ISOLATION AND STRUCTURE ELUCIDATION OF CYTOTOXIC NATURAL PRODUCTS FROM THE RAINFORESTS OF MADAGASCAR AND SURINAME

Brent Jason Yoder

Dissertation submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
in
Chemistry

David G. I. Kingston, Chair
Paul R. Carlier
Harry S. Dorn
Felicia A. Etzkorn
Richard D. Gandour

November 17, 2005
Blacksburg, Virginia

Keywords: Chemistry, Bioorganic, Plants, Cancer

ABSTRACT

ISOLATION AND STRUCTURE ELUCIDATION OF CYTOTOXIC NATURAL PRODUCTS FROM THE RAINFORESTS OF MADAGASCAR AND SURINAME

Brent Jason Yoder

As part of an ongoing investigation of new bioactive metabolites from rainforest flora, extracts from five different plants were determined to have interesting compounds that were new and/or cytotoxic. These phytochemicals were isolated by various separation techniques and then characterized by common spectroscopic methods.

A bark extract of a *Tambourissa* species yielded a new hydroxybutanolide with moderate cytotoxicity. The long hydrocarbon chain in this molecule is unique, and its structure was determined by various NMR techniques.

A fruit extract from *Macaranga alnifolia* yielded four new prenylated stilbenes, one new geranylated dihydroflavanol, and five known compounds. The stilbenoids are highly cytotoxic, and the National Cancer Institute (NCI) further evaluated one of the new compounds.

Bark and leaf extracts from *Cerbera manghas* yielded a known iridoid and a known cardiac glycoside, both of which showed good bioactivity. The cytotoxicity associated with the iridoid is unprecedented, and it also was further evaluated by the NCI.

An extract of a *Cordia* species yielded two known compounds – a naphthoquinone dimer and a triterpene. Both of these structures are new to the *Cordia* genus of plants and showed moderate bioactivity.

An extract of a *Monoporus* species yielded a known triterpene saponin. The compound has been previously located in the same plant family, but it is new to this genus and has no prior record of cytotoxicity.

ACKNOWLEDGEMENTS

This work is the culmination of 5+ years of study at Virginia Tech. I am extremely grateful to the following people for all that they have helped me to accomplish.

Thank you to my wife, Rachel Yoder, for coming back into my life and promising to always stay.

Thank you to my family, Craig, Nan and Brad Yoder, for supporting me and providing more encouragement than I could ever need.

Thank you to my advisor, Dr. David Kingston, for abundant patience and guidance, through all of the successes and failures.

Thank you to my teaching mentor, Dr. Richard Gandour, for leading me through my first semester at the front (and bottom) of the lecture hall.

Thank you to the other members of my committee, Dr. Paul Carlier, Dr. Harry Dorn and Dr. Felicia Etzkorn, for advice, assistance and passing grades.

Thank you to the "three wise men", Dr. Shugeng Cao, Bill Bebout and Tom Glass for knowing which buttons to push and then explaining it all to me.

Thank you to the "cultured" five, Jeannine Hoch, Jennifer Schilling, Becky Guza, Andrew Norris and Peggy Brodie, for giving me something to look forward to on Thursday afternoons.

And thank you to the original, Dr. Jim Yoder, for unknowingly changing my path more than nine years ago.

"Many are the plans in a man's heart, but it is the Lord's purpose that prevails."
Proverbs 19:21

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SCHEMES

LIST OF TABLES

I. GENERAL INTRODUCTION

1.1 The Natural Products Approach to Drug Discovery

Biological organisms produce two distinctly different types of chemical products. The first type, primary metabolites, consists of compounds such as sugars and proteins that are common to most organisms and are essential for functional metabolism. Secondary metabolites, on the other hand, are chemicals unique to a single species or related group of organisms. Not until the 1990s would scientists fully realize that these secondary metabolites are more than mere "leftovers" from an organism's metabolic processes; they actually serve in a wide variety of important roles.[1] These chemicals can function as communications tools, defense mechanisms, or sensory devices.

The biological activity of these chemicals is beneficial to the organism that produces it, but it is often harmful to other species, including humans.[1] This toxicity can adversely affect the functions of the entire human body or only a specific biological process, such as the growth of cancer cells. In this way, certain foreign, naturally produced chemicals can act as powerful drugs when administered at the proper concentration. Natural products have been used by native cultures as a source of remedies for thousands of years, dating back to ancient empires in Mesopotamia, Egypt, China, Greece, and Rome.[2] Now scientists in the modern industrial world are turning to plants, microbes, and marine organisms as a potential storehouse of medicines waiting to be discovered.[3]

Drugs from natural sources may fall into one of three categories of compounds: those that were isolated from biological organisms, those that are modified versions of natural products, and those that are completely synthetic, yet based upon models of natural origin.[4] Today, natural products are responsible for about half of the approved drugs that are currently available.[5] The percentage is even higher for treatment of infection or cancer, as natural products for those illnesses account for approximately 60% of the drugs either in use or awaiting FDA approval between 1989 and 1995.[6] For example, 18 of the 42 new drugs discovered in 1992 are either natural products or synthetic analogs of natural products.[7] Obviously, Nature has had quite an effect on the science of drug discovery, and the role of the chemist has become important for work in

isolation, structure determination, and synthesis of bioactive compounds. The questions for natural products chemists are (1) which biological species produce these compounds, (2) what is the structure of the molecules, and (3) how potent are they as therapeutic agents?

1.1.1 *The Three Sources of Compounds: Microbes, Marine Organisms and Plants*

Of the natural products that have been developed into drugs, many come from plant sources, but there have been a considerable number of important drugs harvested from microorganisms and marine sources.[3] Perhaps the most clinically useful antimicrobial drugs are antibiotics such as penicillin and tetracyclin, immunosuppressant drugs such as cyclosporin A, and anticancer agents such as the bleomycins. Marine environments have also yielded their share of medicines, including manoalide, an analgesic and anti-inflammatory drug, and a variety of anti-fungal compounds.[8] Plants have produced well-known anti-cancer agents such as Taxol®; analgesics such as salicylic acid (the precursor for aspirin), codeine and morphine; anti-malarial drugs such as quinine; pupil dilators such as atropine (which has also shown potential viricidal activity); and cardiac glycosides such as digitalis.[9] Each of these compounds will be discussed in detail, with structures provided, in the following two sections.

Even though most people associate natural products with extracts from roots and leaves, the discovery of natural products is certainly not limited to plant species. Many bioactive compounds, especially antibiotics, have been isolated from microbiological sources. Two of the most well known and often prescribed antibiotic drugs are penicillin and tetracycline. The accidental discovery of penicillin by Alexander Fleming in 1928 is still one of the most important developments in the history of pharmaceutical chemistry.[8] As an inhibitor of the growth of gram-positive bacteria, it became the first natural product to demonstrate that microorganisms, specifically fungi, are a source of medically useful secondary metabolites.[8] The inhibition occurs because penicillin can inhibit a key step in the biosynthesis of the bacterial cell wall.[2] Today, penicillins are a class of over a dozen compounds that can be natural, synthesized, or semi-synthesized.[10] One of the most well-known penicillin molecules is penicillin G (also called benzylpenicillin), which contains the characteristic β-lactam-thiazolidine structure (Figure 1.1).

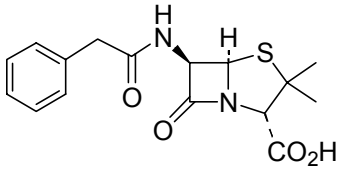


Figure 1.1. Penicillin G.

Tetracyclines are another class of natural (from *Streptomyces sp.*) and semi-synthetic antibiotics that are composed of a polyketide fused tetracyclic structure.[10] After initial discovery almost 50 years ago, many semi-synthetic analogs have been successfully created from the parent molecule.[2] One specific tetracycline that has found use outside of its traditional application as an antibiotic is doxycycline (Figure 1.2), and it is used to aid in the treatment of malaria, often in combination with an alkaloid like quinine.[11] This type of treatment is necessary because of the slow nature of tetracycline's mode of action and its poorly understood mechanism.[12] Both penicillin and tetracycline are the results of 1960s programs that sought to structurally modify and compare bioactivities of several antibiotic natural products.[13]

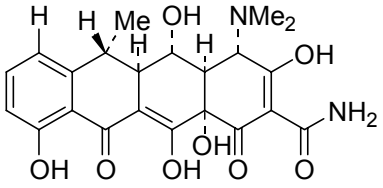


Figure 1.2. Doxycycline.

One type of drug that is often taken for granted and viewed as slightly less glamorous than its pharmaceutical counterparts are medicines that prevent the rejection of organs following surgery to transplant organs. These immunosuppressant compounds are administered not to treat an illness, but to stop the human body from performing a normal function for which one is usually grateful. Cyclosporin A is one such immunosuppressant drug.

This molecule was first isolated from its parent fungus, *Tolypocladium inflatum*, in Switzerland,[14] but a variety of advanced studies were necessary before the efficiency

of the drug was fully realized and the medicine was introduced into the market in 1983. It was the first of its kind, and current worldwide use still places it on the list of the 25 top overall drugs and number one among all immunosuppressive drugs.[8] Even after popular and effective drugs are synthesized in the laboratory, the creation of structurally unique analogues remains a top priority. However, midway through the 1990s, combinatorial analogues of cyclosporin A (Figure 1.3) were still unproduced.[15]

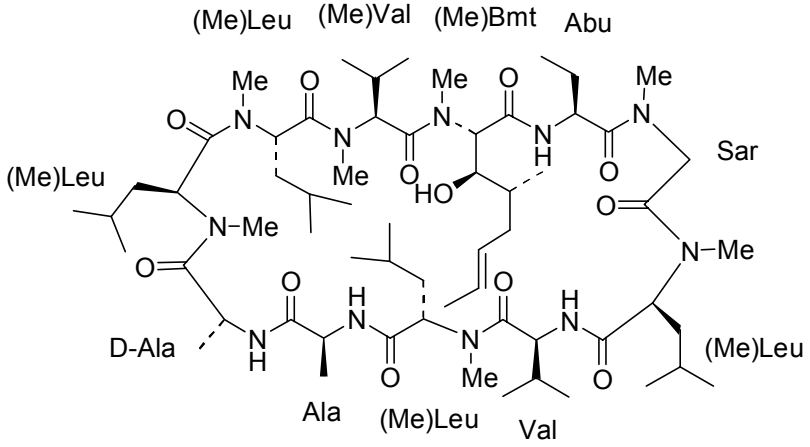


Figure 1.3. Cyclosporin A.

Bleomycins are microbial compounds from *Streptomyces verticillus* that interfere with the replication of DNA by cleaving both single and double strands of genetic material.[16] The two glycopeptides that compose the bleomycin family are bleomycin A_2 (Figure 1.4), which accounts for 55-70% of that isolated, and bleomycin B_2, which accounts for the remaining 30%.[2,10] The enzymes involved in the biosynthesis of these anticancer agents are amide synthases, which create highly complex molecules that contain amino acid, sugar, pyrimidine ring, and dithiazole ring components.[10,17] The bleomycins have found clinical use as treatment for squamous cell cancers of the head, neck, cervix, and lymphomas, all without greatly affecting the patient's supply of bone marrow.[3,10]

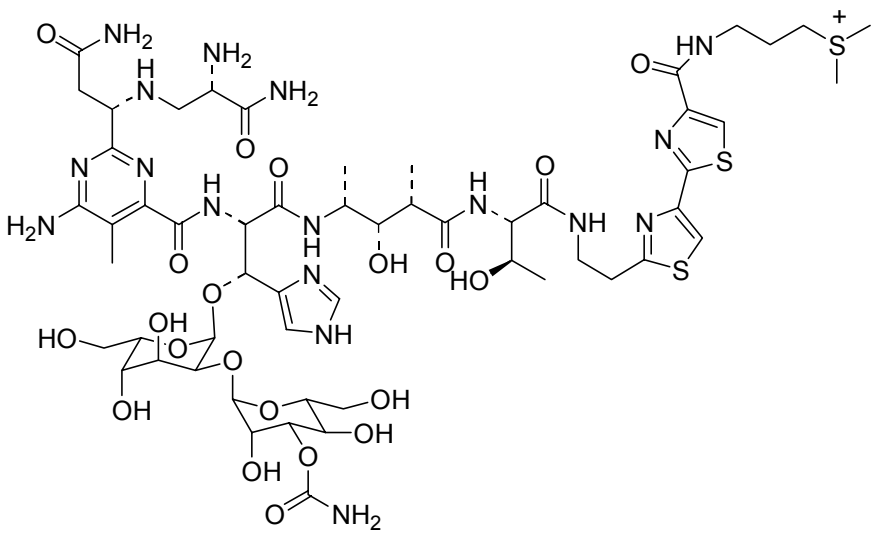


Figure 1.4. Bleomycin A_2.

The prospecting for biologically active molecules in marine organisms is a relatively new and rapidly expanding branch of natural products chemistry. Over the past 30 years alone, the ocean depths have produced some 3000 novel natural products.[3,18] The search includes the examination of sponges, molluscs, corals, and sea-dwelling microorganisms as potential sources of potent drugs. Manoalide (Figure 1.5) is a 25-carbon marine natural product with anti-inflammatory activity towards the cyclooxygenase(COX)-2 enzyme. The COX-2 enzyme has been identified as a catalytic source of prostaglandins (and thus, unwanted inflammation), so drugs that can regulate the enzyme have the ability to control the pain that results from inflammation.[19] Manoalide is isolated from a sponge, *Luffariella variabilis*, but there exist a number of synthetic analogues that may function through irreversible deactivation of phospholipase A_2.[20]

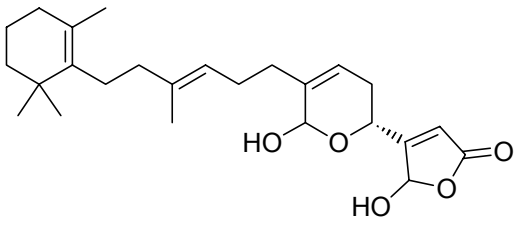


Figure 1.5. Manoalide.

There are quite a few cytotoxic marine natural products that have been isolated and identified in the past two decades, as methods of collection in these remote environments has improved.[3,21] A great majority of these potential medicines are still under investigation in the early stages of clinical trials, but one drug, citarabine, is showing promise as an inhibitor of DNA synthesis in leukemia and lymphomas.[3] Other experimental anticancer medicines include aplidine, which halts the progression of the cell-cycle; bryostatin 1, which was found through the use of a leukemia cell line bioassay; dolastatin 10, a microtubule inhibitor; and ecteinascidin 743, which alkylates specific amino acid components in the minor groove of DNA.[3]

1.1.2 *Medicinal Plant-Derived Compounds*

The realm of drugs obtained from plants is vast, wider than any other source of natural products. They are the basis for the traditional medicine philosophies and practices in China, India, and isolated tribal peoples.[2] It is known that nearly 120 compounds from 90 different plant species were being used around the world as drugs in 1985, and the numbers have certainly grown since then.[22] Approximately 25% of the prescriptions that were filled in the U.S. between 1959 and 1980 are directly tied to extracts of higher plants.[2] Although anticancer agents are the focal point of this review and research occurring in this laboratory, plants provide a multitude of medicines for all types of ailments and diseases.

Taxol® (paclitaxel), a cytotoxic diterpene alkaloid, was first isolated from the bark of the Pacific yew tree *Taxus brevifolia* in the late 1960s.[23] The discovery process involved the screening for anti-cancer bioactivity of over 110,000 compounds from 35,000 different plants by the National Cancer Institute over a 22-year period.[24] *Taxus brevifolia* became one of a number of plant species that was developed into very effective anti-cancer drugs. The anti-tumor activity was originally tested against leukemia cells, but paclitaxel (Figure 1.6) proved to be most effective against breast and refractory ovarian types of cancer. However, it has also been used to treat melanoma and certain types of lung cancers.[3,25]

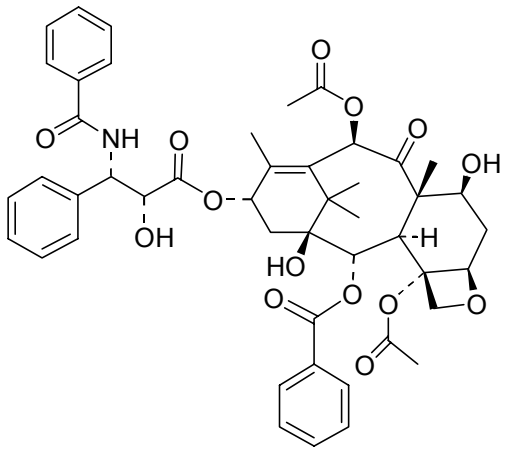


Figure 1.6. Taxol®.

Paclitaxel functions by inhibiting the cancer cell's ability to divide (mitotic arrest), and that inability leads to cell death. The drug binds to and stabilizes the microtubles of a cell, preventing the breakdown of tubulin, which was a very surprising mechanism of action when it was first determined in 1979.[26] Paclitaxel is currently marketed commercially as Taxol® by Bristol-Myers Squibb, which manufactured the compound semisynthetically until 2004, and now produces it by plant tissue culture. In 1998, total worldwide sales of the drug topped the billion-dollar mark.[8,27] It goes without saying that this drug is perhaps the most important anticancer development of the past decade.[28]

After the publication of its structure in 1971, paclitaxel was also isolated from the leaves of *Taxus baccata* (a renewable source of the compound), and bioactive taxoid structures can now be found in a wide variety of other plant parts throughout the *Taxus* genus.[29] There are many analogs of the taxoid structure which are bioactive, including baccatin III (Figure 1.7) and docetaxel (Figure 1.8).[30] The latter analog can actually be synthesized in the chemistry laboratory from the former. However, the direct conversion of paclitaxel to docetaxel (by selective debenzoylation) and other analogs is still a process that hails interest and demands attention.[31] Other semi-synthetic structures can be created by modifying a part of the compound, such as the substitution of an oxygen atom for a sulfur or selenium atom within a ring, even though the resulting molecule may be less biologically active than the original model.[32]

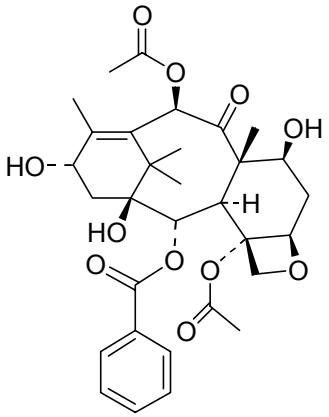


Figure 1.7. Baccatin III.

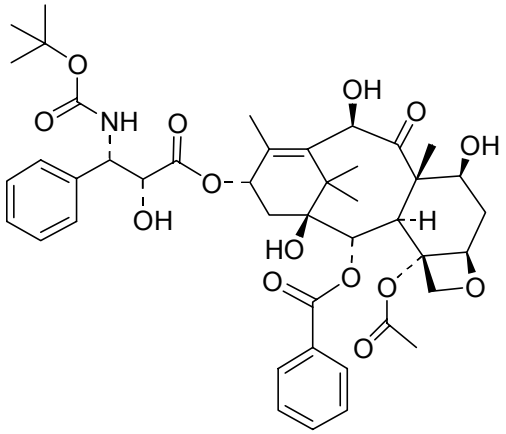


Figure 1.8. Docetaxel.

Aspirin is a powerful synthetic drug that is used to treat a wide variety of ailments. Since its synthesis and initial use in the 1800s, it has come to be known mostly as an anti-inflammatory drug and pain reliever. The natural product that provides the basis for aspirin is salicylic acid, which is isolated from the bark of the willow tree.[1] Use of the willow tree for medicinal purposes dates back nearly 2500 years to the time of the ancient Mediterranean empires.[8] One of the side effects of salicylic acid is gastric discomfort and irritation, so the acetyl derivative of salicylic acid (acetylsalicylic acid or aspirin) is used clinically to partially reduce the side effects.[8] Aspirin (Figure 1.9) functions by inhibiting the COX-1 and COX-2 enzymes and, therefore, the synthesis of human hormones called prostaglandins.[1] It is the production of COX-2 that induces pain within the human body, so inhibition of the enzyme is a biochemical form of pain management. Aspirin also functions as an important preventative treatment against heart disease because of its inhibition of prostaglandins, which affect the clotting of blood.[10] Prostaglandins are vital to many normal biological processes within the human body, so aspirin unfortunately produces many of its own unwanted side effects. Ulcers and other conditions resulting from the loss of stomach lining are due to the unwanted inhibition of COX-1.

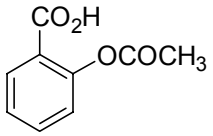


Figure 1.9. Aspirin.

Codeine and morphine are two other well-known and often prescribed analgesics. Both of these similarly structured alkaloids come from unripened seedpods of the opium poppy plant.[1] In fact, the two compounds are so alike that the codeine molecule can be partially synthesized in the laboratory from morphine, which is the more abundant natural product.[10] Use of morphine (Figure 1.10) as a drug dates back many centuries to a time when monks saw the anaesthetic and pain-relieving properties of *Papaver somniferum*, even though morphine was not isolated until 1806 and it was commercially manufactured 20 years later.[8] While codeine (Figure 1.10) is not nearly as effective in its pain-relieving abilities as morphine, it also can be used as a cough suppressant, and it is a considerably less addictive drug, producing fewer effects of euphoria as compared to its narcotic cousin.[10] Each compound includes constipation among its list of side effects, but only morphine leads to additional mental/emotional ailments as well as physical symptoms.[10] In spite of side effects and the possibility of addiction, morphine remains one of the most powerful and effective medicines for intense pain in clinical situations, an advantage that cannot be matched by any human-made compound.[33]

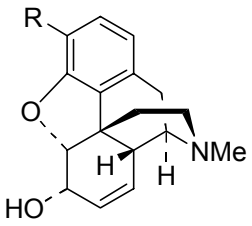


Figure 1.10. Morphine (R = OH) and Codeine (R = OMe).

Quinine is one of the oldest of a number of anti-malarial drugs that are currently available. Only the bark from the *Cinchona* genus of trees, located mainly in South America, is known to be the source of this compound.[11] The first pure form of the active drug (isolated in 1820, nearly 150 years before its structure was determined) is the

precursor of a variety of synthetic analogs that were developed during World War II when the natural supply became too difficult to obtain.[10] Throughout its history, quinine (Figure 1.11) has perhaps saved more lives than any other drug.[11] The mechanism of action of this alkaloid is believed to involve the inhibition of heme polymerization, although debate exists over exactly how the drug operates.[34] Heme is the part of hemoglobin that is left over after the protein part has been digested.[11] Although other quinoline drugs used to treat malaria are known to have few side effects, medical treatment with quinine produces a large number of dangerous side effects, including toxicity to the heart and various sensory and nervous system disorders.[12] With an IC_{50} value of around 100-440 nM, the difference between the toxic and therapeutic doses is very small and difficult to manage in a health care environment.[35] However, a greater concern with quinine (and other anti-malarials) might be the resistance that has developed towards the drug in certain parts of the globe where it is administered.[12] Brazil and Africa have been most affected by the resistance of the malaria parasite *Plasmodium falciparum* to the drug.[36]

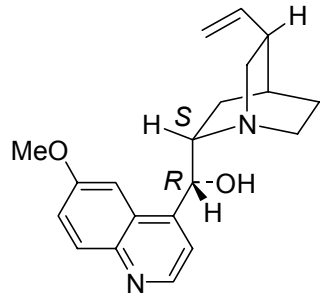


Figure 1.11. Quinine.

Other well-known natural products from plants are the tropane alkaloid atropine from *Atropa belladonna* and digitalis from *Digitalis purpurea*.[8] Atropine has found use as an antitoxin and muscle relaxant, but it is mostly known as a mydriatic (pupil dilator). Use of the *belladonna* fruit juice for such a purpose originates with Italian women who would brighten the eyes of their young females through a practice that Louisa May Alcott details in her book *An Old Fashioned Girl*.[1] "Belladonna" actually means "beautiful woman" or "beautiful lady". Modern doctors use the drug to prepare patients for eye

examinations or surgery because it acts as a local pain reliever by halting the passage of nerve impulses and decreasing sensitivity in the parasympathetic endings.[1] The drug binds to the muscarinic receptor site that is normally occupied by acetylcholine.[10] Atropine is actually a racemic mixture of two compounds, (+)-hyoscyamine (Figure 1.12) and (-)-hyoscyamine (Figure 1.13), although the natural, (-)-enantiomer is considerably more bioactive than the (+)-enantiomer.[10] These tropane alkaloids can be quite addicting, causing dry mouth, sedation (it was historically used during childbirth), or even death.[10] Indeed, the ancient Romans found use for *belladonna* as a poison because there is such a fine line between the dose that is therapeutic and the dose that swiftly kills.[1]

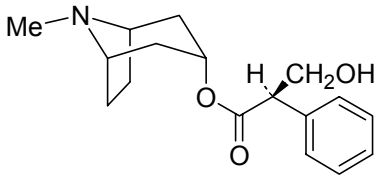


Figure 1.12. (+)-Hyoscyamine.

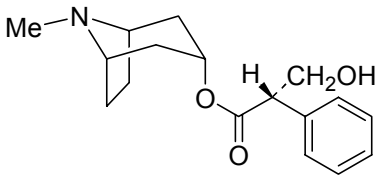


Figure 1.13. (-)-Hyoscyamine.

Digitalis comes from *Digitalis purpurea*, a large flowering herb native to Great Britain. The leaves of the plant produce digitoxin (another name for digitalis), a glycoside prescribed for heart failure and irregular heart rhythm, as well as digoxin, a kidney diuretic, both of which are toxic at high concentrations.[1] The ability of digitoxin (Figure 1.14) to strengthen the muscle contractions of the heart and slow the heart rate has made it a popular natural treatment since its discovery by William Withering in the 18th century.[1] One aspect of this drug that makes it unique in the pharmaceutical industry is that it is still isolated from plants today because the cost of synthesizing the drug in the laboratory is so high.[1] *D. purpurea* has also shown potential as an anticancer agent because of its ability to inhibit protein kinase C in certain yeast bioassays.[37] Many other important compounds have been isolated from this plant, including two cardiac glycosides (gitoxin and gitaloxin), anthraquinones, phenylethanoids, and flavonoid glycosides.[37]

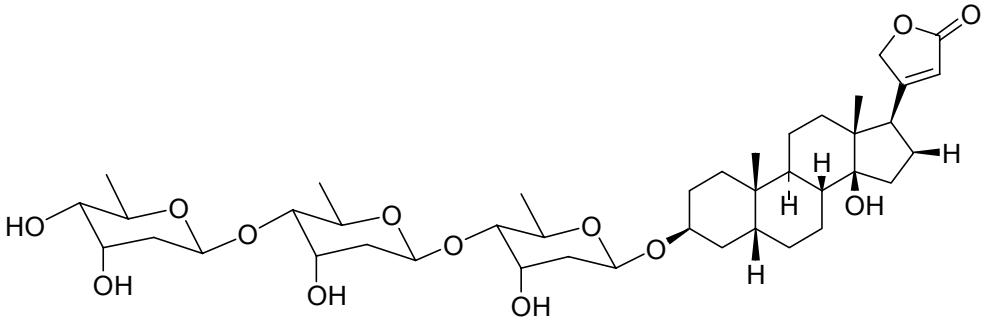


Figure 1.14. Digitoxin.

1.1.3 *Anticancer Drugs Isolated from Plants*

While there are a plethora of novel, bioactive natural products to examine, further discussion will be limited to those that are isolated from botanical sources, specifically phytochemicals with anticancer activity. As previously discussed, Taxol® is currently the world's best-selling anticancer drug available for chemotherapy, and it is one of the most famous of the plant-derived medicines. However, other natural drugs (often alkaloids) play a role in the expanding realm of cancer treatment options. A handful of such compounds are camptothecin, flavopiridol, homoharringtonine, podophyllotoxin, and the *Vinca* alkaloids, vincristine and vinblastine. Each of these compounds will be discussed in detail, with structures provided, in the following section.

Camptothecin comes from the wood and bark of a Chinese tree, *Camptotheca acuminata*, which is a tree well known for its anticancer metabolites.[38] It is a pyrrolo[3,4-*b*]-quinoline alkaloid that was extracted using ethanol from the stem-wood of the plant.[39] Although it was initially discovered in 1966 by Wani and Wall, it is now known that the drug binds to topoisomerase I, making it unique in that most other drugs that interact with topoisomerase do so with topoisomerase II.[23] Cells are unable to replicate when the drug is bound to a complex of topoisomerase I and DNA that has been stabilized.[40] Early chemical studies on camptothecin were performed by a National Cooperative Drug Discovery Group (NCDDG) under the guidance of the National Cancer Institute.[2] Many chemical modifications have been attempted on camptothecin (Figure 1.15), but most have resulted in a loss of efficiency and biological activity. However, the substitution of various functional groups for hydrogen atoms at select locations has led to an increase in the water solubility of the compound and the creation

of two useful analogues, topotecan and irinotecan.[8] These two drugs show bioactivity towards ovarian cancer (topotecan, Figure 1.16) and colorectal cancer (irinotecan, Figure 1.17).[41,42]

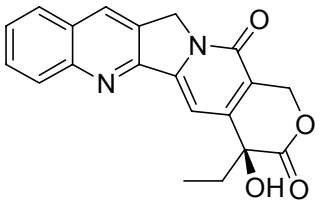


Figure 1.15. Camptothecin.

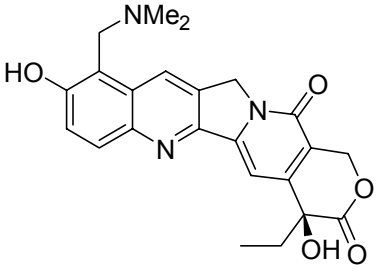


Figure 1.16. Topotecan.

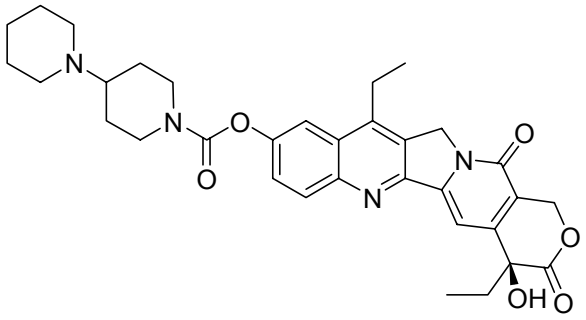


Figure 1.17. Irinotecan.

Flavopiridol (Figure 1.18) is a flavone inhibitor of the cyclin-dependent kinase (CDK) family that was semi-synthesized from rohitukine, a plant natural product.[3] It appears to be non-selective towards any particular CDK. The drug is in the early stages of clinical trials, but it is creating excitement because of its interesting mechanism of action.[2] The progression of the cell cycle is blocked during stages of growth after the compound interferes with the kinase phosphorylation step.[43] The only toxic side effect realized to date is diarrhea.[3]

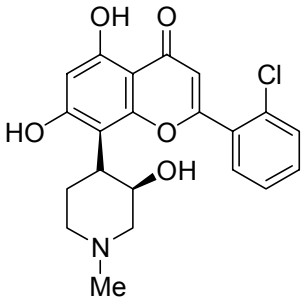


Figure 1.18. Flavopiridol.

