): 3080(w), 2980(w), 1710(s), 1665(s), 1625(s), 1605(s),

1470(m), 1455(m), 1390(m), 1340(m), 1305(s), 1215(s), 1180(s), 1165(s), 1035(s), 985(m), 810(s), 755(m) cm^{-1}.

U.V. $\lambda_{max}(CHCl_3)$: 254(9900), 285(inf.)(4100), 290(4200), 350(8900), 367(6700) n.m.

N.M.R. (100 MHz) τ (TFA): 2.94(1H,s,H-6); 3.29(1H,s,H-3); 5.85(3H,s, O-CH_3); 7.10(3H,s,Ar-CH_3).

M.S. m/e(rel.abundance): 274(M^+)(100), 230(66), 187(33), 185(22), 174 (61), 159(22), 157(22), 131(22), 116(22), 115(22).

High Resolution M.S. molecular ion at m/e = 274.0476; $C_{14}H_{10}O_6$ requires molecular ion at m/e = 274.0477.

Selective Demethylation of O,O,O,-Trimethyllamellicolic Anhydride (53): 4-O-Methyllamellicolic Anhydride (55).

Magnesium iodide-etherate was prepared by adding magnesium turnings (0.67g.) and iodine (3.34g.) to a mixture of dry ether (4.2 ml.) and dry benzene (8.35 ml.). The filtered solution (0.125 ml.) was added to a stirred suspension of O,O,O-trimethyllamellicolic anhydride (40 mg.), in dry benzene (10 ml.), under nitrogen. The mixture was refluxed for three hours, acidified, and chloroform (50 ml.) added. After washing with aqueous sodium metabisulphite and water evaporation gave a product which on P.L.C. (5% methanol-chloroform) yielded material identical in all respects with 4-O-methyllamellicolic anhydride (55), (16 mg., 44%).

Acetylation of Lamellicolic Anhydride.

Lamellicolic anhydride (100 mg.), in acetic anhydride (20 ml.) and pyridine (0.01 ml.), was stirred overnight at room temperature. Ethyl acetate (100 ml.) was added and the solution washed with cold dilute hydrochloric acid (2x50 ml.). After washing to neutrality, evaporation gave a yellow solid which on crystallisation (methanol-chloroform) afforded the triacetate (56) as colourless prisms, m. 177-179°C (73 mg., 49%).

T.L.C. Rf 0.32 (2% methanol-chloroform); U.V.: light blue; $FeCl_3$: negative; Ce^{4+}: brown.

I.R. ν_{max}(KBr): 3060(w), 2960(w), 1775(s), 1730(s), 1598(s), 1580(s), 1468(w), 1370(m), 1355(m), 1278(m), 1172(s), 1158(s), 1075(m), 1058(m), 1035(s), 1010(m), 912(m), 688(w) cm^{-1}.

U.V. λ_{max}(EtOH): 248(5700), 341(2100) n.m.

λ_{max}(EtOH+$^-$OH): 250(inf.)(3900), 316(4600), 372(2700) n.m.

λ_{max}(EtOH+H^+): 252(3700), 292(inf.)(1500), 353(2400), 366(inf.) (1800) n.m.

N.M.R. (60 MHz) τ ($CDCl_3$): 7.18(3H,s,Ar-CH_3); 7.51(9H,s,Ar-O-CO-CH_3). A signal at 2.75τ (H-3 and H-6) was obscured by the chloroform signal.

(60 MHz) τ (d_6DMSO): 2.38(1H,s,H-3); 2.42(1H,s,H-6); 7.16(3H,s, Ar-CH_3). A signal at 7.6τ (Ar-O-CO-CH_3) was obscured by the dimethyl sulphoxide signal.

M.S. m/e(rel.abundance): 386(M^+)(3), 344(9), 302(24), 260(100), 216(4).

High Resolution M.S. molecular ion at m/e = 386.0645; $C_{19}H_{14}O_9$ requires molecular ion at m/e = 386.0638.

Reaction of Lamellicolic Anhydride with Aqueous Sodium Hydroxide: Preparation of Compounds (57) and (58).

Lamellicolic anhydride (106 mg.) was refluxed under nitrogen with 5N sodium hydroxide (5 ml.) for 3 hours. The mixture was acidified and ethyl acetate (50 ml.) added. After washing to neutrality and evaporation the product was seen by T.L.C. to consist of two major components. It was dissolved in methanol and treated with a large excess of ethereal diazomethane (5.14g. nitrosan) and left overnight. Ether (50 ml.) was added and after filtration and evaporation P.L.C. using 50% petrol-chloroform as eluent gave two fractions.

The more polar fraction crystallised from ethyl acetate-hexane giving the ester (57) as colourless prisms m. 126-128°C (47 mg., 39%).

T.L.C. Rf 0.45 (100% chloroform); U.V.: light blue; Ce^{4+}: brown.

I.R. $\nu_{max}(CHCl_3)$: 1720(s), 1620(s), 1590(s), 1455(s), 1350(s), 1070(s), 1045(m), 1030(m), 990(w) cm^{-1}.

U.V. $\lambda_{max}(EtOH)$: 236(17000), 245(inf.)(20000), 249(21000), 307(6200) n.m.: no change observed on the addition of acid or base.

N.M.R. (60 MHz) τ ($CDCl_3$): 3.18(1H,s,irr.7.14→-sharpening,H-6); 3.40(1H, d,J=2Hz,Ar-H); 3.62(1H,d,J=2Hz,Ar-H); 5.96(3H,s,O-CH_3); 6.05(3H,s,O-CH_3); 6.10(6H,s,O-CH_3); 7.14(3H,s,Ar-CH_3).

M.S. m/e(rel.abundance): 290(M^+)(100), 259(88).

Analysis. Found: C, 66.29; H, 6.27%; $C_{16}H_{18}O_5$ requires C, 66.20; H, 6.25%; m.w. = 290.

Crystallisation of the less polar fraction from hexane gave the

trimethoxy compound (58) as colourless prisms m. 93-95°C (13 mg., 13%).

T.L.C. Rf 0.55 (50% chloroform-petrol); Ce^{4+}: olive.

I.R. ν_{max}(KBr): 3000(w), 2940(w), 2830(w), 1615(s), 1595(s), 1250(s), 1205(s), 1160(s), 1055(m), 830(m) cm^{-1}.

U.V. λ_{max}(EtOH): 237(33000), 249(inf.)(26000), 269(2800), 281(3300), 297(3500) n.m.: no change on addition of acid or base.

N.M.R. (100 MHz) τ ($CDCl_3$): 3.10(1H,d,J=3Hz,Ar-H); 3.24(1H,d,J=3Hz,Ar-H); 3.31(1H,d,J=3Hz,Ar-H); 3.61(1H,d,J=3Hz,Ar-H); 6.10(3H,s,O-CH_3); 6.12(6H, s,O-CH_3); 7.20(3H,s,Ar-CH_3).

M.S. m/e(rel.abundance): 232(M^+)(100), 217(12), 189(37), 174(15).

High Resolution M.S. molecular ion at m/e = 232.1099; $C_{14}H_{16}O_3$ requires molecular ion at m/e = 232.1099.

Reaction of O,O,O-Trimethyllamellicolic Anhydride with Methylamine: Preparation of Aminoimides (59) and (60).

O,O,O-Trimethyllamellicolic anhydride (100 mg.) was stirred under nitrogen and heated (ca. 80°C) with 40% aqueous methylamine (10 ml.) for 48 hours. Chloroform (100 ml.) and cold dilute hydrochloric acid (50 ml.) were added. The organic layer was washed to neutrality and evaporated to give a yellow solid which was purified by P.L.C. using chloroform-methanol and multiple elutions.

The diaminoimide (59) crystallised from the least polar fraction as yellow needles (methanol-chloroform) m. 225-228°C (66 mg., 55%).

T.L.C. Rf 0.80 (2% methanol-chloroform); U.V.: blue; Ce^{4+}: brown.

I.R. ν_{max}($CHCl_3$): 3240(br.w), 3005(w), 2935(w), 1635(s), 1605(v.s), 1595(br.s), 1275(w), 1195(w), 1148(w), 1070(w), 818(w) cm^{-1}.

U.V. λ_{max}(EtOH): 246(39000), 265(inf.)(30000), 288(inf.)(12000), 312(7900), 370(inf.)(17000), 390(inf.)(26000), 403(42000) n.m.: no change on addition of acid or base.

N.M.R. (60 MHz) τ ($CDCl_3$): -1.5(2H,br,exchangeable,N-H); 3.61(1H,s,H-6); 4.15(1H,s,H-3); 6.05(3H,s,O-CH_3); 6.50(3H,s,CO-N-CH_3); 6.95(6H,d,J=4Hz, irr.-1.5→s,Ar-N-CH_3); 7.30(3H,s,Ar-CH_3).

M.S. m/e(rel.abundance): 314(22), 313(M^+)(100), 312(22), 298(11), 296(11), 285(28), 269(11).

Analysis. Found: C, 65.15; H, 6.20; N, 13.40%; $C_{17}H_{19}N_3O_3$ requires C, 65.16; H, 6.11; N, 13.41%; m.w. = 313.

The more polar aminoimide (60) crystallised from methanol-chloroform as yellow needles m. 244-246°C (24 mg., 20%).

T.L.C. Rf 0.61 (5% methanol-chloroform); U.V.: green-blue; Ce^{4+}: orange.

I.R. ν_{max}($CHCl_3$): 3230(w), 3005(w), 2930(w), 2850(w), 1660(m), 1620(s), 1582(s), 1270(m), 1160(w), 1100(w), 1062(w), 818(w) cm^{-1}.

U.V. λ_{max}($CHCl_3$): 270(50000), 302(15000), 329(inf.)(14000), 351(19000), 426(22000) n.m.

N.M.R. (100 MHz) τ ($CDCl_3$): -0.3(1H,br,exchangeable,N-H); 3.25(1H,s,H-6); 3.92(1H,s,H-3); 5.92(3H,s,O-CH_3); 6.05(3H,s,O-CH_3); 6.52(3H,s,CO-N-CH_3); 6.95(3H,d,J=4Hz,irr.-0.3→s,Ar-N-CH_3); 7.30(3H,s,Ar-CH_3).

M.S. m/e(rel.abundance): 315(25), 314(M^+)(90), 313(15), 299(25), 297(25), 286(30), 285(100), 271(10), 270(15), 269(10), 268(15), 256(30), 228(15).

High Resolution M.S. molecular ion at m/e = 314.1264; $C_{17}H_{18}N_2O_4$ requires molecular ion at m/e = 314.1266.

The most polar fraction gave starting material (12 mg., 12%) (identified by Rf and spectral data).

2,7-Dihydroxy-5-methyl-1,4-naphthaquinone (65).

After isolation as described above this compound crystallised from methanol-chloroform as brown-red prisms which decomposed slowly above 250°C.

T.L.C. Rf 0.34 (100% ethyl acetate); $FeCl_3$: brown; Ce^{4+}: brown.

I.R. ν_{max}(KBr): 3210(br.w), 1660(s), 1590(s), 1570(s), 1465(m), 1400(m), 1380(m), 1360(m), 1320(m), 1205(m), 1095(m), 1060(s), 1005(m), 880(m), 868(m), 800(m), 740(m), 705(m) cm^{-1}.

U.V. λ_{max}(EtOH): 266(10000), 296(6100), 346(1600) n.m.

λ_{max}(EtOH+$^-$OH): 286(14000), 325(inf.)(4100), 378(2800) n.m.: reverting to EtOH spectrum on acidification.

N.M.R. (100 MHz) τ (CD_3OD): 2.65(1H,d,J=2Hz,Ar-H); 3.10(1H,d,J=2Hz,Ar-H); 7.40(3H,s,Ar-CH_3).

M.S. m/e(rel.abundance): 204(M^+)(100), 176(61), 148(17), 135(78), 107(22).

High Resolution M.S. molecular ion at m/e = 204.0422; $C_{11}H_8O_4$ requires molecular ion at m/e = 204.0423.

2,7-Dimethoxy-5-methyl-1,4-naphthaquinone (67).

The naphthaquinone (65)(35 mg.), in dry acetone (20 ml.), was

treated with anhydrous potassium carbonate (35 mg.) and excess dimethyl sulphate and the mixture refluxed overnight. The brown oil obtained after washing and evaporation gave, on crystallisation from chloroform-petrol, the dimethyl ether (67) as long yellow needles m. 172-173°C (30 mg., 75%).

T.L.C. Rf 0.35 (100% chloroform); $FeCl_3$: negative; Ce^{4+}: red.

I.R. $\nu_{max}(CHCl_3)$: 2970(w), 2940(w), 2840(w), 1680(m), 1640(s), 1625(s), 1595(s), 1560(m), 1365(m), 1345(m), 1305(s), 1275(m), 1155(m), 1115(m), 1080(s), 1035(m), 855(m) cm^{-1}.

U.V. λ_{max}(EtOH): 263(18000), 291(10000), 343(1700), 384(1300) n.m.: no change was observed on addition of acid or base.

N.M.R. (60 MHz) τ ($CDCl_3$): 2.42(1H,d,J=3Hz,H-8); 3.00(1H,d,J=3Hz,H-6); 3.92(1H,s,irr.6.10→sharpening,H-3); 6.05(3H,s,O-CH_3); 6.10(3H,s,O-CH_3); 7.25(3H,s,Ar-CH_3).

M.S. m/e(rel.abundance): 232(M^+)(100), 217(27), 204(20), 203(20), 202(37), 189(13), 175(17), 174(17), 161(47), 146(20), 133(80).

High Resolution M.S. molecular ion at m/e = 232.0731; $C_{13}H_{12}O_4$ requires molecular ion at m/e = 232.0736.

Analysis. Found: C, 67.02; H, 5.20%; $C_{13}H_{12}O_4$ requires C, 67.23; H, 5.21%.

Preparation of the Naphthaquinone (65) from Lamellicolic Anhydride.

Lamellicolic anhydride (30 mg.) was heated (80°C) under nitrogen with aqueous sodium hydroxide (5N, 10 ml.) for 4 hours. The resulting deep red solution was cooled to 0°C under nitrogen and acidified with

dilute hydrochloric acid. The product, after extraction into ethyl acetate (50 ml.), washing and evaporation was a brown oil which was identified by T.L.C. as 1,3,6-trihydroxy-4-methylnaphthalene.

This oil, in methanol (10 ml.) was stirred with 5N sodium hydroxide (0.1 ml.) for 48 hours. Dilute hydrochloric acid was added and the methanol evaporated. Extraction into ethyl acetate (50 ml.) followed by washing to neutrality and evaporation gave a product which was identical in every respect (Rf, spectral data, mixed melting point) with a sample of the naphthquinone isolated as previously described.

4-O-(3,3-Dimethylallyl)-lamellicolic Anhydride (83).

3,3-Dimethylallyl bromide (61 mg.) in acetone (5 ml.) was added to lamellicolic anhydride (100 mg.) and potassium carbonate (100 mg.) in acetone (25 ml.) under nitrogen. The mixture was stirred and refluxed overnight. Chloroform (50 ml.) and cold dilute hydrochloric acid (25 ml.) were added, the organic layer washed to neutrality and evaporated. Crystallisation of the crude product from methanol-chloroform gave the dimethylallyl ether (83) as colourless needles m. 192-193°C (67 mg., 53%).

T.L.C. Rf 0.45 (100% chloroform); $FeCl_3$: brown; Ce^{4+}: grey.

I.R. ν_{max}(KBr): 3100(br.w), 2990(w), 2970(w), 1710(s), 1662(s), 1620(s), 1595(s), 1455(m), 1380(m), 1340(m), 1300(s), 1200(m), 1185(s), 1162(m), 1038(s), 958(m), 808(s), 755(m) cm^{-1}.

U.V. λ_{max}(EtOH): 249(11000), 290(4100), 309(3100), 348(7400), 366(inf.)(6100) n.m.

