Microbial Pyran-2-ones and Dihydropyran-2-ones

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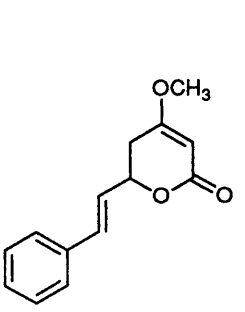
Selectively reviewing the literature published up until December 1991

1 Introduction

The pyran-2-one moiety is found in a large number of natural products, and is responsible for a wide range of biological effects, *e.g.*, antibiotic, antifungal, cytotoxic, neurotoxic, phytotoxic, *etc.* Pyrones thus constitute an important class of compounds. Although certain aspects of individual naturally-occurring pyrones have been discussed within various reviews,[1] there does not appear to have been a recent review devoted entirely to these compounds. It is, therefore, the aim of this article to go some way towards redressing the balance.



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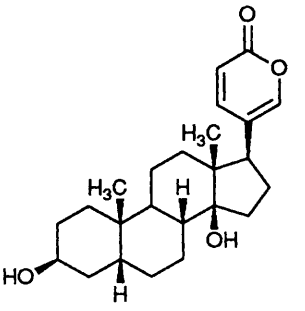

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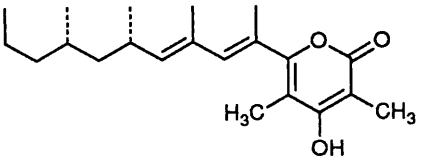

(3)

Pyrones have been isolated from an extensive range of natural sources, such as plants (*e.g.*, kawain (1) from *Piper methysticum*[2]), animals (*e.g.* bufalin (2) from *Bufa vulgaris* (toad)[3]), and marine organisms (*e.g.* diemensin A (3) from *Siphonaria diemensis*[4]). However, in order to keep this review to a sensible length, the present discussion will be restricted to pyrones that have been isolated from microbial sources. Literature coverage is, by necessity, not exhaustive, but will highlight articles published up until the end of 1991.

This review will be divided into two main sections: the first will focus on the fully unsaturated pyran-2-ones, whilst the second will look at the partially saturated dihydropyran-2-ones. Aspects of the isolation, sturcture elucidation, synthesis, biosynthesis, biological activity, and mode of action will be discussed where possible. Fully saturated pyrones (δ-lactones) fall outside the scope of this review.

2 Microbial Pyran-2-ones

2.1 Simple Pyran-2-ones

The simplest pyran-2-ones, from both a structural and a biosynthetic viewpoint, are the 4-hydroxypyrones, triacetic lactone, methyl triacetic lactone, tetraacetic lactone, and dehydroacetic acid.

Methyl triacetic lactone (5) was the first of these to be isolated from a natural source, *Penicillium stipitatum*,[5] and was later isolated from another strain of *P. stipitatum*, along with triacetic lactone (4) and tetraacetic lactone (6).[6] Triacetic lactone (4) had also been isolated from two unstipulated *Penicillium* species.[7] Dehydroacetic acid had been known as a synthetic compound long before its isolation from *Ramaria apiculata*.[8,9] It has also been isolated from *Hypocrea sulphurea*.[10]

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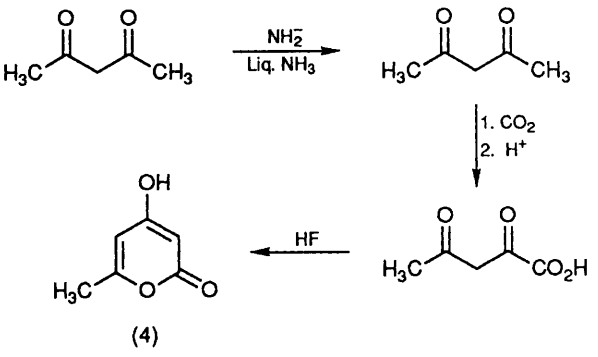
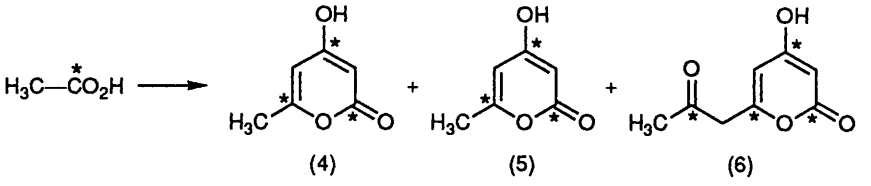
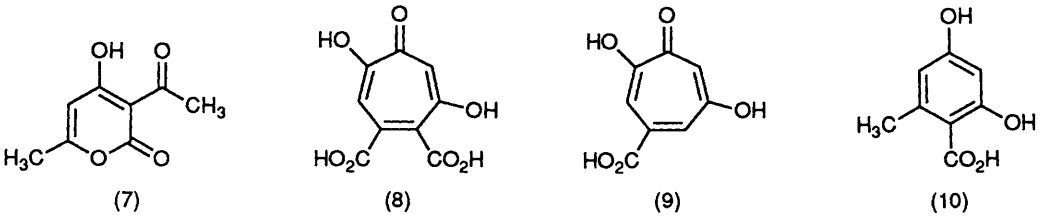
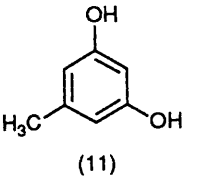


Scheme 1



Scheme 2





Tetraacetic lactone (6) was first synthesized at the end of the last century,[11] and was shown to be converted to triacetic lactone (4) on treatment with sulfuric acid.[12,13] Compound (4) has also been synthesized by treatment of the disodio-derivative of acetylacetone with carbon dioxide, followed by cyclization of the resultant acid with hydrogen fluoride to give the pyrone (Scheme 1).[14] Another route through to triacetic lactone (4) involved condensation of malonic acid or malonyl chloride with ethyl acetoacetate. Use of methylmalonyl chloride allowed the synthesis of methyl triacetic lactone (5).[15,16]

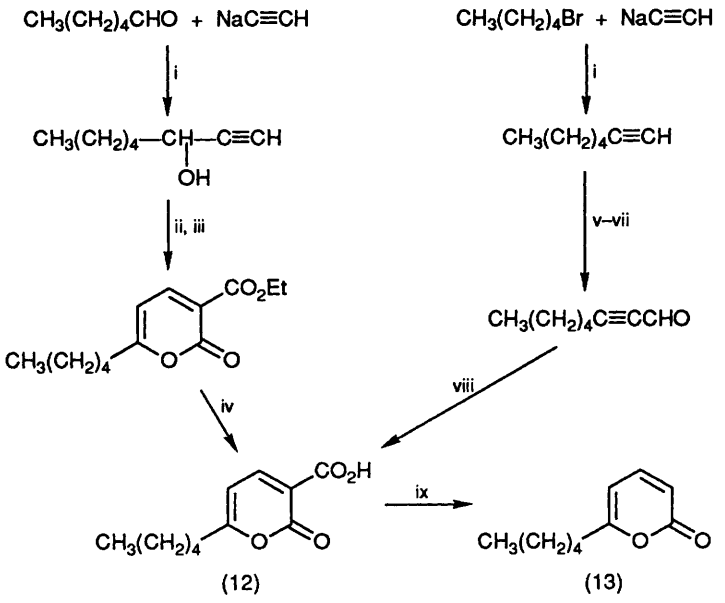
The first synthesis of dehydroacetic acid (7) was also reported at the end of the last century, and was achieved by passing ethyl acetoacetate vapour through a heated iron tube.[17] Other routes include treatment of diketene with sodium phenoxide in benzene to give (7) and 2,6-dimethylpyran-4-one,[18] and also base (sodium bicarbonate)-catalyzed condensation of ethyl acetoacetate.[19]

The biosynthetic origins of the 4-hydroxypyran-2-ones were of considerable interest, particularly because of their apparent relationship to the proposed β-polyketomethylene intermediates on the acetate–methylmalonate pathway. The biosynthesis of triacetic lactone (4), methyl triacetic lactone (5), and tetraacetic lactone (6) was studied in cultures of *Penicillium stipitatum*.[20] This organism normally produces the tropolones stipitatonic acid (8) and stipitatic acid (9). However, addition of ethionine to the culture medium resulted in accumulation of the pyran-2-ones – these were not encountered under normal fermentation conditions. Small amounts of orsellinic acid (10) and orcinol (11) were also isolated.

[1-^{14}C]Acetate was administered to cultures of ethionine-inhibited *Penicillium stipitatum*, and the radioactively-labelled pyrones were isolated. Degradation of tetraacetic lactone (6) gave the expected polyketide-derived labelling pattern (Scheme 2).[20] Although triacetic lactone and methyl triacetic lactone were not degraded, their labelling pattern was assumed to be as shown.

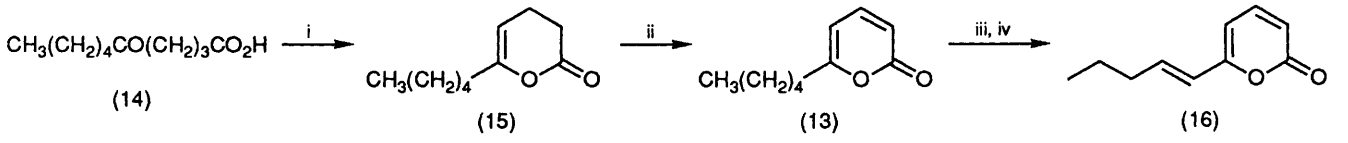
It had been reported that triacetic lactone (4) stimulated the formation of aromatic compounds by *Penicillium urticae*,[21] and increased stipitatic acid (9) formation in *P. stipitatum*.[5] Coupled with the fact that the pyrones accumulated under fermentation conditions where normal tropolone biosynthesis was inhibited, it was suggested that the pyrones could be precursors of stipitatonic and stipitatic acids, and other aromatic metabolites, *e.g.* orsellinic acid, orcinol.[20] However, when radioactively labelled triacetic lactone was incubated with *P. stipitatum* under normal growth conditions, evidence was obtained which indicated that (4) had been broken down into acetate before incorporation into the tropolones, and was not therefore a direct precursor.[20] This was in agreement with other results, which showed that [2-^{14}C]triacetic lactone and [2-^{14}C]tetraacetic acid were degraded to acetate in *Penicillium* sp., before being incorporated into acetate-derived metabolites such as 6-methylsalicylic acid.[7]

The degradation of dehydroacetic acid (7) to triacetic acid *via* triacetic lactone (4) by a *Pseudomonas* species has been reported,[22] as has the breakdown of triacetic lactone to acetoacetic acid and acetic acid *via* triacetic acid by rat liver and kidney, and beef and rabbit liver homogenates.[23,24] The



Reagents: i, Liq. NH_3; 11, CrO_3; 111, $H_2C(CO_2Et)_2$, NaOEt; iv, NaOH; v, EtMgBr; vi, $CH(OEt)_3$; vii, H^+; viii, CH_2CO_2H, AcOH; ix, Δ

Scheme 3



Reagents: i, Ac_2O, AcOH; ii, Pd/C, Δ; iii, NBS, *hv*; iv, LiCl, Li_2CO_3, DMF, Δ

Scheme 4

metabolism of these pyrones therefore appears to be similar in both fungal and animal systems.

The biological activity of (4), (5), and (6) does not appear to have been reported. On the other hand, the use of dehydroacetic acid (7) as a plasticizer, fungicide, and bactericide (in toothpastes),[25] has led to a detailed toxicological study of this compound.[26] It has an LD_{50} (oral) of 1000 mg/kg in rats, whilst its sodium salt has an LD_{50} (oral) of 570 mg/kg. At higher dosage levels toxic effects include loss of appetite, loss of body weight, vomiting, ataxia, and convulsions.

2.2 6-Alkylpyran-2-ones

Other simple pyran-2-ones to have been isolated from microbial sources are the 6-alkyl- and 6-alkenyl-pyrones.

6-Pentylpyran-2-one (13) was the first of these to be identified as a fungal product of *Trichoderma viride*,[27] although it had previously been reported to be a component of peach essence.[28] It has since been isolated from strains of *Trichoderma harzianum*,[29-31] *Trichoderma viride*,[32,33] and an unidentified *Aspergillus* species,[34] from peach[35] and nectarine[36-38] essence, and as an aroma component of roasted beef.[39] In a study on pyrone production by cultures of *Trichoderma viride*, the formation of (13) was found to be affected by the carbon source in the fermentation medium.[32] 6-Pentylpyran-2-one possesses a characteristic coconut odour. Such an odour was noted to be produced by isolates of *Trichoderma viride*, *T. koningii*, and *T. hamatum* during a study on volatile antibiotic production carried out by Dennis and Webster.[40] Although the component responsible was not isolated, it seems probable, in view of the production of 6-pentylpyran-2-one by so many *Trichoderma* species, that (13) was indeed being formed in this case. 6-Pentylpyran-2-one (13) has been produced by a strain of *Trichoderma koningii*, which was also reported to produce 6-heptylpyran-2-one and a 'dehydro' derivative of the latter.[41] The only evidence presented for the latter two compounds, however, was the presence of the corresponding molecular ion peaks in the mass spectrum of partially-purified fungal extracts.

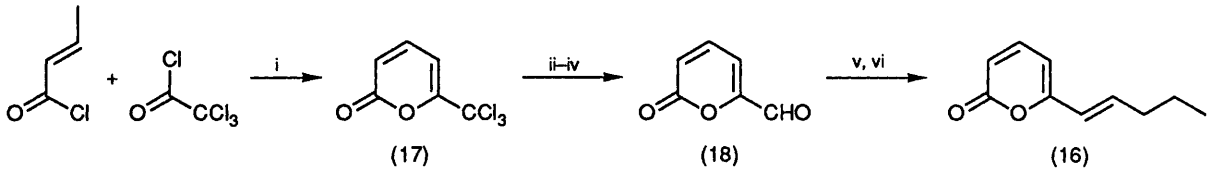
6-Pentylpyran-2-one (13) is a co-metabolite with 6-pentenylpyran-2-one (16) in strains of *Trichoderma harzianum*.[42] Compound (16) has also been isolated from a strain of *Trichoderma viride*,[43] and has been identified as a component of the queen pheromone of the red fire ant, *Solenopsis invicta* (Buren),[44] and of male mandibular gland secretions of the carpenter ants *Camponotus pennsylvanicus*, *C. herculeanus*, and *C. noveboracensis*.[45]

6-Propenylpyran-2-one (19), named sibirinone, has been isolated from *Hypomyces semitranslucens*.[46]

The distinctive coconut aroma of (13) has attracted interest in its use as a possible flavourant in the food industry. As a result of this, its synthesis was developed, in order to ascertain its organoleptic properties, before its first isolation from a natural source. The routes developed[47] are outlined in Scheme 3. In both cases, the key intermediate is the pyrone-acid (12), which is reported to undergo decarboxylation at 200 °C, giving the required product.

A synthesis of (13) has also been described, in which it was an intermediate in the production of the pentenyl derivative (16) (Scheme 4).[45] The keto-acid (14) (formed *via* the reaction of dipentylcadmium with ethyl 4-(chloroformyl)butyrate cyclized to give the dihydropyrone (15), which on dehydrogenation gave (13).[48] The conversion of (13) to (16) was achieved *via* allylic bromination, followed by dehydrobromination.

A second synthesis of (16) has been reported by Rocca *et al.*



Reagents: i, Et_3N; ii, conc. H_2SO_4; iii, $SOCl_2$; iv, H_2, $Pd/BaSO_4$, xylenes; v, Butyl(triphenyl)phosphonium bromide, base; vi, I_2, benzene

Scheme 5

(Scheme 5).[44] [2+4]-Cycloaddition between the ketene derived from crotonyl chloride and trichloroacetyl chloride gave 6-trichloromethylpyran-2-one (17), which was converted to the aldehyde (18). Wittig methodology gave *trans*-6-pentenylpyran-2-one (16). Sibirinone (19) has been synthesized by dimerization of the crotonyl-derived ketene (Scheme 6).[49]

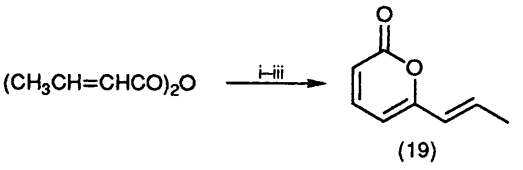
The antifungal properties of 6-pentylpyran-2-one (13) have been demonstrated on a number of occasions. It has been shown to significantly inhibit growth of *Verticillium dahliae*, *V. fungicola*, *Pyranoechaeta lysopersici*, *Phomopsis sclerotioides*,[42] *Gaeumannomyces graminis* (Take-all),[31,42] *Chaetomium cochlioides*, and *C. spinusum*.[30] It is also partially effective against *Rhizoctonia cerealis*, *Fusarium oxysporum*, *Aspergillus niger*,[42] *A. flavus*,[30,42] *Botrytis cinerea*,[29,42] and *Ceratocystis ulmi*.[29] *Pythium ultimum*, *Sclerotinia sclerotiorum*, and *Trichoderma harzianum* showed negligible inhibition,[42] and *Bacillus subtilis* was not affected.[30] Although growth of *Sclerotinia sclerotiorum* was not inhibited, it was found that sclerotial development was significantly affected. This was also noted with *Rhizoctonia cerealis*.[42]

High atmospheric concentrations of 6-pentylpyran-2-one (13) affected germination of lettuce seedlings, and subsequent development.[42] Compound (13) is reported to be non-toxic to greenhouse grown bean plants (*Phaseolus vulgaris*), corn plants (*Zea mays*), or tobacco (*Nicotiana tabacum*), and significantly inhibits growth of etiolated wheat coleoptiles.[30]

Crude extracts of *Trichoderma koningii*, which were reported to contain both 6-pentylpyan-2-one (13) and 6-heptylpyran-2-one, were shown to be active against *Gaeumannomyces graminis*, *Rhizoctonia solani*, *Phytophthora cinnamoni*, *Pythium middletoni*, *Fusarium oxysporum*, and *Bipolaris sorokiniana*.[41] It seems likely that this biological activity was due mainly to the presence of the 6-pentyl derivative (13), particularly as the length of the alkyl chain in 6-alklylpyran-2-ones has been shown to be crucial to the antifungal activity exhibited.[48]

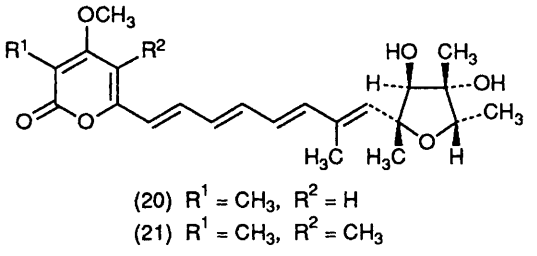
A comparison between 6-pentenylpyran-2-one (16) and the 6-pentyl derivative (13) has shown that the two pyrones display similar antifungal properties, (16) being as effective as (13).[48] It has been reported that (16) was partially responsible for the induction of oospore formation in *Phytophthora cinnamoni*.[43] Its biological role, in combination with other pheromonal components, has been demonstrated with red fire ants, *Solenopsis invicta* (Buren),[44] but its role with respect to Carpenter ants (*Camponotus* sp.), from which it has also been isolated, has not been established.[45]

