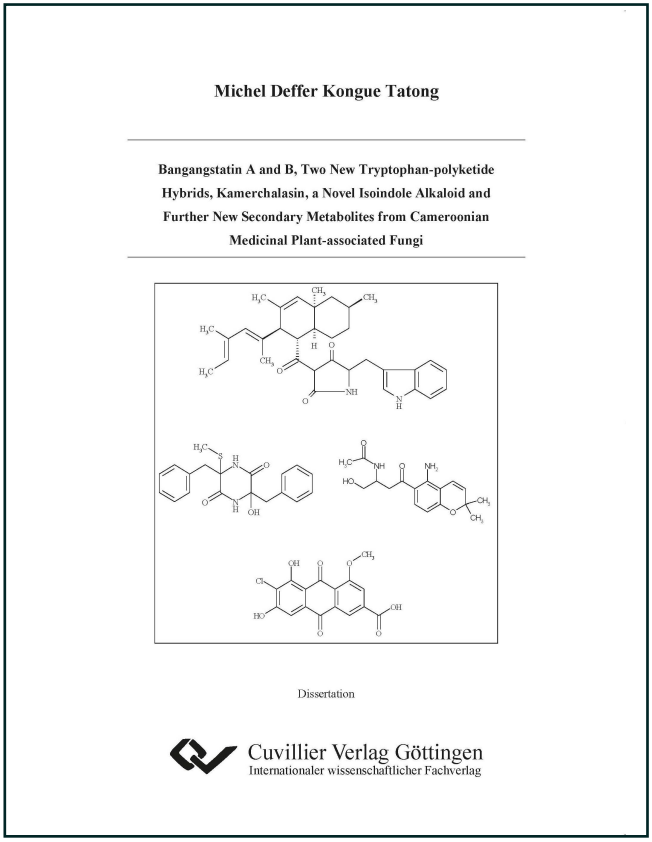
Michel Deffer Kongue Tatong (Autor)

Bangangstatin A and B, Two New Tryptophan-polyketide Hybrids, Kamerchalasin, a Novel Isoindole Alkaloid and Further New Secondary Metabolites from Cameroonian Medicinal Plant-associated Fungi



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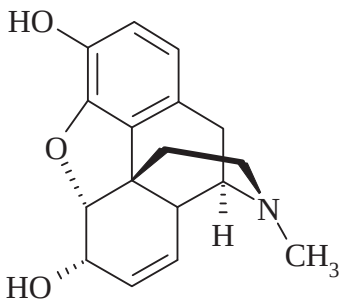
1 Introduction

1.1 Brief history of natural products

For centuries medicine and natural products have been closely linked through the use of traditional medicines. Plants, insects, microorganisms and marine organisms exhibit complex interactions with the environment and produce small molecules (natural products) useful for their survival[1]. Natural products have served mankind as the source of drugs, and higher plants provided most of these therapeutic agents. Today, natural products (their derivatives and analogs) still represent over 50% of all drugs in clinical use, with higher plant-derived natural products representing *ca*. 25% of the total. The World Health Organization estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care, and about 85% of traditional medicine involves the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs[2]. Clinical, pharmacological, and chemical studies of these traditional medicines, which were derived predominantly from plants, were the basis of most early medicines such as the famous analgesic morphine (**1**) isolated from opium, the latex produced by cutting the seed pods of poppy, *Papaver somniferum* and the world-famous aspirin (**2**) with analgesic, anti-inflammatory, and antipyretic properties[3]. The latter is a derivative of salicylic acid, which was first isolated from willow trees *Salix* sp. and has been modified to improve the activity and reduce side-effects. Aspirin (**2**) is one of the best-known examples for the prosperous interaction of natural and synthetic products chemistry.



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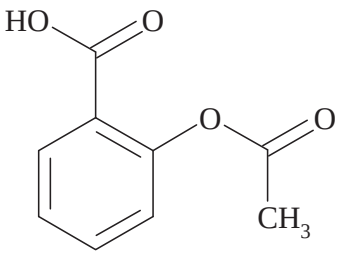

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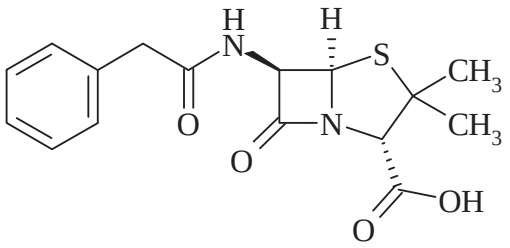
The limitation of plant materials due to environmental degradation, loss of biodiversity, spoilage of land and plant material availability has made it difficult to investigate medicinal plants in some parts of the world. Despite a significantly high number of natural product-derived drugs which continue to be isolated and reported from plants, the ingress to the human population of new diseases and multi-drug-resistant bacteria requires the discovery and development of new drugs to combat them. The need for new and useful compounds to provide assistance and relief in all aspects of the human condition is ever growing[4]. This needs obligated researchers to find out an alternative source for secondary metabolites such as terrestrial plants associated fungi, which have a special adaptability to survive in extreme conditions.

