

Isolation and Structural Elucidation of Compounds from Natural Products

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Abstract

In continuation of the Kingston group's work to identify new compounds from natural products as a part of the International Cooperative Biodiversity Group (ICBG) program and in collaboration with the Institute for Hepatitis and Virus Research (IHVR), the two plants *Neoharmsia baronii* and *Lopholaena cneorifolia* were studied to identify their chemical components. Structural elucidation and characterization of the compounds were done using mass spectrometry, 1D and 2D NMR spectroscopy techniques.

A systematic study of the ethanol extract of the plant *Neoharmsia baronii* Drake from the Madagascar forest led to the isolation of seven compounds, characterized as isoflavones and pterocarpanes. The structures of the compounds were characterized by using 1D NMR and 2D NMR spectra, mass spectroscopy and in one case, x-ray crystallography. The HSQC and HMBC data along with comparison of these data with reported literature values confirmed the structures. The aforementioned isoflavones and pterocarpanes showed varying cytotoxicity to ovarian cancer cell lines, with the isoflavone vogelin E being the most active compound.

The extract of *Lopholaena cneorifolia* was studied as a part of a cooperative project with the IHVR to identify its chemical composition. Fractionation of this extract led to the isolation of three compounds which were characterized as stilbenes. Their structures were elucidated by using 1D NMR and 2D NMR spectra and mass spectroscopic data.

DEDICATION

I would like to dedicate this thesis to my parents Mr. Harishkumar Girdharlal Dengada and Mrs. Nirmala Harishkumar Dengada, my sisters Vedika and Krishna, and my husband Mr. Gajendra Suresh Dolare for their continued love and support.

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I extend my special thanks to my parents for always being there for me and giving me strength to achieve whatever I wish for and always encouraging me to do better. I would also like to thank my husband Mr. Gajendra Dolare for being very supportive of my career and always motivating me to keep going.

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Chapter 1: Introduction

History of natural products

The World Health Organization (WHO) has estimated that over 75% of the world's population still relies on plant derived medicines, usually the ones being traditionally used.¹ Thousands of different preparations made from the extracts of plants have been used in treating various disease conditions since the beginning of civilization, and are still being used in some parts of the world. The first documented use of natural drugs dates from Mesopotamia in 2600 B.C. and reports the use of oils from *Cupressus sempervirens* (Cypress) and *Commiphora* spices (myrrh), which are still used today to treat coughs, colds, and inflammation.² The Egyptian pharmaceutical record the Ebers papyrus documented over 700 plant-based drugs ranging from gargles, pills, and infusions to ointments. The Chinese Materia Medica (1100 B.C.) Wu Shi Er Bing Fang contains 52 prescriptions, and the Shennong Herbals (approximately 100 B.C. 365 drugs) and the Tang Herbals (659 A.D. 850 drugs) are documented records of the uses of natural products, indicating our dependency on natural products. The Greek physician Dioscorides (100 A.D.) recorded the collection, storage and uses of medicinal herbs, and the Greek philosopher and natural scientist Theophrastus (approximately 300 B.C.) dealt with medicinal herbs. The Canon Medicinae, introduced to us by Avicenna, a Persian pharmacist, physician, philosopher and poet, made a significant contribution to the sciences of pharmacy and medicine with the help of the Arabs who became the first to privately own pharmacies in the 8th century.^{2, 3}

Currently many modern medicines are products of nature or derived from natural products, and nature is a promising source of treatments for new and emerging medical conditions. The search for treatments for diseases or infection from nature preceded the discovery of new technologies like ultra-high throughput screening (uHTS), combinatorial chemistry, and genomic technology.⁴

Drug discovery from natural products has however been impacted significantly by the evolution of these new technologies, and there is an emerging perception that the role of natural products is starting to diminish.^{2, 3, 5} In spite of this, plants are still capable of yielding new bioactive compounds, and microbial organisms continue to yield novel structures and novel bioactivities. Since only 10% of the world's biodiversity has been studied for potential curative entities, the remainder is waiting to be welcomed to the world of drug discovery.^{2, 6}

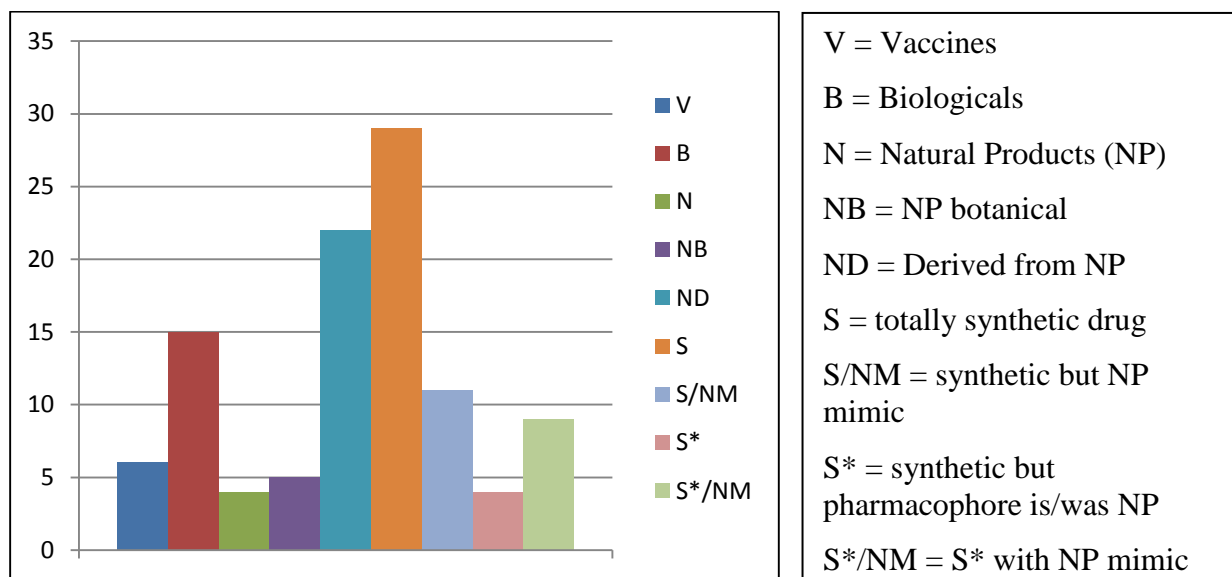


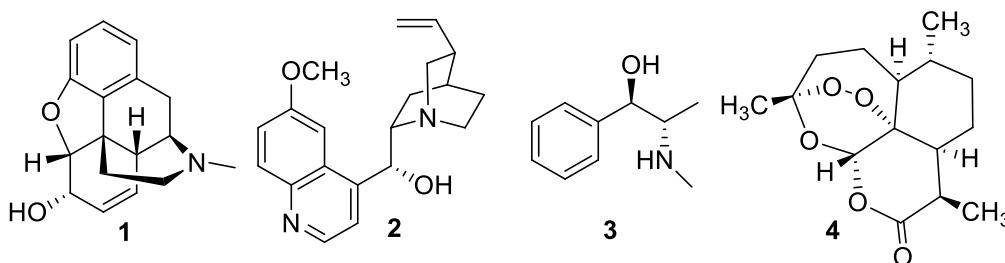
Figure 1. Sources of new drugs 1981-2010.

The importance of natural products as a source of new drugs is shown in Fig. 1, which documents the sources of all new drugs introduced between 1981 and 2010.⁷ The data clearly show the influence of nature in our discovery of new drug entities. About 29% of the drugs were of synthetic origin, and the major portion of the remainder either were from natural products or were derived from natural products or the pharmacophore was from a natural origin. Therefore, the fact that most of the new drug entities are directly or indirectly linked to mother nature cannot be ignored. The linking of products from nature to modern combinatorial synthesis is also on the rise and older drugs with new indications are mainly drugs from natural products or are

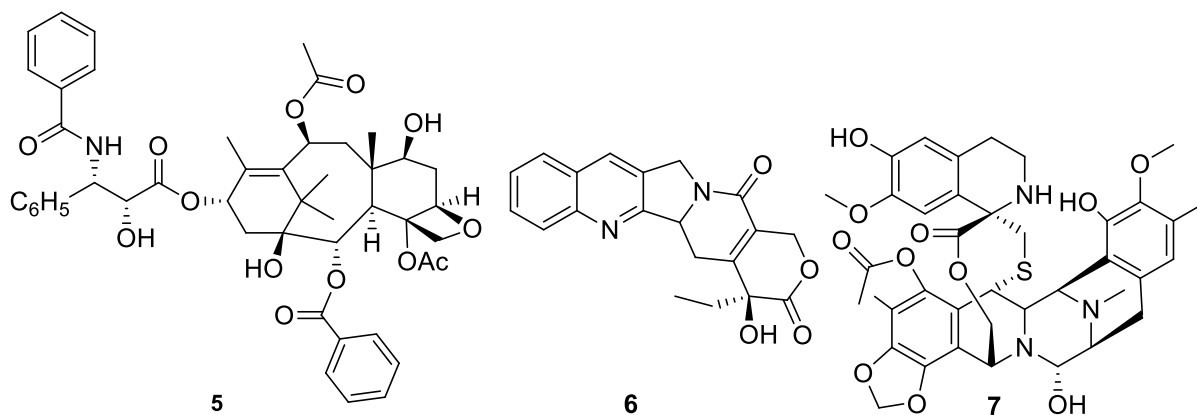
derivatives of natural products.⁸ For example, a study showed that compounds active on tumor cells also exhibited anxiolytic properties and inhibited HIV reverse transcriptase.⁹ The major advantage of screening natural products is that they offer vast structural diversity, as compared with the more modest diversity obtained by combinatorial approaches.

There are three different approaches to drug discovery that have been used and are still being implemented.

A) The first approach is the natural products approach, which can be driven either by ethnomedicine or untargeted collection of biomass. The ethnomedical approach has led to such important medicines as morphine (**1**), quinine (**2**), ephedrine (**3**) and the anti-malarial, artemisinin (**4**).



The untargeted natural product approach has led to new drugs such as paclitaxel (**5**), camptothecin (**6**), and the marine anticancer agent trabectedin (**7**).



B) The second approach is the combinatorial chemistry approach in which a variety of chemical entities are obtained by synthesis. This approach prepares a large number of compounds ranging from tens to thousands. Combinatorial chemistry has become a useful tool for the discovery and optimization of new lead compounds because it is less time consuming and less effort is required to produce a large collection of related compounds but it has been less successful at producing new drug lead compounds.

C) The third approach is the rational design or computational method, which has gained popularity in the modern day world due to the reason that it is a fast and efficient way of doing virtual screening thousands of chemical entities and finding the ones with promising efficacy.⁵

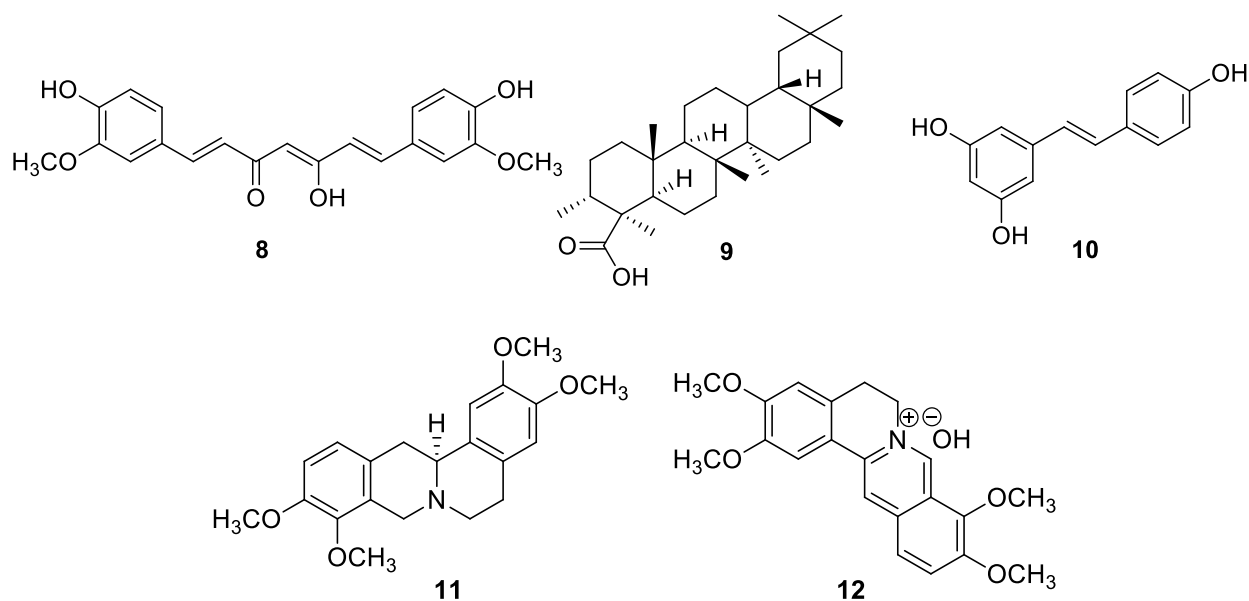
Though the rational approach is gaining popularity, there are many constraints to successfully getting a new drug entity to market.⁵ To successfully overcome all of the challenges faced with the rational drug approach, natural products remains the best option amongst all approaches to date. In an ideal case the natural product sources should be easily available and accessible, and the crude extracts can be separated quickly to find compounds with drug-like properties. Strong de-replication methods also need to be available to avoid re-isolation of known compounds.

Major drug classes of natural products

Anti-inflammatory, anti-pyretic and analgesic compounds

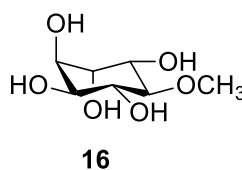
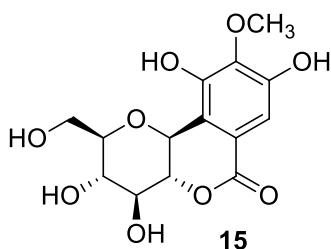
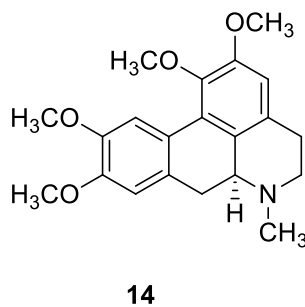
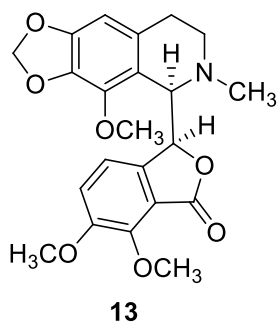
The primary response of the immune system to an infection or irritation is inflammation. Any response not tolerated by our body such as physical damage, UV radiation, or microbial attack, leads to inflammation and subsequently fever, and in some cases pain. A constant inflammation in a particular body part could lead to irritable bowel syndrome, chronic asthma, rheumatoid arthritis, multiple sclerosis or psoriasis.

The only solution before modern drugs became available was the use of natural products, which were easily available and were the only sources known to cure many diseases. Well known examples of natural anti-inflammatory agents include aescin, isolated from the plant *Aesculus hippocastanum* (the horse chestnut), bromelain from *Anans comosus*, curcumin (**8**) from the Indian spice turmeric (*Curcuma longa*),¹⁰ boswellic acid (**9**) isolated from *Boswellia serrata*¹¹ and resveratrol (**10**) from the Japanese knotweed *Fallopia japonica* syn. *Polygonum cuspidatum*.¹² Anti-pyretics and analgesics are another class of drugs which have been used for alleviating fever and pain, and several natural products have these activities. The classic drug morphine (**1**) from *Papaver somniferum* is the best known example of a potent analgesic natural product. Rotundine (**11**), structurally and functionally similar to tetrahydropalmatine (THP), is an alkaloid, isolated from the roots of *Stephania rotunda* Lout. The anti-pyretic alkaloid palmatine (**12**) is an analgesic protoberberine alkaloid found in several plants, including *Phellodendron amurense*, *Rhizoma coptidis* and *Corydalis yanhusuo*; a major source of this alkaloid is *Enantia chlorantha*.¹³



Anti-tussive and expectorant compounds

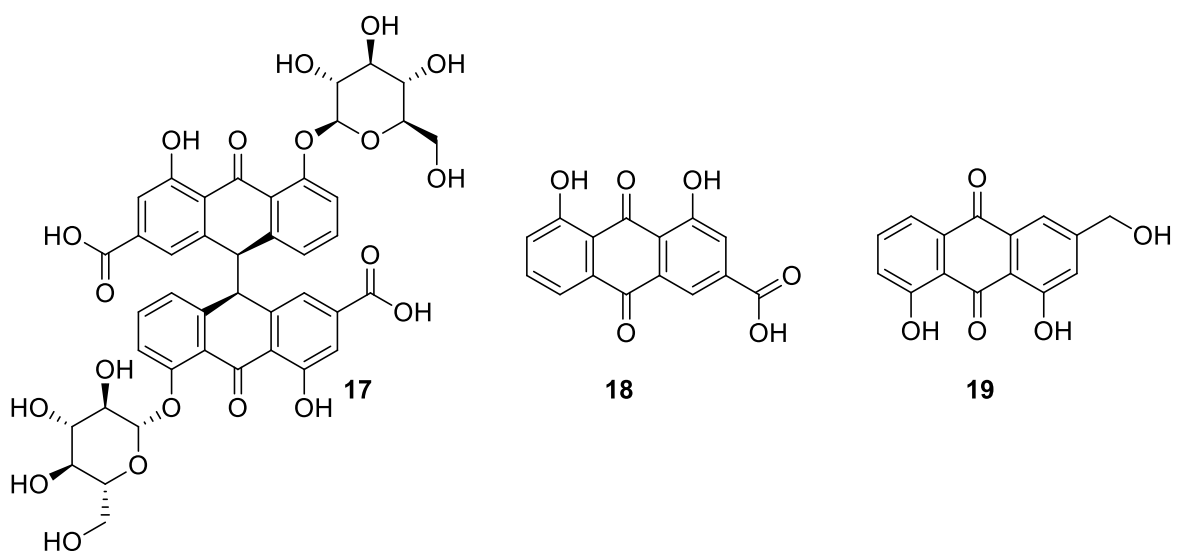
Emerging figures indicate that 95 million units of pediatric drugs are sold for coughs and colds every year in the US. Over the counter (OTC) drugs have the highest number of sales of pediatric and adult cough medications. Many of the non-narcotic anti-tussive agents and narcotic anti-tussive agents were found from natural sources. Many traditional home remedies include ginger, turmeric, liquorice, cardamom and fennel. Expectorants are not curative but alleviate the symptoms of cough and bronchitis. There are more than 70 plant sources that contain this category of drug, and noscapine (**13**) from *Papaver somniferum*, glaucine (**14**) from *Glaucium flavum*, bergenin (**15**) from *Ardisia japonica* and pinitol (**16**) from several plants are a few examples.^{14, 15, 16}



Laxatives

Constipation is a prevalent condition by itself and can also be a side-effect of the treatment of other disease conditions. It is a side-effect of the drugs used during chemotherapy such as certain pain medications and serotonin-based drugs that relieve nausea and vomiting. Extracts of

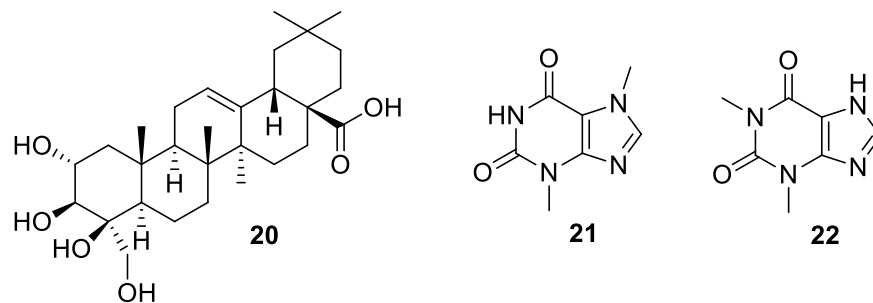
rhubarb, senna leaves, castor oil, aloe and cascara are a few examples of laxatives that are still being used in certain parts of the world. Anthraquinone derivatives are the main constituents of senna and are responsible for its laxative properties. Senna contains sennosides A (**17**), B, C and D, kaempferol, phytosterols, glycosides of rhein (**18**) and chrysophanic acid. Aloe emodin (**19**) found in the gel, sap and leaves of *Aloe vera* is responsible for its laxative action. Daiokanzoto (DKT) is one of the main constituents of rhubarb which is responsible for its effectiveness in relieving constipation.^{17, 18}