Homoharringtonine comes from the seeds of a Chinese evergreen (*Cephalotaxus harringtonia)* widely used in China for traditional medicine and known for efficiency as a cytotoxic anti-leukemia drug.[44,45] As in the case of Taxol®, this drug was a product of discovery through an extensive research program carried out by the National Cancer Institute in the 1960s, and in 1993, it was classified as one of the NCI's investigational new drugs.[16,45] Homoharringtonine (Figure 1.19) is thought to function during the cell cycle when proteins are being elongated by peptidyl transferase.[45] This interruption of protein synthesis leads to "apoptosis and differentiation of cancer cells" because of the loss of cell-cycle progression.[45,46]

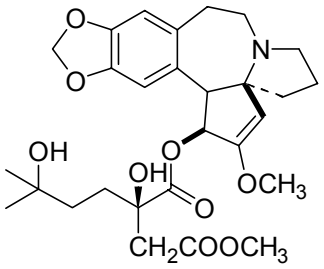


Figure 1.19. Homoharringtonine.

A non-alkaloid bioactive compound from a higher plant that deserves some attention is podophyllotoxin (Figure 1.20). It is isolated from the roots of two different plant species (one from the genus *Podophyllum* and one from the genus *Juniperus)* and identified as an antitumor dimeric lignan in 1880.[47] The epimer of podophyllotoxin is epipodophyllotoxin, giving rise to two semi-synthetic compounds with high activites and clinical applications, etoposide and teniposide (Figure 1.21).[2] These drugs are much less

toxic than their "grandparent" compound (podophyllotoxin). The former is used to battle lung carcinomas and, along with the bleomycins, as a treatment for testicular cancer.[3,48] Like many anticancer drugs, etoposide functions by inhibiting topoisomerase II, during mitosis, which leads to DNA cleavage.[49] Podophyllotoxin, however, causes cells to arrest during metaphase after microtubule assembly interference has occurred.[47]

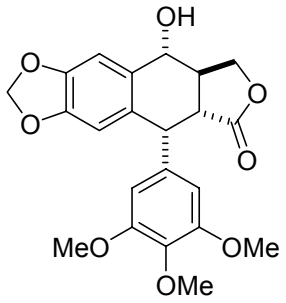


Figure 1.20. Podophyllotoxin.

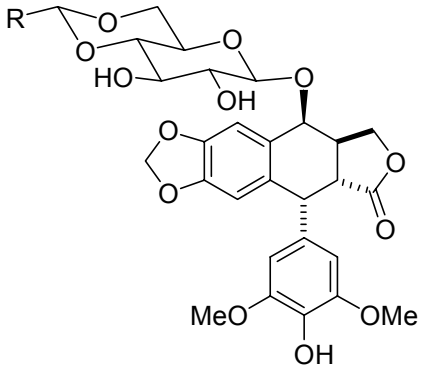
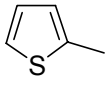


Figure 1.21. Etoposide (R = CH_3) and Teniposide (R = 

).

Vincristine and vinblastine are known as the *Vinca* alkaloids. Both come from *Catharanthus roseus*, a type of periwinkle from the rain forests of Madagscar, and like Taxol®, they target the formation of microtubles to stop the process of cell division at metaphase.[45,50] However, with vincristine and vinblastine, it is the *disassembly* of the microtubles, formed by the polymerization of free tubulin dimers, that halts the formation of spindles and asters necessary for mitosis.[45] Depolymerization begins at metaphase after a dimer of tubulin and one of the *Vinca* alkaloids has bonded to the microtubule.[51]

Vincristine (Figure 1.22) has traditionally been used for acute childhood leukemia and Hodgkin's disease, while vinblastine (Figure 1.22) is a common treatment for lymphoma types of cancer.[45,52] The side effects most commonly seen with vincristine and vinblastine are peripheral neuropathy and depression of bone marrow, respectively.[53] The periwinkle source of these drugs continues to be of great interest to Eli Lilly, the pharmaceutical company that grows it in Texas, and others involved in the search for antitumor compounds. Eli Lilly managed to discover these anticancer agents on the 40th attempt in their program to screen plants with possible antineoplastic activity.[39] Currently, over 500 interesting alkaloids from this plant have been examined and documented.[9]

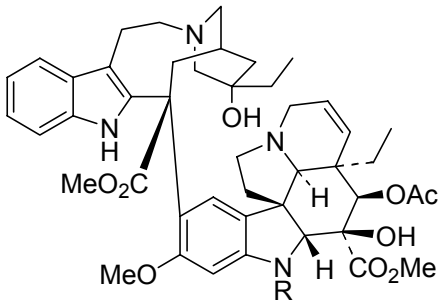


Figure 1.22. Vincristine (R = CHO) and Vinblastine (R = Me).

1.1.4 *Recent Discoveries of Cytotoxic Phytochemicals*

All of the natural products discussed to this point are well known, commercially available drugs that have been used clinically for many years, decades, or even centuries. However, in the past few years, scientists have isolated many phytochemicals that show promise as potential anticancer drugs, but they are awaiting further investigation by pharmaceutical companies. This section of the review briefly describes some of the discoveries made in this area during the last four years (2001-2005).

One plant species that has produced a number of interesting alkaloid structures is *Menispermum dauricum*, a species native to China. Past studies have indicated as many as nine useful alkaloids found in the plant and roots, but two isolated oxoisoaporphine alkaloids have recently shown activity against a human breast cancer cell line.[54] The

compounds that have been isolated, daurioxoisophines A and B, are shown in Figure 1.23 and Figure 1.24, respectively.

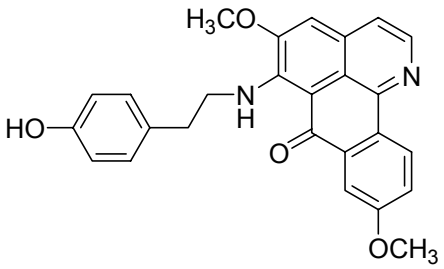


Figure 1.23. Daurioxoisophine A.

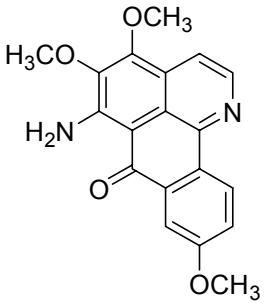


Figure 1.24. Daurioxoisophine B.

Another plant that was recently identified as a source of interesting alkaloids is *Cananga odorata*, from Taiwan. Although this evergreen tree had been traditionally known for its anti-malarial properties and treatment of fever and infection, two novel compounds demonstrated activity against hepatocarcinoma cell lines. Both alkaloids (cananodine, Figure 1.25, and crytpomeridiol 11-α-L-rhamnoside, Figure 1.26) were isolated from the fruit of the plant and bear structural resemblance to other sesquiterpenes that had been previously obtained from the species.[55]

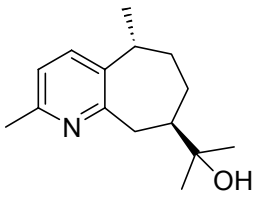


Figure 1.25. Cananodine.

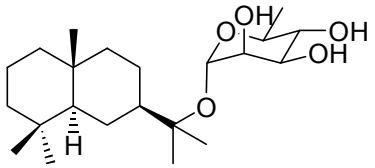


Figure 1.26. Crytpomeridiol 11-α-L-rhamnoside.

Lippsidoquinone (Figure 1.27) is a new naphthoquinone that has been located in *Lippia sidoides*.[56] The compound, extracted with ethanol, is a dimer and has shown activity against a pair of human leukemia cell lines. The plant itself grows in the northeastern part of the country and the oil from its leaves has previously demonstrated antiseptic bioactivity.

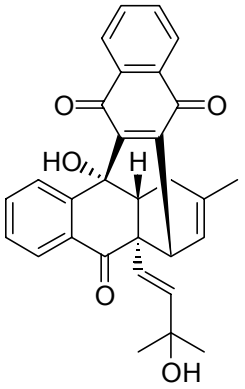


Figure 1.27. Lippsidoquinone.

Another novel molecule that was recently isolated is solavetivone (Figure 1.28). It is produced by the root of a Chinese plant that is popular in Taiwan (*Solanum indicum*). Traditionally used to treat breast cancer, the plant has also been accepted as an anti-inflammatory and anti-toxin source. Cytotoxicity testing of the new compound gives an IC_{50} of 0.1 mM on the OVCAR-3 cell line.[57]

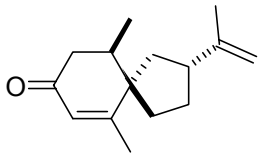


Figure 1.28. Solavetivone.

Yet another shrub from Taiwan that is used in Chinese traditional medicine is *Ventilago leiocarpa*. Its folk uses include treatment for pain and rheumatism, but stem extracts have also shown cytotoxicity towards various cancer cell lines. One of the newest quinones from the dried stems of this plant is 2-hydroxyemodin 1-methyl ether (Figure 1.29). The activity against so many different cell lines is assumed to be partly due to the trihydroxy nature of the anthraquinone.[58]

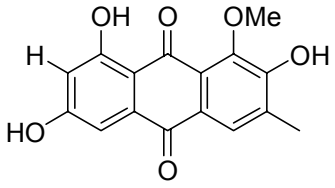


Figure 1.29. 2-Hydroxyemodin 1-methyl ether.

Not all phytochemical compounds of current interest are new discoveries. One molecule that is back in the news is hypericin, an anthraquinone from St. John's wort.[59] *Hypericum perforatum* is an extremely popular over-the-counter remedy for depression, but the active ingredient in this plant is now being examined as an inhibitor of the topoisomerase IIα enzyme in humans. Topoisomerase IIα is an isoform of DNA topoisomerase II enzyme that is regulated by the cell cycle and selectively cleaved during apoptosis of human epithermoid carcinoma cells. Hypericin (Figure 1.30) has shown *in vitro* activity against various leukemia cell lines, making it a potentially interesting drug for cancer patients who also experience depression because of their illness.

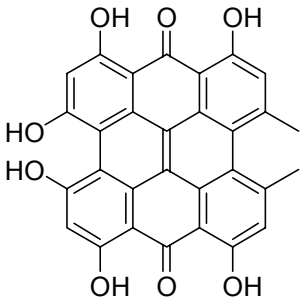


Figure 1.30. Hypericin.

Another Asian plant of interest in traditional medicine, and now cytotoxicity studies, is *Clerodendrum cyrtophyllum*. Folklore has labeled this plant from Taiwan as a form of treatment for a number of illnesses including syphilis and typhoid fever.[60] The most biologically active constituent in terms of cytotoxicity is a methyl ester of a compound found in the related *Clerodendrum calamitosum*. It is a known compound, but it had never been isolated as a plant natural product before this discovery. This structure (Figure 1.31) appears to be potent towards a number of cancer cell lines and has ED_{50} values as low as 0.27 μg/mL.[60]

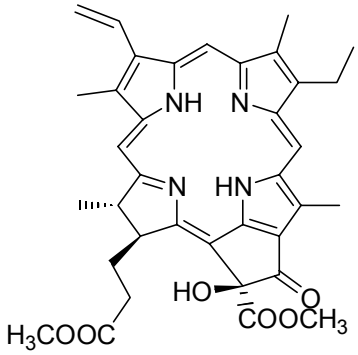


Figure 1.31. Methyl ester from *Clerodendrum calamitosum*.

A chemical class that has not been explored yet in this discussion, but is a viable source of cytotoxic compounds, is that of the acetogenins, which are long molecules with aliphatic chains. One such source of acetogenins is *Annona cherimolia*, a tropical tree from Peru used traditionally to kill insects and parasites.[61] The seeds from this plant have yielded two antitumor compounds from an ethanol extract: annomolin (Figure 1.32) and annocherimolin (Figure 1.33). The former has shown activity against a prostate cancer cell line, while the latter has shown activity against both breast and colon cancer cell lines. Both compounds appear to be 10^4 times as potent as adriamycin.[61]

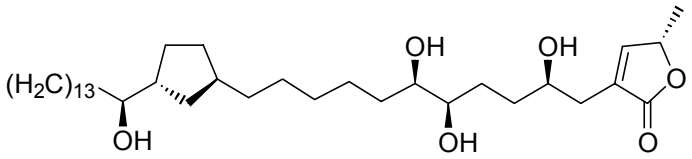


Figure 1.32. Annomolin.

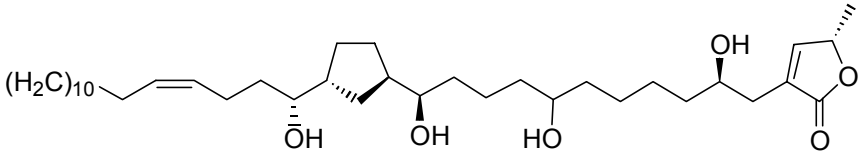


Figure 1.33. Annocherimolin.

Another series of long-chain hydrocarbon compounds with cytotoxic activity are a collection of alkynes from *Ochanostachys amentacea*. The tree is native to South Pacific

islands such as Malaysia and Indonesia, and from its small twigs came two new potent polyacetylenes.[62] (*S*)-17,18-hydroxy-9,11,13,15-octadecatetraynoic acid (Figure 1.34) has indicated activity against oral epidermoid cancer and (*S*)-17-hydroxy-15*E*-octadecen-9,11,13-triynoic acid (Figure 1.35) may be a potential treatment for ovarian and hormone-dependent prostate cancers.

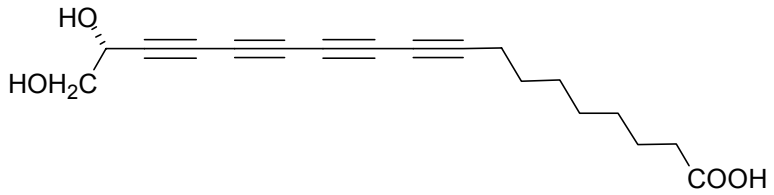


Figure 1.34. (*S*)-17,18-Hydroxy-9,11,13,15-octadecatetraynoic acid.

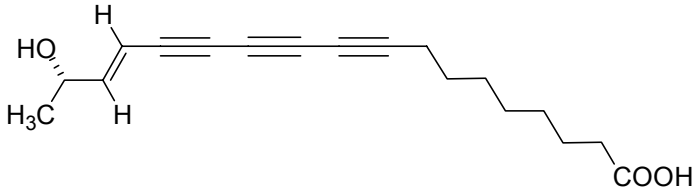


Figure 1.35. (*S*)-17-Hydroxy-15*E*-octadecen-9,11,13-triynoic acid.

Coumarin structures are not unfamiliar in the pharmaceutical industry. The plant *Calophyllum dispar* has been recently identified as a source of 11 coumarin compounds, eight of which are new.[63] These new molecules are 4-phenylfuranocoumarins and are extracted from the fruits and bark of the species. Nearly all of the compounds show cytotoxic activity with IC_{50} values as low as 5 μg/mL. An example of one of these coumarins is provided in Figure 1.36.

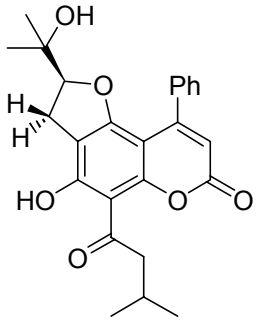


Figure 1.36. Coumarin from *Calophyllum dispar*.

One new natural product to be discussed was a result of the efforts of this laboratory. A benzoquinone, 2-methoxy-6-heptyl-1,4-benzoquinone (Figure 1.37), was isolated from *Miconia lepidota*, a species native to the rainforests of South America and West Africa.[64] The compound discovery was made through fractionation of an ethyl acetate extract from Suriname, and cytotoxicity testing gave an IC_{50} value of 7.9 μg/mL in the A2780 ovarian cancer cell line. This moderate reading indicated the true potency of the molecule, but it was not enough to warrant further examination as an anticancer drug.[64] Other previously isolated and slightly more cytotoxic compounds were also found in this study.

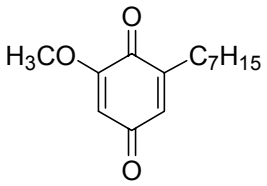


Figure 1.37. 2-Methoxy-6-heptyl-1,4-benzoquinone.

1.2 The ICBG Program

Many bioactive chemical compounds have already been discovered and are being used as clinical drugs. Other, newly discovered molecules have the desired bioactivities, but they require additional testing and understanding of their mechanisms of action before their full potential is known. However, there are many more regions of the world to explore and more medicines to locate in the 21st century. It is estimated that only around 2% of the world's 250,000 higher plant species have been thoroughly examined

for novel drug bioactivity.[65] Many illnesses still do not have cures, and in the case of cancer, it seems unlikely that the desire for a new miracle drug will end anytime soon. Therefore, the search for a "wonder drug" continues at the Virginia Polytechnic Institute and State University as part of an International Cooperative Biodiversity Group (ICBG) project.

The ICBG program, which was begun in 1993, is a joint venture between academic institutions, private industry, the United States government, and organizations in developing countries. The intention is to discover new natural drugs (for a variety of illnesses, not only cancer), while building an inventory of known medicinal plants and encouraging the conservation of biodiversity through the economic development of the host country.[66] The National Science Foundation (NSF), the United States Agency for International Development (USAID), the National Institute of Mental Health, and a number of the National Institutes of Health (NIH) provide the program funding.[4] Current collection sites include a number of countries in Central and South America, as well as nations in Africa. The Kingston laboratory in the Department of Chemistry at Virginia Tech is one member of an ICBG project that includes associates at the Missouri Botanical Garden (MGB), Conservation International (CI), Bedrijf Geneesmiddelen Voorziening in the Republic of Suriname (BGVS), and Bristol-Myers Squibb Pharmaceutical Research Institute (BMS).

One serious concern that the ICBG program attempts to address is a problem that exists throughout the natural products drug discovery industry: deforestation and the loss of biodiversity. Areas of dense plant growth need to be preserved to maintain an environment where potential new medicines may develop and be obtained. The rate of deforestation during the 1980s has been estimated at 170,000 square kilometers per year.[67] Worldwide, the annual loss of tropical rainforest is equivalent to an area the size of the state of Florida.[66] The indigenous peoples and biological resources that inhabit the land cannot be replaced. Biochemical prospecting is not just a scientific process anymore; it is closely tied to economic policy and political rights.

The governments of the countries that contain these ecosystems often undervalue their own land. While industrialized nations that manufacture drugs are interested in preserving rainforests and other similar ecosystems, poorer countries that own the land

but will never have the opportunity to use the medicines often do not share that goal. Each nation is a recognized owner of the plants from which its samples are collected, so it has a right to be financially compensated for the removal of samples that are developed into moneymaking medicines. Sharing revenues of a marketed drug with the country from which it was located through a contractual agreement is an incentive for a local government to preserve its sources of biodiversity.

There are four main steps involved in the ICBG work leading up to and including the chemistry studies in Blacksburg. First, a library of plant samples is harvested and catalogued in the country of their origin. This includes the acquisition of leaves, stems, roots, bark, seeds, or even whole plants. Essentially, the goal at this stage is to collect as many potential sources of drugs as possible. Second, these samples are extracted and sent overseas to the chemistry laboratory. There, they are screened for initial activity using a mammalian cancer cell assay that indicates which plants show the most promise and require further examination. "Hits" are subsequently fractionated with the use of bioassays that guide the process. Bioassays indicate which fractions contain the anticancer activity so that the chemist knows which leads to pursue. Finally, if a pure active compound can be isolated from an extract, its structure is determined with the aid of a number of elucidation techniques (nuclear magnetic resonance and mass spectroscopy, for example) that are common to most branches of chemistry research. The identified compound can then undergo further development by a pharmaceutical company, possibly becoming a new drug.

References

(1) Swerdlow, J. L. *Nature's Medicine: Plants that Heal*; National Geographic Society: Washington, D.C., 2000.

(2) Newman, D. J.; Cragg, G. M.; Snader, K. M. The influence of natural products upon drug discovery. *Nat. Prod. Rep.* **2000,** *17*, 215-234.

(3) da Rocha, A. B.; Lopes, R. M.; Schwartsmann, G. Natural products in anticancer therapy. *Curr. Opin. Pharmacol.* **2001,** *1*, 364-369.

(4) Cragg, G. M.; Boyd, M. R.; Christini, M. A.; Kneller, R.; Mays, T. D.; Mazan, K. D.; Newman, D. J.; Sausville, E. A. Screening of natural products of plant, microbial, and

marine origin: The NCI experience. In *Phytochemical Diversity: A Source of New Industrial Products*; Wrigley, S.; Hayes, M.; Thomas, R.; Chrystal, E., Eds.; The Royal Society of Chemistry: Cambridge, 1997.

(5) Vlietinck, A. J.; Apers, S. Biological screening methods in the search for pharmacologically active natural products. In *Bioactive Compounds from Natural Sources: Isolation, Characterization, and Biological Properties*; Tringali, C., Ed.; Taylor & Francis: New York, 2001.

(6) Cragg, G. M.; Newman, D. J.; Snader, K. M. Natural products in drug discovery and development. *J. Nat. Prod.* **1997,** *60*, 52-60.

(7) Davis, J. *Scrip Review 1992*; PJB Publications: Richmond, 1993.

(8) Grabley, S.; Thiericke, R. The impact of natural products on drug discovery. In *Drug Discovery from Nature*; Grabley, S.; Thiericke, R., Eds.; Springer: Berlin/Heidelberg, 1999.

(9) Duke, J. A.; Bogenschutz-Godwin, M. J. The synergy principle at work in plants, pathogens, insects, herbivores, and humans. In *Natural Products from Plants*; CRC Press: Boca Raton, FL, 1999.

(10) Dewick, P. M. *Medicinal Natural Products: A Biosynthetic Approach*; John Wiley & Sons Ltd.: Chichester, 1997.

(11) Brogger Christensen, S.; Kharazmi, A. Antimalarial natural products. In *Compounds from Natural Sources: Isolation, Characterization, and Biological Properties*; Tringali, C., Ed.; Taylor & Francis: New York, 2001.

(12) Casteel, D. A. Antimalarial agents. In *Burger's Medicinal Chemistry and Drug Discovery 5*; John Wiley & Sons: New York, 1997.

(13) Grafe, U. Secondary metabolites: From past to present. In *Drug Discovery from Nature*; Grabley, S.; Thiericke, R., Eds.; Springer: Berlin/Heidelberg, 1999.

(14) Dreyfuss, M.; Haerri, E.; Hofmann, H.; Kobel, H.; Pache, W.; Tscherter, H. Cyclosporin A and C. New metabolites from *Trichoderma polysporum (Link ex. Pers.) Rifai*. *Eur. J. App. Microbiol.* **1976,** *3*, 125-133.

(15) Angell, Y. M.; Thomas, T. L.; Flentke, G. R.; Rich, D. H. Solid-phase synthesis of cyclosporin peptides. *J. Am. Chem. Soc.* **1995,** *117*, 7279-7280.

(16) Cordell, G. A.; Kinghorn, A. D.; Pezzuto, J. M. Separation, structure elucidation, and bioassay of cytotoxic natural products. In *Bioactive Natural Products: Detection, Isolation, and Structure Determination*; Colegate, S. M.; Molyneux, R. J., Eds.; CRC Press: Boca Raton, FL, 1993.

(17) Sattler, I.; Grabley, S.; Thiericke, R. Structure modification via biological derivitization methods. In *Drug Discovery from Nature*; Grabley, S.; Thiericke, R., Eds.; Springer: Berlin/Heidelberg, 1999.

(18) Schweitzer, J.; Handley, F. G.; Edwards, J.; Harris, W. F.; Grever, M. R.; Schepartz, S. A.; Cragg, G.; Snader, K.; Bhat, A. Summary of the workshop on drug development, biological diversity, and economic growth. *J. Nat. Cancer Ins.* **1991,** *83*, 1294-1298.

(19) Perera, P.; Ringbom, T.; Huss, U.; Vasange, M.; Bohlin, L. Search for natural products which affect cyclooxygenase-2. In *Bioactive Compounds from Natural Sources: Isolation, Characterization, and Biological Properties*; Tringali, C., Ed.; Taylor & Francis: New York, 2001.

(20) De Rosa, M.; Giordano, S.; Scettri, A.; Sodano, G.; Soriente, A.; Pastor, P. G.; Alcaraz, M. J.; Paya, M. Synthesis and comparison of the antiinflammatory activity of manoalide and cacospongionolide B analogues. *J. Med. Chem.* **1998,** *41*, 3232-3238.

(21) Pomponi, A. S. The bioprocess-technological potential of the sea. *J. Biotechnol.* **1999,** *70*, 5-13.

(22) Farnsworth, N. R.; Akerele, O.; Bingel, A. S.; Soejarto, D. D.; Guo, Z. Medicinal plants in therapy. *Bull. World Health Organ.* **1985,** *63*, 965-981.

(23) Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. Plant antitumor agents. I. The isolation and structure elucidation of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. *J. Am. Chem. Soc.* **1966,** *88*, 3888-3890.

(24) Appendino, G. Taxol (paclitaxel): historical and ecological aspects. *Fitoterapia* **1993,** *64*, 5-25.

(25) Suffness, M.; Wall, M. E. Discovery and development of taxol. In *Taxol: Science and Applications*; CRC Press: Boca Raton, FL, 1995.

(26) Horwitz, S. B.; Parness, J.; Schiff, P. B.; Manfredi, J. J. Taxol: a new probe for studying the structure and function of microtubules. *Cold Spring Harbor Symposium on Quantitative Biology* **1982,** *46*, 219-226.

(27) Goodman, J.; Walsh, V. *The Story of Taxol: Nature and Politics in the Pursuit of an Anti-cancer Drug*; Cambridge University Press: USA, 2001.

(28) Kingston, D. G. I. Taxol, a molecule for all seasons. *Chem. Commun.* **2001,** *10*, 867-880.

(29) Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *J. Am. Chem. Soc.* **1971,** *93*, 2325-2327.

(30) Jaziri, M.; Vanhaelen, M. Bioactive taxoid production from natural sources. In *Bioactive Compounds from Natural Sources: Isolation, Characterization, and Biological Properties*; Tringali, C., Ed.; Taylor & Francis: New York, 2001.

(31) Jagtap, P. G.; Kingston, D. G. I. A facile N-debenzoylation of paclitaxel: Conversion of paclitaxel to docetaxel. *Tetrahedron Lett.* **1999,** *40*, 189-192.

(32) Gunatilaka, A. A. L.; Ramdayal, F. D.; Sarragiotto, M. H.; Kingston, D. G. I.; Sackett, D. L.; Hamel, E. Synthesis and biological evaluation of novel paclitaxel (Taxol) D-ring modified analogues. *J. Org. Chem.* **1999,** *64*, 2694-2703.

(33) Harry L. Brielmann, J. *Phytochemicals: The chemical components of plants*; CRC Press: Boca Raton, FL, 1999.

(34) Foley, M.; Tilley, L. Quinoline antimalarials: Mechanisms of action and resistance and prospects for new agents. *Pharmacol. Ther.* **1998,** *79*, 55-87.

(35) Nontprasert, A.; Pukrittayakamee, S.; Kyle, D. E.; Vanijanonta, S.; White, N. J. Antimalarial activity and interactions between quinine, dihydroquinine and 3-hydroxyquinine against Plasmodium falciparum in vitro. *Trans. R. Soc. Trop. Med. Hyg.* **1996,** *90*, 553-555.

(36) Garrett, L. The revenge of the germs or just keep inventing new drugs. In *The Coming Plague, Newly Emerging Diseases in a World Out of Balance*; Farrar, Straus, and Giroux: New York, 1994.

(37) Zhou, B.-N.; Bahler, B. D.; Hofmann, G. A.; Mattern, M. R.; Johnson, R. K.; Kingston, D. G. I. Phenylethanoid glycosides from *Digitalis purpurea* and *Penstemon linarioides* with PKCa-inhibitory activity. *J. Nat. Prod.* **1998,** *61*, 1410-1412.

(38) Tang, W.; Eisenbrand, G. *Chinese Drugs of Plant Origin*; Springer: Berlin/Heidelberg, 1992.

(39) Ghisalberti, E. L. Detection and isolation of bioactive natural products. In *Bioactive Natural Products: Detection, Isolation, and Structure Determination*; Colegate, S. M.; Molyneux, R. J., Eds.; CRC Press: Boca Raton, FL, 1993.

(40) Giovanella, B. C.; Stehlin, J. S.; Wall, M. E.; Wani, M. C.; Nicholas, A. W.; Liu, L. F.; Silber, R.; Potmesil, M. DNA topoisomerase I-targeted chemotherapy of human colon cancer in xenografts. *Science* **1989,** *246*, 1046-1048.

(41) Creemers, G. J.; Bolis, G.; Gore, M.; Scarfone, G.; Lacave, A. J.; Guastalla, J. P.; Despax, R.; Favalli, G.; Kreinberg, R.; Van Belle, S. Topotecan, an active drug in the second-line treatment of epithelial ovarian cancer. *J. Clin. Oncol.* **1996,** *14*, 3056-3061.

(42) Bertino, J. R. Irinotecan for colorectal cancer. *Semin. Oncol.* **1997,** *24*, S18-S23.

(43) Kelland, L. R. Flavopiridol, the first cyclin-dependent kinase inhibitor to enter the clinic: Current status. *Expert Opin. Invest. Drugs* **2000,** *9*, 2309-2911.

(44) Powell, R. G.; Weisleder, D.; C. R. Smith, J.; Rohwedder, W. K. Structures of harringtonine, isoharringtonine, and homoharringtonine. *Tetrahedron Lett.* **1970,** *11*, 815-818.

(45) Warber, S. Modes of action at target sites. In *Natural Products from Plants*; CRC Press: Boca Raton, FL, 1999.

(46) Zhou, D. C.; Zittoun, R.; Marie, J. P. Homoharringtonine: an effective new natural product in cancer chemotherapy. *Bull. Cancer* **1995,** *82*, 987-995.

(47) Kuo, Y.-H.; King, M.-L. Antitumor drugs from the secondary metabolites of higher plants. In *Bioactive Compounds from Natural Sources: Isolation, Characterization, and Biological Properties*; Tringali, C., Ed.; Taylor & Francis: New York, 2001.

(48) Williams, S. D.; Birch, R.; Einhorn, L. H.; Irwin, L.; Greco, F. A.; Loehrer, P. J. Treatment of disseminated germ-cell tumors with cisplatin, bleomycin, and either vinblastine or etopside. *N. Engl. J. Med.* **1987,** *316*, 1435-1440.

(49) Liu, L. F. DNA topoisomerase poisons as antitumor drugs. *Annu. Rev. Biochem.* **1989,** *58*, 351-375.

(50) Noble, R. L. The discovery of the vince alkaloids - chemotherapeutic agents against cancer. *Biochem. Cell Bio.* **1990,** *68*, 1344-1351.