1.2 Fungi as sources of bioactive products

Fungi are widespread, non-photosynthetic microorganisms that play a vital role in the environment, particularly in the biodegradation of organic material. Fungi are remarkable organisms that readily produce a wide range of natural products belonging to the group of so-called secondary metabolites[5]. The study of their metabolites and metabolism has made many contributions to the overall development of chemistry. Although the biosynthetic pathways utilized by the fungi to construct their metabolites have general features in common with those found in bacteria, plants and mammals, they differ in details and the structures of the resultant natural products. The chemical activities of fungi have a long history. Due to the competitive nature of the environment in which they live, many fungi produce antibiotics of varying efficiency. Nearly 3000 years ago the Mayans used fungi to treat intestinal ailments[6]. Without deeper knowledge about the mode of action the transformation by fungi has been used for food production since Neolithic times. The earliest types of fermented food were beer, wine, and leavened bread, followed by the early Chinese who produced fermented soy foods. The discovery of a potent antibiotic active against Gram-positive bacteria, penicillin (**3**) by the biologist and pharmacologist Alexander Fleming (1881-1955) from *Penicillium notatum* in 1928, which was reported in 1929 in the British medical literature, revolutionized medicinal chemistry after a lengthy gestation period. The success of penicillin (**3**) with its derivatives encouraged scientists to search for further antibiotics from microorganisms.



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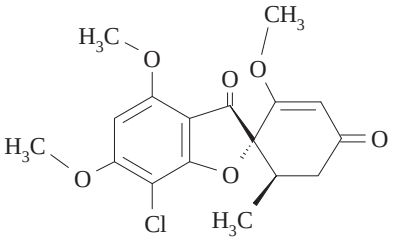
Since then, fungi isolated especially from soil samples have been identified as a rich source of biologically active secondary metabolites. Beside other well known antifungal agents, griseofulvin (**4**) was the first antifungal natural product produced by the filamentous fungi[7]; antimicrobial agents like fusidic acid (**5**) and semisynthetic antifungal drugs like anidulafungin (**6**), whose precursor echinocandin B was isolated from fermentation broth of *Aspergilus nidulans*, are further milestones of success.



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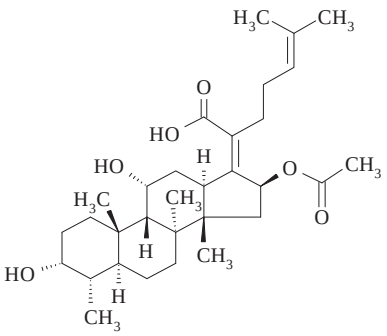

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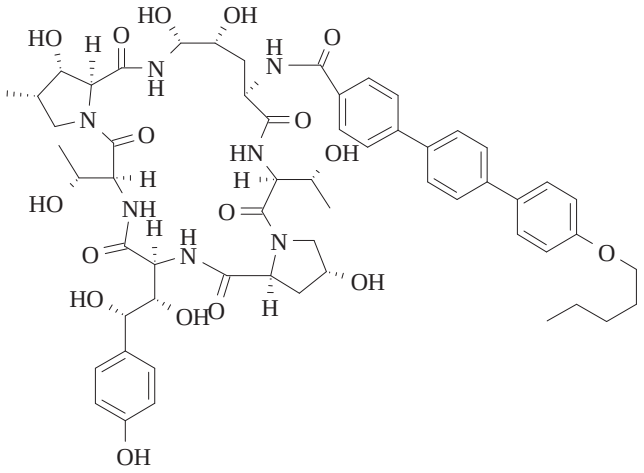

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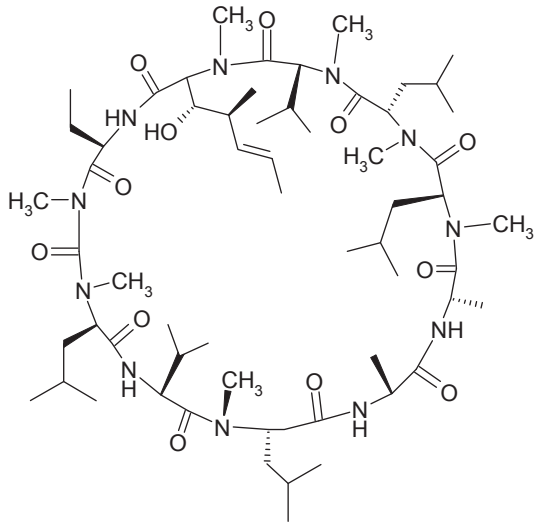
Furthermore, a new era in immunopharmacology began with the discovery of the cyclic undecapeptide cyclosporine (**7**), isolated from *Tolypocladium inflatum*, in 1971. It was the first immunosuppressive drug that allowed selective immunoregulation of T cells without excessive toxicity and was used as an immunosuppressant during organ transplantations[8]. It is now widely exploited in organ and tissue trans-

plant surgery, to prevent rejection following bone marrow, kidney, liver and heart transplants. It has revolutionized organ transplant surgery, substantially increasing survival rates in transplant patients[9]. Improvements in the field of organ transplantations and treatment of autoimmune diseases are still in progress: Another strongly immunosuppressive fungal metabolite that is used for organ transplantations and for treatment of autoimmune diseases is mycophenolic acid[10] (**8**), which was first isolated from a *Penicillium* culture by Gosio in 1896[11]. Cyclosporine (**7**) exhibits, in addition to its potent immunosuppressant activity, pronounced antiviral activity. Therefore it furthermore served as a model for the design of substances like Debio-025, a potential antiviral drug that has successfully passed clinical trials[12,13].



