CHAPTER II

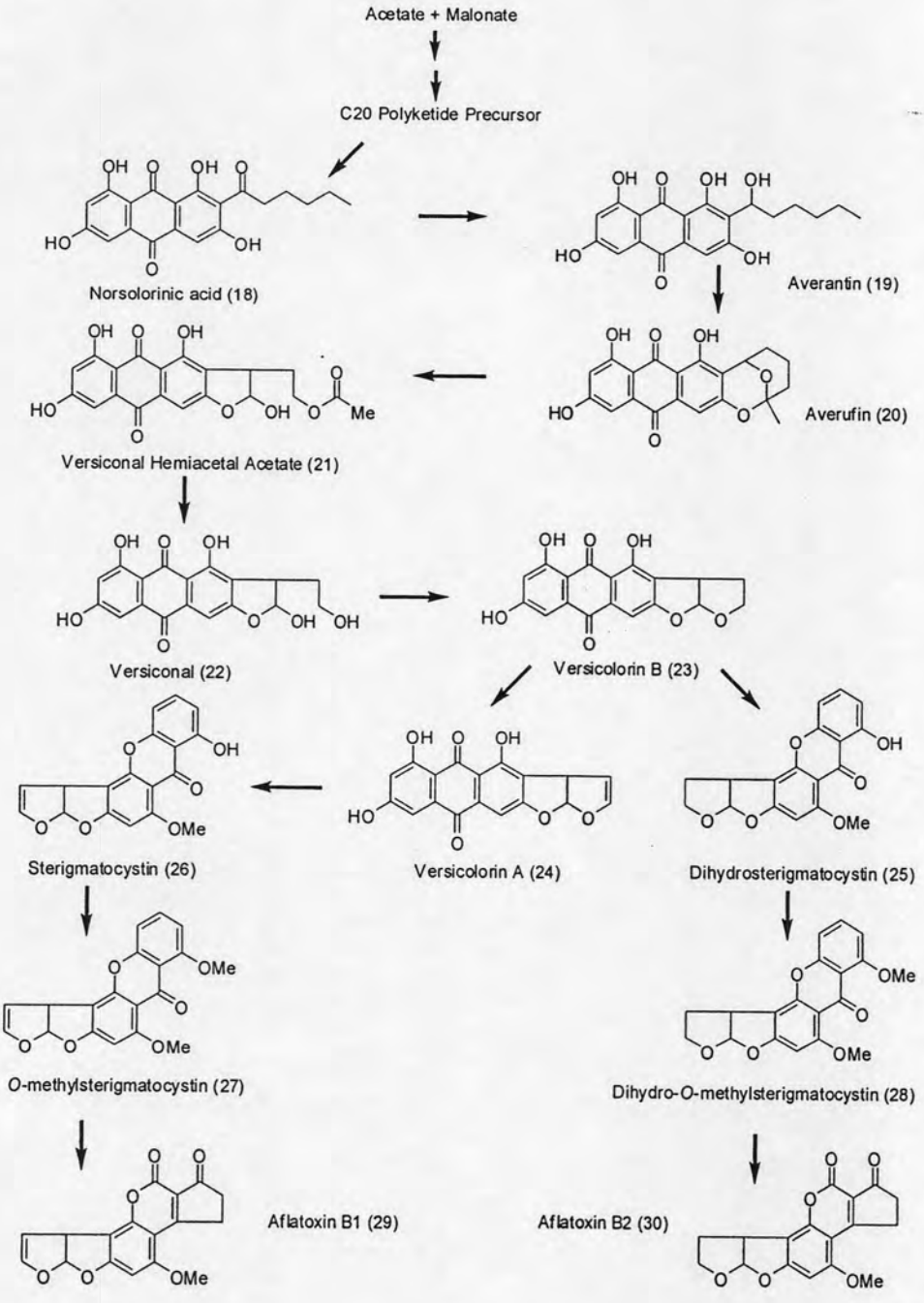
LITERATURE REVIEWS

2.1 Biosynthesis pathway of genus *Aspergillus*

2.1.1 Biosynthesis pathway of Aflatoxin

Even at that time the anamorph genus *Aspergillus* contained species associated with at least eleven telomorph genera within the Eurotiales (Fennell, 1977), as well as species for telomorph connection has been made. The genus *Aspergillus* has provided natural products. The best known secondary metabolites from *Aspergillus* are Aflatoxins which are produced from *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. The alflatoxins are perhaps still the most important mycotoxins in terms of their impact on human health in the tropics and the economy of international trade in food and feeds throughout the world. The most intensively studied group of *Aspergillus* mycotoxins is still the aflatoxins produced by *Aspergillus flavus* Link, *A. parasiticus* Speare and, a more recently described species, *Aspergillus nomius* (Kurtzman et. al., 1987). This family of secondary metabolites has attracted considerable research because of their extreme toxicity, mutagenicity, and carcinogenicity. Aflatoxin biosynthesis is one of the best understood fungal polyketide pathways. The four major naturally occurring are called aflatoxins B1 (29), B2 (30), G1 (31), and G2 (32) based on their characteristic blue or blue-green fluorescence and relative chromatographic mobility. Amount of the four major aflatoxins vary with both the genetic constitution of the strain and with the cultural conditions of fungal growth. Aflatoxins type B are the main congeners produced by *A. flavus* and the most abundant ones produced by *A. parasiticus*. Aflatoxins are formed by head-to-tail condensation of acetyl units to form a cyclised polyketide which is enzymatically altered a series of intermediates (see **Scheme 1**). Mutant blocks have been identified at norsolorinic acid (18), averantin (19), averufin (20), hydroxyversicolorin, versicolorin A (24), and *O*-methyl sterigmatocysin (27). The presense of the esterase inhibitor, dichlorvos leads to the accumulation of versiconal hemiacetal acetate. The overall, accepted scheme for aflatoxin B1 biosynthesis is:

acetate + malonate --> linearised polyketide chain --> anthraquinones (e.g. norsolorinic acid (18), averantin (19), averufin (20), versiconal hemiacetal acetate (21), and versicolorins) --> xanthones sterigmatocystins (26) --> aflatoxin (Hsieh et. al., 1976[a,b]; Lee et. al., 1976; Lin et. al., 1973, Powell et. al., 1994).



Scheme 2.1 Aflatoxin biosynthetic pathway (Powell et. al., 1994).

In Addition, other secondary metabolites produced by different species of *Aspergillus* were reported. A recent reassessment of the toxicological data available for patulin (33) has led to an increased awareness of its occurance and significance in

fresh apple juice. The production of patulin (33) by *Aspergillus clavatus* growing on spent malted barley has been implicated in the poisoning of cattle when this material has been used as a feed additive (Powell et. al., 1994).

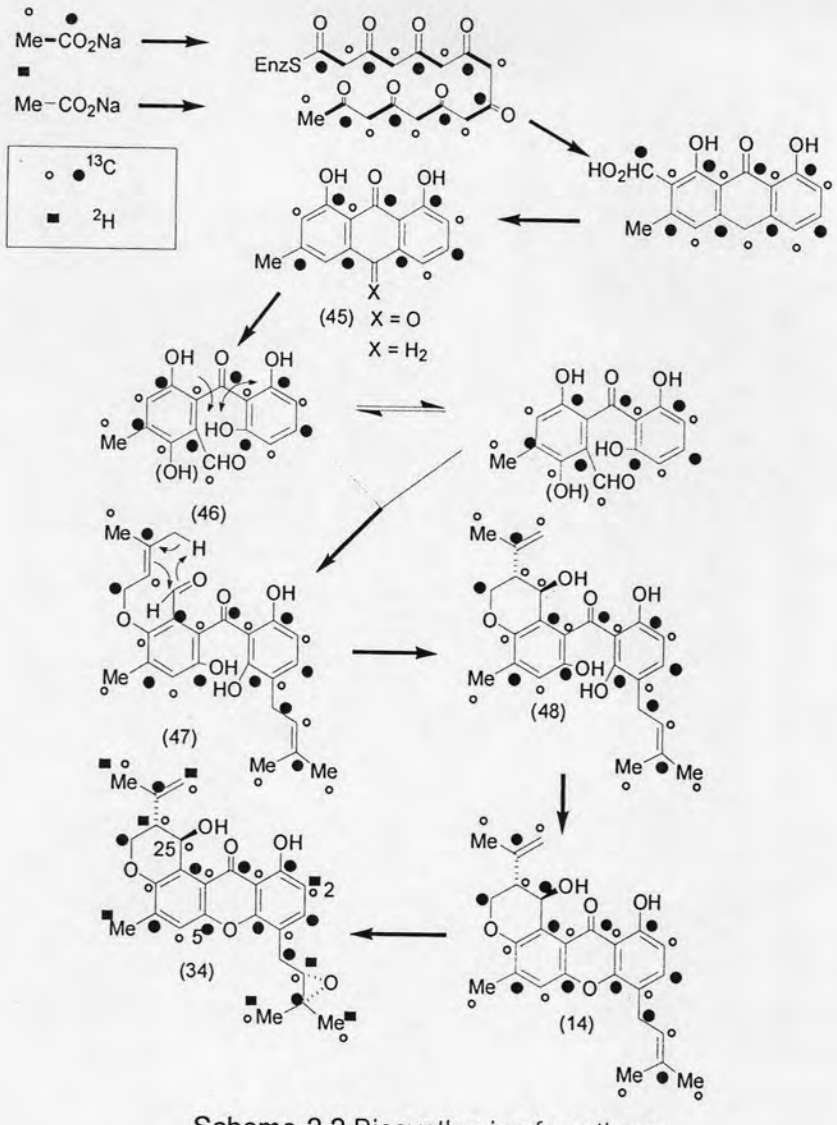
Aspergillus species are widely found in environment. *Aspergillus variecolor* syn. *Aspergillus stellatus*, anamorph of *Emericella variecolor* (Powell et. al., 1994), is one of *Aspergillus* species which produces a variety of natural products. A large number of metabolites in polyketide-terpenoid pathway have been isolated from different strains of *A. variecolor* including terrein (1) (Dunn et. al., 1975; Malmstrom et. al., 2002), 2-methoxy-6-(3,4-dihydroxyhepta-1,5-dienyl)benzyl alcohol (2) (Dunn and Johnestone, 1979; Malmstrom et. al., 2002), bistetrahydrofuran (asteltoxin) (3) (Kruger et. al., 1979; Steyn and Vleggaar, 1984), coumarin (4,7-dimethoxy-5-methylcoumarin) (4) (Chexal et. al., 1975[c]), anthraquinones (5) (Bringmann et. al., 2003), xanthones (Ahmed et. al., 1992, Bringmann et. al., 2003, Chexal et. al., 1975[a], Chexal et. al., 1994) Chexal et. al., 1975[b]; Chexal et. al., 1994; Fujimoto et. al., 2006; Holker et. al., 1974; Kralj et. al., 2006; Malmstrom et. al., 2002; Pornpakakul et. al., 2006) and sesterterpenoids (7) (Bartlett et. al., 1981; Chexal et. al., 1976; Cutler et. al., 1984; Dunn et. al., 1976; Dunn et. al., 1978; Dunn et. al., 1979; Hensens et. al., 1991; Holker and Simpson 1978; Kosemura et. al., 1994; McIntyre et. al., 1982; McIntyre et. al., 1986; Li et. al., 2005; Simpson, 1979; Simpson, 1981; Simpson and Walkinshaw, 1981; Simpson et.al., 1982; Simpson, 1994; Simpson et. al., 1997; Springer et. al., 1979; Wei et. al., 2004). The major groups of the metabolites are xanthones and sesterterpenoids.

2.1.2 Biosynthetic pathway of xanthone

The biosynthsis of xanthones was first investigated by the incorporation of [1-^{13}C] and [2-^{13}C]-acetates into tajixanthone(Ahmed et. al., 1992; Chexal et. al., 1994[a,b,c]). Structure (6) and (14) respectively was proposed for these two metabolites (Ahmed et., 1992). However, a subsequent detailed examination of their spectral and chemical characteristics resulted in reassignment of the original structures (34) and (14) (Chexal et. al., 1994). The similarities between these new structures and those of a group of metabolites first obtained from *Aspergillus rugulosus*, the arugosins A (35), B (36), and

C (37), were apparent, and the subsequent isolation of arugosins A and B from *A. variecolor* provided further circumstantial evidence that this group of metabolites was biogenetically related to tajixanthone and shamixanthone (Chexal et. al., 1975[b]). The same strain of *A. variecolor* also produced three new compounds of related structure, the variecoxanthones A, B and C, namely (38), (39) and (40) respectively, and the seven closely related minor metabolites (Chexal et. al., 1975[a]). Variecoxanthone B has been isolated as emericellin (41) from *Emericella dentate* and shamixanthone from *A.rugulosus*, arugosin E (42) along with arugosin A (35), B (36) and C (37) and the toxic metabolite silvaticamide (43) from *Aspergillus silvaticus* and cycloisomericellin (44) from *Emericella striata* (Ahmed et. al., 1992).