(51) Salmon, S. E.; Sartorelli, A. C. Cancer chemotherapy. In *Basic and Clinical Pharmacology*; Appleton and Lange: Norwalk, CT, 1989.

(52) V. T. DeVita, J.; Serpick, A. A.; Carbone, P. O. Combination chemotherapy in the treatment of advanced Hodgkin's disease. *Ann. Intern. Med.* **1970,** *73*, 881-895.

(53) Pratt, W. B.; Ruddon, R. W.; Ensminger, W. D.; Maybaum, J. *The Anticancer Drugs*. Second ed.; Oxford Unviersity Press: New York, 1994.

(54) Yu, B.-W.; Meng, L.-H.; Chen, J.-Y.; Zhou, T.-X.; Cheng, K.-F.; Ding, J.; Qin, G.-W. Cytotoxic oxoisoaporphine alkaloids from *Menispermum dauricum*. *J. Nat. Prod.* **2001,** *64*, 968-970.

(55) Hsieh, T.-J.; Chang, F.-R.; Chia, Y.-C.; Chen, C.-Y.; Chiu, H.-F.; Wu, Y.-C. Cytotoxic constituents of the fruits of *Cananga odorata*. *J. Nat. Prod.* **2001,** *64*, 616-619.

(56) Costa, S. M. O.; Lemos, T. L. G.; Pessoa, O. D. L.; Pessoa, C.; Montenegro, R. C.; Braz-Filho, R. Chemical constituents from *Lippia sidoides* and cytotoxic activity. *J. Nat. Prod.* **2001,** *64*, 792-795.

(57) Syu, W.-J.; Don, M.-J.; Lee, G.-H.; Sun, C.-M. Cytotoxic and novel compounds from *Solanum indicum*. *J. Nat. Prod.* **2001,** *64*, 1232-1233.

(58) Lin, L.-C.; Chou, C.-J.; Kuo, Y.-C. Cytotoxic principles from *Ventilago leiocarpa*. *J. Nat. Prod.* **2001,** *64*, 674-676.

(59) Peebles, K. A.; Baker, R. K.; Kurz, E. U.; Schneider, B. J.; Kroll, D. J. Catalytic inhibition of human DNA topoisomerase IIa by hypericin, a napthodianthrone from St. John's wort (*Hypericum perforatum*). *Biochem. Pharmacol.* **2001,** *62*, 1059-1070.

(60) Cheng, H.-H.; Wang, H.-K.; Ito, J.; Bastow, K. F. T., Y.; Nakanishi, Y.; Xu, Z.; Luo, T.-Y.; Lee, K.-H. Cytotoxic pheophorbide-related compounds from Clerodendrum calamitosum and C. cyrtophyllum. *J. Nat. Prod.* **2001,** *64*, 915-919.

(61) Kim, D. H.; Ma, E. S.; Suk, K. D.; Son, J. K.; Lee, J. S.; Woo, M. H. Annomolin and annocherimolin, new cytotoxic annonaceous acetogenins from *Annona cherimolia* seeds. *J. Nat. Prod.* **2001,** *64*, 502-506.

(62) Ito, A.; Cui, B.; Chavez, D.; Chai, H.-B.; Shin, Y. G.; Kawanishi, K.; Kardono, L. B. S.; Riswan, S.; Farnsworth, N. R.; Cordell, G. A.; Pezzuto, J. M.; Kinghorn, A. D. Cytotoxic polyacetylenes from the twigs of *Ochanostachys amentacea*. *J. Nat. Prod.* **2001,** *64*.

(63) Guilet, D.; Helesbeux, J.-J.; Seraphin, D.; Sevenet, T.; Richomme, P.; Bruneton, J. Novel cytotoxic 4-phenylfuranocoumarins from *Calophyllum dispar*. *J. Nat. Prod.* **2001,** *64*, 563-568.

(64) Gunatilaka, A. A. L.; Berger, J. M.; Evans, R.; Miller, J. S.; Wisse, J. H.; Neddermann, K. M.; Bursurker, I.; Kingston, D. G. I. Isolation, Synthesis, and Structure-Activity Relationships of Bioactive Benzoquinones from *Miconia lepidota* from the Suriname Rainforest. *J. Nat. Prod.* **2001,** *64*, 2-5.

(65) Farnsworth, N. R.; Soejarto, D. D. Potential consequences of plant extinction in the U.S. on the availability of prescription drugs. *Econ. Bot.* **1985,** *39*, 231-240.

(66) Kingston, D. G. I.; Gunatilaka, A. A. L.; Zhou, B.-N.; Abdel-Kader, M.; Berger, J.; Van der Werff, H.; Evans, R.; Famolare, L.; Mittermeier, R.; Malone, S.; Wisse, J. H. Biodiversity conservation and drug development in Suriname. In *Chemistry, Biological and Pharmacological Properties of Medicinal Plants From the Americas*; Hostettmann, K.; Gupta, M. P.; Marston, A., Eds.; Harwood Academic Publishers: 1999.

(67) Whitmore, T. C.; Sayer, J. A. Deforestation and species extinction in tropical moist forests. In *Tropical Deforestation and Species Extincion*; Whitmore, T. C.; Sayer, J. A., Eds.; Chapman and Hall: London, 1992.

II. TAMBOURANOLIDE, A NEW HYDROXYBUTANOLIDE ISOLATED FROM A *TAMBOURISSA* SPECIES (MONIMIACEAE) FROM MADAGASCAR

2.1 Introduction

Extracts from a species of *Tambourissa* from Madagascar displayed moderate to weak cytotoxicity in the A2780 human ovarian cancer cell line assay. The root extract appeared to be the most bioactive, and it was therefore fractionated and examined for potential anticancer compounds. From this extract, a new hydroxybutanolide was isolated and characterized, using one- and two-dimensional NMR techniques and high-resolution mass spectrometry. The novel compound, tambouranolide (**2.1**), was both the major component and the only compound with significant bioactivity.

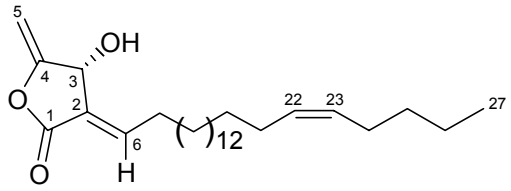
2.1.1 *Previous Investigations of Tambourissa Species*

The *Tambourissa* genus is one of the largest members of the Monimiaceae family of flowering plants, which is typically found in tropical and subtropical areas of the southern hemisphere.[1] Members of the Monimiaceae family are especially common in the vicinity of the Indian Ocean and the South Pacific. More than forty species of *Tambourissa* are known to grow exclusively on the islands of the southwest Indian Ocean, including Madagascar and the Mascarene Islands.[2] Only two published phytochemical investigations, which reveal the presence of a number of volatile terpene compounds (limonene, bergamotene, curcumene, etc.) from *T. leptophylla*[3] and other miscellaneous constituents from *T. quadrifidia*,[4] have been performed on this genus. Those plants were obtained from the islands of the Comores, Réunion and Mauritius. The crude petrol ether extract of *T. leptophylla* was shown to be slightly antifungal, although no individual constituents were specifically examined for biological activity.

2.1.2 *Chemical Investigation of a Tambourissa Species*

Through an ongoing investigation of bioactive compounds from plant collections in the Madagascar rainforest, as part of an International Cooperative Biodiversity Group, the ethanol extract (MG 2090) of an unknown species of *Tambourissa* was investigated by bioassay-guided fractionation. The dry, crude root material yielded an IC_{50} of 22

μg/mL in the A2780 human ovarian cancer cell line bioassay. Fractionation (liquid-liquid partitioning and solid phase extraction) afforded the isolation of a new hydroxybutanolide, tambouranolide (**2.1**, Figure 2-1), whose structure was deduced from ^{1}H NMR, ^{13}C NMR, and MS data. This chapter reports the isolation and characterization of this new compound.



2.1 Tambouranolide

Figure 2.1. Tambouranolide from a *Tambourissa* Species.

2.1.3 *Previous Investigations of Hydroxybutanolides*

Tambouranolide has not been previously isolated, but the compound does belong to a class of γ-lactones called hydroxybutanolides. A hydroxy group at the 3-position of the lactone ring and a long hydrocarbon chain emanating from the 2-position characterize these compounds. Either a methylene or a methyl group may also be present at the 4-position of the ring. Many of the hydroxybutanolides have been isolated from various species of the *Lindera* genus, which is a member of the Lauraceae family of plants commonly found in Japan, but related structures have also been found to exist in other genera of the same family.

The first compound of this type, obtusilactone (**2.2**), was isolated in 1975 from the leaves of *Lindera obtusiloba*,[5] and an additional two obtusilactones were obtained from the same plant later that year.[6] The authors reported cytoxicity associated with these compounds, although no specific data was provided. A total of thirteen new hydroxybutanolides, named the linderanolides, were subsequently obtained from the berries of *Lindera benzoin*[7] and the leaves of *Lindera glauca*.[8] Linderanolide (**2.3**), isolinderanolide (**2.4**) and isolinderenolide (**2.5**) from *L. benzoin* were found to exhibit brine shrimp lethality. Additional study of *L. glauca* also yielded a series of methoxybutanolides.[9] Other related compounds isolated from the Lauraceae family

include the mahubanolides, mahubenolides and mahubynolides from *Licaria mahuba*[10] and *Clinostemon mahuba*,[11] lancifolides from *Actinodaphne lancifolia*,[12] and a butanolide from *Machilus thunbergii*.[13]

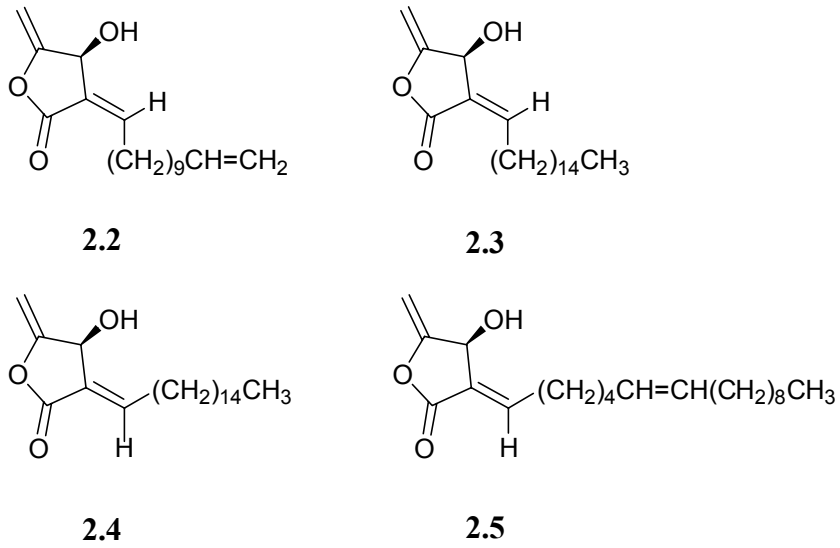


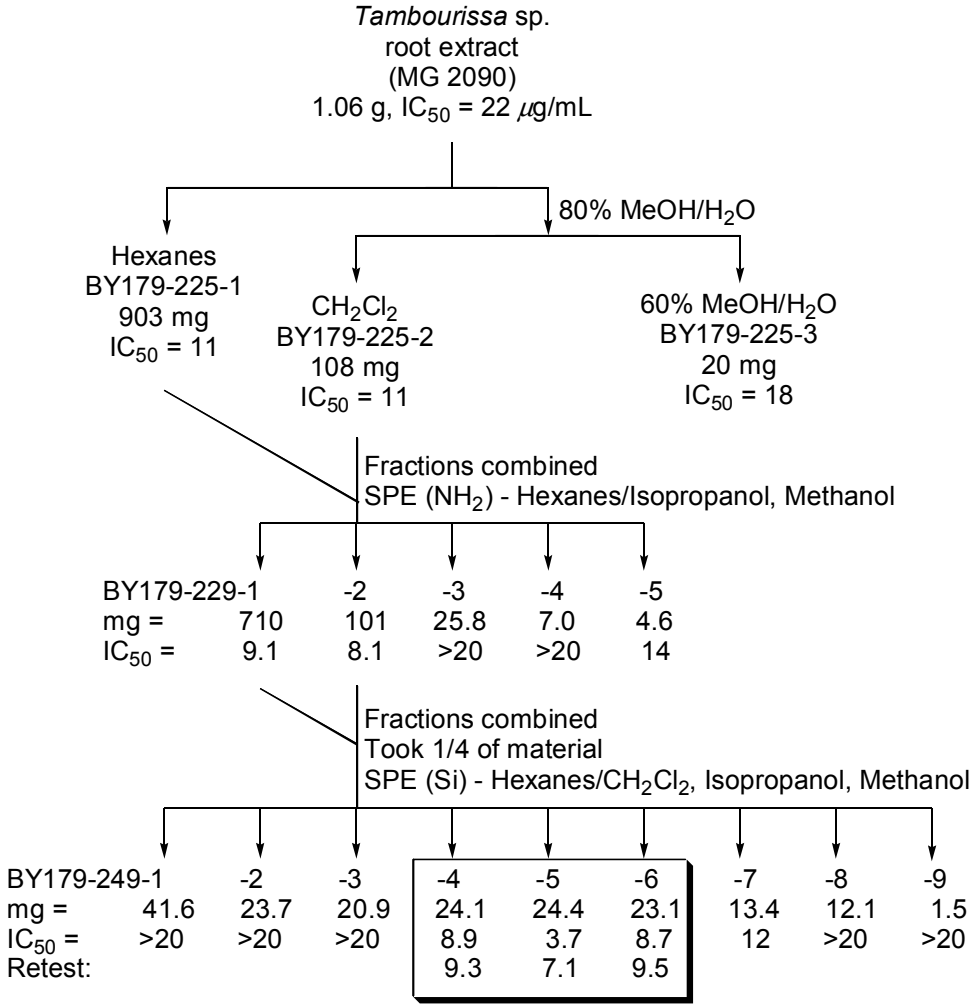
Figure 2.2. Hydroxybutanolides from *Lindera obtusiloba* and *Lindera benzoin*.

2.2 Results and Discussion

2.2.1 *Isolation of a New Hydroxybutanolide from a Tambourissa Species*

Tambouranolide (**2.1**) was isolated as indicated in Scheme 2.1. From the dry root extract (MG 2090), 1.06 g of crude material was taken for liquid-liquid partitioning. An initial partition between hexanes and 80% methanol in water was established. The aqueous layer was diluted to yield a 60% MeOH/H_2O solution and then further partitioned with CH_2Cl_2. All three fractions were subjected to solvent removal by rotary evaporation. Testing of the samples in the A2780 cytotoxicity assay indicated that the hexanes and CH_2Cl_2 fractions were the most active; these were also the samples that contained the majority of the dry weight. The two non-polar fractions were then combined and subjected to fractionation using an NH_2-bonded solid phase extraction (SPE) cartridge. Four fractions were obtained through elution with different percentages of a hexanes/isopropanol mixture. The cartridge was flushed with methanol to ensure the removal of all material. Again, the two most non-polar fractions held the majority of the dry weight and demonstrated the lowest IC_{50} value in the bioassay. The two fractions

were combined and one-fourth of the available material was subjected to fractionation using a silica SPE cartridge. Seven fractions were obtained through elution with different percentages of a hexanes/CH_2Cl_2 mixture, and the cartridge was subsequently flushed with separate volumes of both isopropanol and methanol to obtain two additional fractions. Fractions four, five and six contained the same pure compound **2.1**, totaling nearly 72 mg of product.



Scheme 2.1. Fractionation of a *Tambourissa* Species (Monimiaceae).

2.2.2 *Characterization of a New Hydroxybutanolide from a Tambourissa Species*

2.2.2.1 Structure of Tambouranolide (**2.1**)

Tambouranolide (**2.1**) was obtained as a pale yellow solid. The positive HRFABMS of **2.1** indicated a molecular ion at *m/z* = 419.3486, in agreement with the molecular formula of $C_{27}H_{46}O_3$. A broad doublet at δ_H = 5.25 (H-3), doublets of doublets at δ_H = 4.71 (H-5a) and δ_H = 4.94 (H-5b), and a triplet of doublets at δ_H = 7.07 (H-6) in the ^{1}H-NMR spectrum suggested that the compound was an α,β-unsaturated-γ-lactone with a hydroxyl group (hydroxybutanolide). Signals at δ_C = 166.8 (C-1), 127.4 (C-2), 66.5 (C-3), 157.8 (C-4), 91.4 (C-5), and 150.3 (C-6) in the ^{13}C-NMR spectrum further supported this notion. For the remaining 21 carbons, eight resolved peaks (including two at δ_C = 129.9 and 130.0) and numerous overlapping signals in the region 29.4 – 30.0 ppm were observed. The observations indicated that the compound contained a side chain with several methylenes and one double bond. HMBC data were used to establish the position of the double bond at Δ^{22}, since both H-24 and H-27 showed correlations with C-25 and C-26 and H-24 further correlated with C-22 and C-23, as shown in Figure 2-3.

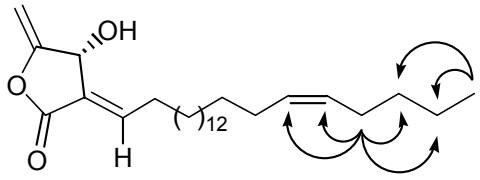


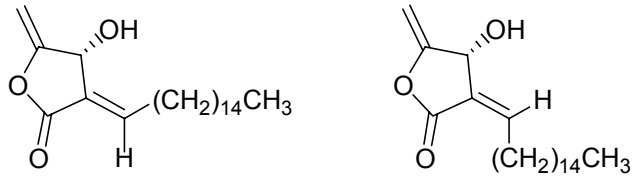
Figure 2.3. Selected HMBC Correlations of **2.1**.

2.2.3 *Determination of the Absolute Configuration of a New Hydroxybutanolide from a Tambourissa Species*

2.2.3.1 Literature Confirmation of the Absolute Configuration of Tambouranolide (**2.1**)

The *Z* stereochemistry of the disubstituted double bond was unequivocally confirmed from the ^{13}C-NMR data of *Z* and *E* isomers of other compounds with long chains.[14,15] The allylic (δ_C = 27.0 and 27.3) and olefinic (δ_C = 129.9 and 130.0) carbon signals of **2.1** were coincident with those typical of *Z* isomers (allylic carbons: δ_C = 27.2, olefinic carbons: δ_C = 129.8 and 129.9), but not those of *E* isomers (allylic carbons: δ_C = 32.6, olefinic carbons: δ_C = 130.3), due to the δ effect. The spectral data of **2.1** resembled

those reported for linderanolides and isolinderanolides from *Lindera benzoin*[7] and *Lindera glauca*,[8] and the ^{13}C-NMR data of these compounds were examined to assign the stereochemistry of the trisubstituted double bond. The allylic (δ_C = 66.5) and olefinic (δ_C = 150.3) carbon signals of **2.1** were coincident with those of isolinderanolide (**2.4**) and isolinderanolide E (**2.6**) (allylic carbons: δ_C = 66.3 and 66.5, olefinic carbons: δ_C = 150.2 and 150.3), which are *E* isomers, but not those of linderanolide (**2.3**) and linderanolide E (**2.7**) (allylic carbons: δ_C = 68.9, olefinic carbons: δ_C = 151.4), which are *Z* isomers. The carbonyl also can be seen to have a deshielding effect on H-6 in the ^{1}H NMR spectrum. Compound **2.1** ($[\alpha]_D$ +20°) was also dextrorotatory, in analogy with linderanolide E and isolinderanolide E, which have (3*R*)-hydroxyl groups, rather than levorotatory in analogy with linderanolide and isolinderanolide, which have (3*S*)-hydroxyl groups. Hence, the structure of **2.1** was assigned as (3*R*,2*E*)-3-hydroxy-4-methylene-2-((17*Z*)-17-docosenylidene)butanolide, as shown. The absolute stereochemical configurations of the first hydroxybutanolides, upon which this assignment was based, were determined through a combination of catalytic hydrogenation experiments and optical rotation measurements.



2.5 **2.6**

Figure 2.4. Hydroxybutanolides from *Lindera glauca*.

2.2.4 *Biological Evaluation of a New Hydroxybutanolide*

Compound **2.1** was tested in the A2780 assay, and it was moderately active with an IC_{50} value of 8 μg/mL, using actinomycin D as a positive control (IC_{50} = 1-3 ng/mL).

2.3 Experimental Section.

General Experimental Procedures. Solid phase extraction was performed with Supelco Discovery DSC-NH_2 and DSC-Si tubes. Optical rotation data was obtained on a

PerkinElmer 241 polarimeter. Mass spectra were obtained on a JEOL JMS-HX-110 instrument. NMR spectra were obtained on either a JEOL Eclipse (at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) or Varian Inova (at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) spectrometer. Chemical shifts are given in δ (ppm) and coupling constants (J) are reported in Hz.

Plant Material. The roots of a *Tambourissa* species (Monimiaceae) were collected by Fidisoa Ratovoson on July 26, 2003. The specimens were collected in the forest of Ampitsahambe, north-west of the village of Androrangabe, around the Natural Reserve of Zahamena in the province of Toamasina, Madagascar. Duplicate voucher specimens have been deposited at the Centre National d'Application des Recherches Pharmaceutiques (CNARP) and the Direction des Recherches Forestieres et Piscicoles Herbarium (TEF) in Antananarivo, Madagascar; the Missouri Botanical Garden in St. Louis, Missouri (MO); and the Museum National d'Histoire Naturelle in Paris, France (P).

Extract Preparation. The roots and bark of a *Tambourissa* species were dried, ground and extracted with ethanol in Madagascar. This yielded extracts labeled MG 2090 (163.5 g) and MG 2091 (47.5 g), respectively.

Cytotoxicity Bioassay.[16,17] The A2780 human ovarian cancer cell line was used to run an *in-vitro* antitumor cytotoxicity assay. First, 200 µL of RPMI media (10% fetal bovine serum) were added to all wells in column 12 of a 96 well tissue culture plate. Also, 20 µL of RPMI media were added to all wells in column 11. Wells A-H in columns 1-11 were then "seeded" with 180 µL of 2.7×10^{5} A2780 DDP-S (Platinol-Sensitive) cells per mL. Plates were incubated for three hours in 5% CO_2 at 37° C to allow cells to begin growing and adhere to well bottoms. Compounds (or fractions) to be tested were prepared and submitted in 50% DMSO / 50% water, at a concentration of 1000 µg/mL. After incubation, 20 µL of the compound sample were added to 80 wells in a 1:10 dilution. Column 11 was left for positive (wells A-D) and negative (wells E-H) control, and column 12 was left for blank control. Actinomycin D served as the positive control

and was run at four dilutions with an IC_{50} ~ 1-3 ng/mL. Plates were incubated for 48 hours in 5% CO_2 at 37° C. Media was removed from the plates and replaced with 200 µL of fresh media and 10% fetal bovine serum containing 1% Alamar Blue solution. The plates were incubated for an additional four hours in 5% CO_2 at 37° C. Finally, the plates were read on a Cytofluor at an emission of 530 nm and an excitation of 590 nm, with a gain of 45, and the IC_{50} values were calculated.

Bioassay-guided Fractionation and Isolation of a New Hydroxybutanolide. The crude bioactive extract MG 2090 (IC_{50} = 22 μg/mL, 1.06 g) was partitioned between hexanes (200 mL) and MeOH-H_2O (4:1, 2 x 100 mL). Water was added to the MeOH-H_2O fraction to yield a MeOH-H_2O solution (3:2) that was subsequently partitioned with CH_2Cl_2. Evaporation of the organic solvents yielded bioactive (IC_{50} = 11 μg/mL) fractions of 903 mg (hexanes) and 106 mg (CH_2Cl_2). The two fractions were combined and subjected to further fractionation through a Discovery DSC-NH_2 solid phase extraction (SPE) cartridge with a mixture of hexanes and isopropanol. The first (hexanes, 710 mg, IC_{50} = 9 μg/mL) and second (hexanes-isopropanol, 19:1, 101 mg, IC_{50} = 8 μg/mL) fractions displayed the greatest cytotoxicity. These two fractions were combined, and one-fourth of the material was subjected to further fractionation through a Discovery DSC-Si SPE cartridge with a mixture of hexanes and CH_2Cl_2. The fourth (hexanes-CH_2Cl_2, 7:3, 24 mg), fifth (hexanes-CH_2Cl_2, 3:2, 24 mg), and sixth (hexanes-CH_2Cl_2, 1:1, 23 mg) fractions all appeared to contain the same bioactive compound **2.1**. Analysis by ^{1}H NMR indicated a high level of purity for all three fractions.

Tambouranolide (2.1): yellow amorphous solid; $[\alpha]_D$ +20^o (*c* 0.11, $CHCl_3$); ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 2-1 and Appendix; HRFABMS *m/z* 419.3486 $[M+H]^+$ (calcd for $C_{27}H_{47}O_3$, 419.3525).

Table 2.1. NMR Spectral Data for Tambouranolide in $CDCl_3$.

position	^1H (*J*, Hz)	^{13}C
1		166.8
2		127.4
3	5.25 *br s*	66.5
4		157.8
5a	4.71 *dd* (2.8, 1.4)	91.4
5b	4.94 *dd* (2.8, 1.7)	
6	7.07 *td* (7.9, 2.2	150.3
7	2.46 *m*	~29.7
8	1.51 *qui* (7.9)	28.4
9-20	1.24 *br s*	29.4-30.0
21	2.00 *m*	27.0
22	5.34 *m*	129.9
23	5.34 *m*	130.0
24	2.00 *m*	27.3
25	1.30 *m*	32.1
26	1.30 *m*	22.4
27	0.88 *br t* (7.2)	14.1
3-OH	2.24 *br s*	

References

(1) Philipson, W. R. A Classification of the Monimiaceae. *Nord. J. Bot.* **1987,** *7*, 25-29.

(2) Lorence, D. H. A Monograph of the Monimiaceae (Laurales) in the Malagasy Region (Southwest Indian Ocean). *Ann. Mo. Bot. Gard.* **1985,** *72*, 1-165.

(3) Gallori, S.; Bilia, A. R.; Mulinacci, N.; Bicchi, C.; Rubiolo, P.; Vincieri, F. F. Identification of Volatile Consituents of *Tambourissa leptophylla*. *Planta Med.* **2001,** *67*, 290-292.

(4) Forgacs, P.; Buffard, G.; Desconclois, J. F.; Jehanno, A.; Provost, J.; Tiberghien, R.; Touche, A. Phytochemical Studies and Biological Activities of Plants Endemic to the Islands of Reunion and Mauritius. *Plantes Medicinales et Phytotherapie* **1981,** *15*, 80-91.

(5) Niwa, M.; Iguchi, M.; Yasamura, S. The Isolation and Structure of Obtusilactone. *Tetrahedron Lett.* **1975,** *19*, 1539-1542.

(6) Niwa, M.; Iguchi, M.; Yamamura, S. The Structures of C_{17}-Obtusilactone Dimer and Two C_{21}-Obtusilactones. *Tetrahedron Lett.* **1975,** *49*, 4395-4398.

(7) Anderson, J. E.; Ma, W.; Smith, D. L.; Chang, C.-J.; McLaughlin, J. L. Biologically Active γ-Lactones and Methylketoalkenes from *Lindera benzoin*. *J. Nat. Prod.* **1992,** *55*, 71-83.

(8) Seki, K.; Sasaki, T.; Wano, S.; Haga, K.; Kaneko, R. Linderanolides and Isolinderanolides, Ten Butanolides from *Lindera glauca*. *Phytochemistry* **1995,** *40*, 1175-1181.

(9) Seki, K.; Sasaki, T.; Haga, K.; Kaneko, R. Two Methoxybutanolides from *Lindera glauca*. *Phytochemistry* **1994,** *36*, 949-951.

(10) V., J. C. M.; Yoshida, M.; Gottlieb, O. R. Six Groups of ω-Ethyl-, ω-Ethenyl- and ω-Ethynyl-α-Alkylidene-γ-Lactones. *Tetrahedron Lett.* **1979,** *12*, 1021-1024.

(11) V., J. C. M.; Yoshida, M.; Gottlieb, O. R. ω-Ethyl-, ω-Ethenyl- and ω-Ethynyl-α-Alkylidene-γ-Lactones from *Clinostemon mahuba*. *Phytochemistry* **1981,** *20*, 459-464.

(12) Tanaka, H.; Nakamura, T.; Ichino, K.; Ito, K. Two Lactonic Compounds, Lancifolide and Isolancifolide, from *Actinodaphne lancifolia*. *Phytochemistry* **1989,** *28*, 626-628.

(13) Karikome, H.; Mimaki, Y.; Sashida, Y. A Butanolide and Phenolics from *Machilus thunbergii*. *Phytochemistry* **1991,** *30*, 315-319.

(14) Sargent, M. V.; Wangchareontrakul, S.; Jefferson, A. The Synthesis and Identification of Alkenyl and Alkadienyl Catechols of Burmese Lac. *J. Chem. Soc. Perkin. 1.* **1989,** *1*, 431-439.

(15) Cao, S.; Schilling, J. K.; Randrianasolo, A.; Andriantsiferana, R.; Rasamison, V. E.; Kingston, D. G. I. New Cytotoxic Alkyl Phloroglucinols from *Protorhus thouvenotii*. *Planta Med.* **2004,** *70*, 683-685.

(16) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. *J. Natl. Cancer Inst.* **1990,** *82*, 1107-1112.

(17) Louie, K. G.; Behrens, B. C.; Kinsella, T. J.; Hamilton, T. C.; Grotzinger, K. R.; McKoy, W. M.; Winker, M. A.; Ozols, R. F. Radiation Survival Parameters of Antineoplastic Drug-sensitive and -resistant Human Ovarian Cancer Cell Lines and Their Modification by Buthionine Sulfoximine. *Cancer Res.* **1985,** *45*, 2110-2115.

III. NEW AND KNOWN PRENYLATED STILBENES AND FLAVONOIDS ISOLATED FROM *MACARANGA ALNIFOLIA* (EUPHORBIACEAE) FROM MADAGASCAR

3.1 Introduction

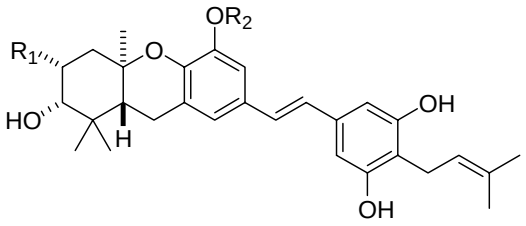
Bioassay-guided fractionation of a fruit extract of *Macaranga alnifolia* (Euphorbiaceae) from Madagascar led to the isolation of four new prenylated stilbenes, schweinfurthins E-H, and one new geranylated dihydroflavonol, alnifoliol. Also isolated were the known prenylated stilbene vedelianin and the known geranylated flavonoids bonanniol A, bonannione A, diplacol and diplacone. Various NMR techniques and mass spectroscopic methods were used to determine the structures. All ten compounds were tested for cytotoxicity in the A2780 human ovarian cancer cell line assay. Vedelianin (IC_{50} = 0.062 μg/mL) exhibited the greatest cytotoxicity among all isolates, while schweinfurthin E (IC_{50} = 0.13 μg/mL) was the most potent of the new compounds.

