LITERATURE REVIEWS

1. Diversity and taxonomic study of *Talaromyces*

1.1 Diversity and distribution of *Talaromyces* in Thailand

Chana (1974) isolated microfungi from paddy and garden soil from 10 provinces in Central, Thailand including Bangkok, Kanchanaburi, Nonthaburi, Samut Songkhram, Prachin Buri, Sing Buri, Ratchaburi, Nakhon Pathom, Nakhon Nayok and Suphan Buri. Alcohol treatment, Glucose Ammonium Nitrate Agar (GAN) and streptomycin were used. *Talaromyces vermiculatus* was found from all soil samples. *T. spiculisporus* was isolated from most soil samples excepted soil from Nakhon Nayok and Kanchanaburi. Whereas, *T. striatus* was reported from garden soil in Samut Songkhram and paddy soil from Sing Buri and Suphan Buri respectively.

Cruesrisawath (1985) studied thermophilic and thermotolerant fungi from soil and debris in Thailand, using modification of Warcup's soil plate method and Yeast Starch Agar (YSA). *Talaromyces dupontii* with *Paecilomyces* - anamorph was found from decomposting starters. The optimum temperature of this fungi is ranging from 45-50^{o}C, but it can grow at 30^{o}C.

Kanjanamaneesathian (1988) reported the diversity of thermophilic and thermotolerant fungi from various substrates in Thailand. Soil plate method, YSA and Yeast Glucose Agar (YGA) with Streptomycin were used. *Talaromyces byssochlamydoides* and *T. emersonii* were recorded from agricultural soil from Prachuap Khiri khan and Chachoengsao respectively.

Six isolates of *Talaromyces* species were isolated from forest soil, Amphur Thong Pa Phum, Kanchanaburi; soil at Kasetsart University, Bangkok; forest soil and strawberry garden soil from Doi Intanonth, Chiang Mai. Alcohol and heat treatment with "GAN" were used (Manoch and Chana ,1996).

Manoch *et al.*, (1997) studied on Ascomycetes and Deuteromycetes from forest, agricultural soil and debris from various locations in Thailand, using alcohol and heat treatment and GAN. They could isolated *Talaromyces bacillisporus* and *Talaromyces* spp. from soil. Manoch *et al.*, (2000) reported 7 isolates of *Talaromyces* spp. from different forest types at Khao Yai National Park, Huay Kha Khang Wildlife Sanctuary, Uthai Thani. Alcohol and heat treatment techniques were employed for isolation.

Sudpro (1999) reported the diversity of soil and plant pathogenic fungi in agricultural soil from Sakhon Nakorn, using the soil plate method and "GAN". Two isolates of *Talaromyces* spp. were recorded from corn and cassava field soils in June and September, respectively.

Wongthong (2001) isolated fungi from mangrove forest at Ranong Coastal Research Station, Ranong Province, using dilution plate method on Glucose-Yeast Extract Agar with 14 ppt of salt. The soil samples were collected in December (winter), April (summer) and July (rainy) during 1999 – 2000. *Talaromyces flavus* was recorded from mangrove soil in December and July. Four isolates of *Talaromyces* spp. were reported from mangrove soil in all season.

Busarakum (2002) reported 3 isolates of *Talaromyces* spp. from rhizosphere soils of *Lusidia discolor*, *Calanthe rosea* and *Spathoglottis plicata* at Queen Sirikit Botanic Garden, Chiang Mai, using alcohol and heat treatment techniques with "GAN".

Manoch *et al.*, (2004) isolated soil fungi from termite mounds in Thailand. Alcohol and heat treatment methods and "GAN" were used. Scanning electron microscope (SEM) photomicrographs showed the ascospores ornamentation of *Talaromyces bacillisporus* from termite mounds in Sakon Nakhon and Trat, whereas, *T. flavus* var. *flavus* was observed from termite mounds in Nakhon Pathom. Sixteen isolates of *Talaromyces* spp. were found from termite mounds from Krabi and Nakron Nayok.

Ito *et al.*, (2001) reported microfungi from mangrove soil in Thailand at Ranong and Phang Nga. *Talaromyces byssochlamydoides* was isolated from rhizosphere soil of *Avicennia alba*, *Rhizophora mucronata*, *Rhizophora apiculata* and *Sonneratin alba*. *Talaromyces flavus* was found from rhizosphere soil of *Ceriops tage* and *Bruguiera sexangula*, whereas *T. wortmannii* was recorded from rhizosphere soil of *Avicennia alba*.

Jeamjitt (2007) studied coprophilous fungi from domestic and animals, using heat treatment and "GAN". *Talaromyces bacillisporus* and *T. rotundus* were recored from rat and buffalo dung in Bangkok respectively.

Manoch *et al.*, (2004) studied teleomorph of *Aspergillus* and *Penicillium* from soil at termite mounds using alcohol and heat treatment and "GAN". *Talaromyces flavus* var. *macrospermus* and *T. trachyspermus* were found from termite mound at Sakhon Nakhon, whilst, *T. wortmannii* were recorded from termite mound in Ratchaburi.

1.2 Diversity and taxonomic study of *Talaromyces*

Satanimi (1971) isolated fungi from soil at Mt. Pelion, Greece using alcohol treatment technique and "GAN". Six isolates of *Talaromyces luteus* were found. Hudson (1973) isolated *Talaromyces thermophilus* from air at Cambridge, England by using 2 % malt extract agar.

Intensive researches on diversity of *Talaromyces* species have been conducted in Japan. New species of *Talaromyces* were found from various substrates including *T. flavus*, *T. helicus* (Udagawa, 1963), *T. wortmannii* (Tokumasu, 1974), *T. trachyspermus* (Horie *et al.*, 1977), *T. derxii*- heterothallic species (Takada and Udagawa, 1988), *T. helicus* var. *boninensis* (Yaguchi *et al.*, 1992), *T. subinflatus* (Yaguchi *et al.*, 1993a), *T. convolutus*, *T. emodensis*, *T. tardifaciens* (Udagawa, 1993) *T. wortmannii* var. *sublevisporus*, *T. trachyspermus* var. *assiutensis*, *T. muroii*

(Yaguchi *et al.*,1994a, b), *T. hachijoensis* (Yuguchi *et al.*, 1996), *T. retardatus* (Udagawa *et al.*, 1993), *T. spectabilis* (Udagawa and Suzuki, 1994) and *T. euchlorocarpius* (Yaguchi *et al.*, 1999).

In the United States, Tansey (1971) reported *Talaromyces emersonii* and *T. thermophilus* from self-heated wood chips. Huang and Schmitt (1975) isolated 7 species of this genus including *T. flavus* var. *flavus, T. helicus, T. luteus, T. trachyspermus, T. udagawae, T. ucrainicus* and *T. wortmannii* form soils of Southern Ohio. In addition, Yuguchi *et al.*, (1993a) described a new species, *T. austrocalifornicus* from soil at Southern California, USA.

Rosenberg (1975) studied the effect of temperature and pH on growth of thermophilic and thermotolerant properties of *Talaromyces emersonii* and *T. thermophilus*. Optimum temperatures and pH of both species are 35-50°C, pH 3.4 – 5.4 and 45-50 °C, pH 7.2 – 8.1, respectively.

Jesenska *et al.*, (1992) reported 6 species of *Talaromyces* from soil at Slovak Republics, including *Talaromyces avellaneus, T. bacillisporus, T. emersonii, T. flavus* var. *flavus, T. trachyspermus* and *T. wortmannii*.

Tzean *et al.*, (1992) isolated *Talaromyces unicus* from soil, in Taiwan. The soil samples was suspended in 60°C aqueous solution for 20 min and placed on dichloram rose bengal chloramphenical agar plate. In addition Yaguchi *et al.*, (1994b) reported *T. eburneus* with *Geosmithia* anamorph from soil in Taipei, Taiwan.

Domsch *et al.*, (1993a, b) studied microfungi from soil and reported five species of *Talaromyces* including *T. emersonii, T. flavus, T. trachyspermus, T. helicus* and *T. wortmannii*. They were found worldwide from various substates such as soil, fertilizer and dungs.

Some species of *Talaromyces* were obtained from Arsenic-polluted soil and able to produce a volatile As-compound from As(III), such as *T. thermophilus*, an

thermopilic fungi was isolated from citrus waste decomposing in Japan (Hiroki and Yoshiwara, 1993; Heerden *et al*., 2002).

Udagawa *et al*, (1996) isolated ascomycetous microfungi from 58 house dust samples from detached house and apartment in Kobe City, by using dilution plate method and potato dextrose agar with chloramphenical. The most frequent species were *Talaromyces flavus, T. trachyspermus, T. macrospermus T. wortmannii and T. helicus*.

Sage *et al.*, (1997) reported 4 species *Talaromyces* including *T. flavus*, *T. helicus*, *T. stipitatus* and *T trachyspermus* from polluted soils, Oued Sebou, Morocco. Soil plate and malt extract agar (MEA) mixed with chloramphenical were used. *Talaromyces flavus* was more often found at heavily polluted sites than at lightly polluted ones.