The biosynthesis of the *A. variecolor* xanthones was first investigated by the incorporatation of [1-^{13}C]- and [2-^{13}C]-acetate into tajixanthone (6). (Holker et. al., 1974) Assignment of the ^{13}C NMR spectrum was accomplished by comparison with the spectra of the several derived compounds, by off-resonance proton decoupling, and by lanthanide-induced shift studies. A labeling pattern was revealed in **Scheme 2.2** that was consistent with the derivation of the xanthone nucleus from an octaketide anthrone or anthraquinone precursor, e.g. chrysophanol (45) with the introduction of two additional C5 (prenyl) unit of mevalonate origin. From the results and analysis of the structure of the known cometabolites, it was suggested that the benzophenone (47), present in its two hemiaetal forms as arugosins A (35) and B (36), could be an intermediate in the biosynthesis of tajixanthone, being formed through oxidative ring cleavage of a suitably substituted anthraquinone. Subsequent cyclodehydration and intramolecular 'ene' cyclization of the *ortho*-prenyloxy aldehyde moiety would provide the xanthone nucleus and the substituted dihydropyran ring respectively (Ahmed et., al., 1992; Holker et., 1974).

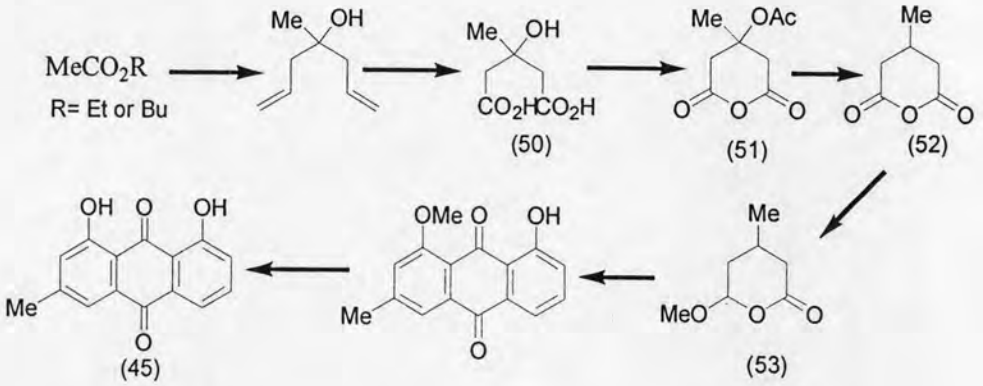


Scheme 2.2 Biosynthesis of xanthone

The structure of tajixanthone is consistent with a derivation through ring cleavage of isolandicin (49), itself presumably formed by hydroxylation of chrysophanol (45). In order to provide definitive evidence for this, the synthesis of these anthraquinones in isotopically labeled form was required. The synthesis of chrysophanol (45) in high yield by Diels-Alder reaction of 6-methoxy-4-methylpyran-2-one (53) with 5-hydroxy-1,4-naphthaquinone has been described by Jung and Lowe. This route as described is not amenable to ready incorporation of isotopic a recently described synthesis of mevalonic acid lactone suggested a different route to the pyrone, a route which would be suitable for introduction of isotopic label. In this synthesis, ethyl acetate was converted by Grignard reaction with allylmagnesium bromide into 3-hydroxy-3-methylglutaric acid (50). Treatment of (50) with acethyl chloride gave 3-acethyl-3-

methylglutaric anhydride (51) which on pyrolysis was converted into 3-methylglutaconic acid anhydride (52). Reaction with diazomethane furnished the required pyrone (53) (Ahmed et. al., 1992).

Isotopically labeled acetate is readily available in a variety of labeled forms as the sodium salt. This can be converted into the ethyl ester in high yield by treatment with an excess of triethyl phosphate. Separation of the ethyl acetate from the triethyl phosphate requires careful fractional distillation. However, by using the higher boiling butyl phosphate, butyl acetate could be more readily obtained in pure form. This gave slightly improved yields in the Grignard reaction leading to the glutaric acid (50). The sequences of reactions leading to labeled chrysophanol are summarized in **Scheme 2.3**. In this way both [methyl-$^{2}H_3$]- and [3-^{14}C]-chrysophanol have been prepared from [2-$^{2}H_3$]- and [1-^{14}C]-acetate respectively. (Ahmed et. al., 1992; Holker et. al., 1974)

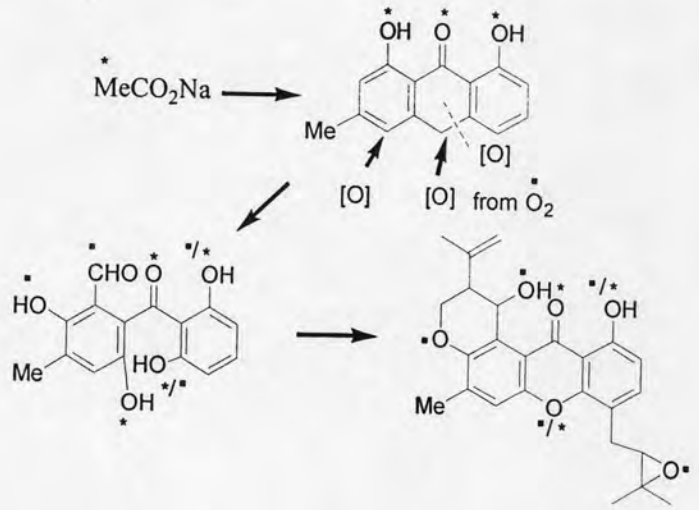


Scheme 2.3 Biosynthesis of Chrysophanol

Biosynthetic pathway of tajixanthone on the biosynthesis of chrysophanol was provided by the results of incorporation of [1,2-$^{13}C_2$]- and [2,2,2-$^{2}H_3$]-acetate into tajixanthone by cultures of *A. variecolor*. The acetate assembly pattern in the xanthone system is entirely consistent with an octaketide precursor fold as shown in **Scheme 2.1**. Decarboxylation of the octaketide precursor occurs after cyclization and aromatization since redeterminating the ^{2}H nmr spectrum of tajixanthone in methylene chloride shows that there is no ^{2}H present on C5, the carbon to which the terminal carboxyl of the octaketide precursor was attached (Chexal et. al., 1994). This contrasts with results

reported by Franck on anthraquinone biosynthesis in *Penicillium isolandicum* in which 14C-labelled diketo naphthol (**54**) was fed to surface cultures to yield radioactive isolandicin (**49**) and skyrin. No degradations to confirm specific incorporation were reported. However, when [2-^{13}C,2,2,2-^{2}H3]acetate was incorporated into rugulosin (**55**) in cultures of *Penicillium brunneum*, deuterium was shown to be present at C1, C3, C8 and the 7-methyl, but not at C6, again consistent with the necessary decarboxylation of the octaketide precursor after formation of the aromatic rings. Chrysophanol is formed by deoxygenenation of emodin (**56**) in *Pyrenochaeta terrestris* presumably through a reduction-elimination process on the keto tautomer of the resorcinol ring of emodin. This result is interesting in light of the now generally accepted ideas that such deoxygenation process in polyketide biosynthesis normally takes place during chain assembly. However, it is clear from this and other work that some deoxygenerations must occur at a post-aromatization stage. Although no further evidence is available at this stage, it would seem likely that the sequence in tajixanthone biosynthesis proceeds via emodin to chrysophanol followed by hydroxylation to give isolandicin (**49**). No deuterium is present on C25, a result which is consistent with the necessary oxidative cleavage occurring on an anthraquinone rather than an anthrone intermediate. C-Prenylation and epoxidation occur with retention of configulation about the double bond of dimethylallyl pyrophosphate. In addition, the lack of randomization of 13C-13C coupling between C22 and C23 indicates that formation of the dihydropyran ring is a stereospecific process. The randomization of 13C-labelling in ring c shows that this ring has been symmetrical and free to rotate around the bond to the carbonyl group at some stage in the biosynthesis. This type of randomization, indicative of the involvement of symmetrical intermediates in biosynthetic pathways, was first observed in similar studies on the biosynthesis of the xanthone revenelin (**57**) in *Helminthosporium ravenelii* (Ahmed et. al., 1992). The observation of a symmetrical intermediate in the case of tajixanthone biosynthesis indicates that the ring cleavage of the carbocyclic precursor must procede the introduction of the C-prenyl residue. Additional evidence for this also comes from the isolation of variecoxanthone A (**38**) in which xanthone formation but no C-prenylation has occurred (Chexal et. al., 1975[a]).

Xanthone ring formation from the symmetrical intermediate **(46)** or **(47)** can occur in two distinct ways: either by nucleophilic attack of the ring-C hydroxyl group on ring A or vice versa. A plausible mechanism for this ring closure would be through a Michael addition-elimination process, followed by loss of hydroxyl as indicated in **Scheme 2.4**. Such reactions are known to occur readily. The alternative pathways can be distinguished by determinating the origin of the xanthone ether oxygen. As shown in **Scheme 2.4**, if ring closure occurred by addition of the ring-A hydroxyl of xanthone ether oxygen would necessarily be derived from acetate. If the alternative ring closure mode operates then the ether oxygen would be derived equally from acetate and from the atmosphere (Ahmed et. al., 1992; Chexal et. al., 1994).



Scheme 2.4 Michael addition-elimination process

In order to obtain more information on this and other aspects of the biosynthetic pathway, the origins of the oxygen atoms in tajixanthone and shamixanthone were investigated by using the technique of ^{18}O-induced isotope shifts in ^{13}C nmr spectra. This metod has already been applied to the xanthones revenelin **(57)** and sterigmatocystin **(26)**. The incorporation of [1-^{13}C, $^{18}O_2$]acetate was investigated in order to determine which oxygen were acetate-derived. However, this met with only limited success. Despite extensive studies to optimize the incorporation of acetate, only low levels of incorporation were achieved. The incorporatation of label from $^{18}O_2$ gas was more successful. *Aspergillus variecolor* was grown as a surface culture by using

apparatus which allowed the circulation of a controlled atmosphere and measurement of consumption of oxygen.

The equal amounts of ^{18}O-labelling at C-1 and C-10 also indicate that on formation of the xanthone nucleus from a benzophenone, e.g. **(46)**, **(47)** or **(48)**, it is the hydroxyl oxygen of ring C, rather than that of ring A, which forms the ether linkage. Cyclization in the reverse manner, whereby the ring-A hydroxyl diplaces the one on ring C, cannot be entirely ruled out because of the experimental error in the intensity measurements, but it can be no more than a minor route.

^{18}O isotope shifts for C1 and C10, although not C11, would have been expected in the experiment with [1-^{13}C,$^{18}O_2$]acetate. That a shift was only seen for C13 probably reflects the reduced intensity of the other signals (assuming roughly equal loss by exchange throughout the molecule) and their smaller shifts, especially in the case of C1.

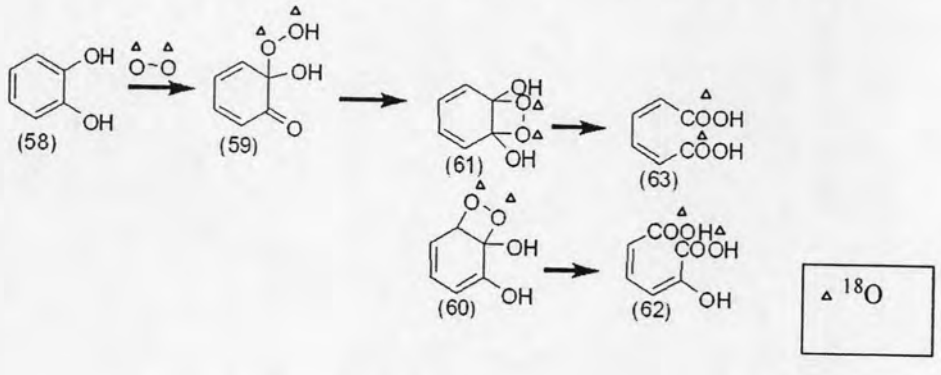
Examination of the ^{13}C spectrum of shamixanthone **(14)** labeled from $^{18}O_2$ confirmed that the same situation prevailed. In this case it was not possible to fully resolve the isotopic shift for C1, and so an accurate assessment of the ^{16}O:^{18}O ratio was not feasible, but the abundance of ^{18}O between C10 and C11 was again about half of that at the other labeled sites in the molecule.

Overall, these results are consistent with similar observations on the related metabolites ravenelins **(57)**. Observations of ^{18}O isotope shifts of ravenelin derived from [1-13C, $^{18}O_2$] acetate showed that the xanthone ring formation occurred with retention of the acetate-derived ^{18}O label of the ring-C hydroxyl. Ravenelin also shows scrambling of the carbon-labelling of the ring and must be considered a good biosynthetic parallel to tajixanthone and shamixanthone.