λ_{max}(EtOH+$^{-}$OH): 248(inf.)(13000), 302(8400), 317(11000), 352(inf.) (4900), 384(9200) n.m.: reverting to EtOH spectrum on acidification.

N.M.R. (100 MHz) τ ($CDCl_3$): -1.50(1H,s,exchangeable,O-H-; -1.25(1H,s, exchangeable,O-H); 3.18(1H,s,H-6); 3.58(1H,s,H-3); 4.50(1H,br.t,J=6Hz, irr.5.30→s,olefinic H); 5.30(2H,d,J=6Hz,irr.4.50→s,O-CH_2); 7.21(3H,s, Ar-CH_3); 8.16(3H,s,vinyl CH_3).

M.S. m/e(rel.abundance): 328(M^+)(7), 313(7), 260(100), 242(7), 231(7), 216(25), 214(14).

High Resolution M.S. molecular ion at m/e = 328.0936; $C_{18}H_{16}O_6$ requires molecular ion at m/e = 328.0931.

Pyrolysis of the Dimethylallyl Ether (83).

1) Pyrolysis in dimethylformamide.

The dimethylallyl ether (83)(35 mg.), in dry dimethylformamide (3 ml.), was heated under nitrogen, at 120°C, overnight. Ethyl acetate (100 ml.) was added, the solution washed with water (3x50 ml.) and evaporated to give a yellow solid which was purified by P.L.C. (80% chloroform-petrol) to yield a product which was identical (Rf, spectral data, mixed melting point) with a sample of the anhydride (82) prepared from herqueinone as described below.

2) Pyrolysis in a sublimation block.

The dimethylallyl ether (83)(50 mg.) was heated in a sublimation tube at 160°C (0.02 mm. Hg) for 5 hours. Some material had sublimed and this, along with the residue, was dissolved in hot ethyl acetate (50 ml.),

the solution filtered and evaporated. P.L.C. (100% chloroform) gave a product which although homogeneous by T.L.C., appeared by N.M.R. to consist of a mixture of the anhydrides (82) and (84).

T.L.C. Rf 0.69 (50% petrol-chloroform); U.V.: blue; $FeCl_3$: blue; Ce^{4+}: green.

I.R. $\nu_{max}(CHCl_3)$: 3150(br.w), 1710(s), 1670(s), 1625(s), 1610(s), 1460(m), 1315(m), 1150(m), 1095(m), 1040(m) cm^{-1}.

U.V. λ_{max}(EtOH): 256(21000), 290(inf.)(6700), 360(11000), 373(inf.) (8200) n.m.

$\lambda_{max}(EtOH+^{-}OH)$: 250(inf.)(17000), 306(12000), 323(13000), 376(inf.) (9500), 392(14000) n.m.: reverting to EtOH spectrum on acidification.

N.M.R. (100 MHz) τ ($CDCl_3$): -1.50(1H,s,exchangeable,O-H); -1.70(1H,s, exchangeable,O-H); 3,15(1H,s,H-6); 5.25($\frac{1}{3}$H,q,J=6Hz,O-CH); 6.62($\frac{2}{3}$H,q, J=6Hz,Ar-CH); 7.20(3H,s,Ar-CH_3); 8.45-8.75(9H,complex group of signals, CH_3).

M.S. m/e(rel.abundance): 328(M^+)(40), 313(100), 295(20), 285(20), 269(20).

Preparation of the Anhydride from Penicillium Herquei (82).

A solution of herqueinone (50 mg.), in pyridine (0.7 ml.) and glacial acetic acid (4 ml.), was shaken with acid washed zinc dust (1g.) for 30 minutes and then filtered. To the filtrate was added firstly 12N sulphuric acid (14 ml.) and then over thirty minutes a solution of chromium trioxide (105 mg.) in a mixture of water (0.4 ml.) and acetic

acid (3.1 ml.). The mixture was heated on the steam bath for 1.5 hours, poured into chloroform, washed, and evaporated to yield a product which was purified by P.L.C. (4% ethanol-benzene) to give the anhydride (82) as colourless needles (methanol-chloroform) which sublimed slowly between 200-250°C (10 mg., 23%).

T.L.C. Rf 0.69 (50% chloroform-petrol); U.V.: blue; $FeCl_3$: blue; Ce^{4+}: green.

I.R. $\nu_{max}(CHCl_3)$: 3280-3000(v.br.w), 1710(s), 1670(s), 1625(s), 1615(s), 1460(m), 1385(m), 1330(m), 1305(s), 1060(m), 1040(s), 865(m) cm^{-1}.

U.V. λ_{max}(EtOH): 256(21000), 290(inf.)(6700), 360(12000), 322(13000), 376(inf.)(9500), 392(14000) n.m.

λ_{max}(EtOH+$^-$OH): 250(inf.)(17000), 306(12000), 322(13000), 376(inf.) (9500), 392(14000) n.m.: reverting to EtOH spectrum on acidification.

N.M.R. (60 MHz) τ ($CDCl_3$): -1.55(2H,m,exchangeable,O-H); 3.20(1H,s,H-6); 5.25(1H,q,J=6Hz,irr.8.45→s,O-CH_3); 7.15(3H,s,Ar-CH_3); 8.40(3H,s,CH_3); 8.45(3H,d,J=6Hz,irr.5.25→s,secondary CH_3); 8.65(3H,s,CH_3).

M.S. m/e(rel.abundance): 328(M^+)(25), 313(100), 295(13), 285(18), 269(18).

High Resolution M.S. molecular ion at m/e = 328.0947; $C_{18}H_{16}O_6$ requires molecular ion at m/e = 328.0931.

This material was identical (Rf, I.R., U.V., M.S.) with a sample supplied by Prof. R. Thomas. There was insufficient of the latter sample to obtain an N.M.R. spectrum.

Reaction of O,O,O-Trimethyllamellicolic Anhydride with Lithium Aluminium Hydride.

In a typical reaction lithium aluminium hydride (19 mg.) was added to a refluxing suspension of O,O,O-trimethyllamellicolic anhydride (75 mg.) in dry tetrahydrofuran (10 ml.). The solution was refluxed for 15 minutes and then stirred for a further 10 minutes. Water (5 ml.) was cautiously added, the mixture stirred for 10 minutes and the tetrahydrofuran evaporated. Chloroform (50 ml.) was added and the solution washed with dilute hydrochloric acid (25 ml.) and water (2x25 ml.) and evaporated. T.L.C. showed that no single compound had been formed in good yield but that a large number of non polar products had been obtained. These compounds were unstable and this in conjunction with the low yields obtained prevented their characterisation.

Reaction of O,O,O-Trimethyllamellicolic Anhydride with Sodium Borohydride.

1) Attempted reaction in tetrahydrofuran.

O,O,O-Trimethyllamellicolic anhydride (30 mg.) suspended in dry tetrahydrofuran (10 ml.) was stirred at room temperature with excess sodium borohydride (10 mg.) overnight. Analytical T.L.C. indicated that no reaction had occurred. The same result was obtained when the reaction was carried out at an elevated temperature or when dimethylformamide was used as solvent.

2) Reaction in ethanol: preparation of compound (94).

A suspension of O,O,O-trimethyllamellicolic anhydride (30 mg.) in

ethanol (5 ml.) was treated with a large excess of sodium borohydride and the mixture stirred at room temperature overnight. T.L.C. indicated that one product (Rf 0.2, 10% methanol-chloroform), i.e. more polar than the starting material (Rf 0.5, 10% methanol-chloroform), had been formed. Chloroform (50 ml.) was added and after filtration through glass paper and evaporation a colourless solid was obtained. When, after P.L.C. (10% methanol-chloroform), the band containing the product (Rf 0.2) was eluted with hot ethyl acetate only starting material (Rf 0.5) was obtained.

The product (Rf 0.2), in methanol (10 ml.), was added to a large excess of ethereal diazomethane (2.57g. nitrosan) and left overnight. After filtration, washing with water and evaporation a product was obtained which on P.L.C. (100% chloroform) and subsequent crystallisation from ethyl acetate-petrol gave the diester (94) as colourless needles m. 96-97°C (29 mg., 81%).

T.L.C. Rf 0.30 (100% chloroform); Ce^{4+}: yellow.

I.R. $\nu_{max}(CHCl_3)$: 1720(s), 1590(s), 1335(s), 1075(s), 1035(s) cm^{-1}.

U.V. $\lambda_{max}(EtOH)$: 254(24000), 320(5000), 341(inf.)(3700) n.m.: no change was observed on the addition of acid or base.

N.M.R. (60 MHz) τ ($CDCl_3$): 3.15(1H,s,H-6); 3.55(1H,s,H-3); 5.70(2H,q, J=6Hz,irr.8.65→s,O-CH_2); 6.10(9H,s,O-CH_3); 6.15(3H,s,O-CH_3); 7.15(3H,s, Ar-CH_3); 8.65(3H,t,J=6Hz,irr.5.70→s,CH_3).

M.S. m/e(rel.abundance): 362(M^+)(75), 331(17), 317(58), 303(84), 289(100), 275(58), 259(50).

Analysis. Found: C, 63.04; H, 6.23%; $C_{19}H_{22}O_7$ requires C, 62.98; H, 6.12%; m.w. = 362.

Reaction of 1,8-Naphthalic Anhydride with Sodium Borohydride: Preparation of the Hemiacetal (93).

Excess sodium borohydride (25 mg.) was added to a suspension of 1,8-naphthalic anhydride (25 mg.) in ethanol (10 ml.) and the mixture stirred overnight at room temperature. Chloroform (50 ml.) was added and after filtration and evaporation the crude product was subjected to P.L.C. (100% chloroform) giving the hemiacetal (93) m. 78-80°C (ethyl acetate-hexane)(17 mg., 71%).

T.L.C. Rf 0.25 (100% chloroform); Ce^{4+}: brown.

I.R. $\nu_{max}(CHCl_3)$: 3570(s), 1060(s), 1025(s), 1010(s), 970(m) cm^{-1}.

N.M.R. (60 MHz) τ ($CDCl_3$): 2.10-2.85(6H,m,Ar-H); 3.80(1H,d,J=5Hz,D_2O-s, Ar-CH-O); 4.60(1H,d,J=14Hz,Ar-C-H); 5.10 (1H,d,J=14Hz,Ar-C-H); 6.60(1H, d,J=5Hz,exchangeable,O-H).

M.S. m/e(rel.abundance): 186(M^+)(90), 179(25), 157(29), 141(100), 139(34), 129(71).

Analysis. Found: C, 77.12; H, 5.38%; $C_{12}H_{10}O_2$ requires C, 77.40; H, 5.41%; m.w. = 186.

Attempted Reduction of the Acid Ester (96) with Lithium Borohydride.

Sodium (0.23g.) was dissolved in ethanol (50 ml.), under nitrogen, and 1 ml. of the solution added to a stirred suspension of O,O,O-tri-

methyllamellicolic anhydride (30 mg.) in ethanol (10 ml.). After 15 minutes T.L.C. showed that the sole product formed was the acid ester (96).

This solution was added to a mixture of sodium borohydride (39 mg.) and lithium chloride (84 mg.) in ethanol (5 ml.) and the resulting mixture stirred and refluxed for 72 hours. Chloroform (50 ml.) was added, the solution washed with dilute hydrochloric acid (25 ml.) and water (2x25 ml.) and evaporated. Analysis of the product by T.L.C. showed that it was largely O,O,O-trimethyllamellicolic anhydride with no other product formed in reasonable yield.

Catalytic Reduction of O,O,O-Trimethyllamellicolic Anhydride.

1) Reaction in ethyl acetate.

A suspension of O,O,O-trimethyllamellicolic anhydride (28 mg.) in ethyl acetate (10 ml.) was shaken overnight, under hydrogen, with Adams catalyst (14 mg.). T.L.C. indicated that no reaction had occurred.

2) Reaction in trifluoroacetic acid: preparation of compounds (98) and (99).

O,O,O-Trimethyllamellicolic anhydride (66 mg.), in trifluoroacetic acid (5 ml.), was shaken overnight, under hydrogen, with Adams catalyst (70 mg.). Chloroform (80 ml.) was added and the mixture filtered and evaporated. The crude product was purified by P.L.C. using methanol-chloroform mixtures as eluents.

Lactone A (98) was obtained as buff-coloured prisms m. 165-166°C

(11 mg., 18%).

T.L.C. Rf 0.50 (2% methanol-chloroform); U.V.: light blue; Ce^{4+}: yellow with an olive halo.

I.R. $\nu_{max}(CHCl_3)$: 2960(w), 2930(w), 2830(w), 1690(s), 1605(s), 1590(s), 1455(s), 1375(m), 1335(s), 1160(m), 1140(w), 1080(s), 1045(s), 1020(w), 1000(w), 960(m) cm^{-1}.

U.V. λ_{max}(EtOH): 240(14000), 259(inf.)(13000), 265(15000), 337(4000), 349(4000), 370(3000) n.m.: no change was observed on the addition of acid or base.

N.M.R. (60 MHz) τ ($CDCl_3$): 3.25(1H,s,H-6); 3.55(1H,s,H-3); 4.55(2H,s, Ar-CH_2-O); 5.90(3H,s,O-CH_3); 6.05(3H,s,O-CH_3); 6.10(3H,s,O-CH_3); 7.25(3H, s,Ar-CH_3).

M.S. m/e(rel.abundance): 288(M^+)(100), 287(33), 273(8), 271(16), 260(16), 259(83), 243(16), 242(16).

Lactone B (99) was obtained as an unstable colourless gum (10 mg., 17%).

T.L.C. Rf 0.55 (5% methanol-chloroform); U.V.: green-blue; Ce^{4+}: brown.

I.R. $\nu_{max}(CHCl_3)$: 2960(w), 2940(w), 2840(w), 1700(s), 1620(m), 1595(s), 1455(s), 1430(w), 1380(w), 1340(s), 1285(s), 1170(w), 1140(m), 1080(s), 1045(s), 995(m) cm^{-1}.

N.M.R. (60 MHz) τ ($CDCl_3$): 3.15(1H,s,Ar-H); 3.50(1H,s,Ar-H); 4.55(2H,s, Ar-CH_2-O); 5.95(3H,s,O-CH_3); 6.05(6H,s,O-CH_3); 7.15(3H,s,Ar-CH_3).

EXPERIMENTAL.

CHAPTER 2.

The Chlorine-containing Metabolite (100).

This compound, isolated as described previously, gave a positive Beilstein test and crystallised from methanol-chloroform as orange prisms which did not melt below 300°C but which sublimed slowly above 250°C (0.03 mm. Hg).

T.L.C. Rf 0.20 (20% methanol-chloroform); $FeCl_3$: brick-red; Ce^{4+}: grey.

I.R. ν_{max}(KBr): 1675(s), 1640(s), 1610(s), 1580(s), 1535(m), 1490(m), 1460(m), 1320(m), 1265(m), 1200(m), 1045(m), 810(m), 735(m) cm^{-1}.

U.V. λ_{max}(EtOH): 270(4000), 322(3000), 400(2000) nm.

λ_{max}(EtOH+$^-$OH): 316(5000), 378(3000) nm.

λ_{max}(EtOH+H^+): 254(7000), 356(4000) nm.

N.M.R. (100 MHz) τ (d_6DMSO): 3.50(1H,s,H-6); 6.30(3H,s,O-CH_3); 7.25(3H,s, Ar-CH_3).

M.S. m/e(rel.abundance): 310(7), 308(M^+-18)(21), 296(33), 294(100), 278(7), 276(21), 264(6), 262(17), 254(12), 252(36), 236(5), 234(14), 196(8), 194(24).

High Resolution M.S. apparent molecular ion at m/e = 308.0085; $C_{14}H_9ClO_6$ requires m.w. = 308.0088.

Reaction of the Chlorine-containing Metabolite with Diazomethane: Preparation of the Diester (102).

A large excess of ethereal diazomethane (5.14g. nitrosan) was added to the chloro compound (100)(30 mg.), in dry methanol (5 ml.), and the mixture set aside overnight. Ether (50 ml.) was added and after filtration and evaporation the crude product was purified by P.L.C.