Diuretics

When the blood flow to the kidneys declines due to illness, diuretics are prescribed to promote the production of urine. This can also be termed forced diuresis. Diuretics are prescribed to treat high blood pressure, congestive heart failure and liver diseases, all of which cause water retention. Diuretics are also abused as inducers of weight loss, and in some cases to meet drug urinalysis criteria before doping tests. The general phytoconstituents responsible for diuretic activity include alkaloids, glycosides, tannins, phenolics, coumarins, triterpenoids, etc. Examples include flavonoids and tannins from *Terminalia arjuna* [arjunolic acid (**20**)],¹⁶ *Acacia suma*, *Camelia sinensis*, *Suscuta reflexa*, and *Mimusops elengi*, and alkaloids from the plants *Aerva*

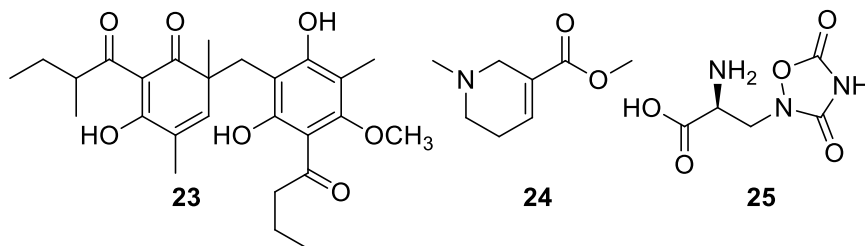
lanata, *Erythrina indica*, *Cordia rothi*, and *Azima tetracantha*. The primary alkaloid theobromine (**21**) found in *Theobroma cocoa* is a well known diuretic. *Theobroma cocoa*, the plant also known as 'food of the Gods' is the source of cocoa powder. Theobromine has also been used to treat cancer, asthma, and cardiovascular diseases, as an antitussive, a vasodilator and as a skin emollient. Theophylline (**22**), also found in cocoa beans, has wide medicinal uses as well as being a diuretic.¹⁹ Coumarins of *Daucus carota*, triterpenes from *Taraxacum officinale* and *Abutilon indicum*, saponins from *Asparagus racemosus* and *Tribulus terrestris*, sesquiterpene lactones from *Taraxacum officinale*, and glycosides from *Opuntia ficus indica* and *Moringa oleifera* are a few more examples of active constituents from various plants that have diuretic effects.¹⁵



Anthelmintics

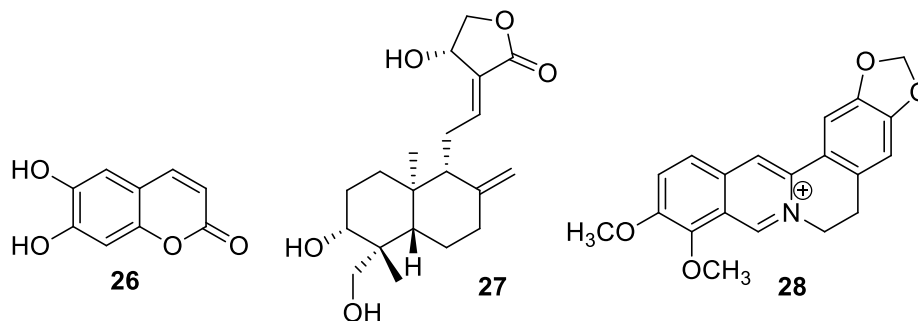
The anthelmintic class of drugs includes a large number of medicinal plants used to treat internal parasites, particularly in tropical developing countries, because of the cost of modern medicines and the easy availability of traditional remedies. An ideal anthelmintic agent should be broad spectrum i.e. treat all helminthes which infect the intestine, including tapeworms, nematodes, roundworms and trematodes or flukes, should be free from toxicity to the host, and should be cost effective. The most common synthetic drugs under this class have shown side effects like nausea, intestinal disturbance and giddiness. Resistance of the parasite is also a concern and hence this class of drugs relies highly on natural product sources. *Ocimum sanctum* Linn.

(Lamiaceae), commonly known as sacred basil (Tulsi), contains volatile oils of which eugenol (5%), β -caryophyllene (37%) and a number of sesquiterpenes and monoterpenes are responsible for the anthelmintic activity. Agrimophol (**23**) is one of the potent anthelmintics from the plant *Agrimonia eupatoria*, and arecolin (**24**) from *Area catechu* and quisqualic acid (**25**) from *Quisqualis indica* are other examples of natural anthelmintics.^{20, 21}



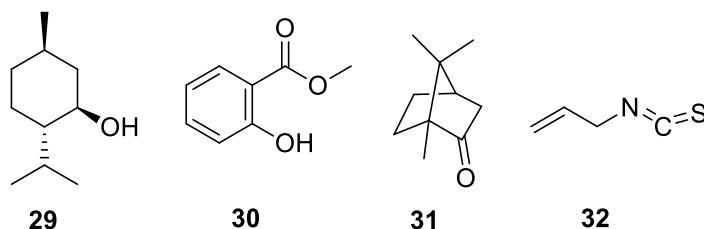
Dysentery

For reasons similar to those mentioned in the anthelmintics category, dysentery is also treated primarily by using natural products in some parts of the world. Active ingredients have been identified from some of the extracts which are more frequently used, e.g. aesculetin (**26**) from the plant *Frazinus rhychophylla*, andrographolide (**27**) from *Andrographis paniculata*, berberine (**28**) from *Berberis vulgaris*, hemsleyadin from *Hemsleya amabiis*, and neoandrographolide from *Andrographis paniculata*. *Musa paradisiaca*, a monoherbacious plant belonging to the family Musaceae, commonly known as plantain, is used to treat many conditions including cancer, diabetes, diarrhea, dysentery, hypertension, marasmus, migraine, psoriasis, small pox, syphilis, tuberculosis, tumor, urticaria and wounds.²² Many of the species of the genus *Clematis* (Ranunculaceae) have been used in various systems of medicine for the treatment of various ailments, one of them being dysentery.^{23, 24}



Rubefacients

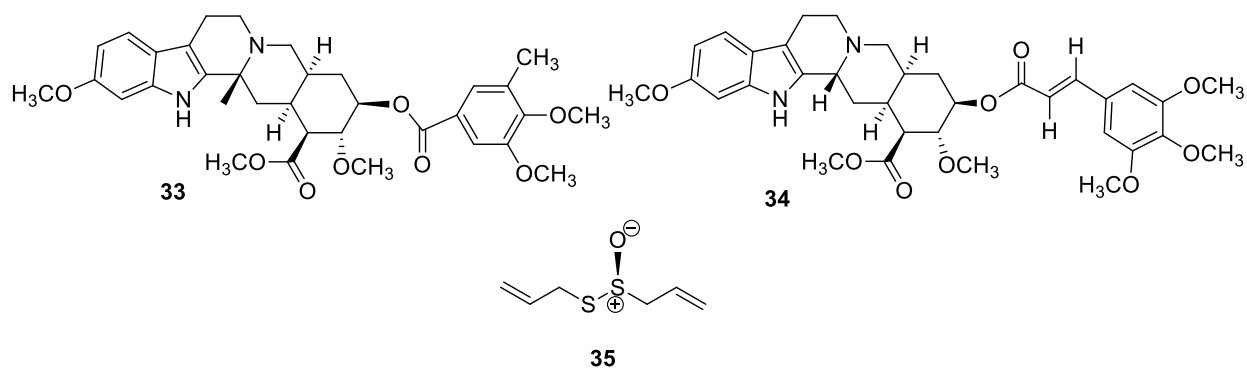
Rubefacients cause redness to the area of application, caused by dilation of capillaries and increased blood flow to the area, which helps relieve pain and stiffness. These are generally topical preparations containing menthol (**29**) from *Mentha piperita*, methysalicylate (**30**) from *Gaultheria procumbens*, camphor (**31**) from *Cinnamomum camphora*, allyl isothiocyanate (**32**) from *Brassica nigra*, and rosemary oil from *Rosmarinus officinalis*. The clove oil used in the Tiger balm preparation is from the plant *Eugenia caryophyllata*.²⁵



Anti-hypertensives

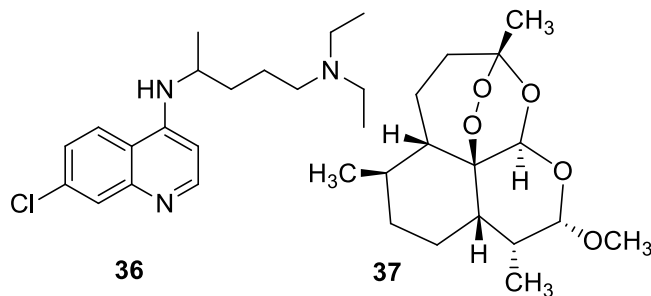
Hypertension has been a commonly observed cardiovascular condition for several decades, resulting from a sedentary life style, genetics, family history, too much alcohol consumption and many other reasons. Hypertension increases the risk of developing a number of cardiovascular diseases such as coronary heart disease, stroke, sudden cardiac death, congestive cardiac disease, renal insufficiency and dissecting aortic aneurysm. Investigations for controlling hypertension are aimed at identifying effective dietary compounds from natural sources.²⁶ Many food items

that have been traditionally used in Pacific and Asian foods have proven to be beneficial to good health and are being incorporated into diets throughout the world. It is estimated that 25% of the drugs prescribed today to treat hypertension are of natural product origin. Desperidine is an ester alkaloid drug isolated from *Rauwolfia canescens* (Apocyanaceae) with antihypertensive properties. Reserpine (**33**) is an indole alkaloid isolated from *Rauwolfia serpentina* used for its antipsychotic and antihypertensive properties, although it is not widely used due to numerous side-effects.²⁷ Another antihypertensive from the same plant, rescinnamine (**34**), is an angiotensin-converting enzyme inhibitor, slightly less toxic compared to reserpine (**33**) at the same dosage. Although both drugs are obsolete, their derivatives are still used. The primary active component that gives garlic, *Allium sativum*, its characteristic odor and its many healing properties is allicin (**35**). Garlic has many cardiovascular benefits including lowering of blood pressure. With many other health benefits, it can also be used as a preventive measure for those at risk of cardiovascular disease and other heart conditions. Other examples of antihypertensives from natural sources are rhomitoxin, a tranquiliser and antihypertensive from the plant *Rhododendron molle*, and tetrandrine, a bis-benzylisquinoline alkaloid, from the plant *Stephania tetrandra* S. Moore, which is a calcium channel blocker and has 'quinidine like' anti-arrhythmic effects.^{28, 29}



Anti-malarials

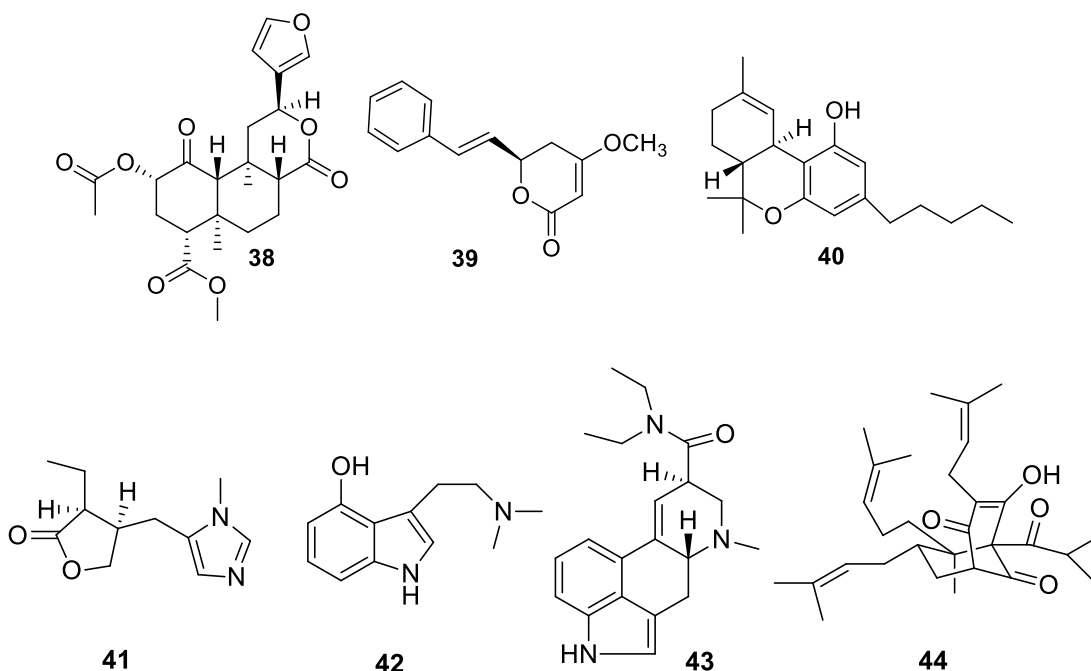
Several hundreds of millions of people suffer from malaria, and with the emergence of resistance to known anti-malarial drugs, developing new drugs in this class is a major challenge.³⁰ Most anti-malarial treatments available are primarily either natural products or products derived from natural products.³¹ Many plant species are used in traditional medicines for the treatment of malaria and only a few of these have been investigated for active anti-malarial compounds. This opens a vast area that should be evaluated and investigated. The marine environment is one of the richest sources of chemical diversity, and alkaloids from this source have been identified as potential antimalarial candidates and some are in clinical trials.³² The Andes Mountains has been a fruitful source of antimalarial agents.³³ The first known antimalarial drug quinine (**2**), was isolated from the bark of the Andean tree *Cinchona spp.* (Rubiaceae) in 1820. Chloroquine (**36**) and mefloquine are two of the main analogs of quinine, widely used today as antimalarials. However, resistance has developed to quinine and its derivatives. Following this, a more potent anti-malarial, artemisinin was discovered.³⁴ Artemisinin (**4**) was isolated from the qinghaosu plant *Artemisia annua*. Artemisinin's two main derivatives artemether (**37**) and artesunate, are also as useful as artemisinin itself, which has poor aqueous solubility and decomposes in other protic solvents resulting in poor bioavailability.^{35, 36}



Central Nervous System (CNS) agents

Modern methods of drug discovery using genomic data and targeted structure-activity relationships (SAR) to find psychoactive agents have not yielded any significant improvements in CNS drug discovery, as these methods have only a 7% probability of reaching the market as compared to an industry average of 15%.³⁷ Investigating naturally occurring CNS agents will improve the chances of productive research and may reduce the time taken for a CNS drug to reach the market. Many of the classical drugs used to treat CNS disorders were developed or modified from basic moieties discovered from nature. Psychoactive natural products have also been used to study the mechanism of various receptor systems in the CNS. A recent example is *Salvia divinorum*, the bioactive constituent of which is salvinorin A (**38**), responsible for its psychotropic effects. This example led to the understanding that activation of the κ -opioid (KOP) receptor and not the serotonin-2A (5-HT_{2A}) receptor is responsible for the observed hallucinogenic effect exerted by salvinorin A. Hence we can conclude that nature is a good source of CNS active compounds and helps in pharmacological characterization as well. Kava pyrones derived from *Piper methysticum* root are used as over-the-counter anxiolytics. Kavalactones, kavain (**39**), dihydrokavin, methysticin, dihydromethysticin, and yangonin are constituents of kava.³⁸ Δ -9-Tetrahydro cannabinol (**40**) (THC) extracted from the plant *Cannabis sativa* is one of the oldest known stimulants.³⁹ Pilocarpin (**41**), psilocin (**42**), and psilocybin have also been used as stimulants; these are isolated from *Pilocarpus microphyllus* Stapf. Lysergamides are a class of hallucinogens. Although the famous lysergic acid diethylamide LSD (**43**) is not found in nature, its analogue lysergic acid amide (LSA), 1/10th as potent as LSD, exists naturally.⁴⁰ LSA is a tryptamine derivative found in seeds of *Ipomoea violacea* and

Arygyreia nervosa. Saint John's wort (SJW) has many active constituents, of which hyperforin (44) seems to be useful as an antidepressant.⁴¹

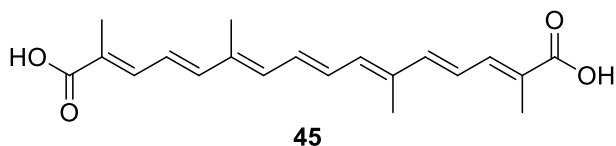


Anti-cancer agents from nature

Natural products have long been a fertile source of treatment for cancer, which is a leading cause of death with 7.6 million people per year dying from it worldwide.⁴² Death due to cancer is seen to be rising worldwide, with the projected figure being 13.1 million deaths in year 2013. The leading cause of cancer is tobacco use, followed by alcohol abuse, high body mass index, low fruit and vegetable intake, and lack of physical exercise. A recent statistical study has shown that a person born in the United States suffers a 41% lifetime risk of being diagnosed with cancer. Such a high figure of the possibility of suffering and the number of deaths reported due to cancer puts tremendous pressure on scientists to identify effective methods to prevent cancer and to find better treatments to cure cancer. Cancer cells show deregulation in multiple cellular signaling pathways, yet the different types of cancer exhibit common capabilities such as genetic instability, dependent growth signals, no response to anti-growth signals, no programmed cell

death phenomenon, multiplying with no control, sustained angiogenesis, tissue invasion and metastasis. Cancer treatments cannot be focused on all of these properties of cancerous cells, as it is very difficult to target all of them, and focusing on one or two of the properties does not cure cancer. Combination chemotherapy, using compounds with different mechanisms of action targeting various processes, can be more promising, but the associated side-effects and toxicity also increase. With the associated toxicity and side effects of currently used procedures to treat cancer, i.e. radiation and chemotherapy, there is a need for treatments free from toxicity and side-effects. There is a continuing need for the development of new anticancer drugs, drug combinations and chemotherapy strategies, by the methodical and scientific exploration of the enormous pool of synthetic, biological and natural products. Taking these things into consideration, one of the best options to explore for anti-cancer agents seems to be from natural sources.

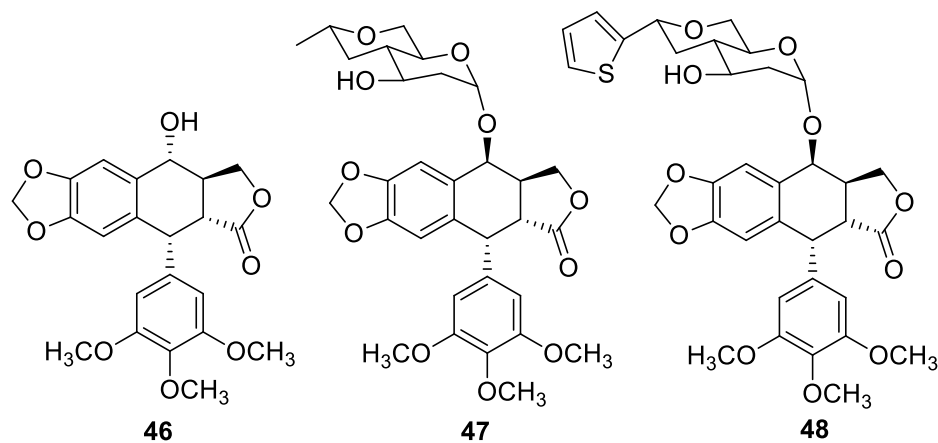
In addition to the use of natural products as a source of FDA approved drugs, natural products are also important in many herbal medicines. There has been a 18% rise in the use of herbal medicines in United States since 2007 and natural products are being used as cancer preventive measures. Crocetin (**45**) from saffron (*Corcus sativus*) and curcumin (**8**), one of the active constituents of turmeric, have both shown growth inhibiting and apoptosis inducing effects, and can be used in combination for either treatment or prevention of cancer without undesired toxicity.⁴³



The potential of using natural products as anticancer agents was recognized in the 1950s by the U.S. National Cancer Institute (NCI), and the NCI has since made major contributions to the

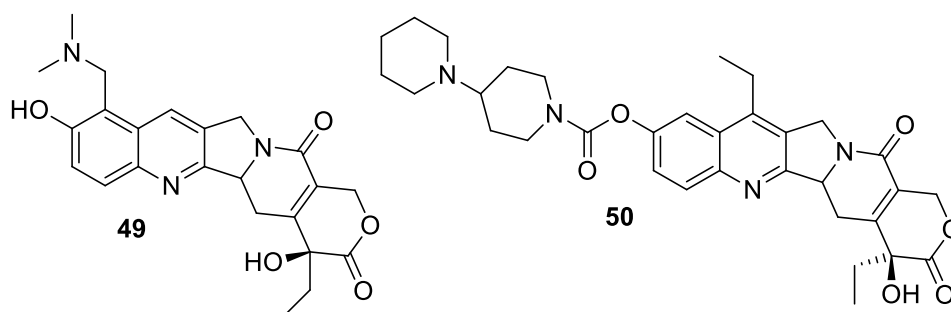
discovery of new naturally occurring anticancer agents. There are at least 2,500,00 species of plants out of which more than one thousand have been found to possess significant anticancer properties.^{44, 45} While many molecules obtained from nature have shown significant usefulness, there is a large number of molecules that still remains to be discovered and brought to potential usefulness.