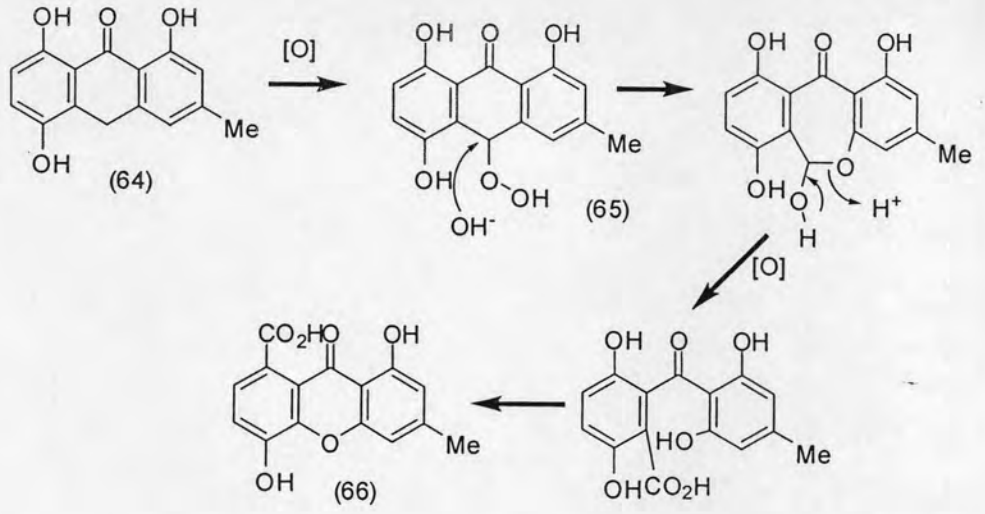
The same Michael addition-elimination mechanism was proposed for the formation of the xanthone ring of sterigmatocystin **(26)** from a suggested benzophenone intermediate containing a symmetrically substituted ring, but, as no scrumbling of ^{13}C

label is observed in this ring, other mechanisms avoiding such symmetry have since been suggested.

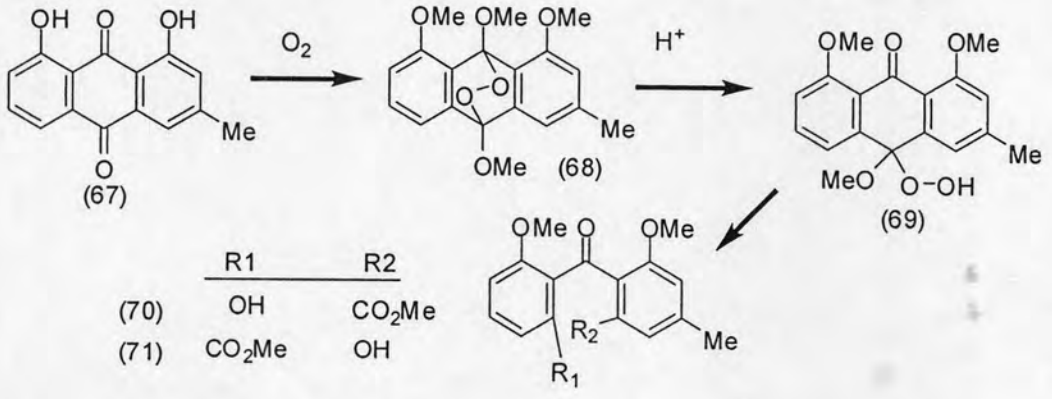
Anthrone and anthraquinone intermediates are widely, although not exclusively, implicated in the biogenesis of fungal xanthone. However, the required fission of the central ring is poorlyunderstand. Biomimetic studies have been widely employed in the general investigation of the ring cleavage of aromatic compounds. Enzymic oxidations of catechol (58) to muconic acid (63) and an isomeric aldehyde (62) are known to result in the incorporation of the two atoms of a labeled oxygen molecule into the carbonyl groups of the products, and this has been rationalized by invoking hydroperoxide (59) and/or dioxetan, (60) and (61), intermediates produced by the action of a dioxygenase enzyme (Scheme 2.5). Following the early suggestion that a hydroperoxide (65) could be implicated in a proposed biosynthesis of the xanthone pinselic acid (66) from helminthosporin anthrone (64) (Scheme 2.5), biomimetic experiments on the ergochromes reslted in the preparation of an endoperoxide (68) from an anthraquinone (67) by using singlet oxygen. An acid-catalysed rearrangement subsequently gave the secoanthraquinones (70) and (71) via the hydroperoxide (69) (Scheme 2.7).



Scheme 2.5 Anthrone and anthraquinone intermediates



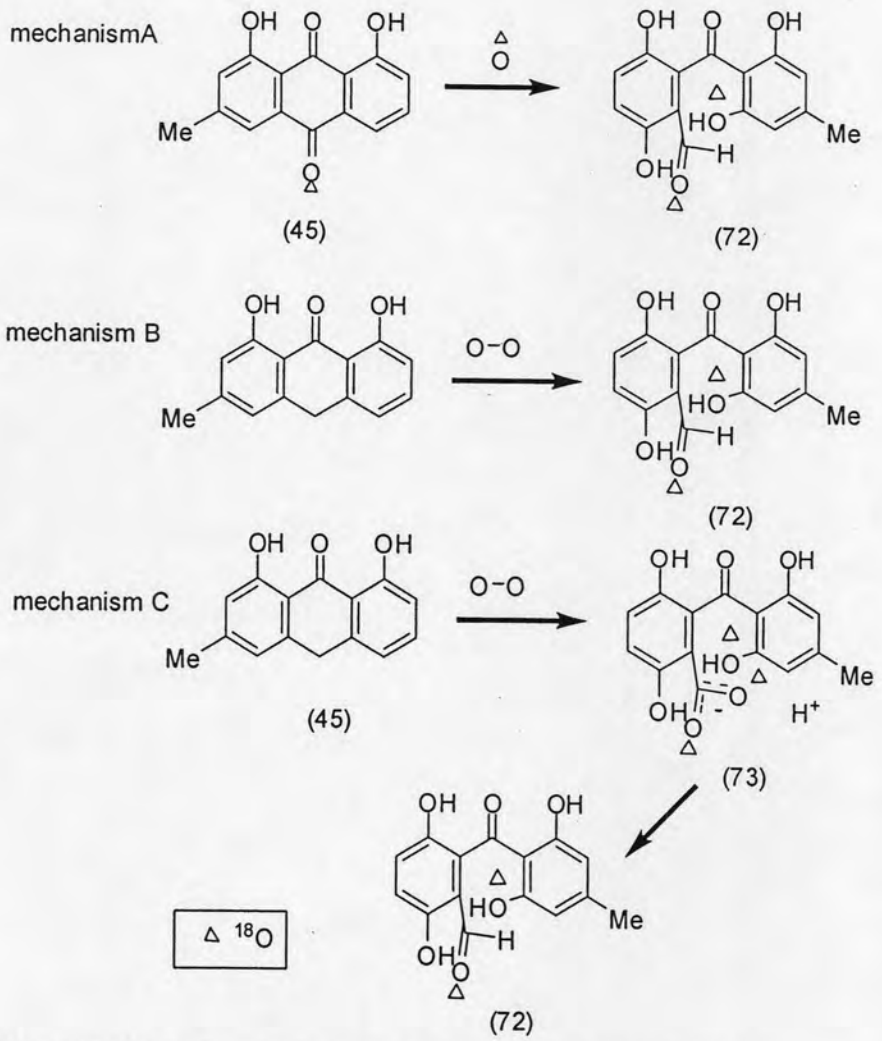
Scheme 2.6 Ergochrome biosynthesis.



Scheme 2.7 An acid-catalysed rearrangement of anthraquinone.

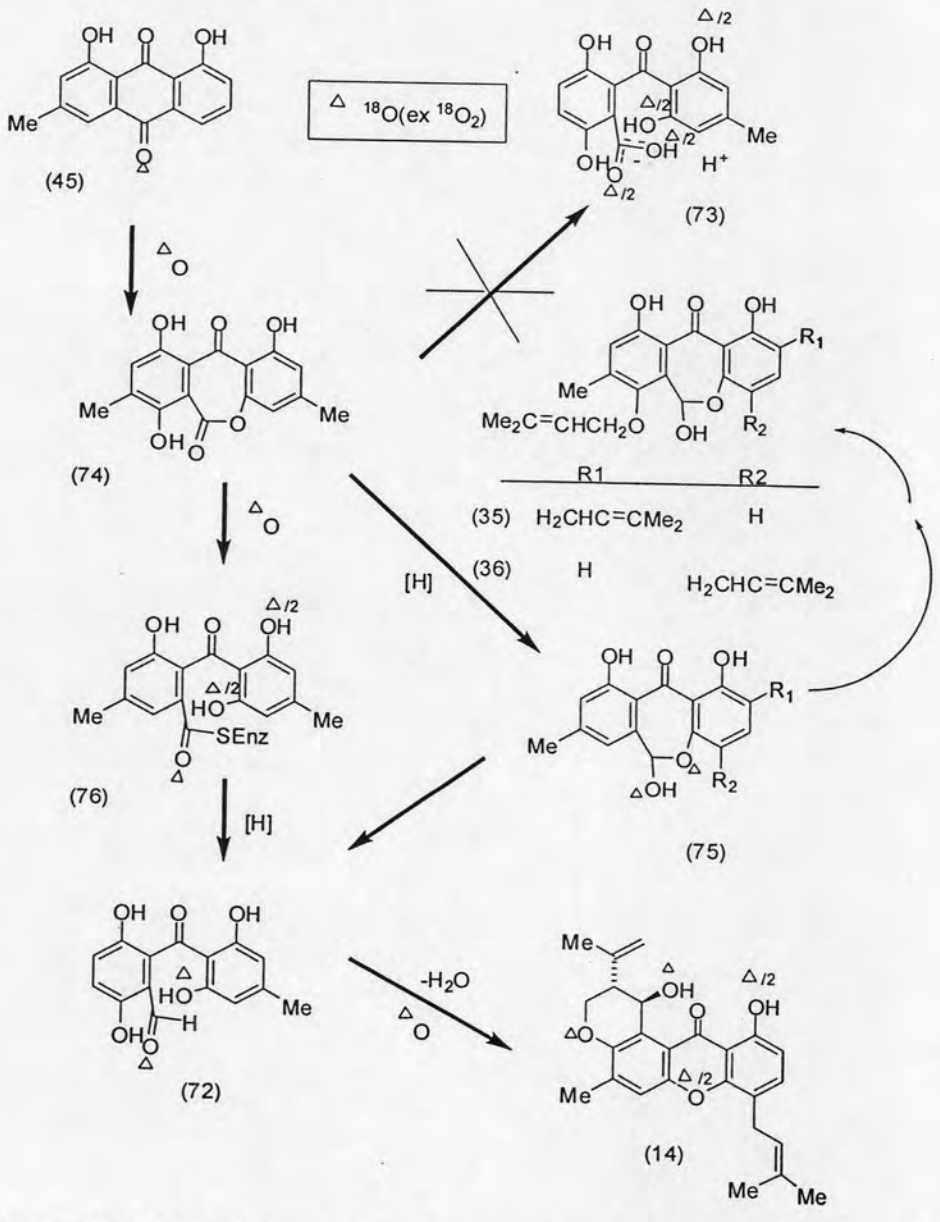
Ergochrome biosynthesis would appear to be closely related to that of tajixanthone (6) and shamixanthone (14). As both of the oxygen atoms introduced in the course of the cleavage of the anthraquinone (45) or corresponding anthrone precursor are, according to the experiment earlier describe, wholly derived from atmospheric oxygen, a mechanism as in Scheme 2.6, where an anthrone hydroperoxide (65) undergoes nucleophilic attack by hydroxide ion is ruled out. Thus, if such an endoperoxide or dioxetan type mechanism is operative in tajixanthone biosynthesis, both of the oxygen atoms finally incorporated might come from the same molecule of atmospheric oxygen (Chexal et. al., 1994).

A. priori, three possible mechanisms, summarized in **Scheme 2.8**, need to be formally considered. These are: A, ring cleavage of an anthraquinone by a monooxygenase; B, a dioxygenase acting on an anthrone (or anthranol); C, a dioxygenase acting on an anthraquinone. The biosynthsis mechanism of tajixanthone **(6)** and shamixanthone **(14)** explained by a biological Baeyer-Villiger type oxidation operating of the corresponding anthrone **(Scheme 2.9)**. The resulting lactone **(74)** would be relatively immune to exchange and, as a possible intermediate, has the additional attraction that direct reduction would give the hemiacetal structure **(75)** found in arugosins A **(35)** and B **(36)**. Direct formation of the putative aldehyde intermediate **(72)** could follow, avoiding the loss of label inevitable if hydrolysis of the lactone **(74)** to the benzoic acid **(73)** was an intermediate step **(Scheme 2.8)**. Benzaldehydes are also less susceptible could proceed without significant loss of ^{18}O label at C25 (Ahmed et. al., 1992).

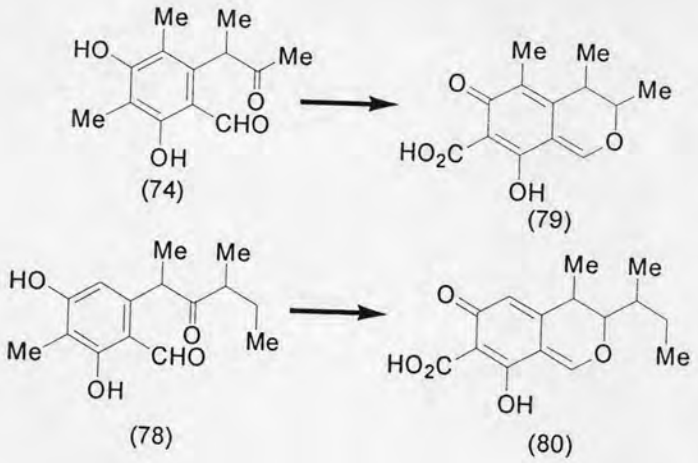


Scheme 2.8 Ring cleavage mechanism of an anthraquinone.