(4% methanol-chloroform) giving the diester (102) as a colourless gum (20 mg., 65%).

T.L.C. Rf 0.58 (2% methanol-chloroform); Co^{4+}: grey.

G.L.C. Retention time of 21.5 minutes on a 1% OV-1 column at a temperature of 220°C.

I.R. $\nu_{max}(CHCl_3)$: 1730(s), 1590(s), 1570(m), 1340(s), 1070(s), 1005(m), 980(m) cm^{-1}.

U.V. λ_{max}(EtOH): 246(57000), 285(inf.)(4600), 298(5700), 311(5700), 344(3100) nm.: no change was observed on the addition of acid or base.

N.M.R. (60 MHz) τ (CCl_4): 3.05(1H,s,irr.7.15→sharpening,H-6); 6.05(6H, s,O-CH_3); 6.10(3H,s,O-CH_3); 6.15(3H,s,O-CH_3); 6.20(3H,s,O-CH_3); 7.15(3H, s,Ar-CH_3).

M.S. m/e(rel.abundance): 384(29), 382(M^+)(86), 353(14), 351(43), 338(5), 336(14), 325(33), 323(100), 310(9), 308(28), 295(19), 293(57).

Reaction of the Chloro Compound with Diethyl Sulphate: Preparation of Compound (104).

The chloro compound (100)(50 mg.), in dry acetone (20 ml.), was stirred and refluxed overnight with potassium carbonate (50 mg.) and excess diethyl sulphate. Chloroform (100 ml.) was added and after washing with dilute hydrochloric acid (50 ml.) and water (3x50 ml.) and evaporation, the product was subjected to P.L.C. (100% chloroform) to give the triethyl ether (104) as colourless prisms which slowly sublimed between 170 and 190°C and finally melted at 195-197°C (20 mg., 38%).

T.L.C. Rf 0.23 (100% chloroform); U.V.: light blue; Ce^{4+}: grey.

I.R. $\nu_{max}(CHCl_3)$: 1760(s), 1725(s), 1595(s), 1570(m), 1375(m), 1345(m), 1340(m), 1095(m), 1030(s), 970(w), 880(w) cm^{-1}.

U.V. λ_{max}(EtOH): 257(14000), 323(inf.)(3100), 339(4000), 366(3300), 384(3000) nm.

$\lambda_{max}(EtOH+^{-}OH)$: 249(17000), 308(3100), 345(inf.)(1900) nm.

$\lambda_{max}(EtOH+H^{+})$: 247(18000), 308(3300), 345(inf.)(2400) nm.

N.M.R. (60 MHz) τ ($CDCl_3$): 2.90(1H,s,H-6); 5.55-5.90(6H,br.m,O-CH_2-); 7.05(3H,s,Ar-CH_3); 8.45(3H,t,J=6Hz,O-C-CH_3); 8.50(6H,t,J=6Hz,O-C-CH_3).

M.S. m/e(rel.abundance): 380(20), 378(M^{+})(60), 362(30), 360(90), 351(33), 349(100), 323(20), 321(60), 296(27), 294(80).

Analysis. Found: C, 60.52; H, 5.16%; $C_{19}H_{19}ClO_6$ requires C, 60.32; H, 5.03%; m.w. = 378.

Preparation and Standardisation of a Solution of Chlorine in Carbon Tetrachloride.

Chlorine, after drying with concentrated sulphuric acid, was bubbled through carbon tetrachloride (50 ml.) until a dark green solution was obtained. It was found that 2.3 ml. of 0.1M sodium thiosulphate solution was required for complete reaction with the iodine produced by the addition of 0.1 ml. of the chlorine solution to 5 ml. of a 0.5M solution of potassium iodide. The molarity of the chlorine solution was, therefore, 1.2M.

3-Chloro-O,O,O-trimethyllamellicolic Anhydride (103).

O,O,O-Trimethyllamellicolic anhydride (30 mg.), suspended in carbon tetrachloride (10 ml.), was stirred overnight with chlorine in carbon tetrachloride solution (0.17 ml.). After adding chloroform (75 ml.), the mixture was washed with aqueous sodium metabisulphite (25 ml.) and water (3x25 ml.) and evaporated. P.L.C. (100% chloroform) afforded 3-chloro-O,O,O-trimethyllamellicolic anhydride (103) which crystallised from chloroform-petrol as pale yellow needles which sublimed slowly above 200°C (15 mg., 44%).

T.L.C. Rf 0.31 (100% chloroform); U.V.: green-blue; Ce^{4+}: brown.

I.R. $\nu_{max}(CHCl_3)$: 1760(s), 1725(s), 1595(s), 1570(s), 1035(s), 965(m), 945(m) cm^{-1}.

U.V. λ_{max}(EtOH): 256(13000), 323(inf.)(3800), 338(4600), 368(inf.)(3800), 386(inf.)(3100) nm.

λ_{max}(EtOH+$^-$OH): 248(18000), 303(4000), 313(4000), 345(2800) nm.

λ_{max}(EtOH+H^+): 247(26000), 303(5000), 313(5000), 345(3800) nm.

N.M.R. (60 MHz) τ ($CDCl_3$): 2.80(1H,s,irr.7.00→sharpening,H-6); 5.85(3H, s,O-CH_3); 5.90(6H,s,O-CH_3); 7.00(3H,s,Ar-CH_3).

M.S. m/e(rel.abundance): 338(33), 336(M^+)(100), 323(27), 321(80), 320(13), 318(40), 305(7), 303(20), 293(20), 291(60).

Analysis. Found: C, 56.94; H, 3.76%; $C_{16}H_{13}ClO_6$ requires C, 56.80; H, 3.84%; m.w. = 338.

Selective Demethylation of 3-Chloro-O,O,O-trimethyllamellicolic Anhydride: 3-Chloro-4-O-methyllamellicolic Anhydride.

Magnesium iodide-etherate was prepared by adding magnesium turnings (0.4g.) and iodine (2.0g.) to dry ether (2.5 ml.) and dry benzene (5 ml.). After filtration, the solution (2 ml.) was added to a stirred suspension of the trimethyl ether (103)(46 mg.) in dry benzene (10 ml.) under nitrogen. The mixture was refluxed for 4 hours, acidified, and chloroform (50 ml.) added. After washing with aqueous sodium metabisulphite (25 ml.) and water (3x25 ml.) evaporation gave a yellow solid which on P.L.C. (4% methanol-chloroform) yielded 3-chloro-4-O-methyllamellicolic anhydride (101) as colourless prisms which slowly decomposed above 250°C (11 mg., 26%).

T.L.C. Rf 0.25 (2% methanol-chloroform); U.V.: blue; Ce^{4+}: grey.

I.R. $\nu_{max}(CHCl_3)$: 1710 (s), 1670(s), 1595(s), 1450(m), 1315(m), 1285(s), 1105(m), 1075(w), 1035(m), 1010(m), 955(m) cm^{-1}.

N.M.R. (60 MHz) τ ($CDCl_3$): 2.85(1H,s,H-6); 5.95(3H,s,O-CH_3); 7.10(3H,s, Ar-CH_3).

Reaction of Lamellicolic Anhydride with Chlorine: 3-Chlorolamellicolic Anhydride (105).

Lamellicolic anhydride (58 mg.), suspended in carbon tetrachloride (10 ml.), was stirred overnight with a standard solution of chlorine in carbon tetrachloride (0.32 ml.). The product was filtered off, washed with more carbon tetrachloride (20 ml.) and crystallised from a large

volume of methanol to give 3-chlorolamellicolic anhydride (105) as colourless needles which decomposed slowly above 250°C (46 mg., 71%).

I.R. ν_{max}(KBr): 1710(s), 1660(s), 1610(s), 1590(s), 1455(s), 1310(m), 1190(s), 1150(m), 1035(s), 805(m), 765(m) cm^{-1}.

U.V. λ_{max}(EtOH): 254, 276(inf.), 290(inf.), 322(inf.), 355, 371(inf.) nm.

λ_{max}(EtOH+$^{-}$OH): 253(inf.), 316, 376 nm.: reverting to EtOH spectrum on acidification.

M.S. m/e(rel.abundance): 296(33), 294(M^{+})(100), 278(7), 276(21), 252(14), 250(42), 196(12), 194(36).

Analysis. Found: C, 52.59; H, 2.32%; $C_{13}H_7ClO_6$ requires C, 53.06; H, 2.45%; m.w. = 294.

Reaction of 3-Chlorolamellicolic Anhydride with Dimethyl Sulphate: 3-Chloro-O,O,O-trimethyllamellicolic Anhydride.

3-Chlorolamellicolic anhydride (35 mg.), suspended in acetone (20 ml.), was stirred and refluxed overnight with, anhydrous potassium carbonate (35 mg.) and excess dimethyl sulphate. Chloroform (100 ml.) was added and after washing with dilute hydrochloric acid (25 ml.) and water (3x25 ml.) and evaporation, P.L.C. (100% chloroform) gave a product identical in all respects (Rf, spectral data and mixed melting point) to a sample of the trimethyl ether (103) prepared as described previously from (54).

Reaction of 3-Chlorolamellicolic Anhydride with Diethyl Sulphate: 3-Chloro-O,O,O-triethyllamellicolic Anhydride.

3-Chlorolamellicolic anhydride (30 mg.), in dry acetone (20 ml.), was stirred and refluxed overnight with a mixture of potassium carbonate (30 mg.) and excess diethyl sulphate. Chloroform (100 ml.) was added and after washing with dilute hydrochloric acid (25 ml.) and water (3x25 ml.) and evaporation, P.L.C. (100% chloroform) gave a product identical in all respects (Rf, spectral data and mixed melting point) to a sample of the triethyl ether (104) prepared from the chlorine-containing metabolite (100).

EXPERIMENTAL.

CHAPTER 3.

4-O-Carbomethoxylamellicolic Anhydride (106).

This compound was isolated, as described above, as colourless prisms from chloroform m. 202-204°C.

T.L.C. Rf 0.25 (5% methanol-chloroform); U.V.: green-blue; $FeCl_3$: red; Ce^{4+}: brown.

I.R. ν_{max}(KBr): 3210-3000(v.br.w), 2980(w), 2920(w), 2850(w), 1765(s), 1725(s), 1675(s), 1615(m), 1605(s), 1460(m), 1435(m), 1270(s), 1190(m), 1165(s), 1065(m), 1040(s), 930(m), 880(w), 870(w), 805(m) cm^{-1}.

U.V. λ_{max}(EtOH): 226(4200), 247(4900), 282(1200), 313(1700), 341(1900), 363(2200), 395(1100) nm.

λ_{max}(EtOH+$^-$OH): 251(inf.)(8000), 314(11000), 372(6000) nm.

λ_{max}(EtOH+H^+): 250(8000), 292(2300), 352(5000), 368(inf.)(4000) nm.

N.M.R.(100 MHz) τ ($CDCl_3$): -1.60(1H,s,exchangeable,Ar-OH); -1.50(1H,s, exchangeable,Ar-OH); 3.02(1H,s,H-3); 3.06(1H,s,H-6); 6.10(3H,s,-OCH_3); 7.30(3H,s,Ar-CH_3).

M.S. m/e(rel.abundance): 318(M^+)(100), 275(13), 274(100), 259(13), 256(13), 230(50), 213(25), 186(13), 184(13), 174(37).

Analysis. Found: C, 56.49; H, 3.24%; $C_{15}H_{10}O_8$ requires C, 56.61; H, 3.17%; m.w. = 318.

4-O-Carbomethoxylamellicolic Anhydride Dimethyl Ether (107).

4-O-Carbomethoxylamellicolic anhydride (106)(58 mg.), in dry acetone (20 ml.), was stirred and refluxed under nitrogen with anhydrous potassium carbonate (58 mg.) and excess dimethyl sulphate for 24 hours.

Chloroform (50 ml.) was added, the mixture acidified with dilute hydrochloric acid and evaporated. P.L.C. (5% methanol-chloroform) gave two products, the more polar of which was identical in all respects (Rf, spectral data) with O,O,O-trimethyllamellicolic anhydride (14 mg., 26%). The less polar fraction yielded the dimethyl ether (107) as colourless prisms (methanol-chloroform) which sublimed slowly between 200 and 210°C (22 mg., 35%).

T.L.C. Rf 0.36 (5% methanol-chloroform); U.V.: light blue; Ce^{4+}: brown.

I.R. $\nu_{max}(CHCl_3)$: 2940(w), 2850(w), 1760(s), 1720(s), 1605(s), 1560(s), 1460(m), 1435(m), 1360(m), 1290(s), 1065(m), 1040(s), 985(m), 935(m), 840(w) cm^{-1}.

U.V. λ_{max}(EtOH): 257(15000), 323(inf.)(2300), 340(3800), 370(3700), 384(3300) nm.

λ_{max}(EtOH+$^-$OH): 249(20000), 304(2900), 312(2800), 345(inf.)(1300) nm.

N.M.R. (60 MHz) τ ($CDCl_3$): 2.82(1H,s,H-3); 2.90(1H,s,H-6); 5.84(6H,s,$-OCH_3$); 5.98(3H,s,$-OCH_3$); 7.18(3H,s,Ar-CH_3).

M.S. m/e(rel.abundance): 346(M^+)(100), 331(33), 302(16), 301(16), 287(16), 259(50), 257(33).

Analysis. Found: C, 58.87; H, 4.03%; $C_{17}H_{14}O_8$ requires C, 58.96; H, 4.08%; m.w. = 346.

Reaction of 4-O-Carbomethoxylamellicolic Anhydride with Acetic Anhydride and Pyridine.

The carbonate (106)(20 mg.) was stirred at room temperature with

acetic anhydride (1 ml.) and dry pyridine (0.05 ml.) for 12 hours. The mixture was poured into cold dilute hydrochloric acid and extracted with ethyl acetate (50 ml.). After washing to neutrality with water, the organic layer was evaporated to yield material identical (Rf, spectral data) with the triacetate of lamellicolic anhydride (56).

Preparation of 4-O-Carbomethoxylamellicolic Anhydride from Lamellicolic Anhydride.

Lamellicolic anhydride (100 mg.), in dry acetone (20 ml.), was stirred and refluxed under nitrogen with anhydrous potassium carbonate (100 mg.) and methyl chloroformate (29 µl.). After 18 hours the mixture was poured into cold dilute hydrochloric acid and extracted with ethyl acetate (50 ml.). The organic layer, after washing to neutrality and evaporation, afforded a yellow solid which on crystallisation from methanol-chloroform gave a product (75 mg., 61%) identical (melting point, Rf, spectral data) to 4-O-carbomethoxylamellicolic anhydride.

Preparation of the Urethane (108).

Methyl chloroformate (1.46 ml.), in dry ether (10 ml.), was added to m-toluidine (4.26g.), in dry ether (50 ml.), the mixture stirred for 1 hour and the resultant precipitate filtered off and recrystallised from hexane to give the urethane as colourless needles m. 68-69°C (2.96g., 88%).
T.L.C. Rf 0.60 (100% chloroform); Ce^{4+}: brown.
I.R. $\nu_{max}(CHCl_3)$: 3435(m), 3010(w), 2950(w), 1735(s), 1615(m), 1595(m),

1530(s), 1490(m), 1445(m), 1310(w), 1295(w), 1265(m), 1070(m) cm^{-1}.

N.M.R. (60 MHz) τ ($CDCl_3$): 2.80-3.40(4H,br.m,Ar-H and N-H); 6.25(3H,s, $-OCH_3$); 7.70(3H,s,Ar-CH_3).

M.S. m/e(rel.abundance): 165(M^+)(40), 133(20), 120(26), 106(13).

Analysis. Found: C, 65.48; H, 6.74; N, 8.38%; $C_9H_{11}O_2$ requires C, 65.44; H, 6.71; N, 8.48%; m.w. = 165.

Reaction of 4-O-Carbomethoxylamellicolic Anhydride with m-Toluidine.

4-O-Carbomethoxylamellicolic anhydride (16 mg.), in acetone (10 ml.), was refluxed with m-toluidine (10 mg.) for 3 days. The urethane (108) could not be detected by T.L.C. in the reaction mixture.

