NATURAL PRODUCTS FROM BACTERIA AND FUNGI

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Summary

Bacteria and fungi are microorganisms known to inhabit almost all ecological niches of the Earth and characterized by their ability to produce secondary metabolites or small-molecule natural products. As a result, many of these secondary metabolites are not directly involved in the normal growth, development or reproduction of the microorganisms in which they occur, but may play an important role in stress tolerance and their ecological interactions with other organisms.

Therefore many of these secondary metabolites exhibit a variety of biological activities and are of interest to the agrochemical, food, and pharmaceutical industries. Bacterial and fungal secondary metabolites originate from a few common biosynthetic pathways, but the resulting intermediates undergo numerous enzyme-catalyzed reactions leading to products with an extremely diverse array of chemical structures. Thus, these secondary metabolites are conveniently classified based on their biosynthetic origin as terpenoids,

polyketides, alkaloids, non-ribosomal peptides, and cytochalasins. This article provides short descriptions of bacteria and fungi, classes of secondary metabolites produced by these organisms, their biosynthetic pathways, and a few important examples of each class of compounds including their bioactivities.

1. Introduction

Bacteria and Fungi belong to a group of minute organisms called microorganisms. A characteristic feature of many bacteria and fungi are their ability to produce secondary metabolites or small-molecule natural products. Microbial secondary metabolites encompass close to 50,000 known compounds with an extremely diverse array of chemical structures. Bacteria are a large group of unicellular prokaryotic organisms that display a wide diversity of sizes and shapes, ranging from spheres to rods and spirals.

They are ubiquitous in every habitat of the Earth, growing in soil, acidic hot springs, radioactive wastes, water, and deep in the Earth's crust, as well as organic matter and the live tissues of most organisms including plants and animals. Bacterial cells are about one tenth the size of eukaryotic cells and are typically 0.5 – 5.0 micrometers in length.

Content of the bacterial cells are surrounded by a lipid membrane, or cell membrane, which act as a barrier to hold nutrients, proteins, and other essential components of the cytoplasm within the cell. Some bacteria play an important role in industry. For example, *Lactobacillus* in combination with yeast and molds, have been used for thousands of years in the preparation of fermented foods such as cheese, pickles, soy sauce, sauerkraut, vinegar, and yoghurt.

Bacteria, which have the ability to degrade a variety of organic compounds, have also been used in waste processing and bioremediation. Some bacteria are capable of digesting hydrocarbons in petroleum and these are used to clean up oil spills. In the case of biological pest control, bacteria can also be used in place of pesticides. Bacteria belonging to the subspecies of *Bacillus thuringiensis* are used as Lepidoptera-specific insecticides under the trade names Dipel® and Thuricide®. Because of their specificity, these pesticides are regarded as environmentally friendly with little or no effect to humans, wildlife, pollinators, and most other beneficial insects.

Ubiquitous soil bacteria, such as *Streptomycetes* and *Myxobacteria*, are especially rich sources of these small molecule natural products. Numerous natural products with antifungal, antibacterial, and anticancer activities have been isolated and characterized from these organisms. In the chemical industry, bacteria are the most important natural source in the production of enantiomerically pure chemicals for use as pharmaceuticals or agrochemicals and/or their synthetic intermediates.

In contrast to bacteria, fungi are eukaryotic organisms known to inhabit almost all ecological niches of the Earth and have the ability to utilize various solid substrates as a consequence of diversity of their biological and biochemical evolution. Thus far, more than 70,000 fungal species have been described. However, some estimates suggest that 1.5 million species of fungi may exist. The fungal kingdom contains some of the most important organisms, both in the terms of their ecological and economic roles and

includes many well-known fungi such as, mushrooms, rusts, smuts, puffballs, truffles, morels, molds, and yeasts. They continue the nutrient cycle of ecosystems by breaking down dead organic matter. In addition, most vascular plants could not grow without the symbiotic fungi called mycorrhizae that inhabit their root systems and supply some essential nutrients, especially phosphates. Fungi are known to cause a number of plant and animal diseases. In humans, ringworm, athlete's foot, and several other diseases are caused by fungi. Plant diseases caused by fungi include rusts, leaf rot, stem rots. These may cause severe damage to important crops.