Scheme 2.9 is an alternative viable pathway whereby the lactone (74) givesthe thioester (76) by tranesterification. Reductive cleavage could then give the aldehyde (72). Again, simple hydrolysis of the lactone or of the thioester is precluded by the retention of ^{18}O label at C25. The direct formation of an aldehyde species by reductive cleavage of a corresponding enzyme-bound thioester, rather than by a hydrolysis-reduction sequence, has been previously suggested. On the basis of incorporation experiments with possible advanced intermediates, the aldehydes (77) and (78) were implicated as the first enzyme-free species in the biosyntheses of citrinin (79) and ascochitine (80) (see Scheme 2.10) (Ahmed et. al., 1992).



Scheme 2.9 Biological Baeyer-Villiger type oxidation of xanthone biosynthesis.

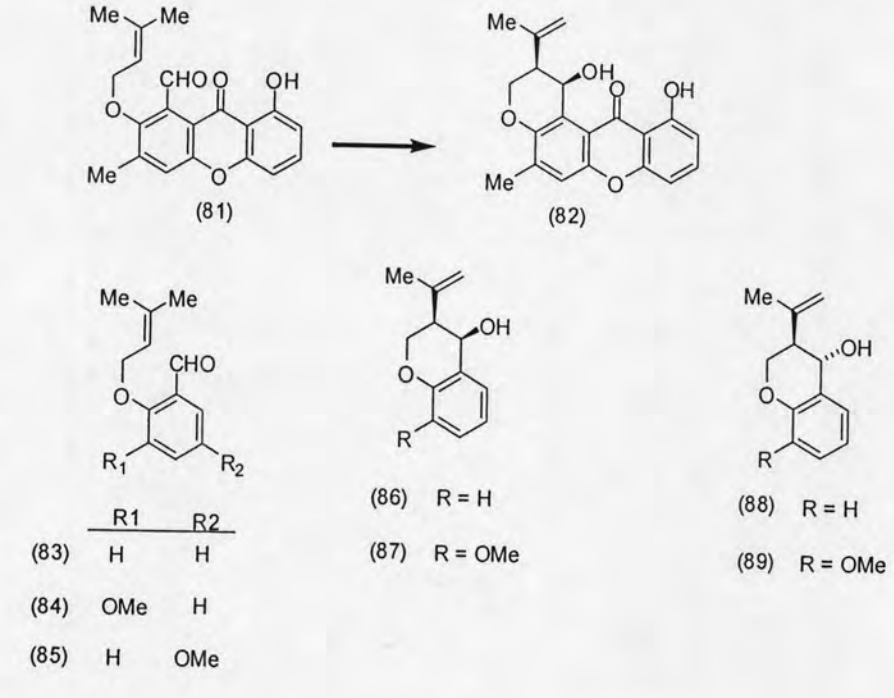


Scheme 2.10 Enzyme-free species in the biosyntheses of citrinin and ascochitine.

Comparison of the cometabolites of tajixanthone suggests that C-prenylation follows the ring cleavage to give the benzophenone **(47)** present in its two hemiacetal forms as arugosin A **(35)** and B **(36)**. The incorporation studies described above required determination of the optimum timefor precursor addition. For this, the time course of the production of tajixanthone and its cometabolites was studied by extraction of the mycelium at regular intervals. Formation of tajixanthone then requires cyclodehydration to form the xanthone nucleus and dihydropyran ring formation through an intramolecular ene reaction of the O-prenyloxy aldehyde moiety. Whereas the isolation of the variecoxanthones might suggest that xanthone formation precedes dihydropyran ring formation, the isolation of arugosin C supports the order of these processes being reverses. Interestingly the benzaldehyde **(81)** obtained by the oxidation of variecoxanthone A **(38)** could be cyclized (see **Scheme 2.11**) by very mild acid treatment (e.g. $CDCl_3$ solution) to give (±) *des*-C-prenylepishamixanthone **(82)** in which the dihydropyran substitutents are in a *cis* relationship in contrast to the trans relationship in shamixanthone and tajixanthone. The stereospecific nature of this conversion was retionalized by postulating a synchronous ene reaction, and consideration of two possible transition states led to the conclusion that the formation of the observed product was favoured due to a electrostatic interaction between the xanyhone and aldehyde carbonyl groups in the transition state required for the trans product. This suggested that in the nucleus, allowing the benzophenone carbonyl to

avoid the aldehyde function by totation out of the plane of the aromatic system (Ahmed et.al., 1992; Chexal et.al., 1975[b]).

In order to obtain more evidence for the timing of the hydropyran ring formation, aseries of model compounds was synthesized, and the ring closure process was examined in vitro. The three model compounds *O*-dimethylallylsalicylaldehyde (83) and the corresponding 3-methoxy- and 5-methoxy-salicylaldehyde (84) and (85) were prepared by alkylation of the corresponding phenols with 1-bromo-3methylbut-2-ene. Attempted cyclization by using a trace of hydrochloric acid in chloroform, which effected cyclization of (81), resulted in Claisen rearrangement products only. However, on treatment with *p*-toluenesulfonic acid in benzene the desired cyclization, but (83) gave the products (86) and (88) in equal amount albeit in very low overall yields. Compound (84) on the other hand gave the cycliation products (87) and (89) again in equal amounts in high yield. The increased yield in this case may be due to buttressing effect of the methoxyl ortho to the *O*-prenyl residue. The fact that the formation of the dihydropyran ring in the variecoxanthone derivative (81) occurs readily under mild conditions (Ahmed et. al., 1992).



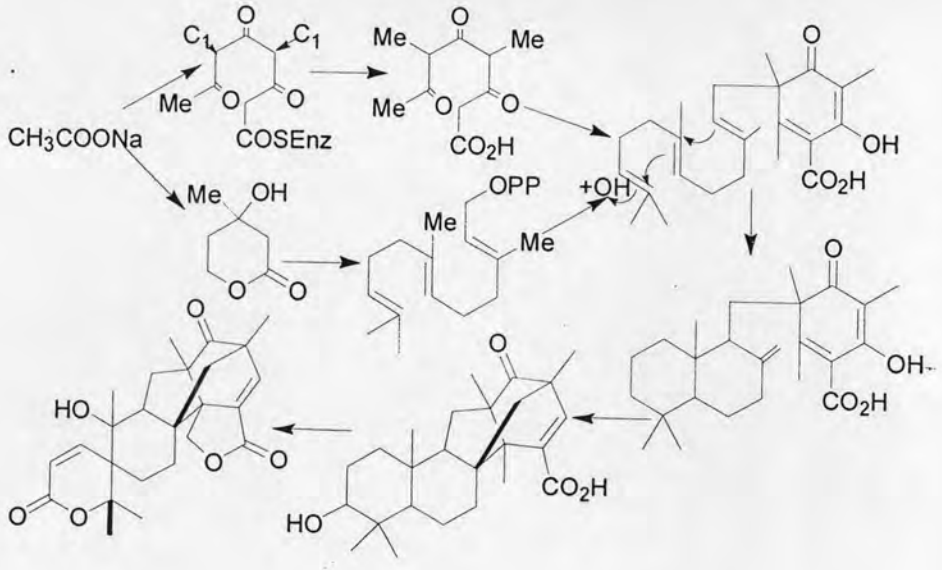
Scheme 2.11 Cyclohydration and dihydropyran ring formation

2.1.3 The biosynthesis pathway of sesterterpoids.

Sesterterpenoids metabolites of *A. variecolor* can be divided into two groups on the biosynthetic pathway. First group of the sesterterpenoids biosynthesis pathway, andibenin B **(90)** and the related metabolitesare formed via a mixed polyketide-terpenoid pathway and are not sesterterpenoid origin and the second group is form via cyclisation and rearrangement of geranylfarnesyl pyrophosphate.

2.1.3.1 The first group of sesterterpenoids biosynthetic pathways of metabolite of *Aspergillus* sp.

Andibenin B **(90)**, two related metabolites, andilesin A **(94)** and andilesin B **(93)** were established the skeletal differences between them and andibenin B (Simpson et. al., 1979). Four structural relationships were andibenin A **(91)** and C **(92)** (Simpson et. al., 1979), andilesin C **(95)** (Simpson et. al., 1979) and anditomin **(96)** (Simpson et. al., 1981; Simpson and Walkinshaw, 1981). The 13C-labelled acetate pattern made the proposed sesterterpenoid origin of andibenin B **(90)** untenable. A subsequently feeding experiment with [methyl-^{13}C]methionine resulted in high enrichment of both 9' and 10' methyls, confirming their origin from the C_1-pool. The labelling pattern shown in **Scheme 12** suggested a mixed polyketide-terpenoid origin in which alkylation of a bis-*C*-methylated tetraketide derived phenol **(97)** with farnesyl pyrophosphate would give cyclohexadienone **(98)**, which after epoxide initiated cyclisation to the triene **(99)** could undergo an intramolecular 4+2 cycloaddition to generate the required carbon skeleton. Subsequent oxidative modifications and elaboration of the spiro-lactone ring system would convert **(100)** to andibenin B **(90)** (Holker and Simpson, 1978).



Scheme 2.12 polyketide-terpenoid pathway of sesterterpenoid.

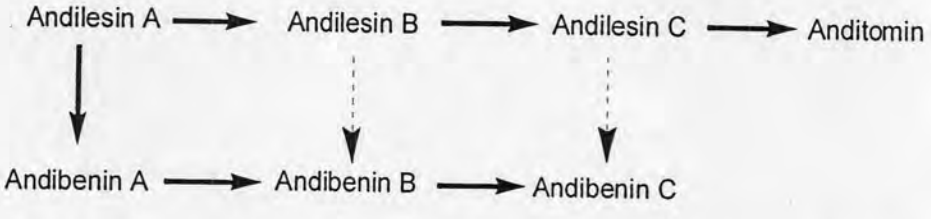
Andibenin B (90) and related metabolites could be as triprenyl-phenols or meroterpenoids. The secondary metabolites of mixed, partially terpenoid were described by biogenesis. Triprenyl-phenols have been isolated from a variety of micro-organisms and marine sources. Simple examples include grifolin (101), in which the farnesyl side chain is unaltered, and siccanin (102), in which it has undergone cyclisation and modification. More examples include the austalides, e.g. (103) from *Aspergillus ustus*, where the sesterterpenoid portion of the molecules has undergone extensive oxidative metabolism, analogous to that observed in andilesin A (94). Another example is mycophenolic acid (104), a metabolite of *Penicillium brevicompactum*, the aromatic nucleus in this case being 5,7-dihydroxy-4-methylphthalide (105). In classic experiments, Birch demonstrated that the side chain was derived from cleavage of a terpenoid moiety. Originally it was assumed to be a geranyl chain, but it was subsequently shown that the corresponding farnesylphthalide is an intermediate in the biosynthesis. Interestingly, studies of the metabolites isolated from a number of a strain of *A. stellatus* have resulted in the isolation of highly oxygenated sesterterpenoid metabolites, and astelloides A (106) and B (107) and stellatin (108) which has been shown, like 3,5-dimethylorsellinate (97), to be derived from a bis-C-methylated tetraketide, a albeit with different sites of methylation (Simpon et. al., 1997).

Prior to the preliminary studies on andibenin B, there were no established examples of biosynthetic Diels-Alder reactions. However, it has now been shown quite clearly that the biosynthesis of the phytotoxin, solanapyrone **(109)** involves a cycloaddition reaction and isolation of a corresponding cell-free activity from *Alternaria solani* has been reported. The degree of skeletal modification required to form the meroterpenoids **(90)-(96)** is unprecedented and so further evidence was sought to substantiate the proposed pathway (Simpson et. al., 1997).

Inspection of the structure of the andibenins and andilesins suggests that the orsellinate **(110)**, 3,5-dimethylorsellinate **(97)** or the corresponding 6-deoxy-compound **(111)** or **(112)** could be the key phenolic intermediate which undergoes alkylation (Simpson et. al., 1979). These compounds were synthsised in ^{14}C labeled from as their ethyl esters and fed to *A. variecolor*. In order to establish the intact incorporation of these phenolic precursors, 3,5-dimethylorsellinate **(113)** was synthesized with the the 5-methyl specifically labeled with deuterium and fed to *A. variecolor*. As the unmethylated compounds **(110)** and **(111)** did not label andibenin B to any significant extent, this confirms that the methionine derived methyl groups must be incorporated into the tetraketide precursor befors aromatization to the phenol. This lends further support to the view that *C*-methylation occur before aromatization and probably concomitant with polyketide chain assembly, whereas prenylation, despite being a similar electrophilic process, occur after aromatization (Holker et. al., 1974; Simpson et. al., 1997).