Heredia *et al.*, (2001) reported *Talaromyces rotundus* and *T. ocotl,* new species with *Saganomella* anamorph from soil planted with *Pinus hartwegii* and *Pinus patula*, using heat and alcohol treatment. For heat treatment, 2-3 g of soil was filled in aluminum foil in hot air oven at 100 ^{o}C for 1hour. Alcohol treatment was by soil sample treated with 60% ethanol, 2% phenol, "GAN" mixed cyclosporin A, streptomycin sulfate and chlortetracycline in a petridish.

Asan (2004) studied *Aspergillus*, *Penicillium* and related species from various substrates in Turkey. Fourteen species of *Talaromyces* were reported from pepper powder, wheat seed, greenhouse and agriculture soil, drug tablets, dust, leather and surgical strings including *T. bacillisporus, T. byssochlamydoides, T. emersonii, T. flavus, T. helicus, T. intermedius, T. luteus, T. leycettanus, T. ohiensis, T. purpureus, T. rotundus, T. stipitatus, T. udagawae* and *T. wortmannii.*

Cavalcanti *et al*., (2002) reported the diversity of microfungi from mangrove in Itamaraca islands, Pernambuco state, Brazil, using a dilution technique and Sabouraud dextrose agar mixed with chloramphenical. *Talaromyces bacillisporus*,

T. trachysperus and *T. flavus* were found. *T. bacillisporus* was reported as a new recorded for Brazil.

Talaromyces flavus and *T. macrosporus* are heat-resistant and reported fairly frequently isolated from heat processed food and pastuerized juice. Ascospores may be soil-born and thus contaminating the fruit (Pitt and Hocking, 1997; Samson *et al*., 2002).

1.3 Study on morphology of *Talaromyces*

Stolk and Samson (1972) studied and revision monograph of *Talaromyces* morphology for identifying and key of 16 species and 2 varieties including *T. bacillisporus, T. byssochlamydoides, T. emersonii, T. flavus* var. *flavus, T. flavus* var. *macrosporus, T. helicus* var. *helicus, T. helicus* var. *major, T. intermedius, T. leycettanus, T. luteus, T. purpurea, T. rotundus, T. stipitatus, T. thermophilus, T. trachyspermus, T. udagawae, T. ucrainicus* and *T. wortmannii*. The main characteristics of *Talaromyces* for identification including **1)** ascomatal initial developing; **2)** ascomatal covering; **3)** shape, size and ornamentation of ascospores under scanning electron microscope and **4)** anamorph (imperfect state).

1) Characteristics of ascomatal initial or young cleistothecia (ascomata) of genus *Talaromyces* shows variable including 1) developing as short branches or as intercalary portions of hyphae which swell considerably, become strongly gnarled and branched profusely (e.g. *T. trachyspermus*) (Figure 1A); 2) consist of a pair of gemetangium (e.g. *T. flavus*) (Figure 1B); 3) consist of swollen cells resembling chlamydospores (e.g. *T. emersonii*) (Figure 1C); 4 coiled hyphae (e.g. *T. leycettanus*) (Figure 1D); 5) swollen irregular septate hyphae producing coiled branches (e.g. *T. wortmannii*) (Figure 2A) and 7) consisting one-celled and chlamydospore-like and produce ascogenous hyphae directly (Figures 2B-C) then the initials become septate and begin to produce branch hyphae for further development.

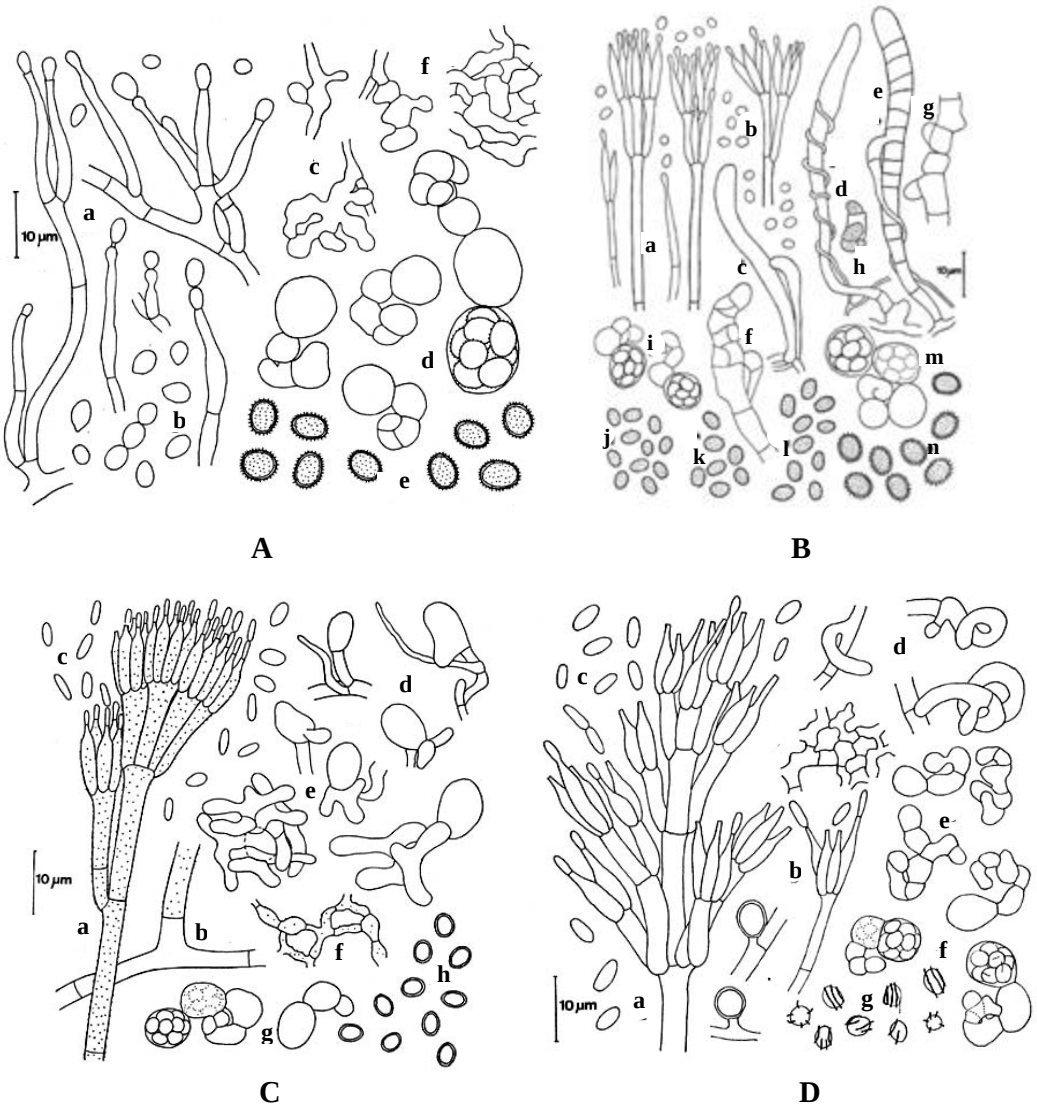


Figure 1 Morphological characteristics of four *Talaromyces* species

A) ***T. intermedius***, a. conidiogenous structures; b. conidia; c. ascomatal initials; d. chains of asci; e. ascospores; f. ascomatal covering on surface view

B) ***T. flavus***, a. conidiogenous structures; b. conidia; c-h. development of ascogonia and antheridia; i-m. chains of asci; j-n. ascospores

C) ***T. emersonii,*** a. conidiogenous structure; b. foot-cell; c. conidia; d. ascomatal initials; e. primordium consisting of profusely branching hyphae; f. fragment of ascomatal covering; g. asci in chains; h. ascospores

D) ***T. leycettanus***, a. complicated conidiogenous structure; b. monoverticillate penicillus; c. conidia; d. ascomatal initials, e-f. chains of asci; g. ascospores; h. fragment of ascomatal covering; i. chlamydospores

Source : Stolk and Samson, (1972)

Figure 2 Morphological characteristics of three *Talaromyces* species
A) ***T. wortmannii***, a. conidiogenous structures; b. conidia; c. ascomatal initials; d. chains of asci; e. ascospores
B) ***T. trachyspermus***, a. conidiogenous structures; b. conidia; c. ascomatal initials; d. chains of asci; e. ascospores; Type collection USDA 5798; f. ascospores; Herbarium specimen 7b. g. conidiogenous structures with conidia; h. ascospores.
C) ***T. bacillisporus***, a. conidiogenous structures; b. conidia; c. swollen cells from which the initials will develop; d. ascomatal initials, producing branches; e. developing asci; f. chain of asci; g. ascospore

Source : Stolk and Samson, (1972)

2) Ascomatal coverings of its species consist of a network of hyphae, differing markedly in their density, 1) covering with scanty and inconspicuous hyphae (e.g. *T. byssochlamydoides*); 2) distinct and loose-textured (e.g. *T. flavus*) and 3) thick-walled and closely knit, becoming pseudoparenchymatous in age (e.g. *T. thermophilus*).

3) Morphology of ascospores which various patterns (Figure 3) including globose, subglobose or ellipsoidal, their walls may be smooth, spinulose or provided with ridges. For a few species, very little variation in the sizes of the ascospores produced in different strains of one *Talaromyces* species.