Many fungi are prolific sources of secondary metabolites. Soon after the World War I, the British scientist, Harold Rainstrick, initiated a systematic study of fungal metabolites for the first time. He and his team made seminal contributions to the recognition of fungi as a major source of natural products. In recent years, studies of fungal metabolites have experienced a tremendous increase due the need for compounds possessing biological activity with possible pharmaceutical and agricultural applications.

Antibiotics, antifungal, immunosuppressive, and cholesterol-lowering agents derived from fungal metabolites have been used in the clinic during the past five decades, contributing significantly to the welfare of the mankind and to the spectacular rise in life expectancy observed in the second half of the twentieth century. Soil-borne, parasitic, and saprophytic fungal sources are relatively well investigated for their secondary metabolites. However, the interest in secondary metabolites of symbiotic fungi that live in association with land plants, lichens, marine organisms, and insects have recently intensified due to the belief that natural products produced by these fungi for their ecological interactions, especially with their hosts, are expected to exhibit biological activities.

It is intriguing that the majority of bacterial and fungal secondary metabolites originate from a few common biosynthetic pathways utilizing precursors (small biosynthetic units or building blocks) formed during primary metabolism. The intermediates resulting from condensation of these small biosynthetic units are further elaborated ('tailored' or 'decorated') by numerous enzyme-catalyzed reactions leading to products with a diversity of structures. Thus, the majority of these secondary metabolites are conveniently classified based on their biosynthetic origin as terpenoids, polyketides, alkaloids, non-ribosomal peptides etc.

2. Terpenoids

Terpenoids, also known as isoprenoids, are a large and varied class of secondary metabolites that include carotenoids, sterols, polyprenyl alcohols, ubiquinone etc. Kekule, in 1880, was the first scientist to name compounds with general empirical formula $C_{10}H_{16}$ as "terpenes", because of their presence in turpentine. Terpenoids occur in nature as pigments, hormones, and signaling molecules and serve a wide range of biological functions such as antibiotics, anti-feedants, or pollinator attractants.

All isoprenoids are biosynthetically derived from the "activated" forms of isoprene namely, isopentenyl pyrophosphate (IPP, also known as isopentenyl diphosphate) and

its allylic isomer, dimethylallyl pyrophosphate (DMAPP, also known as dimethylallyl diphosphate). IPP is usually formed from acetyl-CoA (acetyl-coenzyme A) via the intermediacy of mevalonic acid in the HMG-CoA (3-hydroxy-3-methyl-glutaryl-CoA) reductase pathway in eukaryotes and archeabacteria. However, in many organisms it is synthesized through the recently discovered 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway.

The hydrocarbon chains of terpenoids are constructed one isoprene unit at a time by addition to the allylic moiety of the double bond in isopentenyl pyrophosphate to form the next higher member of the series.

During the formation of terpenoids up to 25 carbons of isoprene units are arranged in a regular pattern. Terpenoids containing 30 carbon atoms are usually formed by the fusion of two smaller terpenoid precursors each containing 15 carbons. Isoprene units often link together in a 'head-to-tail' fashion to form linear chains or may undergo various cyclizations and rearrangements to give cyclic terpenoids.

They can be biosynthetically modified by the loss or addition of carbon atoms. Terpenoids also undergo further modifications (e.g. oxidation, reduction, derivatization etc.) and 'decoration' reactions (e.g. glycosidation, alkylation, acetylation etc.) to give alcohols and their glycosides, ethers, aldehydes, ketones, carboxylic acids and their esters.