The relatively efficient incorporation of isotopic label from the deoxy-compound **(112)** as well as the orsellinate **(97)** was surprising. To obtain more information on its role and the mechanisms of formation of the γ-,δ- and ε-lactone systems in the andibenins and andilesins, the origins of the oxygen atoms were investigated. Sodium [1-^{13}C,$^{18}O_2$]acetate was incorporated into andibenin B and andibesin A. No labeling of C-6' was observed in complimentary experiments in which *A. variecolor* was grown under an $^{18}O_2$ atmosphere so the origin of the 6'-hydroxyl remained obscure. This ambiguity was resolved by the synthesis and incorporation of the ethyl 3,5-dimethylorsellinate **(114)** doubly labeled with ^{13}C and ^{18}O at the ester carbonyl group and C-6. Interestingly, the

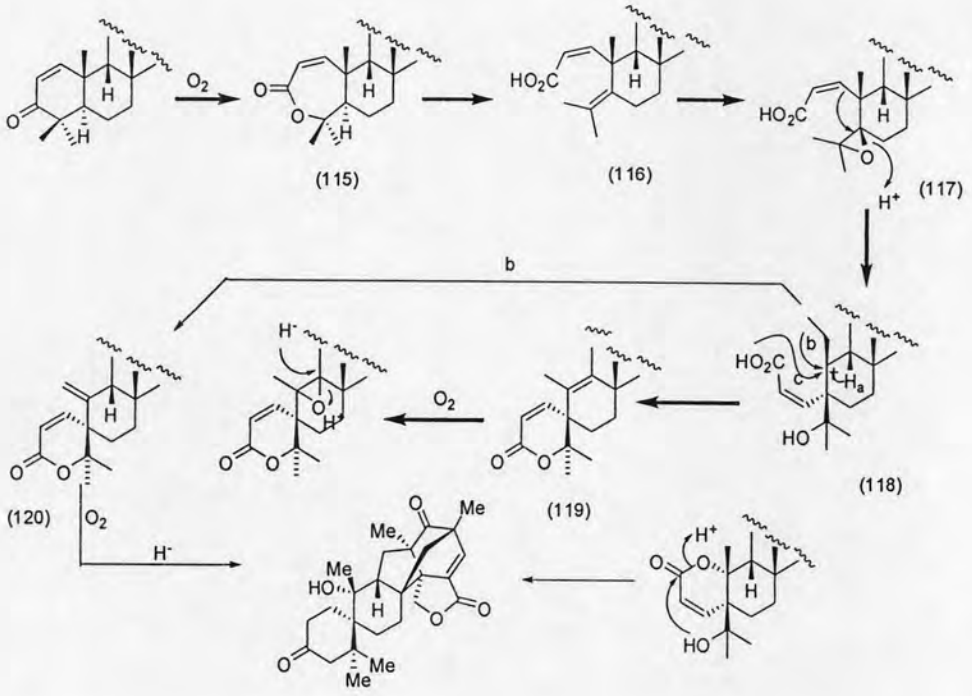
amount of ^{18}O label incorporated at C-8' is approximately half that present at C-6'. Similar results are found for the acetate incorporation experiments which show that the amount of label at C-8' is approximately half that at C-4' in both andibenin and andilesin A. These results suggest that the γ-lactone is formed by attack of a hydroxyl group on C-1' on to C-8' which has existed as a free carboxylate at some stage during biosynthesis. In agreement with this, the ether oxygen of the γ-lactone is derived from atmospheric oxygen. In mycophenolic biosynthesis, it has been clearly demonstrated that the phthalide **(105)** is the substrate for alkylation with farnesyl pyrophosphate, so γ-lactone formation may precede addition of the farnesyl moiety. The derivation of the 6'-hydroxy group from orsellinate **(114)** confirms its role as an obligatory intermediate to andilesin A. The observed incorporation of ^{14}C label from deoxyorsellinate **(112)** is thus probably via prior degradation to acetyl CoA. The lack of labelling of the 6'-hydroxyl by atmospheric oxygen rules out the possibility of deoxyorsellinate **(112)** to orsellinate **(97)**. The results therefore indicate a pathway in which andilesin A **(94)** is the first metabolite to be formed and that it is converted by elimination of water into andilesin B, **(93)** which is then reduced to andilesin C **(95)**. A similar biosynthetic interrelationship can be assumed for andibenins A, B and C **(Scheme13)** (Simpson et. al., 1997).



Scheme 2.13 Biosynthetic pathway interrelationships among the *A. variecolor* meroterpenoids.

The presence of atmosphere oxygen at both C-4 and C-10 suggests possible mechanisms for the biosynthesis of the spiro-lactone system **(Scheme 2.14)**. As the andilesins posses a 7-membered lactone ring, it seems reasonable of propose that the required skeletal rearrangement to form the spiro-lactone occurs subsequent to the introduction of the C-4 oxygen. Similar rearrangements of steroids are observed when a good leaving group is present at C-5. A relatively stable carbocation could be

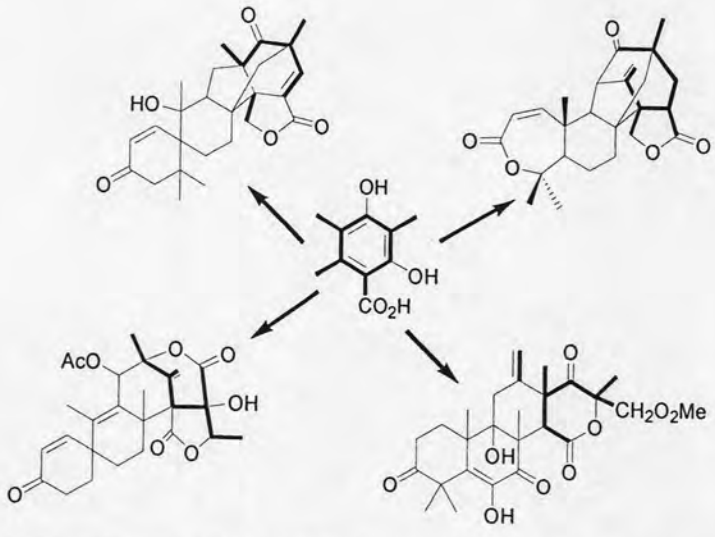
generated from the andilesin lactone ring (115) by alkyl-oxygen cleavage, which under acid conditions is the predominant cleavage mechanism for esters of tertiary alcohols. Loss of the C-5 proton would then give the tetrasubttituted alkene (116). Subsequent oxidation of (116) to epoxide (117) followed by rearrangement would give the carbocation intermediate (118). Three paths for the conversion of (118) to the spirolactone can be envisaged. Elimination to the endocyclic alkenes (119) and (120) and subsequent epoxidation and hydride reduction (paths a and b) would account for the origin of the 10-hydroxyl in atmospheric oxygen. Any subsequent mechanism for the δ-lactone will result in two atoms of atmospheric oxygen in the lactone. The structure of austin (121) which contains both the spirolactone and 9,10-double bond provides some support for this general route (Simpson et. al., 1997).



Scheme 2.14 Possible mechanisms for spiro-lactone formation

Austin (121) is clearly biosynthesis related to andibenin B and previous studies have shown that it is formed the same key intermediate (98) derived by alkylation of 3,5-dimethylorsellinate (97) by farnesyl pyrophosphate. An interesting possibility was that the tetracyclic intermediate (126) involved in austin biosynthesis (Scheme 2.15) is not formed directly from cyclisation of (98) but is formed via the same bicyclofarnesyl

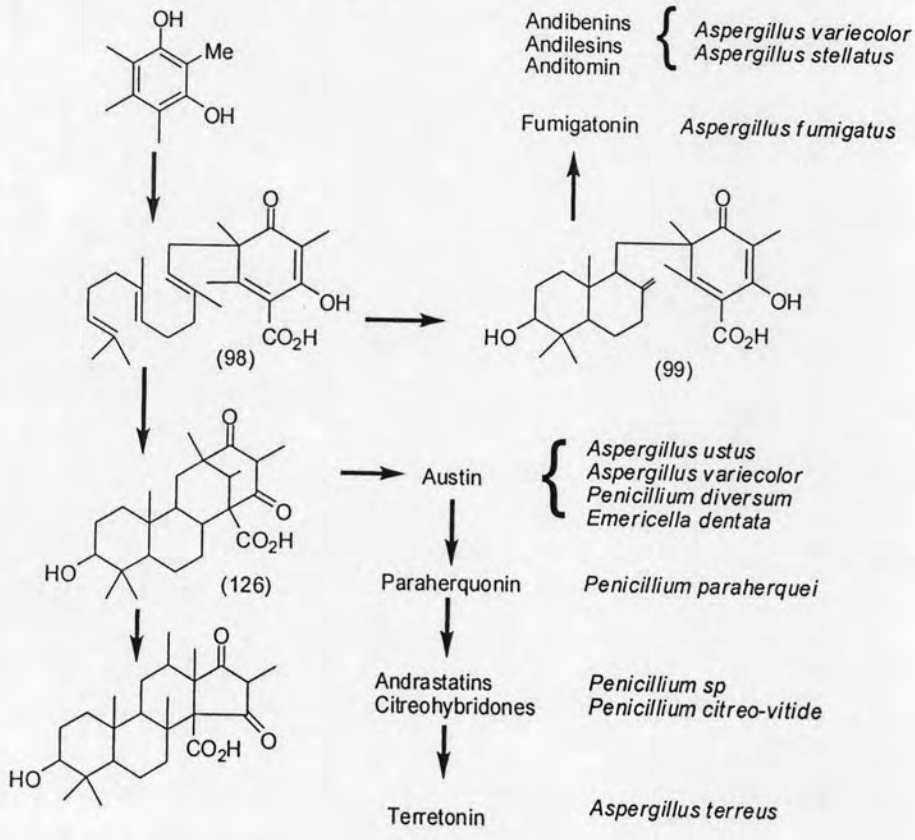
intermediate (99) involved in andibenin biosynthesis. The growth production characteristics of *A. ustus* and the optimum time for feeding labeled precursors had been determined previously. Accordingly, [6-^{13}C, 6-$^{2}H_3$]mevalonate was fed to 5-day old cultures and the resultant enriched austin isolated and its $^{1}H,^{2}H$ noise decoupled ^{2}H nmr spectrum determined. This showed clear isotopically shifted signals corresponding to the incorporatation of two and mainly three deuteriums into the 12-, 13- and 14-methyls. The results for the 12-methyl exclude the possibility of the involvement of the bicyclofarneyl intermediate (99) in austin biosynthesis (Chexal et. al., 1976; Simpson et. al., 1994).



Scheme 2.15 Modification of the orsellinate moiety in meroterpenoid metabolites.

Andilesin C (95) and anditomin (96) have been isolated as co-metabolites in a different strain (CMI 60316) of *A. variecolor* and ^{13}C-labelling studies are consistent with the formation of anditomin from a carbocation-induced rearrangement of the andilesin C skeleton. Anditomin represents an important modification of the meroterpenoid pathway as it was first metabolite in which the carbocyclic ring of the tetraketide-derived moiety had been fragmented. Austin (121) and terretonin (122) which were isolated as toxic metabolites of *Aspergillus ustus* and *Aspergillus terreus* respectively have also been shown to be formed from the meroterpenoid pathway via the common intermediate (98) (Simpson and Walkinshaw, 1981; Simpson et. al., 1982; Simpson et., al.,. 1994). The degree of modification of the orsellinate moiety is even more drastic in these metabolites (Scheme 15). The biosynthetic relationship of austin was supported by the isolation from

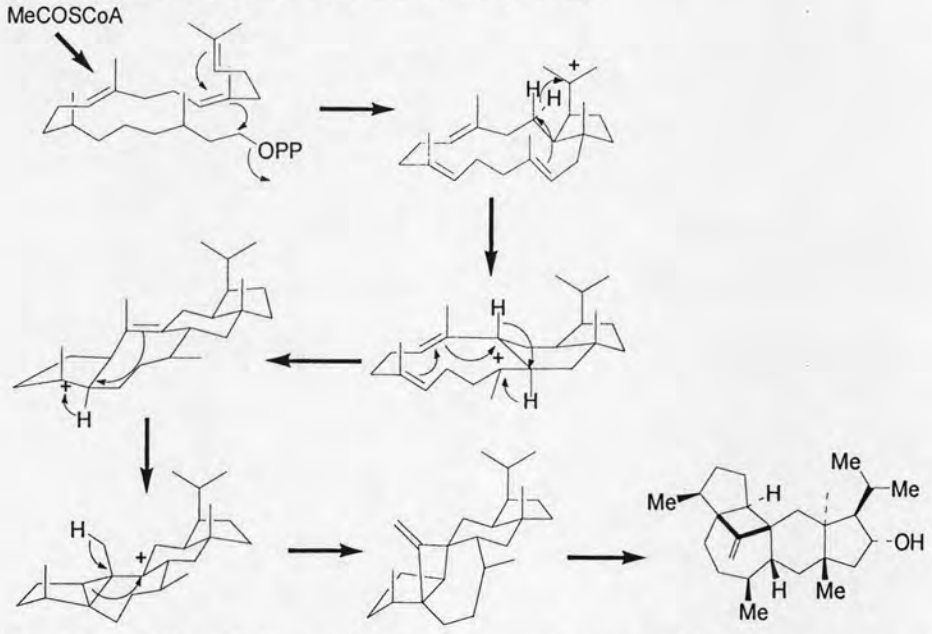
Emericella dentate and *Penicillium diversum* (Simpson et. al., 1982). Other complex metabolites which are almost certainly further products of the meroterpenoid pathway are fumigatonin **(123)** and paraherquonin **(124)** which have been isolated from *Aspergillus fumigatus* and *Penicillium paraherquei* respectively (Okuyama et. al., 1983; Okuyama et. al., 1984). More recently the citreohybridones, e. g. **(125)**, and the closely related andrastins have been isolated from Penicillium citreoviride and Penicillium sp FO-3929 respectively to provide yet further members of the family (Kosemura et. al., 1994). These latter metabolites are clearly related to terretonin **(122)** (McIntyre et. al., 1982; Springer et. al., 1979) and the likely biogenetic relationships amongst these structurally varied metabolites summarized in Scheme 2.16.