3.1.1 *Previous Investigations of Macaranga Species*

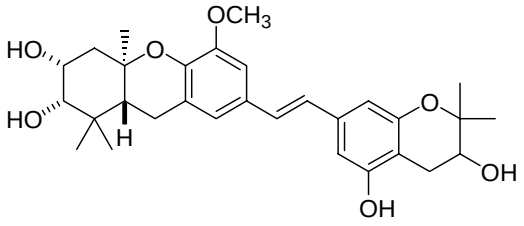
Macaranga is a large genus of the family Euphorbiaceae. Observation of *Macaranga* plants in their natural environment has revealed that they produce thread-like wax crystals on their stems, which make the slippery surfaces impassable for all insects except a species of ants known as "wax runners". Chemical analysis has indicated that terpenoids make up a majority of the wax bloom content that helps maintain this symbiotic relationship between plant and insect.[1] One of the more commonly studied species of this genus is *M. tanarius*, noted for its diterpenoid[2,3] and flavonoid[4-6] content. Work has also been done to obtain terpenes from *M. carolinensis*,[7] flavonoids from *M. conifera*[8] and *M. denticulate*,[9] chromenoflavones from *M. indica*,[10] clerodane diterpenes from *M. monandra*,[11] bergenin derivatives and polyphenols from *M. peltata*,[12,13] prenylflavones from *M. pleiostemona*,[14] a geranyl flavanone from *M. schweinfurthii*,[15] tannins from *M. sinensis*,[16] a rotenoid and other compounds from *M. triloba*,[17] and a geranylflavonol from *M. vedeliana*.[18] No phytochemical studies have been previously reported for *M. alnifolia*.

3.1.2 *Chemical Investigation of Macaranga alnifolia*

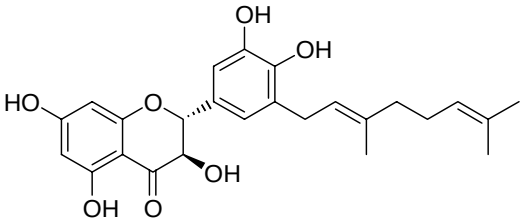
As part of an ongoing search for cytotoxic natural products from tropical rainforests in Madagascar, through the International Cooperative Biodiversity Group (ICBG) program, we obtained an ethanolic fruit extract of *Macaranga alnifolia* for phytochemical investigation. This extract was found to be active in the A2780 ovarian cancer cytotoxicity assay, with an IC_{50} of 3.5 μg/mL. Bioassay-guided fractionation led to the isolation of five new and five known compounds, including four new prenylated stilbenes – schweinfurthins E-H (**3.1**-**3.4**), a new geranylated dihydroflavonol – alnifoliol (**3.5**), a known prenylated stilbene – vedelianin (**3.6**), two known geranylated dihydroflavonols – bonanniol A (**3.7**) and diplacol (**3.8**), and two known geranylated flavanones – bonannione A (**3.9**) and diplacone (or nymphaeol A)(**3.10**). Here we describe the isolation and structure elucidation of these cytotoxic compounds (Figure 3.1).



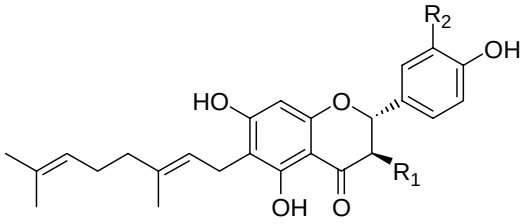
3.1 R_1=OH, R_2=CH_3
3.2 R_1=H, R_2=CH_3
3.3 R_1=R_2=H
3.6 R_1=OH, R_2=H



3.4



3.5

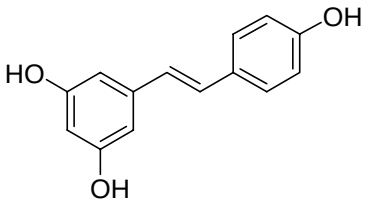


3.7 R_1=OH, R_2=H
3.8 R_1=OH, R_2=OH
3.9 R_1=H, R_2=H
3.10 R_1=H, R_2=OH

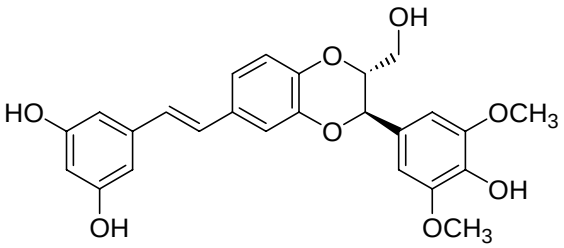
Figure 3.1. Compounds from *Macaranga alnifolia*.

3.1.3 *Previous Investigations of Prenylated Stilbenes*

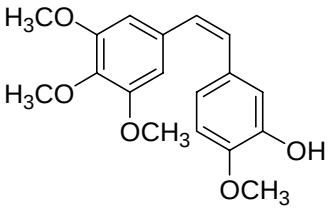
Stilbenes are compounds composed of two benzene rings connected by a double bond. Although simple at the core, stilbene deriviatives have the potential to be highly complex when produced as secondary metabolites. These compounds are not highly prevalent as natural products, but a number of notable stilbenoids have been isolated from various plants. Resveratrol (**3.11**), a component of red wine, has a number of derivatives, and some of those have been obtained from the wood of *Knema austrosiamensis*.[19] The genus *Lonchocarpus* has yielded at least nine stilbenoids – four longistylines from *L. violaceus*[20] and five chiricanines from *L. chiricanus*.[21] Aiphanol (**3.12**), a silbenolignan from *Aiphanes aculeate*, was found to be highly bioactive against cyclooxygenases-1 and -2.[22] The most promising natural stilbenes, in terms of drug candidacy, are the combretastatins, which are compounds isolated from the *Combretum* genus of plants that are currently undergoing clinical trials.[23] Interestingly, many of the combretastatins are cis-isomers, such as combretastatin A-4 (**3.13**). A number of synthetic stilbenes, including the breast cancer drug tamoxifen and some diethyl stilbenoids, are also being studied for their proven or potential pharmaceutical activity.



3.11 Resveratrol



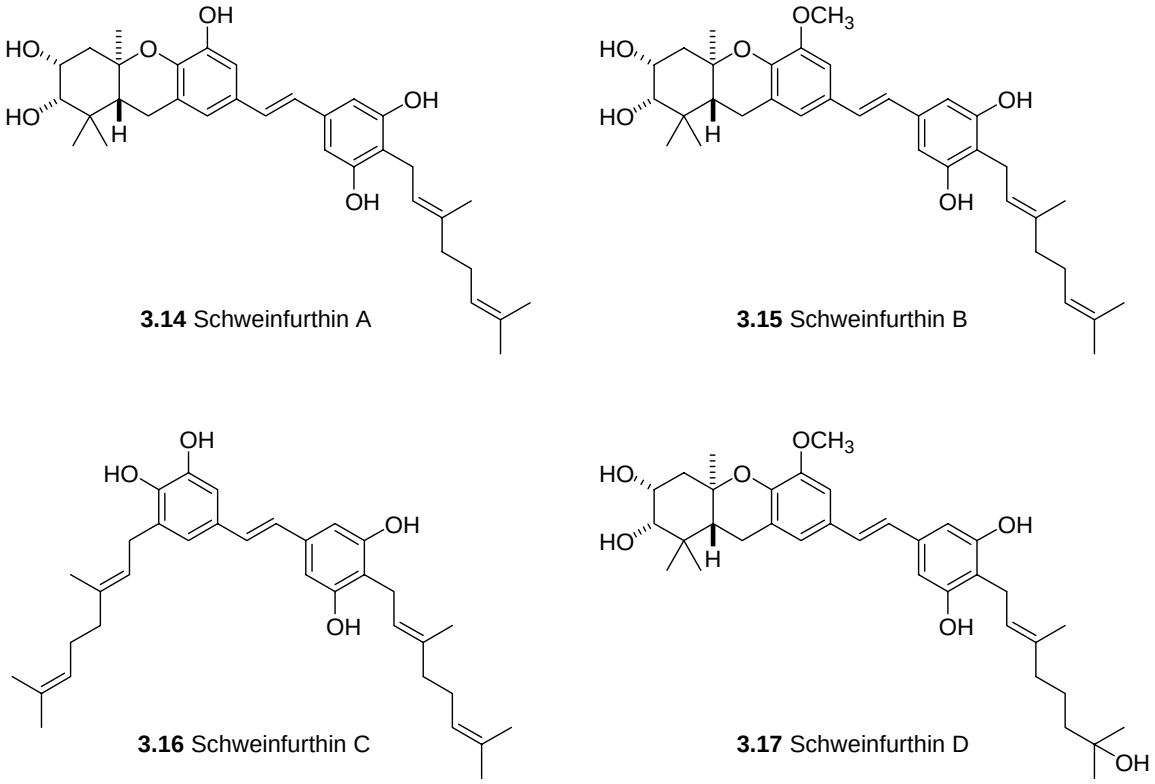
3.12 Aiphanol



3.13 Combretastatin A-4

Figure 3.2. Stilbenes from Various Species.

Perhaps the most interesting biological activity to be discovered through the *Macaranga* genus is the cytoxicity associated with a series of prenylated stilbenes. Schweinfurthins A-D (**3.14-3.17**, Figure 3.3), containing geranyl rather than prenyl substituents, were discovered in *M. schweinfurthii* and subsequently examined in the NCI 60-cell screen.[24,25] Their cytotoxic profile in the NCI screen suggested that the schweinfurthins were mechanistically similar to the stelletins and cephalostatins. Interestingly, schweinfurthin C was found to be much less active than the other three analogues, so the cyclization of the geranyl group must play an important role in the biological activity of these compounds.



3.14 Schweinfurthin A

3.15 Schweinfurthin B

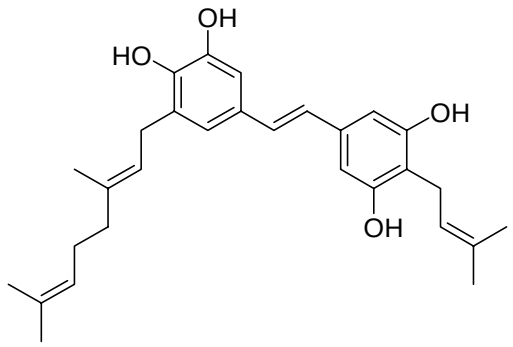
3.16 Schweinfurthin C

3.17 Schweinfurthin D

Figure 3.3. Schweinfurthins A-D from *Macaranga schweinfurthii*.

These schweinfurthins are structurally similar to the novel isolate, vedelianin (**3.6**), which was obtained from *M. vedeliana* seven years prior to the discovery of the schweinfurthins but never examined for biological activity.[26] More recently, a new cytotoxic prenylated stilbene (**3.18**, Figure 3.4) has been isolated from *M. mappa*.[27]

Mappain most closely resembles schweinfurthin C, but it was shown to be cytotoxic to specific lines of ovarian cancer cells (SK-OV-3 and SKVLB-1).



3.18 Mappain

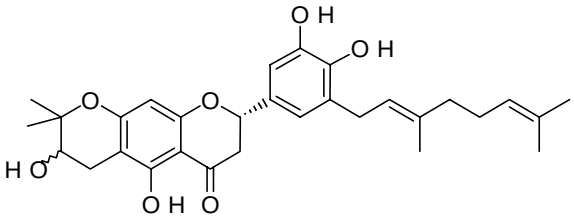
Figure 3.4. Mappain from *Macaranga mappa*.

3.1.4 *Previous Investigations of Flavonoids*

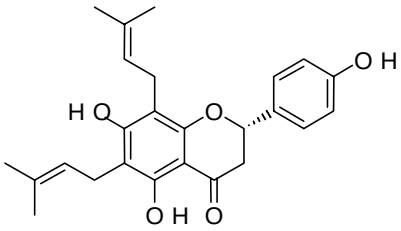
Although perhaps not apparent at first glance, flavonoids are structurally similar to stilbenes. Both classes of compounds are formed through the acetate biosynthetic pathway from 4-hydroxycinnamoyl-CoA precursors. Whereas stilbenes are formed by a Claisen-type cyclization of the poly-*β*- keto chain, flavonoids are formed by an aldol-type cyclization of the same chain.[28] These naturally-occuring compounds are extremely common and well-studied, due to a variety of biological acitivities (most notably, antioxidant activity) that they have exhibited. The chemistry of flavonoids is too vast to describe in detail here, but a review in *Nutritional Biochemistry* summarizes the classification, plant distribution, and therapeutic potential of the more than 4,000 types of flavonoids identified prior to 1996.[29]

As previously mentioned, there are a number of flavonoids that have been specifically isolated from plants of the *Macaranga* genus. Many of these compounds were isolated by bioassay-guided fractionation and, therefore, they have demonstrated bioactivities. Tanariflavanones A (**3.19**) and B, from *M. tanarius*, were found to inhibit radicle growth of lettuce seedlings.[4] *M. tanarius* was also found to contain flavonoids with COX-2 inhibitory activity (nymphaeol B), cytotoxicity (nymphaeol A, **3.10**, and tanariflavanone D), and antioxidant activity (nymphaeol A-C and tanariflavanone D).[6] Also showing significant activity against cyclooxygenase-2 was lonchocarpol A (**3.20**),

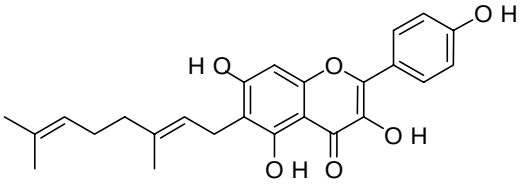
isolated from the leaves of *M. conifera*.[8] Of the flavonoids isolated from *M. denticulata*, macarangin (**3.21**) showed the most potent antioxidant activity,[9] and several flavonoids from *M. pleiostemona* (macarangaflavanone A (**3.22**), macarangaflavanone B and bonannione A, **3.9**) were shown to be antibacterial.[14] Some flavonoids, representing those isolated from the *Macaranga* genus, are shown in Figure 3.5.



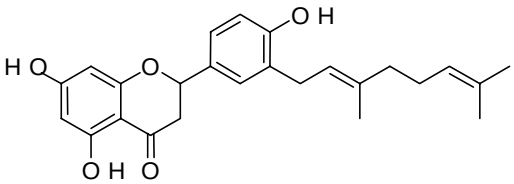
3.19 Tanariflavanone A



3.20 Lonchocarpol A



3.21 Macarangin



3.22 Macarangaflavanone A

Figure 3.5. Prenylated Flavonoids from Various *Macaranga* Species.

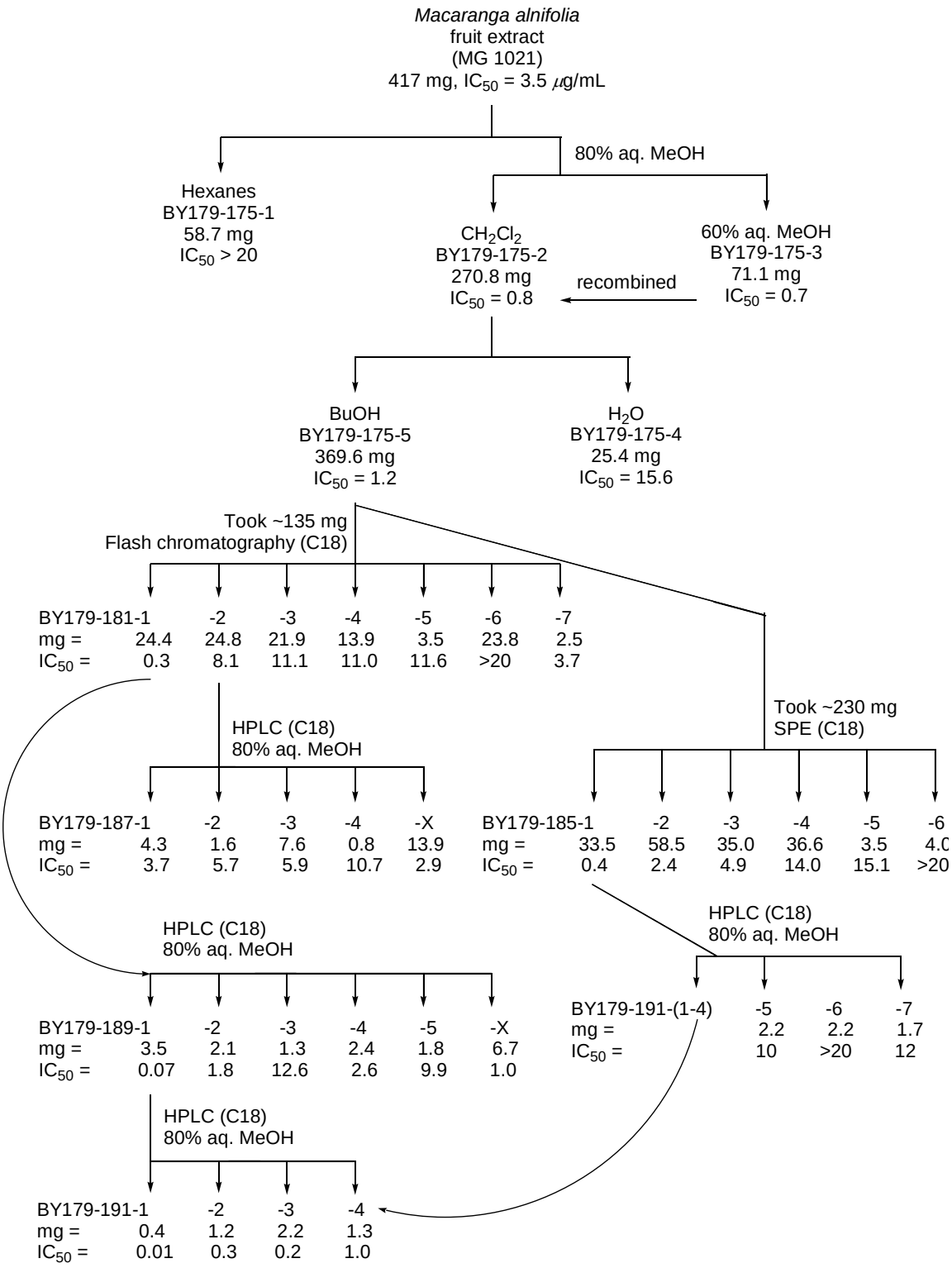
This investigation of *Macaranga alnifolia* was not the only such study to yield both prenylated stilbenes and flavonoids from the same plant. *M. schweinfurthii* was the source of both the schweinfurthins and a novel geranylflavone, isomacarangin, and *M. vedeliana* led to the first discovery of macarangin (**3.21**, a geranylflavonol), in addition to vedelianin (**3.6**). However, neither of these two compounds was initially evaluated for biological activity.

3.2 Results and Discussion

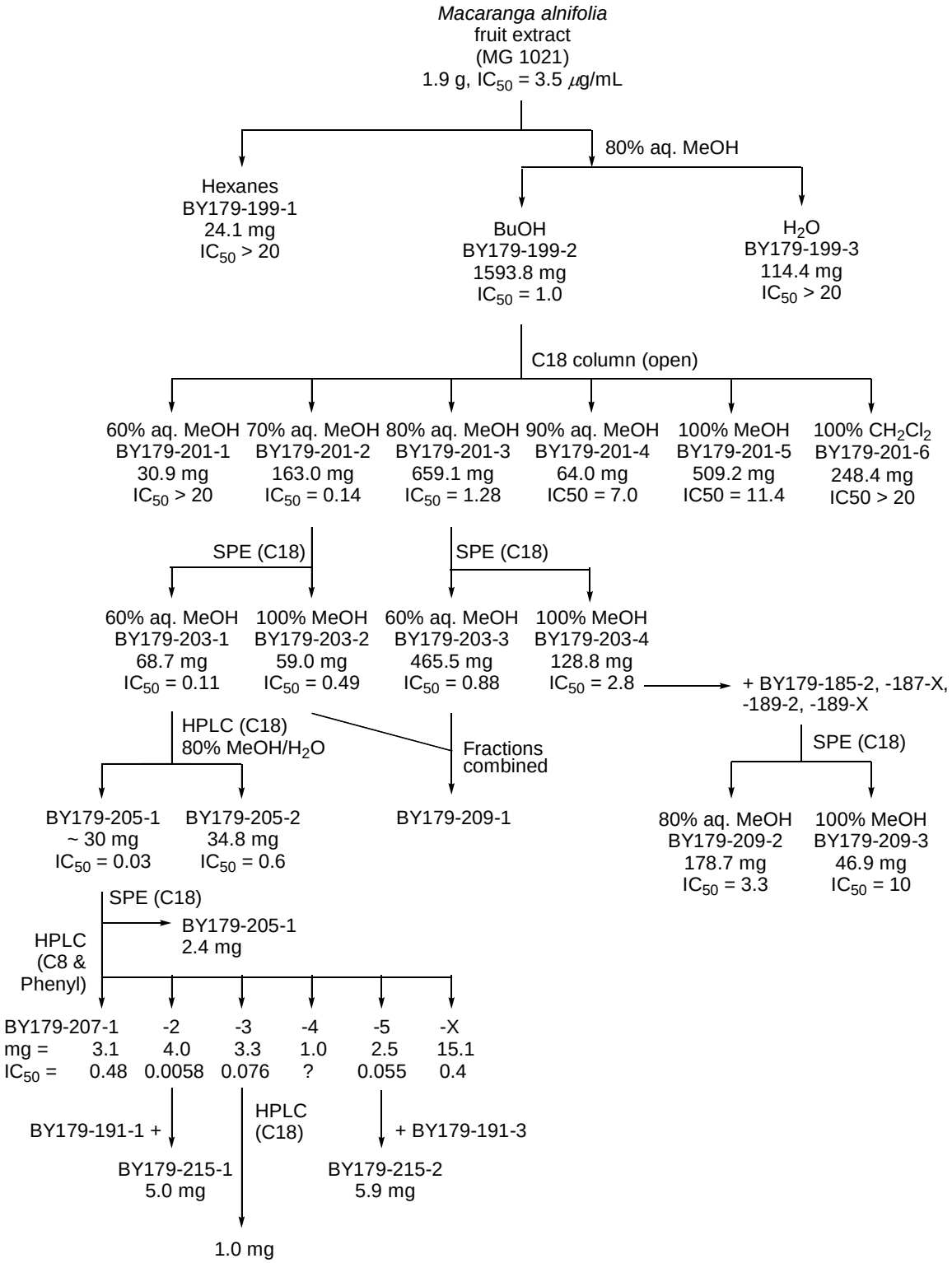
3.2.1 *Isolation of Prenylated Stilbenes and Flavonoids from Macaranga alnifolia*

The ten compounds obtained from *Macaranga alnifolia* were isolated as indicated in Schemes 3.1-3.3. The ethanol extract of *M. alnifolia* was partitioned between hexanes and MeOH-H_2O (4:1), and the aqueous layer was diluted with H_2O to MeOH-H_2O (3:2) and extracted with CH_2Cl_2. Both the CH_2Cl_2 and MeOH-H_2O fractions were active to a similar degree in the A2780 bioassay, and these fractions were recombined and partitioned between BuOH and H_2O. The active BuOH fraction was subjected to RP-C_{18} flash chromatography, eluting with a gradient system of MeOH-H_2O. The earliest fraction (eluted with 70% MeOH-H_2O) showed the greatest improvement in bioactivity. Repeated HPLC, eluting with 80% MeOH-H_2O on a RP-C_{18} column, resulted in a series of fractions with excellent bioactivity but low yield. The initial fractionation process (Scheme 3.1) was therefore repeated (Schemes 3.2-3.3) to acquire additional quantities of the active compounds/mixtures for structural identification purposes.

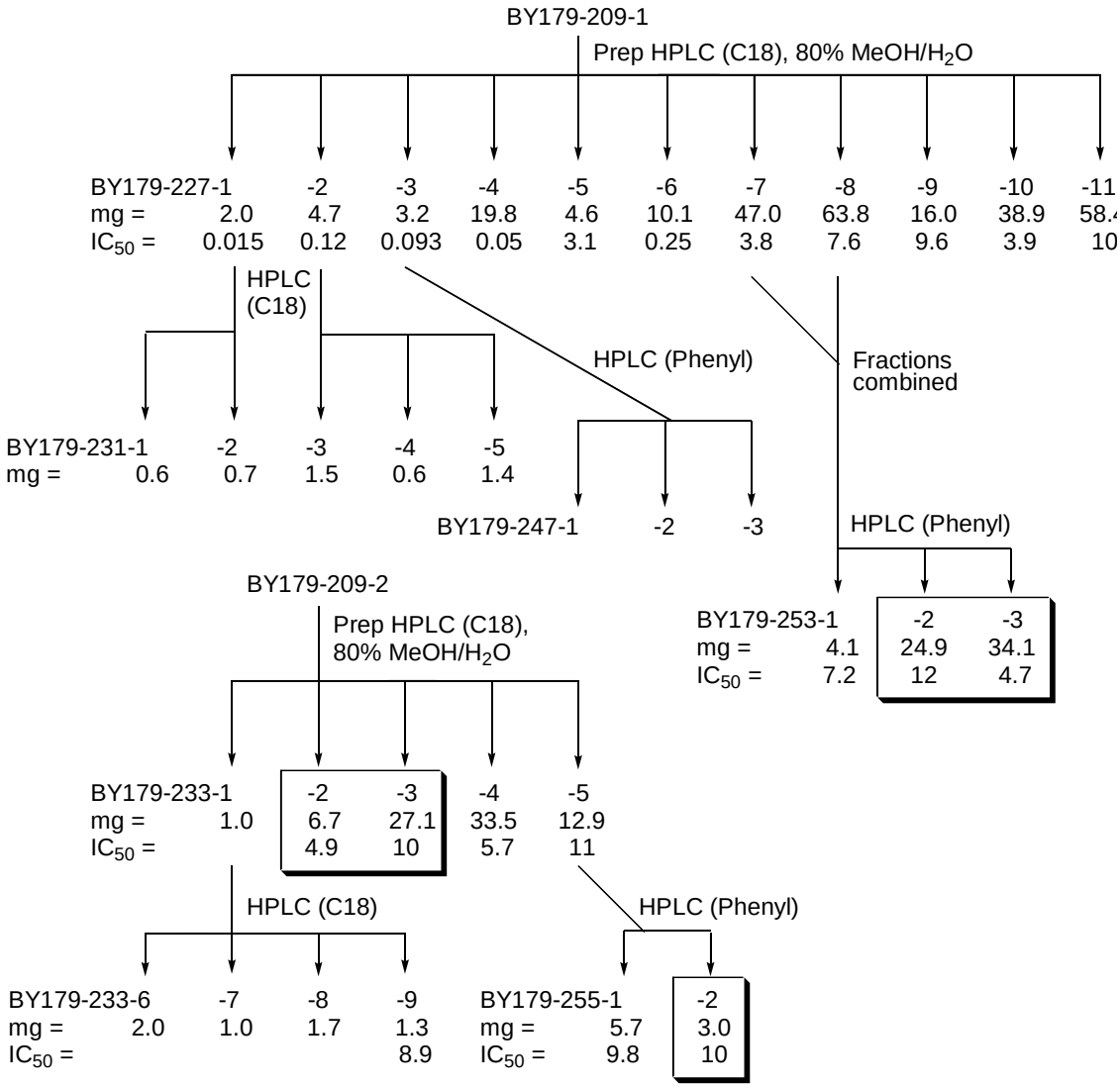
During the second fractionation process, the BuOH fraction from liquid/liquid partitioning was subjected to open-column chromatography with RP-C_{18} as the solid phase. The fractions eluted with 70% and 80% MeOH-H_2O showed the most improved activity and were separately extracted with RP-C_{18} SPE cartridges. Preparative RP-C_{18} HPLC, eluting with 80% MeOH-H_2O, yielded a total of 16 new fractions (A-K and L-P). Semipreparative RP-C18 and RP-phenyl HPLC of the following fractions yielded pure prenylated stilbenes: D (**3.1**, 25.4 mg), A-C (**3.6**, 4.1 mg; **3.3**, 0.9 mg; **3.4**, 1.5 mg), and F (**3.2**,10.6 mg). Semipreparative RP-phenyl HPLC of the following fractions yielded pure flavonoids: G-H (**3.5**, 24.9 mg; **3.10**, 34.1 mg), and M-N and P (**3.8**, 6.7 mg; **3.7**, 27.1 mg; **3.9**, 3.0 mg).



Scheme 3.1. First Fractionation of *Macaranga alnifolia* (Euphorbiaceae).



Scheme 3.2. Second Fractionation of *Macaranga alnifolia* (Euphorbiaceae).



Schweinfurthin mixing

BY179-243-1 (215-1, 231-1)
BY179-243-2 (191-2, 207-4, 231-4, 247-1)
BY179-243-3 (191-2, 207-4, 231-2, 231-4, 231-5, 247-2)
BY179-243-4 (215-2, 227-4, 233-6, 233-7)
BY179-243-5 (233-7)
BY179-243-6 (191-4, 227-6, 233-8, 233-9)

Schweinfurthin cytotoxicities

BY179-243-1 IC_{50} = 0.062
BY179-243-2 IC_{50} = 0.18
BY179-243-3 IC_{50} = 2.3
BY179-243-4 IC_{50} = 0.13
BY179-243-5 IC_{50} = 15
BY179-243-6 IC_{50} = 2.4

Scheme 3.3. Second Fractionation of *Macaranga alnifolia* (Euphorbiaceae) Continued.

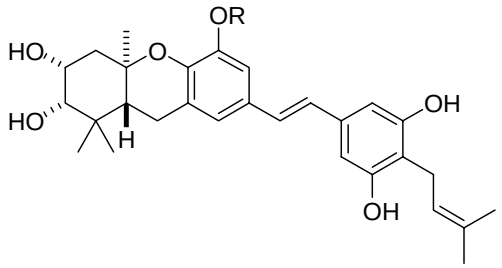
3.2.2 *Characterization of New Prenylated Stilbenes from Macaranga alnifolia*

3.2.2.1 Structure of Schweinfurthin E (**3.1**)

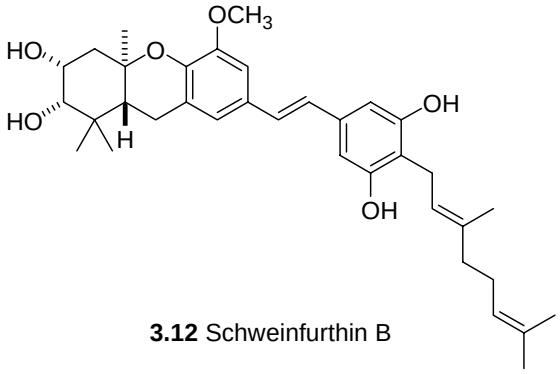
Schweinfurthin E (**3.1**) was isolated as a yellowish solid with a molecular formula of $C_{30}H_{38}O_6$, based on HRFABMS. The ^{1}H NMR spectrum of **3.1** indicated the presence of an asymmetrical stilbene core (δ 6.87 ppm, 1H, d, J = 16, H-1'; δ 6.77 ppm, 1H, d, J = 16.5, H-2') with both an AA' benzene ring system (δ 6.46 ppm, 2H, s, H-4' and -8') and an AB benzene ring system (δ 6.91 ppm, 1H, d, H-6; δ 6.84 ppm, 1H, d, H-8). Proton signals at δ 5.23 (1H, tq, J = 7, 1.5, H-2"), 3.27 (H-1", partially obscured by solvent), 1.76 (3H, s, H-4") and 1.65 ppm (3H, s, H-5") indicated the presence of an isoprenyl group. Also present in the spectrum were three other methyl proton groups at δ 1.40 (3H, s, H-13), 1.10 (3H, s, H-12) and 1.09 (3H, s, H-11) ppm; a methoxy proton group at δ 3.84 ppm (3H, s, 5-OCH_3); and two methine hydrogens bonded to oxygenated carbons at δ 4.14 (1H, q, J = 3.5, H-3) and 3.27 ppm (H-2, partially obscured by solvent).