4) Anamorph-state of *Talaromyces* show much variable. Five genera were reported including *Penicillium*, *Paecilomyces*, *Geosmithia*, *Merimble* and *Sagenomella* (Table 1). In 1972, Stolk and Samson reported anamorph state of *Talaromyces* in 2 genera, *Penicillium* and *Paecilomyces*. *Paecilomyces* is anamorph of *T. leycettanus* and *T. byssochlamydoides*, whereas other *Talaromyces* species have *Penicillium*-anamorph. The genus *Paecilomyces* differs from *Penicillium* by the shape of the phialides, consisting of a cylindrical or swollen basal portion, tapering into a long distinct neck. The conidial structures are usually divergent, verticillate or irregularly arranged.

In the course of a revisionary study of the genus *Penicillium*, Pitt (1979a) recognized that certain species are closely related, but differed significantly in microscopic characters and coloration from other *Penicillium* species. Thus he erected the genus *Geosmithia* gen. nov. comprising 6 species: *G. emersonii* and *G. swiftii* were replaced for *P. emersonii* (teleomorph- *Talaromyces emersonii) and P. bacillisporum* (teleomorph- *Talaromyces bacillisporum*) respectively. *Geosmithia* is distinguished from *Penicillium* primarily by the formation of conidia borne as cylinders from cylindroidal, rough-walled phialides, which commonly lack the distinctive narrowed collula (necks) of *Penicillium* and *Paecilomyces*. In addition, conidia of *Geomithia* viewed on mass are not green: *G. namyslowskii,* an apparent exception, produces grey

conidia with a pale green cast, a color quite distinct from the blue-grey or green-grey characteristic of *Penicillium* (Pitt, 1979b).

Pitt (1979c) reported *Merimble* gen. nov. differs from *Penicillium* by the absence of green in colony colors, and from *Paecilomyces* by the formation of phialides with short straight collula. It differs from both these genera and from *Geosmithia* by the characteristic formation of long, wide, spathulate conidiophores similar to those of *Aspergillus* and *Raperia* (Subramanian and Rajendran, 1975). However, the absence of foot cells and the successive formation of phialides by *Merimbla* provide clear distinctions from both species (Pitt, 1979c).

Heredia *et al.*, (2001) reported *Sagenomella* sp. as an anamorph of *Talaromyces ocotl*. *Sagenomella* is resembled to *Acremonium*, but differs producing a short conidiophore erect from mycelium, simple phialides or a short lateral conidiophore bearing 2-4 whorls of basitonously branched phialides. Phialides acerose, usually tapering apically, but sometimes cylindrical, slightly swollen at the base.

Stolk and Samson, (1972) proposed *Talaromyces* differs from the other perfect penicillate genus *Eupenicillium* by its soft ascomata. *Eupenicillium* is characterized by sclerotioid ascomata that are very hard to gritty when young. *Talaromyces* can easily be distinguished from the related genera in the Eurotiaceae: *Byssochlamys* Westling, *Hamigera* Stolk & Samson and *Thermoascus* Miehe by its catenate asci.

In the Family *Gymnoascaceae*, four genera produce ascomata approximating those of *Talaromyces* including *Arachniotus*, *Narasimhella*, *Amauroascus* and *Arachnotheco*. *Talaromyces* can easily be separated from these genera by its conidial state. In these four gymnoascaceous genera, the imperfect state is lacking or represented by arthro-or aleurioconidia (Stolk and Samson, 1972).

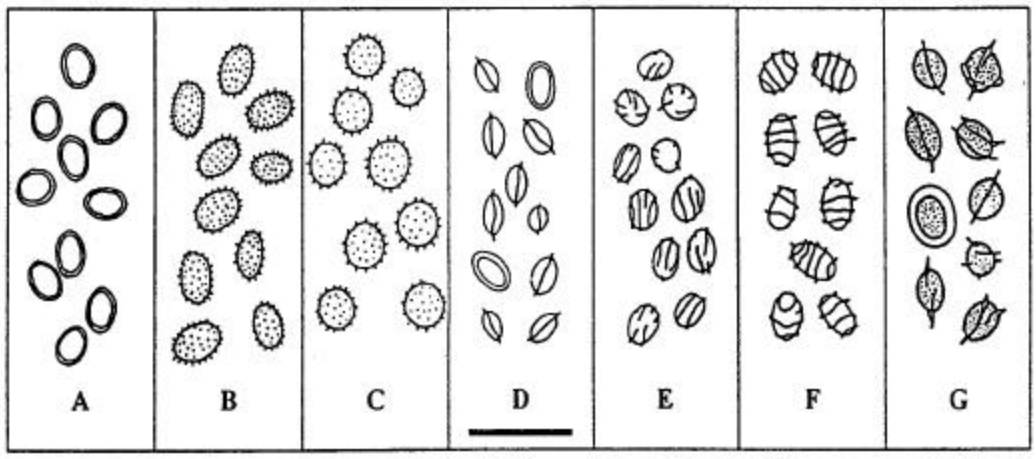


Figure 3 A synoptic illustrations of the ascospores ornamentation of *Talaromyces* species: *T. emersonii* (A), *T. flavus* (B), *T. bacillisporus* (C), *T. stipitatus* (D), *T. thermophilus* (E), *T. udagawae* (F) and *T. unicus* (G). Bar = 10 um.

Source : Stolk and Samson, 1972; Tzean *et al.*, 1992

Table 1 Teleomorph and anamorph connections of *Talaromyces* species

***Talaromyces* (Teleomorph)**	**Anamorph**	**References**
Talaromyces assiutensis	*Penicillium assiutense*	Samson and Abdel-Fattah, 1978
Talaromyces austrocalifornicus	*Penicillium austrocalifornicum*	Yaguchi *et al*.,1993a
Talaromyces bacillisporus	*Penicillium bacillisporum*	Stolk and Samson, 1972
	Geosmithia swiftii Pitt	Pitt, 1979b
Talaromyces barcinensis	*Penicillium barcinense*	Yaguchi *et al*., 1993b
Talaromyces brevicompactus	*Penicillium brevicompacta*	Kong, 1999
Talaromyces byssochlamydoides	*Paecilomyces byssochlamydoides*	Stolk and Samson, 1972
Talaromyces convolutus	*Penicillium convolutum*	Udagawa, 1993
Talaromyces derxii	*Penicillium derxii*	Takada and Udagawa, 1988
Talaromyces dupontii	*Penicillium dupontii*	Stolk and Samson, 1972
Talaromyces eburneus	*Geosmithia eburnea*	Yaguchi *et al*, 1994a
Talaromyces emersonii	*Penicillium emersonii*	Stolk and Samson, 1972
Talaromyces emodensis	*Penicillium emodense*	Udagawa, 1993
Talaromyces euchlorocarpius	*Penicillium euchlorocarpium*	Yaguchi *et al*., 1999
Talaromyces flavus	*Penicillium vermiculatum*	Stolk and Samson, 1972
Talaromyces galapagensis	*Penicillium galapagense*	Pitt, 1979a
Talaromyces gossypii	*Penicillium gossypii*	Pitt, 1979a
Talaromyces hachijoensis	-	Yaguchi *et al*., 1996

Table 1 (Continued)

Talaromyces	**Anamorph**	**References**
Talaromyces helicus var. *boninensis*	*Penicillium spirillum*	Yaguchi *et al*., 1992
Talaromyces helicus var. *helicus*	*Penicillium helicum*	Stolk and Samson, 1972
Talaromyces helicus var. *major*	*Penicillium helicum*	Stolk and Samson, 1972
Talaromyces indigoticus	*Penicillium indigoticum*	Takada and Udagawa, 1993
Talaromyces intermedius	*Penicillium intermedium*	Stolk and Samson, 1972
Talaromyces lagunensis	*Penicillium lagunense*	Udagawa *et al.*, 1994
Talaromyces leycettanus	*Paecilomyces leycettanus*	Stolk and Samson, 1972
Talaromyces luteus	*Penicillium luteum*	Stolk and Samson, 1972
	Penicillium udagawae	Pitt, 1979a
Talaromyces macrosporus	*Penicillium macrosporum*	Frisvad *et al*., 1990
Talaromyces mimosinus	*Penicillium mimosinum*	Pitt, 1979a
Talaromyces muroii	-	Yaguchi *et al*., 1994a
Talaromyces ocotl	*Sagenomella* sp.	Heredia *et al*., 2001
Talaromyces ohiensis	*Penicillium ohiense*	Pitt, 1979a
Talaromyces panasenkoi	*Penicillium panasenkoi*	Pitt, 1979a
Talaromyces purpureus	*Penicillium purpureum*	Stolk and Samson, 1972
Talaromyces retardatus	*Penicillium retardtum*	Udagawa *et al.*, 1993
Talaromyces rotundus	*Penicillium rotundum*	Stolk and Samson, 1972
	Penicillium sphaerum	Pitt, 1979a
Talaromyces spectabilis	*Paecilomyces spectabilis*	Udagawa and Suzuki, 1994
Talaromyces stipitatus	*Penicillium stipitatum*	Stolk and Samson, 1972
	Penicillium emmonsii	Pitt, 1979a
Talaromyces striatus	*Penicillium lineatum*	Pitt, 1979a
Talaromyces subinflatus	*Penicillium subinflatum*	Yaguchi *et al*., 1993a
Talaromyces tardifaciens	*Penicillium tardifaciens*	Udagawa, 1993
Talaromyces thermophilus	*Penicillium dupontii*	Stolk and Samson, 1972
Talaromyces trachyspermus	*Penicillium lehmanii*	Stolk and Samson, 1972
Talaromyces trachyspermus var. *assiutensis*	*Penicillium lehmanii*	Yaguchi *et al*., 1994b
Talaromyces ucrainicus	*Penicillium ucrainicum*	Stolk and Samson, 1972
Talaromyces udagawae	*Penicillium udagawae*	Stolk and Samson, 1972
Talaromyces unicus	*Penicillium unicum*	Tzean *et al.*, 1992
Talaromyces wortmannii	*Penicillium wortmannii*	Stolk and Samson, 1972
Talaromyces wortmannii var. *sublevisporus*	*Penicillium kloeckeri*	Yaguchi *et al*., 1994b

1.4 Identification of *Talaromyces*

Hawksworth *et al.*, (1995) classified *Talaromyces* as below;

Kingdom Eumycota
Phylum Eumycotina
Class Ascomycetes
Order Eurotiales
Family Trichocomaceae
Genus *Talaromyces*

Raper and Thom (1949) studied morphology of the genus *Penicillium*. They found many species of *Penicillium* including *P. wortmanni*, *P. bacillisporus*, *P. vermiculatum*, *P. stipitatum* and *P. luteum* could produce soft ascomata and asci in chains with 8 ascospores.