Scheme 2.16 The fungal meroterpenoid pathway.

2.1.3.1 The second group of sesterterpenoids biosynthetic pathways of metabolite of *Aspergillus* sp.

Second groups of sesterterpenoid biosynthetic studies are reported which indicate that astellatol is formed via cyclisation and rearrangement of geranylfarnesyl pyrophosphate, so confirming its sesterterpenoid origin. The ^{13}C-NMR spectrum of astellatol enriched by feeding [1,2-$^{13}C_2$]acetate to cultures of *A. variecolor* showed a low overall level of enrichment. The observed labeling pattern is consistent with the biosynthetic pathway shown in **Scheme 2.17**, where it is proposed that all-*trans*-geranylfarnesyl pyrophosphate undergoes initial folding and cyclisation. 1,5-Hydride shift from C-2 to C-20 leads to the tricyclic tertiary carbocation. Analogous 1,5-hydride shifts have been observed in the biosynthesis of a number of other sesterterpenoids. Subsequent hydride migrations and cyclisations lead to the cyclopropyl carbocation intermediate. Ring expansion of the cyclopropyl intermediate which contains the cyclobutane ring found in astellatol **(7)**. Stellatic acid **(127)** was found to be a biogenetically class of sesterterpenoid which is expected as the primary cyclization product of this class from all *trans* geranylfarnesyl pyrophosphate. Aspergilloxide **(128)**, a sesterpene epoxide-diol, was isolated from the extract of a cultured marine-derived *Aspergillus* sp (strain CNM-713) (Cueto et. al., 2002).



Scheme 2.17 biosynthetic pathway of sesterterpenoid of astellatol via *trans*-geranylfarnesyl pyrophosphate.

2.2 Study of secondary metabolites from genus *Aspergillus*.

2.2.1 Metabolites from *Aspergillus* sp. (*Emericella* sp.).

Aspergillus spp., most of them known for producing aflatoxins or other mycotoxins, was cultivated on *Aspergillus* differential medium, containing ferric ions. Two red pigments produced by the test-sensitive strains have been isolated and identified as ferriaspergillin **(129)** and ferrineoaspergillin **(130)** (Assante et. al., 1981). Two isomeric linear peptides, aspergillamides A **(131)** and B **(132)**, were isolated from the mycelium of a cultured marine fungus of the genus *Aspergillus*. Aspergillamide A showed moderest *in vitro* cytotoxicity toward the human colon carcinoma cell line HCT-116 (Toske et. al., 1998). A new sesterterpene epoxide-diol, aspergilloxide **(128)**, was isolated from the extract of a cultured marine-derived fungus (strain CNM-713) identified as an undescribed member of the genus *Aspergillus* (Cueto et. al., 2002). Two new chlorinated diphenyl ethers, Methyl dichloroasterrate **(133)** and Methyl chloroasterrate **(134)** have been isolated from the culture broth of an *Aspergillus* species obtained from leaf litter, together with the known metabolites sulochrin **(135)**, the grisandiene geodin **(136)**, diphenyl ether asterric acid **(137)** and methyl asterrate **(138)** (Hargreaves et. at., 2002). Natural product screening and bioassay-guided isolation of an extract of *Aspergillus* sp. led to the identification of three novel 11-membered macrocyclic biphenyl ether lactones, aspercyclides A–C **(139-141)**. Aspercyclide A inhibited the IgE binding with an IC_{50} of 200 μM. (Singh et. al., 2004) Dehydroxychlorofusarielin B **(142)**, a new antibacterial polyoxygenated decalin derivative, and the previously described fusarielins A **(143)** and B **(144)** have been isolated from the broth of a marine isolate of the fungus *Aspergillus*. Compounds 143-145 exhibited a mild antibacterial activity against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and multidrug-resistant *S. aureus*. (Nguyen et. al., 2007) Induction of the production of emericellamides A **(145)** and B **(146)**, by the marine-derived fungus *Emericella* sp., was observed during co-culture with the marine actinomycete *Salinispora arenicola*. Emericellamides A and B show modest antibacterial activities against methicillin-resistant *Staphylococcus aureus* with MIC values of 3.8 and 6.0 μM, respectively (Oh et. al., 2007). Two new xanthones identified as 15-chlorotajixanthone hydrate **(147)** and 14 methoxy-tajixanthone **(148)** were isolated from an *Emericella* sp. strain 25379 along with shamixanthone **(14)** and

tajixanthonehydrate (17). Compound 148, 149, 14 and 17 inhibited the calmodulin-sensitive cAMP phosphodiesterase (PDE1) in a concentration-dependent manner with that of chlorpromazine (CPZ; IC_{50} = 7.26 IM), a well known CaM inhibitor used as a positive control (Figueroa et. al., 2008). Six new prenylated indole alkaloids, named notoamides F-K (149-154), were isolated from a marine-derived *Aspergillus* sp. Their structures, including absolute configurations, were elucidated by spectroscopic methods. Notoamide I (152) showed weak cytotoxicity against HeLa cells (Tsukamoto et. al., 2008).

2.2.2 Metabolites from *Aspergillus aculeatus*

Two new congeners of okaramine, okaramines H (155) and I (156), were isolated from okara fermented with *Aspergillus aculeatus* KF-428. Neither okaramine H nor I showed insecticidal activity against silkworms (Hayashi et. al., 1999).

2.2.3 Metabolites from *Aspergillus arenarius*

Three new terphenyl-type metabolites, arenarins A-C (157-159), have been isolated from the sclerotia of *Aspergillus arenarius* (NRRL 5012). Arenarins A-C exhibited mild activity in feeding assays against the dried-fruit beetle *Carpophilus hemipterus* and cytotoxicity against human tumor cell lines (Oh et. al., 1998).

2.2.4 Metabolites from *Emericella aurantiobrunnea*

An extract from *Emericella aurantiobrunnea* was found to complete with macrophage inflammatory protein (MIP)-1R for binding to human CCR5 in a scintillation proximity assay (SPA). Bioassay-guided fractionation led to the isolation of variecolin (160) and variecolol (161), which had IC_{50} values of 9 and 32 μM, respectively. Also isolated were four new inactive emericolin A (162), B (163), C (164), and D (165) (Yoganathan et. al., 2004).

2.2.5 Metabolites from *Aspergillus candidus*

The great linoleic acid peroxidase system antioxidative components in the broth filtrate of *Aspergillus candidus* (CCRC 31543, three major compounds were isolated and identified as 3,3''-dihydroxyterphenyllin (166), 3-hydroxyterphenyllin (167), and

candidusin B (168). Safety studies showed that these three compounds were neither cyto-nor geno-toxic toward human intestine 407 (INT 407) cells, nor mutagenic toward *Salmonella typhimurium* TA98 and TA100 (Yen et. al., 2001).

2.2.6 Metabolites from *Aspergillus clavatus*

The toxin, Cytochalasin E (169) had been isolated from *A. clavatus* grown on rice. Cytochalasin E killed rats within a few hours after dosing. Death was due to circulatory collapse caused by massive extravascular effusion of plasma. (Buchi et. al., 1973) Tryptoquivaline (170) and Tryptoquivalone (171), two tremorgenic metabolites of *Aspergillus clavatus* collected from a sample of mold-damaged rice (Clardy et. al., 1975).

2.2.7 Metabolites from *Aspergillus falconensis*

Six new hydrogenated azaphilones designated falconensins I-N (172-177), and a new azaphilone, monomethyl-dihydromitorubrin (178), were isolated as minor components from mycelia of *Emericella falconensis* and/or *E. fruticulosa* along with nine azaphilone derivatives, falconensins A-H (179-186) and monomethylmitorubrin (187), and three hopane-type triterpenes (188-190) (Ogasawara and Kawai., 1998).

2.2.8 Metabolites from *Aspergillus flavipes*

Aspergillus flavipes occurring in the rhizosphere of *Ericameria laricifolia* resulted in the isolation of three new cytochalasans, namely, aspochalasins I (191), J (192), and K (193), and four known cytochalasans, aspochalasins C (194), D (195), and E (196) and TMC-169 (197). All compounds exhibited weak to moderate cytotoxicity against NCIH460, MCF-7, and SF-268 cancer cell lines, but none showed significant selectivity (Zhou et. al., 2004).

2.2.9 Metabolites from *Aspergillus flavus*

Parasiticol (198), a metabolite structurally related to the aflatoxins, has been isolated from the strains of the *Aspergillus flavus* series previously shown to be aflatoxin producers. Parasiticol is as acutely toxic to ducklings as aflatoxin B1, but it is only 1/100 as toxic as BI in chick embryo studies (Stubblefield et. al., 1970). Antifungal extracts of

Aspergillus flavus indicated that the primary antifungal compound present was the known aspirochlorine (**199**). New compounds tetrathioaspirochlorine (**200**) and cyclo (D-*N*-methyl-Leu-L-Trp) (**201**). Aspirochlorine was a potent antifungal, superior to three standard compounds against azoleresistant *C. albicans*. Tetrathioaspirochlorine (**200**) was only slightly less potent in the antifungal assay. Aspirochlorine has recently been shown to exert its antifungal activity by inhibition of fungal, but not bacterial or mammalian, protein synthesis (Klausmeyer et. al., 2005).

2.2.10 Metabolites from *Aspergillus fumigatus*

The clavine alkaloids, fumigaclavine A (**202**), a new alkaloid designated fumigaclavine C (**203**), and several tremorgens belonging to the fumitremorgen group were produced by *A. fumigatus* strains isolated from molded silage. Crude extracts of *A. fumigatus* cultures experienced severe diarrhea, irritability, loss of appetite, and postmortem examination showed serous enteritis and evidence of interstitial changes in the lungs (Cole et. al., 1977). Gliotoxin (**204**), a potential etiologic agent which is synthesized by *Aspergillus fumigatus* and other pathogenic fungi, exhibits a variety of immunosuppressive activities. Gliotoxin markedly inhibits both perforin-dependent and Fas ligand-dependent cytotoxic T-lymphocyte (CTL)-mediated cytotoxicity. (Yamada et. al., 2000) A new fumagillin derivative, fumagiringillin (**205**), has been isolated together with fumagillin (**206**) and 12R-fumitremorgin C (**207**) from a strain of *Aspergillus fumigatus* was grown on potato dextrose agar (GibcoBRL) plates (Jiao et. al., 2004).

2.2.11 Metabolites from *Aspergillus fumisynnematus*

Fumimycin (**208**), an unusual metabolite, was isolated from cultures of *Aspergillus fumisynnematus*. Fumimycin was found to inhibit *Staphylococcus aureus* peptide deformylase and also showed antibacterial activity against *S. aureus* (Kwon et. al., 2007).

2.2.12 Metabolites from *Aspergillus glaucus*

Two new metabolites, kotanin (**209**) and desmethylkotanin (**210**) have been isolated from *Aspergillus glaucus* cultures (Buchi et. al., 1971). Eight new aromatic polyketides (**211**, **212-214**, **215**, **216**, **217** and **218**) aspergiolide B (**211**); three naphthyl

ribofuranosides, isotorachrysone-6-*O*-α-D-ribofuranoside (212), 8-methoxy-3-methyl-1-naphthalenol-6-*O*-α-D-ribofuranoside (213), and 8-methoxy-1-naphthalenol-6-α-D-ribofuranoside (214); two anthracene derivatives, isoasperflavin (215) and (+)-variecolorquinones A (216); and two bianthrones, (*trans*)-and (*cis*)-emodin-physcion bianthrone (217 and 218) together with eight known analogues (219, 220, 56, 221-225, and 227) [isotorachrysone (219), asperflavin (220), emodin (56), physcion (221), questin (222), catenarin (223), rubrocristin (224), and physcion bianthrones (225)] were isolated from the marine-derived fungus *Aspergillus glaucus*. The compounds have cytotoxicities against the HL-60 and A-549 cell lines (Tianjiao et. al., 2008). Aspergiolide A (226), a novel antitumor compound, was produced by a marine-derived filamentous fungus *Aspergillus glaucus*. The biosynthesis was determined by feeding experiments using sodium acetate precursors labeling pattern were incorporated in aspergiolide A by polyketide pathway (Tao et. al., 2009).

2.2.13 Metabolites from *Emericrlla heterothullira*

Emeheterone (227), a novel pyrazinone derivative, has been isolated from the Czapek-Dox broth culture filtrate of the fungus *Emericella heterothallica*, along with stellatin (108). (Kawahara et. al., 1988) Emehetin (228), a novel chromane derivative isolated along with emeheterone (227) and stellatin (108) from culture filtrate of the Czapek-Dox culture medium of the fungus *Emericrlla heterothullira* (Kawahara et. al., 1989).

2.2.14 Metabolites from *Aspergillus insulicola*

Insulicolide A (6*β*-[(4-nitrobenzoyl)oxy]-9*R*,14-dihydroxycinnamolide) (229) has been isolated from the chemically unexplored fungus *Aspergillus insulicola* were fermented in one of two liquid media: YES broth and/ or SB . *A. insulicola* is one of the few known marine aspergilli (Rahbaek et. al., 1997).