^{13}C NMR signals at δ 131.1 (C-3"), 124.6 (C-2"), 26.0 (C-5"), 23.3 (C-1") and 17.9 ppm (C-4") confirmed the presence of an isoprenyl group. The other three methyl carbons shifted to δ 29.4 (C-12), 22.0 (C-13) and 16.5 ppm (C-11), and the methoxy carbon shifted to δ 56.5 ppm. Three oxygenated sp^3 carbons (C-2, C-4a and C-3) were present in the spectrum at δ 78.8, 78.1 and 71.8 ppm, respectively, and the carbons of the AA' benzene ring in the stilbene were observed at δ 157.3 ppm for the hydroxylated carbons (C-5' and -7') and δ 105.8 ppm for the hydrogenated carbons (C-4' and -8').

Overall, chemical shifts corresponded closely to those of vedelianin[26] and the schweinfurthins,[24] and the shifts for the hydrogens and carbons of the cyclized geranyl group were nearly identical to the literature values. UV absorbance maxima at λ 331 and 224 nm were also experimentally obtained, and these correlated well with literature values for compounds of this class. The molecular formula of compound **3.1** differed from vedelianin (**3.6**) by CH_2 and from schweinfurthin B (**3.12**) by C_5H_8, which is consistent with a 5-methoxy (^{1}H δ 3.84 ppm and ^{13}C δ 56.4 ppm) derivative of vedelianin.



3.1 Schweinfurthin E (R=CH_3)
3.6 Vedelianin (R=H)

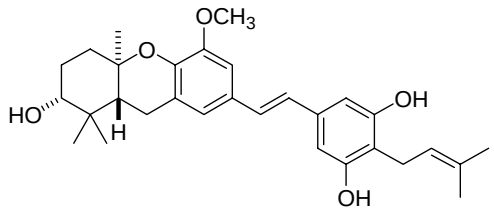


3.12 Schweinfurthin B

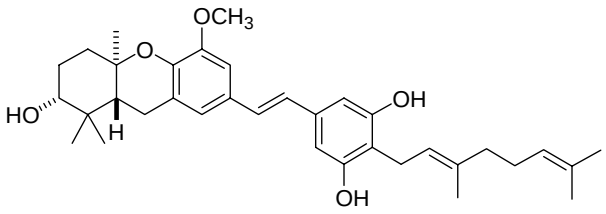
Figure 3.6. Schweinfurthin E and Related Compounds.

3.2.2.2 Structure of Schweinfurthin F (**3.2**)

Schweinfurthin F (**3.2**) was isolated as a yellowish solid with a molecular formula of $C_{30}H_{38}O_5$, based on HRFABMS, differing from **3.1** by a single oxygen. The NMR signals for H- and C-3 were shifted significantly upfield (from δ 4.14 to 2.03 ppm and from δ 71.7 to 39.4 ppm, respectively) when compared to **3.1**, suggesting that **3.2** was a 3-deoxy derivative. This was further confirmed by the upfield shifts for neighboring hydrogens on the α-side of the molecule (H-4, H-11, H-13) and also for adjacent carbons (C-4, C-11, C-12, C-13). A 3-deoxy derivative of schweinfurthin B (**3.23**, Figure 3.7) has now been synthesized and reported, with an activity greater than those of any of the natural products.[30]



3.2 Schweinfurthin F



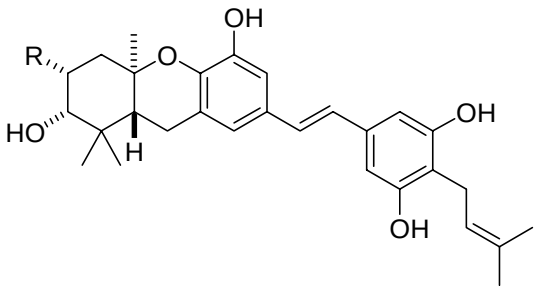
3.23 3-Deoxyschweinfurthin B

Figure 3.7. Schweinfurthin F and 3-Deoxyschweinfurthin B.

3.2.2.3 Structure of Schweinfurthin G (**3.3**)

Schweinfurthin G (**3.3**) was isolated as a yellowish solid. HRFABMS results could not be acquired, despite multiple attempts. ^{1}H and ^{13}C NMR spectra revealed the lack of methoxy signals at δ ~3.8 and ~56 ppm, respectively, which were present in the

spectra of both **3.1** and **3.2**. The signals for H-3 (δ 2.06 ppm), C-3 (δ 39.4 ppm), and proximal atoms also corresponded to those for **3.2**. Therefore, **3.3** was determined to be 3-deoxyvedelianin.

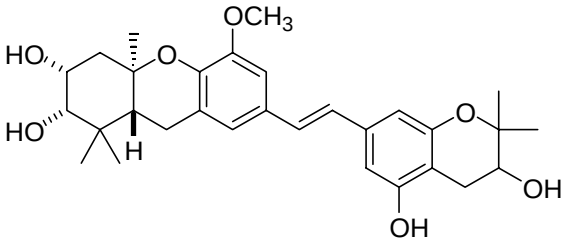


3.3 Schweinfurthin G (R=H)
3.6 Vedelianin (R=OH)

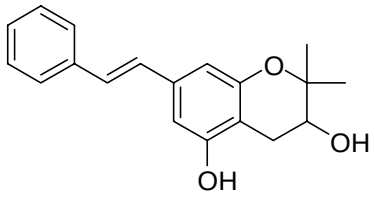
Figure 3.8. Schweinfurthin G and Vedelianin.

3.2.2.4 Structure of Schweinfurthin H (**3.4**)

Schweinfurthin H (**3.4**) was isolated as a pale yellow solid with a molecular formula of $C_{30}H_{38}O_7$, based on HRFABMS, differing from **3.1** by a single oxygen. The ^{1}H NMR spectrum of **3.4** indicated the presence of a different asymmetrical stilbene group with a second, alternate AB benzene ring system rather than an AA' benzene ring system. Signals for H-4' (δ 6.52 ppm) and H-8' (δ 6.44 ppm) appeared as two separate peaks. Within the isoprenyl group, loss of the double bond and cyclization with the C-4' oxygen explained the upfield shifts of H-2" (δ 3.73 ppm), H-4" (δ 1.33 ppm) and H-5" (δ 1.23 ppm), as well as the representation of H-1" as a pair of doublet of doublets at δ 2.90 and 2.53 ppm. The hydroxylation of C-3" was also apparent, due to its ^{13}C chemical shift at δ 76.4 ppm. The final structure was confirmed through NMR comparison with the literature values reported for chiricanine B (**3.24**, Figure 3.9), a tricyclic prenylated stilbene from *Lonchocarpus chiricanus*.[21]



3.4 Schweinfurthin H



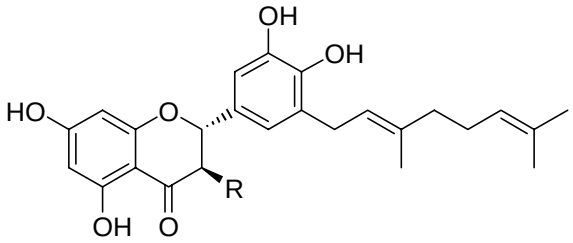
3.24 Chiricanine B

Figure 3.9. Schweinfurthin H and Chiricanine B.

3.2.3 *Characterization of a New Dihydroflavonol from Macaranga alnifolia*

3.2.3.1 Structure of Alnifoliol (**3.5**)

Alnifoliol (**3.5**) was isolated as a yellow-brown solid with a molecular formula of $C_{25}H_{28}O_7$, based on HRFABMS. The ^{1}H NMR spectrum of **3.5** showed four aromatic protons (δ 6.81, d, H-2'; δ 6.74, d, H-6'; δ 5.91, s, H-8; δ 5.87, s, H-6), one oxymethine (δ 4.88, d, H-2), and one methine α to the carbonyl (δ 4.47, d, H-3). This data suggested that **3.5** possessed a dihydroflavanol skeleton. Also present were signals for a geranyl substituent (δ 5.33, m, H-2"; δ 5.10, m, H-7"; δ 3.33, d, H-1"; δ 2.09, td, H-6"; δ 2.02, t, H-5"; δ 1.70, s, H-4"; δ 1.61, s, H-9"; δ 1.56, s, H-10"). The fact that proton signals for both H-6 and H-8 were present suggested that the geranyl group must be located on the B-ring. The splitting patterns for H-2' and H-6' confirmed the location of the geranyl group at C-5. Compound **3.5** is nearly identical to the known component of propolis, isonymphaeol-B (**3.25**, Figure 3.10), except for the presence of the 3-OH group (making it a flavanone, rather than a dihydroflavanol). The spectroscopic literature values[31] were carefully examined to assist in the elucidation of **3.5**.



3.5 Alnifoliol (R=OH)
3.25 Isonymphaeol-B (R=H)

Figure 3.10. Alnifoliol and Isonymphaeol-B.

3.2.4 *Characterization of Known Compounds from Macaranga alnifolia*

Vedelianin (**3.6**), bonanniol A (**3.7**), diplacol (**3.8**), bonannione A (**3.9**) and diplacone (**3.10**, also known as nymphaeol A) were also isolated, and their structures were determined based upon comparison of their ^{1}H NMR, ^{13}C NMR, and HRFABMS spectra to literature values.[26,32-36]

3.2.5 *Biological Evaluation of Compounds from Macaranga alnifolia*

3.2.5.1 A2780 Screening of New and Known Compounds

All ten isolated compounds were tested for cytotoxicity against the A2780 ovarian cancer cell line, and the results are provided in Table 3.1.

Table 3.1. Cytotoxicity Data of *Macaranga alnifolia* Compounds.

Compound	IC_{50} (μg/mL)
Schweinfurthin E (**3.1**)	0.13
Schweinfurthin F (**3.2**)	2.4
Schweinfurthin G (**3.3**)	0.18
Schweinfurthin H (**3.4**)	2.3
Alnifoliol (**3.5**)	12
Vedelianin (**3.6**)	0.062
Bonanniol A (**3.7**)	10
Diplacol (**3.8**)	4.9
Bonannione A (**3.9**)	10
Diplacone (**3.10**)	4.7

3.2.5.2 NCI Screening of Schweinfurthin E (**3.1**)

Schweinfurthin E (**3.1**) was tested in the 60-cell human tumor cancer screen at the National Cancer Institute, and the compound exhibited a mean panel GI_{50} of 0.19 μM. GI_{50} values, like IC_{50} values, are concentrations required to inhibit cell growth by 50%. All lines of the leukemia subpanel were found to be highly sensitive to **3.1**, while all lines of the ovarian cancer subpanel were (surprisingly) somewhat resistant. The most sensitive lines included leukemia (MOLT-4) and CNS (SF-295) and renal (A498 and CAKI-1) cancers, which all gave GI_{50} and TGI values of < 10 nM. Other sensitive lines included leukemia (CCRF-CEM, K-562, and RPMI-8226), melanoma (M14 and UACC-62), and non-small cell lung (A549/ATCC and HOP-62), CNS (SF-539 and U251), renal (786-0), and breast (HS 578T) cancers, which also gave GI_{50} values of < 10 nM, but had TGI values > 10 nM. The NCI cytotoxicity results suggested that schweinfurthin E, similar to the other schweinfurthins, may share a similar mechanism of action with the stelletins. The National Cancer Institute requested an additional 30 mg of sample for further evaluation, but unfortunately, the supply of natural product had been exhausted.

3.3 Experimental Section.

General Experimental Procedures. Solid phase extraction was performed with Supelco Discovery DSC-C_{18} tubes. HPLC was performed using either Shimadzu LC-8A pumps coupled with a Varian Dynamax preparative C_{18} column (250 x 21.4 mm) or Shimadzu LC-10A pumps coupled with a Varian Dynamax semipreparative C_8, C_{18} or phenyl column (250 x 10.0 mm). Both systems employed a Shimadzu SPD-M10A diode array detector. Optical rotation data was obtained on a PerkinElmer 241 polarimeter. UV spectra were measured on a Shimadzu UV-1201 spectrophotometer. Mass spectra were obtained on a JEOL JMS-HX-110 instrument. NMR spectra were obtained on a JEOL Eclipse (at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) spectrometer. Chemical shifts are given in δ (ppm) and coupling constants (J) are reported in Hz.

Plant Material. The fruit of *Macaranga alnifolia* (Euphorbiaceae) was collected by Fidisoa Ratovoson on November 3, 2001. The specimens were collected around the Natural Reserve of Zahamena in the province of Toamasina, Madagascar. Duplicate voucher specimens have been deposited at the Centre National d'Application des Recherches Pharmaceutiques (CNARP) and the Direction des Recherches Forestieres et Piscicoles Herbarium (TEF) in Antananarivo, Madagascar; the Missouri Botanical Garden in St. Louis, Missouri (MO); and the Museum National d'Histoire Naturelle in Paris, France (P).

Extract Preparation. The fruit of *Macaranga alnifolia* was dried, ground and extracted with ethanol in Madagascar. This yielded an extract labeled MG 1021 (2.84 g).

Cytotoxicity Bioassay. The A2780 ovarian cancer cell line cytotoxicity assay was performed at Virginia Polytechnic Institute and State University as previously reported.[37]

Bioassay-guided Fractionation and Isolation of Prenylated Stilbenes and Flavonoids. The crude bioactive extract MG 1021 (IC_{50} = 3.5 μg/mL, 2.32 g) was partitioned between hexanes (200 mL) and MeOH-H_2O (4:1, 200 mL). The aqueous fraction was dried and subsequently partitioned between BuOH and H_2O. The evaporated BuOH fraction (1.96

g) displayed cytotoxicity (IC_{50} = 1.0 μg/mL) and was further separated by repeated RP-C_{18} column chromatography and solid phase extraction. Preparative RP-C_{18} HPLC using MeOH-H_2O (4:1, 1 mL/min) on two separate bioactive fractions yielded a total of 16 new fractions (A-K and L-P). Fraction D was identified as **3.1** (t_R 21.5 min, 25.4 mg), while fractions A-C yielded vedelianin (t_R 17.1 min, 4.1 mg), compound **3.3** (t_R 18.2 min, 0.9 mg) and compound **3.4** (t_R 19.5 min, 1.5 mg), respectively, upon additional purification by semipreparative RP-C_{18} and RP-phenyl HPLC. Fraction F was also identified as **3.2** (t_R 25.9 min, 10.6 mg). Fractions G (t_R 32.6 min) and H (t_R 30-45 min) were combined and purified by semipreparative RP-phenyl HPLC to obtain both **3.5** (24.9 mg) and diplacone (34.1 mg). Additionally, fractions M, N and P yielded diplacol (t_R 19 min, 6.7 mg), bonanniol A (t_R 21 min, 27.1 mg) and bonannione A (t_R 35 min, 3.0 mg). The structures of the known compounds were identified by comparison of their spectral data with literature values.[26,32-36]

Schweinfurthin E (3.1): yellowish solid; $[\alpha]^{22}_D$ +49.2° (*c* 0.13, CH_3OH); UV (MeOH) λ_{max} 331, 211 nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.91 (1H, d, *J* = 1.5, H-6), 6.87 (1H, d, *J* = 16, H-1'), 6.84 (1H, d, H-8), 6.77 (1H, d, *J* = 16.5, H-2'), 6.46 (2H, s, H-4', 8'), 5.23 (1H, tq, *J* = 7, 1.5, H-2"), 4.14 (1H, q, *J* = 3.5, H-3), 3.84 (3H, s, 5-OCH_3), 3.30 (partially obscured by solvent, H-2, 1"), 2.76 (2H, m, H-9), 2.34 (1H, dd, *J* = 14, 3, H-4), 1.93 (1H, dd, *J* = 13.5, 3.5, H-4), 1.76 (3H, s, H-4"), 1.74 (1H, dd, *J* = 12.5, 6, H-9a), 1.65 (3H, s, H-5"), 1.40 (3H, s, H-13), 1.10 (3H, s, H-12), 1.09 (3H, s, H-11); ^{13}C NMR (CD_3OD, 125 MHz) δ 157.3 (C-5', 7'), 150.2 (C-5), 143.4 (C-3'), 137.6 (C-10a), 131.1 (C-3"), 130.8 (C-7), 128.6 (C-1'), 127.7 (C-2'), 124.6 (C-2"), 124.4 (C-8a), 121.7 (C-8), 116.0 (C-6'), 108.3 (C-6), 105.8 (C-4', 8'), 78.8 (C-2), 78.1 (C-4a), 71.8 (C-3), 56.5 (5-OCH_3), 44.8 (C-4), 39.2 (C-1), 29.4 (C-12), 26.0 (C-5"), 24.0 (C-9), 23.3 (C-1"), 22.0 (C-13), 17.9 (C-4"), 16.5 (C-11); HRFABMS *m/z* 494.2646 $[M]^+$ (calcd for $C_{30}H_{38}O_6$, 494.2668).

Schweinfurthin F (3.2): yellowish solid; $[\alpha]^{22}_D$ +50.8° (*c* 0.06, CH_3OH); UV (MeOH) λ_{max} 331, 209 nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.91 (1H, d, *J* = 1.5, H-6), 6.86 (1H, d, *J* = 16.5, H-1'), 6.83 (1H, d, *J* = 1.5, H-8), 6.77 (1H, d, *J* = 16.5, H-2'), 6.46 (2H, s, H-4', 8'), 5.23 (1H, tq, *J* = 7, 1.5, H-2"), 3.83 (3H, s, 5-OCH_3), 3.30 (partially obscured by

solvent, H-2, 1''), 2.72 (2H, m, H-9), 2.03 (2H, m, H-3), 1.79 (1H, m, H-4), 1.76 (3H, s, H-4''), 1.75 (1H, m, H-9a), 1.65 (1H, m, H-4), 1.65 (3H, s, H-5''), 1.21 (3H, s, H-13), 1.09 (3H, s, H-12), 0.87 (3H, s, H-11); ^{13}C NMR (CD_3OD, 125 MHz) δ 157.3 (C-5', 7'), 150.2 (C-5), 143.7 (C-3'), 137.6 (C-10a), 131.2 (C-3''), 130.9 (C-7), 128.6 (C-1'), 127.8 (C-2'), 124.6 (C-2''), 124.1 (C-8a), 121.8 (C-8), 116.0 (C-6'), 108.3 (C-6), 105.8 (C-4', 8'), 78.8 (C-2), 78.2 (C-4a), 56.5 (5-OCH_3), 39.5 (C-3), 39.0 (C-1), 29.0 (C-4), 27.9 (C-12), 26.0 (C-5''), 24.1 (C-9), 23.3 (C-1''), 20.2 (C-13), 17.9 (C-4''), 14.9 (C-11); HRFABMS *m/z* 478.2737 $[M]^+$ (calcd for $C_{30}H_{38}O_5$, 478.2719).

Schweinfurthin G (3.3): yellowish solid; $[\alpha]^{22}_D$ +33.3° (*c* 0.03, CH_3OH); UV (MeOH) λ_{max} 331, 228 nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.80 (1H, d, *J* = 17, H-1'), 6.79 (1H, d, H-6), 6.72 (1H, d, *J* = 1.5, H-8), 6.70 (1H, *J* = 16, H-2'), 6.44 (2H, s, H-4', 8'), 5.23 (1H, tq, *J* = 7, 1.5, H-2''), 3.30 (partially obscured by solvent, H-2, 1''), 2.71 (2H, m, H-9), 2.06 (2H, m, H-3), 1.80 (1H, m, H-4), 1.76 (3H, s, H-4''), 1.75 (1H, m, H-9a), 1.68 (1H, m, H-4), 1.65 (3H, s, H-5''), 1.23 (3H, s, H-13), 1.10 (3H, s, H-12), 0.88 (3H, s, H-11); ^{13}C NMR (CD_3OD, 125 MHz) δ 157.3 (C-5', 7'), 147.0 (C-5), 142.2 (C-3'), 141.3 (C-3''), 137.6 (C-10a), 131.0 (C-7), 128.6 (C-1'), 127.5 (C-2'), 124.6 (C-2''), 124.0 (C-8a), 120.4 (C-8), 115.9 (C-6'), 111.1 (C-6), 105.7 (C-4', 8'), 78.8 (C-2), 78.2 (C-4a), 39.5 (C-3), 38.9 (C-1), 29.0 (C-4), 27.9 (C-12), 26.0 (C-5''), 24.0 (C-9), 23.3 (C-1''), 20.3 (C-13), 17.9 (C-4''), 14.8 (C-11).

Schweinfurthin H (3.4): yellowish solid; $[\alpha]^{22}_D$ +32.4° (*c* 0.04, CH_3OH); UV (MeOH) λ_{max} 330, 210 nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.93 (1H, d, *J* = 1.5, H-6), 6.90 (1H, d, *J* = 16, H-1'), 6.85 (1H, d, *J* = 1, H-8), 6.80 (1H, d, *J* = 16, H-2'), 6.52 (1H, d, *J* = 1.5, H-4'), 6.44 (1H, d, *J* = 1, H-8'), 4.14 (1H, q, *J* = 3.5, H-3), 3.84 (3H, s, 5-OCH_3), 3.73 (1H, dd, *J* = 7.5, 5.5, H-2''), 3.30 (1H, m, H-2), 2.90 (1H, dd, *J* = 17, 5.5, H-1''), 2.76 (2H, m, H-9), 2.53 (1H, dd, *J* = 17, 7.5, H-1''), 2.34 (1H, dd, *J* = 14, 3, H-4), 1.92 (1H, dd, *J* = 14.5, H-4), 1.74 (1H, dd, *J* = 12, 5.5, H-9a), 1.40 (3H, s, H-13), 1.33 (3H, s, H-4''), 1.23 (3H, s, H-5''), 1.10 (3H, s, H-12), 1.09 (3H, s, H-11); ^{13}C NMR (CD_3OD, 125 MHz) δ 157.1 (C-5'), 155.3 (C-7'), 150.2 (C-5), 143.5 (C-3'), 138.5 (C-10a), 130.6 (C-7), 129.1 (C-1'), 127.5 (C-2'), 124.4 (C-8a), 121.9 (C-8), 108.4 (C-4'), 108.4 (C-6), 107.6 (C-6'),

105.0 (C-8'), 78.8 (C-2), 78.1 (C-4a), 77.7 (C-3"), 71.8 (C-3), 70.6 (C-2"), 56.5 (5-OCH_3), 44.8 (C-4), 39.2 (C-1), 29.4 (C-12), 27.4 (C-1"), 25.8 (C-5"), 24.0 (C-9), 22.0 (C-13), 20.8 (C-4"), 16.6 (C-11); HRFABMS *m/z* 510.2579 $[M]^+$ (calcd for $C_{30}H_{38}O_7$, 510.2618).

Alnifoliol (3.5): yellowish-brown solid; $[\alpha]^{23}_D$ +15.3° (*c* 0.25, CH_3OH); UV (MeOH) λ_{max} 292, 210 nm; ^1H NMR (CD_3OD, 500 MHz) δ 6.81 (1H, d, H-2'), 6.74 (1H, d, *J* = 2, H-6'), 5.91 (1H, d, *J* = 2.5, H-8), 5.87 (1H, d, H-6), 5.34 (2H, t, H-2"), 5.10 (2H, t, H-7"), 4.88 (1H, d, H-2), 4.47 (1H, d, *J* = 11, H-3), 3.31 (2H, d, *J* = 7.5, H-1"), 2.09 (2H, q, *J* = 7.5, H-6"), 2.02 (2H, t, *J* = 8, H-5"), 1.70 (3H, s, H-4"), 1.61 (3H, s, H-9"), 1.56 (3H, s, H-10"); ^{13}C NMR (CD_3OD, 125 MHz) δ 197.0 (C-4), 167.4 (C-7), 164.0 (C-5), 163.2 (C-9), 144.5 (C-3'), 143.6 (C-4'), 135.5 (C-3"), 130.9 (C-8"), 128.1 (C-1'), 127.6 (C-5'), 124.1 (C-7"), 122.5 (C-2"), 120.0 (C-6'), 111.8 (C-2'), 100.5 (C-10), 96.0 (C-8), 95.0 (C-6), 84.1 (C-2), 72.4 (C-3), 39.6 (C-5"), 27.8 (C-1"), 26.4 (C-6"), 24.6 (C-9"), 16.4 (C-10"), 14.9 (C-4"); HRFABMS *m/z* 440.1831 $[M]^+$ (calcd for $C_{25}H_{28}O_7$, 440.1835).

Vedelianin (3.6): yellowish solid; $[\alpha]^{22}_D$ +32.9° (*c* 0.07, CH_3OH); UV (MeOH) λ_{max} 331, 224 nm; ^1H NMR (CD_3OD, 500 MHz) δ 6.80 (1H, d, *J* = 16.5, H-1'), 6.79 (1H, d, H-6), 6.72 (1H, d, H-8), 6.70 (1H, d, *J* = 16, H-2'), 6.44 (2H, s, H-4', 8'), 5.23 (1H, tq, *J* = 7, 1.5, H-2"), 4.15 (1H, q, *J* = 3.5, H-3), 3.30 (partially obscured by solvent, H-2, 1"), 2.75 (2H, m, H-9), 2.37 (1H, dd, *J* = 14, 3.5, H-4), 1.96 (1H, dd, *J* = 14.5, 3.5, H-4), 1.76 (3H, s, H-4"), 1.75 (1H, m, H-9a), 1.65 (3H, s, H-5"), 1.42 (3H, s, H-13), 1.11 (3H, s, H-12), 1.09 (3H, s, H-11); ^{13}C NMR (CD_3OD, 125 MHz) δ 157.3 (C-5', 7'), 147.2 (C-5), 137.6 (C-10a), 131.1 (C-3"), 130.9 (C-7), 128.6 (C-1'), 127.4 (C-2'), 124.6 (C-2"), 124.2 (C-8a), 120.4 (C-8), 115.9 (C-6'), 111.1 (C-6), 105.7 (C-4', 8'), 78.9 (C-2), 78.1 (C-4a), 71.8 (C-3), 44.8 (C-4), 39.2 (C-1), 29.4 (C-12), 26.0 (C-5"), 23.9 (C-9), 23.3 (C-1"), 22.0 (C-13), 17.9 (C-4"), 16.6 (C-11); HRFABMS *m/z* 480.2519 $[M]^+$ (calcd for $C_{29}H_{36}O_6$, 480.2512).

Bonanniol A (3.7): yellowish-brown solid; $[\alpha]^{23}_D$ +21.7° (*c* 0.27, CH_3OH); UV (MeOH) λ_{max} 296, 206 nm; ^1H NMR (CD_3OD, 500 MHz) δ 7.33 (2H, d, *J* = 8.5, H-2', 6'), 6.82 (2H, d, *J* = 8.5, H-3', 5'), 5.92 (1H, s, H-8), 5.19 (2H, t, H-2"), 5.05 (2H, t, H-7"), 4.92

(1H, d, J = 11.5, H-2), 4.51 (1H, d, J = 11.5, H-3), 3.21 (2H, d, H-1"), 2.03 (2H, q, H-6"), 1.94 (2H, t, J = 8, H-5"), 1.74 (3H, s, H-4"), 1.61 (3H, s, H-9"), 1.55 (3H, s, H-10"); ^{13}C NMR (CD_3OD, 125 MHz) δ 197.2 (C-4), 165.0 (C-7), 160.9 (C-5), 160.9 (C-9), 157.8 (C-4'), 134.0 (C-3"), 130.7 (C-8"), 129.0 (C-2', 6'), 128.1 (C-1'), 124.2 (C-7"), 122.5 (C-2"), 114.8 (C-3'), 114.8 (C-5'), 108.8 (C-6), 100.3 (C-10), 94.3 (C-8), 83.6 (C-2), 72.4 (C-3), 39.6 (C-5"), 26.4 (C-6"), 24.5 (C-9"), 20.5 (C-1"), 16.4 (C-10"), 14.9 (C-4"); HRFABMS *m/z* 425.1929 $[M+H]^+$ (calcd for $C_{25}H_{29}O_6$, 425.1964).

Diplacol (3.8): yellowish-brown solid; $[\alpha]^{23}_D$ +18.6° (*c* 0.44, CH_3OH); UV (MeOH) λ_{max} 295, 215 nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.95 (1H, s, H-2'), 6.83 (1H, dd, H-5'), 6.79 (1H, d, H-6'), 5.91 (1H, s, H-8), 5.19 (2H, t, H-2"), 5.06 (2H, t, H-7"), 4.47 (1H, d, J = 11.5, H-3), 3.22 (2H, d, J = 7, H-1"), 2.04 (2H, q, H-6"), 1.95 (2H, t, J = 8, H-5"), 1.75 (3H, s, H-4"), 1.61 (3H, s, H-9"), 1.56 (3H, s, H-10"); ^{13}C NMR (CD_3OD, 125 MHz) δ 197.1 (C-4), 165.0 (C-7), 160.9 (C-5), 160.9 (C-9), 145.8 (C-4'), 145.0 (C-3'), 134.1 (C-3"), 130.7 (C-8"), 128.7 (C-1'), 124.2 (C-7"), 122.5 (C-2"), 119.6 (C-6'), 114.8 (C-5'), 114.6 (C-2'), 108.7 (C-6), 100.4 (C-10), 94.2 (C-8), 83.8 (C-2), 72.5 (C-3), 39.6 (C-5"), 26.4 (C-6"), 24.5 (C-9"), 20.5 (C-1"), 16.4 (C-10"), 14.9 (C-4"); HRFABMS *m/z* 425.1948 $[M+H]^+$ (calcd for $C_{25}H_{29}O_6$, 425.1964).