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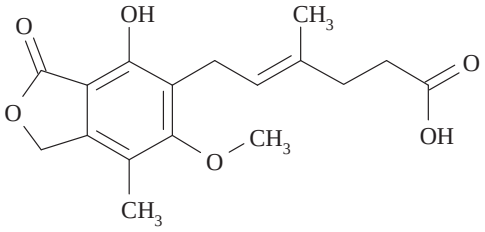

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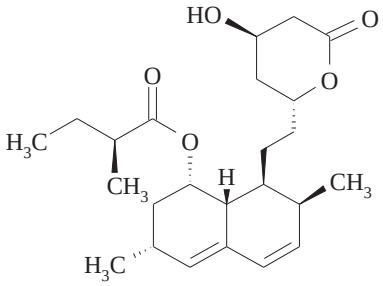
Additionally, one of the most economical important fungal metabolites represent antilipidemic drugs collectively known as statin compounds. Statins are the most potent cholesterol-lowering agents available on the market today. They are either fermentation-derived, for example mevastatin and lovastatin (**9**) from *Penicillium citrinum* and *Aspergillus terreus*, respectively, or synthetic analogues[12].



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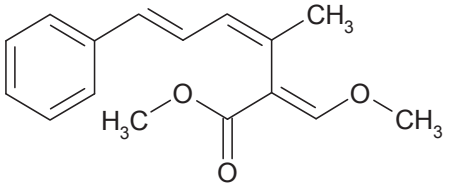
Fungal metabolites have also found applications in agriculture for plant protection as demonstrated by the discovery of the strobilurin A (**10**), from *Strobilurus* sp. that served as a lead compound for synthetic fungicidals such as trifloxystrobin[14] (**11**).



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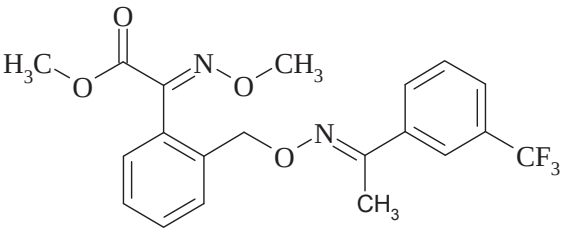

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The growth of fungi depends on the environment in which they occur. Some attack plants, insects and mammals as pathogens, whilst others are saprophytic and grow on dead organic materials. Some live in a positive symbiotic relationship with a host organism. Thus, there are mycorrhizal fungi that are associated with the roots of plants and facilitate the uptake of nutrients by the plant. Others are endophytic organisms that grow within the vascular system of plants. Throughout the natural world there is a chemical language between the fungus and its host, which determines the nature of this relationship. The role of fungal and plant metabolites in this ecological communication is still widely unknown.

1.3 Endophytic fungi as a sources of bioactive natural products

Since the discovery of endophytes in Darnel, Germany in 1904, various investigators have defined endophytes in different ways, which is usually dependent on the perspective from which the endophytes were being isolated and subsequently examined[15]. The term “endophytes” includes a suite of microorganisms that grow intra- and/or intercelullarly in the tissues of higher plants without causing ever symptoms

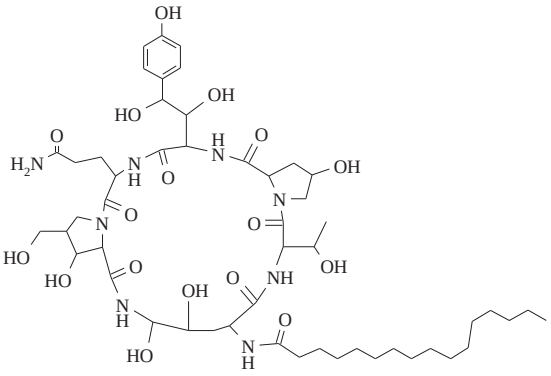
on the plants in which they live. Mutualistic interactions between endophytes and host plants may result in fitness benefits for both partners. The endophytes may provide protection and survival conditions to their host plant by producing a plethora of substances, which once isolated and characterized, may also have potential for use in industry, agriculture, and medicine[16].