In 1955 C.R. Benjamin erected the genus *Talaromyces* with Type species; *T. vermiculatus* (syn. *T. flavus* (Klocker) Stolk & Samson (Stolk and Samson, 1972). For generic description "Ascomata globose to subglobose, soft, superficial, discrete or confluent, of indeterminate growth. Ascomatal covering varying from scanty to dense, consisting of a network of hyphae, which may range from very loosed-textured to closely knit, usually surround by a weft of thin, usually encrusted radiating hyphae, straight or twisted depending on the species. Ascomatal initials of various shape. Asci evanescent, 4-, 6- or 8-spored, globose to subglobose or slightly ellipsoildal, borne in chains. Ascospores globose or ellipsoidal, smooth or showing various ornamentations, yellow, rarely become reddish" (Stolk and Samson, 1972; Pitt, 1979a).

Stolk and Samson (1972) divided *Talaromyces* to 4 sections including section *Talaromyces*, *Thermophila*, *Purpurea* and *Emersonii* (Table 2) showed their different anamorph states as follows;

1. Section *Talaromyces* - conidial state belonging to the *Penicillium* Biverticillate- symmetrical type
2. Section *Emersonii* - conidial state *Paecilomyces* or belonging to the *Penicillium cylindrosporum*-series
3. Section *Thermophila* - conidial state belonging to *Penicillium* asymmetrica-Divaricata, near the *P. janthinellum*-series
4. Section *Purpurea* - conidial state belonging to the *Penicillium restrictum*-series

Section *Talaromyces*

Cleistothecia yellow, occasionally white, creamish, pinkish or reddish. Ascospores yellow, in some strains producing abundant red pigment. Conidial state: *Penicillium* biverticillate–symmetrica type. Phialide usually lanceolate, in a few species showing a wider base. Species belonging to this section show their best development on malt and oatmeal agar. On Czapek's agar (CZA), they develop better than the species belonging to the other sections. The species belonging to this section are mesophillic, with optimum temperatures about 25°C and maximum temperatures not exceeding 40°C. Ascomatal initials are of various types. Paired gemetangia occur in 3 species of this section: *T. flavus*, *T. helicus* and *T. stipitatus*. In the other species initials develop as branches or as intercalary portions of hyphae. Asci are produced in short coiled chains, they are usually 8-spored. Ascospores are generally ellipsoidal and their walls showing various ornamentation.

Section *Talaromyces* comprising 10 species, 2 varieties: *Talaromyces rotundus, T. luteus, T. udagawae, T. stipitatus, T. ucrainicus, T. flavus, T. macrosporus, T. helicus* var. *helicus, T. helicus* var. *major, T. wortmannii, T. trachyspermus* and *T. intermedius*

Section *Emersonii*

Ascomatal coverings scanty to distinct. Ascospores yellow. Imperfect state: *Penicillium cylindrosporum - series* or *Paecilomyces*; thermophilic or thermotolerant. Optimum temperatures of the 4 species are relatively high, ranging from 35°C to 45°C depending on the species. All species grow well at 40°C. Ascomatal initials show different structures. Asci occur in short, curved or coiled chains, usually 8-spored. Ascospores ellipsoidal or globose, smooth wall or showing various ornamentation.

Section *Emersonii* comprising 4 species: *Talaromyces byssochlamydoides*, *T. leycettanus*, *T. emersonii* and *T. bacillisporus*.

Section *Thermophila*

The ascomata have well-developed, thick, parenchymatous walls. Asci are not produced in helicoidal chains as occurring in most species of *Talaromyces*, but in rather straight and branched chains. In addition to the conidial state of the only species is classified in the Asymmetrica–Divaricata near the *Penicillium janthinellum-series*. Ascomata grow well on sterilized oat grains at 45°C.

Section *Thermophila* comprising only one species: *Talaromyces thermophilus*

Section *Purpurea*

Covering of ascomata distinct, consisting of a thin, loose-textured network of hyphae, yellow. Ascospores yellow, becoming reddish from diffusing red pigment. Anamorph state is *Penicillium restrictum – series*.

Section *Purpurea* comprising only one species: *Talaromyces purpureus*

Table 2 Morphological characteristics of different species of *Talaromyces* in four sections and three series (Modified from Stolk and Samson, 1972; Pitt, 1979a)

1. Section *Talaromyces*

Series Flavi	***Series Lutei***	***Series Trachyspermus***
1. *Talaromyces flavus*	1. *Talaromyces luteus*	1. *Talaromyces trachyspermus*
2. *Talaromyces macrosporus*	2. *Talaromyces wortmannii*	2. *Talaromyces gossypii*
3. *Talaromyces helicus*	var. *wortmannii*	3. *Talaromyces ohiensis*
var. *helicus*	var. *sublevisporus*	4. *Talaromyces galapagensis*
var. *major*	3. *Talaromyces rotundus*	5. *Talaromyces mimosinus*
var. *boninensis*	4. *Talaromyces austrocalifornicus*	6. *Talaromyces barcinensis*
4. *Talaromyces stipitatus*	5. *Talaromyces retardatus*	7. *Talaromyces assiutensis*
5. *Talaromyces striatus*	6.*Talaromyces euchlorocarpius*	
6. *Talaromyces panasenkoi*	7. *Talaromyces udagawae*	
7. *Talaromyces muroii*	8. *Talaromyces ucrainicus*	
8. *Talaromyces unicus*	9. *Talaromyces lagunensis*	
9. *Talaromyces intermedius*		

2. Section *Emersonii*	**3. Section *Thermophila***	**4. Section *Purpurea***
1. *Talaromyces bacillisporus*	1. *Talaromyces thermophilus*	1. *Talaromyces purpureus*
2. *Talaromyces leycettanus*		
3. *Talaromyces emersonii*		
4. *Talaromyces byssochlamydoides*		
5. *Talaromyces eburneus*		
6. *Talaromyces spectabilis*		

2. The molecular study of *Talaromyces*

2.1 Microsatellites or simple sequence repeats (SSRs)

Microsatellites or simple sequence repeats (SSRs) are composed of tandemly repeated, simple DNA sequence motif of as many as six nucleotides in length. These loci are commonly found throughout both prokaryotic and eukaryotic genomes and typically are highly polymorphic within species and populations. In addition, these codominant genetic markers are relatively easy to score and have high reproducibility and specificity. As such, microsatellites have become one of the most popular classes of molecular markers and are commonly employed to investigate the population genetics of a diverse range of organisms (Bruford and Wayne, 1993; Goldstein and Schlotterer, 1999; Dettman and Taylor, 2004).

For fungi, SSRs have been also used for phylogenetic analysis and these analyses have examined evolutionary relationships from the kingdom to species level and genetic polymorphism (Chen *et al.*, 2002; Nascimento *et al.*, 2004; Fisher *et al.*, 2004a, b.

White mold and stem rot caused by *Sclerotinia sclerotiorum* is a serious disease in agricultural and native plants at Canada. Sirjusingh and Kohn (2001) was able to show using microsatellites–based analysis the diversity of this fungus. It was also shown successfully amplified the closely related *S. trifoliorum* and *S. minor.*

Paracoccidioides brasiliensis, is a cause of paracoccidioidomycosis (PCM) and widespread in Central and South America, from Mexico to Argentina. Its natural habitat in soil or in plants in areas where PCM endemic. Microsatellite markers were used to elucidate the phylogenetic relationships of the environmental strains and the types of human disease they cause. The result showed no correlation between the clinical form of human PCM and SSR patterns (Fisher *et al.*, 2004a, b; Nascimento *et al.*, 2004) used multilocus microsatellite typing to examine the emergence of *Penicillium marneffei* in human HIV-positive populations.