Bonannione A (3.9): yellowish-brown solid; $[\alpha]^{23}_D$ 0° (*c* 0.08, CH_3OH); UV (MeOH) λ_{max} 294, 207 nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.30 (2H, d, J = 8.5, H-2', 6'), 6.81 (2H, d, J = 8.5, H-3', 5'), 5.92 (1H, s, H-8), 5.30 (1H, dd, H-2), 5.18 (2H, t, H-2"), 5.06 (2H, t, H-7"), 3.20 (2H, d, J = 7, H-1"), 3.09 (1H, dd, J = 13, H-3a), 2.66 (1H, dd, J = 17, 3, H-3b), 2.04 (2H, q, H-6"), 1.94 (2H, t, J = 8, H-5"), 1.74 (3H, s, H-4"), 1.61 (3H, s, H-9"), 1.56 (3H, s, H-10"); ^{13}C NMR (CD_3OD, 125 MHz) δ 196.4 (C-4), 165.0 (C-7), 161.2 (C-5), 161.1 (C-9), 157.7 (C-4'), 133.9 (C-3"), 130.7 (C-8"), 130.0 (C-1'), 127.7 (C-2', 6'), 124.2 (C-7"), 122.7 (C-2"), 115.0 (C-3', 5'), 108.4 (C-6), 101.8 (C-10), 94.2 (C-8), 79.1 (C-2), 42.9 (C-3), 39.6 (C-5"), 26.4 (C-6"), 24.5 (C-9"), 20.5 (C-1"), 16.4 (C-10"), 14.9 (C-4"); HRFABMS *m/z* 409.1813 $[M+H]^+$ (calcd for $C_{25}H_{29}O_5$, 409.2015).

Diplacone (3.10): yellowish-brown solid; $[\alpha]^{23}_{D}$ –13.2° (*c* 0.33, CH_3OH); UV (MeOH) λ_{max} 292, 207 nm; ^1H NMR (CD_3OD, 500 MHz) δ 6.90 (1H, s, H-2'), 6.77 (2H, s, H-5', 6'), 5.93 (1H, s, H-8), 5.23 (1H, dd, H-2), 5.18 (2H, t, H-2''), 5.05 (2H, t, H-7''), 3.20 (2H, d, *J* = 7.5, H-1''), 3.03 (1H, dd, *J* = 17, 13, H-3a), 2.66 (1H, dd, *J* = 17, 3, H-3b), 2.04 (2H, q, *J* = 7.5, H-6''), 1.94 (2H, t, *J* = 8, H-5''), 1.74 (3H, s, H-4''), 1.61 (3H, s, H-9''), 1.55 (3H, s, H-10''); ^{13}C NMR (CD_3OD, 125 MHz) δ 196.5 (C-4), 164.7 (C-7), 161.2 (C-5), 161.2 (C-9), 145.5 (C-4'), 145.2 (C-3'), 133.9 (C-3''), 130.6 (C-8''), 127.7 (C-1'), 124.2 (C-7''), 122.6 (C-2''), 117.9 (C-6'), 114.9 (C-5'), 113.4 (C-2'), 108.4 (C-6), 101.9 (C-10), 94.1 (C-8), 79.1 (C-2), 42.9 (C-3), 39.6 (C-5''), 26.4 (C-6''), 24.5 (C-9''), 20.5 (C-1''), 16.4 (C-10''), 14.9 (C-4''); HRFABMS *m/z* 441.1911 $[M+H]^+$ (calcd for $C_{25}H_{29}O_7$, 449.1913).

NCI 60-Cell Cancer Assay Data. The tumor cell line subpanels are identified as follows: I (leukemia); II (non-small cell lung); III (colon); IV (CNS); V (melanoma); VI (ovarian); VII (renal); VIII (prostate); IX (breast). The subpanel and individual cell-line identifiers are listed, along with the corresponding negative log GI_{50}, TGI, and LC_{50} values (molar) for schweinfurthin E (**3.1**) [I] CCRF-CEM (<8.00, 5.00, >4.00), K-562 (<8.00, 6.16, n/a), MOLT-4 (<8.00, <8.00, >4.00), RPMI-8226 (<8.00, 7.28, >4.00) [II] A549/ATCC (<8.00, 4.90, >4.00), EKVX (5.67, 4.55, >4.00), HOP-62 (<8.00, 7.12, 5.11), HOP-92 (6.10, 5.29, >4.00), NCI-H23 (6.62, 5.63, 4.86), NCI-H322M (6.54, 5.65, 4.86), NCI-H460 (6.82, >4.00, >4.00), NCI-H522 (5.70, >4.00, >4.00) [III] HCC-2998 (5.99, 5.59, 5.19), HCT-116 (7.68, 6.06, 5.16), HCT-15 (6.22, 5.44, 4.57), HT29 (5.89, n/a, >4.00), KM12 (6.34, 5.65, 5.02), SW-620 (6.96, 5.69, >4.00) [IV] SF-268 (5.58, >4.00, >4.00), SF-295 (<8.00, <8.00, <8.00), SF-539 (<8.00, 6.80, n/a), SNB-19 (4.96, >4.00, >4.00), SNB-75 (5.76, 4.70, >4.00), U251 (<8.00, 5.40, 4.49) [V] LOX IMVI (6.19, 5.63, 5.21), M14 (<8.00, 6.54, 5.37), SK-MEL-2 (5.43, >4.00, >4.00), SK-MEL-28 (6.43, 5.28, >4.00), SK-MEL-5 (6.18, 5.65, 5.25), UACC-257 (6.78, 5.44, 4.43), UACC-62 (<8.00, 6.43, 5.39) [VI] OVCAR-3 (5.21, 5.08, 4.62), OVCAR-4 (6.24, 5.03, >4.00), OVCAR-8 (5.60, 5.17, >4.00), SK-OV-3 (5.56, 4.96, 4.03) [VII] 786-0 (<8.00, 5.97, 5.01), A498 (<8.00, <8.00, >4.00), ACHN (6.43, 5.52, 4.88), CAKI-1 (<8.00, <8.00, 6.26), SN12C (5.68, 5.12, 4.46), TK-10 (n/a, 5.58, 4.73), UO-31 (6.98, >4.00, >4.00) [VIII] PC-3 (6.86, 5.02, 4.32), DU-145 (6.33, 4.85, 4.14) [IX] MCF7 (7.21, 4.57, >4.00),

NCI/ADR-RES (5.65, 5.18, 4.30), MDA-MB-231/ATCC (6.07, 5.46, 4.58), HS 578T (<8.00, 6.44, >4.00), MDA-MB-435 (6.91, 5.71, 5.06), BT-549 (5.82, 4.88, >4.00), T-47D (5.49, >4.00, >4.00). This data from the NCI is also presented as mean graphs in Figure 3.11. Dose response curves for the various cell lines are presented in Figure 3.12.

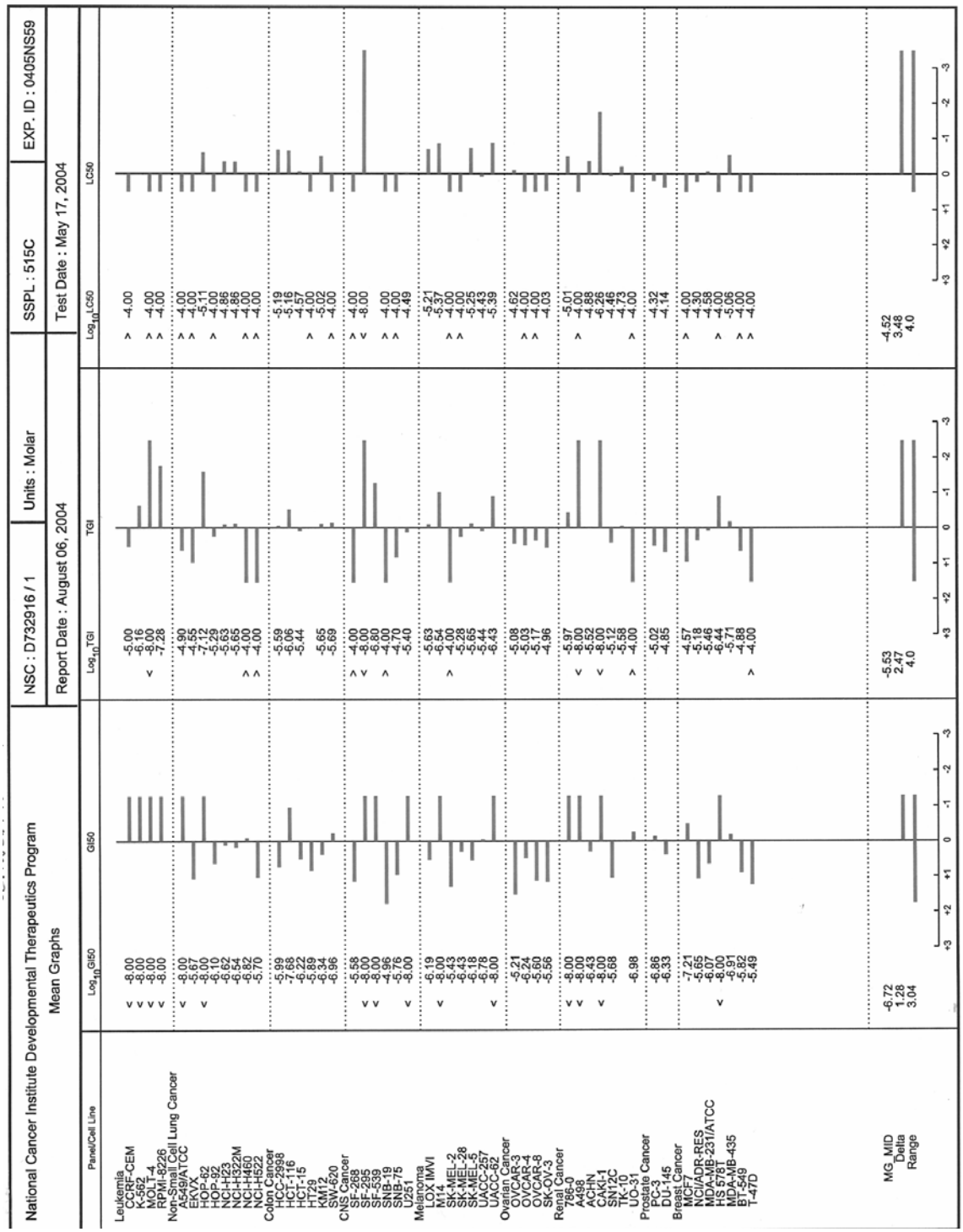


Figure 3.11. NCI Mean Graphs for Schweinfurthin E.

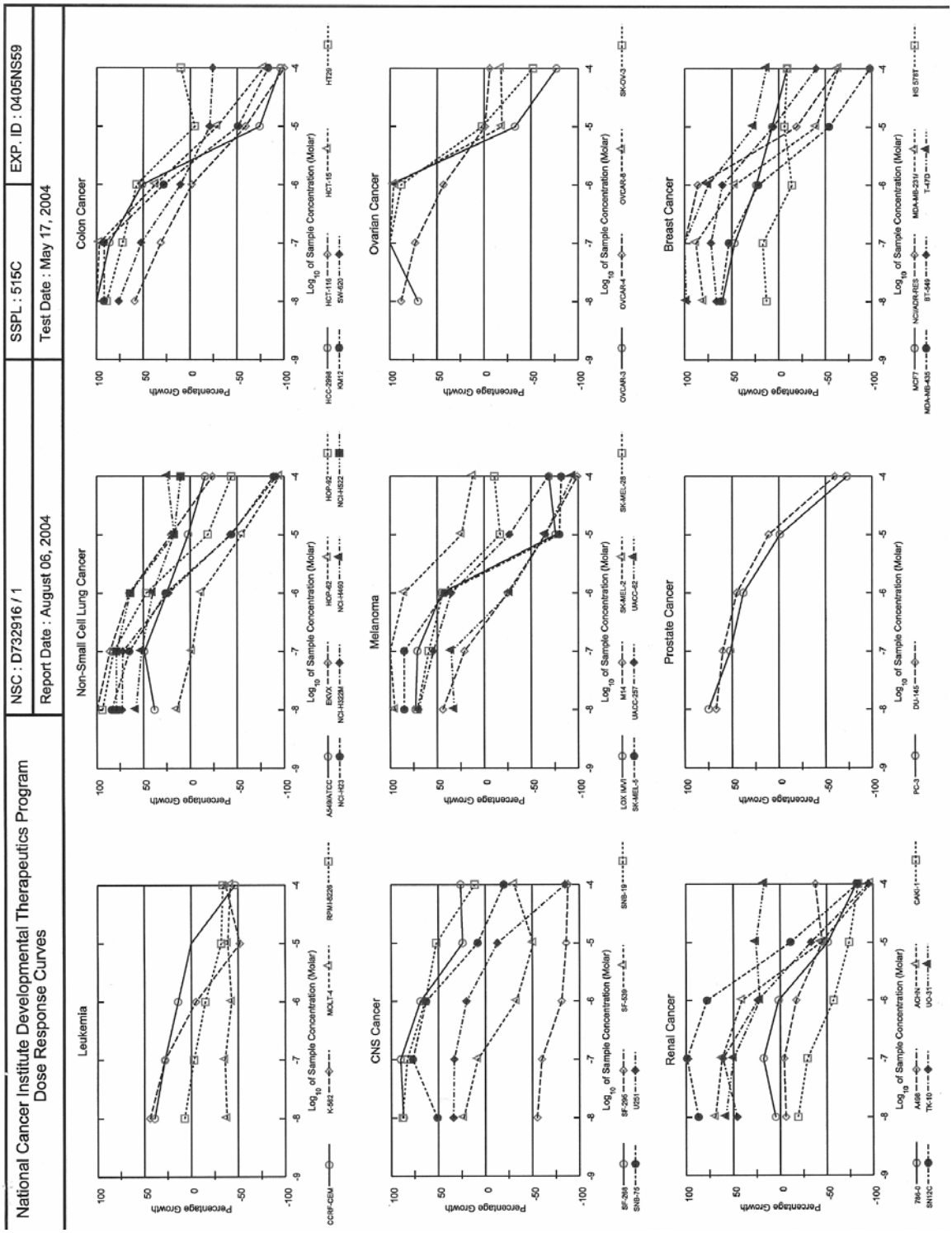


Figure 3.12. NCI Dose Response Curves for Schweinfurthin E.

References

(1) Markstaedter, C.; Federle, W.; Jetter, R.; Riederer, M.; Hoelldobler, B. Chemical Composition of the Slippery Epicuticular Wax Blooms on *Macaranga* (Euphorbiaceae) Ant-plants. *Chemoecology* **2000,** *10*, 33-40.

(2) Hui, W.-H.; Ng, K.-K.; Fukamiya, N.; Koreeda, M.; Nakanishi, K. Isolation and Structure of Macarangonol, a Diterpene Ketol from *Macaranga tanarius*. *Phytochemistry* **1971,** *10*, 1617-1620.

(3) Hui, W.-H.; Li, M.-M.; Ng, K.-K. Terpenoids and Steroids from *Macaranga tanarius*. *Phytochemistry* **1975,** *14*, 816-817.

(4) Tseng, M.-H.; Chou, C.-H.; Chen, Y.-M.; Kuo, Y. H. Alleopathic Prenylflavanones from the Fallen Leaves of *Macaranga tanarius*. *J. Nat. Prod.* **2001,** *64*, 827-828.

(5) Tseng, M.-H.; Kuo, Y.-H.; Chen, Y.-M.; Chou, C.-H. Alleopathic Potential of *Macaranga tanarius* (L.) Muell.-Arg. *J. Chem. Ecol.* **2003,** *29*, 1269-1286.

(6) Phommart, S.; Sutthivaiyakit, P.; Chimnoi, N.; Ruchirawat, S.; Sutthivaiyakit, S. Constituents of the Leaves of *Macaranga tanarius*. *J. Nat. Prod.* **2005,** *68*, 927-930.

(7) Han, G.-q.; Che, C.-t.; Fong, H. H. S.; Farnsworth, N. R.; C. H. Phoebe, J. Chemical Constituents of *Macaranga carolinensis*. *Fitoterapia* **1988,** *59*, 242-244.

(8) Jang, D. S.; Cuendet, M.; Hawthorne, M. E.; Kardono, L. B. S.; Kawanishi, K.; Fong, H. H. S.; Mehta, R. G.; Pezzuto, J. M.; Kinghorn, A. D. Prenylated Flavonoids of the Leaves of *Macaranga conifera* with Inhibitory Activity Against Cyclooxygenase-2. *Phytochemistry* **2002,** *61*, 867-872.

(9) Sutthivaiyakit, S.; Unganont, S.; Sutthivaiyakit, P.; Suksamrarn, A. Diterpenylated and Prenylated Flavonoids from *Macaranga denticulata*. *Tetrahedron* **2002,** *58*, 3619-3622.

(10) Sultana, S.; Ilyas, M. Chromenoflavones from *Macaranga indica*. *Phytochemistry* **1986,** *25*, 953-954.

(11) Salah, M. A.; Bedir, E.; Toyang, N. J.; Kahn, I. A.; Harries, M. D.; Wedge, D. E. Antifungal Clerodane Diterpenes from *Macaranga monandra* (L) Muell. et Arg. (Euphorbiaceae). *J. Ag. Food Chem.* **2003,** *51*, 7607-7610.

(12) Ramaiah, P. A.; Row, L. R.; Reddy, D. S.; Anjaneyulu, A. S. R.; Ward, R. S.; Pelter, A. Isolation and Characterisation of Bergenin Derivatives from *Macaranga peltata*. *J. Chem. Soc. Perkin. 1.* **1979**, 2313-2316.

(13) Asharani, T.; Seetharaman, T. R. Polyphenols from the Leaves of *Macaranga peltata*. *Fitoterapia* **1994,** *65*, 184.

(14) Schuetz, B. A.; Wright, A. D.; Rali, T.; Sticher, O. Prenylated Flavanones from the Leaves of *Macaranga pleiostemona*. *Phytochemistry* **1995,** *40*, 1273-1277.

(15) Beutler, J. A.; McCall, K. L.; Boyd, M. R. A Novel Geranylflavone from *Macaranga schweinfurthii*. *Nat. Prod. Lett.* **1999,** *13*, 29-32.

(16) Lin, J.-H.; Ishimatsu, M.; Tanaka, T.; Nonaka, G.-i.; Nishioka, I. Tannins and Related Compounds. XCVI. Structures of Macaranins and Macarinins, New Hydrolyzable Tannins Possessing Macaranoyl and Tergalloyl Ester Groups, from the Leaves of *Macaranga sinensis* (Baill.) Muell.-Arg. *Chem. Pharm. Bull.* **1990,** *38*, 1844-1851.

(17) Jang, D. S.; Cuendet, M.; Pawlus, A. D.; Kardono, L. B. S.; Kawanishi, K.; Farnsworth, N. R.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. Potential Cancer Chemopreventative Constituents of the Leaves of *Macaranga triloba*. *Phytochemistry* **2004,** *65*, 345-350.

(18) Hnawia, E.; Thoison, O.; Gueritte-Voegelein, F.; Bourret, D.; Sevenet, T. A Geranyl Substituted Flavonol from *Macaranga vedeliana*. *Phytochemistry* **1990,** *29*, 2367-2368.

(19) Gonzalez, M. J. T. G.; Pinto, M. M. M.; Kijjoa, A.; Anantachoke, C.; Herz, W. Silbenes and Other Consittuents of *Knema austrosiamensis*. *Phytochemistry* **1993,** *32*, 433-438.

(20) Monache, F. D.; Marletti, F.; Marini-Bettolo, G. B.; Mello, J. F. D.; Lima, O. G. D. Isolation and Structure of Longistylines A, B, C and D, New Prenylated Stilbenes from *Lonchocarpus violaceus*. *Lloydia* **1977,** *40*, 201-208.

(21) Ioset, J.-R.; Marston, A.; Gupta, M.; Hostettmann, K. Five New Prenylated Stilbenes from the Root Bark of Lonchocarpus chiricanus. *J. Nat. Prod.* **2001,** *64*, 710-715.

(22) Lee, D.; Cuendet, M.; Vigo, J. S.; Graham, J. G.; Cabieses, F.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. A Novel Cyclooxygenase-Inhibitory Stilbenolignan from the Seeds of *Aiphanes aculeata*. *Org. Lett.* **2001,** *3*, 2169-2171.

(23) Pinney, K. G.; Jelinek, C.; Edvardsen, K.; Chaplin, D. J.; Petit, G. R. The Discovery and Development of the Combretastatins. In *Anticancer Agents from Natural Products*; Cragg, G. M.; Kingston, D. G. I.; Newman, D. J., Eds.; Taylor & Francis: Boca Raton, FL, 2005.

(24) Beutler, J. A.; Shoemaker, R. H.; Johnson, T.; Boyd, M. R. Cytotoxic Geranyl Stilbenes from *Macaranga schweinfurthii*. *J. Nat. Prod.* **1998,** *61*, 1509-1512.

(25) Beutler, J. A.; Jato, J.; Cragg, G. M.; Boyd, M. R. Schweinfurthin D, a Cytotoxic Stilbene from *Macaranga schweinfurthii*. *Nat. Prod. Lett.* **2000,** *14*, 399-404.

(26) Thoison, O.; Hnawia, E.; Gueritte-Voegelein, F.; Sevenet, T. Vedelianin, a Hexahydroxanthene Derivative Isolated from *Macaranga vedeliana*. *Phytochemistry* **1992,** *31*, 1439-1442.

(27) Kaaden, J. E. v. d.; Hemscheidt, T. K.; Mooberry, S. L. Mappain, a New Cytotoxic Prenylated Stilbene from *Macaranga mappa*. *J. Nat. Prod.* **2001,** *64*, 103-105.

(28) Dewick, P. M. *Medicinal Natural Products: A Biosynthetic Approach*; John Wiley & Sons Ltd.: Chichester, 1997.

(29) Cook, N. C.; Samman, S. Flavonoids - Chemistry, Metabolism, Cardioprotective Effects, and Dietary Sources. *Nutr. Biochem.* **1996,** *7*, 66-76.

(30) Neighbors, J. D.; Beutler, J. A.; Wiemer, D. F. Synthesis of Nonracemic 3-Deoxyschweinfurthin B. *J. Org. Chem.* **2005,** *70*, 925-931.

(31) Kumazawa, S.; Goto, H.; Hamasaka, T.; Fukumoto, S.; Fujimoto, T.; Nakayama, T. A New Prenylated Flavonoid from Propolis Collected in Okinawa, Japan. *Biosci. Biotechnol. Biochem.* **2004,** *68*, 260-262.

(32) Bruno, M.; Savona, G.; Lamartina, L.; Lentini, F. New Flavonoids from *Bonannia graeca* (L.) Halacsy. *Heterocycles* **1985,** *23*, 1147-1153.

(33) Lincoln, D. E. Leaf Resin Flavonoids of *Diplacus auranticus*. *Biochem. Syst. Ecol.* **1980,** *8*, 397-400.

(34) Wollenweber, E.; Schober, I.; Schilling, G.; Arriaga-Giner, F. J.; Roitman, J. N. A Geranyl *α*-Pyrone from the Leaf Resin of *Diplacus auranticus*. *Phytochemistry* **1989,** *28*, 3493-3496.

(35) Phillips, W. R.; Baj, N. J.; Gunatilaka, A. A. L.; Kingston, D. G. I. *C*-Geranyl Compounds from *Mimulus clevelandii*. *J. Nat. Prod.* **1996,** *59*, 495-497.

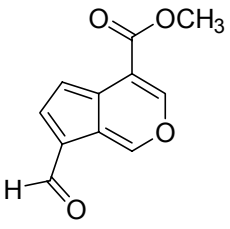
(36) Yakushijin, K.; Shibayama, K.; Murata, H.; Furukawa, H. New Prenylflavones from *Hernandia nymphaefolia* (Presl) Kubitzki. *Heterocycles* **1980,** *14*, 397-402.

(37) Louie, K. G.; Behrens, B. C.; Kinsella, T. J.; Hamilton, T. C.; Grotzinger, K. R.; McKoy, W. M.; Winker, M. A.; Ozols, R. F. Radiation Survival Parameters of Antineoplastic Drug-sensitive and -resistant Human Ovarian Cancer Cell Lines and Their Modification by Buthionine Sulfoximine. *Cancer Res.* **1985,** *45*, 2110-2115.

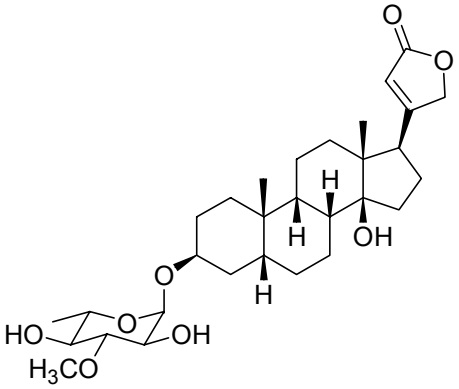
IV. CERBINAL, A KNOWN IRIDOID, AND NERIIFOLIN, A KNOWN CARDIAC GLYCOSIDE, ISOLATED FROM *CERBERA MANGHAS* (APOCYNACEAE) FROM MADAGASCAR

4.1 Introduction

Bark, wood and leaf extracts of *Cerbera manghas* (Apocynaceae) from Madagascar displayed moderate to potent cytotoxicity in the A2780 human ovarian cancer cell line assay. Bioassay-guided fractionation of the bark and wood extracts led to the isolation of cerbinal (**4.1**), a known iridoid previously examined only for antifungal activity. This is the first report of cytotoxicity associated with cerbinal. Bioassay-guided fractionation of the leaf extract led to the isolation of neriifolin (**4.2**), a known cardiac glycoside. Various NMR techniques and mass spectroscopic methods were used to determine the structures. Cerbinal was a major component of the bark and wood extracts (> 1% of the crude material), and it was also the most cytotoxic compound (IC_{50} = 1 μg/mL) observed in that fractionation process. Neriifolin was a minor component of the leaf extract, but it was 100 times more active than cerbinal in the A2780 cytotoxicity assay.



4.1 Cerbinal



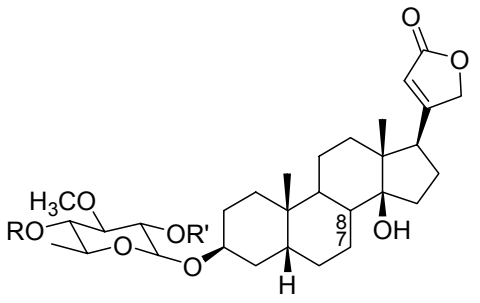
4.2 Neriifolin

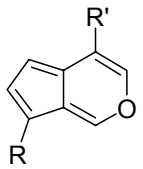
Figure 4.1. Compounds from *Cerbera manghas*.

4.1.1 *Previous Investigations of Cerbera manghas*

Species belonging to the *Cerbera* (Apocynaceae) genus of plants are commonly found on the islands of Southeast Asia and Oceana, and on other lands surrounding the Indian Ocean. The two most frequently encountered species are *C. manghas* and *C. odollam*, which differ only in the color and shape of their respective fruits.[1] *Cerbera manghas*, in particular, is native to the Ryukyu Islands of Japan, where Fumiko Abe and Tatsuo Yamauchi of Fukuoka University have phytochemically investigated the plant for decades. Their partnership is responsible for at least 11 publications on *C. manghas*, which accounts for more than 50% of all reports on its chemical constituents.

The initial investigation of *Cerbera manghas* by Abe and Yamauchi in 1977 revealed the presence of cardiac glycosides (or cardenolides) in the seeds, bark and leaves.[2] These steroidal structures with sugar moieties, including neriifolin (**4.2**), thevetin B (**4.3**), cerberin (**4.4**) and deacetyltanghinin (**4.5**), are a common class of phytochemicals. Also obtained that same year from the stem and root bark material were a number of iridoids, including cerbinal (**4.1**), cerberic acid (**4.6**), cerberinic acid (**4.7**) and baldrinal (**4.8**).[3] Abe and Yamauchi have since reported on the additional isolation of cardenolide glycosides from the leaves and stems,[4-6] lignans from the stems,[7-9] glycosidic iridoids from the leaves[10] and normonoterpenoids and normonoterpenoid glucosides from the leaves.[11,12]

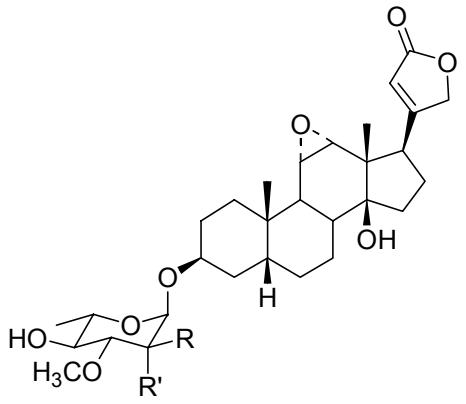




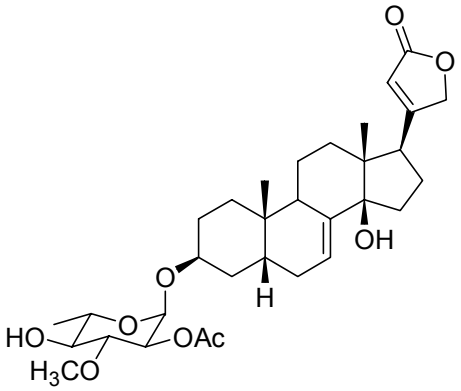
4.3 Thevetin B (R = β-gentiobiosyl, R' = H)
4.4 Cerberin (R = H, R' = Ac)
4.5 Deacetyltanghinin (R = R' = H, $C_{7,8}\beta\beta$-epoxy
4.6 Cerberic acid (R = COOH, R' = $COOCH_3$)
4.7 Cerberinic acid (R = CHO, R' = COOH)
4.8 Baldrinal (R = CHO, R' = CH_2OAc)

Figure 4.2. Cardenolides and Iridoids from *Cerbera manghas*.

Some of the other compounds isolated from *Cerbera manghas* include iridoid glucosides from the leaves and fruit,[13] flavanol glycosides from the leaves,[14,15] and cardenolide glycosides from the seeds and roots.[1,16] Very few of the natural products from this plant were initially examined for biological activity. However, as secondary metabolites have been re-isolated from this and other species, bioassays have played a greater role. The cardenolides obtained from *C. manghas* in recent years have shown a significant level of cytotoxicity. Two new cardenolides (**4.9**, **4.10**) from the roots were found to be both antiproliferative against a human colon cancer cell line (Col2) and antiestrogenic against the Ishikawa cell line.[16] One new cardenolide (**4.11**, 7,8-dehydrocerberin) from the seeds was found to be cytotoxic against oral human epidermoid carcinoma (KB) and human breast cancer (BC).[1]



4.9 (R = H, R' = OH)
4.10 (R = OH, R' = H)



4.11 7,8-Dehydrocerberin

Figure 4.3. Cytotoxic Cardenolides from *Cerbera manghas*.