Endophytic fungi are important components of plant micro-ecosystems[2,16]. Plant endophytic fungi have been found in each plant species examined, and it is estimated that there are over one million fungal endophytes existing in nature[17]. Plant endophytic fungi have been recognized as an important and novel source of natural bioactive products with potential application in agriculture, medicine and food industry[6,18]. Since the discovery of the "gold standard" in anticancer therapy, paclitaxel (taxol) from the endophytic fungus *Taxomyces andreanae* in 1993[19], there has been an increased interest in studying fungal endophytes as potential producers of novel and biologically active compounds. In the past two decades, many valuable bioactive compounds with antimicrobial, insecticidal, antiviral, antimycotic and anticancer activities have been discovered from endophytic fungi. In fact, a recent comprehensive study has indicated that 51% of biologically active substances isolated from endophytic fungi were previously unknown[19,20]. These bioactive compounds could be classified as alkaloids, terpenoids, steroids, quinones, lignans, phenols and lactones[4, 21]. So far, only a small percentage of these metabolites have been carried forward as natural product drugs, nevertheless they represent interesting structures which indicate the great chemical diversity and pharmaceutical potential of endophytic fungi as sources of novel drug leads.

1.3.1 Secondary metabolites from endophytic fungi as antimycotic agents

The vast number and variety of chemotherapeutic agents isolated from microbial natural products and used to treat bacterial infections have greatly contributed to the improvement of human health during the past century. However, only a limited number of antimycotic agents are currently available for the treatment of life-threatening fungal infections[22] indicating the need for new antimycotics.

A unique lipopeptide antimycotic, termed cryptocandin A (**13**), was isolated and characterized from *Cryptosporiopsis quercina*, an endophyte in *Tripterigeum wilfordii*, a medicinal plant belonging to the family Celastraceae that is native to Eurasia[23].



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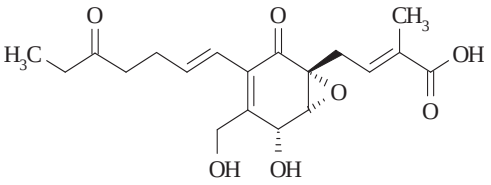

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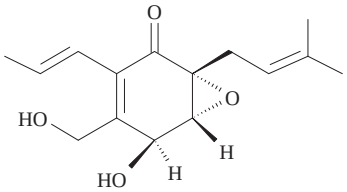

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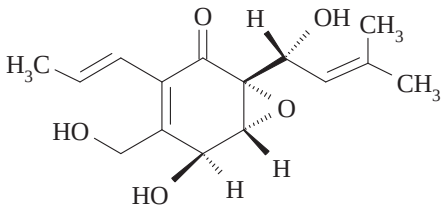
Other fungal metabolites with promising antifungal activity are ambuic acid (**12**), described recently from several isolates of *P. microspore* found in many plants in the world's rainforests[24], as well as jesterone (**14**) and hydroxyjesterone (**15**) from *Pestalotiopsis jesteri*, a newly described species of *Pestalotiopsis*[25]. Furthermore, a new pentaketide antifungal agent, CR377 (**16**), was isolated from the culture broth of an endophytic *Fusarium* sp. from the plant *Selaginella pallescens* collected in Costa Rica. It showed potent activity against *Candida albicans* in agar diffusion assays performed on fungal lawns[26].



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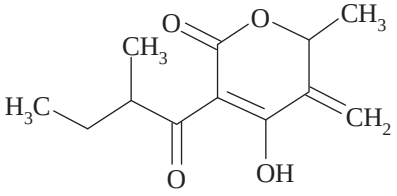

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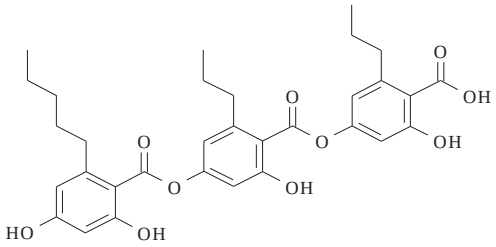
1.3.2 Secondary metabolites from endophytic fungi as antiviral agents

Another fascinating use of antibiotic products from endophytic fungi is the inhibition of viruses. Thus, two novel human cytomegalovirus protease inhibitors, cytonic acid

A (**17**) and B (**18**), have been isolated from the solid-state fermentation of the endophytic fungus *Cytonaema* sp.



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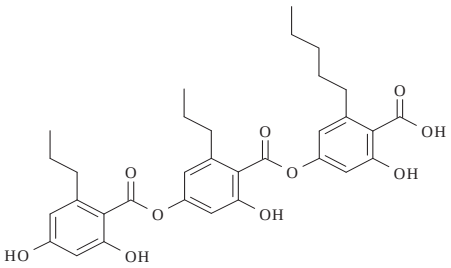