2.2 Phylogenetic study of *Talaromyces*

Ribosomal DNA of 29 species of *Talaromyces* and related genera were examined in their phylogenetic relationships. The variability in the nuclear rDNA repeat unit was studied by the restriction fragments of total DNA that hybridized to the rDNA repeat unit of *Neurospora crassa* (pMF2). The result showed *Talaromyces* species with *Paecilomyces* anamorphic states cluster with *Byssochlamys* and *Thermoascus* species having *Paecilomyces* anamorphic states and not with *Talaromyces* species having *Penicillium* anamorphs. Whereas, the strictly anamorphic *Penicillium* species are not mixed in with the holomorphic species, but are clustered in a group that it well separated from most of the *Talaromyces* species (Taylor *et al.*, 1990).

LoBuglio *et al.*, (1993) was able to reveal the relationship between *Talaromyces* with *Penicillium* anamorphs and strictly mitotic species of *Penicillium* subgenus *Biverticillium* using ribosomal DNA (rDNA) nucleotide characters. They demonstrated that some mitotic *Penicillium* species have a closer phylogenetic relationship to meiotic species than they do to other strictly mitotic *Penicillium* species.

Berbee *et al.*, (1995) concluded that *Penicillium* is not monophyletic based on analysis of the variable ITS region and 5.8, 18S regions of the ribosomal DNA. *Penicillium* species that have a *Eupenicillium* teleomorph grouped with *Aspergillus* whereas *Penicillium* species with *Talaromyces* teleomorphs grouped with *Paecilomyces*. This study, however, only looked at three of *Penicillium* spp. and *Aspergillus* spp. with two *Paecilomyces* species.

Talaromyces ocotl with *Sagenomella* sp.-anamorph described as new species, based on morphological analyses and phylogenetic inferences made from ITS and 28S rDNA sequence alignments. *Talaromyces ocotl* and *T. rotundus* were isolated soil from *Pinus hartwegii* and *Pinus patula* forests at the Volcanic Cordillera in Mexico (Heredia *et al.*, 2001).

Yaguchi *et al.*, (2005) were able to demonstrate, using D1/D2 region of 28 S rDNA sequence analysis, that *Geosmithia argillacea* is the anamorph of *Talaromyces eburneus* as a heat resistant fungus. They are often reported spoilage agents in fruit juices, food and other heat processed fruit based products (Samson *et al*., 2002; Udagawa, 2000).

3. Biological Control of plant pathogenic fungi by *Talaromyces*

Talaromyces flavus (Klöcker) Stolk & Samson (conidial state: *Penicillium vermiculatum* Dangeard) was reported to be antagonistic to many plant pathogenic fungi, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Verticillium albo-atrum*, and *V. dahliae*. A variety of mechanisms have been postulated for biocontrol of pathogens by *T. flavus* including hyperparasitism, competition for nutrients, and antibiosis (Boosalis, 1956; Duo-Chuan *et al.*, 2005; McLaren *et al*., 1986; Kim *et al.*, 1988; Stosz *et al*., 1998).

Boosalis (1956) observed that *T. flavus* invades hyphae of *Rhizoctonia solani* directly by producing penetration pegs. These pegs developing from either a mycelium coiling around the host hyphae or form a hyphae in direct contact with the host. Later, McLaren *et al.*, (1986) demonstrated hyperparasitism of sclerotinia wilt caused by *Sclerotinia sclerotiorum* by *T. flavus*, using light and scanning electron microscopy. The hyphae of *T. flavus* could grow toward and coiled around the host hyphal cells. The coiling effect intensified as the hyphae of *T. flavus* branched repeatedly on the host surface. Tips of hyphal branches often invaded the host by direct penetration of the cell wall without formation of appressoria. Moreover infection of host cells by *T. flavus* resulted in granulation of the cytoplasm and collapse of the cell walls.

Production of the enzymes glucose oxidase has been shown to be involved in the biological control of Verticillium wilt by *T. flavus* (Ayer and Racok, 1990a). Stosz *et al.*, (1998) reported the location of glucose oxidase in *T. flavus* by immunocytochemistry using glucose oxidase-specific polyclonal antibody. The results

showed that glucose oxidase was found in both intracellular and extracellular, both mature and young hyphal cells contained this enzyme, but decreased as the cells aged. Enzyme stability studies confirmed that the glucose oxidase of *T. flavus* is an extremely stable enzyme, retaining 13% of its original activity after 2 weeks at 25°C and retaining activity for several days at temperatures up to 50°C.

Duo-Chuan *et al.*, (2005) purified two chitinases from the culture filtrate of *T. flavus*. By SDS-PAGE, the molecular weight of the two enzymes was estimated to be 41 and 32 kDa respectively. The two chitinases can degrade cell wall of *Verticillium dahliae, Sclerotinia sclerotiorum* and *Rhizoctonia solani*, and inhibited spore germination and germ tube elongation of *Alternaria alternata, Fusarium moniliforme*, and *Magnaporthe grisea*.

Hydrogen peroxide is one of the products of a reaction catalyzed by glucose oxidase in the presence of glucose. Kim *et al.*, (1988) purified and characterized the glucose oxidase from *T. flavus* culture filtrates and determined that this enzyme inhibited germination of *V. dahliae* microsclerotia *in vitro* in the presence of glucose. *In vitro* exposure to hydrogen peroxide or to glucose oxidase in the presence of glucose is lethal to the microsclerotia of *V. dahliae*. (Kim *et al.*, 1988; Stosz *et al.*, 1996).

Nagtzaam and Bollen (1997) reported *Talaromyces flavus* has potential as biocontrol agent against of *Verticillium dahliae*. The fungus could colonize potato and eggplant roots. In pot experiments with field soils, the fungus could growth from potato seed tube or eggplant seeds coated to developing roots, including the root tips. The population of *T. flavus* decreased log-linearly with distance from the seed and was higher with potatoes than with eggplants.

Tjamos and Fraval (1997) reported the distribution and establishment of *T. flavus* in the soil and on roots of solanaceous crops. Ascospores of *T. flavus* were applied to eggplant, tomato and potato as either a drench or in spherical granular (alginate prill). The percentage colonization of roots was greater than that of root tips.

When populations of *T. flavus* were expressed as colony forming units g-1 fresh weight, recovery of *T. flavus* was greater in root tips and superficially associate with the roots. Populations were lower in roots, rhizosphere and non-rhizosphere soils, respectively. Populations of *T. flavus* were greater when it was applied as a drench rather than in alginate prill.

Jun *et al.*, (1999) studied on the antagonism of *T. flavus* against pathogenic fungi infecting cotton in China, including *Verticillium dahliae*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Rhizoctonia solani* and *Colletotrichum gossypii in vitro* on CZA and wheat grain media. Results showed that *T flavus* inhibited the radial growth of *V. dahiae*, *F. oxysporum* f. sp. *vasinfectum*, *R. solani* and *C. gossypii*, and the conidial germination of *Verticillium dahliae*, *Fusarium oxysporum* f. sp. *vasinfectum*, but did not inhibit *R. solani* and *C. gossypii*. The inhibitory activity of *T. flavus* was affected by glucose concentration in Czapek medium, with the highest activity recorded when glucose was at 0.5-4.0%.

In Canada, Inglis and Kawchuk (2002) tested the ability of *Talaromyces flavus* to degrade cell walls of *Pythium ultimum* (Oomycete), *Fusarium equiseti* (Ascomycete) and *Rhizoctonia solani* (Basidiomycete) *in vitro*. The results showed that *T. flavus* could degrade cell walls of *P ultimum* and *F equiseti*. Production of carboxymethyl cellulase, ß- glucanases, chitinases and chitosanases were reported.

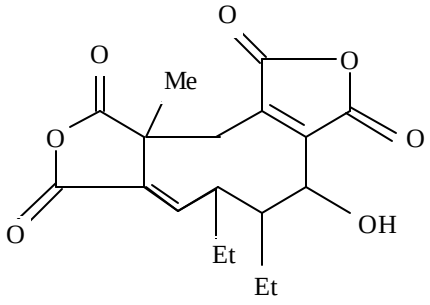
4. Chemistry of the Genus *Talaromyces* C.R. Benjamin

Fungi are know to produce a vast number of bioactive compounds. After the discovery of penicillin G from a *Penicillium* species about 80 years ago that led to many researches were motivated to search and screening large collection of fungi especially antibiotic property for novel drug (Larsen *et al.*, 2005). Until 1995, the top twenty selling prescription medicines worldwide were of fungal origin (Langley, 1997; Peberdy, 1999; Singh and Aneja, 1999; Skehan *et al.*, 1990). *Talaromyces* is an interesting fungus for this purpose.

In recent years, seventeen species of the genus *Talaromyces* including *T. assiutensis, T. austrocalifornicus, T. bacillisporus, T. convolutes, T. derxii, T. emodensis, T. flavus, T. luteus, T. macrosporus, T. mimosinus, T. ohiensis, T. panasenkoi, T. stipitatus, T. tardifaciens, T. trachyspermus, T. udagawae* and *T. wortmannii* have been investigated for their secondary metabolites and its activity (Ayer and Racok, 1990 a,b; Dong *et al.*, 2006; Frisvad *et al.*, 1990; Ishii *et al.*, 1995; Nozawa *et al.*,1995; Phillips *et al.*, 1987; Shiozawa *et al.*, 1995; Suzuki *et al.*, 1992, 1999, 2000; Yamazaki and Okuyama, 1980; Yoshida *et al.*, 1996).

4.1 *Talaromyces assiutensis*

Frisvad *et al.*, (1990) investigated the profiles of secondary metabolites of *Talaromyces* and found that *T. assiutensis* can produce glauconic acid (**1**).