As the chains of isoprene units are built up, the resulting terpenoids are classified sequentially by the size as, hemi-(C_5), mono-(C_{10}), sesqui-(C_{15}), di-(C_{20}), sester-(C_{25}), tri-(C_{30}), tetra-(C_{40}) and poly-(C_{5n} [$n \geq 8$]) terpenoids. Over 40,000 terpenoids have been isolated and characterized from natural sources including fungi and bacteria and hundreds of new structures are reported each year.

2.1 Monoterpenoids

Monoterpenoids are made up of two isoprene units and therefore contain ten carbon atoms (C_{10}). To date over 1,500 monoterpenoids are known, and these constitute acyclic, monocyclic, and bicyclic monoterpenoids. These occur in nature as hydrocarbons, alcohols, aldehydes, carboxylic acids and their esters. Essential oil derived from the fungus, *Ceratocystis virescens*, contains acyclic monoterpenes, linalool, citronellol, geraniol, neral, geranial, citronellyl acetate, and geranyl acetate together with a cyclic monoterpene, α-terpineol (Figure 1). Presence of acyclic monoterpene alcohols, citronellol, nerol, and geraniol in *Trametes odorata* has been reported.

It was shown that several fungal species belonging to *Phellinus* produced acyclic monoterpene alcohol, linalool. Occurrence of acyclic monoterpenes, myrcene and citronellol, monocyclic monoterpenes, limonene, β-phellandrene, and terpinen-4-ol together with bicyclic monoterpenes, α-pinene, β-pinene, camphene, and ^{3}-carene in the volatile fraction of aeciospores of the gall rust fungus *Cronartium fusiforme*, have been reported. Some representative examples of monoterpenoids are provided in Figure 1.