Sibirinone (19) appears to be biologically inert, and only its inactivity against *Staphylococcus aureus* has been noted.[46]

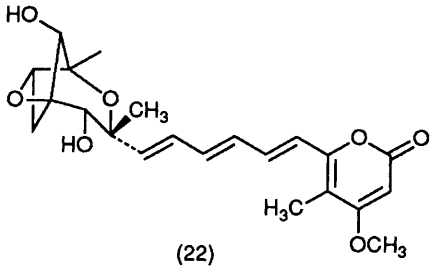


Reagents: i, 550 °C, 0.1 Torr; ii, [4+2] dimerization; iii, $NaHCO_3$ or *p*-TsOH

Scheme 6



(20) $R^1 = CH_3$, $R^2 = H$
(21) $R^1 = CH_3$, $R^2 = CH_3$



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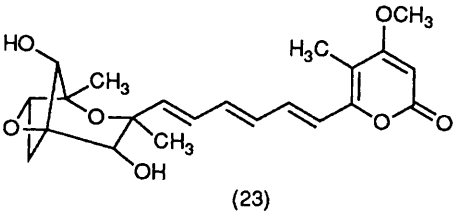
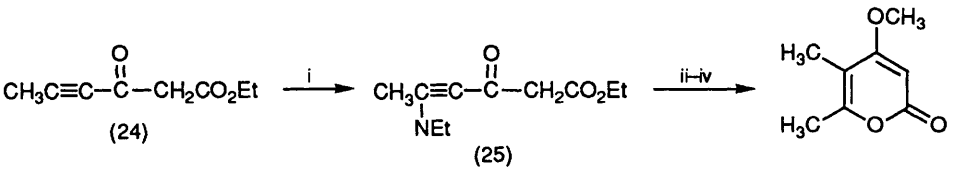


(23)

2.3 Citreoviridin and Derivatives

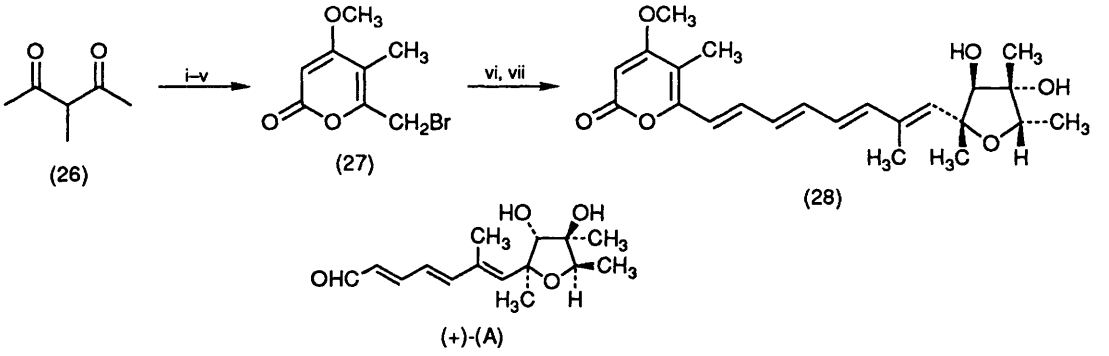
In the early part of this century, the occurrence of cardiac beriberi in East Asia reached epidemic-like proportions. Mouldy rice was found to be responsible for the observed symptoms, the principal mould being identified as *Penicillium citreoviride* (*P. toxicarium*).[50] The mould was found to produce a toxic principle, named citreoviridin (28), which exhibited the biological effects attributed to the micro-organism itself.[51] Since then, citreoviridin (28) has been found to occur in culture filtrates of *Penicillium ochrosalmoneum*,[52,53] *P. pulvillorum*,[53] *P. pedemontanum*,[54] *P. citreoviride*,[55] *P. charlesii*,[56] and *Aspergillus terreus*.[57] This latter organism also produced a number of very similar metabolites to citreoviridin, referred to as citreoviridins B, C, D, E, and F. The structures of citreoviridins C (20) and D (21) were assigned on the basis of spectroscopic data. Isocitreoviridin, which is isomeric about the C-13,C-14 double bond as compared to citreoviridin, has also been isolated from culture filtrates of *Penicillium pulvillorum*,[53] but was shown to be an artefact after pure citreoviridin was found to be converted to a mixture of citreoviridin and isocitreoviridin under simulated fermentation and extraction conditions. In addition to citreoviridin (28), the related metabolites, citreoviridinol (22), isocitreoviridinol (23), secocitreoviridin (30), and citreoviral (29), have also been isolated from *Penicillium* species.[58–60]

The synthesis of citreoviridin (28) has been the subject of a



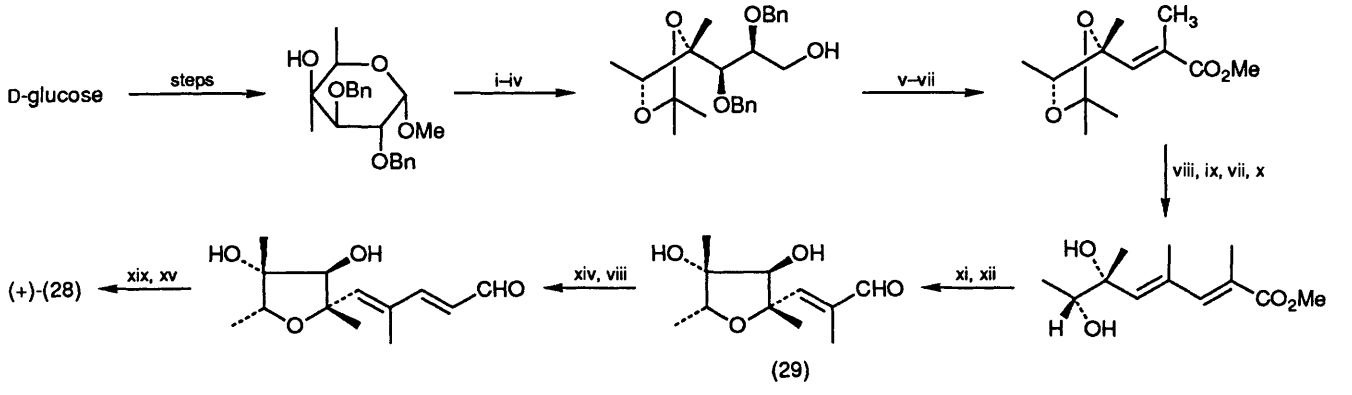
Reagents: i, $HNEt_2$; ii, MeI; iii, H_2O; iv, polyphosphoric acid

Scheme 7



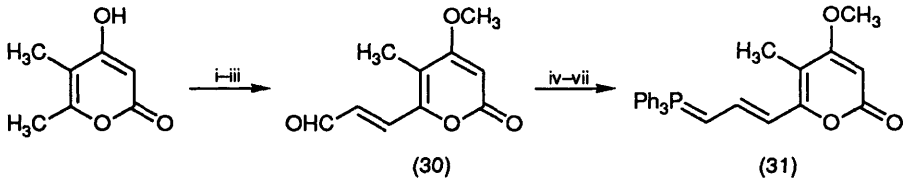
Reagents: i, $NaNH_2$, NH_3; ii, CO_2, Ether; iii, HF; iv, DMSO, K_2CO_3, 2-butanone; v, NBS, peroxide, CCl_4; vi, $(CH_3O)_3P$, toluene; vii, (+)-(A), LDA, THF, HMPA

Scheme 8



Reagents: i, Ac_2O–$BF_3.Et_2O$, 0 °C; ii, 0.4 M NaOMe, MeOH, r.t.; iii, $NaBH_4$, H_2O:MeOH (1:2), r.t.; iv, *p*-TsOH-Drierite, acetone, r.t.; v, H_2, Pd/C, MeOH, r.t.; vi, $NaIO_4$, MeOH:H_2O (1:1), r.t.; vii, Ph_3=C(Me)CO_2Me, benzene; viii, DIBAL-H, toluene, −78 °C; ix, PDC, DMF, 0 °C; x, Amberlite, MeOH:H_2O (2:1), r.t.; xi, mCPBA, CH_2Cl_2, 0 °C; xii, CsOH, CH_2Cl_2, r.t.; xiii, MnO_2, CH_2Cl_2, r.t.; xiv, Ph_3P=$CHCO_2Et$, benzene, r.t.; xv, (31), benzene

Scheme 9

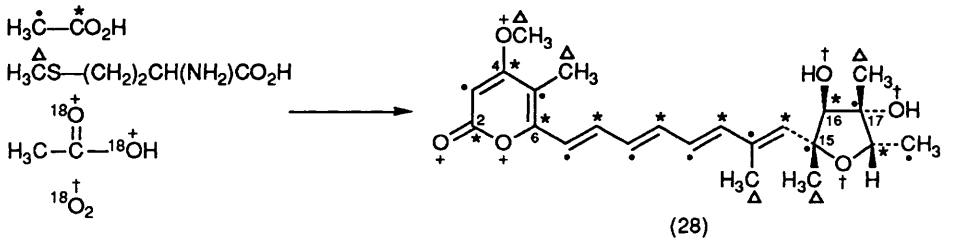


Reagents: i, DMSO, K_2CO_3; ii, SeO_2, dioxan, Δ; iii, Ph_3=CH-CHO; iv, DIBAL-H, THF; v, MsCl, pyridine, CH_2Cl_2; vi, Ph_3P, benzene; vii, NaH, THF

Scheme 10

number of papers, and considerable success has been achieved. The synthesis of the pyrone moiety from γ,δ-acetylenic-β-oxo-ester (24) *via* the enaminoester (25) has been reported (Scheme 7).[61] A second approach towards the synthesis of the pyrone moiety is outlined in Scheme 8.[62] Methylacetylacetone (26) was converted to 6-bromomethylpyrone (27), in five steps. Wittig methodology gave (−)-citreoviridin (28). The tetrahydrofuran portion of the molecule was prepared from (+)-citreoviral (29), for which a number of syntheses have been reported.[63–67] The first total synthesis of citreoviridin (28), starting with D-glucose, was reported in 1985,[68] and is outlined in Scheme 9. The absolute configurations of both citreoviridin (28) and citreoviral (29) were established based on D-glucose.

Pyrone-phosphorane (31), widely used in the synthesis of citreoviridin (28), the aurovertins, and related compounds, was prepared from secocitreoviridin (30), the total synthesis of which had already been reported.[69] The route devised, and the subsequent conversion of (30) into (31), are outlined in Scheme 10. This synthesis enabled the structure of (30) to be established unequivocally – previously two possible structures had been



(28)

Scheme 11

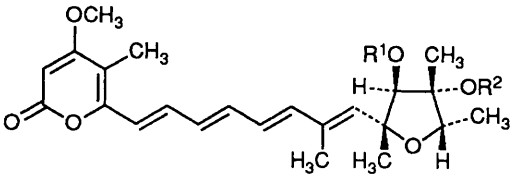
proposed.[58,68] The total synthesis of citreoviridinol (22) has also been reported.[70]

Initial studies into the biosynthetic origins of citreoviridin (28) in *Penicillium pulvillorum* suggested that the molecule was derived from nine molecules of acetic acid and five molecules of methionine,[71] although degradation of the [^{14}C]-labelled metabolite did not enable confirmation of all the positions of incorporation. Subsequent studies with [1-^{13}C]- and [2-^{13}C] acetate in *Aspergillus terreus*, demonstrated the incorporation of nine acetate units, as shown (Scheme 11).[57] Oleic acid was suggested as a possible biosynthetic precursor of citreoviridin, however, feeding with [1-^{14}C]oleic acid resulted in a low incorporation, the distribution being consistent with degradation of the acid to acetic acid. Labelling studies with [1-^{13}C]- and [2-^{13}C]acetate in *Penicillium pulvillorum* showed a similar incorporation pattern to that demonstrated in *A. terreus*.[72] In addition to this, the remaining methyl groups were shown to be methionine-derived, and an acetate-starter unit was demonstrated by feeding experiments with [1-^{13}C,2-^{2}H$_3$]acetate. Feeding experiments with [1-^{13}C,^{18}O$_2$]acetate (*P. pulvillorum*) led to upfield isotope shifts for the C-2, C-4, and C-6 resonances in the ^{13}C NMR spectrum of (28), indicating that the corresponding carbon–oxygen bonds had remained intact throughout the biosynthetic pathway. Fermentation of cultures under an ^{18}O$_2$ atmosphere, and simultaneous addition of [1-^{13}C]acetate demonstrated the origin of the tetrahydrofuran ring oxygens from oxidative processes.[73]

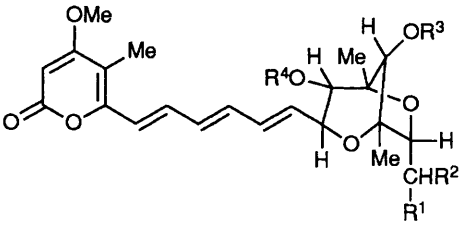
The discovery of citreoviridin (28) was as a result of the search for the cause of acute cardiac beriberi, which was prevalent in rice-eating countries at the beginning of the century.[50] Citreoviridin induced acute poisoning in cats and dogs (intraperitoneally (i.p.), subcutaneously (s.c.)), early symptoms of which were progressive paralysis of the hind legs, vomiting, and convulsions. Respiratory distress appeared gradually, whilst cardiovascular disturbance and hypothermia were marked in the advanced stages. The final stage of toxicity was characterized by dyspnoea, gasping, and Cheyne–Stokes respiration, followed by respiratory arrest. Subacute administration in cats led to induced damage of the central nervous system. The LD_{50} in male mice has been determined as 11 (s.c.), and 7.5 (i.p.) mg/kg, whilst in female rats it is 3.6 mg/kg (s.c.). Pretreatment of test animals with vitamin B_1 reduced acute intoxication, whilst vitamin C led to an increase in the life-span of poisoned mice. Because of the similarity of the clinical manifestations of acute cardiac beriberi in humans with the toxicological effects of citreoviridin in poisoned animals, the mycotoxin was presumed to be the cause of the cardiac beriberi that swept East Asia.

The toxicity of citreoviridin (28) had been reported previously,[53] where it was noted that a single dose of 100 mg/kg (s.c.) was sufficient to kill all the experimental animals within two hours. Isocitreoviridin, on the other hand, had no effect at all. Citreoviridin monoacetate (32) is less potent than citreoviridin (28), whilst the diacetate (33) is virtually ineffective.[74,75] Hydrogenation of the monoacetate reduced its potency considerably.[75]

The phytotoxic activity of citreoviridin (28) has been examined to a lesser degree. The compound was found to inhibit growth of wheat coleoptiles at concentrations of 10^{-3} M,



(32) R^1 = Ac, R^2 = H
(33) R^1 = R^2 = Ac



(34) R^1 = H, R^2 = CH_3, R^3 = R^4 = COMe
(35) R^1 = R^2 = R^4 = H, R^3 = COMe
(36) R^1 = OH, R^2 = CH_3, R^3 = COMe, R^4 = H
(37) R^1 = R^3 = R^4 = H, R^2 = CH_3

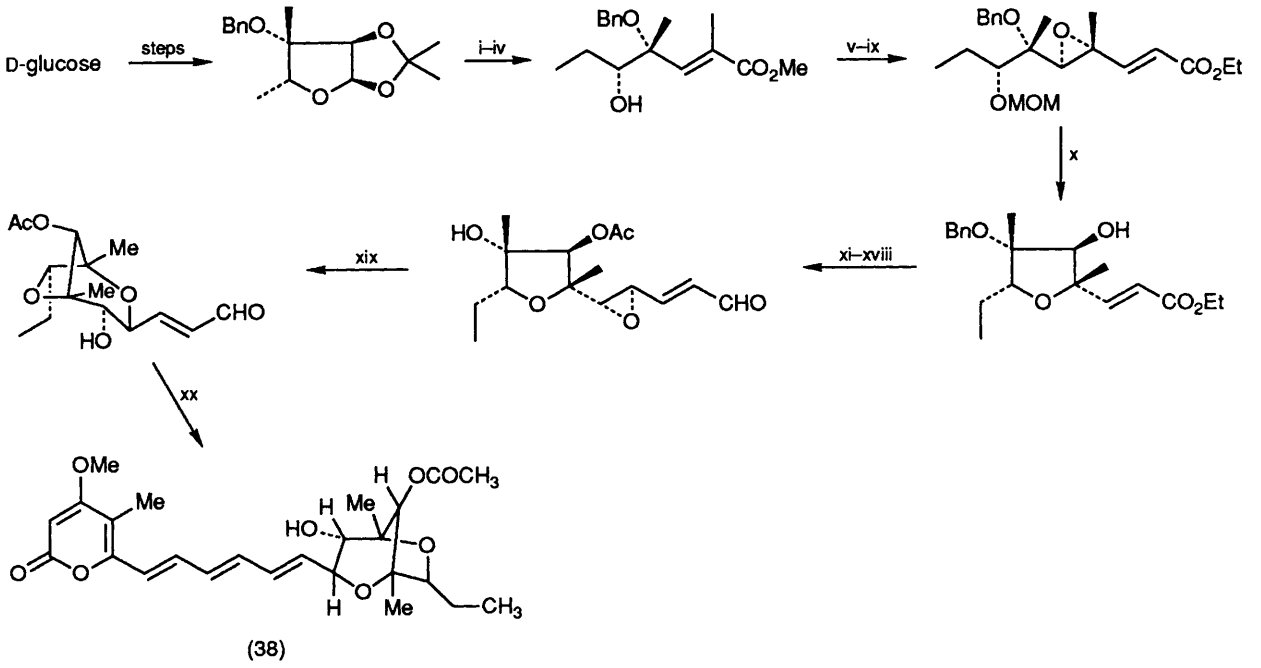
and of corn seedlings, with the inhibitory effects on the latter still in evidence two months after treatment at concentrations of 10^{-2} M. On the other hand, tobacco seedlings were not visibly affected by citreoviridin (28), indicating a degree of selectivity in its phytotoxic activity.