2.2.15 Metabolites from *Aspergillus janus*

Two compounds janoxepin (230) and brevicompanine B (231) were isolated from the fungus *Aspergillus janus*. Both compounds were tested in antimicrobial assays

and found to be active against the malaria parasite *Plasmodium falciparum*. However, no activity was observed in antifungal or antibacterial assays (Sprogoe et. al., 2005).

2.2.16 Metabolites from *Aspergillus japonicus*

A new paralytic alkaloid, Asperparaline A (232), has been isolated from *Aspergillus japonicus* JV-23 which exhibited paralysis in silkworm *(Bombyx mori)*. Asperparaline A (232) was examined against fourth in star larvae of silkworm (Hayashi et. al., 1997).

2.2.17 Metabolites from *Aspergillus karnatakaensis*

Karnatakafurans A (233) and B (234), two novel dibenzofurans, have been isolated from the Specie Novum *Aspergillus karnatakaensis* Frisvad. The compounds were tested for antimicrobial and antimalarial activity and proved to be moderately active against *Plasmodium falciparum* (Manniche et. al., 2004).

2.2.18 Metabolites from *Aspergillus nidulans*

Three new dimeric naphthopyrones, asperpyrones A (235), B (236), and C (237), together with two known compounds, fonsecinone A (238) and aurasperone A (239), have been isolated from okara that was fermented with *Aspergillus niger* JV-33-48. Compounds 237, 240, and 241 showed inhibitory activity on *Taq* DNA polymerase (Akiyama et. al., 2003). 5'-Acetyl-3'-deoxyadenosine (240), a new nucleoside derivative, has been isolated from *Emericella niduluns* var.*lata* (Kawahara et. al., 1992).

The fungus *Emericella nidulans* var. *acristata* was isolated as an endophyte from a Mediterranean green alga. Two new compounds, arugosins G (241) and H (242), together with the known metabolites, Arugosin A (35), Arugosin B (36), Shamixanthone (14), Emericellin (41), Emindole DA (243), Microperfuranone (244), and Sterigmatosystin (245). Arugosins (242, 35, 36) are benzophenone derivatives, biosynthetically related to the xanthones 14, 41, and 245. The indole alkaloid 243 displayed antitumor activity in a panel of 36 human tumor cell lines. Furthermore, compounds 243 and 244 showed moderate antitumor activity toward individual tumor cell lines. None of compounds 242-

246 exhibited any immunostimulatory activity assessed as the capacity to induce cytokines in PBMCs from healthy donors (Kralj et. al., 2006).

2.2.19 Metabolites from *Aspergillus niger*

Asperazine (247), an unusual unsymmetrical diketopiperazine dimer, obtainedby saltwater culture of *Aspergillus niger* derived from a Caribbean *Hyrtios* sponge culture in sea water liquid medium. It was apparent that 255 was potentially related to isomeric terrestrial fungal natural products WIN 64821, $C_{40}H_{36}N_6O_4$ (248) and ditryptophenaline, $C_{40}H_{36}N_6O_4$ (249). Asperazine (247) showed significant leukemia selective cytotoxicity. (Varoglu et. al., 1997) Eight novel bioactive farnesylated epoxy cyclohexenones from an *Aspergillus niger* isolate obtained from tissue homogenates of an orange *Aplidium* sp. ascidian. The compounds were given the following trivial names yanuthone A (250), B (251), C (252), D (253), E (254), 1-hydroxyyanuthone A (255), 1-hydroxyyanuthone C (256), and 22-deacetylyanuthone A (257). (Bugni et. al., 2000) The new diketopiperazine dimer (258), as well as the known compounds TMC-256A1 (259), TMC-256C1 (260), and demethylkotanin (261), were isolated from a culture of *Aspergillus niger* (Ovender et. al., 2004).

2.2.20 Metabolites from *Aspergillus novofumigatus*

Novofumigatonin (262), a new metabolite, has been isolated from *Aspergillus novofumigatus* cultured on 200 YES agar Petri dishes at 25 °C for 14 days (Rank et. al., 2008).

2.2.21 Metabolites from *Aspergillus nomius*

Nominine (263), a new indole diterpenoid biogenetically related to the aflavinines, has been isolated as the major organic-soluble component of the sclerotia of the fungus *Aspergillus nomius* (NRRL 13137). Nominine exhibits potent activity against the widespread crop pest *Heliothis zea*. (Gloer et. al., 1989) Aspernomine (264), a new cytotoxic antiinsectan fungal metabolite with a previously undescribed ring system has been isolated from a pentane extract of the sclerotia of *Aspergillus nomius* (Staub et. al., 1992).

2.2.22 Metabolites from *Aspergillus ochraceus*

A new metabolite from an ochratoxin-producing isolate of *Aspergillus ochraceus* has been isolated and identified as 4-hydroxymellein **(265)**. The chemical of this compound have moiety of the ochratoxins implicates it as a possible biosynthetic precursor of the ochratoxins (Cole et. al., 1971). The metabolite 3-(1,2-epoxypropyl)-5,6-dihydro-5-hydroxy-6-methylpyran-2-one **(266)** has been isolated from the culture medium of an ochratoxin-producing isolate of *Aspergillus ochraceus*. This metabolite was also produced in trace amounts on corn and rye (Moore et. al., 1974). New diketopiperazine-containing metabolites *N*-methylepiamauromin **(267)**, epiamauromine **(268)** and cycloechinulin **(269)** have been isolated from the sclerotia of *Aspergillus ochraceus* (NRRL *3519*) by chromatography on Sephadex LH-20 and reversed-phase HPLC. All three compounds because moderate reduction in weight gain in assays against the lepidopteran crop pest *Helicwea zea*. (de Guzman et. al., 1992) Four new prenylated bis-indolyl benzenoid metabolites ochrindoles A-D **(270-273)** were isolated from antiinsectan organic extracts of the sclerotia of *Aspergillus ocbraceus* (NRFU *3519)*. These compounds displayed moderate activity in feeding assays against the corn earworm *Helicoverpa zea* and the dried fruit beetle *Carpopbihs hemipteusr.* Compounds **270-273** also exhibited activity against gram-positive bacteria (de Guzman et. al., 1994). Three new benzodiazepine alkaloids, Circumdatin A **(274)**, B **(275)** and C **(276)** have been isolated as minor constituents of culture extracts of a terrestrial strain of the fungus *Aspergillus ochraceus* (Rahbaek et. al., 1999). The crude extract of the broth of *Aspergillus ochraceus* was found to inhibit the final stage of polyprotein processing during hepatitis C virus replication. Bioassay-guided fractionation led to the isolation of the known compound mellein as the active component of the extract. Also isolated were circumdatin F **(277)** and a new alkaloid, circumdatin G **(278)** (Dai et. al., 2001). Diaporthin **(279)** and orthosporin **(280)** were characterized from the fungus *Aspergillus ochraceus* D2306. The biosynthetic origin of diaporthin was demonstrated by incorporation of [1-^{14}C] acetate and [methyl-^{14}C] methionine administered in early idiophase (Harris and Mantle., 2001). Two new antitumor alkaloids, stephacidin A **(281)** and B **(282)**. Two compounds 285 and 286, produced by *Aspergillus ochraceus* WC76466, are structurally related to the cytotoxic marine natural product,

avrainvillamide (283), isolated first from a marine fungal strain of *Aspergillus* sp. and later from the fermentation broth of *Aspergillus ochraceus*. Both compounds 289 and 290 demonstrated in vitro cytotoxic activity against various human tumor cell lines, but 290 exhibited more potent and selective antitumor activities, especially against testosterone-dependent prostate LNCaP cell line with an IC_{50} value of 0.06 μM (Qian-Cutrone et. al., 2002).

2.2.23 Metabolites from *Aspergillus ostianus*

Three new pentaketides, aspinotriols A (284) and B (285) and aspinonediol (286), were isolated together with two known compounds, aspinonene (287) and dihydroaspyrone (288), from the marine fungus *Aspergillus ostianus* strain 01F313, which was collected in Pohnpei and cultured with bromine-modified artificial seawater (Kito et. al., 2007). Three new 14-membered macrolides, named aspergillides A, B, and C (289-291), were isolated from marine-derived fungus *Aspergillus ostianus* strain 01F313, cultured in a medium composed of bromine-modified artificial seawater. The new compounds showed cytotoxic activity against mouse lymphocytic leukemia cells (L1210) (Kito et. al., 2008).

2.2.24 Metabolites from *Aspergillus parasiticus*

A new metabolite, 1,3,6,8-tetrahydroxy-2-(1'-hydroxy-3'-oxobutyl)-anthra quinone, designated as asparasone A (292), was isolated from an *Aspergillus parasiticus* mutant (ATCC 20979), and its structure was deduced from spectral data. Several wild-type isolates of *A. parasiticus* also produced 296. Asparasone A (292) is structurally related to intermediates in the aflatoxin biosynthetic pathway (Sobolev et. al., 1997).

Sequoiatones A (293) and B (294) were isolated from the fungus *Aspergillus parasiticus*, an endophytic fungus of the coast redwood, *Sequoia sempervirens*. The compounds were isolated because of their brine shrimp lethality. Sequoiatones A (293) and B (294) are provided herein, along with the test results from the NCI human tumor 60 cell-line screen. (Stierle et. al., 1999) *Aspergillus parasiticus*, a fungal isolate from a coast redwood tree (*Sequoia sempervirens*), has been shown to produce four new

compounds, sequoiatones C-F (295-298). The structures of these compounds, all of which are cytotoxic to brine shrimp (Stierle et. al., 2001).

Bioassay-guided fractionation of an organic extract of the broth from the marine-derived fungus culture of *Aspergillus parasiticus* led to the isolation and subsequent structural elucidation of a new gabosine derivative, parasitenone (299), and two known benzyl alcohols, 3-chloro-4,5 dihydroxy- benzyl alcohol (300) and gentisyl alcohol (301). The benzyl alcohols (300 and 301) were identified as the principal free radical scavenging components. Parasitenone (299) also showed moderate activity in the free radical scavenging assay (Son et. al., 2002).

Aspergillus parasiticus, a fungal isolate from the bark of a redwood tree (*Sequoia sempervirens*), has been shown to produce the antitumor metabolites sequoiatones A and B and more recently the sequoiatones C-F (295-298). The sequoiamonascins A-D (302-305) were isolated by brine shrimp lethalityguided fractionation and were submitted to the NCI for anticancer evaluation (Stierle et. al., 2003).

2.2.25 Metabolites from *Emericella purpurea*

Two new sesterterpenes, designated variecolol (161) and variecolactone (306), were isolated from the mycelium of *Emericella purpurea*, along with a sesterterpene, variecolin (160), which was recently isolated from *Emericella variecolor* as an angiotensin II receptor binding inhibitor, and an indoloditerpene, emindole PA (Takahashi et. al., 1999).

Three new type indoloditerpenes, emindoles PA (307), PB (308), and PC (309), were isolated from the mycelium of *Emericella purpurea* along with the sesterterpenes variecolol and variecolactone, and the dicyanide derivatives epurpurins A to C. Emindoles PA (307), PB (308), and PC (309) are the indoloditerpenes having a new type of carbon skeleton (Hosoe et. al., 2006).

2.2.26 Metabolites from *Emericella quadrilineata*

Emericedins A (310), B (311), and C (312), new betaines having inhibitory activity of long chain fatty acid oxidation, were isolated from the culture broth of

Emericella quadrilineata IF0 5859. (Shinagawa et. al., 1987) Five metabolites, nidulalin A (312), nidulalin B (314), 1-hydroxy-3-methylxanthone (315), 1,9a-Dihydronidulalin A (316), Sordarial (317) from Gelasinospora santi-florii, and four metabolites, Microperfuranone (318), Pinselin (319), (4*S*,4a*R*,9a*R*)-4a-carbomethoxy-1,4,4a,9a-tetrahydro,4,8-dihydroxy-6-methylxanthone (320), 9-Hydroxymicroperfuranone (321) together with 313-317 from *Emericella quadrilineata* have been isolated in a screening study on immunomodulatory fungal constituents. Among these nine metabolites, EQ-7 and 8 have been unknown. On bioassay, a dihydroxanthone, nidulalin A (313), a hexaketide, sordarial (317), and a xanthone, pinselin (319) have displayed significant immunosuppressive activities (Fujimoto et. al., 2006).

2.2.27 Metabolites from *Aspergillus sclerotiorum*

Scleramide (322), a new cyclic hexapeptide, and a new oxidized bisindolyl benzenoid derivative, 2''-oxoasterriquinol D methyl ether (323) were isolated from extracts of the sclerotia of *Aspergillus sclerotiorum* (NRRL 5167) (Whyte et. al., 2000).

2.2.28 Metabolites from *Aspergillus sulphureus*

Extracts from the sclerotia of *Aspergillus sulphureus* have yielded four new antiinsectan compounds of the paspaline/penitrem class, Sulpinine A (324), Sulpinine B (325), Sulpinine C (326), Secopenitrem B (327), Penitrem B (328). These metabolites were determined primarily by analysis of various NMR experiments and also by comparison to the structurally related penitrems. Sulpinine C contains an unusual eight-membered-ring lactam and possesses a ring system. The three indole metabolites (328, 329, and 331) exhibit potent activity against the lepidopteran crop pest *Helicoverpa zea* (Laakso et. al., 1992).