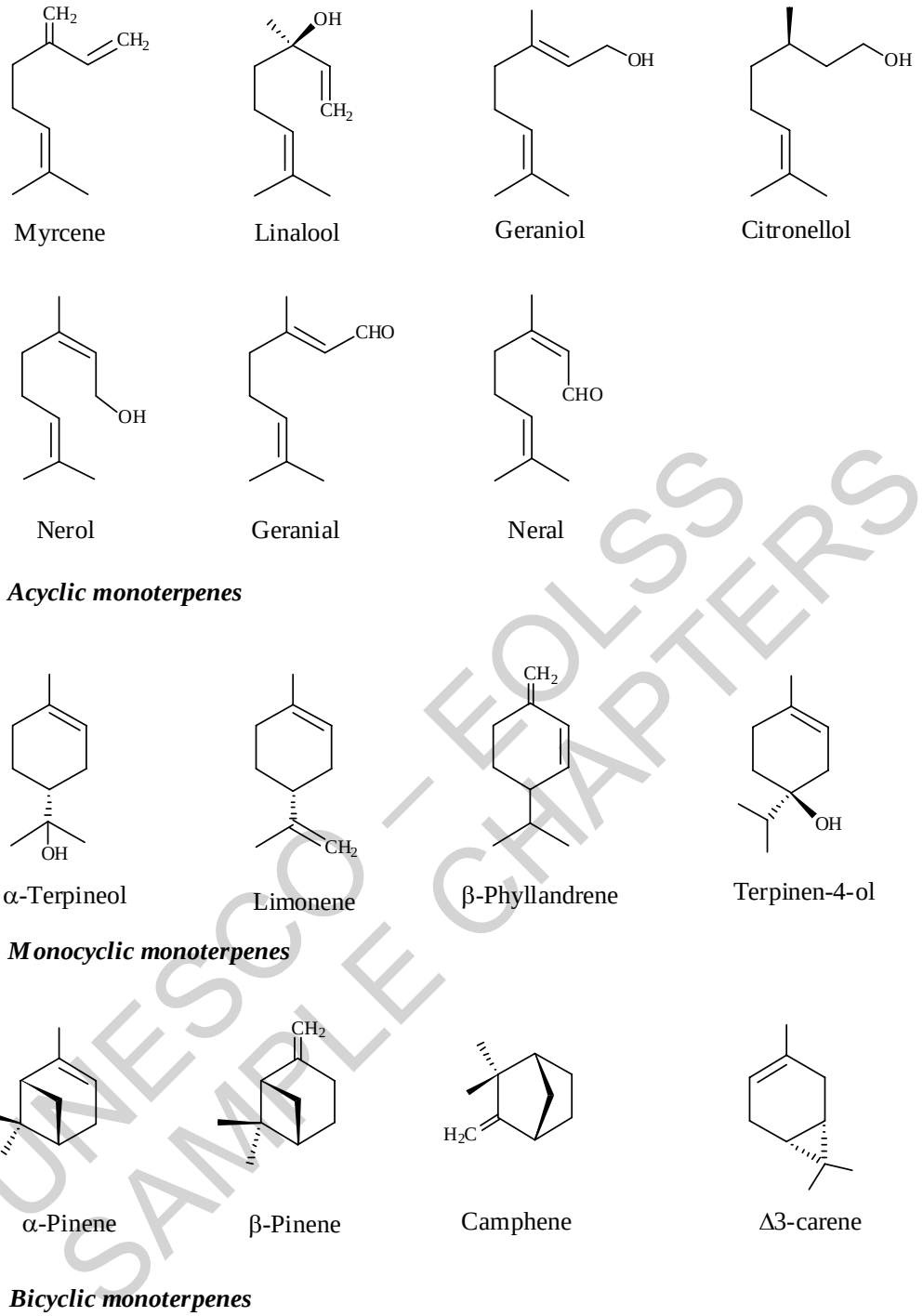

Figure 1. Some examples of monoterpenoids encountered in fungi.

2.2. Sesquiterpenoids

Sesquiterpenoids are terpenoids containing fifteen carbons (C_{15}) derived by the assembly of three isoprenoid units. They are the most diverse group of terpenoids in nature and to date about 10,000 sesquiterpenoids are known. They are mainly found in higher plants but a number of them are also found in bacteria and fungi. In plants, they function as pheromones and juvenile hormones. The sesquiterpenes, albaflavenone, *epi*-

isozizaene, (4*S*)-albaflavenol, and (4*R*)-albaflavenol in *Streptomyces coelicolor* A3, germacrane-type sesquiterpenes, 1(10)*E*,5*E*-germacradiene-11-ol, 1(10)*E*,5*E*-germacradiene-3,11-diol, and 1(10)*E*,5*E*-germacradiene-2,11-diol from an endophytic *S. griseus* and germacrene D, germacradienol, dihydroagarofurane, β-elemene, bicyclogermacrene, cadinene, and calerene from *S. citreus* CBS 109.60 represent some examples of bacterial sesquiterpenoids.