2.4 The Aurovertins

The aurovertins are a group of mycotoxins related to citreoviridin (28). To date, the structures of five aurovertins, A (34), B (38), C (35), D (36), and E (37), have been reported. The first isolation of an aurovertin was from *Calcarisporium arbuscula*[76] – later evidence showed this to be aurovertin D (36). Aurovertin B (38) was also isolated from *C. arbuscula*,[77] and its structure determined.[78] At least nine aurovertins were reported in the mycelial extracts of this organism,[77] although, as mentioned, structures have only been proposed for aurovertins A through to E.[74]

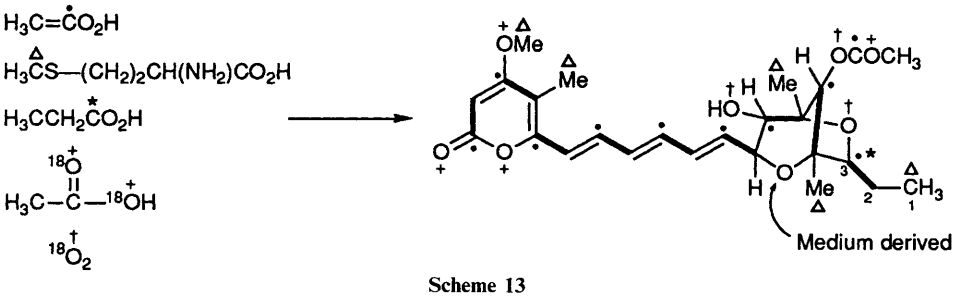
The total synthesis of aurovertin (38) has been reported,[79] and the route is outlined in Scheme 12. As with citreoviridin, D-glucose was used as starting material, and so the absolute configuration of aurovertin B (38) was established on the basis of its derivation from this sugar.

The biosynthetic origins of the aurovertins have been determined. Feeding experiments with [1-^{13}C]acetate in *C. arbuscula*, demonstrated the incorporation of acetate units into aurovertin B (38), as shown in Scheme 13.[80] Five units of [Me-^{13}C]methionine were also incorporated. Incorporation of [1,2-^{13}C]acetate indicated that C(2) and C(3) of the ethyl side chain were derived from an intact acetate unit. These results thus



Reagents: i, 80% aq. AcOH; ii, $NaIO_4$, MeOH–H_2O; iii, $Ph_3P{=}C(Me)CO_2Me$, benzene; iv, K_2CO_3, MeOH; v, $CH_2(OMe)_2$-P_2O_5, $CHCl_3$; vi, DIBAL-H, THF; vii, mCPBA, CH_2Cl_2; viii, $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2; ix, $Ph_3P{=}CHCO_2Et$, benzene; x, CF_3CO_2H, CHC_3; xi, DIBAL-H, THF; xii, Trityl chloride, pyridine, CH_2Cl_2; xiii, Ac_2O, pyridine, r.t.; xiv, *p*-TsOH, MeOH; xv, BU^tOOH, $Ti(OPr^i)_4$, D-(−)-DET, CH_2Cl_2; xvi, H_2/Pd black, MeOH; xvii, $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2; xviii, $Ph_3P{=}CHCHO$, benzene; xix, CSA, CH_2Cl_2; xx, (31), THF

Scheme 12



Scheme 13

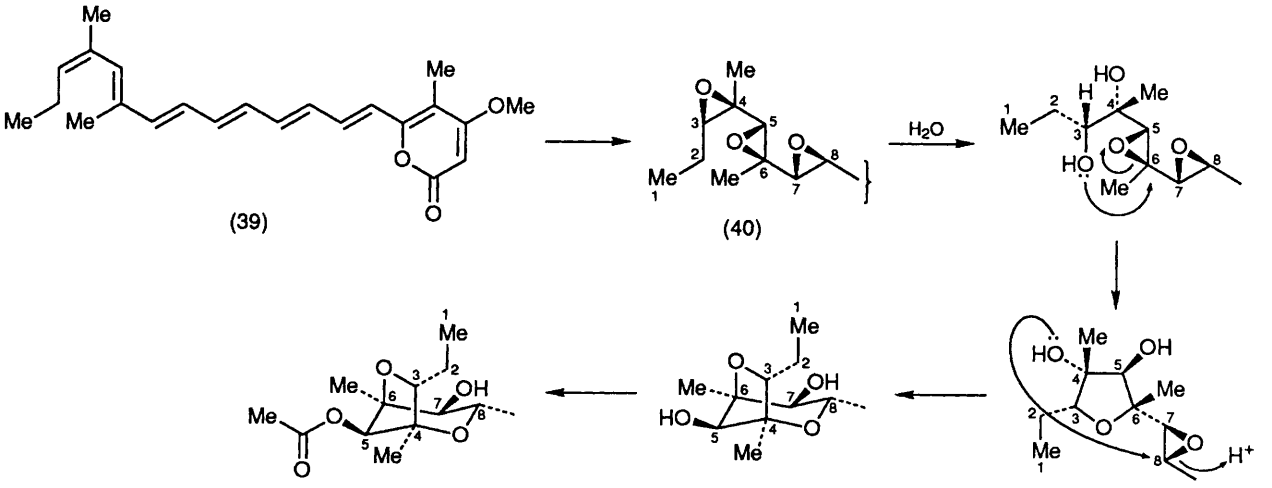
suggested that aurovertin B was derived from a C_{18}-polyketide precursor with the introduction of a methyl group from the C_1-pool onto the methyl carbon atom of the chain-initiating acetate unit. However, feeding experiments with [1-^{13}C]propionate demonstrated that this ethyl side chain could also be propionate-derived. Similar results were found for aurovertin D (36).[81] Further labelling studies confirmed that aurovertins B (38) and D (36) were, in fact, derived from a C_{20}-polyketide which was methylated at C_{18} of this precursor, followed by the loss of the starter acetate unit through a retro-Claisen cleavage.[81] This was shown by the incorporation of [2-^{13}C]malonate with high enhancement at the positions also labelled by [2-^{13}C]acetate – all atoms were labelled to a similar extent. The previous results with [1-^{13}C]propionate still held, and so the question was raised as to whether both pathways operated simultaneously. This was shown to be the case. It appeared that aurovertin C (35) was derived only *via* the single pathway using the acetate–polymalonate–methionine route.

Addition of [1-^{13}C,$^{18}O_2$]acetate to cultures of *C. arbuscula* demonstrated that, as in the case of citreoviridin (28), the pyrone oxygens of aurovertins B (38) and D (36) were acetate-derived.[82] Fermentation of cultures under an $^{18}O_2$ atmosphere, and simultaneous addition of [1-^{13}C]acetate established the derivation of all the remaining oxygens by oxidative processes, with the exception of the C(4)-oxygen atom, which was presumably derived from the medium.

With the above results in hand, a detailed mechanism was proposed for the formation of the 2,6-dioxabicyclo[3.2.1]octane moiety, from the postulated polyene precursor (39) (Scheme 14).[82] The polyene is postulated to undergo monooxygenase-mediated epoxidation using molecular oxygen to give the (3*R*, 4*R*, 5*R*, 6*R*, 7*R*, 8*S*)-triepoxide (40). Nucleophilic attack by water at C-4 leads to a series of ring closures to generate, eventually, the 2,6-dioxabicyclo[3.2.1]octane moiety of aurovertin B (38).

The biological activity, and particularly the mode of action of the aurovertins has been studied in considerable detail. Whilst there is no apparent antibiotic activity against bacteria or pathogenic fungi, toxicity against a number of animals has been noted.[74,76] Pharmacological effects include brief stimulation followed by depression. Hypotension has also been noted, along with a marked diuretic effect and enhanced secretion of Na^+ ions. The LD_{50} for mice is 1.65 mg/kg. Intravenous injection of 1 mg/kg caused death in rabbits in less than 15 minutes, and in dogs in less than 50 minutes.[76] Aurovertin does not appear to be significantly metabolized in the body.[74]

Aurovertins B (38) and D (36) have been shown to inhibit oxidative phosphorylation, $^{32}P_i$-ATP exchange, and exchange of ^{18}O between P_i and water in rat liver mitochondria. They also enhanced ATP hydrolysis induced by selenite, selenate, and deoxycholate. The hydrolysis of ATP induced by DNP and



Scheme 14

agents that uncouple oxidative phosphorylation were partially inhibited. It was concluded[83,84] that aurovertin inhibited at a site located on the coupling factor F_1 of ATPase.

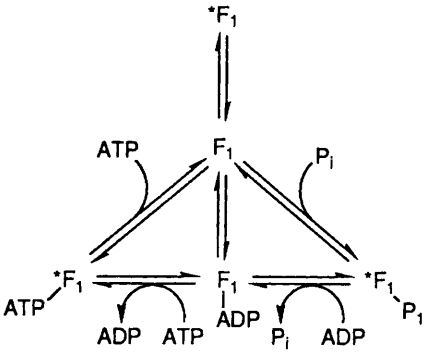
Enzymatic activity of highly purified mitochondrial ATPase from rat liver was found to be dependent on the anion of the buffer used. However, aurovertins decreased enzymic activity to the same level, regardless of the anion present.[85]

Aurovertins B (38), C (35), and D (36) were reported to have similar levels of activity, whilst aurovertin E (37) was only a weak inhibitor of enzyme activity. Aurovertin A (34) was anomalous in its effects, in that it was a powerful inhibitor of ADP-stimulated respiration, but was impotent as an inhibitor of ATPase activity.[74] The inhibition of ATPase activity of soluble *Escherichia coli* coupling factor isolated from wild-type *E. coli* K-12 by aurovertin D (36) was found to be totally lost on acetylation or saponification of this metabolite.[86] Aurovertin-resistant mutants of *E. coli*, generated by nitrosoguanidine mutagenesis,[87] were shown to be altered in the β-subunit, suggesting that this was the position of the aurovertin binding site. Further evidence indicated that aurovertin B (38) and citreoviridin (28) bound to nonidentical subunits on the β-subunit of yeast F_1-ATPase.[88] The binding of citreoviridin was noncompetitive with respect to aurovertin, and F_1-ATPase obtained from aurovertin-resistant mutants was partly inhibited by citreoviridin.

The ATPase activity, and ATP-induced energization of photosynthetic membranes from *Rhodopseudomonas capsulata*, a facultative photosynthetic bacterium, were reported to be stimulated by phosphate – aurovertins completely inhibited the activity elicited by this anion.[89] They also inhibited the energy transfer reactions of *R. rubrum*, and the membrane-bound and soluble ATPases of this organism.[90] The *R. rubrum* coupling factor was suggested to be the site of action. Inhibitory action by aurovertins on chloroplast functions has not been found.[74,91]

The aurovertins fluoresce weakly in solutions of methanol, ethanol, or alcohol–water mixtures, when irradiated with light at 370 nm, with an emission maximum at 470 nm. At lower temperatures, or in glycerol solution, an enhanced fluorescent intensity is observed.[74] Aurovertin has found use as a fluorescent probe, in following structural changes of membrane components.[92] Thus, when aurovertin was added to fragmented rat liver mitochondria, the fluorescence was found to decrease on the addition of ADP. These changes were interpreted in terms of environmental constraints of the probe binding site.

The formation of a complex between aurovertin and soluble mitochondrial ATPase (F_1) was accompanied by a 55-fold enhancement of fluorescence.[93] This was partially quenched by ATP, as previously noted for mitochondrial fragments, and by Mg^{2+}. Two binding sites were found on F_1 in the presence of ATP, and one site in the presence of ADP, Mg^{2+}, and dilute buffer. It was thus proposed that of the two binding sites for



Scheme 15

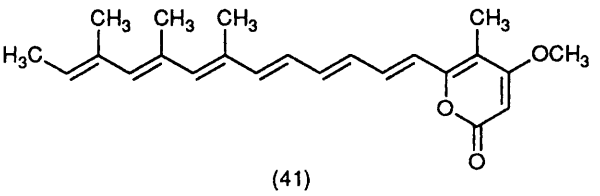
aurovertins, only one participated in inhibition of ATPase activity.

The fluorescence maxima was found to be during State-3 respiration, and was partially quenched on anaerobiosis, or on addition of respiratory inhibitor, oligomycin, or uncoupler, suggesting that aurovertin bound cooperatively to State-3 mitochondria.[94] Aurovertin thus induced conformational change in F_1 binding sites in two ways: firstly, by directly acting as an allosteric effector of an oligomeric system; and secondly, indirectly by inhibiting State-3 respiration which changes the allosteric constant of the oligomeric system.

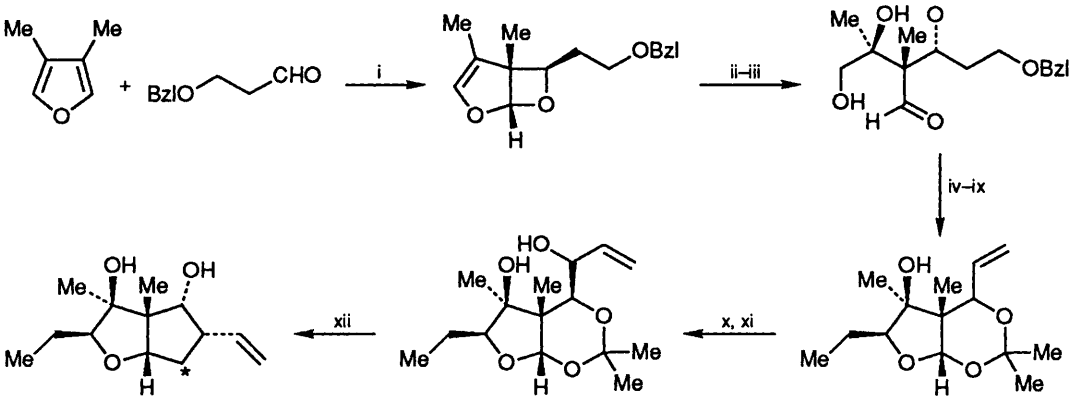
Of the two binding sites for aurovertin, one had high affinity binding, and the other low affinity. A model was presented (Scheme 15) in which changes of the aurovertin fluorescence reflected conformational changes of the ATPase induced by its ligands.[95] The two conformations, termed F_1 and $*F_1$, are in equilibrium. F_1 contained one binding site per mole of enzyme, which had a high affinity, whilst $*F_1$ exposed two binding sites, one of high affinity and one of low affinity. Changes in the fluorescent properties of aurovertins were explained by a shift in equilibrium between the two conformations, induced by binding of ligands to the enzyme. ATP and phosphate induce conformation $*F_1$, whilst ADP stabilizes conformation F_1. Further evidence has been obtained in support of these conformational changes.[96,97] The rate of change of fluorescence on addition of the various ligands was found to be pH dependent.[98]

2.5 Asteltoxin and Citreomontanin

Asteltoxin (44), a mycotoxin structurally related to both citreoviridin (28) and the aurovertins, was first isolated from toxic maize meal cultures of *Aspergillus stellatus*, and its structure confirmed by single crystal *X*-ray crystallography.[99]



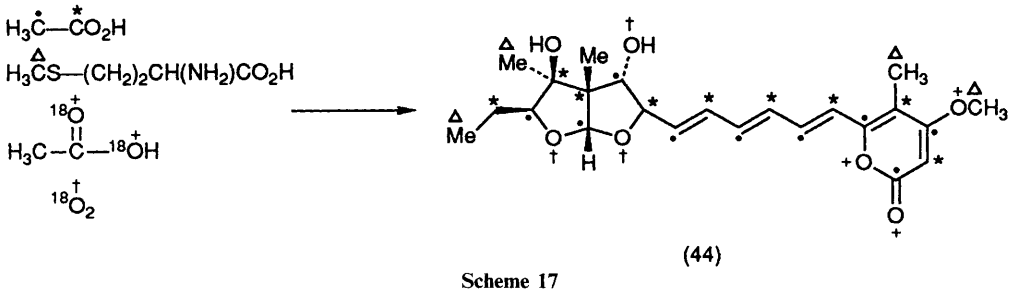
(41)



(43) (42)

Reagents: i, *hν*; ii, mCPBA, $NaHCO_3$, CH_2Cl_2; iii, THF; 3N HCl (3:1); iv, Me_2NNH_2, CH_2Cl_2, $MgSO_4$; v, EtMgBr, THF; vi, CSA, acetone, $CuSO_4$; vii, Li, NH_3, Et_2O; viii, *o*-$NO_2C_6H_4SeCN$, Bu_3P, THF; ix, H_2O_2, THF; x, O_3, CH_2Cl_2, MeOH; xi, CH_2=CHMgBr, THF; xii, Ac_2O, Et_3N, DMAP

Scheme 16



(44)

Scheme 17

The polyene-pyrone citreomontanin (41) has been isolated from *Penicillium pedemontanum*, and also from a citreoviridin-producing strain of *P. pulvillorum*.[100] The structure of this compound has also been determined by means of *X*-ray crystallography, and confirmed that the double bonds possessed an all-*E* configuration.[101]

The synthesis of the bistetrahydrofuran moiety of asteltoxin (44) has been reported, and the route is illustrated in Scheme 16.[102] Rearrangement of alcohol (42) gave the bistetrahydrofuran moiety of asteltoxin (43), with the correct relative stereochemistry.

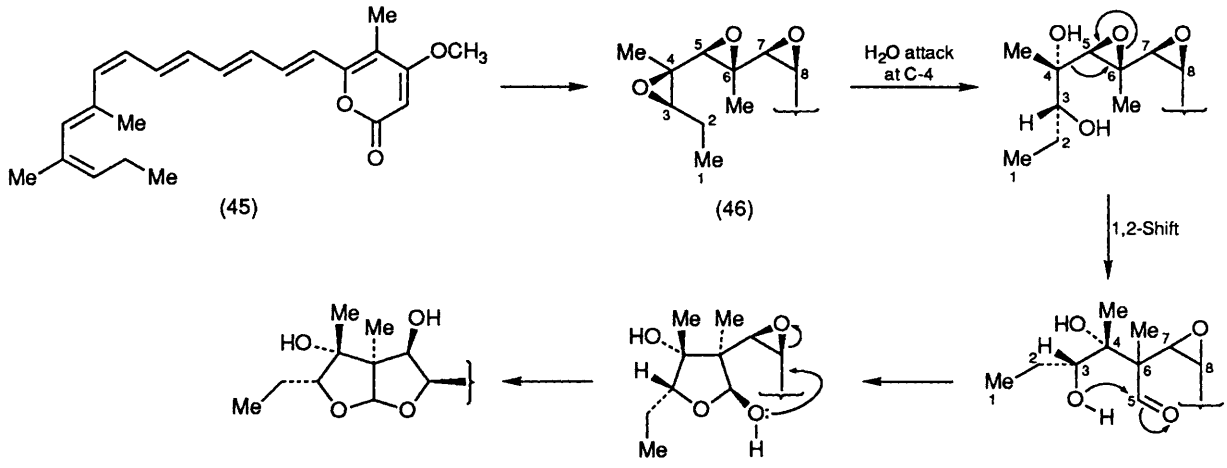
Biosynthetic studies of the origin of asteltoxin in *Aspergillus stellatus* have demonstrated the incorporation of acetate with the position of label as shown in Scheme 17.[103] Feeding with [1,2-$^{13}C_2$]acetate proved the presence of eight intact acetate units, and also indicated the cleavage of a ninth unit *via* a 1,2-bond migration. It was suggested that this cleavage occurred *via* a pinacol or epoxide rearrangement to generate a branched aldehyde which was subsequently utilized in the formation of the tetrahydrofuran moiety.