Attempted Photo-oxidation of Lamellicolic Anhydride.

1) Reaction using methylene blue as photo-sensitizer.

Lamellicolic anhydride (60 mg.) and methylene blue (5 mg.), in methylene chloride (40 ml.) and methanol (10 ml.), were stirred at room temperature under oxygen, in a strong light, for 36 hours. After evaporation the starting material was recovered unchanged (T.L.C.).

2) Reaction under irradiation with ultraviolet light.

Lamellicolic anhydride (50 mg.), in methylene chloride and methanol (100 ml.) and (10 ml.), was irradiated for 8 hours in a Hanovia 1L photochemical reactor and the solvent evaporated. T.L.C. investigation of the resultant yellow solid revealed that extensive decomposition to very polar products had occurred.

Feeding of Labelled Precursors to Cultures of V. Lamellicola.

a) Feeding of [^{14}C-methyl]-methionine.

[^{14}C-methyl]-methionine (50 μCi) was dissolved in 5 ml. of sterile distilled water and the solution was added to the culture filtrates of a 2 day old surface culture of V. lamellicola (5 Roux bottles). The cultures were harvested after a further 8 days growth.

b) Feeding of [1-^{14}C, 2-^{3}H]-acetate.

[1-^{14}C]-sodium acetate (0.2 mCi) and [2-^{3}H]-sodium acetate (5 mCi) were dissolved in sterile distilled water (10 ml.) and 8 ml. of this solution was added to the culture filtrates of a 2 day old surface culture of V. lamellicola (4 Roux bottles). The cultures were harvested after a further 8 days growth.

Isolation of Labelled Lamellicolic Anhydride and 4-O-Carbomethoxy-lamellicolic Anhydride.

The culture filtrate was extracted with methylene chloride (100 ml.) for 8 hours in a Soxhlet apparatus and the extract evaporated. Inactive 4-O-carbomethoxylamellicolic anhydride (100 mg.) was added and reisolated by crystallisation from chloroform. Chemically pure 4-O-carbomethoxy-lamellicolic anhydride was obtained after two crystallisations and the material was then crystallised to constant activity.

Inactive lamellicolic anhydride (100 mg.) was added to the combined residues of these crystallisations, reisolated by P.L.C. (2% methanol-chloroform) and crystallised to constant activity from methanol-chloroform.

EXPERIMENTAL.

CHAPTER 4.

Pritchard's Complete Medium.

Peptone.	1 g.
Yeastrel.	1 g.
Casamino acids.	1 g.
Glucose.	10 g.
Aqueous solution of biotin.	1 ml.
Aqueous solution of trace elements.	0.5 ml.
Aqueous vitamin solution.	0.5 ml.
Water.	1 litre.
Growth temperature.	= 30°C.

Trace Element Solution.

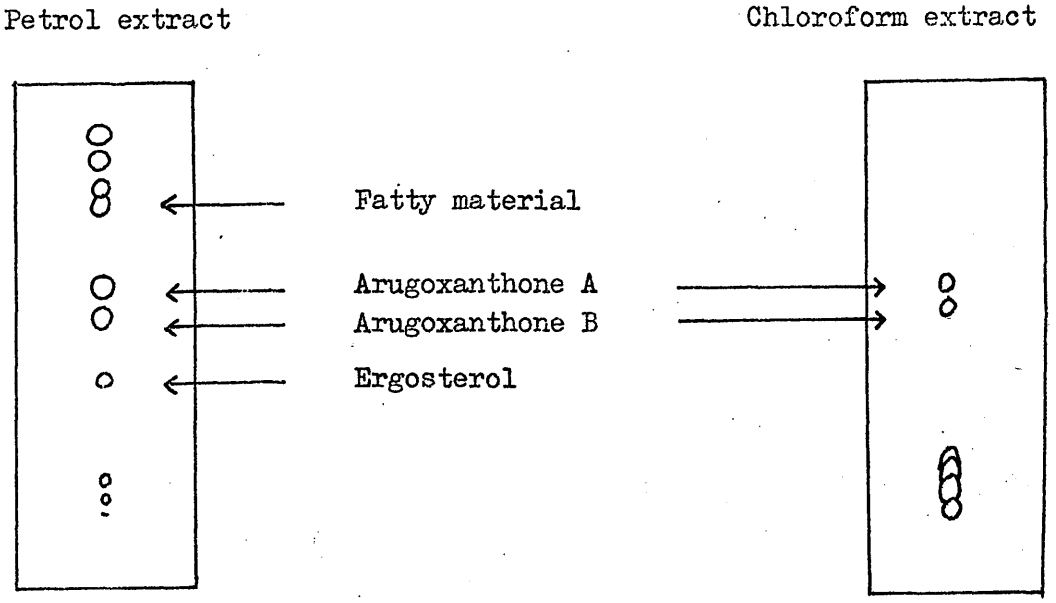
Copper sulphate.	393 mg.
Ferric sulphate.	910 mg.
Sodium molybdate.	50 mg.
Zinc sulphate.	4403 mg.
Manganese chloride.	72 mg.
Sodium borate.	88 mg.
Ethylenediaminetetra-acetic acid.	5 g.
Water.	1 litre.

Vitamin Solution.

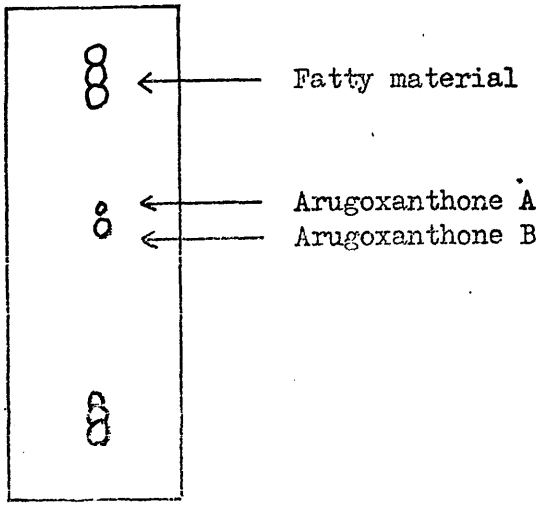
Riboflavin.	0.2 g.
Nictotinamide.	0.2 g.
p-Aminobenzoic acid.	0.02 g.
Pyridoxin hydrochloride.	0.05 g.
Aneurin hydrochloride.	0.1 g.
Biotin.	2 phials.
Water.	200 ml.

Mutant.	Weight of dried spores /Petri dish (mg.)
Yellow.	10.
Green.	48.

T.L.C. of Crude Extracts of the Yellow Mutant (100% chloroform).



T.L.C. of Crude Extract of the Green Mutant (100% chloroform).



Growth of the Spores of the Mutants of Aspergillus Nidulans.

The white, yellow, green and fawn spore producing mutants used were strains of Aspergillus nidulans grown for some time in this department and were maintained on 2% malt agar slants. Spores from a slant were used to innoculate 40 flat-bottomed Petri dishes containing approximately 20 ml. of Pritchard's complete medium. After 3 days the spores produced were scraped from the surface of the medium and transferred to a round-bottomed flask containing diethyl ether. The solvent was evaporated under reduced pressure and residual water removed azeotropically with benzene.

Yellow Spores.

Dried yellow spores (30g.) were ground in a mortar with sand (5g.) and extracted for 24 hours in a Soxhlet apparatus with light petroleum. Evaporation of the solvent gave a viscous yellow oil (500 mg.) which on P.L.C. using chloroform-light petroleum mixtures as eluents gave pure samples of two non polar, yellow, crystalline compounds- arugoxanthone A (10 mg.) and arugoxanthone B (10 mg.). A pure sample of ergosterol (5 mg.)(identified by comparison with an authentic sample) was also obtained.

Extraction of the spores for 24 hours in a Soxhlet apparatus with chloroform and evaporation gave a brown oil (1g.). This oil was taken up in methanol (100 ml.) and left at $0^{\circ}C$ overnight. The resulting precipitate was filtered off and recrystallised to give mannitol (200 mg.). Examination of the filtrate by T.L.C. showed the presence of arugoxanthones A and B in smaller amounts relative to the other constituents when

compared with the light petroleum extracts.

Green Spores.

Dried green spores (30g.) were ground in a mortar with sand (5g.) and extracted for 24 hours in a Soxhlet apparatus with light petroleum, evaporation of the solvent then gining a yellow oil (510 mg.) in which small quantities of arugoxanthones A and B could be detected by T.L.C. No other pigmented material was evident in the extract. The spores were then extracted successively for 24 hours with benzene, chloroform, ethanol and water. The extracts obtained were red-brown in colour and T.L.C. investigation of them failed to reveal the presence of any green pigmented material. The only compound obtained in a pure state was mannitol.

Arugoxanthone A (114).

This compound was isolated as described above and crystallised from chloroform-hexane as yellow prisms m. 149-151°C.

T.L.C. Rf 0.77 (100% chloroform); $FeCl_3$: blue; Ce^{4+}: green.

I.R. ν_{max}(KBr): 2970(w), 2920(m), 2850(w), 1635(s), 1595(s), 1575(s), 1470(s), 1425(s), 1355(m), 1240(s), 1195(m), 1115(w), 1075(m), 1045(m), 900(m), 855(s), 825(s) cm^{-1}.

ν_{max}($CHCl_3$): 3460(w), 1635(s), 1595(s), 1570(s), 1470(s), 1350(m), 1115(w), 1070(w), 1045(m) cm^{-1}.

U.V. λ_{max}(EtOH): 242(25000), 257(inf.)(23000), 270(inf.)(30000), 276(32000), 297(inf.)(12000), 394(6500) nm.

λ_{max}(EtOH+$^-$OH): 244(33000), 281(27000), 322(inf.)(6100), 425 (6300) nm.: reverting to EtOH spectrum on acidification.

λ_{max}(EtOH+$AlCl_3$): 242(34000), 269(inf.)(19000), 290(35000), 319 (13000), 346(inf.)(2500), 450(5300) nm.

Gibbs Test. λ_{max}: 660 nm. (ε = 30000).

N.M.R. (100 MHz) τ ($CDCl_3$): -2.55(1H,s,exchangeable,Ar-OH); 2.55(1H,d, J=8Hz,Ar-H); 2.72(1H,s,irr.7.72→sharpening,Ar-H); 3.27(1H,d,J=8Hz,Ar-H); 4.65(1H,m,H geminal to O); 4.75(1H,br.t,irr.8.25→t,J=6Hz,irr.6.57→s, 3,3-dimethylallyl olefinic H); 5.02(1H,d,J=3Hz,irr.4.65→s,exchangeable, O-H); 5.26(1H,s,irr.8.25→sharpening,terminal methylene H trans to vinyl CH_3); 5.47(1H,s,terminal methylene H cis to vinyl CH_3); 5.69(2H,d,J=2Hz, irr.7.35→s,O-CH_2); 6.57(2H,d,J=6Hz,irr.4.75→s,Ar-CH_2); 7.35(1H,m, irr.5.69→d,J=2Hz,irr.5.47→sharpening,irr.4.65→sharpening,allylicH);

7.72(3H,s,Ar-CH_3); 8.21(3H,s,vinyl CH_3); 8.26(3H,s,irr.4.75→-sharpening, vinyl CH_3); 8.30(3H,s,irr.4.75→-sharpening,vinyl CH_3).

M.S. m/e(rel.abundance): 406(M^+)(63), 391(20), 388(36), 376(18), 373(40), 363(24), 338(80), 337(98), 310(49), 295(100).

m^* at m/e = 359, 348, 284, 282, 266.

Analysis. Found: C, 73.81; H, 6.39%; $C_{25}H_{26}O_5$ requires C, 73.87; H, 6.45%; m.w. = 406.

High Resolution M.S. molecular ion at m/e = 406.1780; $C_{25}H_{26}O_5$ requires molecular ion at m/e = 406.1780.

Methylation of Arugoxanthone A: Preparation of the Methyl Ether (116).

Arugoxanthone A (38 mg.), in dry acetone (10 ml.), was stirred and refluxed overnight with anhydrous potassium carbonate (36 mg.) and excess dimethyl sulphate. The mixture was poured into ethyl acetate (50 ml.), washed to neutrality, evaporated and purified by P.L.C. (100% chloroform) to give the methyl ether (116) as colourless needles (ethyl acetate) m. 164-166°C, (25 mg., 65%).

T.L.C. Rf 0.32 (100% chloroform); U.V.: light blue; $FeCl_3$: negative; Ce^{4+}: brown.

I.R. ν_{max}(KBr): 2970(m), 2910(m), 1635(s), 1585(s), 1475(s), 1455(s), 1420(s), 1350(w), 1340(w), 1270(s), 1215(w), 1200(w), 1185(w), 1100(s), 1065(w), 1050(m), 1030(w), 965(w), 895(w), 855(m), 815(m), 800(m) cm^{-1}.

U.V. λ_{max}(EtOH): 243(41000), 248(42000), 290(16000), 372(12000) nm.: no change was observed on the addition of acid or base.

N.M.R. (100 MHz) τ ($CDCl_3$): 2.53(1H,d,J=8Hz,Ar-H); 2.74(1H,s,Ar-H); 3.25(1H,d,J=8Hz,Ar-H); 4.45(1H,d,J=3Hz,exchangeable,irr.4.65→s,O-H); 4.65(2H,m,irr.6.50,7.30 and 8.40→sharpening,3,3-dimethylallyl olefinic H and H geminal to O); 5.26(1H,s,irr.8.40→sharpening,terminal methylene H trans to vinyl CH_3); 5.42(1H,s,irr.7.30→sharpening,terminal methylene H cis to vinyl CH_3); 5.60(2H,d,J=3Hz,irr.7.30→s,O-CH_2); 6.00(3H,s,O-CH_3); 6.50(2H,d,J=6Hz,irr.4.65→s,Ar-CH_2); 7.30(1H,m,irr.4.65 and 5.60→sharpening,allylic H); 7.70(3H,s,Ar-CH_3); 8.41(9H,s,vinyl CH_3).

M.S. m/e(rel.abundance): 420(M^+)(25), 402(38), 387(20), 351(100), 324(20), 295(30).

Analysis. Found: C, 74.36; H, 6.82%; $C_{26}H_{28}O_5$ requires C, 74.26; H, 6.71%; m.w. = 420.

Hydrogenation of Arugoxanthone A: Preparation of Compound (130).

Arugoxanthone A (63 mg.), in ethyl acetate (25 ml.), was stirred for 20 hours with 10% palladium-charcoal (64 mg.) under hydrogen. Hot ethyl acetate (25 ml.) was added, the mixture filtered through glass paper, the residue washed with hot ethyl acetate (25 ml.) and the combined filtrate and washings evaporated. P.L.C. (70% chloroform-petrol) gave the hydrogenolysis product (130) as a yellow oil (17 mg., 28%).

T.L.C. Rf 0.75 (70% chloroform-petrol); Ce^{4+}: brown.

I.R. ν_{max}(CCl_4): 2950(m), 2920(w), 2860(w), 1640(s), 1600(m), 1585(m), 1475(s), 1465(s), 1440(m), 1405(m), 1380(w), 1335(w), 1260(w), 1230(s), 1215(m), 1190(m), 1125(w), 1100(w), 1075(w), 1025(w), 1005(w), 855(w) cm^{-1}.

U.V. λ_{max}(EtOH): 242(30000), 254(28000), 266(inf.)(33000), 273(39000), 294(inf.)(10000), 322(inf.)(3900), 392(7500) nm.

λ_{max}(EtOH+$^{-}$OH): 243(35000), 266(inf.)(26000), 275(29000), 330(4300), 409(5100) nm.: reverting to EtOH spectrum on acidification.