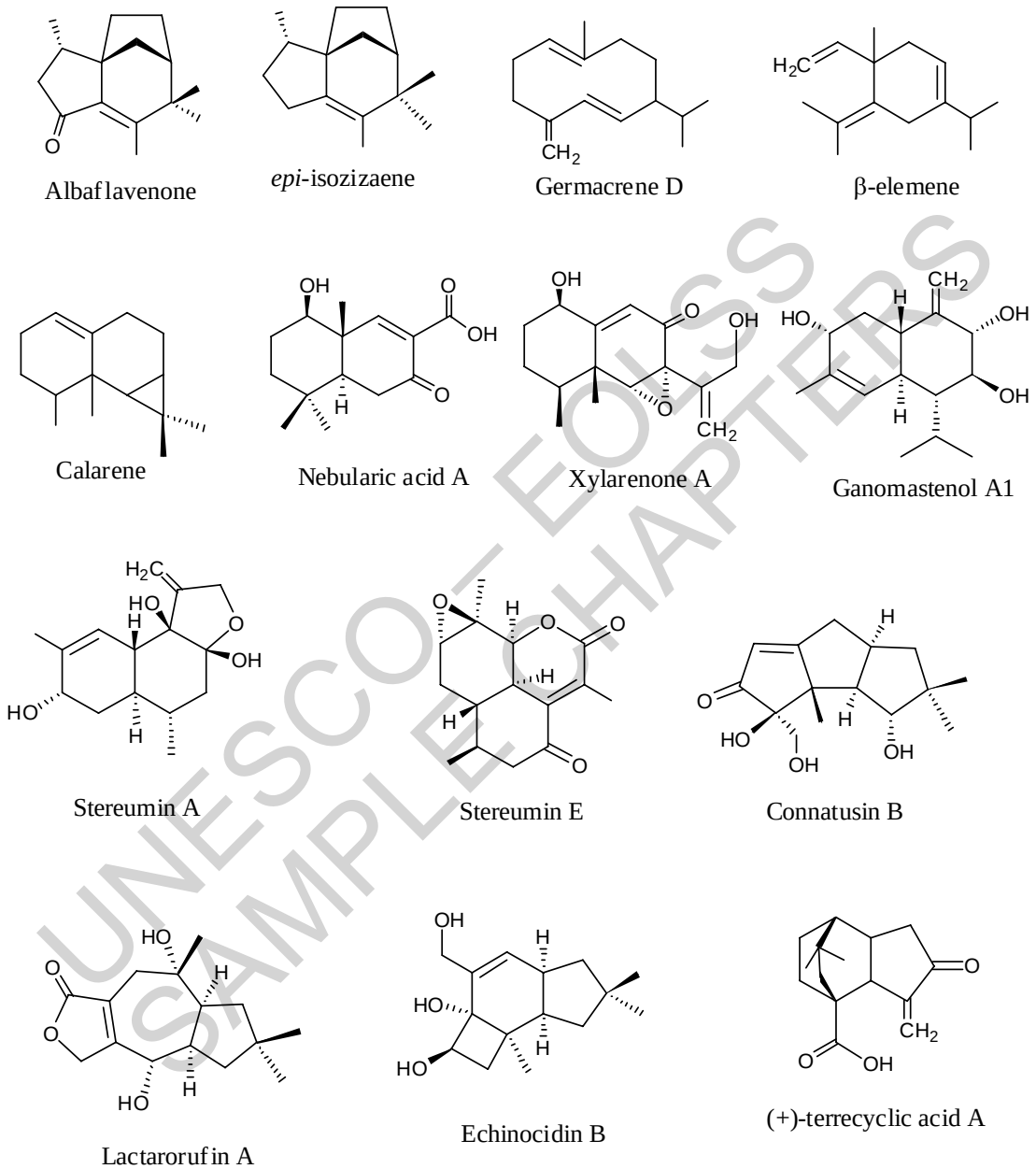

Figure 2. Examples of sesquiterpenoid natural products encountered in bacteria and fungi.

Sesquiterpenoids reported from fungal sources also occur as acyclic, mono-, bi-, tri-, and tetra-cyclic systems. Presence of acyclic sesquiterpenoid, β-farnesene in *Cronartium fusiforme* has been reported. Hymenoic acid isolated from *Hymenochaetaceae* sp. is an example of monocyclic sesquiterpene fungal metabolite.