Feeding of (2*S*)-[methyl-^{13}C]methionine led to incorporation of label at the C-4 and C-5 methyl groups, and C-1 of the bistetrahydrofuran moiety, and at the two methyl groups of the pyrone ring. [1-^{13}C]Propionate was also incorporated, with the signal assigned to C-3 of the bistetrahydrofuran moiety in the ^{13}C NMR spectrum being enhanced. This suggested that, as in the case of the aurovertins, two biosynthetic pathways were in operation – the first in which C-1–C-3 was derived from methionine-malonate, and the second in which these atoms were propionate-derived.

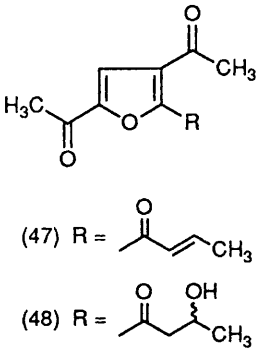
In order to investigate the mechanism of the proposed 1,2-shift, labelling studies with [1-^{13}C,$^{18}O_2$]acetate and with $^{18}O_2$ were carried out.[104] Again, as with both citreoviridin (28) and the aurovertins, the pyrone ring oxygen atoms were found to be acetate-derived. The remaining oxygen atoms, with the exception of that attached to C(4) of the bistetrahydrofuran system, are O_2 derived, suggesting that this latter oxygen is derived from the medium (Scheme 17).

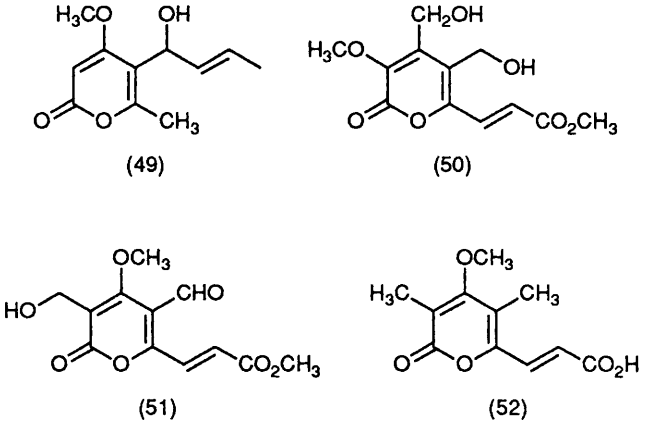
The mechanism proposed for the formation of asteltoxin (44) in *A. stellatus* is outlined in Scheme 18.[104] Thus, the polyene (45) was postulated to undergo monooxygenase-mediated epoxidation to give the (3*R*, 4*R*, 5*R*, 6*R*, 7*S*, 8*S*)-triepoxide (46). Nucleophilic attack at C-4 by water would then initiate a series of reactions, including the 1,2-shift, resulting in the formation of the bistetrahydrofuran moiety as shown.

Both asteltoxin (44) and citreomontanin (41) were both tested for their effect on ATPase activity in *Escherichia coli* BF_1.[105] Whilst citreomontanin (41) was found to be completely inactive, asteltoxin (44) was found to inhibit enzyme activity with a potency intermediate between citreoviridin (28) and aurovertin B (38). Asteltoxin (44) showed a large enhancement of fluorescence upon interaction with BF_1, analogous to the



Scheme 18





aurovertins, and showed similar increase in intensity on treatment with ADP, and was quenched on addition of Mg^{2+}. However, unlike the aurovertins, asteltoxin did not enhance the binding affinity of BF_1 for inorganic phosphate.

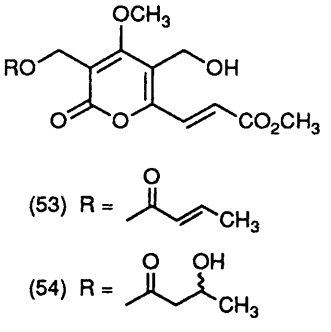
2.6 The Pyrenocines and Macommelins

As well as citreoviridin, *Penicillium citreoviride* has been the source of citreopyrone (58),[106] and the novel dihydrothiopyrone, citreothiolactone (56).[107] From a biogenetic point of view, these may be derived from a common intermediate, as will be discussed later. Citreopyrone (58) has also been isolated from *Pyrenochaeta terrestris*, the causal agent of onion pink root disease, and was named pyrenocine A. This latter name will be used in reference to structure (58) in the remainder of this review. The structurally-related pyrenocine B (55) was a co-metabolite.[108,109] Pyrenocines A and B were originally erroneously assigned furan structures (47) and (48),[108] respectively, but were reassigned as (58) and (55) on the basis of an *X*-ray crystal structure determination of pyrenocine A.[109] Pyrenocine C (49) has also been isolated from *P. terrestris*.[110]

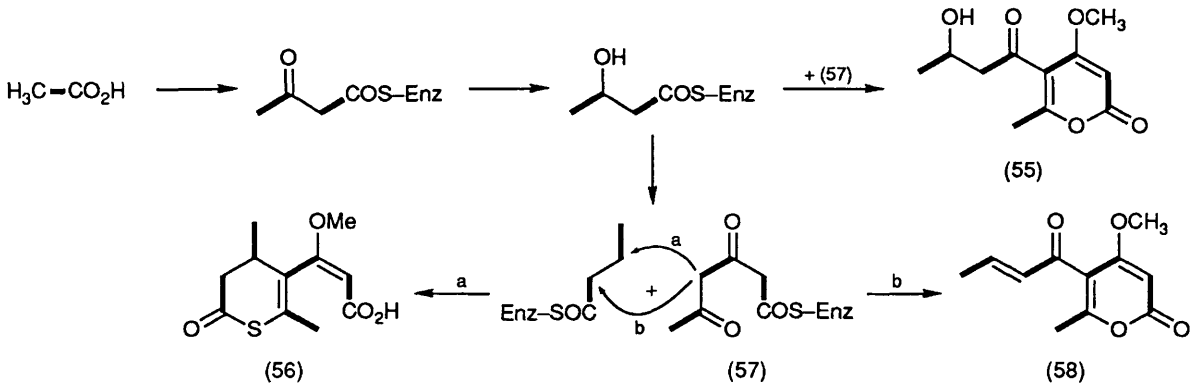
The macomellins (64), (59)—(61), which differ from the pyrenocines only with respect to the structure of the side chain at C-5, have been isolated from *Macrophoma commelinae* (fruit rot disease of apple and other plants).[111] Rosellisin (114) was a co-metabolite of the macommelins,[111] but had also previously been isolated from *Hypomyces rosellus*.[112] Its structure was originally misassigned as (50), but was later revised to (68), on the basis of biogenetic studies.[113] Rosellisin aldehyde (51) has also been isolated from *Hypomyces rosellus*.[113]

Compounds related to rosellisin have been reported. Macrophin (53) has been obtained from *Macrophoma commelinae*, along with macrophic acid (52).[114] Islandic acid (54) was isolated from *Penicillium islandicum*.[115]

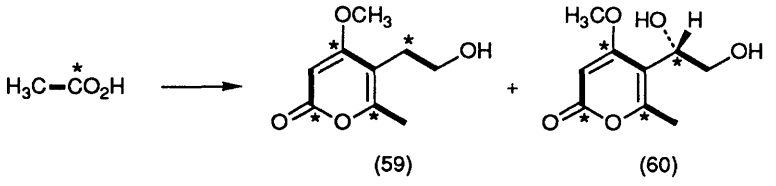
The biogenesis of pyrenocine A (58) and citreothiolactone (56) has been investigated in cultures of *Penicillium citreoviride*.[116] On addition of cysteine or methionine to the fermentation medium, yields of the two metabolites decreased significantly. However, addition of [1,2-$^{13}C_2$]acetate led to the isolation of enriched (58) and (56), as well as pyrenocine B (55), a metabolite not normally isolated from this organism. A possible biogenetic route was proposed (Scheme 19), in which all three intermediates were formed *via* a common intermediate (57). It was suggested that the sulfur atom in citreothiolactone was derived from enzyme-bound thiocrotonate, although there is no evidence to support this.



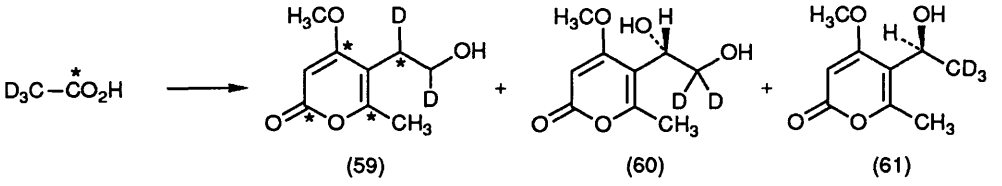
Feeding experiments with [1-^{13}C]- and [1,2-$^{13}C_2$]acetate in *Macrophoma commelinae* resulted in formation of macommelins (61) and (60), with a labelling pattern as shown (Scheme 20).[114] Cultivation of the organism with diethyl [2-^{13}C]malonate gave high enrichment at positions C-3, C-5, and C-7 of macommelinol (61), but only low incorporation at C-9. This result precluded the possibility that the macommelins were derived from a branched tetraketide chain,[116] but supported the



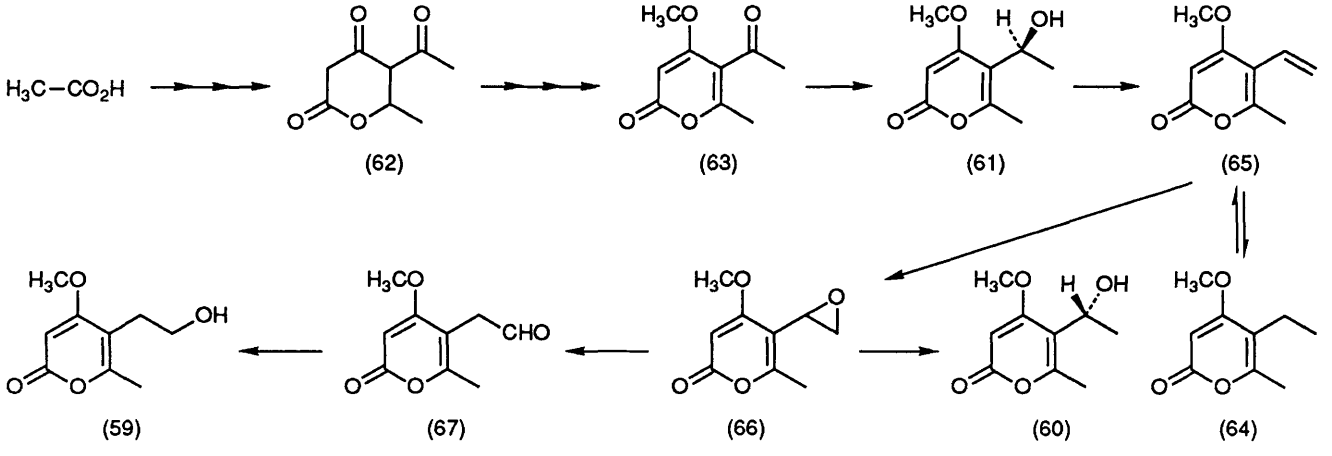
Scheme 19



Scheme 20



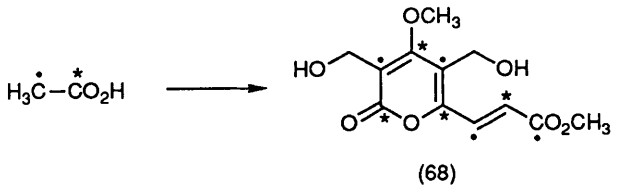
Scheme 21



Scheme 22

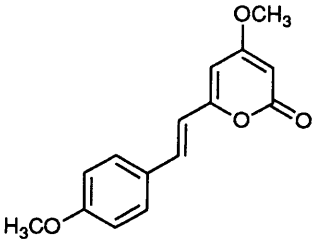
theory proposed by Turner and Aldridge[117] that an aromatic intermediate was formed, with subsequent oxidative cleavage of the benzene ring, followed by rearrangement. When [1-^{13}C,2-$^{2}H_3$]acetate was administered to *M. commelinae*, deuterium was incorporated into the C-5 side chain of (59), (60), and (61) as shown in Scheme 21.[114]

A biogenetic pathway, on the basis of the above results, was suggested to be as shown in Scheme 22. The epoxide (66) was postulated as intermediate in which a 1,2-hydride shift could occur, thus accounting for the presence of a deuterium atom α to the pyrone ring in (59). [Methoxy-^{14}C] (63) was prepared,[114] and shown to be incorporated into (59), (60), and (61). Compound (61) was only formed from (63), suggesting that this compound was at an early stage of the biosynthetic pathway. [Methoxy-^{14}C] (59) was converted to radioactively-labelled postulated intermediates (64), (67), (65), and (66). All were transformed into (59), with the aldehyde (67) showing an extremely high incorporation (91.7%), suggesting that it is an immediate precursor. The incorporation of epoxide (66) into (59) was low (2.5%), with most of the radioactivity being taken into (60). The reduction of the alkene (65) to macommelin (64) was not detected by these experiments. Compound (64), however, was incorporated into (55). It was suggested that conversion of (65) to (64) was reversible.

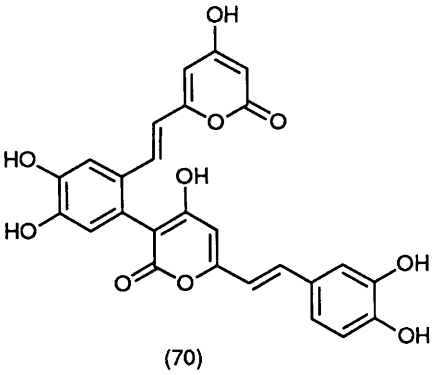


(68)

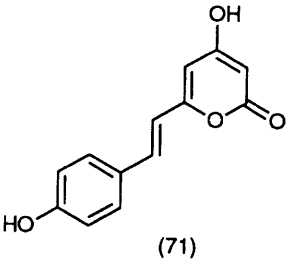
Scheme 23



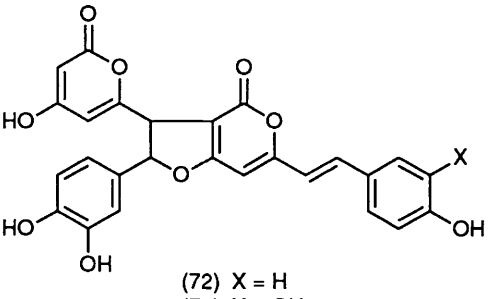
(69)



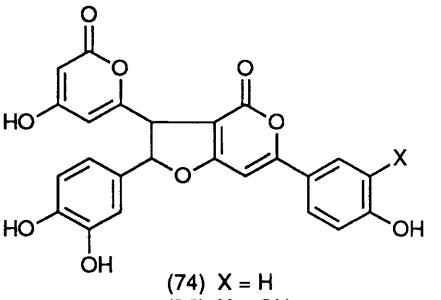
(70)



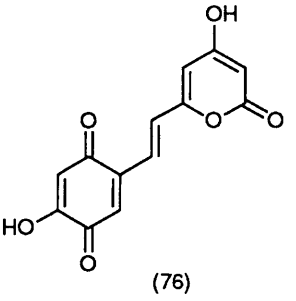
(71)



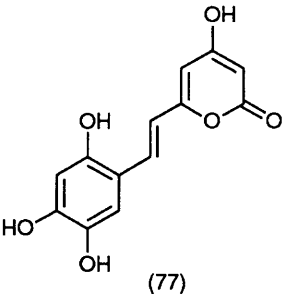
(72) X = H
(73) X = OH



(74) X = H
(75) X = OH



(76)



(77)

The biosynthesis of rosellisin (68), a co-metabolite of the macommelins, has been investigated in *Hypomyces rosellus*.[113] It was found that incorporation of [1-^{13}C]acetate and [2-^{13}C]acetate was as shown in Scheme 23. The branch carbons of the hydroxymethylene groups did not show any incorporation of label, and so it was assumed that these were derived from the C_1 pool.