One of the first known anticancer drugs is podophyllotoxin (**46**). This was used for the treatment of warts and as a purgative, and was later found to act as an anti-cancer agent by irreversibly binding to tubulin.⁴⁶ Synthetic modification of this molecule led to the development of etoposide (**47**) and teniposide (**48**), known to be effective for small cell cancers of the lungs and testes.⁴⁷ These modified drugs act by inhibiting topoisomerase II, thus disrupting the enzyme-DNA complex and causing cell death.⁴⁸

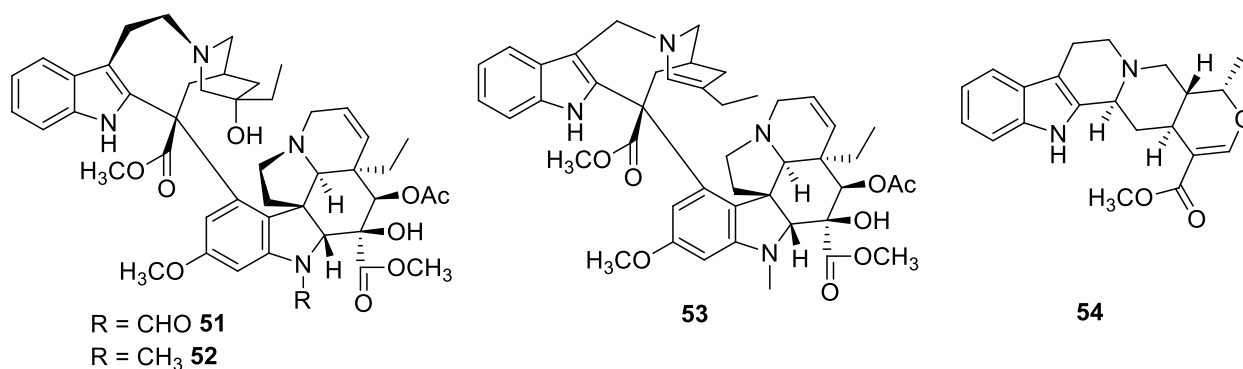


Camptothecin (**6**) is an extensively studied natural alkaloid isolated from the Chinese tree *Camptotheca acuminata*.⁴⁹ The mechanism of action of camptothecin is by inhibition of topoisomerase I, thus disrupting DNA-enzyme interaction and causing cell death. Camptothecin (**6**) has low bioavailability and is poorly water soluble, hence structural modification was aimed

at developing more water-soluble derivatives. Topotecan (**49**) and irinotecan (**50**) have solved the problem and are being used as successfully.⁴⁹

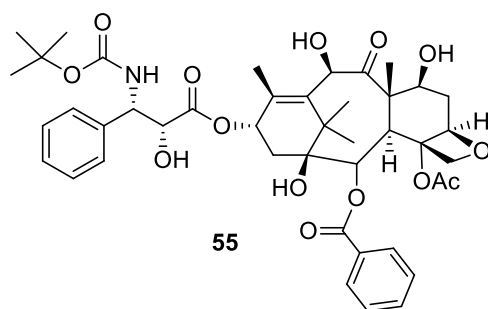


The Madagascar periwinkle plant *Catharanthus roseus* from the family Apocynaceae contains the vinca alkaloids vincristine (**51**) and vinblastine (**52**), which were the first natural product anticancer agents to enter clinical trials. Vincristine (**51**) and vinblastine (**52**) are both known to irreversibly bind to tubulin, preventing mitosis and eventually causing cell death. Vinorelbine (**53**) is a synthetic analogue of vincristine (**51**), with an eight-membered rather than a nine-membered C-ring and a dehydrated D ring. The other traditional use of this plant is as an antihypertensive, due to ajmalicine (**54**), and for treating diabetes by enhancing the production on insulin in the body.

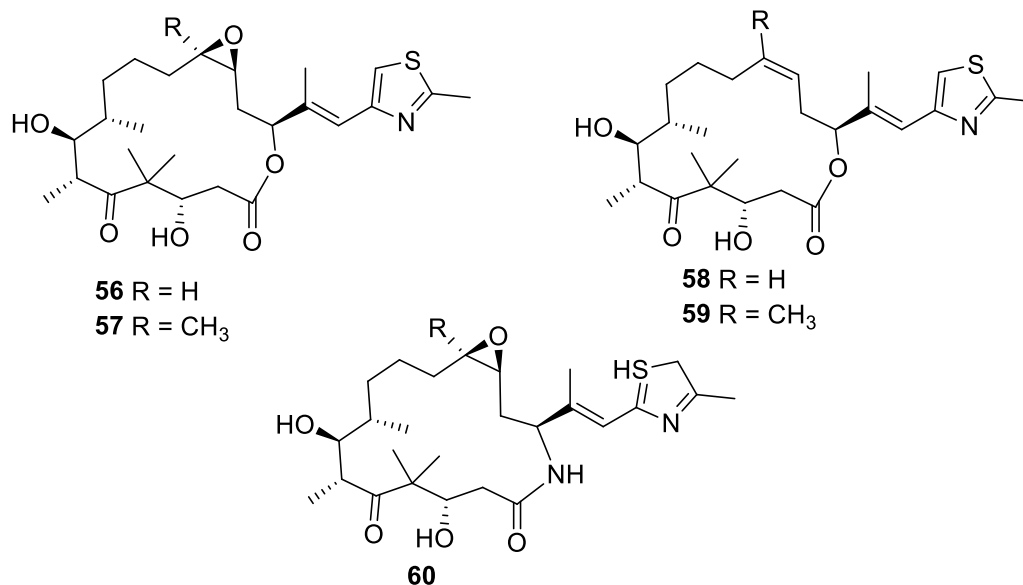


A most important discovery in this class of drugs is paclitaxel from the bark of the Pacific yew, *Taxus brevifolia* Nutt. (Taxaceae).⁵⁰ Paclitaxel (**5**) has a unique mode of anticancer action,

promoting the assembly of microtubules and consequently inhibiting mitosis. Since isolation of paclitaxel required the sacrifice of the tree to obtain its bark, a semi-synthetic route to production was developed, and this led to the development of the paclitaxel analogue docetaxel (**55**).³⁵ Docetaxel is a semi-synthetic derivative of paclitaxel, and has shown good clinical results for the treatment of breast cancer and was approved by the FDA in 1996. It is also in clinical trials for the treatment of other cancer types. Docetaxel is synthesized from the more readily available 10-deacetyl-baccatin III, which is extracted from the European yew tree *Taxus baccata* L.⁴⁵



Epothilones (**56**, **57**, **58** and **59**), are bacterial natural products with potent microtubule stabilizing and anti-proliferative activity, and are the first non-taxane based microtubule stabilizers to be developed clinically. They were isolated from the mycobacterium *Sorangium cellulosum*, and they have been synthesized and their semi-synthetic analogues have been used to understand their SAR. Six of the many analogues are in clinical trials and many other promising compounds are entering clinical studies for their properties. Ixabepilone (**60**) is one analogue that has recently been approved by the FDA.⁵¹

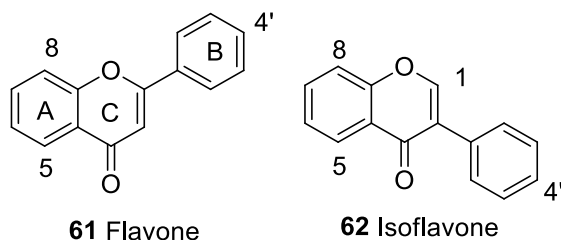


The previous sections have reviewed some of the significant bioactivities of natural products. Another way of understanding the importance of natural product is to classify them by structural type. The next sections will review the natural product classes of flavonoids and stilbenes, since these were the two classes encountered in this research.

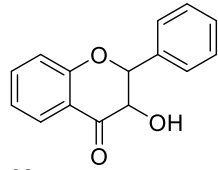
Structure and chemistry of flavonoids

Flavonoids are widespread in the plant kingdom and are plant secondary metabolites. They exhibit a broad range of biological activities including antibacterial, antifungal, antiviral, antiallergenic, anti-inflammatory, anti-proliferative and antioxidant activities.^{52, 53} They can have either flavone (**61**), isoflavone (**62**) or neoflavonoid skeletons and are usually polyphenolic in nature. Flavonoids exist either as free aglycones or as glycosidic conjugates. The type of flavonoid and its chemical properties are determined on the basis of the positions of the groups substituting rings A, B, and C. The distinction between flavonoids and isoflavonoids is in the position at which ring B is attached to the 4-benzopyrone core of the molecule. In flavonoids

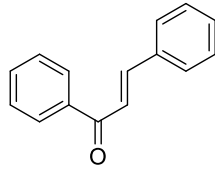
ring B is bound to position 2 of ring C and in isoflavonoids, ring B is found at position 3 of ring C.⁵⁴



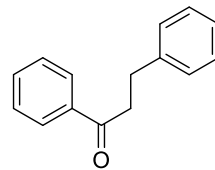
Different types of flavonoids are shown below and include flavones, flavonols, isoflavones, flavanones, dihydroflavonols, aurones and chalcones. Over 4000 flavonoids have been identified, and occur mostly in fruits and vegetables. Many flavonoids are still yet to be uncovered for their potential bioactivity. Numerous studies have been carried out to determine the biological importance of this class of compounds. Apart from the previously mentioned activities, flavonoids can be used for conditions such as diabetes, hypercholesterolemia, obesity and hypertension.⁵⁵ Their antibacterial activity in particular is observed to be in the order flavonoids>polymethoxyflavonoids>isoflavonoids.⁵⁶ The diversity of structural patterns has resulted in flavonoids being recognized as a rich source of compounds with good anticancer potential. The ability of some flavonoids to block the cell cycle, induce apoptosis, and disrupt mitotic spindle formation or inhibit angiogenesis makes them potential anti-cancer agents.⁵⁷



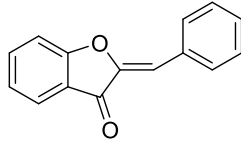
63 Leucoanthocyanidin



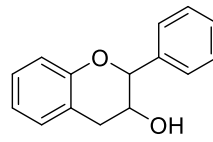
64 Chalcone



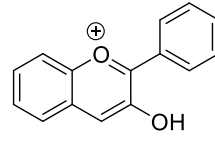
65 Dihydrochalcone



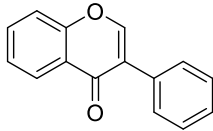
66 Aurone



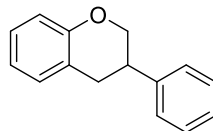
67 Flavan-3-ol



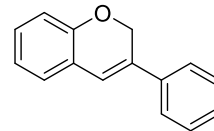
68 Anthocyanidin



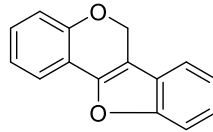
69 Isoflavone



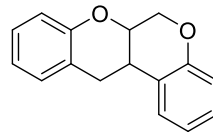
70 Isoflavan



71 Isoflaven



72 Pterocarpan

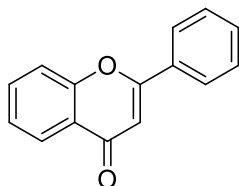


73 Rotenoid

Basic skeletons of some flavonoids

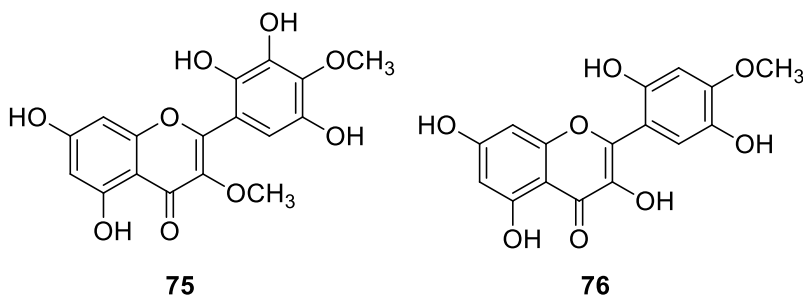
Flavones

These are a class of flavonoids based on the backbone of 2-phenylchromen-4-one.



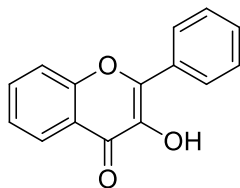
74 Flavone skeleton

Flavones are mainly found in cereals and herbs. Some flavones such as primetin, chrysin and luteolin show vasorelaxing, anti-oxidative and chemo-preventive effects respectively.⁵⁸ The total flavones from the leaves of *Choerospondias axillaris* (Roxb.) have shown activities including anti-arrhythmic action, hypoxic tolerance, myocardial ischemic protection, platelet congregation inhibition, and hemorheology improvement.⁵⁹ Flavones isolated from *Callistemon lanceolatus* DC have exhibited anti-diabetic activity.⁶⁰ Robusflavones A (75) and B (76) and other flavones isolated from the twigs of *Erisema robustum* (Fabaceae) have shown anti-oxidant activity.⁶¹



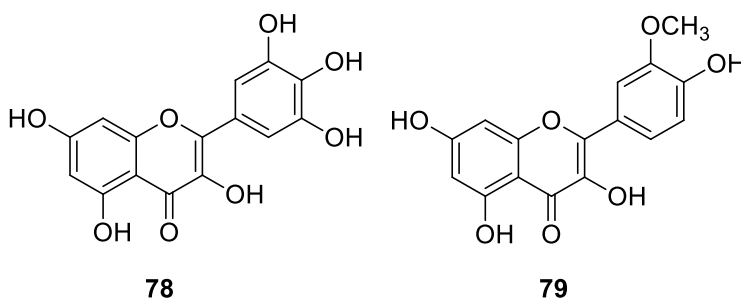
Flavonols

Flavonols have a 3-hydroxyflavone structure, and are unstable to light as they undergo photo-oxidation resulting in fading or formation of darker colors. This can explain why onions (*Allium cepa*) and marigolds (*Tagetes* spp.) which are rich in flavonols, are highly colored.



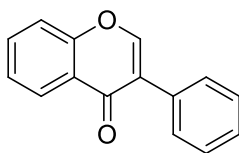
77 Flavonol skeleton

Flavonols are present in a wide variety of fruits and vegetables and find many applications in the dye and pharmaceutical industries.⁶² Flavonols benefit human health by displaying antioxidant and anti-carcinogenic properties, and protecting against coronary heart disease. Quercetin, kaempferol, myricetin (**78**) and isorhamnetin (**79**) are commonly found flavonols.⁶³ Dorsmanin C, isolated from the plant *Dorstenia mannii* (Moraceae), has antioxidant properties.⁵⁵



Isoflavones and isoflavonones

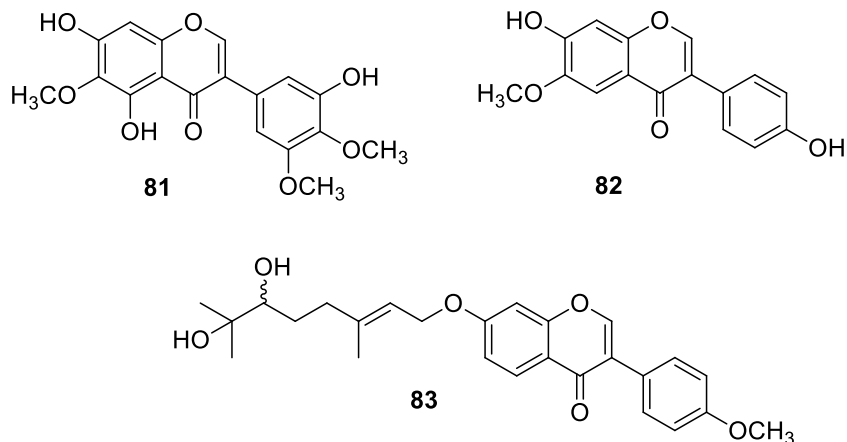
These are naturally occurring compounds generally acting as phytoestrogens in humans. These are produced almost exclusively by the Fabaceae family (Leguminosae). Isoflavones differ from flavones (2-phenyl-4*H*-1-benzopyr-4-one) in the location of the phenyl group.



80 Isoflavone skeleton

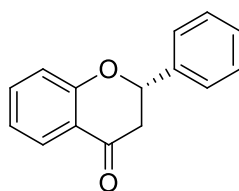
Isoflavones are major products in soy. Soy products contains diadzen, genistein and glycitein, their respective β -glycosides and their respective acetyl glycosides.⁶⁴ Irogenin (**81**), glycitein

(**82**), 8-hydroxyglycitein and licoisoflavone have been extracted from *Nicotiana tabacum*.⁶⁵ Genistein has been isolated from the *Bolusanthus speciosus* (Fabaceae), *Ficus chlamydocarpa* (Moraceae), *Erythrina latissima* (Fabaceae), *E. indica* (Fabaceae), and exhibits antimicrobial activity. Griffonianone D (**83**) from *Millettia griffoniana* exhibits cytotoxic activity, enzyme inhibition, estrogenic properties and anti-inflammatory activity.^{55, 66}



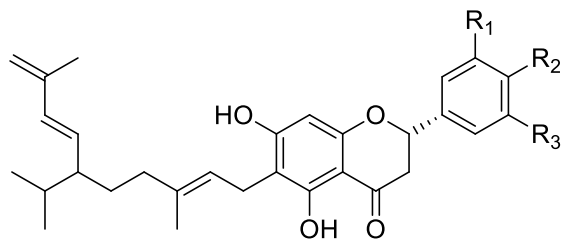
Flavanones

The flavanone class of flavonoids is found almost exclusively in citrus plants.⁶⁷



84 Flavanone skeleton

In citrus fruits, flavanones account for about 95% of the total flavonoids.⁶⁸ Flavanones have been shown to exhibit anti-fungal, anti-diabetic, anti-bacterial, anti-inflammatory, anti-proliferative, anti-platelet aggregating, antioxidant and aldose reductase inhibitory activities. Japonicasins A (**85**) and B (**86**) isolated from *Sophora japonica* L. are isoprenylated flavanones.⁶⁹



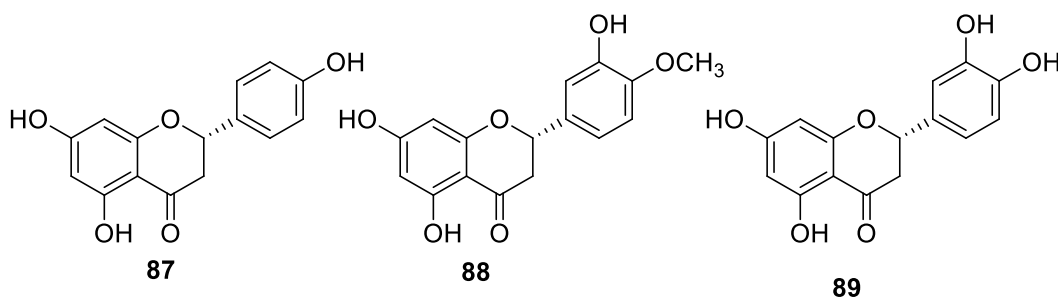
85 $R_1 = R_3 = \text{OH}$, $R_2 = \text{H}$

86 $R_1 = R_3 = \text{H}$, $R_2 = \text{OH}$

Citrus flavanones are often glycosylated, examples includes naringin, hesperidin, and eriocitrin.