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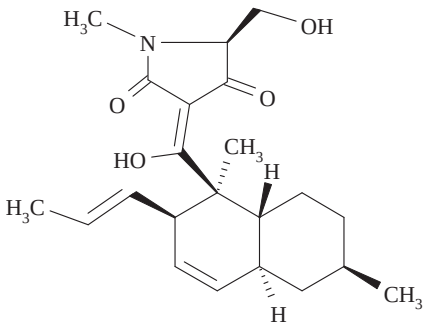
A tetramic acid, equisetin (**19**), was reported to be produced by a number of *Fusarium* spp. Interest in equisetin (**19**) emerged with reports of its inhibitory activity against human immunodeficiency virus type 1 (HIV-1) integrase *in vitro* having a mode of action quite different from those of previously described inhibitors[27]. Equisetin (**19**) inhibits 3'-end-processing and strand transfer as well as disintegration catalysed by either the full-length enzyme or the truncated integrase core. It was also reported as the first natural inhibitor of integrase[28]. Screening of natural product extracts against recombinant HIV-1 integrase led to the discovery of several classes of natural product inhibitors including integracins, integric acid, and oxygenated tetracyclic triterpenoids integracides A-D (**21**a-c) from *Fusarium* sp. Among them, the most active compound was found to be the sulphated integracide A[29].



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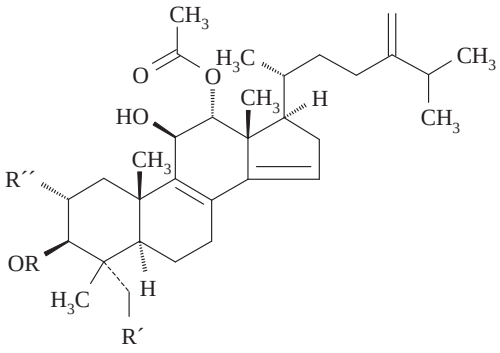


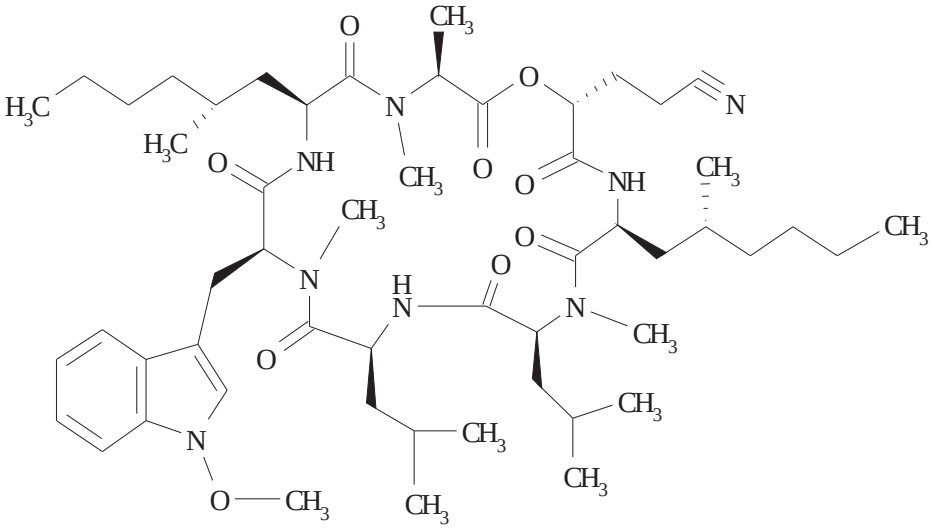
21a: R = SO_3H, R' = H, R'' = OH
21b: R = R' = H, R'' = OH
21c : R = H, R' = R'' = OH

1.3.3 Secondary metabolites from endophytic fungi as antibiotics

Pasteur was one of the first scientists to recognize the antagonism between microorganisms, which led to the use of the term ‘antibiote’ in 1889 by the French biologist

Vuillemin to describe the substances involved. Waksman redefined the term antibiotic much later in 1941 to describe a natural product formed by a microorganism that inhibited the growth of or killed another microorganism[30]. Matabolites bearing antibiotic activity can also be defined as law-molecular weight organic natural substances made by microorganisms that are active at low concentrations against other microorganisms[31]. The discovery of novel antimicrobial metabolites from endophytes is an important alternative to overcome the increasing levels of drug resistance by plant and human pathogens[32]. The antimicrobial compounds can be used not only as drugs by mankind but also as food preservatives in the control of food spoilage and food-borne diseases, which poses serious concerns in the world food chain[33].

The cyclopeptolide antibiotic HUN-7293, pestahivin (**22**), was purified from *Pestalotiopsis* sp. RF5890[34]. The antibiotic was reported as a naturally-occurring inhibitor of inducible cell adhesion molecule expression and the prototypical lead of a new class of potential therapeutics for the treatment of chronic inflammatory disorders or autoimmune diseases. It potently suppressed cytokine-induced expression of VCAM-1 on human endothelial cells[34,35].



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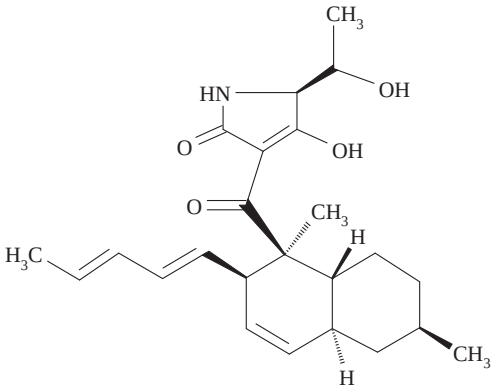
A novel antibacterial antibiotic, altersetin (**23**) was isolated from the culture broth of two endophytic *Alternaria* species. Altersetin (**23**) showed strong activities in the serial agar dilution assay against various human pathogenic bacteria[36].