Some bicyclic sesquiterpenoids produced by fungi include, nebularic acid A and nebularic acid B occurring in *Lepista nebularis*, xylarenone A and xylarenone B in *Xylaria* sp. NCY2, strobiluric acid in *Strobilurus stephanocystis*, cadinane sesquiterpenes, ganomastenols A – D in *Ganoderma mastoporum*, diastereoisomeric 3,9,12-trihydroxycalamenenes, 2,12-dihydroxycalamenene, 3,12-dihydroxycadalene, and 3,11,12-trihydroxycadalene in *Phomopsis cassiae*, and stereumin A – E in *Stereum* sp. CCTCC AF 207024. Hirsutane sesquiterpenes, connatusins A and B, hypnophilin and dihydrohypnophilin have been reported from the fungus, *Lentinus connatus* BC 8996, secolactarane-type sesquiterpenes, 3,8-oxa-13-hydroxy-lactar-6-en-5-oic acid, lactarorufin A, lactarorufin D, lactarorufin E, blennin, echinocidin B from *Strobilurus stephanocystis*, and protoilludane related sesquiterpene, repraesentin F from *Lactarius repraesentaneus* are examples of some of tricyclic sesquiterpenes. Terrecyclic acid A, and (+)-5,6-dihydro-6-hydroxyterrecyclic acid A isolated from the rhizosphere fungus, *Aspergillus terreus*, represent tetracyclic sesquiterpenoids. Depicted in Figure 2 are some sesquiterpenoid natural products of microbial origin.

2.3. Diterpenoids

Diterpenoids consist of natural products having twenty carbon atoms (C_{20}). Over 5,000 naturally occurring diterpenoids belonging to 20 major structural types are known mostly from higher plants and microorganisms. Physiologically active compounds having diterpenoid skeleton include phytohormones, gibberellins which are major constituents of the fungus, *Gibberella fujikuroi*, the fungal hormone, trisporic acid, the phytoalexins, casbene and podocarpic acid, and the natural cannabinoids. Neoverrucosan-5β,9β-diol, neoverrucosan-5β,18-diol, neoverrucosan-5β,9β,18β-triol, and neoverrucosan-5β,9β-diol-4-carboxaldehyde from gliding bacterium *Saprospira grandis*, a rare diterpene with 3,6,6,5-tetracyclic ring system, (–)-verrucosan-2β-ol, from the phototropic bacterium *Chloroflexus aurantiacus*, and viguiepinone and oxaloterpins A – E from *Streptomyces* sp. KO-3988, are a few examples of the bacterial diterpenoids.

Among the diterpenoids known from fungal sources, baccatin III, which is the diterpenoid fragment of the well-known anticancer drug, paclitaxel (Taxol®) may be considered as the most important. Despite the original belief that paclitaxel occurs only in plants of the genus *Taxus* (yew), isolation of this natural product from the endophytic fungus, *Taxomyces andreanae*, inhabiting *Taxus brevifolia*, led to its search from fungal sources. As a result the occurrence of paclitaxel in a number of endophytic fungal species belonging to genera *Pestalotia*, *Pestalotiopsis*, *Fusarium*, *Alternaria*, *Pithomyces*, and *Monochaetia* have been reported. However, it is noteworthy that the endophytic fungus *T. andreanae* has not been isolated from any of the yew trees investigated other than the original tree from Glaciar National Park, Montana, USA. Isolation of monocyclic diterpenoid, moreloriol, has been reported form the fungus, *Morchella conica*. Periconicin A and periconicin B from an endophytic fungus *Periconia* sp., (+)-pimara-8,15-diene, isopimara-8,15-diene, 8β-pimara-9(11),15-diene, pimara-7,15-diene, and pimara-8(14),15-diene from *Phoma betae*, fusicoccin A and fusicoccin J from the phytopathogenic fungus *Phomopsis* (*Fusicoccum*) *amygdali*, and heptemerone A and heptemerone G from *Coprinus heptemerus* represent a few examples of tricyclic diterpenoids of fungal origin. Tetracyclic diterpenoids occurring in

fungi include, among others, heptemerone B – F isolated from *Coprinus heptemerus*, aphidicolin, aphidicolan-16β-ol, aphidicol-15-ene, aphidicol-16-ene and stemar-13-ene from *Phoma betae*, and gibberellins GA40, GA54, GA55, GA56 and GA57 isolated from *Gibberella fujikuroi*. Some examples of microbial diterpenoids are presented in Figure 3.