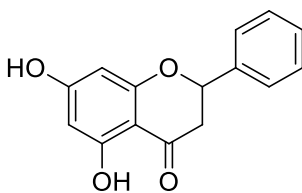
The aglycones of these glycosides are naringenin (**87**), hesperitin (**88**), and eridictyol (**89**).⁷⁰

Other flavanones includes sigmoidins A and B from *Erythrina sigmoidea* (Fabaceae), tephrocandidin A from *Tephrosia candida* (Fabaceae), and burttinone from *Erythrina caffra* Thunb (Fabaceae).⁵⁵



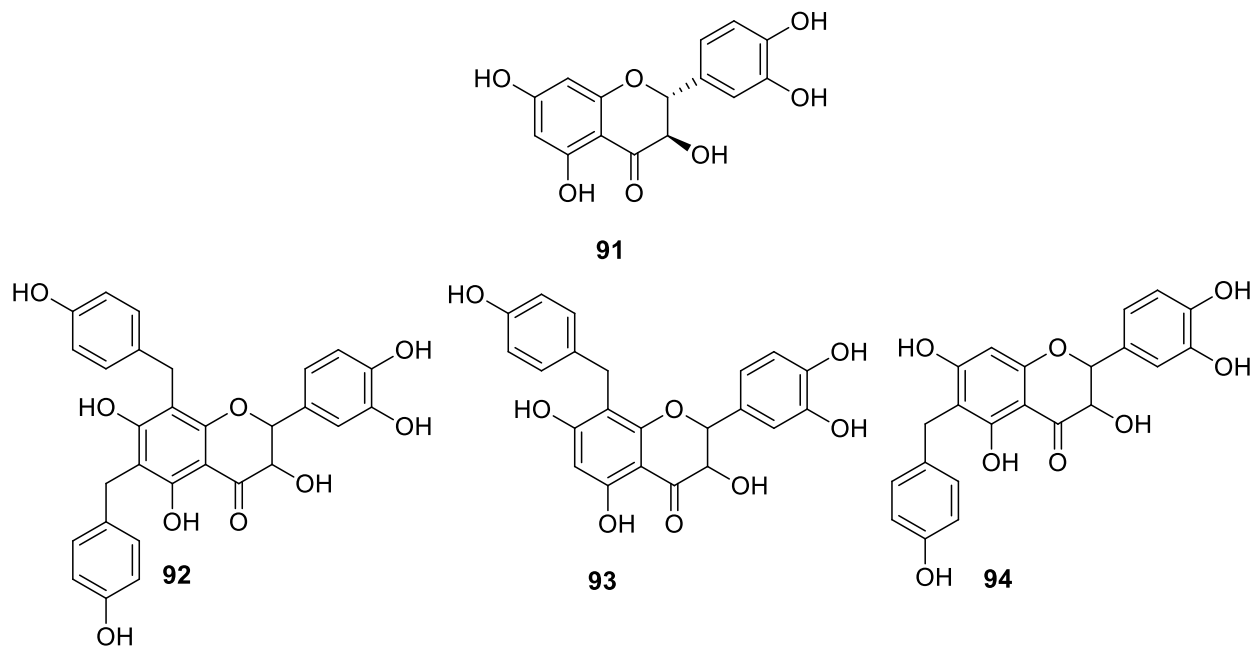
Dihydroflavonols

Dihydroflavonols are an important class of flavonoids which exhibit a wide spectrum of pharmacological properties. They generally have a structure confined to the 5,7-dihydroxyflavone skeleton. They are found in woody plants and may also occur in herbaceous species in minor amounts.



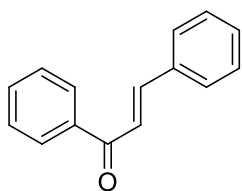
90 Dihydroflavonol skeleton

Dihydroflavonols such as taxifolin (**91**) exhibit a wide spectrum of pharmacological properties including antioxidative, antifungal, hepatoprotective and gastro protective and neoplastic activities.⁷¹ Gericudranin A, B and C (**92**, **93**, and **94**) isolated from *Cudrania tricuspidata* has shown notable cytotoxicity against human cancer cell lines.⁷²



Chalcones

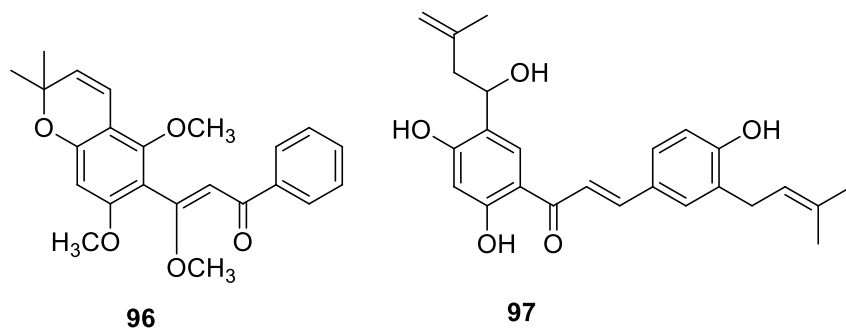
Chalcones are aromatic enones that form the central core for a variety of important biological compounds which are also known as chalconoids. Benzylidene acetophenone is the parent member of this series.



95 Chalcone skeleton

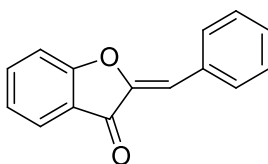
Members of this class of flavonoids exhibit varying pharmacological activities such as antimicrobial, anti-plasmodial, anti-inflammatory, anti-diabetic, anti-obesity, antibacterial and

anti-reverse transcriptase. With such a broad spectrum of activities, this class is a very important class of flavonoids. Praecansone A (**96**), B and demethylpraecansone isolated from *Tephrosia aequilata* (Fabaceae),⁷³ and bartericin A (**97**) from *Dorstenia barteri* var. *subtriangularis* (Moraceae) are examples of complex chalcones.⁷⁴



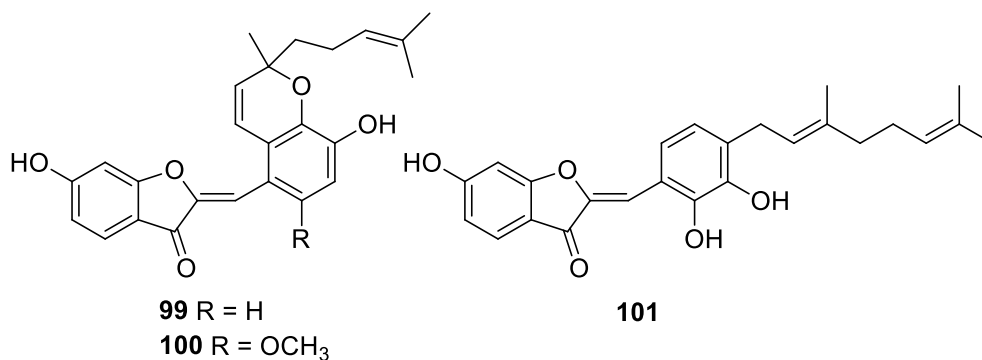
Aurones

This is unique type of flavonoid which contains a benzofuran ring with a benzylidene group linked at position 2.



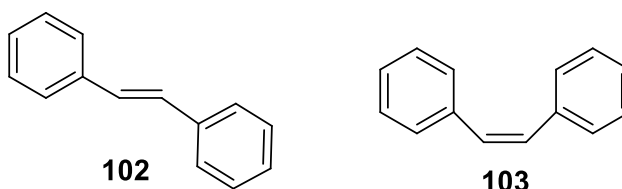
98 Z-Aurone skeleton

In aurones, a chalcone-like group is closed into a 5-membered ring instead of the 6-membered ring more typical of flavonoids. Aurones are plant flavonoids that provide a yellow color to the flowers of many popular ornamental plants. Altilisin H (**99**), I (**100**), and J (**101**) isolated from the extract of the leaves of *Artocarpus altitis* (Moraceae) and leptosidin from *Coreopsis grandiflora* are examples of aurones.⁷⁵



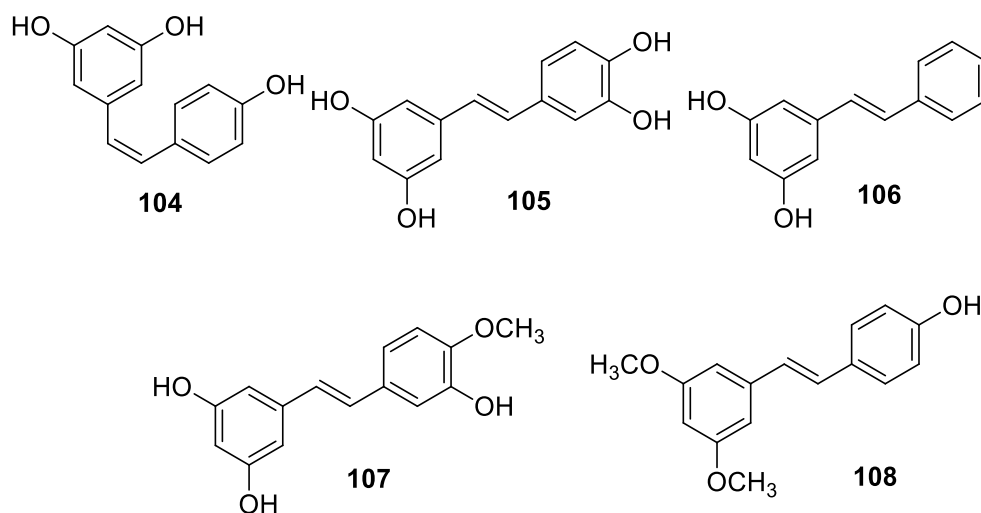
Structure and chemistry of stilbenes

Stilbenes are 1,2-diphenylethylene skeletons with two isomeric forms, *E*-stilbene (**102**) (trans) which is not sterically hindered and *Z*-stilbene (**103**) (cis), which is sterically hindered and therefore less stable. The *E* and the *Z* forms of stilbenes elicit different pharmacological responses and also have different potencies.⁷⁶ Stilbenes are generally observed to possess anticancer and antioxidant properties.⁷⁷ Hydroxylated derivatives of the stilbenes (stilbenoids) are secondary products of heartwood formation in trees and can act as phytoalexins.⁷⁸ Stilbenes act as natural protective agents to defend the plant against viral and microbial attack, excessive ultra violet exposure and disease. More than 200 stilbenes are known from nature, and are often found in the heartwood of trees.⁷⁸

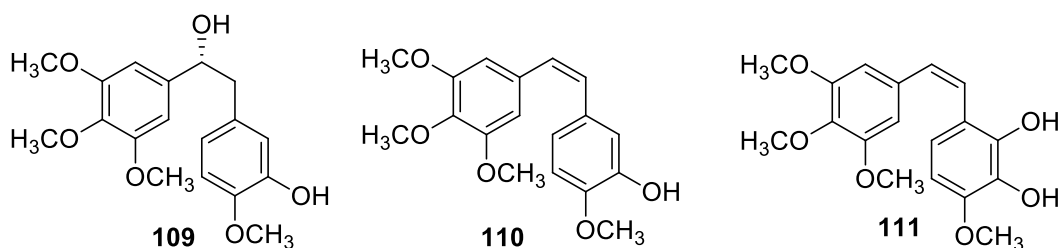


Diethylstilbestrol, hexestrol and dienesterol (estrogenic hormones) are stilbenes. Another extensively studied stilbene, resveratrol (**6**), along with having numerous neurological benefits,⁷⁹ has been shown to exhibit anticancer, anti-inflammatory and antioxidant activities. Studies have proved trans-resveratrol (**10**) to be 10 times more potent in its ability to induce apoptosis compared to cis-resveratrol (**104**). Resveratrol is produced by *Vitis vinifera* grapevines as a

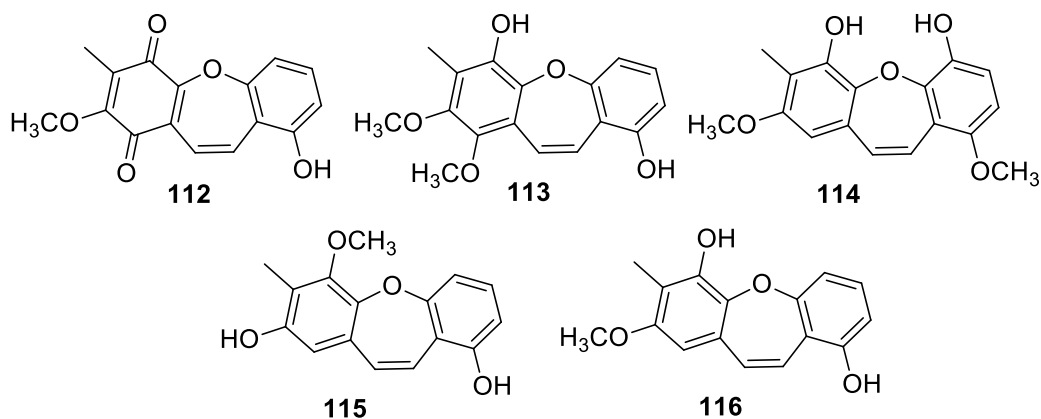
defense in response to fungal infection or other environmental stressors. Resveratrol has also been isolated from many species of the Dipterocarpaceae family.⁸⁰



Piceatannol (**105**) is a naturally occurring stilbene present in sugar cane berries, peanuts, red wines and the skin of grapes, and was first isolated from *Euphorbia lagascae* and is also found in *Cassia garrettiana*. Pinosylvin (**106**) is yet another potent stilbene known to exhibit anticancer, anti-inflammatory and antioxidant properties. It is found in many pine tree wood extracts and eucalyptus. Rhapontigen (**107**), a stilbene found in Korean rhubarb rhizomes, is most abundantly found in the *Rhei undulatum* species. It is used in treatment of Oketsu, a condition characterized by poor circulation, pain and chronic inflammation, and has been recommended to treat allergies. Pterostilbene (**108**) has been identified as a phenolic compound in darakchasava, a traditional Ayurvedic medicinal drink to treat cardiovascular diseases and other conditions. Pterostilbene (**108**) is also found in deer berry and rabbiteye blueberries, unripe Pinot noir grapes and in *Botrytis vinifera* infected chardonnay grapes and immature berries of Pinot and Gamay varieties. Pterostilbenes are also found in *Pterocarpus marsupium*, a tree whose heartwood is used to treat diabetes.⁷⁶



Another very potent class of anticancer agents that comes from the stilbenes, is that of the combretastatins (**109-111**). Combretastatins were first isolated from the bark of the South African tree *Combretum caffrum*.⁸¹ The mechanism of action of these stilbene is that they inhibit tubulin polymerization by targeting the colchicine-binding domain of tubulin,⁸² and they are cytotoxic against a wide variety of human cancer cell lines including multi-drug resistant cancer cell lines. Combretastatin A-4 (**110**) was found to be a potent anti-angiogenic agent in its pre-clinical trial begun in November 1998.⁸³ Combretastatins consist of a cis-stilbene skeleton along with methoxy and hydroxy groups on the aromatic rings.⁸¹ Structure activity relationship studies have shown that the (*Z*)-olefin and phenolic functional groups are important, as they bind to the colchicine binding site and inhibit tubulin polymerization.^{84, 85} The bauhiniastatins 1-4 (**112-115**) from the plant *Bauhinia purpurea*, which resemble the (*Z*)-stilbene geometry of the combretastatin series, also have anticancer activity.^{86, 87} These are related to pacharin (**116**) which is a cancer cell growth inhibitor.⁸⁸



Bioassay

Bioassays forms an important step for the successful isolation of active compounds from various natural sources. The usual method for isolation of active components is bioassay-guided fractionation. Several bioassays are available to evaluate the of bioactivities of different types of compounds.⁸⁷ The assays can be chosen based on the nature and type of activity that is to be isolated. An ideal bioassay would be highly sensitive to small amounts of active material, selective to the specific bioactivity, cost effective and easy to run and maintain.^{89, 90}

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Chapter 2: Isoflavone and pterocarpans from *Neoharmsia baronii* from the Madagascar forest.

Introduction

A systematic study of an ethanolic extract of the plant *Neoharmsia baronii* Drake (figure 2), from the Madagascar forest led to the isolation of isoflavones and pterocarpans. All the isoflavones showed varying antiproliferative activity to the A2780 ovarian cell line, with vogelin-E showing the most potent activity.

Because of the need for natural products based drug discovery combined with biodiversity conservation, as discussed in the previous chapter, our group has focused on discovering antiproliferative natural products from the forests of Madagascar over the past 15 years as a part of the International Cooperative Biodiversity Group (ICBG) program.¹ In the course of this research, an ethanolic extract of the wood of the tree *Neoharmsia baronii* (Drake) (Fabaceae) R. Vig. was found to have antiproliferative activity against the A2780 ovarian cancer cell line, with an IC₅₀ value of 12 µg/mL.² This extract was thus selected to study its chemical components.



Figure 2. *Neoharmsia baronii*
Photography by : Fidy Ratovoson (efloras.org)

Neoharmsia baronii is found in highly restricted localities in the northern tip of Madagascar. It is an important coastal species, falling in the critically endangered species category of the IUCN Red list of Threatened Spices.³ The Fabaceae family is well known, and includes various herbaceous perennial plants, found growing throughout the world in many different environments and climatic conditions.⁴ Many of the plants in this family are economically important as agricultural and food plants. The general chemical constituents of this family include alkaloids, flavonoids, and tannins. The *Neoharmsia* genus of legume is found exclusively in Madagascar.⁵ No previous studies have been made on the chemical constituents of any *Neoharmsia* species.

Results and discussion:

The ethanolic extract of *Neoharmsia baronii* was subjected to bioassay-guided fractionation against the A2780 cell line. The initial step was a liquid-liquid extraction to give an active dichloromethane fraction with a IC_{50} of 16 $\mu\text{g/mL}$. Various solid phase extractions and normal phase silica gel chromatography on a Biotage flash chromatography column, together with C-18 reverse phase chromatographic separations, were used to obtain seven isolated compounds. Characterization of the compounds was done on the basis of their physiochemical properties and spectroscopic data and comparison with literature data.

The first compound **A** was isolated as yellow crystals, with a composition of $C_{17}H_{14}O_6$, confirmed by its negative ion HR ESIMS data with an $[M-H]^-$ ion at 313.2895. The ^1H NMR spectrum for compound **A** showed signals in the aromatic region and two 3-proton singlets at approximately 3 ppm assigned to methoxy protons. The presence of a carbonyl group was confirmed by an IR spectrum, which showed a strong absorption at 1734 cm^{-1} , and by a ^{13}C NMR resonance at δ_{C} 178.1. The ^1H NMR spectrum (figure 3) showed a singlet at δ_{H} 8.17,

correlating to the ^{13}C signal at δ_{C} 154.2 in HSQC; this signal is characteristic of the H-2 resonance in isoflavones, thus suggesting the presence of an isoflavone skeleton. Two aromatic ring doublets at δ_{H} 7.77 and δ_{H} 6.93 with coupling of 8.9 Hz indicated an *ortho* di-substituted benzene ring.

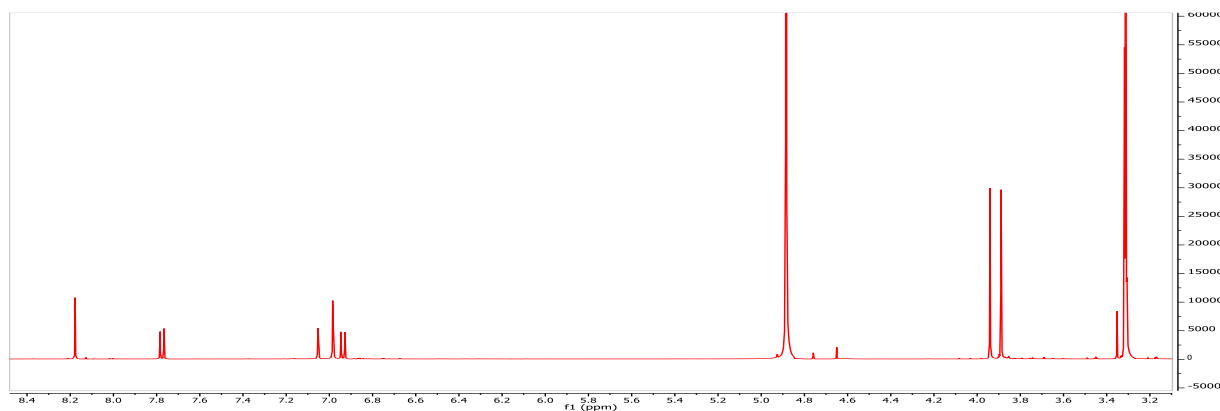


Figure 3. $^1\text{H-NMR}$ spectrum of compound A

These data suggested possible partial structures **117**, **118**, **119**, **120**, and **121** as shown in figure 4.