Moreover, guanacastepenes, exemplified by guanacastepene A (**24**), represent highly diverse diterpenoids produced by an unidentified endophytic fungus isolated from *Daphnopsis americana* tree. The compounds exhibited pronounced antibiotic activity against drug-resistant strains of *Staphylococcus aureus* and *Enterococcus faecium*[37].



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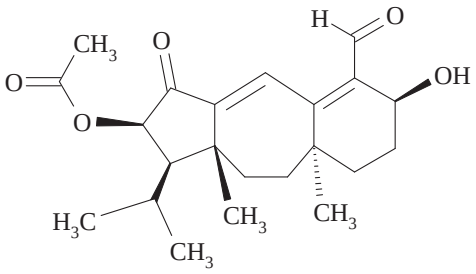

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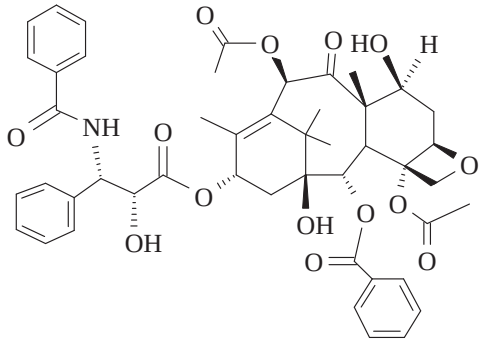
1.3.4 Secondary metabolites from endophytic fungi as anticancer agents

The anticancer properties of several secondary metabolites from endophytes have been investigated recently. Following, some examples of the potential of endophytic fungus on the production of anticancer agents are cited.

Not only is the discovery of new compounds from endophytes promising but also the re-discovery of known compounds from these new biological sources indeed serves as a benchmark for improved yields of such important drugs in clinical use. One such example is the well known plant metabolite paclitaxel (**25**), the "world's first billion-dollar anticancer compound"; it was originally isolated from the bark of the endemic Pacific yew tree, *Taxus brevifolia*[6]. Paclitaxel (**25**), a highly functionalised diterpenoid, and some of its derivatives represent the first major group of anticancer agents recently discovered also to be produced by endophytes. Taxol (**25**) has generated more attention and interest than any other new drug since its discovery, possibly due to its unique mode of action. The mode of action of paclitaxel is to preclude tubulin molecules from depolymerising during the processes of cell division[38]. In fact, tubulin molecules in taxol-sensitive plant pathogenic fungi were found to be affected in the same manner as human cancer cells, which indicated that taxol, in nature, may provide a defensive role for the yew tree (*Taxus* sp.) from which it originates[39].

FDA (Food and Drug Administration) has approved Paclitaxel (**25**) for the treatment of advanced breast cancer, lung cancer and refractory ovarian cancer[40].



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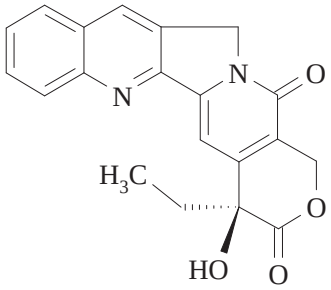

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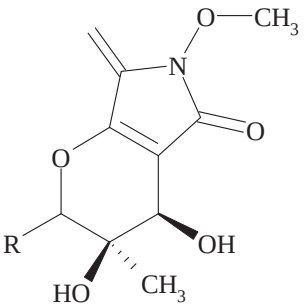
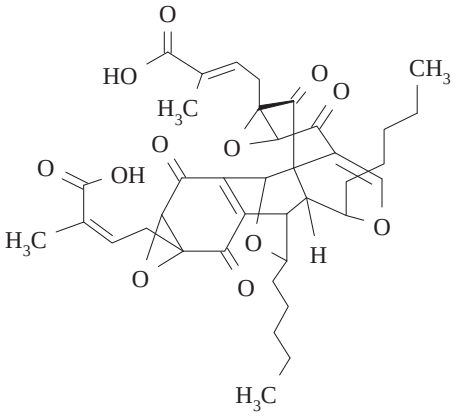
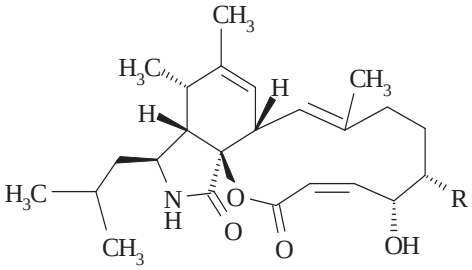
Another important anticancer compound is the alkaloid camptothecin (**26**), a potent antineoplastic agent, which was firstly isolated from the wood of *Camptotheca acuminata* Decaisne (Nyssaceae) in China. Camptothecin (**26**) and 10-hydroxycamptothecin are two important precursors for the synthesis of the clinically useful anticancer drugs, topotecan, and irinotecan[41]. Despite its crucial value in medical applications, the unmodified camptothecin (**26**) suffers drawbacks that compromise its applications due to very low solubility in aqueous media and high toxicity[42]. Furthermore, phaeosphoramide A (**27**) and B (**28**) are two new carbon skeleton derivatives, from the endophytic fungus *Phaeosphaeria avenaria*[43]. A selectively cytotoxic quinone dimer torreyanic acid (**29**) was reported as a potential anticancer agent, isolated from *Pestalotiopsis microspora.* This strain was originally obtained as an endophyte associated with the endangered tree *Torreya taxifolia* (Florida torreya). Torreyanic acid (**29**) was tested in several cancer cell lines, and it demonstrated 5 to 10 times more potency than taxol (**25**) and more potent cytotoxicity in lines that are sensitive to protein kinase C agonists; it causes cell death by apoptosis. Recently, torreyanic acid has been successfully synthesized by a biomimetic oxidation/dimerization cascade[44]. Phaeosphoramide A (**27**) was found to be an inhibitor of the signal transducer and activator of transcription (STAT)-3, which plays a vital role in regulating cell growth and survival, constituting a target for anticancer therapy[43].