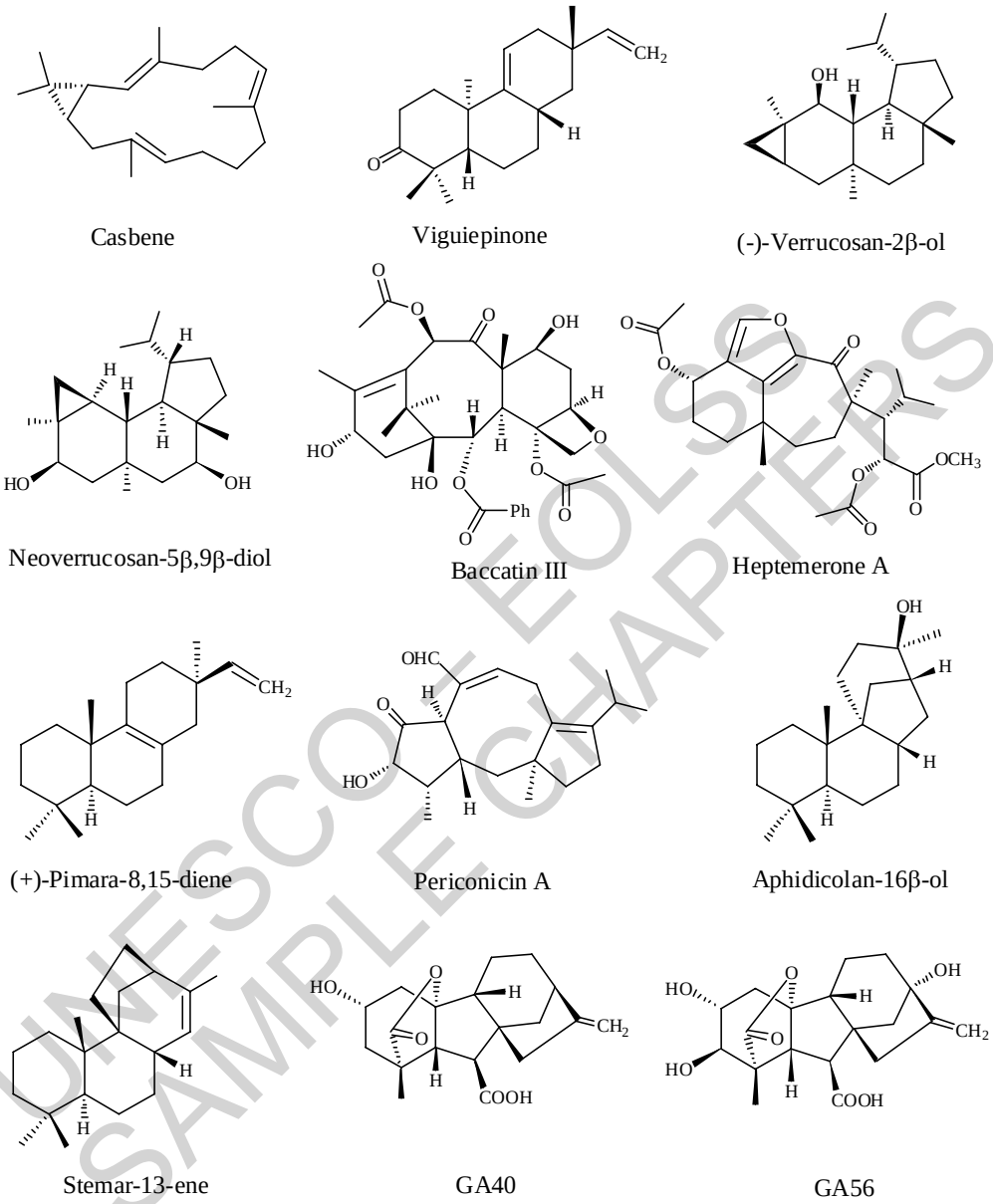

Figure 3. Some examples of microbial diterpenoids.