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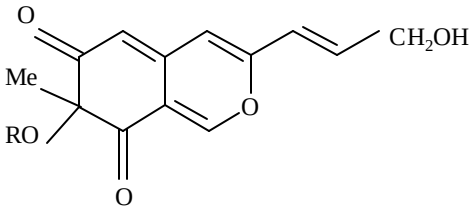
4.2 *Talaromyces austrocalifornicus*

Suzuki *et al.*, (1999) reported the isolation of (-)- mitorubrinal (**2**) and (-)- mitorubrinic acid (**3**) from yellow pigments on ascomata of *T. austrocalifornicus*.



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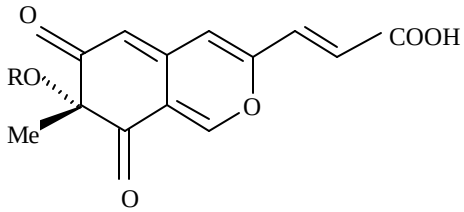

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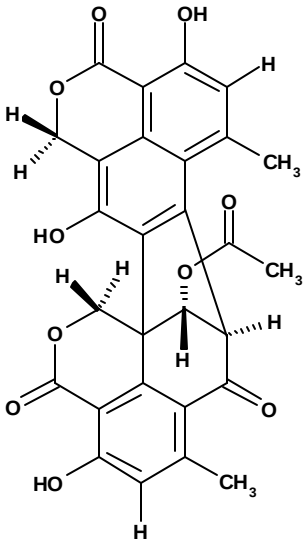
4.3 *Talaromyces bacillisporus*

Oxaphenalenone dimers and xanthone carboxylic acid were isolated from *T. bacillisporus* as bacillosporins A (**4**), B (**5**), C (**6**), pinselin and pinselic acid. Compound **4** exhibited antibacterial activity against *Bacillus subtilis* and *Sarcina lutea* (Yamazaki and Okuyama, 1980).



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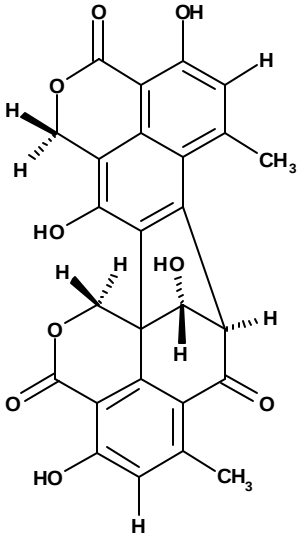

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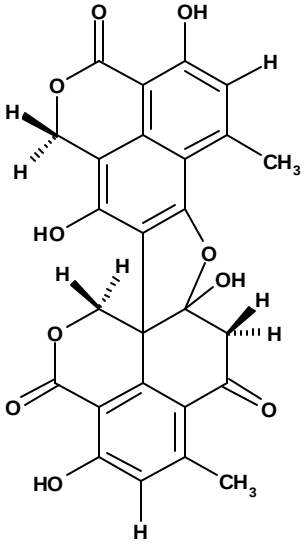

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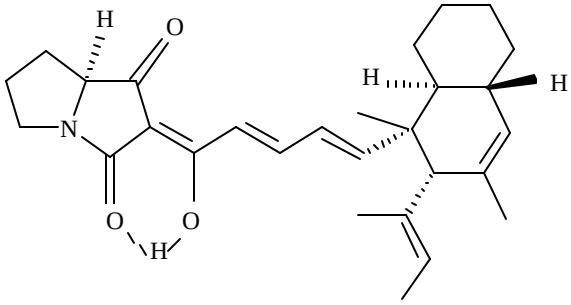
Furthermore, Ishii *et al.*, (1995) isolated talarotoxin (**7**) from *T. bacillisporus* that showed cytotoxic activity inhibited the proliferation of both mouse myeloma X63.Ag8.6.5.3 cells and BALB/3T3 mouse fibroblasts.



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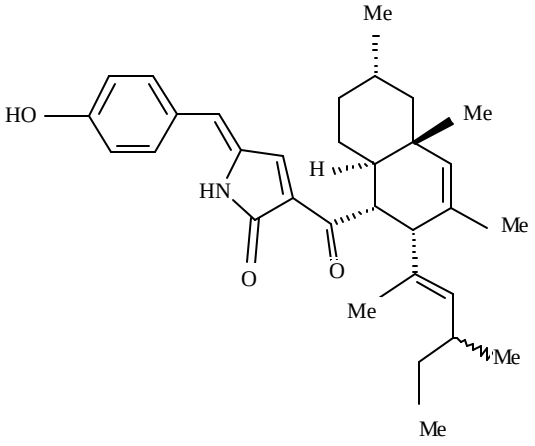
4.4 *Talaromyces convolutus*

The fungus contained the secondary metabolites: talaroconvolutins A (**8**), B (**9**), C (**10**) and D (**11**). Compounds **8** and **9** were reported to inhibit he growth of *Aspergillus fumigatus*, *A. niger* and *Candida albicans* (Suzuki *et al.*, 2000).



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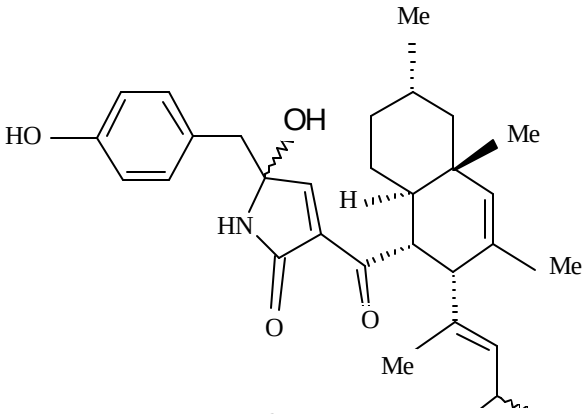

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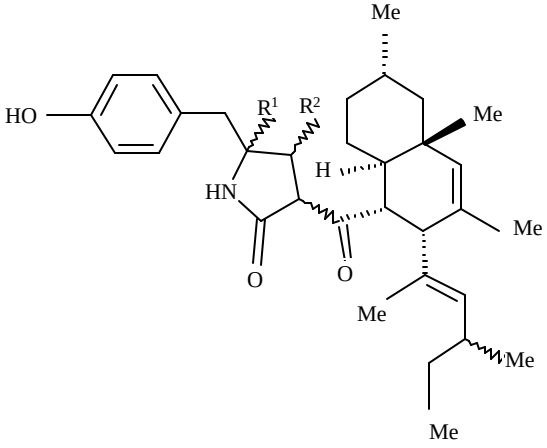
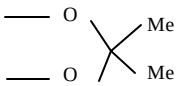


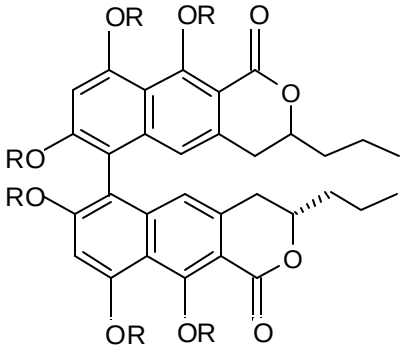
10 R^1 = OH, R^2 = OEt

11 R^1, R^2 = 

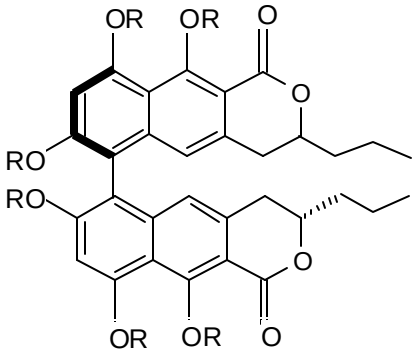
Moreover, Suzuki *et al.*, (1999) have reported the isolation of (-)-mitorubrinal (**2**) and (-)- mitorubrinic acid (**3**) from yellow pigment on ascomata of *T. convolutus*.