N.M.R. (100 MHz) τ (CCl_4): -2.65(1H,s,exchangeable,Ar-OH); 2.78(1H,d, J=8Hz,Ar-H); 3.08(1H,s,irr.7.82→sharpening,Ar-H); 3.50(1H,d,J=8Hz,Ar-H); 5.70(1H,br.d,J=10Hz,irr.6.40→s,irr.8.45→sharpening,O-C-H); 6.40(2H, br.m,complex changes on irradiation at 5.70, 7.05 and 8.45,O-C-H and Ar-C-H); 7.05(1H,br.d,J=10Hz,Ar-C-H); 7.35(2H,t,J=6Hz,irr.8.45→s, Ar-CH_2); 7.82(3H,s,Ar-CH_3); 8.45(5H,m,O-C-C-H and O-C-C-C-H); 9.00(12H, m,CH_3).

M.S. m/e(rel.abundance): 394(M^+)(64), 352(21), 351(100), 337(28), 279(14), 267(14).

m^* at m/e = 313.

Attempted Oxidation of Arugoxanthone A.

Arugoxanthone A (20 mg.), in acetone (2 ml.), was stirred at room temperature for 15 minutes with Jones reagent (0.3 ml.) and the mixture poured into water (20 ml.). Ethyl acetate (50 ml.) was added, the organic layer washed to neutrality and evaporated to give a yellow gum which on P.L.C. (50% chloroform-petrol) yielded only starting material (18 mg., 90%).

Reaction of Arugoxanthone A with p-Toluenesulphonic Acid.

1) Reaction at room temperature.

Arugoxanthone A (20 mg.), in dry benzene (5 ml.), was stirred at room temperature with p-toluenesulphonic acid (1 mg.). After 48 hours no reaction could be detected by T.L.C.

2) Reaction in refluxing benzene.

Arugoxanthone A (40 mg.) and p-toluenesulphonic acid (1 mg.), in dry benzene (10 ml.), were refluxed overnight. T.L.C. indicated that a single product, less polar than the starting material had been formed. The solvent was evaporated and the yellow gum obtained subjected to P.L.C. (100% chloroform). Extensive decomposition occurred forming a number of polar compounds. Neither these nor the initial non polar product could be obtained in a pure state.

Arugoxanthone B (133).

Arugoxanthone B, isolated as previously described, crystallised as yellow prisms m. 109-110°C (chloroform-hexane).

T.L.C. Rf 0.68 (100% chloroform); $FeCl_3$: blue; Ce^{4+}: brown.

I.R. ν_{max}(KBr): 3470(m), 2970(w), 2930(m), 1640(s), 1600(s), 1575(s), 1480(s), 1425(m), 1380(m), 1345(m), 1330(m), 1260(m), 1240(s), 1215(s), 1080(m), 1005(s), 975(m), 955(s), 820(s) cm^{-1}.

$\nu_{max}(CHCl_3)$: 3500(w), 1640(s), 1600(s), 1580(s), 1475(s), 1380(m), 1345(m), 1105(m), 1075(m), 1005(s) cm^{-1}.

U.V. λ_{max}(EtOH): 236(20000), 268(20000), 290(6700), 380(4400) nm.

λ_{max}(EtOH+$^{-}$OH): 277(20000), 314(8200), 420(4600) nm.: reverting to EtOH spectrum on acidification.

Gibbs Test. λ_{max}: 670 nm. (ε = 28000).

N.M.R. (100 MHz) τ ($CDCl_3$): -1.5(1H,s,exchangeable,Ar-OH); 2.54(1H,d, J=8Hz,irr.6.52→sharpening,Ar-H); 2.65(1H,s,irr.→sharpening,Ar-H); 3.25(1H,d,J=8Hz,Ar-H); 4.38(1H,br.t,irr.8.20→t,J=6Hz,irr.5.55→s, O-3,3-dimethylallyl olefinic H); 4.67(1H,br.t,irr.8.20→t,J=7Hz, irr.6.52→s,C-3,3-dimethylallyl olefinic H); 4.91(2H,d,J=7Hz,irr.5.55→s, D_2O→s,Ar-CH_2-O); 5.55(3H,d,D_2O→2H,d,J=6Hz,irr.4.38→br.s,Ar-O-CH_2 and O-H); 6.52(2H,d,J=7Hz,irr.4.67→s,Ar-CH_2-C); 7.54(3H,s,Ar-CH_3); 8.20(6H, s,vinyl CH_3); 8.24(3H,s,vinyl CH_3); 8.28(3H,s,vinyl CH_3).

M.S. m/e(rel.abundance): 408(M^+)(2), 340(64), 339(75), 322(98), 307(100), 295(12), 254(22).

m^* at m/e = 305, 293.

Analysis. Found: C, 73.13; H, 6.93%; $C_{25}H_{28}O_5$ requires C, 73.51; H, 6.91%; m.w. = 408.

High Resolution M.S. molecular ion at m/e = 408.1936; $C_{25}H_{28}O_5$ requires molecular ion at m/e = 408.1937.

Reaction of Arugoxanthone B with Dimethyl Sulphate: Preparation of the Methyl Ether (134).

A mixture of arugoxanthone B (25 mg.), potassium carbonate (25 mg.) and excess dimethyl sulphate was stirred and refluxed in acetone (20 ml.) for 2 hours. Ethyl acetate (50 ml.) was added and after washing to

neutrality evaporation followed by P.L.C. (100% chloroform) gave the monomethyl ether (134) as an unstable colourless oil (22 mg., 85%).

T.L.C. Rf 0.31 (100% chloroform); U.V.: blue; $FeCl_3$: negative; Ce^{4+}: brown.

I.R. $\nu_{max}(CCl_4)$: 3480(w), 2960(w), 2920(m), 1645(s), 1610(m), 1585(m), 1480(s), 1450(m), 1430(m), 1420(m), 1375(m), 1325(w), 1275(s), 1260(s), 1200(m), 1185(w), 1100(s), 1020(m), 970(m) cm^{-1}.

U.V. λ_{max}(EtOH): 238(27000), 257(26000), 288(9000), 362(6500) nm.: no change was observed on the addition of acid or base.

N.M.R. (60 MHz) τ (CCl_4): 2.74(1H,d,J=8Hz,Ar-H); 2.90(1H,s,Ar-H); 3.25(1H,d,J=8Hz,Ar-H); 4.50(1H,t,J=6Hz,O-3,3-dimethylallyl olefinic H); 4.80(1H,t,J=7Hz,C-3,3-dimethylallyl olefinic H); 5.25(2H,br.s,Ar-CH_2-O), 5.60(2H,d,J=6Hz,Ar-O-CH_2); 6.15(3H,s,Ar-O-CH_3); 6.20(1H,br.s,exchangeable, O-H); 6.60(2H,d,J=7Hz,Ar-CH_2); 7.65(3H,s,Ar-CH_3); 8.30(12H,br.s,vinyl CH_3).

M.S. m/e(rel.abundance): 422(M^+)(4), 367(4), 354(66), 353(100), 336(49), 325(6), 308(8), 307(10), 295(50), 293(40).

m^* at m/e = 319, 283, 278, 256.

Acetylation of Arugoxanthone B: Preparation of the Mono and Diacetates of Arugoxanthone B.

Arugoxanthone B (20 mg.) was allowed to stand at room temperature for 48 hours in acetic anhydride (1 ml.) and pyridine (0.05 ml.). The solution was poured into chilled dilute hydrochloric acid (50 ml.) and

ethyl acetate (50 ml.) added. The organic layer was washed to neutrality and evaporated and the resultant yellow gum purified by P.L.C. (90% chloroform-petrol).

The monoacetate (135)(6 mg., 27%) was obtained as a yellow oil.

T.L.C. Rf 0.75 (100% chloroform); $FeCl_3$: blue; Ce^{4+}: brown.

I.R. $\nu_{max}(CHCl_3)$: 3150(br.w), 2960(m), 2930(m), 2870(m), 1730(s), 1640(s), 1600(s), 1470(br.s), 1360(m), 1075(m), 1030(m), 960(br.m), 865(w) cm^{-1}.

N.M.R. (100 MHz) τ ($CDCl_3$): -2.70(1H,s,exchangeable,Ar-OH); 2.58(1H,d, J=8Hz,Ar-H); 2.62(1H,s,Ar-H); 3.33(1H,d,J=8Hz,Ar-H); 4.26(2H,s,Ar-CH_2-O); 4.38(1H,t,J=6Hz,irr.5.65→s,O-3,3-dimethylallyl olefinic H); 4.67(1H,t, J=7Hz,irr.6.55→s,C-3,3-dimethylallyl olefinic H); 5.65(2H,d,J=6Hz, irr.4.38→s,Ar-O-CH_2); 6.55(2H,d,J=7Hz,irr.4.67→s,Ar-CH_2-C); 7.56(3H,s, Ar-CH_3); 7.99(3H,s,O-CO-CH_3); 8.24(6H,s,vinyl CH_3); 8.30(3H,s,vinyl CH_3); 8.35(3H,s,vinyl CH_3).

The diacetate (136)(13 mg., 54%) was obtained as an unstable colourless oil.

T.L.C. Rf 0.44 (100% chloroform); $FeCl_3$: negative; Ce^{4+}: brown.

I.R. $\nu_{max}(CHCl_3)$: 2970(m), 2920(m), 1760(s), 1730(s), 1655(s), 1615(m), 1605(s), 1470(s), 1440(s), 1360(s), 1100(w), 1075(m), 1030(s), 960(s), 900(w), 865(w) cm^{-1}.

N.M.R. (60 MHz) τ ($CDCl_3$): 2.65(1H,d,J=8Hz,Ar-H); 2.85(1H,s,Ar-H); 3.30(1H,d,J=8Hz,Ar-H); 4.45(2H,s,Ar-CH_2-O); 4.50(1H,t,J=6Hz,O-3,3-dimethylallyl olefinic H); 4.67(1H,t,J=7Hz,C-3,3-dimethylallyl olefinic

H); 5.75(2H,d,J=6Hz,Ar-O-CH_2); 6.50(2H,d,J=7Hz,Ar-CH_2-C); 7.60(3H,s, Ar-CH_3); 7.70(3H,s,Ar-O-CO-CH_3); 8.05(3H,s,Ar-C-O-CO-CH_3); 8.20(9H,s, vinyl CH_3); 8.35(3H,s,vinyl CH_3).

Oxidation of Arugoxanthone B: Preparation of Anhydroarugosin (141).

1) Reaction with manganese dioxide.

Arugoxanthone B (20 mg.), in benzene (5 ml.), was stirred under nitrogen with manganese dioxide (200 mg.). No reaction could be detected by T.L.C. after 48 hours.

2) Reaction with Jones reagent.

Arugoxanthone B (56 mg.), in acetone (5 ml.), was stirred at 0°C for 15 minutes with Jones reagent (0.5 ml.). Water (50 ml.) was added and the acetone evaporated. After extraction into ethyl acetate (50 ml.), washing to neutrality and evaporation a yellow gum was obtained. P.L.C. of this (60% chloroform-petrol) gave anhydroarugosin (141) as yellow needles from benzene-petrol m. 131-132°C (15 mg., 27%).

T.L.C. Rf 0.68 (100% chloroform); $FeCl_3$: blue; Ce^{4+}: yellow.

I.R. ν_{max}($CHCl_3$): 3100(br.w), 1705(s), 1645(s), 1595(s), 1475(s), 1375(m), 1355(m), 1070(m), 980(m), 945(m) cm^{-1}.

U.V. λ_{max}(EtOH): 236(27000), 265(30000), 295(inf.)(11000), 380(5100) nm.

λ_{max}(EtOH+$^-$OH): 278(24000), 305(inf.)(9300), 316(inf.)(8700), 432(5900) nm.: reverting to EtOH spectrum on acidification.

N.M.R. (100 MHz) τ ($CDCl_3$): -2.50(1H,s,exchangeable,Ar-OH); -0.74(1H,s, Ar-CHO); 2.50(1H,d,J=8Hz,Ar-H); 2.85(1H,s,Ar-H); 3.22(1H,d,J=8Hz,Ar-H);

4.60(1H,br.t,J=7Hz,O-3,3-dimethylallyl olefinic H); 4.62(1H,br.t,J=6Hz, C-3,3-dimethylallyl olefinic H); 5.50(2H,d,J=7Hz,Ar-O-CH_2); 6.65(2H,d, J=6Hz,Ar-CH_2); 7.60(3H,s,Ar-CH_3); 8.30(12H,br.s,vinyl CH_3).

M.S. m/e(rel.abundance): 406(M^+)(7), 338(53), 337(26), 310(26), 309(13), 295(100), 242(26).

m^* at m/e = 284, 281.

$C_{25}H_{26}O_5$ requires m.w. = 406.

Reaction of Arugoxanthone B with Acid: Preparation of Compound (148).

Arugoxanthone B (100 mg.) was stirred for 25 minutes in a 3% solution of 5N sulphuric acid in glacial acetic acid (5 ml.) and the mixture poured into ethyl acetate (50 ml.). The yellow oil obtained after washing to neutrality and evaporation was purified by P.L.C. (100% chloroform) to give the alcohol (148) as long yellow needles m. 151-153°C (49 mg., 59%).

T.L.C. Rf 0.29 (2% methanol-chloroform); Ce^{4+}: orange.

I.R. ν_{max} (KBr): 2960(w), 2920(w), 1640(s), 1600(s), 1575(s), 1480(s), 1460(m), 1430(s), 1375(w), 1335(m), 1270(m), 1240(s), 1170(m), 1075(m), 1025(w), 995(m), 825(m), 815(m) cm^{-1}.

U.V. λ_{max}(EtOH): 242(9500), 255(9200), 268(inf.)(11000), 275(12000), 297(4000), 394(2800) nm.

λ_{max}(EtOH+$^-$OH): 265(13000), 283(inf.)(11000), 343(inf.)(2200), 432(3400) nm.: reverting to EtOH spectrum on acidification.

N.M.R. (100 MHz) τ ($CDCl_3$+CD_3OD): 2.65(1H,d,J=8Hz,Ar-H); 2.82(1H,s,Ar-H);

3.35(1H,d,J=8Hz,Ar-H); 4.60(2H,s,Ar-CH_2-O); 4.70(1H,br.t,J=6Hz,3,3-dimethylallyl olefinic H); 6.55(2H,d,J=6Hz,Ar-CH_2-C); 7.62(3H,s,Ar-CH_3); 8.20(6H,s,vinyl CH_3).

M.S. m/e(rel.abundance): 340(M^+)(16), 324(33), 322(50), 310(66), 309(66), 307(100), 295(100), 269(16), 267(16), 255(33), 254(33), 242(33).

$C_{20}H_{20}O_5$ requires m.w. = 340.

Preparation of Deisopentenylanhydroarugosin (142).

The alcohol (148)(18 mg.), in dry chloroform (5 ml.), was stirred under nitrogen for 1 hour with manganese dioxide (186 mg.). The mixture was poured into chloroform (50 ml.), filtered, and the filtrate evaporated to give a yellow oil which on crystallisation from benzene-petrol gave deisopentenylanhydroarugosin as yellow microcrystals m. 187-189°C (7 mg., 38%).

T.L.C. Rf 0.58 (100% chloroform); Ce^{4+}: brown.

I.R. ν_{max}(KBr): 2960(w), 2910(w), 2850(w), 1640(s), 1600(m), 1570(m), 1470(s), 1370(m), 1340(m), 1285(m), 1215(m), 1185(m), 1105(m), 1060(m), 1025(m), 965(m), 810(m), 800(w) cm^{-1}.

U.V. λ_{max}(EtOH): 234(2000), 247(22000), 267(22000), 300(17000), 408(52000) nm.

N.M.R. (100 MHz) τ ($CDCl_3$): -3.32(1H,s,exchangeable,Ar-OH); -2.80(1H,s, exchangeable,Ar-OH); -1.39(1H,s,Ar-CHO); 2.60(1H,s,Ar-H); 2.64(1H,d, J=8Hz,Ar-H); 3.26(1H,d,J=8Hz,Ar-H); 4.72(1H,m,3,3-dimethylallyl olefinic H); 6.60(2H,d,J=6Hz,Ar-CH_2); 7.65(3H,s,Ar-CH_3); 8.30(6H,s,vinyl CH_3).