2.2.29 Metabolites from *Aspergillus sydowi*

Three new diketopiperazine alkaloids, 6-methoxyspirotryprostatin B (329), 18-oxotryprostatin A (330), and 14-hydroxyterezine D (331), with an oxaspiro[4.4]lactam moiety, 14-norpseurotin A (332), and the 29-nordammarane triterpenoid 6β,16β-diacetoxy-25-hydroxy-3,7-dioxy-29-nordammara-1,17(20)-dien-21-oic acid (333), as well as 12 known compounds (334-341), were isolated from the ethyl acetate extract of a

marine-derived fungal strain, *Aspergillus sydowi* PFW1-13. Compounds **329-331** exhibit weak cytotoxicity against A-549 cells. Compounds **332** and **333** display significant antimicrobial activities against *Escherichia coli*, *Bacillus subtilis*, and *Micrococcus lysoleikticus* (Zhang et. al., 2008).

2.2.30 Metabolites from *Aspergillus terreus*

Terpeptin **(338)**, a novel peptide having cell cycle inhibitory activity, was isolated from the cultured broth of *Aspergillus terreus*. Terpeptin **(334)** inhibited the cell cycle progression of mouse tsFI210 cells in the G2/M phase. (Kagamizono et. al., 1989) Terrein **(1)** and *E-4-(*1-propen-1-yl)-cyclopenta-1,2diol **(335)** were isolated from standing cultures of *Aspergillus terreus*. Both metabolites inhibit the growth of wheat take-all fungus (Ghisalberti et. al., 1990). A strain of *Aspergillus terreus* inhibited the growth of the plant pathogen *Phytophthora cinnamomi*, produces an antifungal metabolite when grown in liquid culture. This metabolite was isolated by bioassay-guided fractionation and identified as terrecyclic acid A **(336)** and terrecyclodiol **(337)** (Almassi et. al., 1996). A novel chiral dipyrrolobenzoquinone derivatives, terreusinone **(338)**, have been isolated as a potent UV-A protectant from the marine algicolous fungus *Aspergillus terreus*. Compound 342 exhibited a UV-A absorbing activity (Lee et. al., 2003). A novel cyclopentenedione, asterredione **(339)**, two new terrecyclic acid A derivatives, (+)-5(6)-dihydro-6- methoxyterrecyclic acid A **(340)** and (+)-5(6)-dihydro-6-hydroxyterrecyclic acid A **(341)**, and five known compounds, (+)-terrecyclic acid A **(342)**, (-)-quadrone **(343)**, betulinan A **(344)**, asterriquinone D **(345)**, and asterriquinone C-1 **(346)**, were isolated from *Aspergillus terreus* occurring in the rhizosphere of *Opuntia versicolor*, using bioassay-guided fractionation. All compounds were evaluated for cytotoxicity in a panel of three sentinel cancer cell lines, NCI-H460 (non-small cell lung cancer), MCF-7 (breast cancer), and SF-268 (CNS glioma), and were found to be moderately active. (Wijeratne et. al., 2003) The screening efforts led to the isolation of two alkaloids from *Aspergillus terreus*: alantrypinone **(347)** and serantrypinone **(348)**. Both compounds showed insecticidal activity against *Myzus persicaein*. Binding assay-guided screening should provide significant opportunities for the identification of novel and selective insecticides (Kiriyama et. al., 2004). A novel metabolite, named terreinol **(349)** was

isolated from *Aspergillus terreus*. The labeling pattern indicated a predominantly polyketide origin 14 involving six acetyl/malonyl units added by CoA. The labeling of the aromatic methyl, adjacent to another labeled position, may indicate alkylation of the polyketide intermediate by methionine (Macedo, et. al., 2004). Four new sesterterpenoids, terretonins A-D **(350-353)**, and a new alkaloid, asterrelenin **(354)** were isolated from the ethyl acetate extract of a PDA solid-state fermented culture of *Aspergillus terreus* (Li et. al., 2005). A new seven-membered lactone type meroterpenoid, isoterreulactone A **(355)**, was isolated from the solid state fermentation of *Aspergillus terreus*. Isoterreulactone A inhibited acetylcholinesterase (Yoo et. al., 2005). New bioactive metabolites from microbial resources, *Aspergillus terreus* (HKI0499) was examined by chemical metabolite profiling. Together with the known butyrolactone I **(356)**, the unusual sulfate derivatives butyrolactone I 3-sulfate **(357)** and butyrolactone I 4''-sulfate **(358)** were discovered. The chemical structures were determined by NMR and MS data analyses. All compounds were tested on CDK1/cyclin B, CDK5/p25, DYRK1A, CK1, and GSK-3R/_ kinases; compounds 361 and 362 were also evaluated for their cytotoxic and antiproliferative activities. Butyrolactone I 3-sulfate **(357)** exhibited specific inhibitory activity against CDK1/cyclin B and CDK5/p25, yet 15–30-fold less than butyrolactone I **(356)**. Likewise, butyrolactone I 3-sulfate **(357)** exhibited moderate cytotoxicity solely against HeLa cells (CC50) 80.7 μM (Niu et. al., 2008). Two aromatic butenolides, aspernolides A **(359)** and B **(360)** along with the known metabolites, butyrolactone I, terrein and physcion were isolated from the fermentation broth of a soft coral derived fungus *Aspergillus terreus*. The structures of these metabolites were assigned on the basis of detailed spectroscopic analysis. Biogenetically aspernolides A and B must be derived from butyrolactone I, a well known specific inhibitor of cyclin dependent kinase (cdk) from *A. terreus*. When tested, aspernolide A exhibited mild cytotoxicity against cancer cell lines (Parvatkar et. al., 2008).

2.2.31 Metabolites from *Aspergillus tubingensis*

Investigation of the fungus *Aspergillus tubingensis* has led to the isolation and identification of two dihydrocarbazole-containing compounds, Dihydrotubingensin A

(361) and Dihydrotubingensin B (362), with two previously reported metabolites, tubingensins A (363) and B (364). This is the first known report of dihydrocarbazole-containing compounds to be isolated from a living system (Singh et. al., 2001).

2.2.32 Metabolites from *Emericella unguis*

Unguisin A (365) and B (366), the first cyclic heptapeptides containing GABA in the ring, were isolated from a marine-derived strain of *Emericella unguis* was grown on solid YES medium (Malmstrom et. al., 1999). Besides the known unguisins A (365) and B (366), a new cyclic heptapeptide, unguisin C (367), containing a GABA-derived moiety in the ring, was isolated from the fungus *Emericella unguis.* Precursor-directed biosynthesis of the unguisins was performed by supplementation of the culture medium with amino acids (Ala, Ser, Phe and Leu) (Malmstrom et. al., 2002).

2.2.33 Metabolites from *Aspergillus ustus*

Two new metabolites, ophiobolin G (368) and ophiobolin H (369), were isolated from *Aspergillus ustus.* Both inhibited growth of *Bacillus subtilis* cultures, but ophiobolin H was a more potent inhibitor at rates than ophiobolin G. Neither inhibited growth of *Escherichia coli* (Cutler et. al., 1984).

(—)-Phenylahistin (370) is a fungal diketopiperazine metabolite culture from *Aspergillus ustus* NSC-F038 consisting of L-phenylalanine and isoprenylated dehydrohistidine, and it showed an inhibitory activity on the cell cycle progression of P388 cells in the G2/M phase (Kanoh et. al., 1997).

2.2.34 Metabolites from *Aspergillus versicolor*

From the marine sponge *Xestospongia exigua* collected in Indonesia the fungus *Aspergillus versicolor* was isolated. Following cultivation in a seawater-based medium seven new angular tricyclic chromone derivatives aspergillitine (371), aspergione A-F (372-377) was obtained from the mycelia and culture filtrate. Aspergillitine (371) displayed only moderate antibacterial activity against *Bacillus subtilis*, 31 while it was inactive against *Escherichia coli* and *Saccharomyces cerevisiae*. In the same assay systems, aspergiones C (374) and E (376) were inactive (Lin et. al., 2003). Seven new

aroyl uridine derivatives (kipukasins A-G); 378-384) were isolated from solid-substrate fermentation cultures of two different Hawaiian isolates of *Aspergillus versicolor*. The bioactivity of the original *A. Versicolor* extracts was accounted for mainly by the presence of the known metabolite sterigmatocystin, but kipukasins A (378) and B (379) showed modest activity in assays against Gram-positive bacteria (Jiao et. al., 2007).

2.2.35 Metabolites from *Aspergillus viridi-nutans*

Besides the antifungal agents, variotin (385), wasabidienone B (386), and phomaligin A (387), two new butinactive metabolites, viriditin (388) and *O*-methylviriditin (389), were isolated from extracts of the culture filtrate of liquid cultures of a strain of *Aspergillus viridi-nutans*. In addition, wasabidienone B_1 (390) was isolated (Omolo et. al., 1990).

2.2.36 Metabolites from *Emericella variecolor* (*Aspergillus variecolor*)

A new sesterterpenoid, variecolin (160) was isolated from fermentation of *Aspergillus variecolor* MF138. It was shown to be an angiotensin I1 receptor binding inhibitor. Variecolin was shown to have a novel ring skeleton, a hybrid of the ophiobolin and ceriferene class of sesterterpenoids (Hensens et. al., 1991). From a marine-derived strain of the fungus *Emericella variecolor*, varitriol (388), varioxirane (392), dihydroterrein (393), and varixanthone (394) were isolated. In the NCI's 60-cell panel, varitriol (391) displayed increased potency toward selected renal, CNS, and breast cancer cell lines. Varixanthone (394) showed antimicrobial activity (Malmstrom et. al., 2002). From a strain of the fungus *Emericella variecolor* derived from the marine sponge *Haliclona valliculata*, a new anthraquinone evariquinone, 1,2,3-trihydroxy-6-methoxyanthra quinone (395), and a known 1,2,3,8-tetrahydroxyanthraquinone (7-hydroxyemodin) (396), as well as the new prenylxanthone isoemericellin (397), accompanied by the biosynthetically related known metabolite shamixanthone. Furthermore, the *C*-glycosidic depside stromemycin (13) was isolated from the fungus was grown on WSA liquid medium. Evariquinone showed antiproliferative activity towards KB and NCI-H460 cells (Bringmann et. al., 2003). Two new ophiobolins, 6-*epi*-ophiobolin G (8) and 6-*epi*-ophiobolin N (9), and six known ophiobolins, ophiobolin G (372), ophiobolin H (373), 6-

epi-ophiobolin C (**398**), ophiobolin C (**399**), 6-*epi*-ophiobolin K (**400**), ophiobolin K (**401**) from the culture MG medium broth of of the marine derived fungus, *Emericella variecolor* GF10. Ophiobolin K showed cytotoxic activity against various tumor cell lines, including adriamycin-resistant mouse leukemia cells (P388) (Wei. H., et. al., 2004). Shimalactone A (**10**), a novel polyketide having bicycle [4.2.0] octadiene andoxabicyclo [2.2.1] heptane units, was isolated from a cultured marine fungus of *Emericella variecolor* GF10 in the MG medium. Shimalactone A (**10**) induced neuritogenesis at 10 mg/mL against neuroblastoma Neuro 2A cells (Wei et. al., 2005). Twelve new compounds, variecolorins A-L (**402-413**), together with eleven known analogues, dihydroxyisoechinulin A (**414**), isoechinulin A (**415**), neoechinulin A (**416**), echinulin (**417**), tardioxopiperazine B (**418**), tardioxopiperazine A (**419**), preechinulin (**420**), cryptoechinuline G (**421**), alkaloid E-7 (**422**), isoechinulin B (**423**), and neoechinulin B (**424**), resectively were isolated from the broth of a halotolerant fungus *Aspergillus variecolor*. Compounds **402-413**, **415-416** and **421-424** exhibited weak radical scavengering activity against DPPH. The new compounds **402-413** were essentially non toxic against the P388, HL-60, BEL-7402, and A-549 cell lines (Wang, et. al., 2007).

Since the secondary metabolites of *E. variecolor* obtained from different culture media are different it is suggested that the secondary metabolites produced by *E. variecolor* should depend on culture media. Recently, metabolites of an endophytic fungus, *Emericella variecolor*, of *Croton oblongifolius* were investigated. Four xanthones including shamixanthone (**14**), 14-methoxytajixanthone-25-acetate (**15**), tajixanthone methanoate (**16**) and tajixanthone hydrate (**17**) were isolated from mycelia of *E. variecolor*. All of them showed moderate activities and 14-methoxytajixanthone-25-acetate and tajixanthone hydrate were selective against gastric carcinoma and breast carcinoma (Pornpakakul et.al., 2006). Comparison of metabolites of the endophytic fungus, *E. variecolor* with others terrestrial *E. variecolor* were not exactly the same. Thus, the endophytic fungus, *E. variecolor* cultured in different media may produce a variety of metabolites and provide novel biologically active compounds due to endophytic fungi are the rich sources of novel compounds.

Therefore, this research will investigate the effect of culture media on secondary metabolites production of an endophytic fungus, *E. variecolor* Berk & Br. of *Croton oblongifolius* and biological activities of the isolated metabolites.