4.5 *Talaromyces derxii*

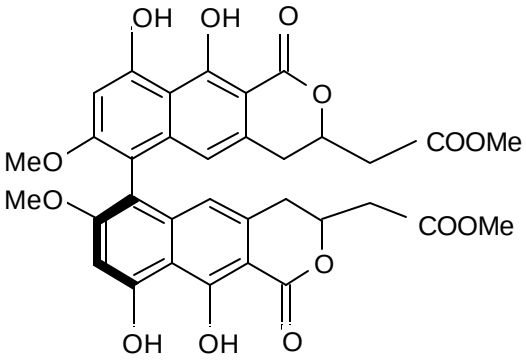
Secondary metabolites isolated from *T. derxii* including; talaroderxines A (**12**) and B (**13**), viriditoxin (**14**), vioxanthin (**15**), semiviriditoxin (**16**), hexamethyl ether (**17**) and vioxanthin (**18**) have strong antibacterial activity against *Bacillus subtilis* (Suzuki *et al.*,1992)



12 R = H
17 R = Me



13 R = Ac



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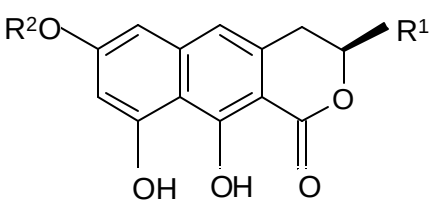
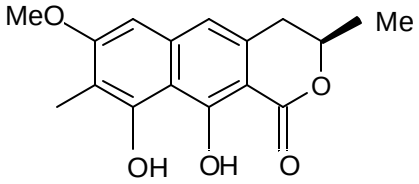


15 R^1 = Me, R^2 = H

16 R^1 = CH_2COOMe, R^2 = Me



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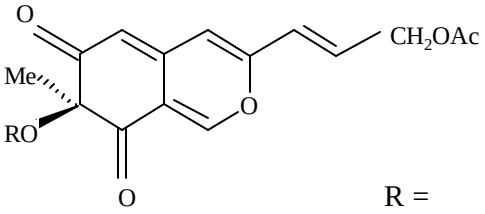
4.6 *Talaromyces emodensis*

Suzuki *et al.*, (1999) isolated (+)- mitorubrinol acetate (**19**) from yellow pigment on ascomata of *T. emodensis.*



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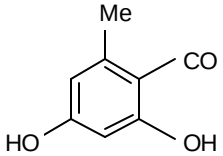
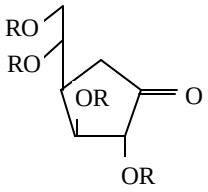
R =

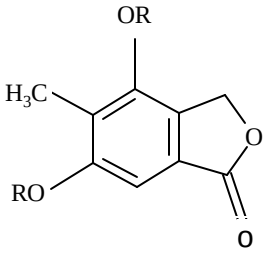


4.7 *Talaromyces flavus*

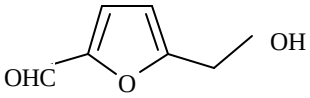
A number of secondary metabolites have been isolated from *T. flavus* including D-glucono-1,4-lactone (**20**), acetylation of D-glucono-1,4-lactone (**21**), 4,6 –dihydroxy-5-methylphthalide (**22**), methylation of 4,6 –dihydroxy-5-methylphthalide (**23**), 5-hydroxymethylfurfural (**24**), 7-hydroxy-2,5-dimethylchromone (**25**), methylation of 7-hydroxy-2,5-dimethylchromone (**26**), methyl 4-carboxy-5-hydroxyphthaladehydate (**27**), (**28**), 3-hydroxymethyl-6,8-dimethoxycoumarin (**29**), altenusin (**30**), dehydroaltenusin (**31**), dehydroaltenusin diacetate (**32**), desmethyldehydroaltenusin (**33**), acetylation of desmethyldehydroaltenusin (**34**), talaroflavone (**35**), acetylation of talaroflavone (**36**), deoxytalaroflavone (**37**),



20 R = H
21 R = $COCH_3$



22 R = H
23 R = CH_3



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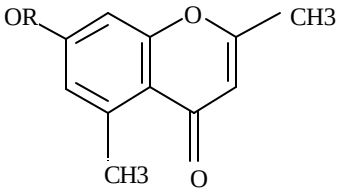
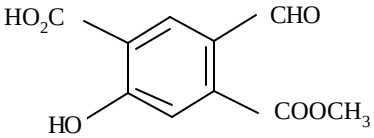


25 R = H
26 R = CH_3



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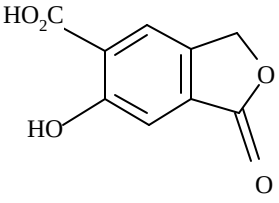

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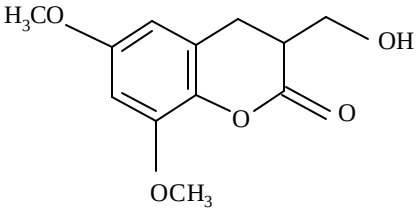

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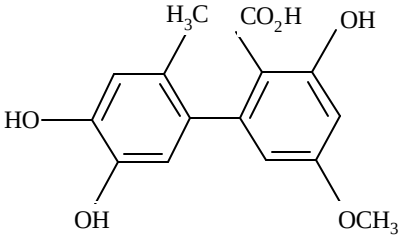

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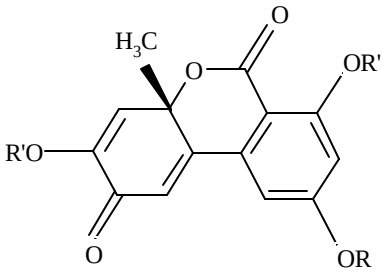
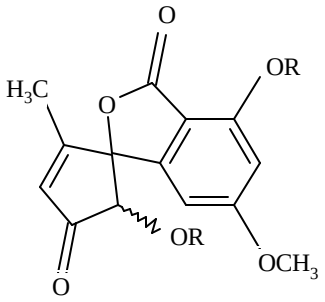
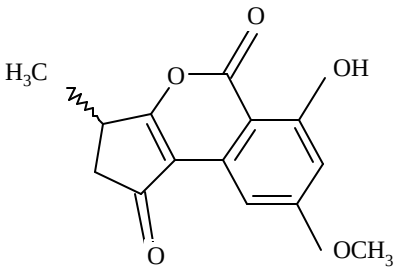


31 R = CH_3, R' = H
32 R = CH_3, R' = $COCH_3$
33 R = H, R' = H
34 R, R' = $COCH_3$

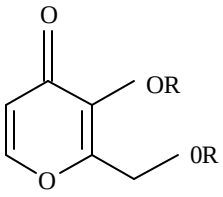


35 R = H
36 R = $COCH_3$

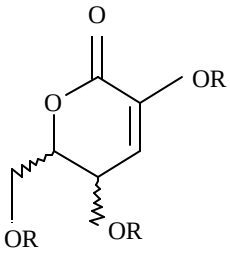


37

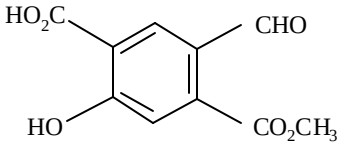
hydroxymethylmaltol (**38**), acetylation of hydroxymethylmaltol (**39**), 6-hydroxymethyl-2H-pyran-2-one (**40**), acetylation of 6-hydroxymethyl-2H-pyran-2-one (**41**), methyl 4carboxy-5-hydroxyphthalaldehydate (**42**), vermiculine (**43**) and wortmannin (**44**) (Ayer and Racok, 1990 a,b; Frisvad *et al.*, 1990).



38 R = H
39 R = $COCH_3$



40 R = H
41 R = $COCH_3$



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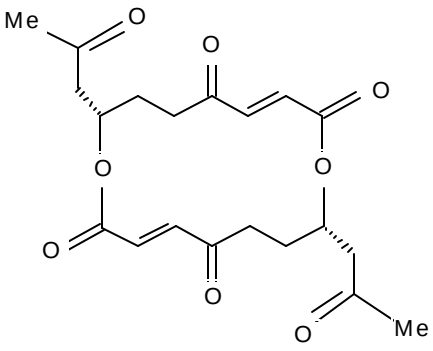

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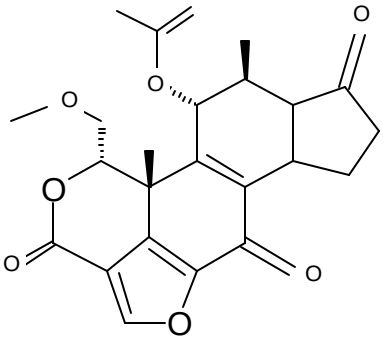
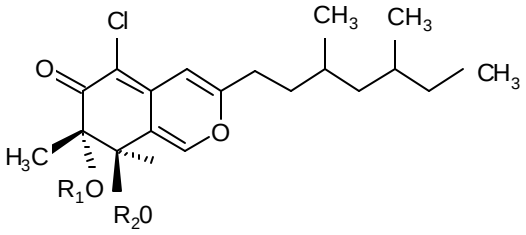


44

4.8 *Talaromyces luteus*

Yoshida *et al.*, (1996) have isolated three new azaphilones, luteusins A (**45**), B (**46**), C (**47**), D (**48**) and E (**49**) from *T. luteus*.