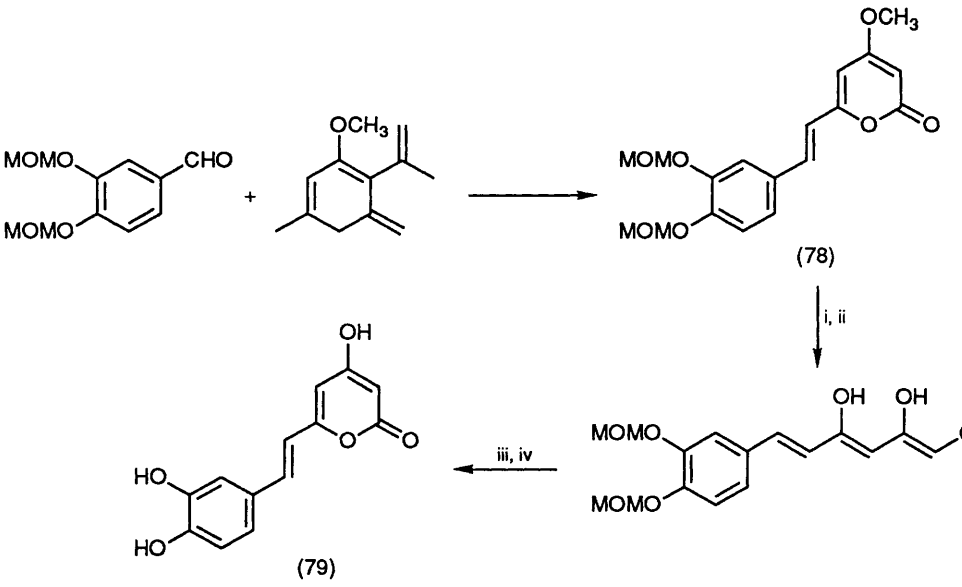
Both rosellisin (68) and rosellisin aldehyde (51) have been shown to be active against *Staphyloccoccus aureus* at low concentrations.[112,113] The structurally related islandic acid (54) has exhibited cytotoxicity against Yoshida sarcoma cells in tissue culture, and inhibited the transfection of *Bacillus* phage M_2.[115]

Pyrenocines A (58) and B (55) were phytotoxic, preventing germination of lettuce seeds, and inhibiting root elongation in seedlings.[108] Inhibition of root elongation was also demonstrated in rice and onion seedlings. Compound (58) showed the greater activity. Pyrenocine C (49) was reported to be only weakly phytotoxic.[110]

2.7 The Styryl-Pyrones and Related Compounds

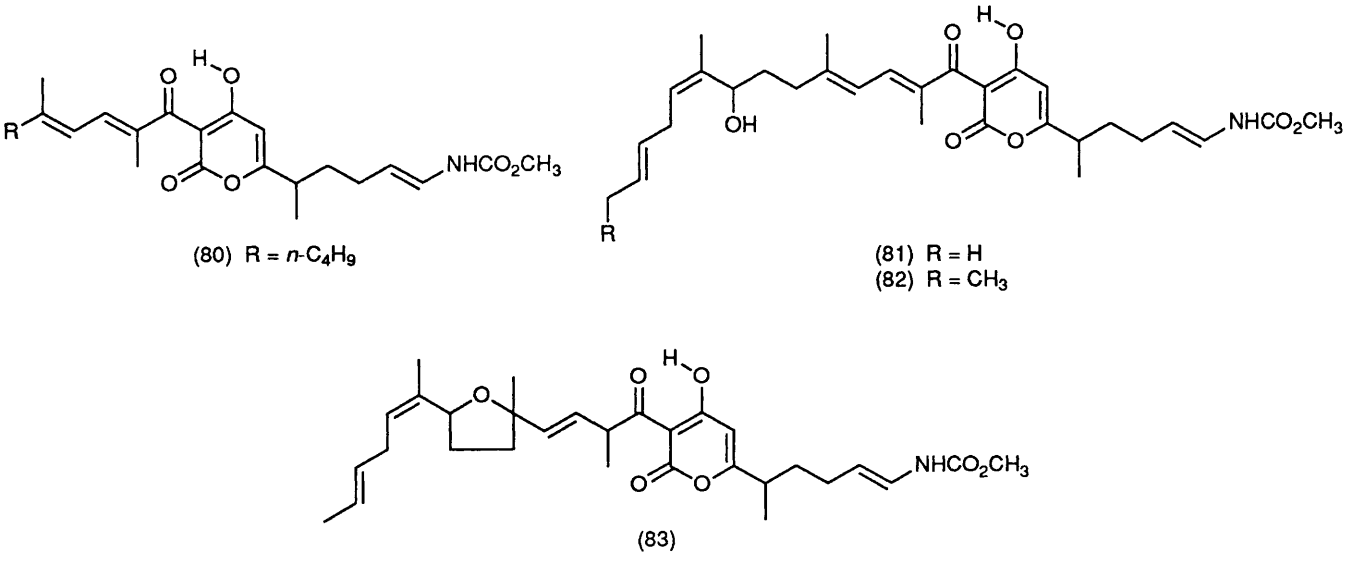
A number of conjugated arylpyrones, *e.g.* yangonin (69), have been isolated from plant sources such as *Piper*, *Aniba*, *Alpinia*, and *Ranunculus*. The first report of a styrylpyrone from a micro-organism, however, concerned the isolation of hispidin (79) from *Polyporus hispidus*,[118,119] a lignin-attacking white rot, parasitic chiefly on ash (*Fraxinus excelsior*). Hispidin has since been isolated from a number of other species, including *Polyporus schweinitzii*,[120] *Phellinus pomaceus*, where it is a co-metabolite with 3,14′-bishispidinyl (70),[121] *Phellinus igniarius*,[122] and *Gymnopilus* species.[123] Bisnoryangonin (71) was a co-metabolite in this latter organism, and has also been reported to occur in *Pholiota squarroso-adiposa*,[124] *Gymnopilus spectablis*,[125] and *G. decurrens*.[126] A systematic study of the distribution of styrylpyrones and derivatives has been carried out among the *Strophariaceae* and related genera.[127] Hispidin (79) and bisnoryangonin (71) were found to occur in *Hypholoma*, *Flammula*, *Philiota*, and *Gymnopilus* species. Hypholomines A (72) and B (73), and fasciculines A (74) and B (75) were also found to be distributed amongst these same species. These compounds were also isolated from the fruiting bodies of *Hypholoma fasciculare* (Agaricales) ('Sulfur Tuft').[128] Other related compounds to have been isolated from micro-organisms were hymenoquinone (76) and leucohymenoquinone (77), both metabolites of the fruiting bodies of *Hymenochaete mougestii* (Poriales).[129]

The synthesis of hispidin (79) has been reported on occasions, and involves condensation of an aromatic aldehyde with the



Reagents: i, EtOH, KOH; ii, H^+; iii, Ac_2O; iv, aq. H_2SO_4, EtOH, Δ

Scheme 24



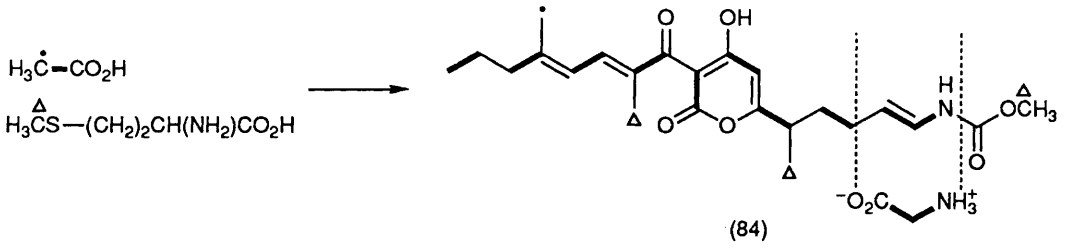
methyl ether of dehydroacetic acid to give a protected hispidin analogue (78). Deprotection followed by recyclization of the pyrone ring gave hispidin (79) (Scheme 24).[119,130]

Biosynthetic studies on the styrylpyrones have concentrated on the formation of hispidin (79) in cultures of *Polyporus hispidus*. Early studies demonstrated the incorporation of [U-^{14}C]phenylalanine and [1-^{14}C]acetate, and so suggested that hispidin was formed by condensation of a phenylpropanoid moiety with two acetate units.[131] Conversion of phenylalanine to cinnamic acid in cultures of *P. hispidus* was also demonstrated and the presence of an enzyme capable of effecting hydroxylation of cinnamic, *p*-coumaric and benzoic acids was indicated.[132] *p*-Coumaric acid and caffeic acid were incorporated efficiently into hispidin (79).[133]

The hydroxylase enzyme from *P. hispidus* was isolated and shown to catalyse hydroxylation of *p*-coumaric acid and, even more readily, hydroxylation of bisnoryangonin (71) to yield hispidin (79). NADH, NADPH, and ascorbate were found to serve as electron donors.[133,134] It was also noted that blue light (430—530 nm) stimulated pigment formation. The effect of light on growth, pigmentation and enzymic activity in the biosynthesis of hispidin (79) was studied in more detail. It was found that conversion of cinnamic acid to *p*-coumaric acid was enhanced, whilst *p*-coumaric acid was converted to caffeic acid only in cultures exposed to light.[134] Action spectrum studies showed that the system responded to light of 380 and 440 nm. Cycloheximide was found to block light-induced activity.[135] As the fruiting bodies of *P. hispidus* matured, the amount of hispidin present decreased. Simultaneously, the fruit-bodies became tougher and their fibrous 'woody' structure more pronounced. Oxidase enzymes isolated from cultures of *P. hispidus* were found to act on hispidin *in vitro* to bring about rapid oxidative polymerization,[118] and so a role for hispidin as a precursor of toughening polymers in Basidiomycetes had been suggested. No firm evidence either for or against this hypothesis has been reported, although the presence of hispidin in other non-woody Basidiomycetes suggested that this might not be the case.[133]

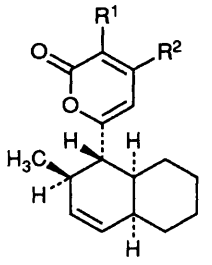
2.8 Pyran-2-ones from the Gliding Bacteria

Gliding bacterium *Myxococcus fulvus* Mx f50 has been the source of the novel N-alkenylcarbamate pyrones, myxopyronins A (84) and B (80),[136] whilst the structurally related corallopyronins A (81), B (82), and C (83) have been isolated from *Corallococcus coralloides* Cc c127.[137]

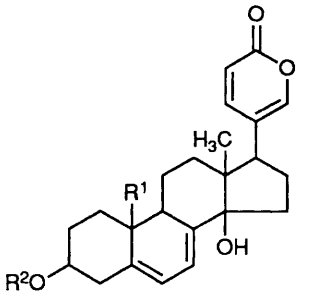


(84)

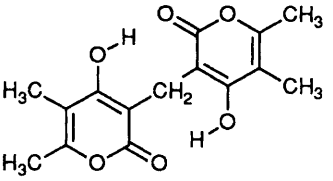
Scheme 25



(85) R^1 = CHO, R^2 = OCH_3
(86) R^1 = CH_2OH, R^2 = OCH_3
(87) R^1 = CHO, R^2 = —$NHCH_2CH_2OH$



(88) R^1 = CHO, R^2 = xylose
(89) R^1 = CH_3, R^2 = (glucose)$_2$–ribose



(90)

Biosynthetic studies on myxopyronin A have shown it to be derived from two polyketide chains, with glycine being incorporated as a starter unit in one of the chains.[138] Two of the remaining methyl groups, and the carbamate methyl ester, are incorporated from methionine, with the third methyl group being derived from the C-2 of acetate (Scheme 25).

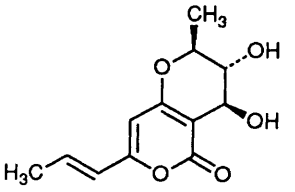
Myxopyronins A (84) and B (80) were found to be active against a range of bacteria, *i.e. Corynebacterium mediolanum*, *Arthrobacter simplex*, *Bacillus megaterium*, *B. subtilis*, *Brevibacterium ammoniagenes*, *Staphylococcus aureus*, *Micrococcus luteus*, *Acinetobacter calcoaceticus*, and *Agrobacterium tumefaciens*. Activity was marked for Gram-positive bacteria. However, Gram-negative bacteria were hardly affected, and yeasts and moulds were totally resistant.[139]

Incorporation studies[139] with *Staphylococcus aureus* suggested that the myxopyronins inhibited RNA synthesis and thus, with a certain delay, protein synthesis. This was confirmed when DNA-dependent RNA polymerase, isolated from *Escherichia coli*, was found to be strongly inhibited by the myxopyronins. Inhibition with both whole cells (*S. aureus*) or isolated enzyme (*E. coli*) was not, however, inhibited completely. The inhibitory effect was not seen for wheat germ RNA polymerase II, and so the biological activity appeared to be restricted to prokaryotic RNA polymerases. Myxopyronins had no acute toxicity for mice up to 100 mg/kg (s.c.). In general, myxopyronin B (80) appeared to be more active than myxopyronin A (84).

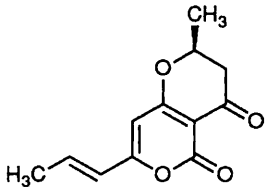
The corallopyronins (81)—(83) were found to exhibit similar biological activity to the myxopyronins,[140] with the mechanism of action also being similar, *i.e.*, inhibition of bacterial RNA polymerases. However, whereas the myxopyronins only partially inhibited RNA synthesis, corallopyronin A (81) gave total inhibition both in whole cells, and with the isolated enzyme. RNA synthesis was inhibited even after chain elongation had started. Once again, eukaryotic cells were completely resistant.

2.9 The Phytotoxic Pyran-2-ones

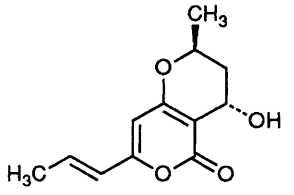
A number of pyran-2-ones have been isolated from microorganisms which were pathogenic on plants, and the pyrones themselves were found to exhibit some of the phytotoxic activity attributed to the organism. Such compounds include radicinin (94), the solanopyrones A (85), B (86), and C (87), poaefusarin (88), sporofusarin (89), and colletopyrone (90). These will be discussed in this section.



(91)



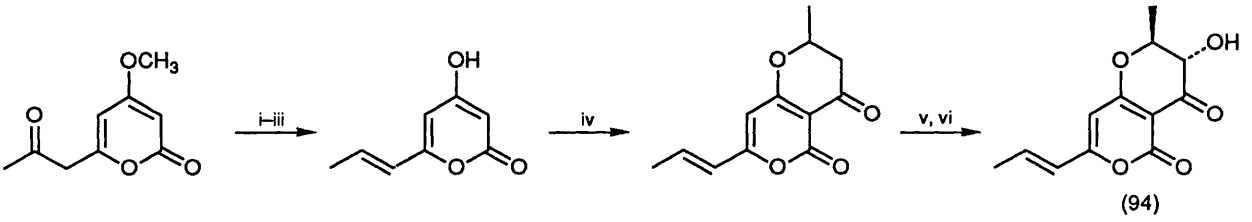
(92)



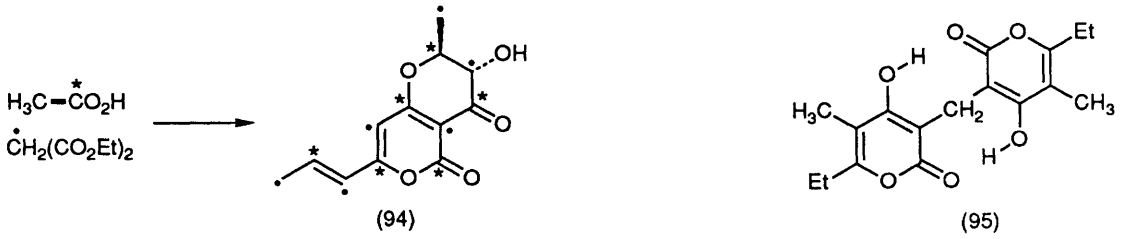
(93)

2.9.1 *Radicinin and Related Compounds*

Radicinin (94), also named stemphylone, was first reported in culture filtrates of *Alternaria radicina*,[141] and has been isolated from *Stemphylium radicinum*.[142–145] This latter organism has been shown to be identical with *A. radicina*.[145] Radicinin (94) has also been found in culture filtrates of *Curvularia lunata*, *C. inequalis*, *C. coisis*, and *C. trifolii*,[146] and as a co-metabolite with the structurally related radicinol (91) in culture filtrates of *Cochliobolus lunata*,[147] and *Alternaria chrysanthemi*.[148] The *X*-ray crystal structure of radicinin (94) has been reported, whilst the absolute configuration of the 4-O-*p*-bromobenzoyl ester of radicinin, and by analogy, of radicinin itself, has been determined.[148] Two other closely related metabolites, deoxyradicinin (92) and 3-epideoxyradicinol (93) have been isolated from *Alternaria helianthi*.[149,150]



Scheme 26



Scheme 27

The syntheses of (±)-radicinin (94) and the dihydro analogue have been reported.[151—153] The route through to radicinin is outlined in Scheme 26.[151] Construction of the second ring of radicinin was achieved *via* the Lewis acid-mediated condensation of crotonyl chloride with the hydroxy-pyrone.

Biosynthetic studies on radicinin have been carried out, and its derivation from acetate established. Labelling studies with [1-^{14}C]acetate and [2-^{14}C]malonate led to distribution of label, as shown in Scheme 27, with evidence suggesting that radicinin (94) was formed by the condensation of two acetate-malonate-derived chains.[154]

Feeding studies with [1,2-$^{13}C_2$]acetate confirmed these results.[155] Incorporation of [1-^{13}C]- and [2-^{13}C]acetate into deoxyradicinin (92) was shown to give a similar labelling pattern to that demonstrated for radicinin.[150] Two pathways are biosynthetically possible for the formation of radicinin. The first would require the condensation of a C_8-unit with a C_4-unit, whilst the second would require the condensation of two C_6-units. Although the former is biosynthetically preferable, no evidence has been obtained that favours one pathway over the other.

Radicinol (91) was produced some time after the first production of radicinin (94) in cultures of *Alternaria chrysanthemi*, and with concomitant decrease in concentration of the latter, thus suggesting that radicinin was converted to radicinol by this organism.[148]

Radicinin (94) has been shown to inhibit the germination of seeds of *Lepidum sativum* (cress) at concentrations of 5×10^{-5} M. At concentrations which permitted seed germination, considerable damage to root development was noted, in that the roots were shorter than normal, discoloured, and devoid of root hairs.[142] Stems and seed-leaves were not affected. Radicinin has been found to be a constituent of necrotic lesions on chrysanthemum leaves that were artificially infected with *Alternaria chrysanthemi*. This suggested a causal role for the metabolite in foliar disease caused by this organism. Radicinol (91) has been shown to exhibit similar phytotoxic effects to that demonstrated for radicinin (94), and caused interveinal necrosis of cuttings of Canada thistle.[148] 3-Epideoxyradicinol (93) when applied over needle punctures on sunflower leaves caused small necrotic spots in less than 24 hours. These were enlarged after 24 hours and surrounded by chlorotic halos. These symptoms were also typical of the leaf spot phase of the disease caused by *Alternaria helianthi*. Although this suggested a causal role for 3-epideoxyradicinol in disease produced by the organism, it has not been identified as a constituent of *A. helianthi*-induced sunflower leaf lesions. Deoxyradicinin (91) has been identified, however.[150]

Radicinin has been reported to be inhibitory towards the growth of several Gram-positive bacteria, such as *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium* species,[142,146,148] and antagonistic towards *Phytophthora erythroseptica*.[141] In general, however, its antifungal properties are negligible.

Experiments to determine the mode of action of radicinin (94) showed that it increased the rate of dehydrogenation of isopropyl alcohol by *Fusarium lini*. However, there was no effect on the quantity or degree of desaturation of fatty acid synthesized.[144]

2.9.2 The Solanopyrones

The solanopyrones A (85), B (86), and C (87) have been isolated from *Alternaria solani*, the causal organism of early blight disease of tomato and potato. Solanopyrone A (85) induced a necrotic lesion on the leaf of potato at concentrations of 100 μg/μl (methanol).[156]

2.9.3 Poaefusarin and Sporofusarin

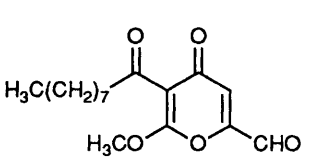
Poaefusarin (88) has been isolated from *Fusarium poae*, and sporofusarin (89) from *Fusarium sporotrichiella*.[157] These *Fusarium* species have been identified as the causal organism of alimentary toxic aleukia. The toxins themselves have marked phytotoxic effects, as well as exhibiting mammalian toxicity.[157] Phytotoxic symptoms include the death of branches of peas, beans, and tomatoes, accompanied by extreme loss of turgor. The germination of pea, beans, and barley seeds was completely inhibited.

In mammals, the toxins caused temporary inflammation of the skin, and oedematous, haemorrhagic, or leukocytorric reactions. The final stages of toxicity resulted in leucopenia and agranulocytosis, necrotic angina, haemorrhagic diathesis, sepsis and necrosis of various parts of the alimentary tract, especially the throat, and exhaustion of the bone marrow.