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Biographical Sketches

Leslie Gunatilaka was born in Kolonnawa, Sri Lanka, and obtained his undergraduate training in chemistry at the University of Peradeniya in Sri Lanka. He then proceeded to Imperial College of Science and Technology, University of London, where he obtained his Ph.D. in organic chemistry and one year of postdoctoral research experience in isolation and synthesis of natural products under the guidance of Professor Sir Derek Barton, F.R.S. He returned to his academic position at the University of Peradeniya in 1974 where he served as the Professor of Organic Chemistry (1984–1989) and the Chairman of the Department of Chemistry (1985–1989). During the period 1979–1985 he obtained additional research

experience in natural products as a Visiting Scholar in the laboratories of Professors Carl Djerassi (Stanford University, USA), Anthony G. M. Barrett (Imperial College, London), and David G. I. Kingston (Virginia Tech, USA). He returned to Virginia Tech in 1989 as a Visiting Professor and a Senior Research Scientist in Professor David Kingston's group. In 1997 he joined the University of Arizona where he currently serves as a Professor at the School of Natural Resources and the Environment and the Director of the Southwest Center for Natural Products Research and Commercialization. His current research interests are in discovery, structure-activity relationship studies, and molecular target identification of novel and biologically active natural products from plants, and microorganisms. In 1983, he was awarded the Institute of Chemistry, Ceylon Gold Medal for his outstanding scientific contributions and in 1987 he was awarded the Sri Lankan Presidential Gold Medal for creating a center of excellence in natural products research at the University of Peradeniya. In 2003 he received the Research Faculty of the Year Award from the University of Arizona College of Agriculture and Life Sciences. He was honored by the Asian American Faculty, Staff and Alumni Association of the University of Arizona by presenting the Outstanding Faculty Award in 2005. He is a Fellow of the Institute of Chemistry Ceylon, National Academy of Science (Sri Lanka), and the Third World Academy of Sciences. He is the author of over 200 research publications and book chapters.

Kithsiri Wijeratne was born in Mandugoda, Sri Lanka, and obtained his undergraduate training in Chemistry at the University of Peradeniya in Sri Lanka. Then he joined the Peradeniya Center of the World Health Organization (WHO) multi-collaborative research project on isolation and characterization of fertility regulating agents from endemic/indigenous plants of Sri Lanka. At the same time he continued his graduate studies under the supervision of Professors Leslie Gunatilaka and Ratnayake Bandara and obtained his Ph. D. in Natural Products Chemistry from the University of Peradeniya, Sri Lanka. During his graduate training he was awarded the UNESCO fellowship by Tokyo Institute of Technology, Japan, where he obtained his diploma in Chemistry and Chemical Engineering (by research) under the guidance of Professor Hironobu Hashimoto. After completing his Ph. D. he joined the Medical Research Institute, Sri Lanka as a Research Officer in Natural Products Chemistry (1991 – 1999). During the period 1987 – 1988 he visited Japan under JICA (Japan International Cooperation Agency) program and obtained additional research experience in natural products chemistry from Professor Tohru Kikuchi's laboratory in Toyama Medical and Pharmaceutical University, Japan and some experience in synthesis from Professor Hajime Katayama's laboratory in Niigata College of Pharmacy, Japan. In 1999 he joined the Professor Leslie Gunatilaka's research group as a research associate at the Southwest Center for Natural Products Research and Commercialization, University of Arizona, USA, where he is currently employed as an assistant research scientist. In 2004 he was invited by Professor Katsuko Komatsu of Toyama Medical and Pharmaceutical University, Japan, as a Visiting Professor to establish a database on medicinal plants. He is a member of the Institute of Chemistry, Ceylon, and American Chemical Society. He is the author of over 40 research publications.