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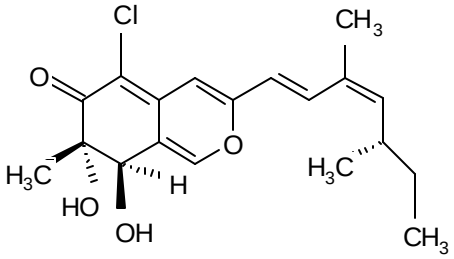

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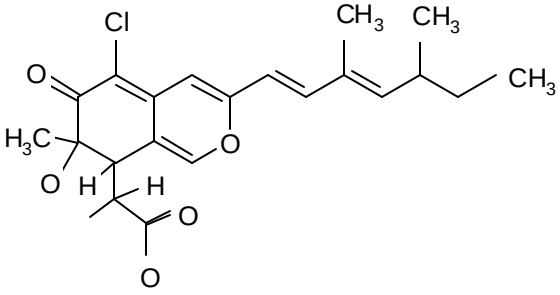

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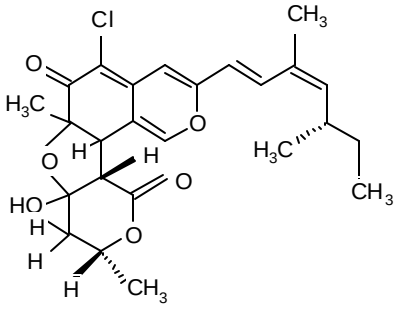

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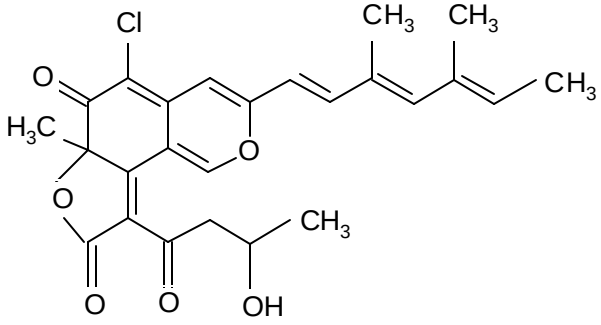
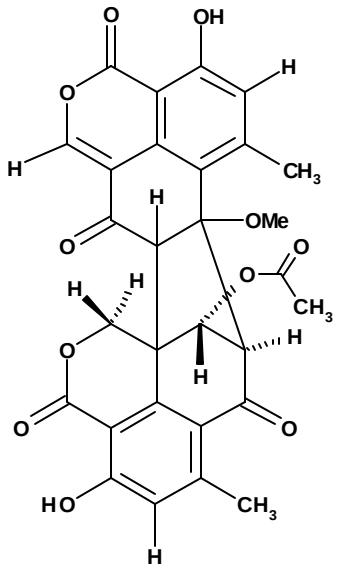


49

4.9 *Talaromyces macrosporus*

Frisvad *et al.*, (1990) reported the isolation of duclauxin (**50**) from this fungus.



50

4.10 *Talaromyces mimosinus*

Frisvad *et al.*, (1999) have reported the isolation of (-)- mitorubrinal (**2**) and (-)- mitorubrinic acid (**3**) from yellow pigments on ascomata of this fungus.

4.11 *Talaromyces ohiensis*

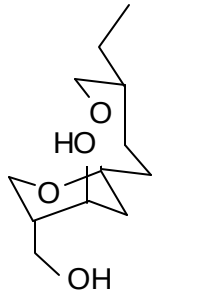
Frisvad *et al.*, (1990) reported glauconic acid (**1**) and vermiculine (**43**) from this fungus.

4.12 *Talaromyces panasenkoi*

Frisvad *et al.*, (1990) reported glauconic acid (**1**) from this fungus.

4.13 *Talaromyces stipitatus*

Phillips *et al.*, (1987) reported four new spiroketal talaromycins derived, talaromycins C (**51**), D (**52**), E (**53**) and F (**54**) from *T. stipitatus* as well as the known talaromycins A, B from *Talaromyces flavus*. Later, Frisvad *et al.*, (1990) reported more compounds from this fungus including duclauxin (**50**), catenarin (**55**), emodin (**56**), stipitatic acid (**57**) and erythroglaucin (**58**).



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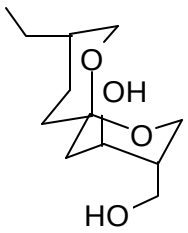

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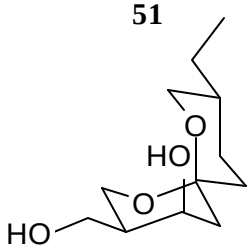

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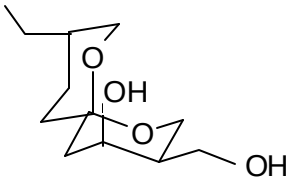

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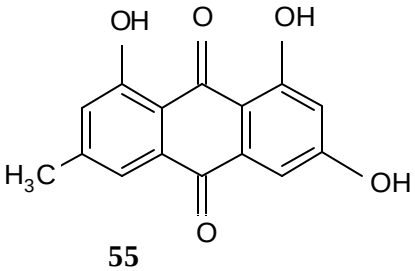

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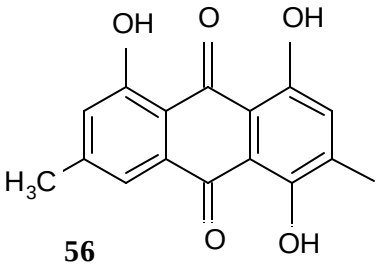

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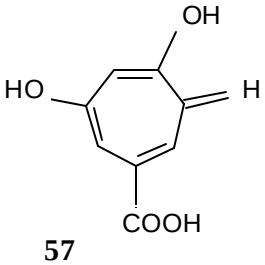

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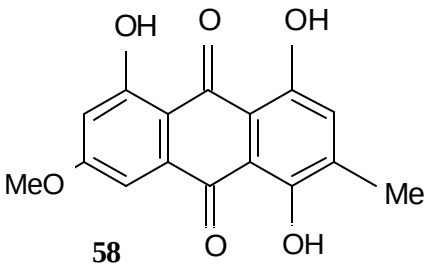
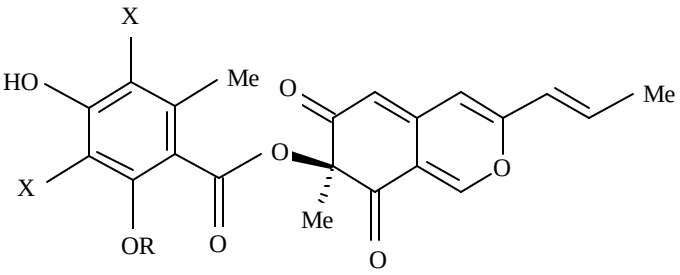


58

4.14 *Talaromyces tardifaciens*

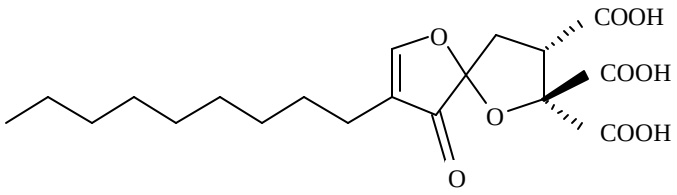
The monomethyl-(+)–mitorubrin (**59**), (+)–mitorubrin (**60**) and falconensin H (**61**) have been isolated from this fungus (Nozawa *et al.*,1995).



59 R = Me, X = H
60 R = H, X = H
61 R = Me, X = Cl

4.15 *Talaromyces trachyspermus*

Shiozawa *et al.*, (1995) reported a new metabolites, trachyspic acid (**62**) from *T. trachyspermus* that inhibits tumor cell heparanase.



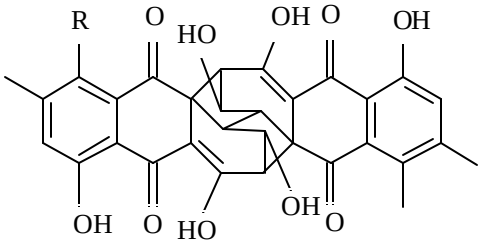
62

4.16 *Talaromyces udagawae*

Frisvad *et al.*, (1990) reported the isolation of (-)- mitorubrinal (**2**) and (-)- mitorubrinic acid (**3**) from this fungus.

4.17 *Talaromyces wortmannii*

Frisvad *et al.*, (1990) isolated mitorubrin (**2**), mitorubrinol acetate (**19**), rugulosin (**63**) and skyrin (**64**) from this fungus. Later, Dong *et al.*, (2006) reported four new compounds including wortmanilactones A (**65**), B (**66**), C (**67**) and D (**68**) from *T. wortmannii*. All compounds were screened for cytotoxic activities against a panel of five human cell lines (HCT-5, HCT-115, A549, MDA-MB-231, and K562). The IC50 values of the compounds range from 28.7 to 130.5 µM.



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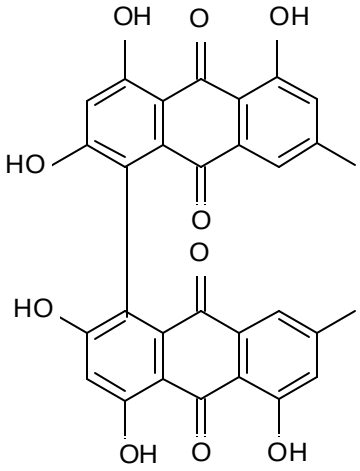

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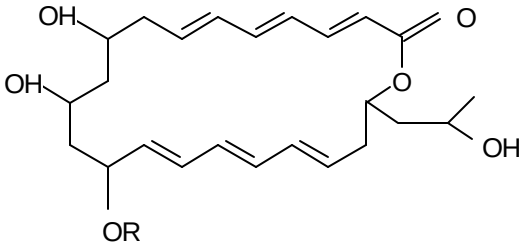
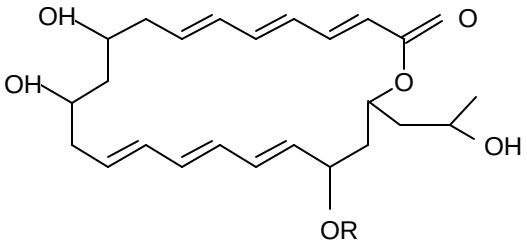


65 R = H
66 R = CH_3



67 R = H
68 R = CH_3