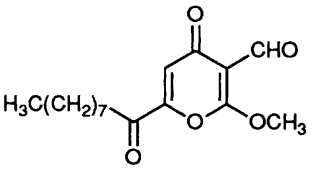
2.9.4 Colletopyrone

Colletopyrone (90) has been isolated from *Colletotrichum nicotianae*, a pathogenic fungus causative of tobacco anthracnose.[158] It is related, in structure, to helipyrone (95), a dipyran-2-one that has been isolated from a higher plant, *Helichrysum italicum*.[159]

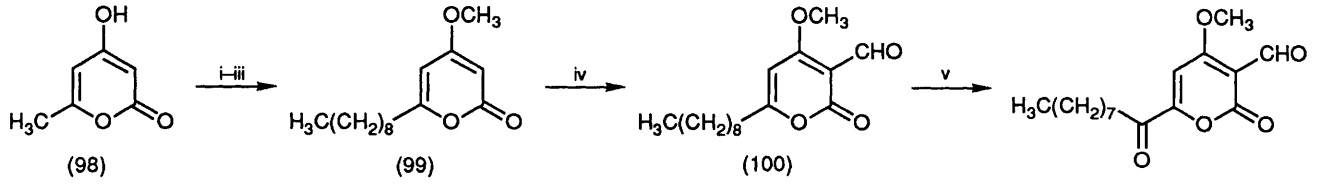
When solutions of colletopyrone (90) were placed on young tobacco leaves that had been pricked with a needle, brown necrotic spots appeared within three days; the symptoms were analogous to those caused by the pathogenic fungus.[158]



(96)



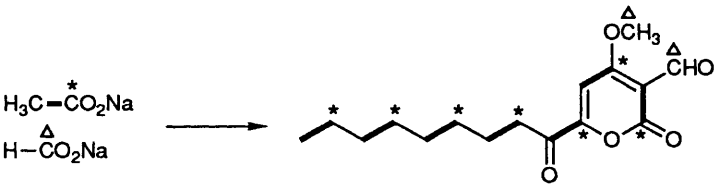
(97)



(98) (99) (100) (101)

Reagents: i, 2 BuLi; ii, $H_3C(CH_2)_7Br$; iii, $(CH_3)_2SO_4$, K_2CO_3; iv, $MeOCH_2Cl$, $TiCl_4$; v, SeO_2

Scheme 28



(101)

Scheme 29

2.10 Other Pyran-2-ones

2.10.1 Phacidin

Phacidin (101) has been isolated from the canker fungus *Potebniamyces balsamicola*, causal organism of bark disease in *Abies grandis*.[160,161] Phacidin was originally assigned the pyran-4-one structure (96),[161] which was revised to that of structure (97) on the basis of spectroscopic evidence.[162] However, the actual structure was finally confirmed as the pyran-2-one (101), by an unambiguous total synthesis, the route for which is outlined in Scheme 28.[163] Thus pyrone (98) was *O*-methylated under conditions that had been shown to produce only 4-methoxy-2H-pyran-2-ones to give methyl ether (99), which was then converted into phacidin *via* the formyl derivative (100).

Labelling studies with [1-^{13}C]- and [1,2-$^{13}C_2$]acetate demonstrated that phacidin (101) was derived from seven acetate units, with two carbons derived from the C_1 pool.[163] The distribution of label is shown in Scheme 29.

Phacidin (101) showed potent antifungal properties, and inhibited the growth of fungi in all the major groups. Fungi that were particularly affected were *Phytophthora cactorum*, *Xenomeris abietis*, *Acremonium tsugae*, *Sirococcus strobilinus*, *Conisphora puteana*, *Lenzites saepiaria*, *Polyporus schweinitzii*, *P. sulphureus*, *Poria placenta*, *P. subacida*, *P. weirii*, and *Serpula himantioides*.[160] Although in most cases inhibition by the control antibiotic, nystatin, was greater, phacidin was considerably more inhibitory against species of *Pythium*, *Phycomycetes*, and *Phytophthora*.

The effect of phacidin (101) on fungi and yeasts that cause superficial and deep mycoses in man was also investigated.[164] It was found that, amongst yeasts, species of *Candida*, *Saccharomyces*, *Trichosporon*, and *Torulopsis* were inhibited. Phacidin was also inhibitory against dermatophytes such as *Epidermophyton floccosum*, *Trichophyton mentagrophytes*, and *T. rubrum*, its *in vitro* effectiveness being similar to that reported for griseofulvin. Its activity against systemic dimorphic fungi, *e.g.*, *Histoplasma capsulatum*, *Sporothrix schenckii*, *Blastomyces dermatitidis*, and *Coccidioides immitis* was, in general, greater than that of the known antifungal agent 5-fluorocytosine. By comparison, phacidin (101) was comparatively ineffective against opportunistic fungi such as *Aspergillus* species.

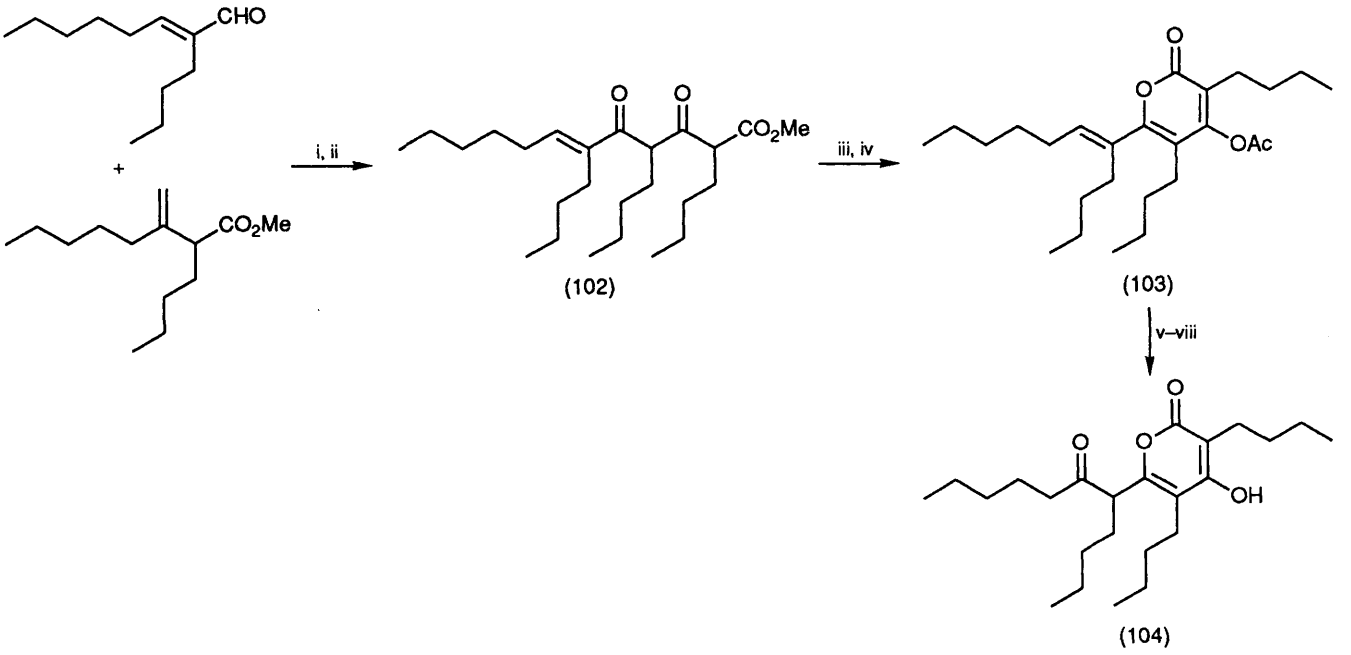
2.10.2 Elasnin

Elasnin (104) was isolated from *Streptomyces norboritoensis*.[165] The synthesis of elasnin has been reported, and the route followed is outlined in Scheme 30.[166] Treatment of diketone (102) with a catalytic amount of acid, and simultaneous azeotropic removal of water, gave pyrone (103, R = H), which was then converted into elasnin (104).

Original biosynthetic studies using [1,2-$^{13}C_2$]acetate as the labelled precursor suggested that elasnin (104) was derived from twelve molecules of acetate.[167] This was confirmed in subsequent studies, with the distribution of label as shown in Scheme 31.[168]

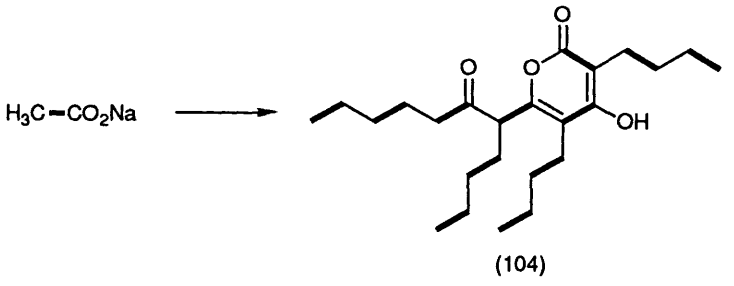
Elasnin (104) was originally isolated because of its inhibitory effect on human sputum (leukocyte) elastase, an enzyme that has been implicated in many inflammatory disease states such as pulmonary emphysema, acute arthritis, and destruction of connective tissue. 50% inhibition of this enzyme was achieved by elasnin at concentrations of 1.3 μg/ml.[165,169] The compound appeared to be specific in its activity, with considerably higher concentrations required in order to achieve 50% inhibition of pancreatic elastase. Chymotrypsin, trypsin, thermolysin, and papain were unaffected.

A number of analogues of elasnin have been prepared, with the aim of developing more specific inhibitors of greater potency. The phenyl derivative (105) was as active as elasnin against human sputum elastase,[170] and more effective against porcine pancreatic elastase or chymotrypsin, whilst the octyl derivative (106) was thirty times more active than elasnin.[171]

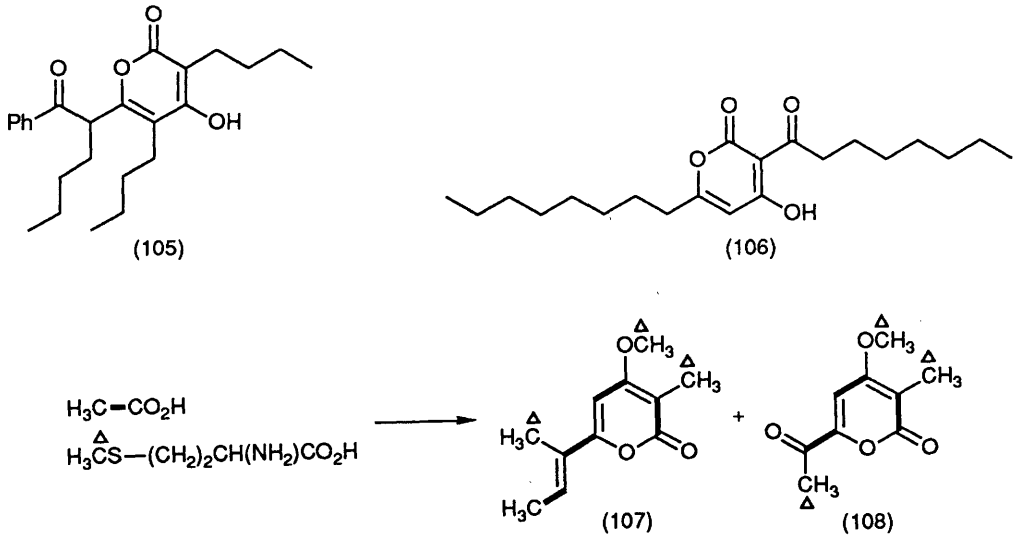


Reagents: i, Base condensation; ii, DDQ, dioxan; iii, TsOH (cat.), toluene; iv, Ac_2O, pyridine; v, mCPBA, ArSAr; vi, 10% Pd–C, EtOH; vii, Jones reagent; viii, conc. H_2SO_4, 0 °C

Scheme 30



Scheme 31



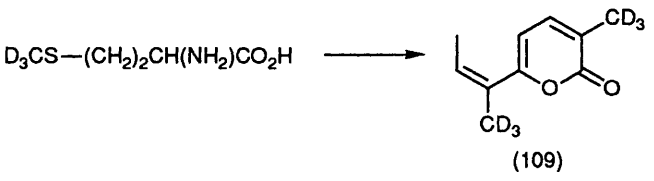
Scheme 32

Studies on the effects of substituents on biological activity have also been carried out.[171]

Elasnin (104) was reported to be of low toxicity, with an LD_{50} in mice 290 mg/kg (i.p.), and > 1000 mg/kg (orally).[165] Elasnin has no antibacterial or antifungal activity.[169]

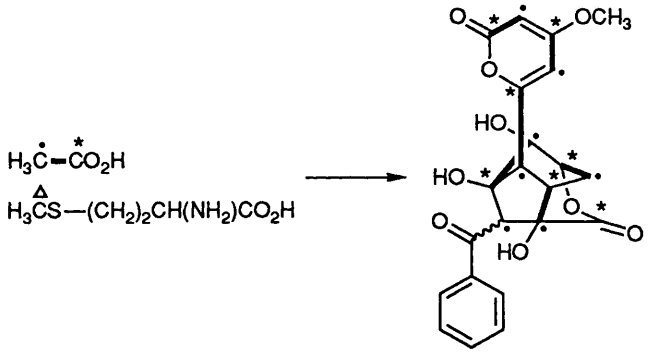
2.10.3 Nectriapyrone and Related Compounds

Nectriapyrone (107) has been isolated from *Gyrostroma missouriense*, the imperfect stage of *Thyronectria missouriensis*,[172] and more recently from *Gliocladium vermoesenii*.[173] Vermopyrone (108) was a co-metabolite in this latter organism.



(109)

Scheme 33



(110) X = H
(111) X = F

Scheme 34

A closely related compound, fusalanipyrone (109), has been isolated from *Fusarium solani*.[174]

A route through to substituted 2-pyrones, which involves the condensation of two molecules of α,β-unsaturated acid chlorides with loss of two molecules of hydrogen chloride, has been developed. Fusalanipyrone (109) was one of the pyrones synthesized by this method.[175]

When nectriapyrone (107) was first isolated, it was reported to incorporate [2-^{14}C]mevalonic acid, and the conclusion was drawn that nectriapyrone was, in fact, a monoterpene.[172] However, there was no evidence from degradation studies to support this claim. Recent studies using [1,2-$^{13}C_2$]acetate and [Me-^{13}C]methionine have demonstrated that this metabolite is acetate-derived, with the extra methyl groups being introduced from methionine (Scheme 32).[173] The labelling pattern for vermopyrone (108) suggested that the latter may have arisen through cleavage of nectriapyrone. The fact that vermopyrone was not isolated from shorter fermentations provided some support for this hypothesis.

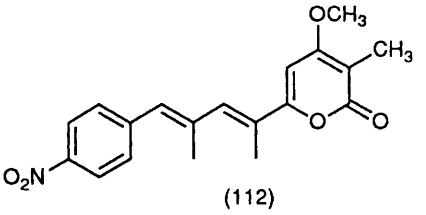
Fusalanipyrone (109) also appeared to follow the isoprene rule, and the question was raised as to whether this might be a monoterpene. However, feeding studies with [$^2H_3$C]methionine demonstrated that the C-3 and the C-1′methyl groups were methionine-derived, and that, presumably, fusalanipyrone (109) was biosynthesized *via* a polyketide which was subsequently methylated (Scheme 33).[176]

Nectriapyrone (107) has been reported to display antibacterial activity against *Staphylococcus aureus* at a concentration of 30 ppm.[172] Fusalanipyrone was, in contrast, inactive against *Staphylococcus* and *Escherichia coli*, although it displayed weak antibiotic activity against *Candida albicans*, *Mucor* and *Trichoderma koningii*.[174]

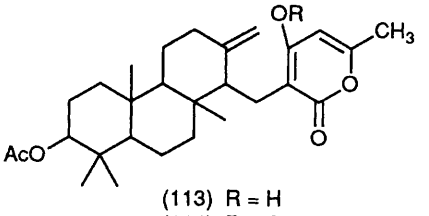
2.10.4 *Vulgamycin*

Vulgamycin (110), also named enterocin, has been isolated from *Streptomyces candidus* var. *enterostaticus*, *S. viridochromogenes*,[177] and *S. hygroscopicus*.[178] An *X*-ray crystal structure determination of the *m*-bromobenzoyl ester of vulgamycin has been carried out.[179]

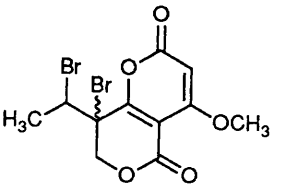
Biosynthetic feeding studies with [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]acetate demonstrated that vulgamycin was derived from



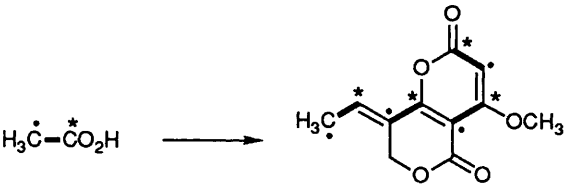
(112)



(113) R = H
(114) R = CH_3



(115)



(116)

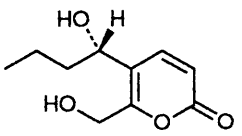
Scheme 35

seven acetate units, with the distribution of label as shown in Scheme 34.[178] The methoxyl carbon of the pyrone ring was derived from methionine. [U-^{14}C]Benzoate was incorporated specifically into the benzoyl portion of vulgamycin. It was thus proposed that vulgamycin was biosynthesized from methionine and seven acetate units with benzoate as the starter unit.