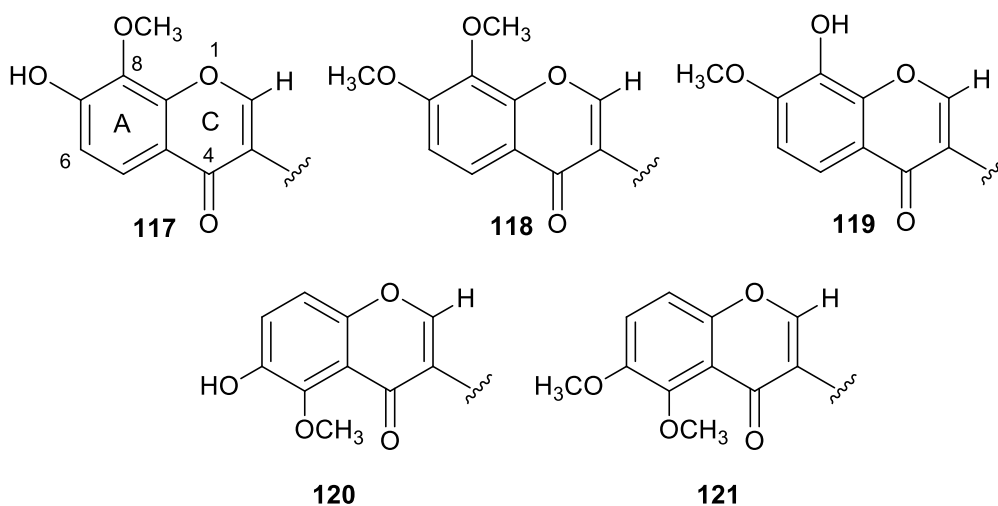


Figure 4. Possible structures of ring A

The NOE spectrum was then obtained to assist in the assignment of the ring A and ring C. The H-2 proton showed a weak NOE cross peak to the protons of a methoxy group, which suggested that a methoxy was in proximity to H-2, so the methoxy group was assigned to C-8, and this result eliminates structures **119**, **120** and **121**. No correlations were obtained between the other methoxy group and the protons of ring A, and this fact helped exclude structures **118** and **119**. The carbons for rings A and ring C were then assigned using HSQC as well as HMBC to confirm the previously determined positions. The singlet at 8.17 assigned as H-2 showed correlation with the peak at δ_C 154.2 in HSQC, and also showing correlation with the peak at δ_C 178.1 in HMBC. The ring A protons assigned at δ_H 7.77 and δ_H 7.69 showed clear correlations with the peaks at δ_C 122.2 and δ_C 118.8 in HSQC spectrum which were thus confirmed as C-5 and C-6. Both the protons H-5 and H-6 showed correlations to δ_C 161.5, a peak which did not appear in the ^{13}C -NMR spectrum. This allowed the assignment of C-7 to 161.5. These data put together determined the substitution on the rings A and C.⁶ The remaining peaks in the proton NMR spectrum with δ_H 7.05 (1H, t, $J = 1.2$), 6.98 (2H, d, $J = 1.2$) and signals for a methoxy group were assigned to ring B.

The three ^1H resonances in the aromatic region of ring B appeared as a triplet at δ_H 7.05 ($J = 1.2$) and a two proton doublet at 6.98 ppm with $J = 1.2$. The peak with δ_H 7.05 (1H, t, $J = 1.2$) showed a correlation with the peak at δ_C 117.5 in the HSQC spectrum, and the doublet for the 2 protons at δ_H 6.98 (d, 2H, $J = 1.2$) showed correlations with δ_C 112.6 and δ_C 121.6 in the HSQC spectrum.⁷ This left us with three possibilities shown in figure 5.

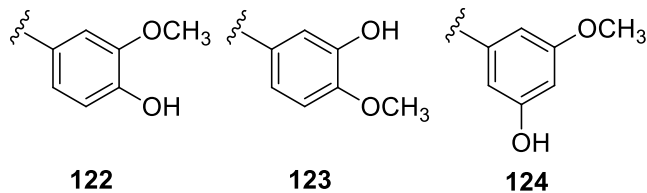


Figure 5. Possibilities for ring B

The next important step was to determine the position of at least one of the protons in ring B, most importantly the triplet proton. The H-2 proton showed weak correlations in the NOE spectrum to the triplet peak and thus determined the triplet to be due to H-2' or H-6'. To confirm this the HMBC correlations were studied, which confirmed that H-2' showed a three bond correlation with the C-3 carbon at δ_C 125.3.

The methoxy protons at δ_H 3.89 also showed weak NOE correlations with the proton at δ_H 7.05, and suggested the methoxy group was in proximity to H-2', which was consistent with structure **124**. To confirm this structure, a comparison was done between the literature values for ring B with those for compound **125**. Since the candidate structure **124** was not reported in the literature only the chemical shifts of ring B were compared to literature values. The following table depicts the comparison of the data for compound A with those for compound **125**.⁸ These NMR comparison showed good agreement between the data for the ring B carbons and protons of compound A with those of compound **125**. The NMR spectra for A and for **125** were both obtained in methanol-*d*₄ (500 MHz).

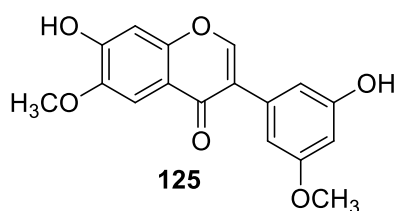


Table 1. NMR spectroscopic data for comparison of rings B for compound **A** with **125**

A		125		
position	δ_{H}	δ_{C}	δ_{H}	δ_{C}
C1	-	-	-	-
C2	8.17 s	154.2	8.12 s	154.6
C3	-	125.3	-	126.5
C4	-	178.1	-	177.7
C5	7.77 d (8.9)	122.2	7.55 s	105.5
C6	6.93 d (8.9)	118.8	-	148.8
C7	-	161.5	-	155.3
C8	-	136.8	6.92 s	104
C9	-	153.2	-	154.4
C10	-	116.8	-	117.8
C1'	-	126.4	-	125.3
C2'	7.05 t (1.2)	117.5	7.05 br s	117.5
C3'	-	147.4	-	147.5
C4'	6.98 d (1.2)	121.6	6.97 br s	121.7
C5'	-	149.1	-	149.2
C6'	6.98 d (1.2)	112.6	6.97 br s	112.7
6-OCH ₃	-	-	3.95 s	56.7
8-OCH ₃	3.94 s	61.5	-	-
5'-OCH ₃	3.89 s	56.4	3.88 s	56.5

^a NMR in *d*-ethanol

This information, coupled with the composition C₁₇H₁₄O₆, is consistent with a *meta* disubstituted ring B, as in structure **124**. The only other possibility would be structures **122** or **123**, but these structures would require an unusual equivalence of coupling constants for *ortho*, *meta*, and *para* coupled protons, and was not initially considered. The further evidence of structure **124** came from a comparison of the ¹H NMR spectrum of ring C with that of the known compound **125**. These spectra matched with ring C well (Table **1**) and appeared to confirm the structure of **A** having ring C as **124**.

In spite of the good agreement of NMR signals noted above the other possible structure for compound **A** *i.e.* **122** and **123** was not ruled out, because the key HMBC correlations in ring B supported the positions of the protons and methoxy and hydroxy groups.

The NOE spectrum of **A** was thus re-examined to verify the correlation between H-2 and H-2'/H-6'. To our surprise, H-2 was found to correlate to the proton that appeared as a triplet at 7.05 ppm and so this proton must be H-2' or H-6'. This evidence is not compatible with structure **124** but is consistent with structures **122** and **123**.

The H-2' proton showed a two bond correlation with δ_C 149.1, and H-5' showed a two bond correlation with δ_C 147.4. The proton at H-6' showed a two bond correlation with δ_C 126.4 and a three bond correlation with δ_C 117.5, as shown in Fig. 6.

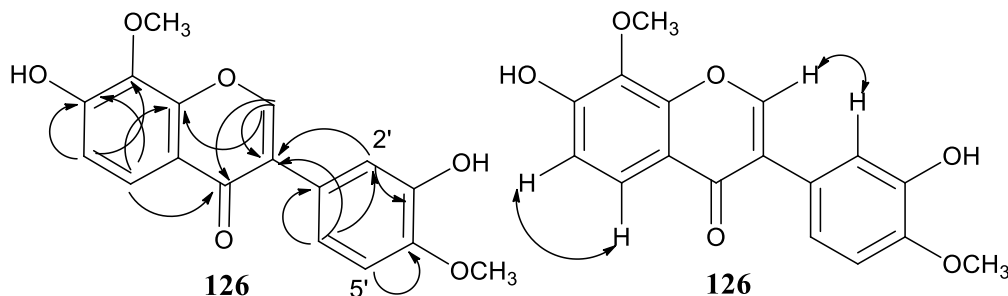


Figure 6. HMBC (left) and NOESY (right) correlations of compound **126**

Because of the ambiguity in the structure of compound **A** it became necessary to carry out a crystal structure determination. A small crystal of compound **A** was grown from methanol : water (7:3) and was found to be suitable for X-ray crystallography which was carried out by Dr. Carla Slebodnick, and confirmed that structure **126** was correct (Fig.7).

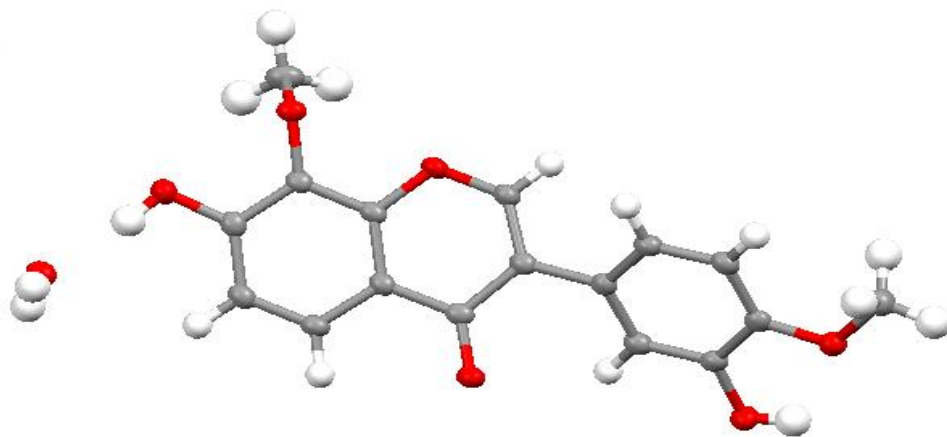


Figure 7. X-ray crystallography structure of compound **A**

Structure **126** was shown to be correct by comparison between the ^1H NMR and ^{13}C NMR data of compound **126** and the same compound from the literature.⁹ The NMR spectra for **A** and for **126** were both obtained in methanol- d_4 .

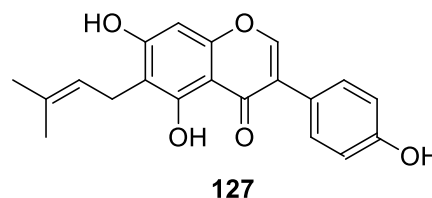
Table 2. NMR spectroscopic data for comparison of compound **A** with literature values for **126**.⁹

Position	A		Literature Value for 126	
	δ_{H} (J in Hz)	δ_{C} , type	δ_{H} (J in Hz)	δ_{C} , type
C1	-		-	
C2	8.17 s	154.2	8.16 s	154.6
C3	-	125.3	-	125.6
C4	-	178.1	-	178
C5	7.77 d (8.9)	122.2	7.77 d (8.9)	122.3
C6	6.93 d (8.9)	118.8	6.96 d (8.9)	112.5
C7	-	161.5	-	156.5
C8	-	136.8	-	136.2
C9	-	153.2	-	152.6
C10	-	116.8	-	119.1
C1'	-	126.4	-	126.1
C2'	7.05 t (1.2)	117.5	7.02 br s	117.4
C3'	-	147.4	-	147.4
C4'	-	149.1	-	149.2
C5'	6.98 d (1.2)	121.6	6.97 br s	116.5
C6'	6.98 d (1.2)	112.6	6.93 br s	121.6
8-OCH ₃	3.94 s	61.5	3.93 s	61.8
4'-OCH ₃	3.89 s	56.4	3.84 s	56.4

The second compound was identified as erythrinin B (**127**). The molecular formula of erythrinin B (**127**) was established as $C_{20}H_{18}O_5$ by HREIMS (m/z 339.1226) $[M+H]^+$ calcd. for $C_{20}H_{19}O_5^+$ as 339.1227). The aromatic singlet proton at δ_H 8.00 in the 1H NMR spectrum (Table 3) of **127** indicated that it might be an isoflavone. Furthermore the two doublet proton signals at δ 7.39 (2H, d, $J = 8.7$, H-2', H-6'), and 6.86 (2H, d, $J = 8.7$, H-3', H-5'), indicated the presence of a 4-hydroxy substituted B ring. The remaining aromatic peak at δ 6.40 (1H, s, H-8) was concluded to be in ring A. The peaks at δ 5.25 (1H, tq, H-2''), 1.80 (3H, s, H-4''), and at 1.68 (3H, s, H-5'') indicated the presence of a prenyl group which was concluded to be located on ring A. The final structure was confirmed by comparing the NMR values of **127** with those of the literature.¹⁰ The NMR spectra for **127** and the literature values were both obtained in methanol- d_4 (500 MHz).

Table 3. NMR spectroscopic data for comparison of compound **127** with literature values.

Position	127	Literature Value
2	8.0 s	8.01 s
8	6.40 s	6.36 s
2'	7.39 d (8.7)	7.35 dd (2.0, 8.5)
3'	6.86 d (8.7)	6.83 dd (2.0, 8.5)
5'	6.86 d (8.7)	6.83 dd (2.0, 8.5)
6'	7.39 d (8.7)	7.35 dd (2.0, 8.5)
1''	*	3.31 d (7.0)
2''	5.25 tq	5.22 m
4''	1.80 s	1.77 s
5''	1.68 s	1.65 s



* indicates the peak concealed in solvent peak.

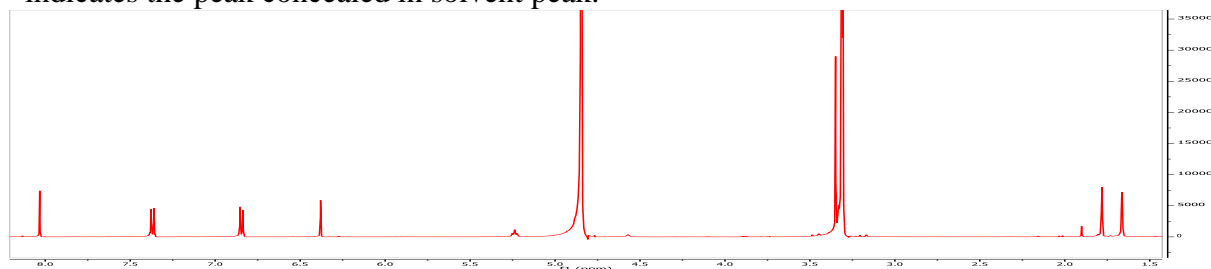


Figure 8. 1H -NMR spectrum of compound **127**

The third compound was identified as genistein (**128**). The molecular formula of genistein was established as C₁₅H₁₀O₅ by HREIMS (*m/z* 269.0479 [M-H]⁻, calcd. for C₁₅H₉O₅⁻ as 269.0455). The aromatic singlet proton at δ 8.04 in the ¹H NMR spectrum (Table 4) of **128** indicated that it might be an isoflavone. Furthermore the two doublet proton signals at δ 7.37 (2H, d, *J* = 8.7, H-2', H-6'), and 6.84 (2H, d, *J* = 8.7, H-3', H-5'), indicated the presence of a 4-hydroxy substituted B ring. The other remaining aromatic peak at δ 6.30 (1H, d, *J* = 2.0, H-8) and 6.19 (1H, d, *J* = 2.0, H-8) indicated *meta* coupled protons in ring A. The final structure was confirmed by comparing the NMR data of **128** with the literature data.¹¹ The NMR spectra for **128** was obtained in methanol-*d*₄ and the literature values were obtained in DMSO.

Table 4. NMR spectroscopic data for comparison of compound **128** with literature values.

Position	128	Literature Value
2	8.04 s	8.28 s
6	6.19 d (2.0)	6.28 d (2.1)
8	6.30 d (2.0)	6.42 d (2.1)
2'	7.37 d (8.7)	7.32 d (8.8)
3'	6.84 d (8.7)	6.95 d (8.8)
5'	6.84 d (8.7)	6.95 d (8.8)
6'	7.37 d (8.7)	7.32 d (8.8)

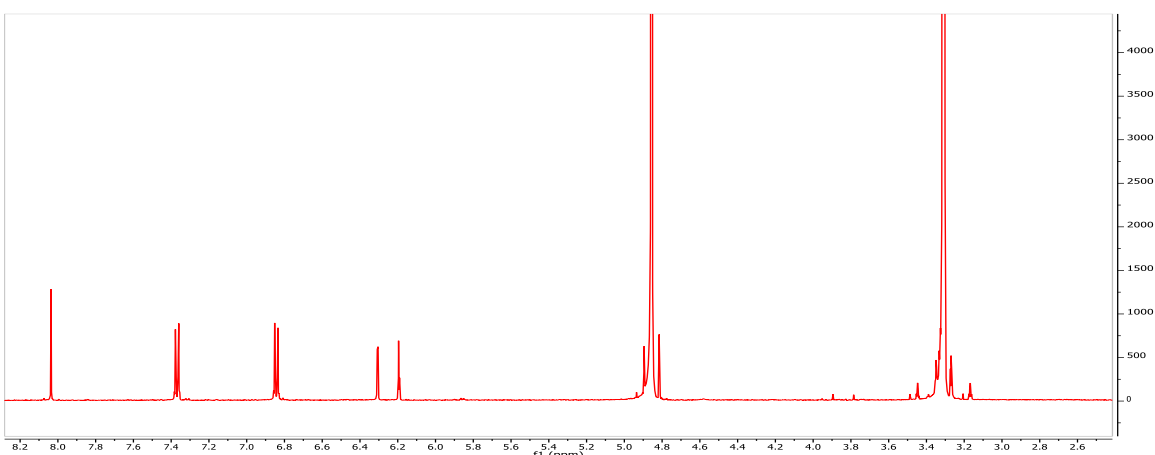
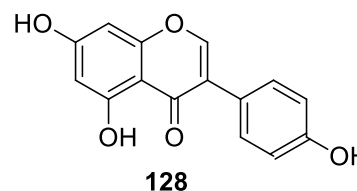
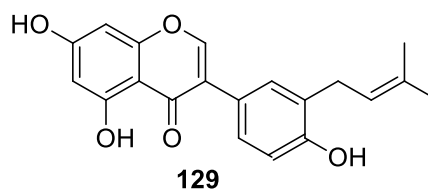


Figure 9. ¹H-NMR spectrum of compound **128**

The fourth compound was identified as isowighteone (**129**). The molecular formula of isowighteone was established as $C_{20}H_{18}O_5$ by HREIMS (m/z 337.1097 $[M-H]^-$, calcd. for $C_{20}H_{17}O_5^-$ as 337.1081). The aromatic singlet proton at δ 7.94 in the 1H NMR spectrum (Table 5) of **129** indicated that it might be an isoflavone. Furthermore the two doublet protons signals at δ 7.20 (1H, d, $J = 2.1$, H-2'), and 6.80 (1H, d, $J = 8.7$, H-5'), and one doublet of doublets 7.16 (1H, dd, $J = 8.7, 2.1$, H-6') indicated the presence of three protons, two of which were *meta* coupled and one was *ortho* coupled. The remaining aromatic peaks at δ 6.23 (1H, d, $J = 2.1$, H-8) and 6.14 (1H, d, $J = 2.1$, H-6) were *meta* coupled to each other, suggesting the possibility of being in ring A only. The peaks at δ 5.35 (1H, tq, H-2''), 1.73 (3H, s, H-4''), and at 1.73 (3H, s, H-5'') indicated the presence of prenyl group, which was again concluded to be located on ring B. The final structure was confirmed by comparing the NMR values of **129** with the literature values.¹² The NMR spectra for **129** was obtained in methanol- d_4 and the literature values were obtained in $CDCl_3$.

Table 5. NMR spectroscopic data for comparison of compound **129** with literature values.

Position	129	Literature Value
2	7.94 s	7.86 s
6	6.14 d (2.1)	6.32 brs
8	6.23 d (2.1)	6.38 brs
2'	7.20 d (2.1)	7.28 d (2.2)
5'	6.80 d (8.2)	6.8 d (8.8)
6'	7.16 dd (8.2, 2.1)	7.26 dd (8.8, 2.2)
1''	*	3.41 d (7)
2''	5.35 tq	5.37 tq (7, 11)
4''	1.73 s	1.81 s
5''	1.73 s	1.81 s



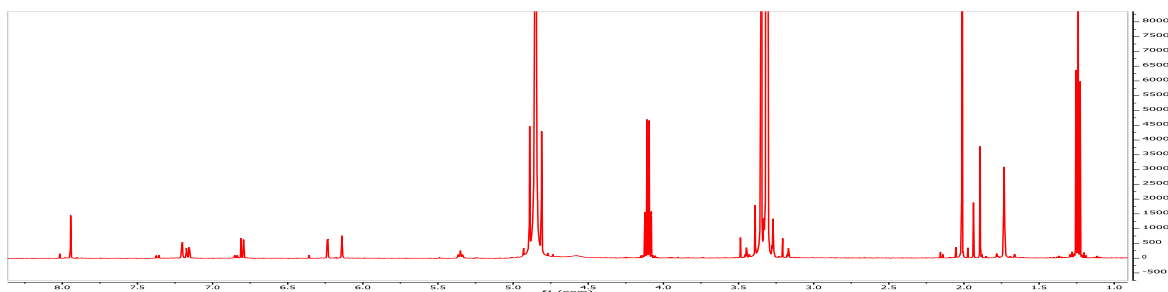
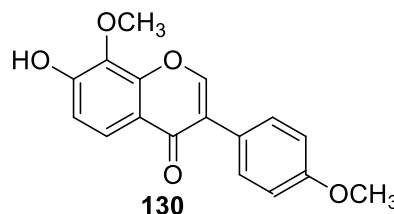


Figure 10. ^1H -NMR spectrum of compound **129**

The fifth compound was identified as 8-*O*-methylretusin (**130**). The molecular formula of 8-*O*-methylretusin was established as $\text{C}_{17}\text{H}_{14}\text{O}_5$ by HREIMS (m/z 299.0912) $[\text{M}+\text{H}]^+$, calcd. for $\text{C}_{17}\text{H}_{15}\text{O}_5^+$ as 299.0914. The aromatic singlet proton at δ 8.17 in the ^1H NMR spectrum (Table 6) of **130** indicated that it might be an isoflavone. Furthermore the two doublet proton signals at δ 7.47 (2H, d, $J = 8.7$, H-2', H-6'), and 6.98 (2H, d, $J = 8.7$, H-3', H-5'), indicated the presence of a 4-hydroxy substituted B ring. The other remaining aromatic peaks at δ 7.75 (1H, d, $J = 9.0$, H-5), and 6.90 (1H, d, $J = 9.0$, H-6), indicated the presence of *ortho* coupled protons which were concluded to be a part of ring A. The upfield protons at δ 3.92 (3H, s, H-8-OCH₃) and 3.83 (3H, s, H-4'-OCH₃) were assigned to rings A and ring B. The final structure was confirmed by comparing the ^1H NMR values of **130** with the literature values.¹³ The NMR spectra for **130** was obtained in methanol- d_4 and the literature values were obtained in DMSO.