M.S. m/e(rel.abundance): 338(M^+)(42), 310(16), 295(100), 280(10), 267(5), 255(16), 242(21).

$C_{20}H_{18}O_5$ requires m.w. = 338.

The Gibbs Test.

.5 ml. of a freshly prepared solution of Gibbs reagent in pyridine (20-30 mg./15 ml.) was added to the substance under test (1-3 mg.), in pyridine (1 ml.), and the mixture was diluted to 25 ml. with sodium borate buffer and well mixed. The absorption spectrum was determined between 10 and 20 minutes after mixing, a precisely similar solution of the chloroimide in pyridine-borate being used in the solvent cell of the spectrophotometer. Any necessary dilution was carried out using the aqueous buffer solution.

Mannitol (189).

Mannitol was obtained as colourless prisms from ethanol m. 162-163°C (lit. 166°C).

I.R. ν_{max}(KBr): 3500-3000(v.br.s), 2940(m), 1450(s), 1375(m), 1325(m), 1085(s), 1050(m), 1020(s), 960(m), 930(s), 885(m), 870(m) cm^{-1}.

N.M.R. (60 MHz) τ (D_2O): 6.30.

M.S. m/e(rel.abundance): 146(M^+-36)(3), 133(28), 121(1), 115(7), 103(100).

m^* at m/e = 99.5, 42.5, 30.5.

Analysis. Found: C, 39.30; H, 7.79%; calculated for $C_6H_{14}O_6$ C, 39.56; H, 7.75%; m.w. = 182.

Mannitol Hexaacetate (190).

The compound (85 mg.) obtained from the spore extracts and thought to be mannitol was stirred for 48 hours with acetic anhydride (2 ml.) and pyridine (0.25 ml.). The mixture was poured into cold dilute hydrochloric acid (50 ml.) and extracted with ethyl acetate (2x50 ml.). After washing to neutrality and evaporation a colourless oil was obtained which gave, on crystallisation from ethyl acetate-petrol, material identical in all respects (Rf, spectral data, mixed melting point) with an authentic sample of mannitol hexaacetate (190) m. 122-123°C (120 mg., 59%).

T.L.C. Rf 0.43 (100% chloroform); Ce^{4+}: grey.

I.R. $\nu_{max}(CHCl_3)$: 1745(s), 1365(s), 1030(m) cm^{-1}.

N.M.R. (60 MHz) τ ($CDCl_3$): 4.45-5.95(8H,m,O-C-H); 7.92(6H,s,O-CO-CH_3); 7.95(6H,s,O-CO-CH_3); 7.98(6H,s,O-CO-CH_3).

Analysis. Found: C, 49.64; H, 5.95%; calculated for $C_{18}H_{26}O_{12}$ C, 49.77; H, 5.99%.

3,3-Dimethylallyl Bromide.

Freshly distilled isoprene (42g.) was added dropwise at 0°C to a cooled solution of hydrogen bromide in acetic acid (113 ml., 45% w./v. HBr). The mixture was set aside in the dark for 48 hours and then poured into ice cold water (400 ml.). The green oil which separated out was washed with water and dried with potassium carbonate and magnesium sulphate. Distillation under reduced pressure gave 3,3-dimethylallyl

bromide as a colourless oil (56g., 61%)(b.pt. 36-38°C/10 mm. Hg).

I.R. ν_{max}(liquid film): 3020(w), 2970(m), 2930(m), 2910(m), 2850(w), 1660(m), 1440(m), 1370(m), 1220(w), 1195(s), 1135(w), 1095(w), 1085(w), 1010(w), 835(m), 755(w) cm^{-1}.

N.M.R. (60 MHz) τ ($CDCl_3$): 4.45(1H,br.t,J=8Hz,olefinic H); 6.00(2H,d, J=8Hz,CH_2-Br); 8.25(3H,s,vinyl CH_3); 8.30(3H,s,vinyl CH_3).

2-O-(3,3-Dimethylallyl)-3-methoxybenzaldehyde (149).

3,3-Dimethylallyl bromide (2.2g.), in acetone (20 ml.), was added to a refluxing mixture of freshly distilled 2-hydroxy-3-methoxybenzaldehyde (1.5g.) and potassium carbonate (1.5g.) in acetone (50 ml.). After 2 hours ethyl acetate (100 ml.) was added, the mixture washed with water and evaporated to give a green oil which after treatment with decolourising charcoal afforded the dimethylallyl ether (149) as a colourless oil (2.1g., 95%)(b.pt. 80°C/0.03 mm. Hg).

T.L.C. Rf 0.54 (100% chloroform); U.V.: green-blue; Ce^{4+}: brown.

I.R. ν_{max}($CHCl_3$): 2980(m), 2950(m), 2880(m), 2850(w), 1690(s), 1600(m), 1585(s), 1480(s), 1380(m), 1075(s), 955(m) cm^{-1}.

N.M.R. (60 MHz) τ ($CDCl_3$): -0.4(1H,s,-CHO); 2.7(3H,br.m,Ar-H); 4.45(1H, br.t,J=8Hz,irr.5.35→s,olefinic H); 5.35(2H,d,J=8Hz,irr.4.45→s,O-CH_2-); 6.10(3H,s,O-CH_3); 8.25(3H,s,vinyl CH_3); 8.40(3H,s,vinyl CH_3).

M.S. m/e(rel.abundance): 220(M^+)(2), 152(100), 151(12), 149(11), 106(33). m^* at m/e = 118, 99.5, 84, 74.

Analysis. Found: C, 71.02; H, 7.48%; $C_{13}H_{16}O_3$ requires C, 70.89;

H, 7.32%; m.w. = 220.

Attempted Reaction of the Dimethylallyl Ether (149) with Acetic Anhydride and Pyridine.

The dimethylallyl ether (149)(20 mg.) was stirred at room temperature with acetic anhydride (2 ml.) and pyridine (0.05 ml.) After 48 hours no reaction could be detected by T.L.C.

Reaction of the Dimethylallyl Ether (149) with p-Toluenesulphonic Acid: Preparation of Compounds (150) and (151).

The dimethylallyl ether (149)(2g.), in dry benzene (20 ml.), was stirred, under nitrogen, with a trace of p-toluenesulphonic acid for 72 hours and the solvent evaporated. Analysis by P.L.C. (petrol-chloroform mixtures) gave 4 products of which product 1, the least polar proved to be unstable.

Product 2. This compound (800 mg., 40%) was identical (Rf, N.M.R.) with 2-hydroxy-3-methoxybenzaldehyde.

Product 3. Crystallisation of this material from hexane gave the alcohol (150) as colourless prisms m. 73-75°C (520 mg., 26%).

T.L.C. Rf 0.21 (100% chloroform); Ce^{4+}: brown.

I.R. ν_{max}(KBr): 3520(s), 1645(w), 1590(m), 1485(s), 1260(s), 1215(s), 1100(s), 880(s), 730(s), 610(s) cm^{-1}.

N.M.R. (100 MHz) τ ($CDCl_3$): 3.10(3H,br.m,Ar-H); 4.90(1H,s,irr.7.35 and 8.05→sharpening,olefinic H); 5.25(2H,br.s,irr.7.35 and 8.05→sharpening,

D_2O→two close singlets,olefinic H and Ar-C-H); 5.65(2H,br.m,irr.7.35→s, O-CH_2-); 6.10(3H,s,O-CH_3); 7.35(1H,m,irr.5.65→br.s,allylic H); 8.05(4H, s,D_2O→3H,s,vinyl CH_3 and O-H).

M.S. m/e(rel.abundance): 220(M^+)(64), 152(100), 151(32), 109(40), 106(94).

Analysis. Found: C, 71.07; H, 7.53%; $C_{13}H_{16}O_3$ requires C, 70.89; H, 7.32%; m.w. = 220.

Product 4. The most polar product, the alcohol (151), was obtained as colourless needles from hexane m. 94-95°C (480 mg., 24%).

T.L.C. Rf 0.20 (100% chloroform); Ce^{4+}: brown.

I.R. ν_{max}(KBr): 3350(m), 3080(w), 2950(w), 2840(w), 1640(m), 1580(m), 1480(s), 1435(m), 1260(s), 1240(s), 1220(s), 1040(s), 890(s), 785(m), 745(s) cm^{-1}.

N.M.R. (100 MHz) τ ($CDCl_3$): 3.10(3H,br.m,Ar-H); 5.05(1H,s,irr.8.20→ sharpening,olefinic H); 5.20(1H,s,olefinic H); 5.23(1H,d,J=5Hz,irr.7.45→ s,Ar-C-H); 5.65,5.85(2H,m,O-CH_2-) and 7.45(1H,m,allylic H)(ABX system, J_{AB}=11Hz,J_{AX}=3Hz,J_{BX}=9Hz); 6.20(3H,s,O-CH_3); 8.05(1H,s,exchangeable,O-H); 8.20(3H,s,vinyl CH_3).

M.S. m/e(rel.abundance): 220(M^+)(33), 202(38), 161(38), 152(100), 106(66).

Analysis. Found: C, 71.00; H, 7.44%; $C_{13}H_{16}O_3$ requires C, 70.89; H, 7.32%; m.w. = 220.

Oxidation of the Alcohol (150).

The alcohol (150)(38 mg.) and Jones reagent (0.15 ml.) were stirred in acetone (2 ml.) at 0°C, for 1 minute and water (20 ml.) and ethyl

acetate (50 ml.) added. The organic layer, after washing, drying and evaporation, gave an oil which was purified by P.L.C. (80% chloroform-petrol) to yield the ketone (154) as colourless rods (ethyl acetate-hexane), m. 72°C (32 mg., 89%).

T.L.C. Rf 0.53 (100% chloroform); Ce^{4+}: brown.

I.R. $\nu_{max}(CCl_4)$: 1695(s), 1640(w), 1605(m), 1580(m), 1490(s), 1260(s), 1055(m), 895(m) cm^{-1}.

N.M.R. (60 MHz) τ (CCl_4): 2.59(1H,dd,$J_{5,6}$=7Hz,$J_{5,7}$=3Hz,H-5); 3.10(2H,m, H-6 and H-7); 5.05(2H,br.s,olefinic H); 5.45(2H,d,J=6Hz,O-CH_2); 6.19(3H, s,O-CH_3); 6.80(1H,t,J=6Hz,allylic H); 8.19(3H,s,vinyl CH_3).

M.S. m/e(rel.abundance): 218(M^+)(50), 203(5), 175(5), 150(66), 122(100). m^* at m/e = 99.

Analysis. Found: C, 71.44; H, 7.44%; $C_{13}H_{14}O_3$ requires C, 70.89; H, 7.32%; m.w. = 218.

Oxidation of the Alcohol (151).

The alcohol (151) was oxidised under conditions identical to those described for the alcohol (150). The product, isolated in the same manner, was found to be identical in all respects (Rf, spectral data, mixed melting point) to the ketone (154) obtained from the previous reaction.

Reduction of the Ketone (154).

The ketone (154)(20 mg.) obtained by oxidising the alcohol (150),

in ethanol (5 ml.), was stirred with an excess of sodium borohydride (10 mg.) for 1 hour. Water (20 ml.) was added and the ethanol evaporated. After extraction with ethyl acetate (50 ml.) and washing of the organic layer with water evaporation yielded a product (15 mg.) in which the alcohols (150) and (151) could be detected by T.L.C.

Catalytic Reduction of the Alcohol (150): Preparation of Compounds (152) and (153).

The alcohol (150)(52 mg.), in ethyl acetate (15 ml.), was stirred under hydrogen with 5% palladium-charcoal (20 mg.) for 16 hours. Hot ethyl acetate (25 ml.) was added, the mixture filtered and the residue washed with more hot solvent (25 ml.). The combined filtrate and washings was evaporated and the resultant colourless oil purified by P.L.C. (100% chloroform). Elution of the least polar band gave the hydrogenolysis product (152) as a colourless oil (30 mg., 60%).

T.L.C. Rf 0.68 (100% chloroform); Ce^{4+}: brown.

I.R. $\nu_{max}(CHCl_3)$: 1580(m), 1480(s), 1095(s), 1075(s), 880(w) cm^{-1}.

N.M.R. (60 MHz) τ (CCl_4): 3.45(3H,m,Ar-H); 5.70(2H,br.m,O-CH_2-); 6.25(3H,s,O-CH_3); 7.15(2H,br.d,J=8Hz,Ar-CH_2-); 8.30(2H,br.m,methine H); 9.0(6H,d,J=4Hz,-CH_3).

M.S. m/e(rel.abundance): 206(M^+)(100), 191(13), 163(25), 138(63).

The hydrogenation product (153) was obtained from the more polar band as a colourless oil (20 mg., 34%).

T.L.C. Rf 0.28 (100% chloroform); Ce^{4+}: yellow-brown.

I.R. $\nu_{max}(CHCl_3)$: 3600(m), 1585(m), 1485(s), 1255(s), 1080(m), 1035(m), 995(m) cm^{-1}.

N.M.R. (60 MHz) τ (CCl_4): 3.30(3H,m,Ar-H); 5.25(1H,m,D_2O→-d,J=4Hz, Ar-C-H); 5.85(2H,d,J=3Hz,O-CH_2-); 6.25(3H,s,O-CH_3); 8.15(1H,m,exchangeable,O-H); 8.50(2H,m,methine H); 9.05(6H,d,J=4Hz,-CH_3).

M.S. m/e(rel.abundance): 222(M^+)(27), 206(13), 204(33), 189(13), 161(100), 152(33).

$C_{13}H_{18}O_3$ requires m.w. = 222.

EXPERIMENTAL.

CHAPTER 5.

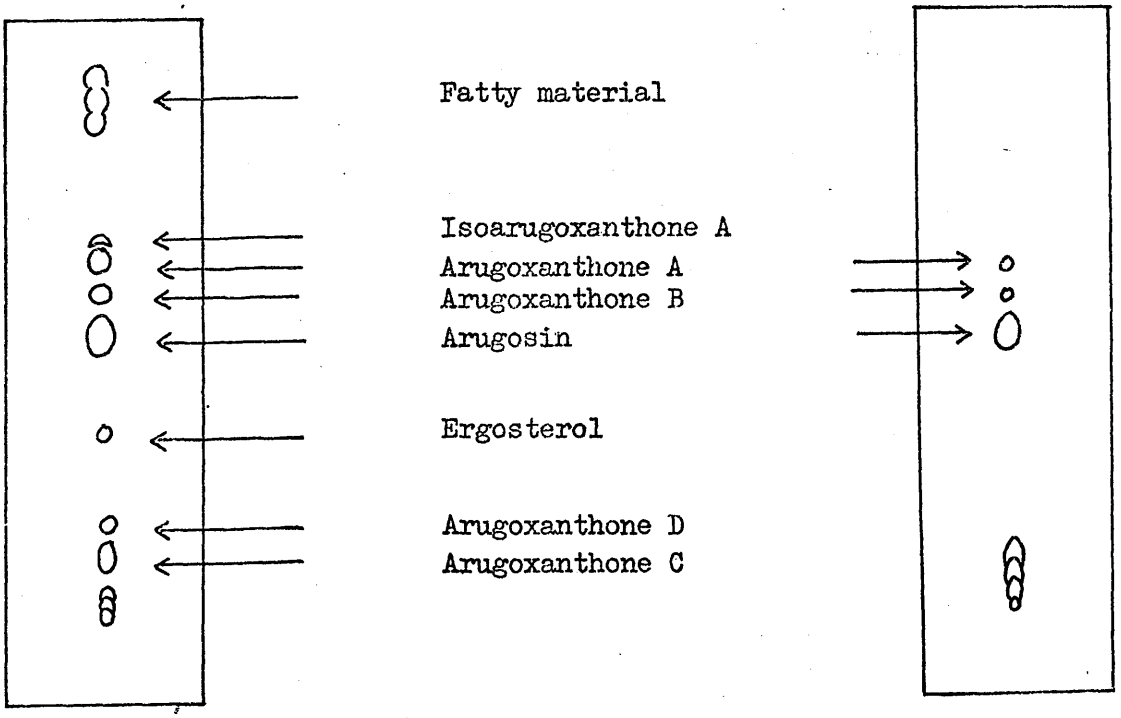
Culture Medium for Yellow Mutant of Aspergillus Nidulans.