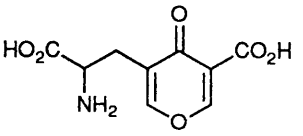
Vulgamycin (110) was bacteriostatic against both Gram-positive and Gram-negative bacteria, such as *Escherichia coli*, and species of *Proteus*, *Sarcina*, *Staphylococcus*, and *Corynebacterium*. Derivatives of vulgamycin were prepared in which the aromatic moiety was substituted with fluorine at various positions – these derivatives were formed by addition of the fluorinated benzoic acid to fermentations of *Streptomyces hygroscopicus*.[180] Although *p*-fluorovulgamycin (111) showed stronger activity than the parent compound against *Micrococcus luteus*, the fluorinated derivatives displayed very little difference in their antimicrobial spectrum compared to vulgamycin itself. Vulgamycin had no activity against fungi and yeasts, and its mammalian toxicity was low.[177]

2.10.5 *Luteoreticulin*

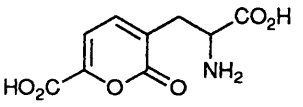
Luteoreticulin (112) has been isolated as a toxic metabolite of *Streptomyces luteoreticuli*.[181]



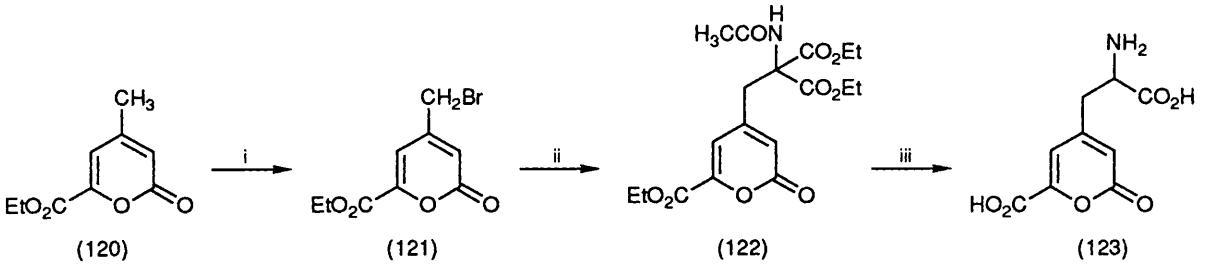
(117)



(118)



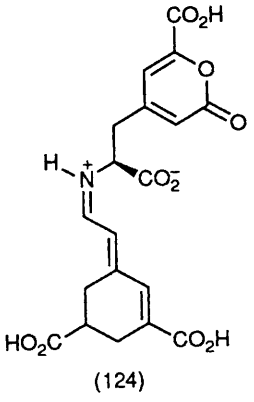
(119)



(120) (121) (122) (123)

Reagents: i, NBS, CCl_4, *hν* or peroxide; ii, Na^+ $C(NHCOCH_3)(CO_2Et)_2$; iii, HCl/CH_3CO_2H, 100 °C, sealed tube

Scheme 36



(124)

2.10.6 Aszonapyrone A

Aszonapyrone A (113) has been isolated from *Aspergillus zonatus*,[182] and the crystal structure of the monomethyl ether (114) determined by *X*-ray analysis.[183] It has been suggested that aszonapyrone A is biosynthesized by a combination of both the mevalonate–geranylgeranyl–pyrophosphate route, and the acetate–polyketide route.[182]

Aszonapyrone A showed antibacterial activity, with an MIC of 6.3 μg/ml, against *Staphylococcus aureus*.[182]

2.10.7 Coarctatin

Coarctatin (116) has been isolated as an inactive metabolite of the fungus *Chaetomium coarctatum*[184] and its structure was determined by spectroscopic means. Confirmation of the structure was obtained by *X*-ray crystallography studies on the dibromo-derivative (115).[185]

A number of possible biosynthetic pathways have been proposed for the formation of coarctatin. However, feeding studies with [1,2-$^{13}C_2$]acetate demonstrated the incorporation of four intact acetate units into the structure (Scheme 35). The methyl carbon of [2-^{13}C]acetate was also incorporated into the three remaining carbons, as shown, indicating that these were derived from the C_1 pool.[186]

2.10.8 Taiwapyrone

Taiwapyrone (117) has been isolated from the fungus *Cercospora taiwanensis*, a plant pathogen, and its structure determined by spectroscopic means.[187]

2.10.9 Stizolobic Acid and Stizolobinic Acid

Stizolobic acid (123) was first isolated from the cut surface of the epicotyl tips of etiolated seedlings of *Stizolobium hassjoo* (Velvet bean), and its structure reported to be that of the pyran-4-one (118) on the basis of chemical degradations.[188] It was also found to be present in other *Stizolobium* species and in *Mucuna irukanda* (Leguminoseae). The structure of stizolobic acid was later reassigned to that of the pyran-2-one (123), whilst the structure of a co-metabolite in *S. hassjoo*, named stizolobinic acid, was shown to be the related pyran-2-one (119).[189] Both of these metabolites have since been isolated from the fungus *Amanita pantherina*, a frequent cause of non-fatal mushroom poisoning in the Pacific Northwest.[190]

The syntheses of stizolobic acid (123) and stizolobinic acid (119) have been reported.[189] The synthesis of the former is illustrated in Scheme 36. Thus ethyl 4-methylpyran-2-one-6-carboxylate (120) is treated with *N*-bromosuccinimide to give the bromomethyl derivative (121), which condenses with sodio diethyl acetamidomalonate to give pyrone (122). Hydrolysis of this compound gave the target diacid (123). Stizolobinic acid (119) was synthesized in an analogous manner, taking ethyl 3-methylpyran-2-one-6-carboxylate as the starting pyran-2-one.

Biosynthetic studies utilizing the plants *S. hassjoo* and *Mucuna deeringiana* demonstrated the derivation of both stizolobic acid (123) and stizolobinic acid (119) from tyrosine *via* DOPA, with extradiol cleavage of the aromatic ring of DOPA being invoked in order to explain the formation of the heterocyclic rings of the pyrone-acids.[191–194] The enzymes responsible for this conversion have been isolated and purified.[193,195] Feeding studies with [U-^{14}C]DOPA utilizing *Amanita pantherina* demonstrated the incorporation of this amino acid into stizolobic acid, thus indicating that a similar biosynthetic pathway was acting in the fungus as had been demonstrated for higher plants.[196]

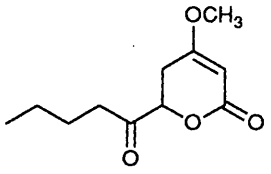
2.10.10 Muscaurin II

Muscaurin II (124) is one of a group of orange pigments to have been isolated from the caps of *Amanita muscaria* (Fly Agaric).[197] It is structurally related to stizolobic acid (123), and its synthesis from the latter has been reported.[197]

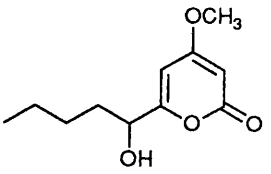
3 Microbial Dihydropyran-2-ones

3.1 Pestalotin and Related Compounds

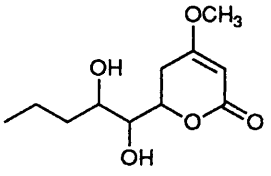
Pestalotin (also designated LL-P880α) (129) was originally isolated from the culture broth of *Pestalotia cryptomeriaecola*,



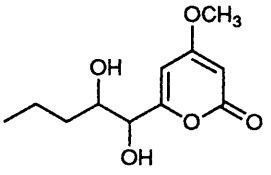
(125)



(126)



(127)



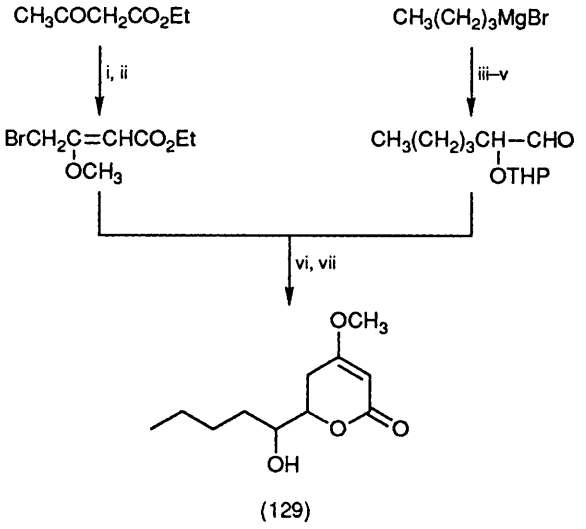
(128)

a fungus pathogen of *Cryptomeria japonica* (Japanese Cedar).[198] It has since been isolated from an unidentified *Penicillium* species,[199] and an unidentified fungus which was believed to be neither a *Pestalotia* nor a *Penicillium* species.[200] The keto-analogue (125) was a co-metabolite in this latter organism. Both pestalotin (129) and the keto analogue (125) have been isolated from another unidentified *Penicillium* species, along with the fully unsaturated pyran-2-one dehydropestalotin (126).[201] The dihydroxy analogues LL-P880β (127) and LL-P880γ (128) have also been isolated from an unidentified *Penicillium* species.[202] The absolute configuration of pestalotin (129) at C-6 and C-1′ has been determined as (*S*).[199]

The structure of pestalotin (129), established by spectroscopic means,[203] has been confirmed by total synthesis,[204] the route for which is illustrated in Scheme 37. The pyrone ring was constructed from ethyl acetoacetate, whilst the side chain was synthesized from acrolein and butylmagnesium bromide. Reformatsky methodology yielded pestalotin (129), though in poor yield.

The polyketide origin of pestalotin (129) has been determined from biosynthetic studies with labelled acetate. Thus, addition of [1,2-$^{13}C_2$]acetate to fungal cultures led to isolation of pestalotin with the distribution of label as shown in Scheme 38.[205] Related metabolites are also, presumably, polyketide derived.

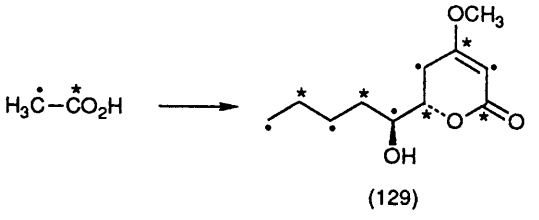
Pestalotin (129) was originally isolated because of its role as a gibberellin synergist. Thus it was found that when pestalotin alone was applied to rice seedlings, no effect was observed. However, when it was applied in combination with gibberellic acid (GA_3), the stimulative effect of this plant growth hormone was considerably enhanced. It was demonstrated that this enhancement was due to an increase in the promotive effect of GA_3 on α-amylase synthesis.[198] Pestalotin alone was also found to be capable of inducing sugar release, although its activity was much lower than that of GA_3.



(129)

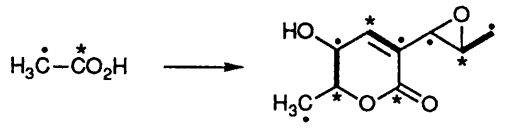
Reagents: i, $CH(OCH_3)_3$, H_2SO_4 (cat.); ii, NBS, CCl_4, Δ; iii, acrolein; iv, O_3; v, Dihydropyran, H^+; vi, Zn; vii, H^+

Scheme 37



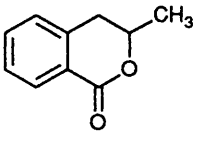
(129)

Scheme 38



(130)

Scheme 39



(131)

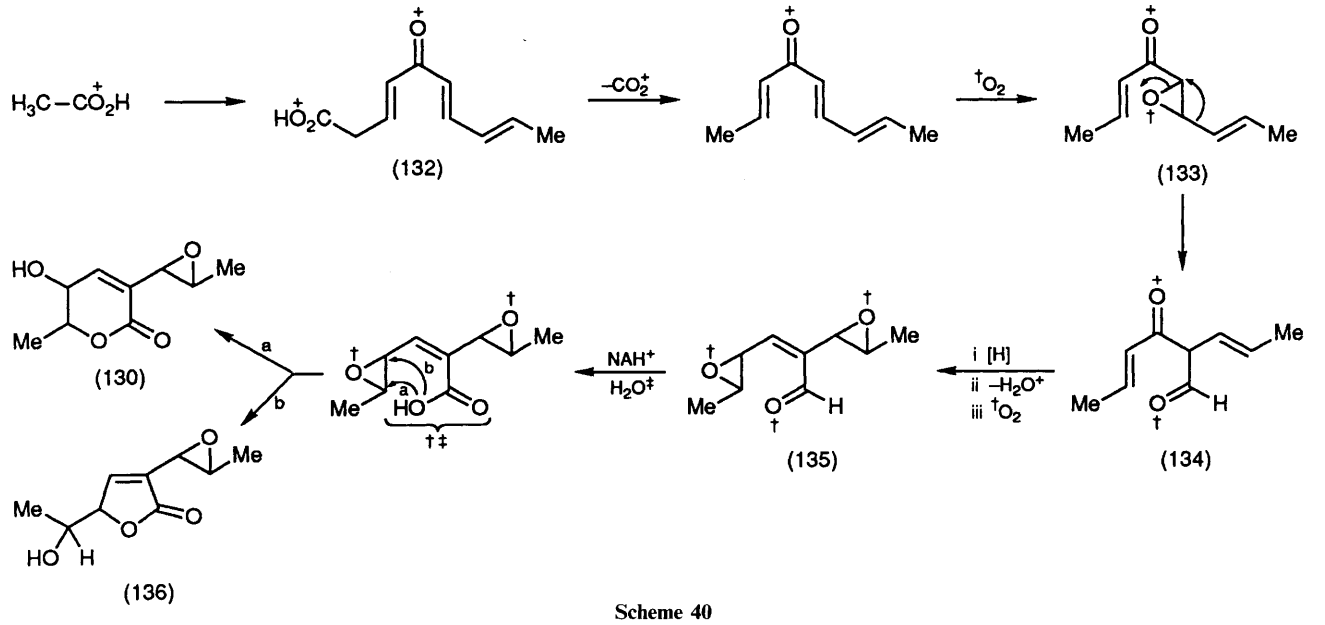
3.2 Aspyrone and Related Compounds

3.2.1 Aspyrone

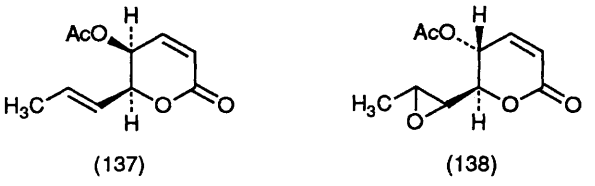
Aspyrone (130) was originally isolated from *Aspergillus melleus*,[206] and has since been isolated from *A. elegans*,[207] *A. ochraceus*,[208] and another unidentified *Aspergillus* species.[209] Its structure has been determined by spectroscopic means, and by *X*-ray crystallographic studies.[210]

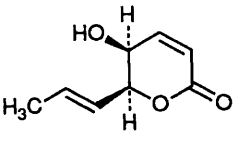
Considerable work has been carried out in elucidating the biosynthetic origins of aspyrone (130). Feeding studies with [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]acetate established the labelling pattern to be as shown in Scheme 39.[211] It was proposed that the pyrone was formed *via* a pentaketide precursor which underwent a Favorskii-type rearrangement. Cleavage of an originally intact acetate unit would account for loss of 1,2-coupling in the ^{13}C NMR spectrum as observed for the C-6 methyl group of [1,2-$^{13}C_2$]acetate enriched aspyrone.[212–214] When a ^{13}C NMR spectrum was obtained at 500 MHz, a small coupling of 6.2 Hz between C-1 and C-8 was observed, thus demonstrating that these carbons were originally derived from the same acetate unit.[212] Incorporation of [2-^{14}C]malonate into aspyrone demonstrated lower levels of label at C-10, thus identifying C-9—C-10 as the starter unit. Any possibility that aspyrone may have been derived from an aromatic intermediate such as mellein (131) (a co-metabolite) was discounted on the basis of feeding studies with [2-$^3H_3$]acetate.[215] Tritium was retained at C-7, whereas in the formation of mellein or other aromatic metabolites all three hydrogens would be removed in the formation of the aryl ring.

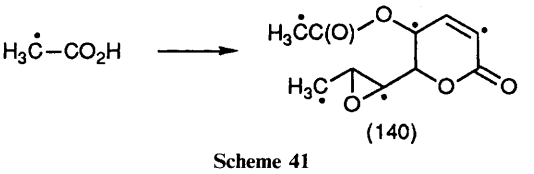
Asperlactone (136), a co-metabolite of aspyrone (130), showed a similar incorporation pattern to that observed for the latter. This could be accounted for if both metabolites were derived from a common (post-Favorskii rearrangement) intermediate.[216] Feeding studies using ^{18}O-labelled precursors



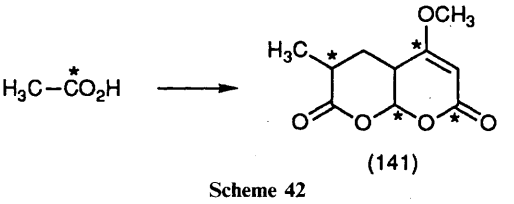
Scheme 40







Scheme 41



Scheme 42

have allowed further elaboration of the biosynthetic pathway to aspyrone. When [1-^{13}C,$^{18}O_2$]acetate was investigated as a precursor, it was found that there were no ^{18}O-isotope induced shifts in the ^{13}C NMR spectrum, indicating that none of the oxygen atoms of aspyrone were acetate derived. Growth of organisms under an atmosphere of $^{18}O_2$ gas and simultaneous addition of ^{13}C-labelled acetate demonstrated that the epoxide and C-5-hydroxy oxygen atoms were derived from the atmosphere. In addition to this, the C-2 and C-6 signals were also shifted but the intensity of their signals was approximately half that of the carbons attached to the hydroxyl and epoxide oxygen atoms. It was suggested that ^{18}O had been introduced onto C-2 from the atmosphere, and that this was incorporated equally into both the carbonyl and ether oxygen of the pyrone ring. The remaining oxygen at C-2 would, therefore, be derived from the medium. Similar results were observed for asperlactone (136).

A proposed biosynthetic pathway that takes into account all the above results has been postulated,[216–220] and is illustrated in Scheme 40. Thus the epoxide (133), derived from the trienone intermediate (132), would undergo rearrangement to give the aldehyde (134). Further epoxidation and NAD$^+$-mediated oxidation would then give the epoxycarboxylic acid (135). Ring closure to either end of the epoxide moiety would finally yield either aspyrone (130) or asperlactone (136).

3.2.2 *Asperline, Phomalactone, and their Derivatives*

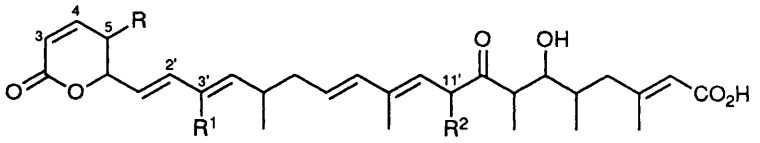
Asperline (U-13,933) (140) was originally isolated from *Aspergillus nidulans*,[221,222] and has since been isolated from *A. carneus*[207] and *A. caespitosus*.[223] The epimer of asperline (137) was a co-metabolite in this latter organism, as was the propenyl-derivative (138). Phomalactone (139) has been isolated from an unidentified *Nigrospora* species,[224] and from an unidentified *Phoma* species.[207]

Feeding studies with [2-^{13}C]acetate have demonstrated that asperline was polyketide-derived, with the distribution of label as shown in Scheme 41.[225]

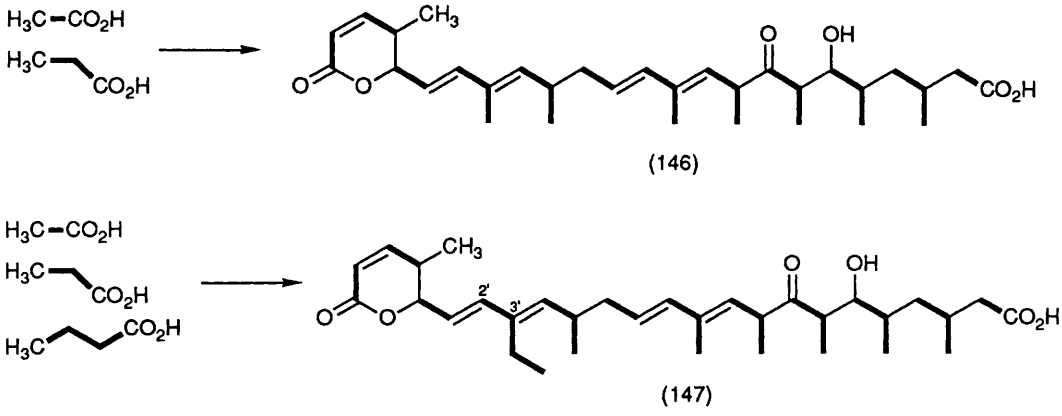
Asperline (140) displayed antibiotic activity against *Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella gallinarum*, *Bacillus cereus*, *Sarcina lutea*, *Mycobacterium avium*, *Salmonella pullorum*, *Rhodopseudomonas spheroides*, and *Chromobacterium violaceum in vitro*, although it was inactive in *P. vulgaris*-infected mice when they were treated subcutaneously at the maximum tolerated dose.[221,226] Asperline was also found to exhibit antifungal activity against *Trichophyton violaceum*, *T. rubrum*, *Homodendrum compactum*, *Coccidioides immitis*, *Blastomyces dermatitidis*, and *Nocardia asteroides*,[226] but was inactive against *Fusarium inoculiform* and *Verticillium alboatrum*.[223] Activity against *Candida albicans* was also noted. The related metabolites have been reported to exhibit a similar antimicrobial spectrum.[207,223]

3.2.3 *Astepyrone*

Astepyrone (141) has been isolated from cultures of *Aspergillus terreus*, and has been shown to be a polyketide.[227] The distribution of label derived from [1-^{13}C]acetate was as shown in Scheme 42.