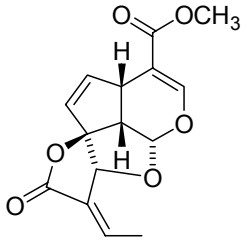
4.1.2 *Chemical Investigation of Cerbera manghas*

As part of an ongoing search for cytotoxic natural products from tropical rainforests in Madagascar, through the International Cooperative Biodiversity Group (ICBG) program, we obtained ethanolic bark, wood and leaf extracts of *Cerbera manghas* for phytochemical investigation. All extracts were found to be active in the A2780 ovarian cancer cytotoxicity assay, but the leaf extract was approximately 40 times more active (IC_{50} = 0.3 μg/mL) than the wood extract (IC_{50} = 12 μg/mL). The extracts

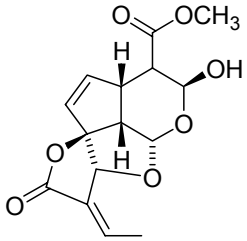
were fractionated separately, guided by bioassay, and each yielded one compound of interest. From the bark and wood extracts was isolated the known iridoid cerbinal (**4.1**), and from the leaf extract was isolated the known cardiac glycoside neriifolin (**4.2**). Here we describe the isolation and structure elucidation of these cytotoxic compounds (Figure 4.1).

4.1.3 *Previous Investigations of Iridoids*

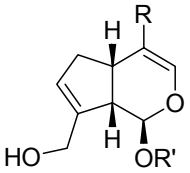
Iridoids are monoterpenoid-derived molecules that contain a six-membered oxygen heterocycle fused to a cyclopentane ring.[17] While not a major class of natural products, they are common, and both glycosides and aglycons of this type are known for their cytotoxicity. Many of the iridoids that have shown promising biological activity belong to the plumeria and allamanda family of compounds, including plumericin (**4.12**) and allamandin (**4.13**).[18] These compounds, along with a host of other analogues, are found in the bark of *Plumeria rubra* and known to be generally cytotoxic to a variety of leukemia and cancer cell-types.[19]



4.12 Plumericin



4.13 Allamandin



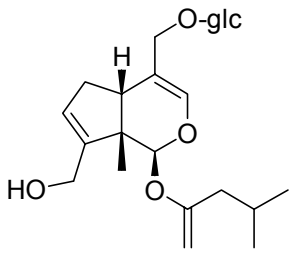
4.14 Genipin (R = CO_2CH_3, R' = H)
4.15 Tarennoside (R = CHO, R' = glc)

Figure 4.4. Known Cytotoxic Iridoids.

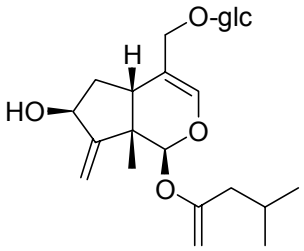
Genipin (**4.14**) is one of the most famous iridoids and also one of the most cytotoxic. Various glycosidic analogues of genipin, including tarennoside (**4.15**), have been obtained from a variety of sources, including *Tarenna gracilipes*, *Gardenia jasminoides* and *Genipa americana*. Genipin and tarennoside have both proven to be anti-tumor promoting when tested against 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced Epstein-Barr virus (EBV) activation.[20,21] Geniposide, along with aucubin, was

recently found to be a potential poison of DNA topoisomerase I, but not topoisomerase II.[22] It is rare for a compound to be able to stabilize the complex of DNA and the type I enzyme, so this discovery has raised interest in the possibility of using iridoids as clinical anticancer agents.

Other iridoid glycosides have also shown promising potential as anticancer agents through their biological activity in various bioassays. Penstemide (**4.16**) and serrualatoloside (**4.17**) from *Penstemon serrulatus* have been reported to inhibit [^{3}H]-thymidine incorporation into DNA.[23] The novel compound, 8-acetylharpagide, from *Ajuga decumbens* has demonstrated *in vivo* cancer chemoprevention against mouse hepatic tumors.[24] A series of luzonosides (iridoid glucosides), luzonoids (aglycons) and luzonials (iridoid aldehydes) from *Viburnum luzonicum* were found to be inhibitory against HeLa S3 cancer cells.[25,26] Scrophuloside B_4 (**4.18**), an uncharacteristically large iridoid glycoside obtained from *Scrophularia ningpoensis*, is slightly active on K562 and Bowes cells.

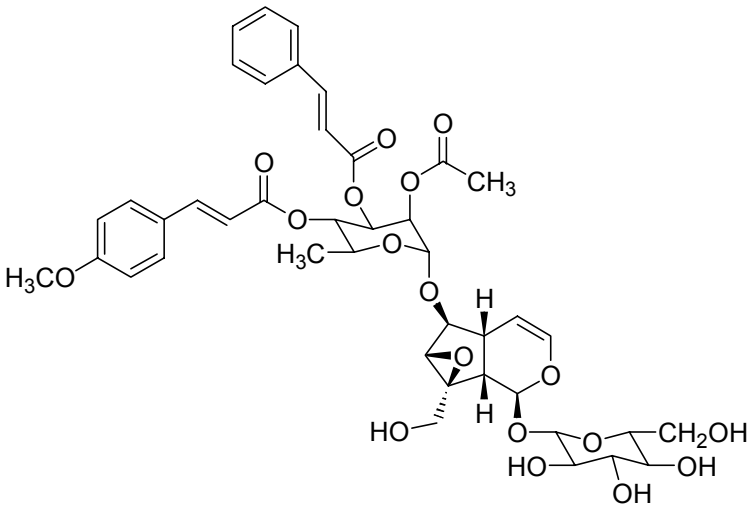


4.16 Penstemide



4.17 Serrulatoloside

Figure 4.5a. Known Cytotoxic Iridoid Glycosides.



4.18 Scrophuloside B_4

Figure 4.5b. Known Cytotoxic Iridoid Glycosides.

4.2 Results and Discussion

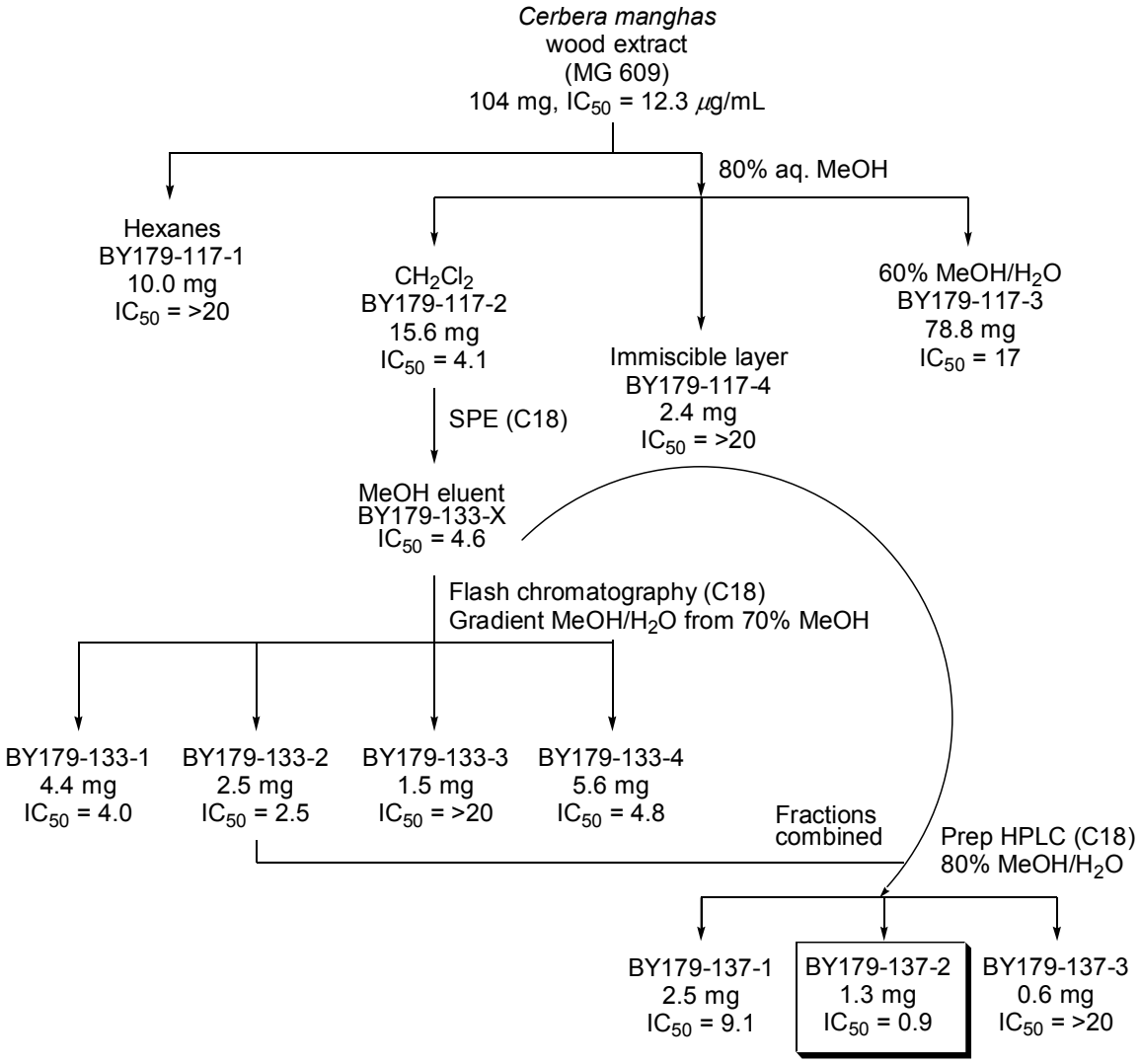
4.2.1 *Isolation of Compounds from Cerbera manghas*

4.2.1.1 Isolation of Cerbinal from the Bark and Wood of *Cerbera manghas*

As part of our ongoing ICBG program to isolate cytotoxic compounds from rainforest plants, the ethanol extract of the wood of *Cerbera manghas* was found to have an IC_{50} value of 12 μg/mL in the A2780 assay. Cerbinal was subsequently isolated from this extract, as indicated in Scheme 4.1. Cerbinal was also later obtained from the bark extract in a similar manner.

A sample of the wood extract (104 mg) was partitioned between hexanes and 80% methanol-water. The aqueous fraction was then diluted with water (to 60% methanol-water) and extracted with dichloromethane. An immiscible layer between these two fractions was also collected separately. All four layers were subjected to solvent removal by rotary evaporation and bioassay. The dichloromethane layer was the most active fraction, and that material was "purified" by dissolving it in methanol and passing it through a RP-C18 solid phase extraction cartridge. The MeOH eluent was further chromatographed using a flash system with a RP-C18 column and eluting with a 70% methanol-water to 100% methanol gradient, collecting four fractions. The second fraction displayed the most improved bioactivity, and it was purified by preparative

HPLC to obtain 1.3 mg of **4.1**. An additional 5.1 mg was obtained by repeating the entire fractionation process; therefore, the total yield of **4.1** was 6.4 mg.

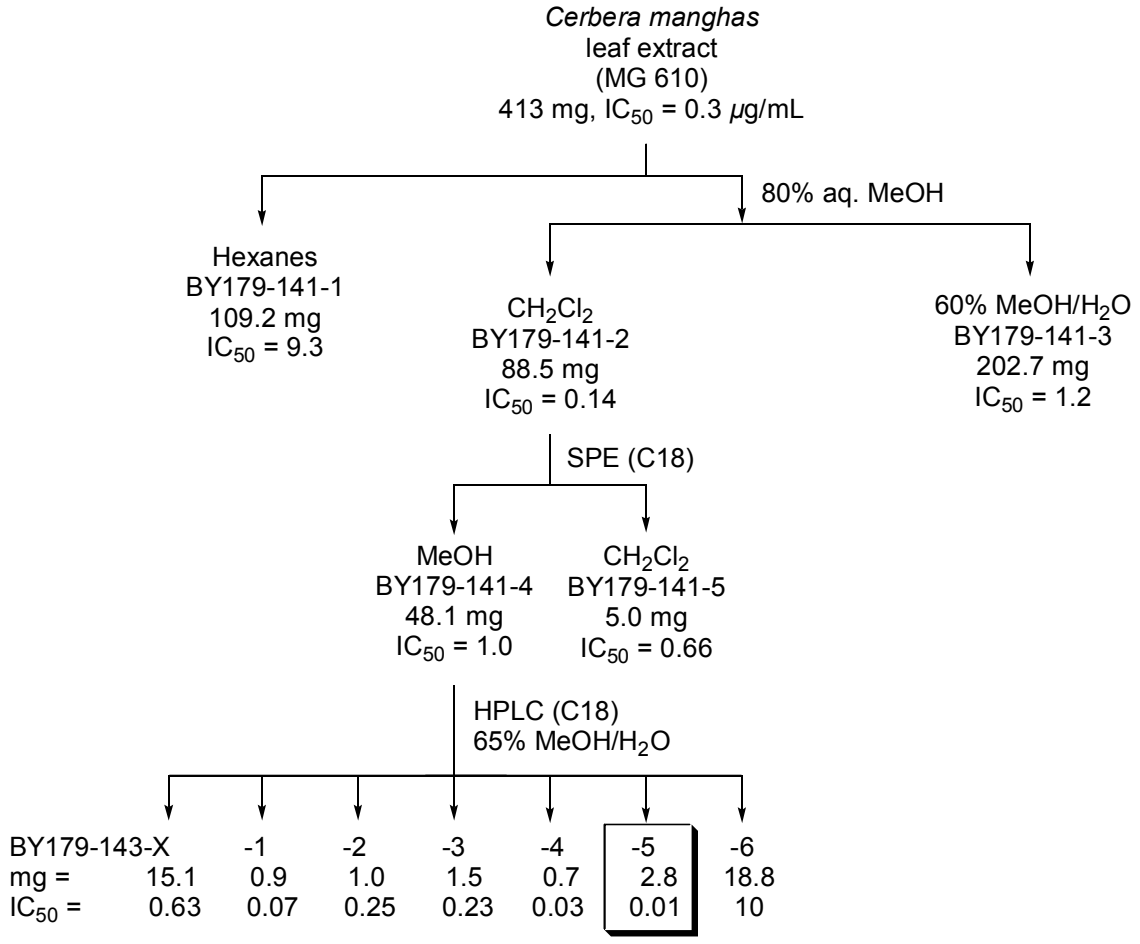


Scheme 4.1. Fractionation of *Cerbera manghas* (Apocynaceae) Wood.

4.2.1.2 Isolation of Neriifolin from the Leaves of *Cerbera manghas*

As part of our ongoing ICBG program to isolate cytotoxic compounds from rainforest plants, the ethanol extract of the leaves of *Cerbera manghas* was found to have an IC_{50} value of 0.3 μg/mL in the A2780 assay. Neriifolin was subsequently isolated from this extract, as indicated in Scheme 4.2.

A sample of the leaf extract (413 mg) was partitioned between hexanes and 80% methanol-water. The aqueous fraction was then diluted with water (to 60% methanol-water) and extracted with dichloromethane. All three layers were subjected to solvent removal by rotary evaporation and bioassay. The dichloromethane layer was the most active fraction, and that material was purified by dissolving it in methanol and passing it through a RP-C18 solid phase extraction cartridge. Dichloromethane was used to flush out remaining material that proved to be insoluble in methanol. The MeOH eluent was further chromatographed by HPLC with a RP-C18 column and eluting with 65% methanol-water, collecting six fractions. The fifth fraction (2.8 mg) displayed the most improved bioactivity, and it was determined to be pure **4.2**. An additional 0.7 mg was obtained by repeating the entire fractionation process; therefore, the total yield of **4.2** was 3.5 mg.



Scheme 4.2. Fractionation of *Cerbera manghas* (Apocynaceae) Leaves.

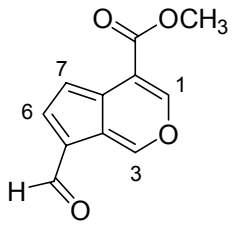
4.2.2 *Characterization of Compounds from Cerbera manghas*

4.2.2.1 Structure of Cerbinal (**4.1**)

During the HPLC purification process (guided by a UV/visible detector), it became apparent that **4.1** was a highly conjugated molecule with a high percentage of double bonds. The absorbance profile showed a strong peak at 252 nm, two moderate peaks at 280 and 288 nm, and two short, broad peaks at 327 and 428 nm. As expected for a molecule with an absorbance (428 nm) in the visible region of the light spectrum, **4.1** was a bright yellow solid.

^{1}H NMR confirmed the presence of an aromatic iridoid chromophore. Present in the spectrum were singlets for an aldehyde proton at δ 9.95 ppm and two separate protons on an aromatic heterocycle at δ 9.17 (H-1) and 8.51 (H-3) ppm. Also present were doublets at δ 7.94 (H-6, J = 3.2) and 7.13 (H-7, J = 3.2) ppm, representing neighboring protons on an unsaturated cyclopentane ring. All of these signals integrated to 1H. A singlet for the protons of a methyl ester, which integrated to 3H, was also present at δ 4.00 ppm.

The structure of cerbinal (**4.1**) was confirmed by comparison of experimental spectroscopic values to those reported in the literature. UV/Vis and ^{1}H NMR data are reported for cerbinal isolated from both *Cerbera manghas*[3] and *Gardenia jasminoides*,[3,27] and all values are nearly identical to the experimental data obtained. A six-step synthesis of cerbinal from (+)-genipin has also been reported in the literature.[28]



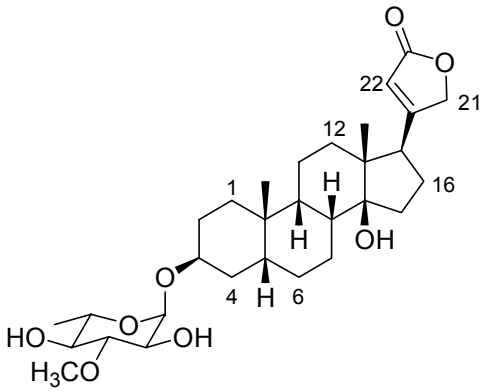
4.1 Cerbinal

Figure 4.6. Cerbinal from *Cerbera manghas*.

4.2.2.2 Structure of Neriifolin (**4.2**)

HRFABMS of **4.2** indicated a M+ molecular ion at 535.3262, suggesting a molecular formula of $C_{30}H_{46}O_8$. ^1H and ^{13}C NMR shifts also led to the conclusion that the compound was a glycoside derivative of digitoxigenin. In the proton spectrum, oxygenated methines were observed at δ 3.96 (m, H-3), 3.73 (dq, H-5'), 3.58 (dd, H-2'), 3.24 (t, H-3') and 3.14 (t, H-4') ppm, and an anomeric proton was observed at δ 4.85 ppm (d, H-1'). A methoxy singlet was observed δ 3.68 ppm, and methyl singlets were observed at δ 1.24 (H-6'), 0.96 (H-19) and 0.87 (H-18) ppm. A vinylic proton was observed as a triplet at δ 5.87 ppm (H-22), and additional doublets of doublets were observed at δ 4.98 (H-21β), 4.80 (H-21α) and 2.78 (H-17) ppm. All chemical shifts were within δ 0.02 ppm of their reported literature values.[29] Neriifolin was actually isolated nearly sixty years ago, but the NMR spectra were not reported until more recently.

In the carbon spectrum, an ester carbonyl was observed at δ 174.5 ppm (C-23), and a vinylic carbon was observed at δ 117.9 ppm (C-22). The other, quaternary vinylic carbon (C-20) is reported to occur at δ 174.6 ppm, overlapping with C-23, but it was not observed. The carbons of the 3-*O*-methyl rhamnose sugar were observed at δ 97.3 (C-1'), 84.7 (C-3'), 74.8 (C-4'), 73.0 (C-2'), 67.6 (C-5') and 17.6 (C-6') ppm. Additional oxygenated carbons were observed for C-14 (δ 85.6 ppm) and C-21 (δ 73.4 ppm), and additional methyl carbons were observed for C-19 (δ 24.0 ppm) and C-18 (δ 15.8 ppm). All chemical shifts were within δ 0.1 ppm of their reported literature values.[29]



4.2 Neriifolin

Figure 4.7. Neriifolin from *Cerbera manghas*.

4.2.3 *Biological Evaluation of Compounds from Cerbera manghas*

4.2.3.1 A2780 Screening of Cerbinal and Neriifolin

Cerbinal and neriifolin were tested for cytotoxicity against the A2780 ovarian cancer cell line. Repeated testings suggested that cerbinal had an IC_{50} value of approximately 1.0 μg/mL, while neriifolin had an IC_{50} of 0.01 μg/mL in the assay.

4.2.3.2 NCI Screening of Cerbinal (**4.1**)

Cerbinal (**4.1**) was tested in the 60-cell human tumor cancer screen at the National Cancer Institute. No subpanels were found to be either uniformly sensitive or uniformly resistant to **4.1**. However, the non-small cell lung cancer and renal cancer lines appeared to be largely sensitive, while the colon cancer and breast cancer lines appeared to be largely resistant. The most sensitive lines included non-small cell lung (A549/ATCC), ovarian (OVCAR-3) and renal (ACHN and CAKI-1) cancers. Other moderately sensitive lines included non-small cell lung (EKVX) and CNS cancers (SF-539), melanoma (MALME-3M), and ovarian (OVCAR-8), renal (SN12C and TK-10), and prostate (DU-145) cancers. Overall, cerbinal (**4.1**) appeared to be generally cytotoxic, and no further action was deemed appropriate by the NCI.

4.3 Experimental Section.

General Experimental Procedures. Solid phase extraction was performed with Supelco Discovery DSC-C_{18} tubes. HPLC was performed using Shimadzu LC-10A pumps coupled with a Varian Dynamax semipreparative C_{18} column (250 x 10.0 mm) and employed a Shimadzu SPD-M10A diode array detector. Optical rotation data was obtained on a PerkinElmer 241 polarimeter. UV spectra were measured on a Shimadzu UV-1201 spectrophotometer. Mass spectra were obtained on a JEOL JMS-HX-110 instrument. NMR spectra were obtained on a JEOL Eclipse (at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) spectrometer. Chemical shifts are given in δ (ppm) and coupling constants (J) are reported in Hz.

Plant Material. The bark, wood and leaves of *Cerbera manghas* (Apocynaceae) were collected by Stephan Rakotonandrasana on October 20, 2000 around the Natural Reserve of Zahamena in the province of Toamasina, Madagascar. Duplicate voucher specimens have been deposited at the Centre National d'Application des Recherches Pharmaceutiques (CNARP) and the Direction des Recherches Forestieres et Piscicoles Herbarium (TEF) in Antananarivo, Madagascar; the Missouri Botanical Garden in St. Louis, Missouri (MO); and the Museum National d'Histoire Naturelle in Paris, France (P).

Extract Preparation. The bark, wood and leaves of *Cerbera manghas* were dried, ground and extracted with ethanol in Madagascar. This yielded extracts labeled MG 608, (1.47 g), MG 609 (1.06 g) and MG 610 (1.02 g), respectively.

Cytotoxicity Bioassay. The A2780 ovarian cancer cell line cytotoxicity assay was performed at Virginia Polytechnic Institute and State University as previously reported.[30]

Bioassay-guided Fractionation and Isolation of Cerbinal. The crude bioactive extract MG 609 (IC_{50} = 12 μg/mL, 104 mg) was partitioned between hexanes (100 mL) and MeOH-H_2O (4:1, 2 x 100 mL). Water was added to the MeOH-H_2O fraction to yield a MeOH-H_2O solution (3:2) that was subsequently partitioned with CH_2Cl_2. An immiscible layer between these two fractions formed and was collected separately. Evaporation of the organic solvents yielded a single bioactive (IC_{50} = 4.1 μg/mL) fraction of 15.6 mg (CH_2Cl_2). That fraction was passed through a RP-C18 solid phase extraction cartridge, and the MeOH eluent was further chromatographed using a flash system with a RP-C18 column and a MeOH-H_2O (7:3) to MeOH elution gradient. Four fractions were collected, but the second fraction (2.5 mg, IC_{50} = 2.5 μg/mL) displayed the most improved bioactivity. Final purification by preparative HPLC yielded **4.1** (1.3 mg**,** IC_{50} = 0.9 μg/mL). Repeating the entire fractionation process, but beginning with approximately 700 mg of crude extract, led to the isolation of an additional 5.1 mg. The total yield of **4.1** was therefore 6.4 mg.

Bioassay-guided Fractionation and Isolation of Neriifolin. The crude bioactive extract MG 610 (IC_{50} = 0.3 μg/mL, 413 mg) was partitioned between hexanes (200 mL) and MeOH-H_2O (4:1, 2 x 200 mL). Water was added to the MeOH-H_2O fraction to yield a MeOH-H_2O solution (3:2) that was subsequently partitioned with CH_2Cl_2. Evaporation of the organic solvents yielded a potent bioactive (IC_{50} = 0.14 μg/mL) fraction of 88.5 mg (CH_2Cl_2) and a moderate bioactive (IC_{50} = 1.2 μg/mL) fraction of 202.7 mg (MeOH-H_2O). The CH_2Cl_2 fraction was dissolved in MeOH and passed through a RP-C18 solid phase extraction cartridge, flushing with CH_2Cl_2. The MeOH fraction was slightly less active (IC_{50} = 1.0 μg/mL), but contained a greater quantity of material (48.1 mg) than the CH_2Cl_2 fraction. The MeOH fraction was further chromatographed by HPLC (RP-C18, 65% methanol-water), and six fractions were collected. The fifth fraction (2.8 mg, IC_{50} = 0.01 μg/mL) displayed the most improved bioactivity, and it was determined to be pure **4.2**. Repeating the entire fractionation process, but beginning with 107 mg of crude extract, led to the isolation of an additional 0.7 mg. The total yield of **4.2** was therefore 3.5 mg.

Cerbinal (4.1): bright orange-yellow solid; UV (MeOH) λ_{max} 428, 327, 288, 280, 252 nm, Lit. λ_{max} 428, 326, 288, 277, 249 nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.95 (1H, s, -CHO), 9.17 (1H, s, H-1), 8.51 (1H, s, H-3), 7.94 (1H, d, J = 3.2, H-6), 7.13 (1H, d, J = 3.2, H-7), 4.00 (3H, s, -$COOCH_3$); ^{13}C NMR (CD_3OD, 125 MHz) δ 185.5 (-COH), 164.6 (-COOR), 150.1 (C-3), 149.0 (C-1), 148.5 (C-5), 131.1 (C-6), 125.4 (C-7), 124.3 (C-4), 114.8 (C-8), 113.2 (C-9), 51.5 (-$ROOCH_3$).

Neriifolin (4.2): white solid; $[\alpha]^{22}_D$ –16.7^o (*c* 0.06, CH_3OH); ^{1}H NMR (CD_3OD, 500 MHz) δ 5.87 (1H, t, H-22), 4.98 (1H, dd, J = 18.2, H-21β), 4.85 (1H, d, J = 4.4, H-1'), 4.80 (1H, dd, H-21α), 3.96 (1H, m, H-3), 3.73 (1H, dq, H-5'), 3.68 (3H, s, -OCH_3), 3.58 (1H, dd, H-2'), 3.24 (1H, t, H-3'), 3.14 (1H, t, H-4'), 2.78 (1H, dd, H-17), 2.08-2.19 (2H, m, H-16α,β), 1.25 (3H, d, H-6'), 0.96 (3H, s, H-19), 0.87 (3H, s, H-18); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.4 (C-23), 117.9 (C-22), 97.3 (C-1'), 85.6 (C-14), 84.7 (C-3'), 74.8 (C-4'), 73.5 (C-21), 73.4 (C-3), 73.0 (C-2'), 67.6 (C-5'), 60.7 (-OCH_3), 51.0 (C-17), 49.7 (C-13), 41.9 (C-8), 40.1 (C-12), 37.0 (C-5), 35.8 (C-9), 35.3 (C-10), 33.3 (C-15),

30.7 (C-1), 30.0 (C-4), 26.9 (C-16), 26.6 (C-2), 26.6 (C-6), 24.0 (C-19), 21.4 (C-11), 21.3 (C-7), 17.6 (C-6'), 15.8 (C-18); HRFABMS *m/z* 535.3262 $[M+H]^+$ (calcd for $C_{30}H_{47}O_8$, 535.3271).

NCI 60-Cell Cancer Assay Data. The tumor cell line subpanels are identified as follows: I (leukemia); II (non-small cell lung); III (colon); IV (CNS); V (melanoma); VI (ovarian); VII (renal); VIII (prostate); IX (breast). The subpanel and individual cell-line identifiers are listed, along with the corresponding negative log GI_{50}, TGI, and LC_{50} values (molar) for cerbinal (**4.1**) [I] CCRF-CEM (5.65, >4.00, >4.00), HL-60(TB) (5.90, 5.45, >4.00), K-562 (<5.65, 5.24, >4.00), MOLT-4 (6.30, 5.50, >4.00), RPMI-8226 (5.33, >4.00, >4.00), SR (6.34, n/a, >4.00) [II] A549/ATCC (6.54, 6.13, 5.56), EKVX (6.04, 5.51, 5.00), HOP-62 (5.82, 5.37, 4.81), HOP-92 (5.49, 4.77, >4.00), NCI-H226 (5.56, 5.22, 4.68), NCI-H23 (6.16, 5.58, n/a), NCI-H322M (5.64, 5.21, 4.52), NCI-H460 (5.69, 5.11, >4.00), NCI-H522 (6.12, 4.81, >4.00) [III] COLO 205 (5.19, 4.40, >4.00), HCT-116 (5.86, 5.22, 4.50), HCT-15 (5.69, 5.25, 4.40), HT29 (5.45, >4.00, >4.00), KM12 (5.41, 4.33, 4.00), SW-620 (5.41, 4.31, >4.00) [IV] SF-268 (5.96, 5.41, 4.62), SF-295 (5.71, 5.25, >4.00), SF-539 (5.87, 5.43, 4.94), SNB-19 (5.43, 4.82, 4.28), SNB-75 (5.32, 4.37, >4.00), U251 (5.55, 4.95, 4.32) [V] LOX IMVI (5.84, 5.38, 4.59), MALME-3M (5.72, 5.39, 5.06), M14 (5.61, 5.20, 4.43), SK-MEL-2 (5.27, >4.00, >4.00), SK-MEL-28 (5.47, 4.80, 4.03), UACC-257 (5.50, 5.18, 4.46), UACC-62 (5.66, 5.30, 4.74) [VI] IGROV1 (5.33, >4.00, >4.00), OVCAR-3 (6.67, 6.14, 5.04), OVCAR-4 (5.57, 4.83, 4.05), OVCAR-5 (5.39, 4.69, >4.00), OVCAR-8 (5.73, 5.35, 4.89), SK-OV-3 (5.33, >4.00, >4.00) [VII] 786-0 (5.83, 5.28, 4.56), A498 (5.55, 5.14, >4.00), ACHN (6.41, 5.79, 5.15), CAKI-1 (6.20, 5.63, 5.12), RXF 393 (5.63, >4.00, >4.00), SN12C (6.29, 5.60, 4.13), TK-10 (6.31, 5.29, 4.20), UO-31 (5.76, >4.00, >4.00) [VIII] PC-3 (5.59, 4.22, >4.00), DU-145 (6.37, 5.66, 4.96) [IX] MCF7 (5.73, 4.49, >4.00), NCI/ADR-RES (5.86, 5.37, 4.55), MDA-MB-231/ATCC (5.30, 4.61, >4.00), HS 578T (5.47, >4.00, >4.00), MDA-MB-435 (5.50, 4.96, 4.24), BT-549 (5.84, 5.34, 4.24), T-47D (5.50, 5.14, >4.00). This data from the NCI is also presented as mean graphs in Figure 4.8. Dose response curves for the various cell lines are presented in Figure 4.9.