Table 6. NMR spectroscopic data for comparison of compound **130** with literature values.

Position	130	Literature Value
2	8.17	8.43 s
5	7.75 d (9.0)	7.74 d (8.8)
6	6.90 d (9.0)	7.05 d (9.0)
2'	7.47 d (8.7)	7.53 d (8.8)
3'	6.98 d (8.7)	7.00 d (8.8)
5'	6.98 d (8.7)	7.00 d (8.8)
6'	7.47 d (8.7)	7.53 d (8.8)
8-OCH ₃	3.92 s	3.88 s
4-OCH ₃	3.83 s	3.79 s



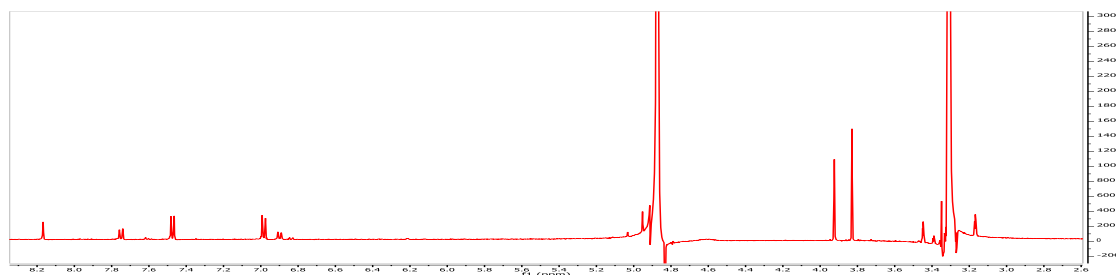
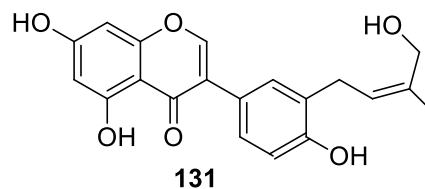


Figure 11. ^1H -NMR spectrum of compound **130**

The sixth compound was identified as vogelin E (**131**). The aromatic singlet proton at δ 7.93 in the ^1H NMR spectrum (Table 7) of **131** indicated that it might be an isoflavone. Furthermore the two doublet proton signals at δ 7.25 (1H, d, $J = 2.3$, H-2'), and 6.83 (1H, d, $J = 8.2$, H-5'), and one doublet of doublets 7.20 (1H, dd, $J = 8.2, 2.3$, H-6') indicated the presence of three protons, of which two were *meta* coupled and one *ortho* coupled. The other remaining aromatic peaks at δ 6.23 (1H, d, $J = 2.1$, H-8) and 6.14 (1H, d, $J = 2.1$, H-6) were *meta* coupled to each other, suggesting the possibility of being in ring A. The peaks at δ 5.66 (1H, tq, H-2''), 3.98 (2H, s, H-4''), and at 1.79 (3H, s, H-5'') indicated the presence of a 3-methylbut-2-enyl group which was concluded to be located on ring B. The final structure was confirmed by comparing the NMR values of **131** with the literature values.¹⁴ The NMR spectra for **131** and the literature values were both obtained in methanol- d_4 .

Table 7. NMR spectroscopic data for comparison of compound **131** with literature values.

Position	131	Literature Value
2	7.93 s	8.00 s
6	6.14 d (2.1)	6.20 d (1.9)
8	6.23 d (2.1)	6.32 d (1.9)
2'	7.25 d (2.3)	7.24 d (1.9)
5'	6.83 d (8.2)	6.80 d (8.3)
6'	7.20 dd (8.2, 2.3)	7.19 dd (1.9, 8.3)
1''	3.40 d (7.2)	3.42 d (7.32)
2''	5.66 tq	5.40 t (7.32)
4''	3.98 s	3.95 s
5''	1.79 s	1.80 s



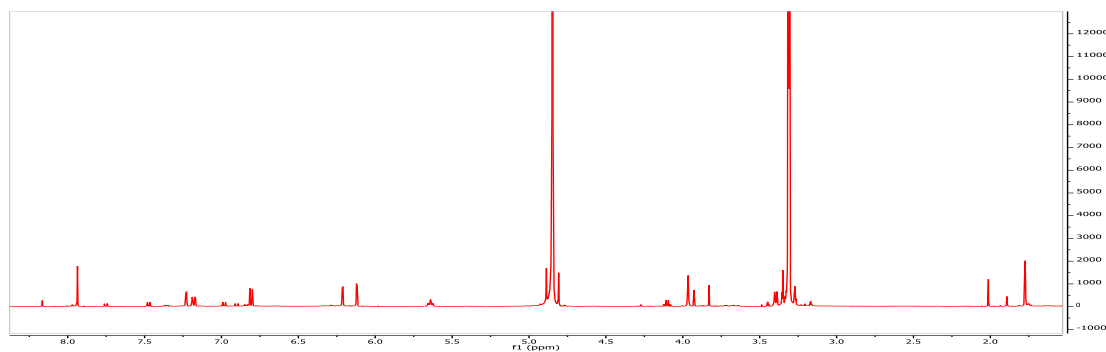
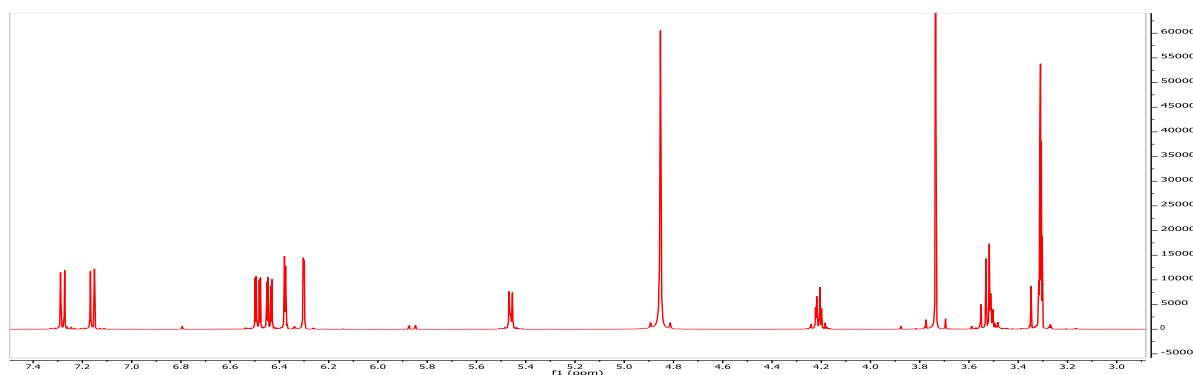
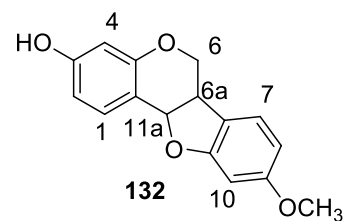


Figure 12. ^1H -NMR spectrum of compound **131**

The seventh compound was identified as (6*S*, 11*S*)-medicarpin (**132**). The molecular formula of (6*S*, 11*S*)-medicarpin was established as $\text{C}_{16}\text{H}_{18}\text{O}_4$ by HREIMS (m/z 271.0958) $[\text{M}+\text{H}]^+$, calcd. for $\text{C}_{16}\text{H}_{19}\text{O}_4^+$ as 271.0965). The ^1H NMR spectrum (Table 8) of (**132**) showed a set of proton signals at δ 3.53-3.50 (2H, m, H-6a, H β -6), 4.21 (1H, dd, $J = 5.9, 0.7$, H α -6), and 5.46 (1H, d, $J = 6.2$, H-11a), characteristic of a pterocarpan skeleton. The spectrum also exhibited the presence of two sets of ABX-type aromatic resonances at δ 6.37 (1H, d, $J = 2.3$, H-4), 6.48 (1H, dd, $J = 8.4, 2.4$, H-2), and 7.28 (1H, d, $J = 8.4$, H-1) assigned to the A ring protons and at δ 6.43 (1H, dd, $J = 8.2, 2.3$, H-8), 6.29 (1H, d, $J = 2.4$, H-10), and 7.16 (1H, d, $J = 8.2$, H-7) assigned to the B ring protons. The ^1H NMR spectrum also showed a peak at δ 3.73 (3H, s, H-9-OCH $_3$) which was assigned at H-9. The final structure was confirmed by comparing the NMR values of **132** with the literature values.¹⁵ The NMR spectra for **132** and the literature values were both obtained in methanol- d_4 .

Table 8. NMR spectroscopic data for comparison of compound **132** with literature values.

Position	132 ¹ H NMR	¹ H NMR lit.	132 ¹³ C NMR	¹³ C NMR lit.
1	7.28 d (8.4)	7.31	133.2	133.2
2	6.48 dd (8.4, 2.4)	6.52	110.7	110.7
3	-	-	160.1	160.1
4	6.37 d (2.3)	6.33	104	104.1
4a	-	-	158	158
6 α	4.21 dd (5.9, 0.7)	4.23	40.9	40.9
6 β	3.35-3.5 m	3.59	120.9	120.9
6a	3.35-3.5 m	3.57	67.6	67.9
7	7.16 d (8.2)	7.18	126	126
8	6.43 d (8.2, 2.3)	6.46	107.2	107.3
9	-	-	162.6	162.6
10	6.29 d (2.4)	6.4	97.5	97.6
10a	-	-	162	162
11a	5.46 d (6.2)	5.4-8	80.1	80.1
11b	-	-	112.9	112.9
9-OCH ₃	3.73 s	3.76	55.9	55.9

**Figure 13.** ¹H-NMR spectrum of compound **132**

Biological evaluation

Biological evaluation was done for all the compounds against the A2780 ovarian cancer cell line.

The compounds 8-*O*-methylretusin (**130**) and vogelin E (**131**) showed modest inhibition of the proliferation of A2780 ovarian cancer cells. The compounds 3',7-dihydroxy-4',8-dimethoxyisoflavone (**126**), erythrinin-B (**127**), genistein (**128**), isowighteone (**129**), and 6*S*,

11S)-medicarpin (**132**) exhibited weak activity against these cell lines. The active fraction with an IC₅₀ value of 2.6 µg/mL, from which compound **126** was isolated, showed the presence of two compounds in its HPLC separation. This suggests there might be a possibility of a synergistic effect shown by the compounds in the fraction. The other compound from the same fraction could not be studied further as the amount isolated was less than the minimum needed to do the required analysis.

Table 9. Antiproliferative activities (µg/mL) of compounds isolated from *Neoharmsia baronii*.

Compound	A2780 (IC ₅₀)
124	19 ± 0.3
125	17 ± 0.3
126	16 ± 0.3
127	2.3 ± 0.3
128	1.7 ± 0.3
129	12 ± 0.3

Experimental section

NMR spectra were recorded in methanol-*d*₄ on a BrukerAvance 500 spectrometer. The chemical shifts are given in δ (ppm), and coupling constants (*J*) are reported in Hz. Mass spectra were obtained on an Agilent 6220 LC-TOF-MS in the positive and negative ion mode. UV and IR spectroscopic data were measured on Shimadzu UV-1201 spectrophotometer and a MIDAC M-series FTIR spectrophotometer, respectively.

Antiproliferative bioassays

Antiproliferative activities were obtained by Ms. Peggy Brodie at Virginia Polytechnic Institute and State University against the drug-sensitive A2780 human ovarian cancer cell line using the following protocol.¹⁶

Human ovarian cancer cells (A2780) grown to 95% confluence were harvested and re-suspended in growth medium (RPMI1640 supplemented with 10% fetal bovine serum and 2 mM L-glutamine). Cells were counted using a hemacytometer and a solution containing 2.5×10^5 cells per ml was prepared in growth media. Eleven columns of a 96 well microtiter plate were seeded with 199 μ L of cell suspension per well, and the remaining column contained media only (one hundred percent inhibition control). The plate was incubated for 3 hours at 37° C/5%CO₂ to allow the cells to adhere to the wells. Following this incubation, potential cytotoxic agents, prepared in DMSO, were added to the wells in an appropriate series of concentrations, 1 μ l per well. One column of wells was left with no inhibitor (zero percent inhibition control), and 4 dilutions of a known compound (taxol or actinomycin) was included as a positive control. The plate was incubated for 2 days at 37° C/5%CO₂, then the media gently shaken from the wells and replaced with reaction media (supplemented growth medium containing 1% alamarBlue), and incubated for another 3 hours. The level of alamarBlue converted to a fluorescent compound by living cells was then analyzed using a Cytofluor Series 4000 plate reader (Perseptive Biosystems) with an excitation wavelength of 530 nm, an emission wavelength of 590 nm, and gain of 45. The percent inhibition of cell growth was calculated using the zero percent and one hundred percent controls present on the plate, and an IC₅₀ value (concentration of cytotoxic agent which produces 50% inhibition) was calculated using a linear extrapolation of the data which lie either side of the 50% inhibition level. Samples were analyzed in triplicate on at least two separate occasions to produce a reliable IC₅₀ value.¹⁷

Plant material

The plant material was collected from a tree 5 m high and 10 cm diameter at breast height, with orange red petals. Its vernacular name is Manangona. The tree was growing in dry forest on limestone (Tsingy) 1 km east of the village of Mahamasina in the Diana locality, coordinates 12°57'40"S 049°08'57"E, in northern Madagascar.

Extraction and isolation

Dried stems woods of *Neoharmsia baronii* (Drake) (Fabaceae) were ground in a hammer mill, then extracted with EtOH by percolation for 24 h at room temperature to give crude extract MG 4371. 3.16 g was shipped to Virginia Tech for bioassay-guided isolation. A 1 g sample of MG 4371 with IC₅₀ of 12 µg/mL was suspended in aqueous MeOH (MeOH:H₂O, 9:1, 100 mL), and extracted with hexanes (3×100 mL portions) to separate the water-insoluble portion of the extract. The hexanes were evaporated under vacuum to leave 256 mg of material with IC₅₀ > 20 µg/mL. The aqueous layer was then diluted to 60% MeOH (v/v) with H₂O and extracted with DCM (3×150 mL portions). The residue from the DCM fraction (455 mg) had an IC₅₀ of 9.9 µg/mL, and the remaining aqueous MeOH fraction had an IC₅₀ > 20 µg/mL. Reverse phase C-18 open column chromatography of the DCM fraction was used to obtain five fractions, of which the most active fraction F2 (317 mg) had an IC₅₀ of 4 µg/mL. To further fractionate the active fraction, a Biotage silica gel column was deployed with elution with hexane : ethylacetate; 4:6 to give twelve fractions of which nine fractions were active with varying IC₅₀ values. The most active fractions amongst the nine fractions were then separated by reverse phase C-18 HPLC to yield the seven pure compounds.

3',7-dihydroxy-4',8-dimethoxyisoflavone (**126**): colorless crystal; ¹H NMR (500 MHz, *d*₄-MeOH) δ: 8.17 (1H, s, H-2), 7.77 (1H, d, *J* = 8.9, H-5), 7.69 (1H, d, *J* = 8.9, H-6), 6.98 (2H, d, *J* = 1.2,

H-5', H-6'), 7.02 (1H, t, $J = 1.2$, H-2'), 3.94 (3H, s, H-8-OCH₃), 3.89 (3H, s, H-4'-OCH₃). ¹³C (500 MHz, *d*-MeOH) δ : 154 (C-2), 125 (C-3), 178 (C-4), 122 (C-5), 118 (C-6), 161 (C-7), 136 (C-8), 153 (C-9), 116 (C-10), 126 (C-1'), 117 (C-2'), 147 (C-3'), 149 (C-4'), 121 (C-5'), 112 (C-6'), 61 (C-8), 56 (C-4'). HRESIMS m/z 313.0742 [M-H]⁻ (calcd for C₁₇H₁₃O₆⁻, 313.0718).

X-ray crystallography: X-ray crystallography was carried out by Dr. Carla Slebodnick as follows. A colorless rod (0.06 x 0.07 x 0.34 mm³) was centered on the goniometer of an Agilent Nova diffractometer. The data collection routine, unit cell refinement, and data processing were carried out with the program CrysAlisPro.¹⁸ The Laue symmetry and systematic absences were consistent with the orthorhombic space groups *Pnma* and *Pna2*₁. A satisfactory solution could not be obtained in *Pnma*. Therefore, the space group *Pna2*₁ was chosen. The structure was solved using SHELXS-2013¹⁹ and refined using SHELXL-2013¹⁹ via OLEX2.²⁰ The final refinement model involved anisotropic displacement parameters for all non-hydrogen atoms. A riding model was used for the alkyl H-atoms. The H-atoms of the methyl and hydroxyl groups and the water molecules were located from the residual electron density map and refined as rotating groups. The isotropic displacement parameters of all H-atoms were constrained to 1.2 times U_{eq} (aromatic) or 1.5 times U_{eq} (methyl, hydroxyl, water) the heteroatom to which they are bonded. The structure was refined as a 2-component inversion twin with the Flack x refining to 0.37(16).²¹

Erythrinin B (**127**): colorless crystal; ¹H NMR (500 MHz, *d*-MeOH) δ : 8.0 (1H, s, H-2), 7.39 (2H, d, $J = 8.7$, H-2', H-6'), 6.86 (2H, d, $J = 8.7$, H-3', H-5'), 6.40 (1H, s, H-8), 5.25 (1H, tq, H-2''), 1.80 (3H, s, H-4''), 1.68 (3H, s, H-5''). HRESIMS m/z 339.1226 [M+H]⁺ (calcd for C₂₀H₁₉O₅⁺, 339.1227).

Genistein (128): white powder; $^1\text{H NMR}$ (500 MHz, d_4 -MeOH) δ : 8.04 (1H, s, H-2), 7.37 (2H, d, $J = 8.7$, H-2', H-6'), 6.84 (2H, d, $J = 8.7$, H-3', H-5'), 6.30 (1H, d, $J = 2.0$, H-8), 6.19 (1H, d, $J = 2.0$, H-6). HRESIMS m/z 269.0479 $[\text{M-H}]^-$ (calcd for $\text{C}_{15}\text{H}_9\text{O}_5^-$, 269.0455).

Isowighteone (129): yellow powder; $^1\text{H NMR}$ (500 MHz, d_4 -MeOH) δ : 7.94 (1H, s, H-2), 7.20 (1H, d, $J = 2.1$, H-2'), 7.16 (1H, dd, $J = 8.7, 2.1$, H-6), 6.80 (1H, d, $J = 8.7$, H-5), 6.23 (1H, d, $J = 2.1$, H-8), 6.14 (1H, d, $J = 2.1$, H-6), 5.35 (1H, tq, H-2''), 1.73 (3H, s, H-4''), 1.73 (3H, s, H-5''). HRESIMS m/z 337.1097 $[\text{M-H}]^-$ (calcd for $\text{C}_{20}\text{H}_{17}\text{O}_5^-$, 337.1081).

8-O-methylretusin (130): colorless crystals; $^1\text{H NMR}$ (500 MHz, d_4 -MeOH) δ : 8.17 (1H, s, H-2), 7.75 (1H, d, $J = 9.0$, H-5), 7.47 (2H, d, $J = 8.7$, H-2, H-6), 6.98 (2H, d, $J = 8.7$, H-3', H-5'), 6.90 (1H, d, $J = 9.0$, H-6), 3.92 (3H, s, H-8-OCH₃), 3.83 (3H, s, H-4'-OCH₃). HRESIMS m/z 299.0912 $[\text{M+H}]^+$ (calcd for $\text{C}_{17}\text{H}_{15}\text{O}_5^+$, 299.0914).