(142) R = CH_3, R^1 = CH_2CH_3, R^2 = CH_2OH
(143) R = R^1 = CH_3, R^2 = CH_2OH
(144) R = H, R^1 = R^2 = CH_3
(145) R = H, R^1 = CH_2CH_3, R^2 = CH_3



(146)

(147)

Scheme 43

Astepyrone was reported to exhibit antiulcerogenic activity in rat, but also showed considerable toxicity.

3.3 The Leptomycins, Kazusamycins, and Anguinomycins

A group of structurally-related cytotoxic dihydropyran-2-ones, isolated from *Streptomyces* species, are leptomycins A (146) and B (147), kazusamycins A (142) and B (143), and anguinomycins A (144) and B (145).

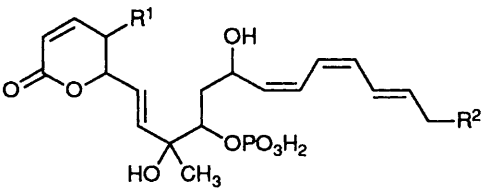
Leptomycin A (146) (also named PD 118,607) was isolated from *Streptomyces* sp.,[228,229] and from another *Streptomyces* species,[230] with leptomycin B (147) as a co-metabolite. The latter compound, which is identical to PD 114,720 and CI-940, has also been isolated from an unidentified Actinomycete.[231,232] Leptomycins A and B differ only in the nature of the alkyl group at C-3′ of the polyene side chain. The kazusamycins are analogous to the leptomycins, but bear a hydroxymethyl substituent at C-11′ of the same side chain. Kazusamycin A (142) (identical to PD 114,721), has been isolated from the same unidentified Actinomycete, as a co-metabolite of leptomycin B,[231,232] and from a *Streptomyces* sp.[233,234] Kazusamycin B (143) (PD 124,895) was also isolated from this latter organism,[233] as well as from another unspecified *Streptomyces* sp.[236] Anguinomycins A (144) and B (145), isolated from a *Streptomyces* sp.,[237] differ from the leptomycins only with respect to the methyl substituent at C-5 of the dihydropyrone ring, this group being absent in the former.

Incorporation studies with labelled precursors ([1-^{13}C]-acetate, [1-^{13}C]propionate, and [1-^{13}C]butyrate) demonstrated that leptomycin A (146) was derived from four acetate units, and eight propionate units, with the distribution of label as shown in Scheme 43. Leptomycin B, however, was derived from four acetate units, and seven propionate units, with C-2′, C-3′, and the C-3′ ethyl substituent being butyrate derived.[238] The biosynthetic origins of the kazusamycins and anguinomycins have not been determined, but are presumably analogous to the leptomycins

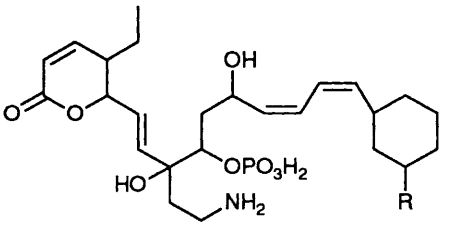
As already mentioned, all these compounds have been shown to be cytotoxic, and as such their biological activity has been explored on a number of occasions. The leptomycins (146 and 147) have been shown to cause hyphal curling of *Mucor racemosus*, *M. rouxianus*, and *Trichophyton mentagrophytes*, and to cause cell elongation of *Schizosaccharomyces pombe*.[229,230] Their activity against other micro-organisms is, however, less well pronounced. They showed weak activity against *Rhizopus* and *Rhodotorula*,[230] whilst leptomycin B (147) was also active against *Alcaligenes viscolactis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *S. pneumoniae*, and *Bacillus cereus*.[232] With these exceptions, most other fungi or yeasts were insensitive, as were Gram-positive and Gram-negative bacteria, *e.g.* species of *Aerobacter*, *Bacillus*, *Mycobacterium*, *Corynebacterium*, *Staphylococcus*, *Pseudomonas*, *Aspergillus*, *Penicillium*, *Paecilomyces*, and *Candida*.[230] Kazusamycin A (142) was active against fungi and some yeasts, but was inactive against Gram-positive and Gram-negative bacteria.[234] The antimicrobial spectrum of kazusamycin B (143) is similar to that of kazusamycin A.[235]

The mode of action of leptomycin B (147) with *Schizosaccharomyces pombe* was examined.[228] Low concentrations caused inhibition of cell division, leading to the production of elongated cells with morphologically altered nuclei. High concentrations of leptomycin B inhibited nucleic acid synthesis. It appeared, from the evidence obtained, that the antibiotic inhibited a specific step, possibly in the M phase, just prior to nuclear division.

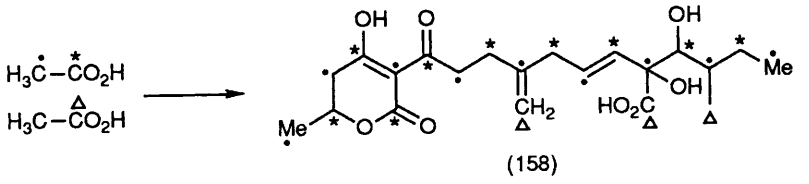
Leptomycin A (146) was highly active against murine B16 melanoma *in vivo*, and showed anticancer activity at very low dosages.[239] Leptomycin B (147) was active *in vitro* against a number of human and mouse tumour lines and, *in vivo*, against murine experimental tumour systems such as P388 leukaemia, L1210 leukaemia ($IC_{50} = 3 \times 10^{-10}$ M), Ridgeway osteogenic and M5076 sarcomas, and mammary adenocarcinoma 16/C.[231,239] Growth of HeLa cells was also inhibited, and the life-span of mice bearing Ehrlich ascite carcinoma, or Lewis lung carcinoma was increased.[240] Kazusamycin A (142) was also strongly active against murine tumours *in vivo*,[234,241] as were the anguinomycins.[237] Anguinomycin B (145) was found to be more potent than anguinomycin A (144).[237]



(148) $R^1 = H$, $R^2 = OH$
(149) $R^1 = R^2 = H$
(150) $R^1 = R^2 = OH$



(151) R = $OC(O)CH(CH_3)_2$
(152) R = H
(153) R = $OC(O)CH_2CH(CH_3)_2$
(154) R = $OC(O)CH_2CH_2CH(CH_3)_2$
(155) R = OC(O)-(cyclohexyl)
(156) R = $OC(O)CH_2CH_2CH(CH_3)CH_2CH_3$
(157) R = $OC(O)CH(CH_3)CH_2CH_3$



(158)

Scheme 44

3.4 The Phoslactomycins and Related Compounds

The phoslactomycins are a group of biologically active dihydropyran-2-ones containing a phosphate ester moiety. A number of structurally related compounds have also been isolated, and these will be discussed together in this section.

CI-920 (PD 110,161) (148), PD 113,270 (149), and PD 113,271 (150) were the first of this group of metabolites to be isolated, in 1983, from *Streptomyces pulveraceus* subsp. *fostreus*.[242] Their structures were determined by a combination of spectral and chemical studies.[243] The phoslactomycins, A (151), B (152), C (153), D (154), E (155), and F (156) were isolated as a complex from culture broth of *Streptomyces nigrescens*.[244] Their structures have been shown to differ only with respect to the nature of the substituent on the cyclohexane ring.[245] Phoslactomycin B (152) is identical to phospholine, which has been isolated from *Streptomyces hygroscopicus*,[246,247] whilst phoslactomycin C (153) is identical to phosphazomycin C_1, isolated from *Streptomyces* sp.[248] Phosphazomycin C_2 (157) was a co-metabolite in this latter organism,[248] as was phosphazomycin A.[249] A structure has not been assigned to this metabolite, although it appears to belong to the phoslactomycin class of antitumour antibiotics.

CI-920 (148) has been shown to exhibit cytotoxic activity against murine P388 lymphocytic and L1210 lymphoid leukaemia, as have PD 113,270 (149) and PD 113,271 (150) with the former being the most active.[242] When CI-920 (148) was administered to mice (25 mg/kg i.p.), bearing approximately 10^7 L1210 leukaemic cells, it was found to be curative in about 10% of the mice, whilst the lifespan of mice that eventually died was increased typically in excess of 150%. The lactone and phosphate moieties were shown to be essential for anti-tumour activity, whereas ring hydroxylation or removal of the terminal hydroxyl group had little effect. CI-920 (148) was inactive when given orally or subcutaneously, and also failed to show activity against murine M5076 sarcoma, B16 melanoma, or Ridgeway osteogenic sarcoma.[250] CI-920 (148), PD 113,270 (149), and PD 113,271 (150) were all devoid of antimicrobial activity when tested against a range of micro-organisms including *Bacillus subtilis*, *Escherichia coli*, *Penicillium avellaneum*, and *Staphylococcus aureus*.[242]

The phoslactomycins (151)–(156) showed strong antifungal activity against a range of organisms, including *Botrytis cinerea*, *Alternaria* sp., *Chaetomium globosum*, *Verticillium albo-atrum*, and *Pseudocercosporella herpotrichoides*.[244] Antibacterial activity was weak although there was a slight inhibitory effect against some Gram-positive bacteria. A comparison of activity between phoslactomycins A to F showed that they had almost the same antimicrobial spectrum, and so it was thought that the substituent on the cyclohexane ring was unimportant for activity.[244] On the other hand, CI-920 (148), although structurally similar, was devoid of antifungal activity, suggesting that the aminomethyl and cyclohexadienyl moieties may be required for expression of antifungal activities of the phoslactomycins. Although the phoslactomycins have not been tested as a group for cytotoxic activity, phoslactomycin B (152) has been shown to be active against L1210, P388, and EL-4 leukaemias.[246]

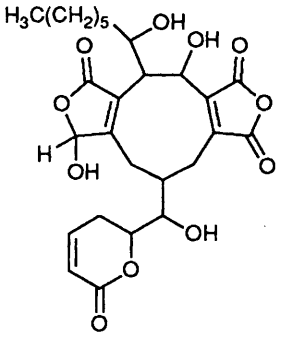
Phosphazomycin C_2 (157), along with phoslactomycin C (phosphazomycin C_1) (153) has been shown to exhibit strong antifungal activity against *Aspergillus* sp., *Trichophyton mentagrophytes*, *Colletotrichum lagenarium*, *Glomerella cingulata*, and *Alternaria mali*, amongst others.[248] Phosphazomycin A has also been shown to inhibit fungal growth, being effective against the above named organisms and against yeasts (*Saccharomyces cerevisiae*, *Candida albicans*).[249] The inhibition of fungal growth by phosphazomycin A was found to be accompanied by swelling of the mycelia, and in *Saccharomyces cerevisiae* β-1,3-glucan synthetase was inhibited at concentrations of 100 μg/ml. Pot tests demonstrated that the metabolite prevented infection by cucumber grey mould disease and cucumber anthracnose at concentrations of 25 ppm. The LD_{50} in mice was 19 mg/kg when administered orally.

3.5 Other Polyketide-derived Dihydropyran-2-ones

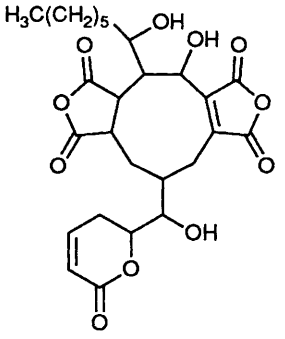
3.5.1 Alternaric Acid

Alternaric acid (158) has been isolated from the plant pathogen *Alternaria solani*,[251,252] and its structure elucidated by a combination of chemical degradation and spectroscopic studies.[253–257]

Biosynthetic studies using [1-^{14}C]- and [2-^{14}C]acetate demonstrated the incorporation of nine acetate units into the molecule and thus established alternaric acid as a polyketide.[258] The remaining carbons were derived from the C_1-pool (Scheme 44). The possibility that alternaric acid may have been



(159)



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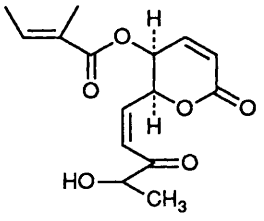

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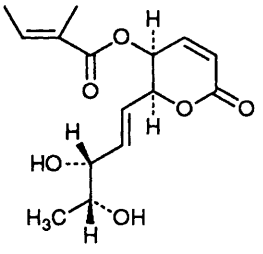


(162)

propionate-derived was precluded, due to the low incorporation of [1-^{14}C]propionate into the molecule.

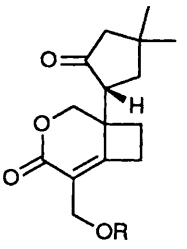
Alternaric acid did not display antibacterial activity, but was shown to inhibit spore germination in a number of fungi (*e.g.*, *Absidia glauca*, *Myrothecium verrucaria*, *Mucor mucedo*, *Thamnidium elegans*) at concentrations of 1 μg/ml or less. Spore germination of other fungi was unaffected. However, at high concentrations (100 μg/ml) the extension of germ-tubes after germination was retarded markedly in *Botrytis allii*, *Aspergillus tamari*, and *Stemphylium* species.[251,252]

3.5.2 The Rubratoxins

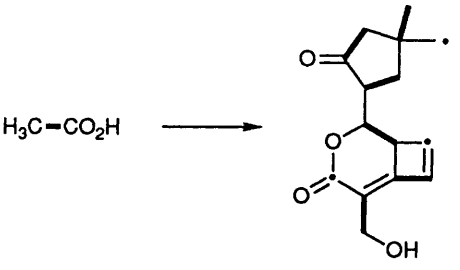
Rubratoxins A (159) and B (160) have been isolated from cultures of *Penicillium rubrum* and *P. purpurogenum*, moulds responsible for diseases induced by infected corn. The isolation, biosynthesis, and biological activity of these metabolites has been reviewed,[259] and so these aspects will only be summarized here. The effect of medium on toxin production has since been studied in *P. rubrum*.[260,261]

Biosynthetic studies using ^{14}C-labelled precursors have established that [1-^{14}C]acetate, [2-^{14}C]malonate, [1-^{14}C]glucose, [1,5-$^{14}C_2$]citrate, [1-^{14}C]hexanoate, and [U-^{14}C]glucose were all incorporated into the rubratoxins, although the position of label has not been established. The incorporation of both acetate and malonate provided evidence for the theory that these metabolites were polyketides.[262,263]

The biological activity of the rubratoxins has been examined in detail.[259] Rats treated with these compounds developed, within a short period, anorexia, diarrhoea, and a porphyrin



(163) R = *p*-$BrC_6H_4CONHCO$
(164) R = (–)-Camphanyl



(165)

Scheme 45

discharge from the eyes, ears, and nose. Post-mortem examination of these animals revealed extensive liver damage, which consisted mainly of massive haemorrhagic necrosis with entire sections of the organ destroyed. The toxicity of the rubratoxins decreased when administered orally, and rubratoxin B (160) displayed greater activity than any of its derivatives. The LD_{50} of rubratoxin B in rats was 0.36 mg/kg (i.p.). Rubratoxin B was also found to exhibit antiprotozoal activity, inhibiting growth of *Tetrahymena pyriformis in vitro* at concentrations of 25 μg/ml.[264]

3.5.3 Phomopsolides A and B

The fungus *Phomopsis oblonga* has been shown to act as a biocontrol agent for the control of *Scolytus scolytus*, the insect vector of Dutch elm disease.[265] Phomopsolides A (161) and B (162) were isolated from this organism,[266] and shown to act as boring/feeding deterrents against adult Scolytid beetles *in vitro*.[267] The structures of these metabolites were assigned on the basis of chemical and spectroscopic studies.

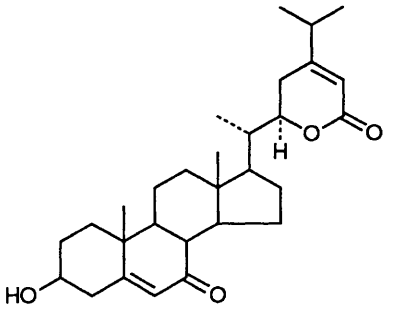
3.6 Non-polyketide Derived Dihydropyran-2-ones

3.6.1 Fomannosin

Fomannosin (165) has been isolated from the wood-rotting fungus *Fomes annosus*, and its structure determined on the basis of chemical and spectral data.[268] An *X*-ray crystal structure of the *p*-bromobenzoylurethane derivative of dihydrofomannosin (163) has been determined,[269] as has that of the camphanate ester (164).[270]

In direct contrast to other fungal pyrones, fomannosin (165) has been shown to be a sesquiterpene, derived from mevalonalactone *via trans,trans*-farnesyl pyrophosphate and humulene. Feeding studies with [1,2-$^{13}C_2$]acetate have demonstrated the incorporation of label to be as shown (Scheme 45).[271,272] The possibility that 1,2-hydride shifts may be occurring in the conversion of humulene to fomannosin was discounted on the basis of evidence obtained from feeding studies with [5,5-$^{2}H_2$]mevalonate.[273]

Fomannosin has been shown to be phytotoxic, affecting seedlings of *Pinus tadea* and *Chlorella pyrenoidosa*, thus



(166)

suggesting a role for this metabolite in diseases caused by *Fomes annosus*. Antibacterial activity has also been demonstrated.[268]

3.6.2 23-Deoxyantheridiol

23-Deoxyantheridiol (166) has been isolated from culture filtrates of the water moulds *Achyla bisexualis* and *A. ambisexualis*.[274]

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