Glucose	50 g.
Sodium nitrate	2 g.
Potassium chloride	1 g.
Magnesium sulphate	1 g.
Potassium hydrogen phosphate	0.5 g.
Ferrous sulphate	0.01 g.
Yeast extract	1 g.
Distilled water	1 litre.

Chromatographic Separation of the Metabolites Present in the Petrol Extract of the Yellow Spores.

Fractions.	Eluting solvent.	Constituents.
1-19	Petrol, 1 and 2% ethyl acetate-petrol.	colourless oils.
20-23	5% ethyl acetate-petrol	isoarugoxanthone A, arugoxanthone A, arugoxanthone B and arugosin.
24-26	15% ethyl acetate-petrol	arugosin and ergosterol.
27-32	30% ethyl acetate-petrol	arugoxanthone C and arugoxanthone D.
33-37	40-100% ethyl acetate-petrol.	polar oils.

T.L.C. of Crude Extracts of the Yellow Mutant (50% petrol-chloroform).



Surface Cultures of Aspergillus Nidulans.

The mutants of Aspergillus nidulans were grown on surface culture using the same technique as that described previously for the growth of Verticillium lamellicola.

Yellow Mutant.

This strain was grown on the Czapek Dox and yeast medium shown opposite, at 75% humidity and 25°C and cropped off after 24 days. The broth was decanted off, the mycelium killed by the addition of a little chloroform, dried at 40°C and then extracted in a Soxhlet apparatus for 24 hours with first light petroleum and then chloroform. Evaporation yielded the crude extracts.

Chromatographic Fractionation of the Light Petroleum Extract.

Crude light petroleum extract (12.5g.) in chloroform (100 ml.) was adsorbed on silica (25g.), evaporated under reduced pressure, finely powdered and introduced onto a column of silica (400g., 120x5 cm.) made up in light petroleum (450 ml.). The column was eluted with light petroleum, 1, 2, 5, 15, 20, 40, 60% ethyl acetate-light petroleum mixtures and finally ethyl acetate using one column volume of each successively. Fractions (150 ml.) were collected and moitered by T.L.C.

Fractions 1-19, containing non polar fatty oils, were set aside and no further work was carried out on them. Fractions 20-23 were combined and separated by P.L.C. (using petrol-chloroform mixtures and multiple elution) into 3 major yellow bands. Crystallisation of the least polar

of these bands, from hexane-chloroform, gave arugoxathone A (200 mg.) - identified by comparison with a sample obtained from the spore extracts. P.L.C. (50% petrol-chlorcform) of the crystallisation residues gave more arugoxanthone A (20 mg.) and also isoarugoxanthone A (20 mg.).

Crystallisation of the more polar second band afforded material which was identified by comparison with a sample obtained from spore extracts as arugoxanthone B (150 mg.). P.L.C. (40% petrol-chloroform) of the most polar band gave arugosin (750 mg.).

More arugosin (250 mg.) was obtained by P.L.C. (50% petrol-chloroform) of the material in fractions 24-26. Ergosterol (5 mg.) was also obtained and identified by comparison (Rf, spectral data) with an authentic sample.

Crystallisation of fractions 27-32 from hexane-ethyl acetate gave a yellow solid which could be separated by P.L.C. (2% methanol-chloroform) into arugoxanthone C (50 mg.) and arugoxanthone D (10 mg.).

Chloroform Extract.

Analytical T.L.C. revealed that arugoxanthones A, B, C and D and arugosin were present in this extract but that it mainly consisted of more polar material from which no pure compounds have so far been isolated.

Green Mutant.

Unsuccessful attempts were made to grow the green strain on surface

culture using the Czapek Dox and yeast medium mentioned above After 24 days only a very poor yield of fungus was obtained and very little pigmented material had been produced. A more effective culture medium has not as yet been found.

Isoarugoxanthone A (114).

This metabolite was isolated as described previously and crystallised from chloroform-hexane as yellow prisms m. 115-116°C.

T.L.C. Rf 0.78 (100% chloroform); $FeCl_3$: blue; Ce^{4+}: brown.

I.R. $\nu_{max}(CHCl_3)$: 3500(br.w), 1645(s), 1605(m), 1580(m), 1485(s) cm^{-1}.

U.V. λ_{max}(EtOH): 242(18000), 256(16000), 269(inf.)(20000), 275(22000), 296(6800), 394(4100) nm.

λ_{max}(EtOH+$^-$OH): 245(21000), 281(18000), 320(4400), 330(inf.)(4300), 426(3800) nm.: reverting to EtOH spectrum on acidification.

N.M.R. (100 MHz) τ ($CDCl_3$): -2.50(1H,s,exchangeable,Ar-OH); 2.60(1H,d, J=8Hz,Ar-H); 2.75(1H,s,Ar-H); 3.30(1H,d,J=8Hz,Ar-H); 4.53(1H,m,D_2O→d, J=3Hz,H geminal to O); 4.68(1H,br.t,J=6Hz,irr.6.52→s,3,3-dimethylallyl olefinic H); 4.95(1H,br.s,irr.7.98→sharpening,terminal methylene H trans to vinyl CH_3); 5.20(1H,s,terminal methylene H cis to vinyl CH_3); 5.45(1H,m,exchangeable,O-H); 5.60(2H,m,O-CH_2); 6.52(2H,d,J=6Hz,irr.4.68 →s,Ar-CH_2-); 7.50(1H,m,irr.4.53→d.d,J_1=4Hz,J_2=8Hz,allylic H); 7.65(3H, s,Ar-CH_3); 7.98(3H,s,vinyl CH_3); 8.20(6H,s,vinyl CH_3).

M.S. m/e(rel.abundance): 406(M^+)(60), 391(25), 388(36), 376(20), 373(35), 363(24), 338(70), 337(95), 310(50), 295(100).

$C_{25}H_{26}O_5$ requires m.w. = 406.

Arugosin (143 and 144).

Arugosin, isolated in the manner described above, was obtained as a viscous yellow oil.

T.L.C. Rf 0.58 (100% chloroform); Ce^{4+}: brown.

I.R. $\nu_{max}(CHCl_3)$: 3570(m), 3340(br.m), 1625(s), 1585(s), 1475(s), 1410(s), 1340(s), 1095(m), 1030(m), 985(m), 820(m) cm^{-1}.

U.V. λ_{max}(EtOH): 280(9300), 368(7200) nm.

λ_{max}(EtOH+$^-$OH): 243(inf.)(38000), 275(inf.)(19000), 385(8700), 476(8500) nm.

N.M.R. (60 MHz) τ (CCl_4): -3.37($\frac{2}{3}$H,s,exchangeable,Ar-OH); -2.78($\frac{1}{3}$H,s, exchangeable,Ar-OH); -1.09($\frac{1}{3}$H,s,exchangeable,Ar-OH); -0.87($\frac{2}{3}$H,s, exchangeable,Ar-OH); 2.76(1H,d,J=8Hz,Ar-H); 3.20(1H,s,Ar-H); 3.41($\frac{1}{3}$H,d, J=8Hz,Ar-H); 3.61($\frac{2}{3}$H,d,J=8Hz,Ar-H); 3.46(1H,d,J=5Hz,D_2O--s,Ar-CH-O); 4.48(1H,m,O-3,3-dimethylallyl olefinic H); 4.73(1H,m,C-3,3-dimethylallyl olefinic H); 5.00(1H,m,exchangeable,O-H); 5.66(2H,d,J=7Hz,Ar-CH_2-); 6.74(2H,d,J=8Hz,Ar-O-CH_2-); 7.68(3H,s,Ar-CH_3); 8.25(12H,br.s,vinyl CH_3).

M.S. m/e(rel.abundance): 424(M^+)(14), 406(7), 356(52), 338(69), 327(31), 323(34), 321(28), 311(14), 300(21), 295(34), 283(66), 271(100).

Analysis. Found: C, 71.38; H, 5.40%; $C_{20}H_{18}O_5$ requires C, 71.00; H, 5.36%; m.w. = 424.

Methylation of Arugosin: Preparation of the Dimethyl Ether (158).

1) Reaction with dimethyl sulphate.

Arugosin (76 mg.), in dry acetone (20 ml.), was stirred and refluxed for 18 hours with potassium carbonate (76 mg.) and excess dimethyl sulphate. Chloroform (50 ml.) was added and the solution washed with water (2x50 ml.) and evaporated. P.L.C. (40% chloroform-petrol) gave the

dimethyl ether (158) as pale yellow rods (chloroform-hexane) m. 117-118°C (49 mg., 61%).

2) Reaction with methyl iodide.

Arugosin (119 mg.), potassium carbonate (1.5g.) and methyl iodide (20 ml.) were stirred, in acetone (20 ml.), for 24 hours and then poured into ether (150 ml.). After filtration and evaporation P.L.C. (100% chloroform) gave material identical (Rf, spectral data) to that obtained from the previous reaction i.e. (158).

T.L.C. Rf 0.14 (50% chloroform-petrol); $FeCl_3$: blue; Ce^{4+}: brown.

I.R. $\nu_{max}(CCl_4)$: 2960(m), 2920(m), 2850(m), 1690(s), 1610(s), 1400(s), 1310(m), 1220(s), 1195(s), 1095(s), 985(s) cm^{-1}.

U.V. λ_{max}(EtOH): 274(16000), 346(9000) nm.

λ_{max}(EtOH+$^-$OH): 288(8600) nm.: reverting to EtOH spectrum on acidification.

Gibbs Test. λ_{max} 630 nm. (ε = 30000).

N.M.R. (100 MHz) τ (CCl_4): -3.15(1H,s,exchangeable,Ar-OH); -0.05(1H,s, -CHO); 2.95(1H,d,J=8Hz,Ar-H); 3.10(1H,s,Ar-H); 3.96(1H,d,J=8Hz,Ar-H); 4.55(1H,m,O-3,3-dimethylallyl olefinic H); 4.78(1H,m,C-3,3-dimethylallyl olefinic H); 5.65(1H,d,J=6Hz,Ar-O-CH_2-); 6.36(3H,s,-OCH_3); 6.74(3H,s, -OCH_3); 6.76(2H,d,J=6Hz,Ar-CH_2-); 7.69(3H,s,Ar-CH_3); 8.25(6H,s,vinyl CH_3); 8.32(3H,s,vinyl CH_3); 8.41(3H,s,vinyl CH_3).

M.S. m/e(rel.abundance): 452(M^+)(5), 434(5), 421(5), 403(5), 384(20), 353(100), 335(15), 297(40), 217(15), 215(15), 201(10), 196(10), 193(15), 163(30).

m^* at m/e = 324.5.

Analysis. Found: C, 71.53; H, 7.27%; $C_{27}H_{32}O_6$ requires C, 71.66; H, 7.13%; m.w. = 452.

Reaction of the Dimethyl Ether (158) with p-Toluenesulphonic Acid.

The dimethyl ether (158)(10 mg.), in dry benzene (5 ml.), was stirred with a trace of p-toluenesulphonic acid for 8 hours. Evaporation of the solvent gave a black gum from which no pure compound could be isolated by P.L.C.

Preparation of the Pseudo-acid (161).

The dimethyl ether (158)(28 mg.) was stirred for 10 minutes, under nitrogen, with 2% ethanolic potassium hydroxide (10 ml.) and the mixture acidified with dilute hydrochloric acid. Ethyl acetate (50 ml.) was added, the organic layer washed to neutrality and evaporated to give a brown gum which on P.L.C. (3% methanol-chloroform) afforded the pseudo-acid (161) as colourless prisms from carbon tetrachloride m. 135-136°C (8 mg., 61%).

T.L.C. Rf 0.31 (5% methanol-chloroform); U.V.: blue; Ce^{4+}: grey.

I.R. $\nu_{max}(CCl_4)$: 3380(br.s), 1750(s), 1670(w), 1600(w), 1485(s), 1460(m), 1440(m), 1410(w), 1375(m), 1345(w), 1325(m), 1225(s), 1185(w), 1150(w), 1125(w), 1070(s), 950(br.s), 870(m) cm^{-1}.

U.V. λ_{max}(EtOH): 227(14000), 243(8800), 312(5000) nm.

λ_{max}(EtOH+$^-$OH): 232(14000), 266(5700), 340(3600) nm.: reverting to

EtOH spectrum on acidification.

N.M.R. (100 MHz) τ ($CDCl_3$): 3.20(1H,s,Ar-H); 3.40(1H,br.s,D_2O→-sharpening,Ar-CH-O); 4.48(1H,br.t,irr.8.20→t,J=6Hz,irr.5.45→s,O-3,3-dimethylallyl olefinic H); 5.45(2H,d,J=6Hz,irr.4.48→s,Ar-CH_2-); 6.06(3H,-OCH_3); 7.62(3H,s,Ar-CH_3); 8.15(3H,s,vinyl CH_3); 8.25(3H,s,vinyl CH_3).

M.S. m/e(rel.abundance): 210(M^+-68)(63), 192(100), 182(30), 163(53). m^* at m/e = 175.5, 140, 128.5.

Analysis. Found: C, 64.90; H, 6.55%; $C_{15}H_{18}O_5$ requires C, 64.74; H, 6.55%; m.w. = 278.

Reactions of Arugosin Under Acidic Conditions.

1) Attempted reaction with ethereal sulphuric acid.

Arugosin (20 mg.) was stirred with diethyl ether (2 ml.) and 5N sulphuric acid (0.1 ml.). No reaction could be detected by T.L.C. after 48 hours.

2) Reaction with p-toluenesulphonic acid.

Arugosin (20 mg.), in benzene (2 ml.), was stirred with a trace of p-toluenesulphonic acid for 18 hours and the reaction monitored by T.L.C. A slow conversion to a large number of products was observed.

3) Reaction with 3% sulphuric acid in acetic acid.

Arugosin (300 mg.) was stirred for 30 minutes with 3% sulphuric acid in acetic acid (10 ml.). The mixture was poured into ethyl acetate (50 ml.), washed to neutrality with water and evaporated to give a yellow gum which was purified by P.L.C. (chloroform-petrol mixtures).

The least polar product- the cyclic compound (162)- crystallised from benzene-petrol as yellow prisms m. 128°C (24 mg., 8%).

T.L.C. Rf 0.58 (100% chloroform); Ce^{4+}: red.

I.R. $\nu_{max}(CCl_4)$: 3615(w), 3580(m), 3400-3100(br.), 2980(m), 2925(m), 2855(w), 1610(m), 1585(m), 1485(s), 1415(s), 1385(w), 1375(w), 1360(w), 1345(w), 1320(w), 1285(w), 1275(w), 1210(s), 1170(w), 1140(w), 1125(w), 1055(s), 1025(m), 990(w), 965(w), 905(m), 875(w) cm^{-1}.

U.V. λ_{max}(EtOH): 270(7600), 290(7400), 307(7600), 405(5500) nm.

λ_{max}(EtOH+$^-$OH): 290(8500), 450(4200) nm.: reverting to EtOH spectrum on acidification.

Gibbs Test. λ_{max} 632 nm. (ε = 31000).

N.M.R. (100 MHz) τ ($CDCl_3$): -4.00(1H,s,exchangeable,Ar-OH); -0.65(1H,s, exchangeable,Ar-OH); 2.75(1H,d,J=8Hz,Ar-H); 3.20(1H,s,Ar-H); 4.75(1H, br.t,irr.8.25→t,J=6Hz,C-3,3-dimethylallyl olefinic H); 4.95(1H,d,J=4Hz, irr.7.95→s,Ar-CH-O); 5.40-5.90(2H,br.m,irr.7.95→complex changes, Ar-O-CH_2-); 6.70(2H,d,J=6Hz,irr.4.75→s,Ar-CH_2-); 7.30(1H,br.s,exchangeable,O-H); 7.75(3H,s,Ar-CH_3); 7.95(1H,m,Ar-C-C-H); 8.23(3H,s,vinyl CH_3); 8.26(3H,s,vinyl CH_3); 8.60(3H,s,CH_3 geminal to O); 8.65(3H,s,CH_3 geminal to O).