Vogelin-E (131): amorphous yellow powder; $^1\text{H NMR}$ (500 MHz, d_4 -MeOH) δ : 7.93 (1H, s, H-2), 7.25 (1H, d, $J = 2.3$, H-2'), 7.20 (1H, dd, $J = 8.2, 2.3$, H-6'), 6.83 (1H, d, $J = 8.2$, H-5'), 6.23 (1H, d, $J = 2.1$, H-8), 6.14 (1H, d, $J = 2.1$, H-6), 5.66 (1H, tq, H-2''), 3.98 (2H, s, H-4''), 1.79 (3H, s, H-5'').

(6S, 11S)-Medicarpin (132): white solid; $^1\text{H NMR}$ (500 MHz, d_4 -MeOH) δ : 7.28 (1H, d, $J = 8.4$, H-1), 7.16 (1H, d, $J = 8.2$, H-7), 6.48 (1H, dd, $J = 8.4, 2.4$, H-2), 6.43 (1H, dd, $J = 8.2, 2.3$, H-8), 6.37 (1H, d, $J = 2.3$, H-4), 6.29 (1H, d, $J = 2.4$, H-10), 5.46 (1H, d, $J = 6.2$, H-11a), 4.21 (1H, dd, $J = 5.9, 0.7$, H-6 α), 3.73 (3H, s, H-9-OCH₃), 3.53-3.50 (2H, m, H-6a, H-6 β). ^{13}C (125 MHz, MeOH) δ : 162 (C-9), 162 (C-10a), 160 (C-3), 158 (C-4a), 133.2 (C-1), 126 (C-7), 121 (C-6 β), 113 (C-11b), 110 (C-2), 107.2 (C-8), 104 (C-4), 97 (C-10), 80 (C-11a), 67 (C-6a), 56 (MeO-9), 41 (C-6 α). HRESIMS m/z 271.0958 $[\text{M+H}]^+$ (calcd for $\text{C}_{16}\text{H}_{15}\text{O}_4^+$, 271.0965).

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Chapter 3: Combretastatins from *Lopholaena cneorifolia*

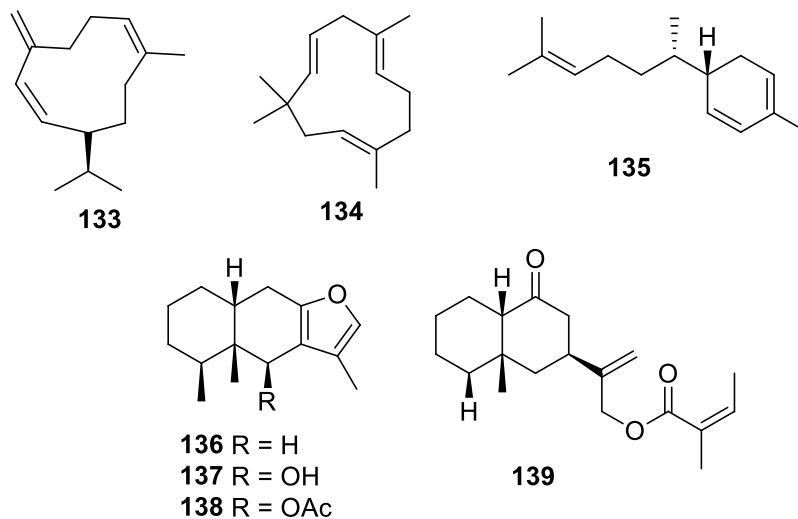
Introduction

The Merck repository is considered to be one of the most diverse and organized natural product extract libraries in the world.^{1, 2} In 2008, Merck halted their in-house natural product research after discovering exceptional products like the anti-fungal agent Cancidas, the antibacterial agent Mefoxin (cefoxitin), the statin Mevacor, and many other natural products with medicinal properties. The Merck repository of natural products extracts was transferred to the Institute of Hepatitis and Viral research (IHVR) and the associated Natural Products Discovery Institute.³ To study the plant extracts in the Merck repository, the Kingston group was able to initiate a collaborative project with IHVR to find potential anti-proliferative and anti-malarial agents. As a part of this collaboration, the extract 1000585-11H, obtained from the above ground parts of the South African plant *Lopholaena cneorifolia*, was found to have an $IC_{50} = 0.7 \mu\text{g/mL}$ against the A2780 ovarian cancer cell line.

Background

The Asteraceae family is a large family of flowering plants with over 1620 genera and 12 sub-families. The name Asteraceae comes from the most prominent genus name in the family Aster, meaning star-like as the inflorescence is star shaped form in most plants in this family. Plants in the Asteraceae family are most commonly found in arid and semi-arid regions of subtropical and low temperature latitudes.⁴ This is an economically important family as it provides cooking oils, sunflower seeds, artichokes, herbal teas, a coffee substitute and also sweetening agents.⁵ The plants of this family show anti-bacterial, antitrypanosomal, antiprotozoal, wound healing, anti-inflammatory, neurological effects, spasmolytic effects, gastric protection, and anti-tumor and analgesic properties.^{6, 7, 8} The genus *Lopholaena* with about 20 species is restricted to southern

and tropical Africa.⁹ Of the 20 species of this genus only two have been studied chemically, namely *L. coriifolia* and *L. segmentata*. No previous work has been reported on *L. cnerifolia*. Plants in the *Lopholaena* genus contain eremophilanes and furanoeremophilanes,¹⁰ which are known for their antioxidant, antifungal, herbicidal and anti-radical properties.¹¹ Other medicinal uses include alleviating gastric ulcers, kidney ailments, bladder infections, prostrate problems, chest pain and diabetes. The compounds isolated from the aerial parts of *Lopholaena coriifolia* include germacrene D (**133**), bicyclogermacrene, α -humulene (**134**), caryophyllene, polyisoprene, α -zingiberene (**135**), α -curcumene, caryophyllene 1,10-epoxide and furanoeremophilanes.¹² The aerial parts of *Lopholeana segmentata* afforded germacrene D (**133**) and α -humulene (**134**), while furanoeremophilanes (**136-139**) were isolated from the roots.¹³ *Lopholaena cneorifolia* is a plant generally 0.2-0.5 m tall and is found in rocky areas of South Africa.



Isolation and structure determination of compounds from *Lopholaena cneorifolia*

As previously noted, the search for potential anticancer agents from the extract 1000585-11H was undertaken as a result of the finding the crude extract being active against the A2780 human ovarian cancer cell line with $IC_{50} = 0.7 \mu\text{g/mL}$. The ethanol extract of 1000585-11H was subjected to a liquid-liquid partition between hexane ($3 \times 100 \text{ mL}$) and aqueous MeOH (MeOH:H₂O, 9:1, 100 mL). The aqueous layer was then diluted to 50% MeOH (v/v) and extracted with CH₂Cl₂ ($3 \times 150 \text{ mL}$). The CH₂Cl₂ fraction displayed the highest activity ($IC_{50} = 3.1 \mu\text{g/mL}$) and subsequently was selected to isolate active compounds. This fraction was fractionated through a reverse phase SPE-C₁₈ column and eluted with a gradient elution of 70% MeOH: H₂O to 100% MeOH and a final CH₂Cl₂ wash. A total of five fractions were collected of which the first two were active with $IC_{50} = 1.9 \mu\text{g/mL}$ and $2.4 \mu\text{g/mL}$, and a third fraction had $IC_{50} = 16 \mu\text{g/mL}$. The fraction with $IC_{50} = 1.9 \mu\text{g/mL}$ was further separated using a C-18 open column to yield three active fractions with $IC_{50} = 13$, 3.7 and $3.2 \mu\text{g/mL}$ respectively. The three active fractions were purified by column chromatography on a C₁₈ reverse phase HPLC column using a gradient elution of 50% MeOH:H₂O to 100% MeOH. The three compounds collected had $IC_{50} = 0.6 \mu\text{g/mL}$, $IC_{50} = 0.6 \mu\text{g/mL}$, and $IC_{50} = 0.8 \mu\text{g/mL}$.

Structural elucidation of compounds

Compound **B** was isolated as solid yellowish compound. Its molecular formula was deduced as C₂₄H₃₀O₁₁ by HREIMS, which gave a sodiated molecular ion peak at m/z 517.1786 [M+Na]⁺. It gave signals for 14 sp² carbons in its ¹³C NMR spectrum. The UV spectrum showed absorbance at λ_{max} 220 and 295 nm. Its ¹H and ¹³C NMR spectra indicated that the compound belonged to the stilbene class of compounds. The ¹H NMR spectrum in methanol-*d*₄ showed that the compound had four methoxy groups with δ_{H} 3.62 (3H, s), 3.62 (3H, s), 3.72 (3H, s), and 3.81

(3H, s). The olefinic doublets at δ_{H} 6.48 (1H, d, $J = 12.1$) and δ_{H} 6.85 (1H, d, $J = 12.1$) gave a strong indication for it being a *Z*-stilbene derivative. The large coupling constant ($J = 12.1$) for the vinyl protons of *Z*-stilbene is well documented in the literature.¹⁴ The ^{13}C NMR spectrum of compound **B** showed 24 signals, with signals for four oxygenated carbons, one methylene, eleven methines, and eight quaternary carbon peaks.

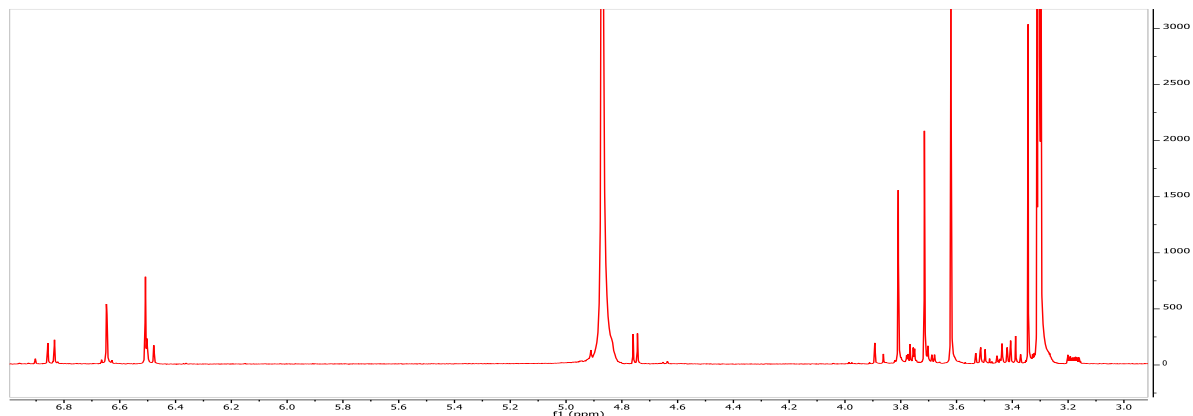
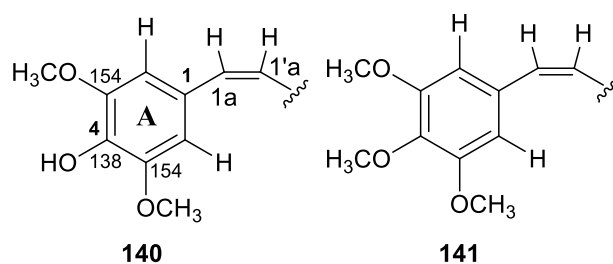


Figure 14. ^1H -NMR spectrum of compound **B**

From the HSQC and HMBC correlations, the proton-carbon pairs were connected to each other and J_{CH} correlations were determined to confirm the stilbene skeleton. The H-1a and H-1'a protons were confirmed as δ_{H} 6.48 (1H, d, $J = 12.1$) and δ_{H} 6.85 (1H, d, $J = 12.1$) and the assignment for the attached carbons was done with the help of HSQC and they were assigned as C-1a δ_{C} 130.6 and C-1'a δ_{C} 127.7. The ^1H δ_{H} 4.7 (1H, d, $J = 7.8$) and ^{13}C NMR δ_{C} 106.9 signals in methanol- d_4 showed typical signals for an anomeric proton^{15, 16} and its respective carbon, indicating the presence of a sugar moiety. The sugar was identified as glucose by comparing the δ_{H} and δ_{C} values with literature data.¹⁶ The singlet at δ_{H} 6.51 (2H, s) suggested the presence of two aromatic protons in identical environments. The HSQC spectrum helped in identifying the carbons attached to the respective protons. The signal at δ_{H} 6.51 (2H, s) correlated with carbon signals at δ_{C} 107.5 for two carbons. The doublet proton at δ_{H} 6.48 (1H, d, $J = 12.1$) which forms the bridge between two rings showed HMBC correlations to the carbon at δ_{C} 107.5 which

indicated that the protons at 6.51 are *ortho* to the bridge. To further confirm the bridge, HMBC correlations between H-1a at δ_{H} 6.85 (1H, d, $J = 12.1$) with C-1'a δ_{C} 127.7 and that between H-1'a at δ_{H} 6.48 (1H, d, $J = 12.1$) with C-1a δ_{C} 130.6 were observed. The observation of NOE correlations between H-1a at δ_{H} 6.48 and H1'a at δ_{H} 6.85 in the NOE difference spectrum further supported this result. In addition to the signals of aromatic and vinyl protons, the signals of the four methoxy and one hydroxy groups suggest that the structure of ring A is either **140** or **141**.



HMBC correlations for δ_{H} 6.51 with carbons at δ_{C} 154 and 138 are consistent with both structures. The carbons at δ_{C} 138.1 was shown to be a methoxylated carbon by an HMBC correlation from methoxy protons at δ_{H} 3.72 with δ_{C} 138.1; this is only consistent with structure **141**. HMBC correlations were observed for the singlet protons at δ_{H} 6.5 (2H, s) with the methoxy carbons at δ_{C} 154.0 and δ_{C} 138.1 and also with C-1a and C-1'a helped confirm the structure of ring A and the protons representing the bridge at H-1a and H-1'a.

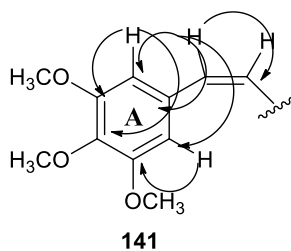


Figure 15. Key HMBC correlations in ring A for compound **B**

The substitution of ring B, also an aromatic ring, was determined by using ^1H and ^{13}C NMR spectra, and HSQC, HMBC and NOE-DIFF spectra. Signals for two other aromatic protons were

observed at δ_{H} 6.65 as a doublet with two peaks separated by 0.8 Hz. This signal must be due to two protons on a benzene ring with almost identical chemical shifts, so that the outer signals of the expected AB quartet are lost in the noise. The spectrum also had a signal for another methoxy group δ_{H} 3.81 (3H, s). The H-1'a proton with δ_{H} 6.85 (1H, d, $J = 12.1$) showed a long range HMBC correlation with C-2' (δ_{C} 144.8), and the HMBC correlation between the anomeric proton δ_{H} 4.75 and C-2' at δ_{C} 144.8 confirmed the position of attachment of the glucosyl moiety to be C-2' on ring B. To complete ring B, a hydroxyl group was added to comply with HREIMS data. There were two possibilities to be considered for the ring B substitution. The first possibility is where the protons are *ortho* coupled and the second possibility is where the protons are *meta* coupled.

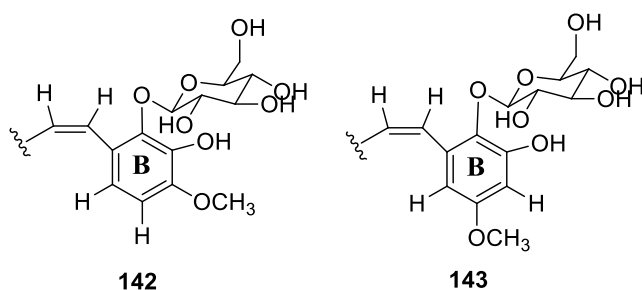


Figure 16. Possible structures for compound **B**

These results could be explained by one other structure for compound **B**, namely structure **143**. Structure **143** is supported by the proton NMR spectrum with an apparent doublet at δ_{H} 6.65 (2H, d, $J = 0.8$). However a study of H2BC correlations eliminated **143** as a candidate (Fig. 17). The two bond correlations between δ_{H} 6.65 and C-5' (δ_{C} 149.6) and δ_{H} 6.65 and C-3' (δ_{C} 139.9) that would have been observed for structure **143** were not observed.

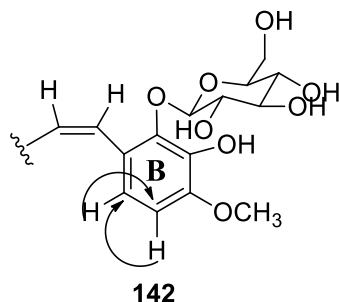


Figure 17. Key H2BC correlations in ring B for compound **B**

The important HMBC correlations between H-5' δ_{H} 6.65 (H, d, $J = 0.8$) and H-6' δ_{H} 6.65 (H, d, $J = 0.8$) with C-1' (δ_{C} 125.9), C-3' (δ_{C} 139.9), C-2' (δ_{C} 144.8) and C-4' (δ_{C} 149.6) are shown in the Fig. 18. The HMBC correlation between the methoxy group at δ_{H} 3.81 (3H, s) with C-4' (δ_{C} 149.6) was key to confirm this structure. The ^1H NMR signals for H-5' and H-6' appeared as an apparent 2H doublet due to accidental chemical shift near equivalence; the corresponding ^{13}C NMR signals appeared as separate singlets at δ_{C} 109.4 and δ_{C} 121.0.

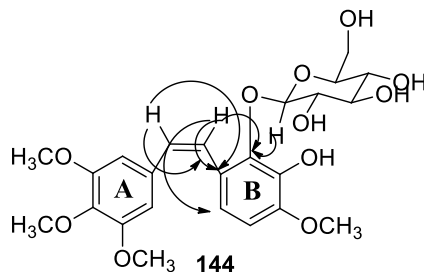


Figure 18. Key HMBC correlations in ring B for compound **B**

A comparative study was done with literature values and the structure of compound **144** was confirmed (Table 10).^{17, 18} The NMR spectra for **144** and the literature values were both obtained in methanol- d_4 .

Table 10. NMR spectroscopic data for comparison of compound **144** with literature values.^{17, 18}

Position	¹ H-NMR for		¹³ C-NMR for	
	compound 144	Lit. value	compound 144	Lit. value
1	-	-	134.6	134.6
2	6.51 (1H, s)	6.50 (1H, s)	107.5	107.5
3	-	-	154	154
4	-	-	138.1	138.2
5	-	-	154	154
6	6.51 (1H, s)	6.50 (1H, s)	107.5	107.5
1'	-	-	125.9	125.9
2'	-	-	144.8	144.8
3'	-	-	139.9	140.9
4'	-	-	149.6	149.6
5'	6.65 (2H, d, 0.8)	6.65 (1H, s)	109.4	109.5
6'	6.65 (2H, d, 0.8)	6.65 (1H, s)	121	121.1
1a	6.48 (1H, d, 12.1)	6.49 (1H, d, 12.12)	130.6	130.6
1'a	6.85 (1H, d, 12.1)	6.84 (1H, d, 12.12)	127.7	127.7
G1	4.77 (1H,d, 7.8)	4.75 (1H,d, 7.91)	106.9	106.9
G2	3.51 (1H, dd, 9.0,7.9)	3.51 (1H, dd, 9.0,7.9)	75.4	75.4
G3	3.39 (1H, m)	3.38 (1H, dd, 9.01,9.19)	77.9	77.9
G4	3.41 (1H, m)	3.44 (1H, dd, 9.19,7.9)	71	71
G5	3.19 (1H, m)	3.18 (1H, m)	78.5	78.5
G6	3.69 (1H, d, 4.8)	3.69 (1H, dd, 4.8, 11.95)	62.3	62.3
	3.78 (1H, m)	3.75 (1H, m)		
3-OCH ₃	3.62 (3H, s)	3.61 (3H, s)	56.3	56.3
4-OCH ₃	3.72 (3H, s)	3.71 (3H, s)	56.8	56.8
5-OCH ₃	3.62 (3H, s)	3.61 (3H, s)	56.3	56.3
4'-OCH ₃	3.81 (3H, s)	3.81 (3H, s)	61.1	61.1

Structure elucidation of compound 145.

Compound **145** had a molecular weight 482 which was deduced by the positive molecular ion peak at m/z 505.1778 $[M+Na]^+$ suggesting the molecular composition as C₂₃H₃₀O₁₁. The UV spectrum showed absorbance with λ_{\max} 215 and 275 nm which was similar to that of compound

143, but the shift to shorter wavelengths was consistent with a loss of conjugation.