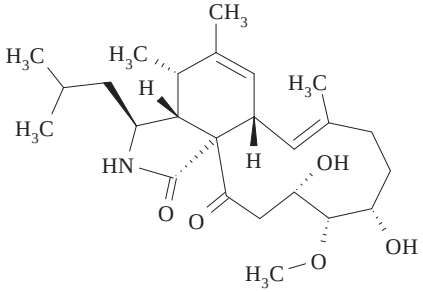




27 R = βC_6H_{11} **28** R = αC_6H_{11} **29**

Cytochalasins form a large group of fungal metabolites possessing antitumor and antibiotic activities, but due to their cellular toxicity they have not been developed into pharmaceuticals[45]. Chaetoglobosins are fungal metabolites also belonging to the family of cytochalasins. Some chaetoglobosins have been isolated recently from endophytic *Chaetomium globosum* and were shown to exhibit cytotoxic activities against the human nasopharyngeal epidermoid tumour KB cell line[46]. Recently, three new cytotoxic cytochalasans, aspochalasins I, J, and K (**30**a-c), were isolated from endophytic fungus *Aspergillus flavipes* from the southwestern desert regions of the United States[47].





30a: R = OH
30b: R = H

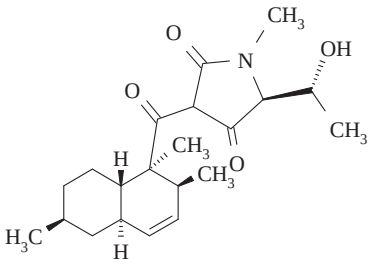
30c

1.3.5 Potential secondary metabolites from endophytic fungi in agriculture

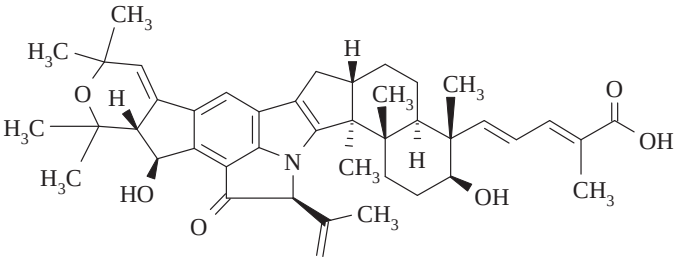
Another important aspect of this investigation is the search of fungal metabolites for inhibition of plant pathogens being relevant in the agricultural fields.

Many endophytic fungi species produce antibiotic substances, which can be useful as alternative ways to control farm pests.

One example is the unique tetramic acid, known as cryptocin (**31**), which was produced by *Cryptosporiopsis quercina* endophytic in the medicinal plant *Tripterigeum wilfordii*. It showed potent activity against *Pyricularia oryzae*, causal agent of rice blast, one of the most important plant diseases on earth, and is currently being examined as a natural chemical control agent for rice blast[48]. Another interesting finding consisted of the discovery of nodulisporic acid A (**32**), which was isolated from a *Nodulisporium* sp. endophytic in *Bontia daphnoides*. Nodulisporic acid A (**32**) was found to exhibit potent insecticidal properties against the larvae of the blowfly[49].