M.S. m/e(rel.abundance): 424(M^+)(33), 366(27), 349(7), 310(7), 293(13), 205(13), 203(19), 188(100), 162(54), 149(33).

m^* at m/e = 316.

Analysis. Found: C, 70.44; H, 6.77%; $C_{25}H_{28}O_6$ requires C, 70.74; H, 6.65%; m.w. = 424.

The more polar cyclic compound (163) was obtained as a yellow oil (23 mg., 8%).

T.L.C. Rf 0.50 (100% chloroform); Ce^{4+}: red.

I.R. $\nu_{max}(CCl_4)$: 3620(m), 2970(w), 2920(w), 2855(w), 1615(s), 1585(s), 1480(s), 1415(s), 1360(s), 1330(w), 1285(w), 1215(s), 1165(w), 1125(w), 1060(s), 1000(w), 930(w), 915(w), 870(w) cm^{-1}.

U.V. λ_{max}(EtOH): 270(8900), 291(9100), 312(9600), 404(8200) nm.

λ_{max}(EtOH+$^-$OH): 290(9800), 434(5700) nm.: reverting to EtOH spectrum on acidification.

Gibbs Test. λ_{max} 635 nm. (ε = 32000).

N.M.R. (100 MHz) τ ($CDCl_3$): -3.75(1H,s,exchangeable,Ar-OH); -0.6(1H,s, exchangeable,Ar-OH); 2.75(1H,d,J=8Hz,Ar-H); 3.20(1H,s,Ar-H); 3.62(1H,d, J=8Hz,Ar-H); 4.70(1H,br.t,irr.8.25→t,J=6Hz,irr.6.70→s,C-3,3-dimethyl-allyl olefinic H); 4.95(1H,d,J=4Hz,irr.7.60→s,Ar-CH-O); 5.65, 5.82 and 7.60(1H,1H,1H,ABX system,J_{AB}=12Hz,J_{AX}=4Hz,J_{BX}=6Hz,Ar-O-CH_2- and Ar-O-C-C-H); 6.70(2H,d,J=6Hz,Ar-CH_2-); 7.75(3H,s,Ar-CH_3); 8.22(3H,s,vinyl CH_3); 8.25(3H,s,vinyl CH_3); 8.35(1H,br.s,exchangeable,O-H); 8.66(3H,s,CH_3 geminal to O); 8.72(3H,s,CH_3 geminal to O).

M.S. m/e(rel.abundance): 424(M^+)(44), 366(15), 349(8), 310(9), 293(15), 271(6), 205(16), 203(26), 188(100), 162(57), 149(33).

m^* at m/e = 333, 316, 277, 262.5, 246, 188, 161, 136.5, 108.5, 96.5, 71.5.

The most polar product- deisopentenylarugosin (170)- was obtained as yellow prisms from ethyl acetate-petrol m. 118-120°C (135 mg., 52%).

T.L.C. Rf 0.10 (100% chloroform); Ce^{4+}: green.

I.R. $\nu_{max}(CHCl_3)$: 3580(m), 3440(br.m), 1650(m), 1620(s), 1590(s), 1040(s), 985(s) cm^{-1}.

U.V. λ_{max}(EtOH): 246(11000), 270(11000), 290(inf.)(5700), 386(2300) nm.

λ_{max}(EtOH+$^-$OH): 240(inf.)(13000), 280(9700) nm.

N.M.R. (100 MHz) τ ($CDCl_3$): -3.60(1/5H,s,exchangeable,Ar-OH); -3.02(1/5H, s,exchangeable,Ar-OH); -1.80(3/5H,s,exchangeable,Ar-OH); 0.30(3/5H,s, Ar-CHO); 1.20(3/5H,s,exchangeable,Ar-OH); 1.60(2/5H,s,exchangeable,Ar-OH); 2.05(6/5H,s,exchangeable,Ar-OH); 2.65-3.70(19/5H,br.m,Ar-H and Ar-CH-O); 4.80(1H,br.t,C-3,3-dimethylallyl olefinic H); 4.85(4/5H,m,exchangeable, O-H); 6.78(2H,d,Ar-CH_2-); 7.70(3H,s,Ar-CH_3); 8.25(3H,s,vinyl CH_3); 8.28(3H,s,vinyl CH_3).

M.S. m/e(rel.abundance): 356(M^+)(46), 338(100), 323(46), 295(70), 283(100), 271(70), 267(46), 255(55), 179(61), 149(84).

$C_{20}H_{20}O_6$ requires molecular ion at m/e = 356.

Preparation of the Trimethyl Ether (173).

Deisopentenylarugosin (170)(40 mg.), in acetone (20 ml.), was stirred and refluxed overnight with potassium carbonate (40 mg.) and excess dimethyl sulphate. Chloroform (50 ml.) was added, the mixture acidified with dilute hydrochloric acid, washed to neutrality with water and evaporated. P.L.C. (50% petrol-chloroform) gave the trimethyl ether (173) as light yellow needles m. 158-160°C (benzene-petrol)(21 mg., 48%).

T.L.C. Rf 0.14 (50% chloroform-petrol); Ce^{4+}: olive.

I.R. ν_{max}($CHCl_3$): 3180(br.w), 1690(s), 1610(s), 1460(m), 1385(m), 1370(m), 1310(s), 1095(s), 995(m), 965(w), 955(w) cm^{-1}.

U.V. λ_{max}(EtOH): 272(23000), 346(12000) nm.

λ_{max}(EtOH+$^-$OH): 263(25000) nm.

Gibbs Test. λ_{max} 630 nm. (ε = 23000).

N.M.R. (60 MHz) τ ($CDCl_3$): -3.80(1H,s,exchangeable,Ar-OH); -0.80(1H,s, Ar-CHO); 2.85(1H,s,Ar-H); 3.05(1H,d,J=8Hz,Ar-H); 3.95(1H,d,J=8Hz,Ar-H); 4.80(1H,t,J=6Hz,3,3-dimethylallyl olefinic H); 6.25(3H,s,Ar-OCH_3); 6.35(3H,s,Ar-OCH_3); 6.65(2H,d,Ar-CH_2-); 6.75(3H,s,Ar-OCH_3); 7.65(3H,s, Ar-CH_3); 8.30(6H,br.s,vinyl CH_3).

M.S. m/e(rel.abundance): 398(M^+)(49), 383(20), 380(15), 369(20), 367(100), 365(8), 353(5), 349(5), 342(15), 339(13), 327(8), 323(5), 311(46), 207(43), 163(41), 161(5).

Analysis. Found: C, 69.62; H, 6.47%; $C_{23}H_{26}O_6$ requires C, 69.33; H, 6.58%; m.w. = 398.

Arugoxanthone C (176).

After isolation in the manner described above this pigment crystallised from ethyl acetate-hexane as yellow prisms m. 169-171°C.

T.L.C. Rf 0.21 (2% methanol-chloroform); U.V.: yellow; Ce^{4+}: yellow.

I.R. ν_{max}($CHCl_3$): 3530(br.w), 1645(s), 1610(s), 1590(s), 1455(s), 1385(m), 1325(m), 1305(m), 1125(s), 1065(s), 985(s) cm^{-1}.

U.V. λ_{max}(EtOH): 238(34000), 281(63000), 319(inf.)(13000), 380(8100) nm.

: no change was observed on the addition of acid or base.

N.M.R. (60 MHz) τ ($CDCl_3$): 2.75(1H,d,J=8Hz,Ar-H); 2.85(1H,s,Ar-H); 3.20(1H,d,J=8Hz,Ar-H); 3.70(1H,d,J=10Hz,olefinic H); 4.40(1H,d,J=10Hz, olefinic H); 5.05(3H,br.s,D_2O→2H,s,Ar-CH_2-O and O-H); 5.90(2H,t,J=6Hz, irr.7.95→s,Ar-O-CH_2-); 7.30(1H,m,exchangeable,O-H); 7.60(3H,s,Ar-CH_3); 7.95(2H,t,J=6Hz,irr.5.90→s,CH_2-C-O-); 8.45(6H,s,CH_3-); 8.65(6H,s,CH_3-).

M.S. m/e(rel.abundance): 424(M^+)(11), 406(11), 391(6), 377(11), 337(100), 305(18).

Analysis. Found: C, 70.87; H, 6.72%; $C_{25}H_{28}O_6$ requires C, 70.74; H, 6.72%; m.w. = 424.

Arugoxanthone D (178).

This metabolite, purified as described previously, was a yellow oil.

T.L.C. Rf 0.30 (2% methanol-chloroform); $FeCl_3$: negative; Ce^{4+}: brown.

I.R. ν_{max}($CHCl_3$): 3560(br.m), 1700(s), 1650(s), 1590(s), 1460(s), 1380(m), 1360(m), 1320(m), 1300(m), 1125(m), 1110(m), 1075(s) cm^{-1}.

N.M.R. (60 MHz) τ ($CDCl_3$): -0.77(1H,s,Ar-CHO); 2.70(1H,d,J=8Hz,Ar-H);

2.72(1H,s,Ar-H); 3.15(1H,d,J=8Hz,Ar-H); 3.65(1H,d,J=10Hz,olefinic H); 4.35(1H,d,J=10Hz,olefinic H); 5.85(2H,t,J=6Hz,Ar-O-CH_2-); 7.30(1H,m, exchangeable,O-H); 7.55(3H,s,Ar-CH_3); 8.00(2H,t,J=6Hz,CH_2-C-O); 8.45(6H, s,CH_3-); 8.65(6H,s,CH_3-).

M.S. m/e(rel.abundance): 422(M^+)(8), 407(8), 404(8), 389(4), 376(16), 361(24), 353(8), 335(68), 321(40), 308(72), 293(100).

$C_{25}H_{26}O_6$ requires m.w. = 422.

EXPERIMENTAL.

CHAPTER 6.

3-Carboxy-3-nonenoic Acid (184).

Diethyl succinate (17.4g.) and n-hexanal (10.0g.), in dry tertiary butanol (25 ml.), were added over 15 minutes to a refluxing solution of potassium (3.9g.) in dry tertiary butanol (80 ml.), the mixture refluxed overnight, acidified with dilute hydrochloric acid and the solvent evaporated. Ether (100 ml.) was added and the mixture washed firstly with water (2x50 ml.) and then extracted with 10% aqueous sodium carbonate (2x50 ml.). The alkaline solution was acidified with concentrated sulphuric acid, chilled and washed with ether (2x50 ml.). After washing to neutrality with water the organic layer was evaporated to give a red oil which was heated at 80°C in 1N sodium hydroxide (180 ml.), for 6 hours. The solution was cooled to 0°C, acidified with dilute sulphuric acid and ether (200 ml.) added. The organic layer was washed to neutrality and evaporated to give a brown oil which when taken up in benzene and kept at 0°C overnight gave the acid (183) as colourless needles m. 139-140°C (lit. 141-142°C)(12.5g., 63%).

T.L.C. Rf 0.85 (benzene, dioxan, acetic acid, 50:45:5); Ce^{4+}: brown.

I.R. ν_{max}(nujol mull): 3400-2500(br.m),1720(s), 1700(s), 1655(m) cm^{-1}.

N.M.R. (60MHz) τ (d_6DMSO): -3.6(2H,m,exchangeable,$-CO_2-H$); 3.20 (1H,t,J=7Hz,H-4); 7.80(2H,m,H-2); 8.65(8H,m,H-5,6,7 and 8); 9.05 (3H,m,CH_3).

Analysis. Found: C, 60.23; H, 8.14%; calculated for $C_{10}H_{16}O_4$ C, 59.98; H, 8.05%.

3-Carboxy-3,4-dibromononanoic Acid (185).

3-Carboxy-3-nonenoic acid (2g.), suspended in carbon tetrachloride (2 ml.), and bromine (0.78 ml.) were irradiated with a wide spectrum ultraviolet lamp for 6 hours. The solid was filtered off and washed with hexane (50 ml.). Recrystallisation from toluene gave the dibromo compound (185) as colourless needles m. 139-141°C (lit. 140-141°C) (3.3g., 92%).

T.L.C. Rf 0.76 (benzene, dioxan, acetic acid, 50:45:5); Ce^{4+}: grey.

I.R. ν_{max}(nujol mull): 3400-2500(br.m), 1730(s) cm^{-1}.

N.M.R. (60 MHz) τ (d_6DMSO): 1.0(2H,m,exchangeable,CO_2-H); 5.7(1H,m,H-4); 7.9(2H,m,H-2); 8.6(8H,m,H-5,6,7 and8); 9.1(3H,m,CH_3).

5-n-Pentylaconic Acid (186).

The dibromo compound (185)(7.4g.) was stirred at room temperature for 10 minutes with 1N sodium hydroxide (8 ml.), the mixture cooled to 0°C and acidified with dilute hydrochloric acid. The oil which was precipitated crystallised slowly and after filtration it was recrystallised to give the acid (186) m. 133-134°C (lit. 134°C)(1.5g., 26%).

T.L.C. Rf 0.80 (benzene, dioxan, acetic acid, 50:45:5); Ce^{4+}: brown.

I.R. ν_{max}(nujol mull): 3400-2600(br.m), 1740(s), 1710(s), 1630(s), 695(m), 675(m) cm^{-1}.

N.M.R. (60 MHz) τ ($CDCl_3$): -0.7(1H,s,exchangeable,CO_2-H); 3.2(1H,d,J=2Hz, olefinic H); 4.7(1H,m,H-5); 8.6(8H,m,H-6,7,8 and 9); 9.1(3H,m,CH_3).

Analysis. Found: C, 60.62; H, 7.15%; calculated for $C_{10}H_{14}O_4$ C, 60.59; H, 7.12%.

Methyl Isocanadensate (182).

A large excess of ethereal diazomethane (2.6g. nitrosan) was added to 5-n-pentylaconic acid (186)(500 mg.) in dry methanol (10 ml.). After 1.5 hours anhydrous magnesium sulphate was added, the mixture filtered and evaporated to give a colourless oil which was heated, under nitrogen, at 90°C for 8 hours. The resultant oil was purified by P.L.C. (100% chloroform) to give methyl isocanadensate (182) (445 mg., 78%)(b.pt. 68°C/0.05 mm. Hg) as a colourless oil. This material was identical (Rf, spectral data) with methyl isocanadensate obtained from natural sources.

T.L.C. Rf 0.62 (100% chloroform); Ce^{4+}: brown.

I.R. ν_{max}(liquid film): 1770(s), 1728(s), 1218(m), 1117(m), 1095(m), 1045(m) cm^{-1}.

U.V. λ_{max}(EtOH): 229(13500) n.m.

N.M.R. (100 MHz) τ ($CDCl_3$): 4.94(1H,m,irr.7.83→dd,J_1=8Hz,J_2=1.5Hz,H-5); 6.14(3H,s,OCH_3); 7.83(3H,d,J=2Hz,irr.4.94→s,vinyl CH_3); 8.3(2H,m,H-6); 8.6(6H,m,H-7,8 and 9); 9.12(3H,m,CH_3).

M.S. m/e(rel.abundance): 226(M^+)(15), 197(53), 156(100), 128(48), 127(32), 124(37), 123(19), 99(27).

Analysis. Found: C, 63.72; H, 7.78%; calculated for $C_{12}H_{18}O_4$ C, 63.70; H, 8.62%; m.w. = 226.

Attempted Preparation of Methyl Dihydroisocanadensate (188).

Methyl isocanadensate (182)(160 mg.) and activated zinc dust (170 mg.) were stirred and refluxed in acetic acid (10 ml.) for 8 hours

and chloroform (50 ml.) added. The mixture was washed with aqueous sodium hydrogen carbonate (2x25 ml.) and water (3x25 ml.) and evaporated to give material identical (Rf, spectral data) with the starting material.

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