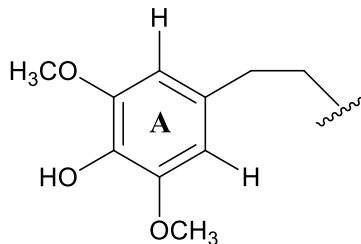


Figure 19. Ring A for compound **145**

The ^1H spectrum in methanol- d_4 showed that the compound was missing the stilbene cis doublet protons and instead showed three multiplets, one with two protons and two others with one proton each. In agreement with this finding, unlike the ^{13}C spectrum for compound **144** which showed just one methylene signal, compound **145** had three methylene signals in the ^{13}C spectrum with the remaining signals being three oxygenated carbons, nine methines and eight quaternary carbon signals.

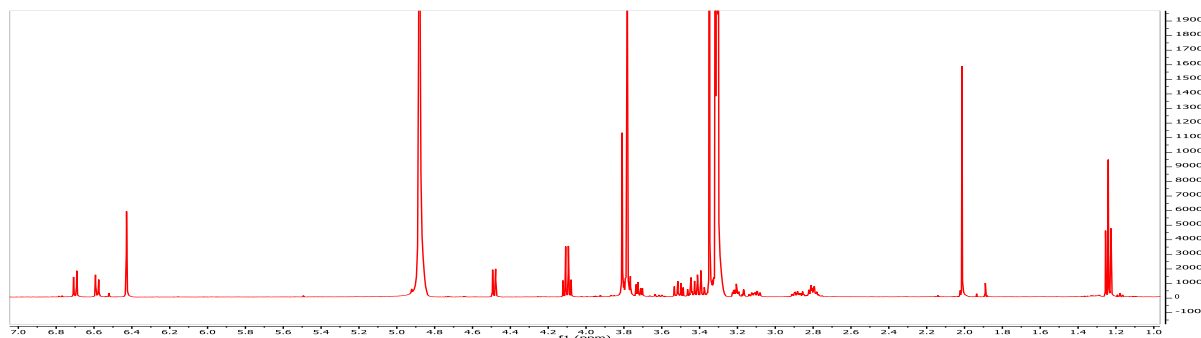


Figure 20. ^1H -NMR spectrum of compound **145**

The ^1H and ^{13}C NMR spectrum signals in methanol- d_4 showed typical signals for an anomeric proton and its respective carbon, indicating the presence of a sugar moiety with $\delta_{\text{H}} 4.7$ (1H, d, $J = 7.9$). The aromatic region protons suggested a similar ring A structure as found for compound **144**, with $\delta_{\text{H}} 6.42$ (2H, s). The corresponding carbon shifts at $\delta_{\text{C}} 107$ for two carbons confirmed the structure of ring A for compound **145**. The presence of two methylene signals at $\delta_{\text{H}} 3.10$ (1H, m), $\delta_{\text{H}} 2.92$ (1H, m), and $\delta_{\text{H}} 2.8$ (2H, m) helped in determining the change in the bridged carbons

and its protons. The bridge was confirmed as containing two saturated carbons appearing as methylene signals in the ^{13}C spectrum at δ_{C} 32.8 and δ_{C} 38.08.

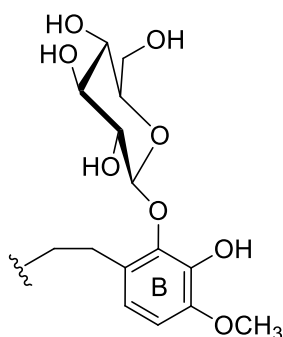


Figure 21. Ring B for compound **145**

The ^1H spectrum of **145** in methanol- d_4 showed that the compound had three methoxy groups with δ_{H} 3.77 (3H, s), 3.77 (3H, s), and 3.80 (3H, s), but the molecular formula and the molecular weight indicated the presence of two hydroxyl groups which did not give signals in the ^1H spectrum. The presence of the two hydroxyl groups was confirmed by ^{13}C spectroscopic data showing the presence of signals at δ_{C} 134.4 and δ_{C} 139.9. In addition to this, the signals for two *ortho*-coupled protons δ_{H} 6.69 (1H, d, $J = 8.4$) and δ_{H} 6.57 (1H, d, $J = 8.4$) along with the other glucosyl moiety peaks were seen upfield. The anomeric proton G-1 showed $J = 7.9$ Hz, indicating that the sugar in compound **145** had the β -configuration. To confirm the data, a comparative study was done with literature values and the structure of compound **145** was confirmed as the dihydro analogue of **144** (Table 11).¹⁴ The NMR spectrum for **145** was obtained in methanol- d_4 (500 MHz) and the NMR for literature values was reported in DMSO- d_6 (200 MHz).

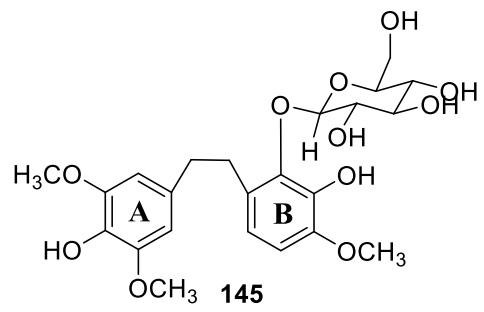


Figure 22. Structure for compound **145**

Table 11. NMR spectroscopic data for comparison of compound **145** with literature values.¹⁴

Position	¹ H-NMR for compound 145	¹ H-NMR lit. value	¹³ C-NMR for compound 145	¹³ C-NMR lit. value
1	-	-	132.88	132.43
2	6.42 (1H, s)	6.47 (1H, s)	107.05	105.96
3	-	-	148.88	147.77
4	-	-	134.4	133.34
5	-	-	148.88	147.77
6	6.42 (1H, s)	6.47 (1H, s)	106.91	105.96
1'	-	-	129.81	128.22
2'	-	-	145.21	143.95
3'	-	-	139.09	139.29
4'	-	-	148.37	146.9
5'	6.57 (1H, d, 8.4)	6.56 (1H, d, 8)	109.97	109.1
6'	6.69 (1H, d, 8.4)	6.67 (1H, d, 8)	120.44	118.68
1a	2.8 (1H, m)	2.87 (2H, m)	37.93	36.58
1'a	2.92 (1H, m) 3.10 (1H, m)	2.87 (2H, m)	32.59	31.35
G1	4.47 (1H, d, 7.9)	4.52 (1H, d, 7.9)	106.83	105.79
G2	3.51 (1H, dd, 9.1, 7.9)	3.52 (1H, dd, 9.1, 7.9)	75.47	74.04
G3	3.39 (1H, m)	3.40 (1H, dd, 8.00, 9.10)	77.77	76.14
G4	3.39 (1H, m)	3.42 (1H, dd, 8.00, 6.89)	70.86	69.71
G5	3.2 (1H, m)	3.23 (1H, m)	78.32	77.44
G6	3.71 (2H, m)	3.65-3.90 (2H, m)	61.53	60.89
3-OCH ₃	3.77 (3H, s)	3.78 (3H, s)	56.69	56
5-OCH ₃	3.77 (3H, s)	3.78 (3H, s)	56.69	56
4'-OCH ₃	3.8 (3H, s)	3.80 (3H, s)	56.84	56

Structure elucidation of compound **146**.

The UV spectrum of compound **146** showed absorbance with λ_{max} 215 and 275 nm which was similar to compound **144**, and its ^1H -NMR spectrum indicated that it had a similar structure to **144**.

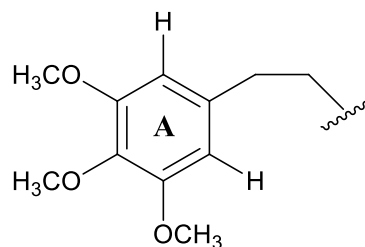


Figure 23. Ring A for compound **146**

The ^1H spectrum of **146** in methanol-*d*₄ showed that the compound was missing the stilbene cis doublet protons of **146** and instead showed three multiplets, one with two protons and two others with one proton each. In agreement with this finding, unlike the ^{13}C spectrum for compound **144** which showed just one methylene signal, compound **145** had three methylene signals in the ^{13}C spectrum with the remaining signals being four oxygenated carbons, nine methines and eight quaternary carbon signals.

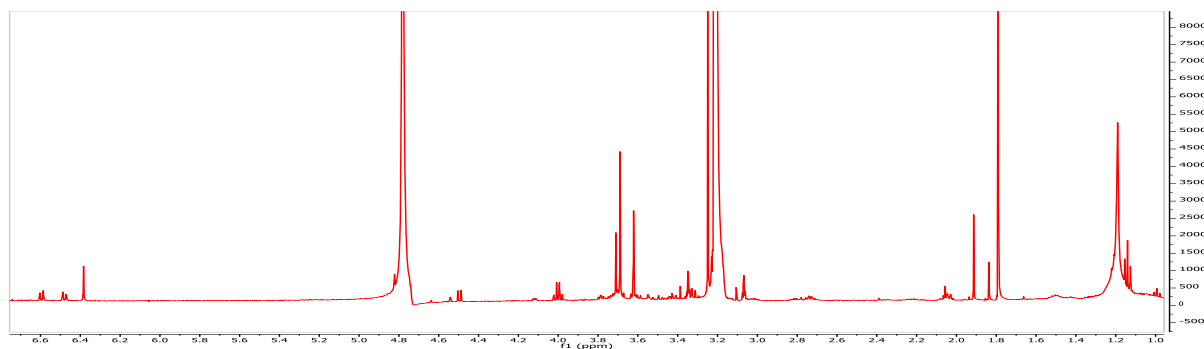


Figure 24. ^1H -NMR spectrum of compound **146**

The ^1H and ^{13}C NMR spectrum signals in methanol-*d*₄ showed typical signals for an anomeric proton and its respective carbon, indicating the presence of a sugar moiety with δ_{H} 4.7 (1H, d, $J =$

7.9). The aromatic region protons suggested a similar ring A structure as found for compound **144**, with the δ_{H} 6.48 (2H, s). The presence of two methylene signals at δ_{H} 3.10 (1H, m), δ_{H} 2.92 (1H, m), and δ_{H} 2.8 (2H, m) helped in determining the change in the bridged carbons and its protons. The bridge contained two methylene groups which appeared at δ_{C} 32.8 and δ_{C} 38.08 in the ^{13}C NMR spectrum in Fig. **24**.

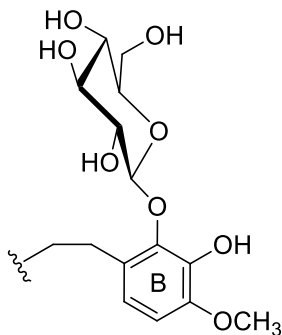


Figure 25. Ring B for compound **146**

The ^1H spectrum of **146** in methanol- d_4 showed that the compound had four methoxy groups with δ_{H} 3.79 (6H, s), 3.72 (3H, s), and 3.81 (3H, s), and the molecular formula and the molecular weight indicated the presence of an additional hydroxyl group. In addition to this, there were signals for two *ortho*-coupled aromatic protons at δ_{H} 6.69 (1H, d, $J = 8.4$) and δ_{H} 6.58 (1H, d, $J = 8.4$) along with peaks for a glucosyl moiety. The anomeric proton G-1 showed $J = 7.9$ Hz indicating that the compound **146** had a β -*L*-glucose unit. To confirm the data, a comparative study was done with literature values and the structure of compound **146** was confirmed (Table 12).¹⁷ The NMR spectra for **146** and the literature values were both obtained in methanol- d_4 .

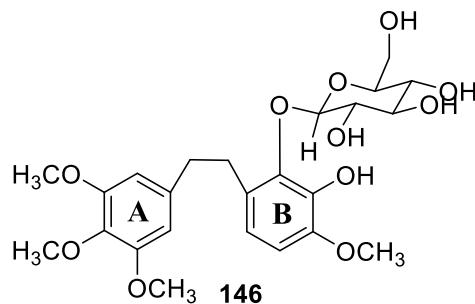


Figure 26. Structure for compound **146**

Table 12. NMR spectroscopic data for comparison of compound **146** with literature values.¹⁷

Position	¹ H-NMR for compound 146	Lit. value
1	-	-
2	6.48 (1H, s)	6.47 (1H, s)
6	6.48 (1H, s)	6.47 (1H, s)
5'	6.69 (1H, d, 8.4)	6.69 (1H, d, 8.46)
6'	6.58 (1H, d, 8.4)	6.58 (1H, d, 8.46)
1a	2.84 (1H, m)	2.84 (1H, m)
1'a	2.91 (1H, m) 3.10 (1H, m)	2.92 (1H, m) 3.11 (1H, m)
G1	4.6 (1H, d, 7.9)	4.59 (1H, d, 7.9)
G2	3.51 (1H, dd, 9.1, 7.9)	3.52 (1H, dd, 9.1, 7.9)
G3	3.39 (1H, m)	3.40 (1H, dd, 8.00, 9.10)
G4	3.39 (1H, m)	3.42 (1H, dd, 8.00, 6.89)
G5	3.2 (1H, m)	3.23 (1H, m)
G6	3.71 (2H, m)	3.65-3.90 (2H, m)
3-OCH ₃	3.79 (3H, s)	3.78 (3H, s)
4-OCH ₃	3.72 (3H, s)	3.71 (3H, s)
5-OCH ₃	3.79 (3H, s)	3.78 (3H, s)
4'-OCH ₃	3.81 (3H, s)	3.80 (3H, s)

Unfortunately the sample was lost before its HRESIMS could be obtained, but the NMR evidence is conclusive for the assigned structure.

Bioassay

A biological evaluation was done for all the compounds against the A2780 ovarian cancer cell line. The compounds **144**, **145** and **146** showed strong inhibition of the proliferation of the A2780 ovarian cancer cell line. As already known, these isolated compounds are recognized for their anti-angiogenic activity.

General experimental procedures

NMR spectra were recorded in methanol-*d*₆ on BrukerAvance 500 spectrometer. The chemical shifts are given in δ (ppm), and coupling constants (*J*) are reported in Hz. Mass spectra were obtained on an Agilent 6220 LC-TOF-MS in the positive and negative ion mode. UV and IR spectroscopic data were measured on Shimadzu UV-1201 spectrophotometer and a MIDAC M-series FTIR spectrophotometer, respectively.

Antiproliferative bioassays

Antiproliferative activities were obtained at Virginia Tech as previously described against the drug-sensitive A2780 human ovarian cancer cell line.¹⁹

Plant material:

The dried above-ground parts of *Lopholaena cneorifolia* was collected on November 2002 at 1294 m altitude in the savanna in the Postmasburg District, Northern Cape; 27:58 S, 23:21 E in South Africa by Johan H. Venter. The dried plant material was exhaustively extracted with ethanol. After defatting with three portions of hexanes, the extract was diluted to 1:1 water : ethanol and partitioned three times with methylene chloride. Evaluation of the methylene chloride extract at Virginia Tech indicated that it had an IC₅₀ value of 3.5 μ g/ml against the A 2780 cell line.

Extraction and isolation

Based on the initial activity of the methylene chloride fraction of *Lopholaena cneorifolia*, a resupply of a the original ethanol extract was obtained from IHVR. 1.0 g was shipped to Virginia Tech for bioassay-guided isolation. A 900 mg sample of 1000585-11H with an IC_{50} value of 16 $\mu\text{g/ml}$ was suspended in aqueous MeOH (MeOH:H₂O, 9:1, 100 mL), and extracted with hexanes (3 \times 100 mL portions) to separate the water insoluble portion of the extract. The hexane was evaporated under vacuum to leave 84 mg of material with $IC_{50} > 20 \mu\text{g/ml}$. The aqueous layer was then diluted to 60% MeOH (v/v) with H₂O and extracted with DCM (3 \times 150 mL portions). The CH₂Cl₂ fraction displayed the highest activity ($IC_{50} = 3.1 \mu\text{g/mL}$) and subsequently was selected to isolate active compounds. This fraction was fractionated through a reverse phase SPE-C₁₈ column and eluted with a gradient elution of 70% MeOH: H₂O to 100% MeOH and final CH₂Cl₂ wash. A total of five fractions were collected of which the first two were active with $IC_{50} = 1.9 \mu\text{g/mL}$ and 2.4 $\mu\text{g/mL}$. A third fraction had $IC_{50} = 16 \mu\text{g/mL}$. The fraction with $IC_{50} = 1.9 \mu\text{g/mL}$ was further separated using a C-18 open column to yield three active fractions with $IC_{50} = 13, 3.7$ and 3.2 $\mu\text{g/mL}$ respectively. The three active fractions were purified by column chromatography on a C₁₈ reverse phase HPLC column using a gradient elution of 50% MeOH:H₂O to 100% MeOH. The chromatograms of the fractions were similar and varying amounts of the same compounds were collected. The three compounds collected had $IC_{50} = 0.6 \mu\text{g/mL}$, $IC_{50} = 0.6 \mu\text{g/mL}$ $IC_{50} = 0.8 \mu\text{g/mL}$.

Compound **144**: ^1H NMR (500 MHz, d_4 -MeOH) δ : 6.85 (1H, d, $J = 12.1$, H-1'a), 6.65 (2H, s, H-5', H-6'), 6.51 (2H, s, H-2, H-6), 6.48 (1H, d, $J = 12.1$, H-1a), 4.77 (1H, d, $J = 7.8$, G-1), 3.81 (3H, s, 4'-OCH₃), 3.78 (1H, m, G-6), 3.72 (3H, s, 4-OCH₃), 3.69 (1H, d, $J = 4.8$, G-6), 3.62 (6H, s, 3-OCH₃, 5-OCH₃), 3.51 (1H, dd, $J = 9.0, 7.9$, G-2), 3.41 (1H, m, G-4), 3.39 (1H, m, G-3), 3.19 (1H, m, G-5). ^{13}C (125 MHz, d_4 -MeOH) δ : 154 (C-3, C-5), 149.6 (C-4'), 144.8 (C-2'), 139.9 (C-3'), 138.1 (C-4), 134.6 (C-1), 130.6 (C-1a), 127.7 (C-1'a), 125.9 (C-1'), 121 (C-6'), 109.4 (C-5'), 107.5 (C-2, C-6), 106.9 (G-1), 78.5 (G-5), 77.9 (G-3), 75.4 (G-2), 71.0 (G-4), 62.3 (G-6), 61.1 (4'-OCH₃), 56.8 (4-OCH₃), 56.3 (3-OCH₃, 5-OCH₃). HRESIMS m/z 517.1786 [M+Na]⁺ (calcd for C₂₄H₃₀O₁₁+Na, 517.1681).

Compound **145**: ^1H NMR (500 MHz, d_4 -MeOH) δ : 6.69 (1H, d, $J = 8.4$, H-5'), 6.57 (1H, d, $J = 8.4$, H-6'), 6.42 (2H, s, H-2, H-6), 4.47 (1H, d, $J = 7.9$, G-1), 3.8 (3H, s, 4'-OCH₃), 3.77 (6H, s, 3-OCH₃, 5-OCH₃), 3.51 (1H, dd, $J = 9.1, 7.9$, G-2), 3.39 (2H, m, G-3, G-4), 3.2 (1H, m, G-5), 3.10 (1H, m, H-1'a), 2.92 (1H, m, H-1'a), 2.8 (1H, m, H-1a). ^{13}C (500 MHz, MeOH) δ : 148.8 (C-3, C-5), 148.4 (C-4'), 145.2 (C-2'), 139.1 (C-3'), 138.1 (C-4), 134.4 (C-1), 129.8 (C-1'), 120.4 (C-6'), 109.9 (C-5'), 106.9 (C-2, C-6), 107.1 (G-1), 78.4 (G-5), 78.0 (G-3), 75.6 (G-2), 71.7 (G-4), 62.3 (G-6), 62.4 (4'-OCH₃), 56.7 (3-OCH₃, 5-OCH₃), 38.1 (C-1a), and 32.8 (C-1'a). HRESIMS m/z 505.1778 [M+Na]⁺ (calcd for C₂₃H₃₀O₁₁+Na, 505.1681).

Compound **146**: ^1H NMR (500 MHz, d_4 -MeOH) δ : 6.69 (1H, d, $J = 8.4$, H-5'), 6.58 (1H, d, $J = 8.4$, H-6'), 6.48 (2H, s, H-2, H-6), 4.6 (1H, d, $J = 7.9$, G-1), 3.81 (3H, s, 4'-OCH₃), 3.79 (6H, s, 3-OCH₃, 5-OCH₃), 3.72 (3H, s, 4-OCH₃), 3.51 (1H, dd, $J = 9.1, 7.9$, G-2), 3.39 (2H, m, G-3, G-4), 3.2 (1H, m, G-5), 3.10 (1H, m, H-1'a), 2.92 (1H, m, H-1'a), and 2.8 (1H, m, H-1a).

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