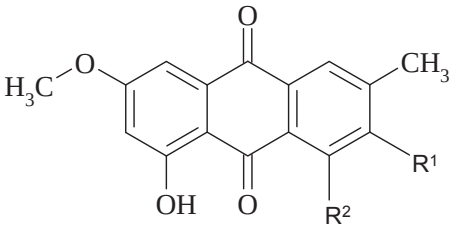


31

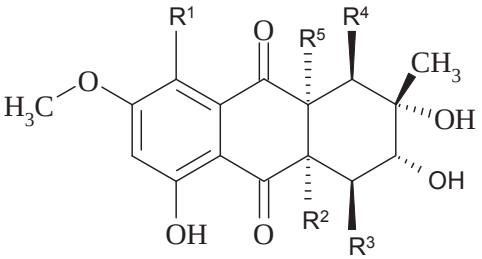


32

More recently, seven anthraquinones, **92–98**, with potent cytotoxic activities against human colon (SW1116) and leukaemia (K562) cancer cell lines were separated from *Pleospora* sp. IFB-E006 associated with *Imperata cylindrical* (*Gramineae*)[4,50].



33 : $R^1 = R^2 = OH$
34 : $R^1 = H$, $R^2 = OH$
35 : $R^1 = OH$, $R^2 = H$



36 : $R^1 = R^2 = R^3 = R^4 = R^5 = H$
37 : $R^1 = OH$, $R^2 = R^3 = R^4 = R^5 = H$
38 : $R^1 = R^2 = R^3 = R^4 = H$, $R^5 = OH$
39 : $R^1 = H$, $R^2 = R^3 = R^4 = R^5 = OH$

Endophytic fungi represent an abundant and dependable source of bioactive and chemically novel compounds with potential for exploitation in a wide variety of medical, agricultural, and industrial arenas. There has been a monotonic increase in the number of US patents filed on endophytic fungi producing important metabolites with diverse biological activities. The annual progress of cumulative number of US

patents on endophytic fungi producing natural products from 1990-2010 is shown in Figure 1.

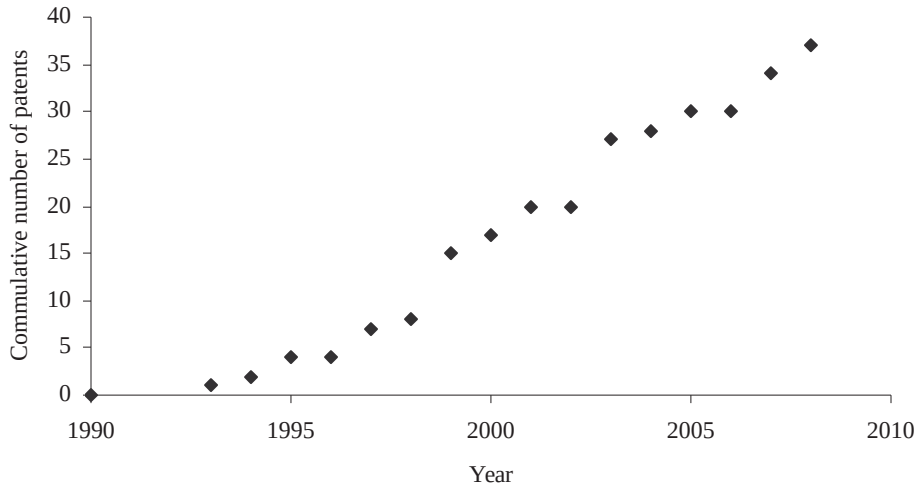


Figure 1: Cumulative number of US patents granted on endophytic fungi producing important metabolites and exhibiting biologically important activity[51].

1.3.6 Selection of promising sources for the isolation of endophytic fungi

When working with endophytic fungi in order to discover bioactive metabolites, the choice of the host is of particular importance. The exploration of endophytic fungi is still an emerging field and all plants seem to deliver fungi with auspicious contents and activities. However, certain microbial metabolites seem to be characteristic of certain biotopes[52] and a rationale for selecting promising plant sources has recently been proposed[6]. Of particular interest are plants which themselves are used as medicinal plants or are known to produce bioactive metabolites. Other favourable sources of endophytic fungi include plants that populate distinct biotopes and have to cope with extreme living conditions like cold, heat or multitudinous competing organisms in their natural environment, for example inhabitants of rainforests or mangrove forests. In these ecosystems, where competition for survival is most pronounced and requires constant innovations by the plants, i. e. morphological and physiological adaptations as well as chemical variation, the chance of obtaining novel compounds with high bioactivities is most probable[6].

On the other hand, tropical and subtropical rainforests are found nearer to north or south of the equator. They are common in Asia, Australia, Africa, South America, Central America, Mexico and on many of the Pacific Islands. Rainforests are home to half of all the living animal and plant species on the Earth. As tropical and subtropical climate harbour most of the worlds plants diversity, endophytic diversity in these geographical zones is expected to be very high since almost all vascular plant species examined to date are found to posses endophytic bacteria and fungi. Moreover, in tropical rainforests are great competition, resources are limited and selection pressure is at its peak. This gives rise to a high probability that rainforests are a source of novel molecular structures and biologically active compounds[53]. And the majority of undescribed fungal diversity lies within the tropical plant-associated fungi, yet the diversity and ecological role of endophytes in tropical angiosperms are almost entirely unexplored[54, 55]. Additionally, tropical rain forests are also called the world's largest pharmacy because over one-quarter of modern medicines originate from plants found therein[56].