Figure 4.8. NCI Mean Graphs for Cerbinal.

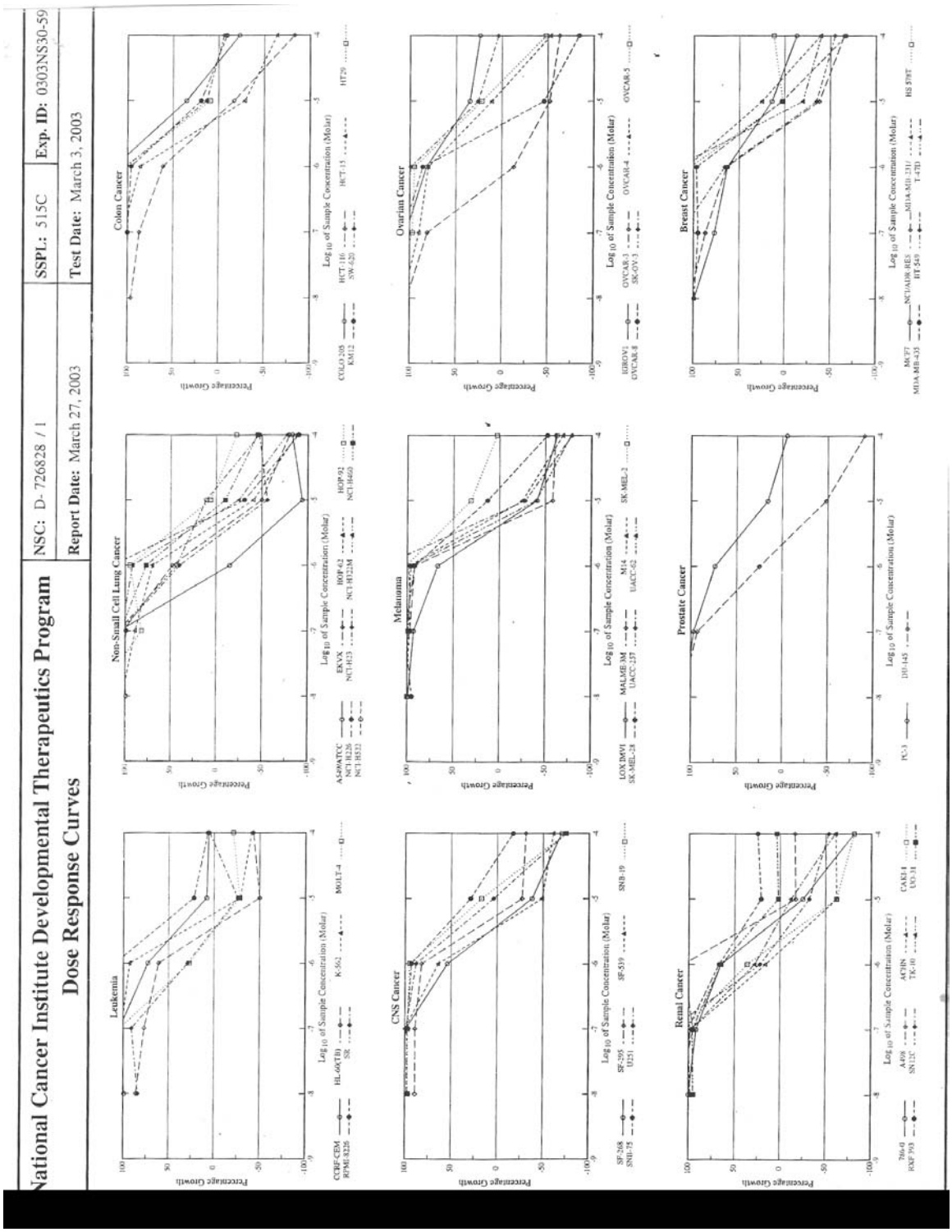


Figure 4.9. NCI Dose Response Curves for Cerbinal.

References

(1) Cheenpracha, S.; Karalai, C.; Rat-a-pa, Y.; Ponglimanont, C.; Chantrapromma, K. New Cytotoxic Cardenolide Glycoside from the Seeds of *Cerbera manghas*. *Chem. Pharm. Bull.* **2004,** *52*, 1023-1025.

(2) Abe, F.; Yamauchi, T. Studies on *Cerbera*. I. Cardiac Glycosides in the Seeds, Bark, and Leaves of *Cerbera manghas* L. *Chem. Pharm. Bull.* **1977,** *25*, 2744-2748.

(3) Abe, F.; Okabe, H.; Yamauchi, T. Studies on *Cerbera*. II. Cerbinal and Its Derivatives, Yellow Pigments in the Bark of *Cerbera manghas* L. *Chem. Pharm. Bull.* **1977,** *25*, 3422-3424.

(4) Yamauchi, T.; Abe, F.; Wan, A. S. C. Cardenolide Monoglycosides from the Leaves of *Cerbera odollam* and *Cerbera manghas* (*Cerbera*. III). *Chem. Pharm. Bull.* **1987,** *35*, 2744-2749.

(5) Yamauchi, T.; Abe, F.; Wan, A. S. C. Studies on Cerbera. IV. Polar Cardenolide Glycosides from the Leaves of *Cerbera odollam* and *Cerbera manghas*. *Chem. Pharm. Bull.* **1987,** *35*, 4813-4818.

(6) Yamauchi, T.; Abe, F.; Wan, A. S. C. Studies on Cerbera. V. Minor Glycosides of 17*α*-Digitoxigenin from the Stems of Genus *Cerbera*. *Chem. Pharm. Bull.* **1987,** *35*, 4993-4995.

(7) Abe, F.; Yamauchi, T.; Wan, A. S. C. Lignans Related to Olivil from Genus *Cerbera* (*Cerbera*. VI). *Chem. Pharm. Bull.* **1988,** *36*, 795-799.

(8) Abe, F.; Yamauchi, T.; Wan, A. S. C. Sesqui-, Sester- and Trilignans from Stems of *Cerbera manghas* and *C. odollam*. *Phytochemistry* **1988,** *27*, 3627-3631.

(9) Abe, F.; Yamauchi, T.; Wan, A. S. C. Cerberalignans J-N, Oligolignans from *Cerbera manghas*. *Phytochemistry* **1989,** *28*, 3473-3476.

(10) Yamauchi, T.; Abe, F.; Wan, A. S. C. 10-*O*-Benzoyltheveside and 10-Dehydrogeniposide from the Leaves of *Cerbera manghas*. *Phytochemistry* **1990,** *29*, 2327-2328.

(11) Abe, F.; Yamauchi, T.; Wan, A. S. C. Normonoterpenoids and Their Allopyranosides from the Leaves of *Cerbera* Species (Studies on *Cerbera*. VIII). *Chem. Pharm. Bull.* **1989,** *37*, 2639-2642.

(12) Abe, F.; Yamauchi, T. 10-Carboxyloganin, Normonoterpenoid Glucosides and Dinormonoterpenoid Glucosides from the Leaves of *Cerbera manghas* (Studies on *Cerbera*. 10). *Chem. Pharm. Bull.* **1996,** *44*, 1797-1800.

(13) Inouye, H.; Nishimura, T. Irridoid Glucosides of *Cerbera manghus*. *Phytochemistry* **1972,** *11*, 1852.

(14) Sakushima, A.; Nishibe, S.; Hisada, S. A New Flavonol Glycoside from *Cerbera manghas*. *Phytochemistry* **1980,** *19*, 712-713.

(15) Sakushima, A.; Hisada, S.; Ogihara, Y.; Nishibe, S. Studies on the Constituents of Apocynaceae Plants. Gas Chromatography-Mass Spectrometric Determination of New Flavonoid Triglycosides from the Leaves of *Cerbera manghas* L. (2). *Chem. Pharm. Bull.* **1980,** *28*, 1219-1223.

(16) Chang, L. C.; Gillis, J. J.; Bhat, K. P. L.; Luyengi, L.; Farnsworth, N. R.; Pezzuto, J. M.; Kinghorn, A. D. Activity-Guided Isolation of Constituents of *Cerbera manghas* with Antiproliferative and Antiestrogenic Activities. *Bioorg. Med. Chem. Lett.* **2000,** *10*, 2431-2434.

(17) Dewick, P. M. *Medicinal Natural Products: A Biosynthetic Approach*; John Wiley & Sons Ltd.: Chichester, 1997.

(18) Trost, B. M.; Balkovec, J. M.; Mao, M. K.-T. A Total Synthesis of Plumericin, Allamcin, and Allamandin. 2. A Biomimetic Strategy. *J. Am. Chem. Soc.* **1986,** *108*, 4974-4983.

(19) Kardono, L. B. S.; Tsauri, S.; Padmawinata, K.; Pezzuto, J. M.; Kinghorn, A. D. Cytotoxic Constituents of the Bark of *Plumeria rubra* Collected in Indonesia. *J. Nat. Prod.* **1990,** *53*, 1447-1455.

(20) Ueda, S.; Iwahashi, Y.; Tokuda, H. Production of Anti-Tumor-Promoting Iridoid Glucosides in *Genipa americana* and Its Cell Celtures. *J. Nat. Prod.* **1991,** *54*, 1677-1680.

(21) Kapadia, G. J.; Sharma, S. C.; Tokuda, H.; Nishino, H.; Ueda, S. Inhibitory effect of iridoids on Epstein-Barr virus activation by a short-term in vitro assay for anti-tumor promoters. *Cancer Lett.* **1996,** *102*, 223-226.

(22) Galvez, M.; Martin-Cordero, C.; Ayuso, M. J. Iridoids as DNA Topoisomerase I Poisons. *J. Enzyme Inhib. and Med. Chem.* **2005,** *20*, 389-392.

(23) Wysokinska, H.; Skrzypek, Z.; Kunert-Radek, J. Studies on Iridoids of Tissue Cultures of *Penstemon serrulatus*: Isolation and Their Antiproliferative Properties. *J. Nat. Prod.* **1992,** *55*, 58-63.

(24) Konoshima, T.; Takasaki, M.; Tokuda, H.; Nishino, H. Cancer chemopreventative activity of an iridoid glycoside, 8-acetylharpagide, from *Ajuga decumbens*. *Cancer Lett.* **2000,** *157*, 87-92.

(25) Fukuyama, Y.; Minoshima, Y.; Kishimoto, Y.; Chen, I.-S.; Takahashi, H.; Esumi, T. Iridoid Glucosides and *p*-Coumaroyl Iridoids from *Viburnum luzonicum* and Their Cytotoxicity. *J. Nat. Prod.* **2004,** *67*, 1833-1838.

(26) Fukuyama, Y.; Minoshima, Y.; Kishimoto, Y.; Chen, I.-S.; Takahashi, H.; Esumi, T. Cytotoxic Iridoid Aldehydes from Taiwanese *Viburnum luzonicum*. *Chem. Pharm. Bull.* **2005,** *53*, 125-127.

(27) Ohashi, H.; Tsurushima, T.; Ueno, T.; Fukami, H. Cerbinal, a Pseudoazulene Iridoid, as a Potent Antifungal Compound Isolated from *Gardenia jasminoides* Ellis. *Agric. Biol. Chem.* **1986,** *50*, 2655-2657.

(28) Ge, Y.; Isoe, S. An Efficient Synthesis of Cerbinal, a 10 π Aromatic Iridoid. *Chem. Lett.* **1992**, 139-140.

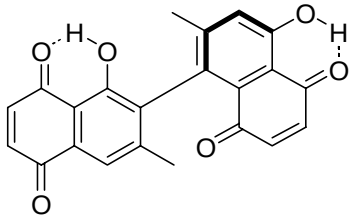
(29) Jolad, S. D.; Hoffmann, J. J.; Cole, J. R.; Tempesta, M. S.; Bates, R. B. 3'-*O*-Methylevomonside: A New Cytotoxic Cardiac Glycoside from *Thevetia ahouia* A. DC (Apocynaceae). *J. Org. Chem.* **1981,** *46*, 1946-1947.

(30) Louie, K. G.; Behrens, B. C.; Kinsella, T. J.; Hamilton, T. C.; Grotzinger, K. R.; McKoy, W. M.; Winker, M. A.; Ozols, R. F. Radiation Survival Parameters of Antineoplastic Drug-sensitive and -resistant Human Ovarian Cancer Cell Lines and Their Modification by Buthionine Sulfoximine. *Cancer Res.* **1985,** *45*, 2110-2115.

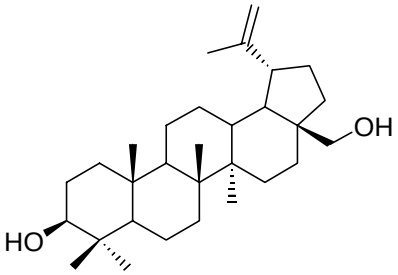
V. ISODIOSPYRIN, A KNOWN NAPHTHOQUINONE DIMER, AND BETULIN, A KNOWN TRITERPENE, ISOLATED FROM A *CORDIA* SPECIES (BORAGINACEAE) FROM SURINAME

5.1 Introduction

A stem extract of a *Cordia* species (Boraginaceae) from Suriname displayed moderate cytotoxicity in the A2780 human ovarian cancer cell line assay. Bioassay-guided fractionation led to the isolation of isodiospyrin (**5.1**), a known naphthoquinone dimer from species of *Euclea* and *Diospyros* (Ebenaceae). This is the first report of isodiospyrin obtained from this plant family. Bioassay-guided fractionation also led to the isolation of betulin (**5.2**), a known triterpene. Various NMR techniques and mass spectroscopic methods were used to determine the structures.



5.1 (*R*)-Isodiospyrin



5.2 Betulin

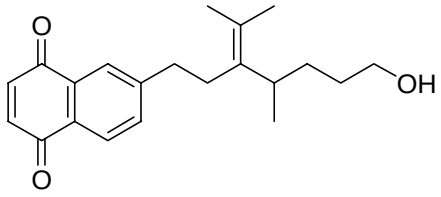
Figure 5.1. Compounds from a Species of *Cordia*.

5.1.1 *Previous Investigations of Cordia Species*

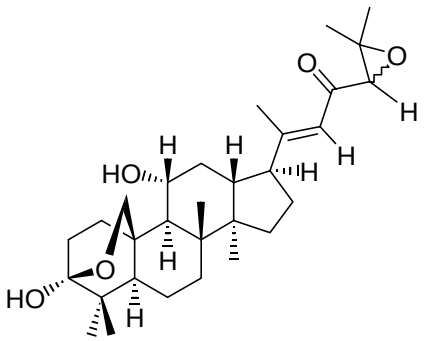
Cordia is a genus consisting of many different trees and shrubs found in tropical areas throughout Central and South America.[1] They are known for their practical use as sources of timber, but more and more, specific species are being investigated for their phytochemical, and possibly medicinal, constituents.

The size of the *Cordia* genus is reflected in the number of different species that have been studied for secondary metabolites. The heartwood of *Cordia alliodora* is known to be the source of geranylated quinones,[2] and the leaves of the same species contain oleanoic acid triterpene derivatives with ant-repellent activity.[3] Meroterpenoid

naphthoquinones, including cordiaquinone A (**5.3**), are common in the roots of *Cordia corymbosa*,[4,5] and other terpenoid quinones can be found in the heartwood of both *Cordia elaeagnoides*[6] and *Cordia millenii*.[1] The roots of *Cordia obliqua* have led to the isolation of a triterpene glycoside,[7] and triterpene aglycons, including cordialin A (**5.4**), have been isolated from the leaves of both *Cordia spinescens*[8] and *Cordia verbenacea*.[9]



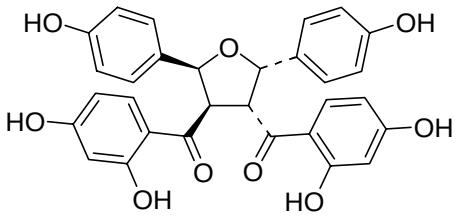
5.3 Cordiaquinone A



5.4 Cordialin A

Figure 5.2. Compounds from *Cordia corymbosa* and *Cordia verbenacea*.

Cordia verbenacea is one of a handful of species from this genus that have been examined for biological activity. The crude leaf extract of *C. verbenacea* has demonstrated anti-inflammatory activity in various rat experiments.[10] Meroterpenoid naphthoquinones from the roots of both *Cordia curassavica* and *Cordia linnaei* have shown antifungal and larvicidal activity.[11,12] A series of polyphenols, including cordigone (**5.5**), from the stem bark of *Cordia goetzei* have also shown antifungal activity.[13]



5.5 Cordigone

Figure 5.3. Cordigone from *Cordia goetzei*.

5.1.2 *Chemical Investigation of a Cordia Species*

As part of a former search for cytotoxic natural products from tropical rainforests in Suriname, through the ICBG program, we obtained an ethyl acetate stem extract of a species of *Cordia* for phytochemical investigation, which was found to be active in the A2780 ovarian cancer cytotoxicity assay. Bioassay-guided fractionation yielded two compounds of interest, the known naphthoquinone dimer (*R*)-isodiospyrin (**5.1**) and the known triterpene betulin (**5.2**). Here we describe the isolation and structure elucidation of these cytotoxic compounds (Figure 5.1).

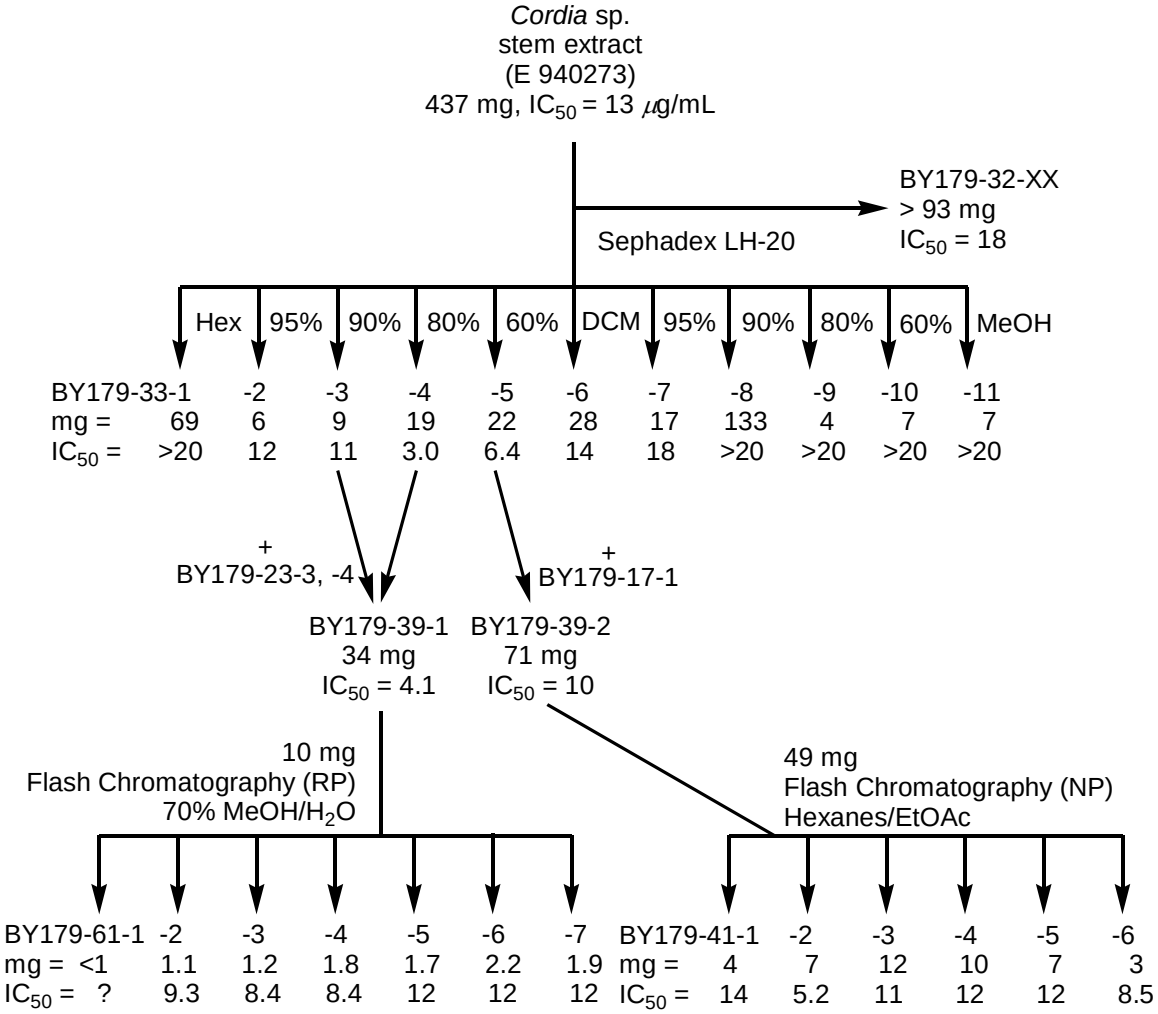
5.2 Results and Discussion

5.2.1 *Isolation of Compounds from a Cordia Species*

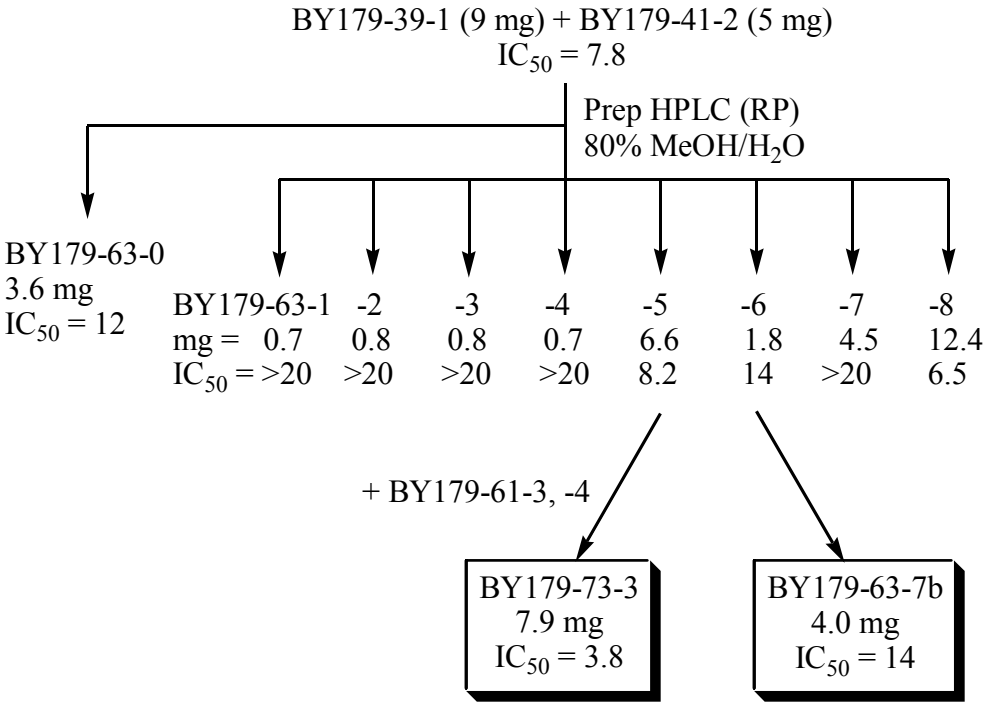
As part of our ongoing ICBG program to isolate cytotoxic compounds from rainforest plants, the ethyl acetate extract of the stems of a species of *Cordia* was found to have an IC_{50} value of 13 μg/mL in the A2780 assay. Isodiospyrin (**5.1**) and betulin (**5.2**) were subsequently isolated from this extract.

A sample of the stem extract (589 mg) was initially partitioned between hexanes and 80% methanol-water. The aqueous fraction was then diluted with water (to 60% methanol-water) and extracted with dichloromethane. All three layers were subjected to solvent removal by rotary evaporation and bioassay. The dichloromethane layer was the most active fraction, and that material was further chromatographed through an open column, using Sephadex LH-20 as a solid phase and CH_2Cl_2-MeOH as a mobile phase. All ten fractions that were collected were found to be inactive (IC_{50} > 20 μg/mL).

The fractionation process was repeated with a fresh sample of crude extract (437 mg), as indicated in Schemes 5.1 and 5.2. Sephadex LH-20 separation (collecting eleven fractions from 100% hexanes to 100% MeOH) led to three fractions (totaling 50 mg) with IC_{50} values of 11 μg/mL or less. The first two fractions (eluted with 90% and 80% hexanes in CH_2Cl_2) were combined and subjected to reversed-phase flash chromatography with 70% MeOH-H_2O. The third fraction (eluted with 60% hexanes in CH_2Cl_2) was subjected to normal-phase flash chromatography with 80% hexanes-ethyl acetate. The most active fractions from these separate processes were combined and subjected to reversed-phase HPLC with 80% MeOH-H_2O (collecting nine fractions). The fifth fraction displayed the strongest cytotoxicty, and it was purified by HPLC to obtain 7.9 mg of **5.1**. The seventh fraction was also purified by HPLC to obtain 4.2 mg of **5.2**.



Scheme 5.1. Fractionation of a *Cordia* Species (Boraginaceae).



Scheme 5.2. Purification of Isodiospyrin and Betulin from a *Cordia* Species.

5.2.2 *Characterization of Compounds from a Cordia Species*

5.2.2.1 Structure of Isodiospyrin (**5.1**)

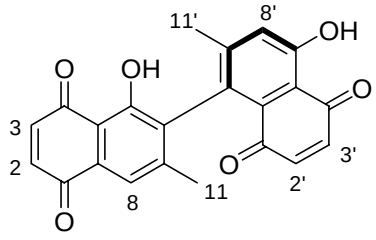
Compound **5.1** was isolated as a red-orange solid with a molecular formula of $C_{22}H_{14}O_6$, based upon HRFABMS. Consistent with the brightly colored nature of the solid, the UV absorption spectrum indicated a λ_{max} value at 431 nm in the visible region of the light spectrum. Also present were λ_{max} values at 252 and 219 nm.

The ^{1}H NMR spectrum confirmed that **5.1** was almost entirely aromatic, with all but two proton signals present at $\delta > 6.0$ ppm. Present in the spectrum were two singlets at δ 12.43 and 12.05 ppm for a pair of phenolic –OH protons that each formed an intramolecular hydrogen bond with a carbonyl oxygen. Six aromatic protons were observed – two as singlets at δ 7.61 and 7.30 ppm and four as doublets at δ 6.95, 6.93, 6.91 and 6.72 ppm, all with J coupling constants of 10 Hz. Two sets of methyl protons were also present at δ 2.03 and 2.01 ppm.

The pairing of signals in the ^{13}C NMR spectrum reinforced the notion of **5.1** as an aromatic, unsymmetrical dimer. Again, all but two carbon signals were present at $\delta >$ 110 ppm. Only methyl shifts at δ 20.7 and 20.5 ppm appeared to belong to non-sp^2

carbons. Four signals belonging to carbonyl carbons that have been shifted upfield due to their presence in an α,β-unsaturated ketone (or quinone) system were present at δ 190.4, 190.1, 185.0 and 184.5 ppm. Two signals belonging to aromatic oxygenated carbons were present at δ 162.0 and 158.7 ppm. Twelve other shifts for aromatic carbons appeared in the spectrum between δ 140.2 and 113.2 ppm. A DEPT spectrum confirmed the presence of only CH and CH_3 groups in **5.1**. Overall, all ^{13}C NMR chemical shifts were within ± 1 ppm of those reported in the literature.[14]

The compound was determined to be a naphthoquinone dimer of 7-methyljuglone, 1',4-dihydroxy-2,3'-dimethyl[1,2'-binaphthalene]-5,5',8,8'-tetrone, more commonly known as isodiospyrin (Figure 5.2). The molecule was first discovered from the stem and stem-bark of *Diospyros chloroxylon* in 1967.[15] Since that time, it has been reported to be present in miscellaneous plant parts from many *Diospyros* and *Euclea* species, all members of the family Ebenaceae.[16] There are no prior reports of isodiospyrin obtained from the Boraginaceae family. Isodiospyrin occurs naturally in its $(R)_{axial}$-form, and the presence of the identical atropisomer was confirmed by comparison of the levorotatory optical rotation to literature values of synthetic isodiospyrin and other axially chiral binapthoquinones.[17,18]



5.1 (*R*)-Isodiospyrin

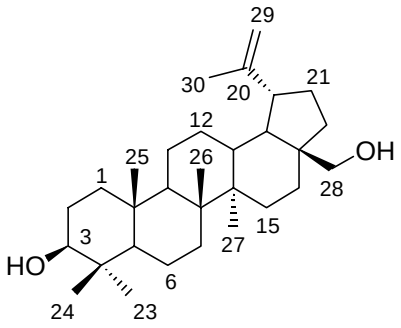
Figure 5.4. Isodiospyrin from a *Cordia* Species.

5.2.2.2 Structure of Betulin (**5.2**)

Compound **5.2** was isolated as a white solid with a molecular formula of $C_{30}H_{50}O_2$, based on HRFABMS. The ^1H NMR spectrum indicated the presence of six methyl groups at δ 1.67, 0.99, 0.97, 0.96, 0.80 and 0.75 ppm. A doublet of doublets was

present at δ 3.17 ppm, which is characteristic for an α-oriented hydrogen at C-3 of a 3β-hydroxy triterpene. Doublets for geminal protons at δ 4.70 and 4.58 ppm, along with the methyl group at δ 1.67 ppm, suggested that **5.2** was a lupeol-type triterpene derivative. Another pair of doublets at δ 3.79 and 3.33 ppm, rather than a seventh methyl singlet around δ 0.8 ppm, confirmed the presence of a second hydroxy group at C-28.

The ^{13}C NMR spectrum further established **5.2** as a lupeol-type triterpene derivative. The characteristic pair of sp^2 carbons comprising the double bond of lupeol[19] were observed as shifts at δ 150.6 and 109.8 ppm. Oxygenated carbon shifts for C-3 and C-28 were observed at δ 79.2 and 60.6 ppm, respectively. Compound **5.2** was therefore determined to be the known structure 20(29)-lupene-3,28-diol, more commonly known as betulin (Figure 5.3). Experimental NMR data was compared to that reported in the literature,[20,21] and all ^{13}C shifts were within ± 0.3 ppm.



5.2 Betulin

Figure 5.5. Betulin from a Cordia Species.

5.2.3 *Biological Evaluation of Compounds from a Cordia Species*

Isodiospyrin and betulin were tested for cytotoxicity against the A2780 ovarian cancer cell line. Repeated testings suggested that isodiospyrin had an IC_{50} value of approximately 3.8 μg/mL, while betulin had an IC_{50} of 14 μg/mL in the assay.

5.3 Experimental Section.

General Experimental Procedures. HPLC was performed using either Shimadzu LC-8A pumps coupled with a Varian Dynamax preparative C_{18} column (250 x 21.4 mm) or Shimadzu LC-10A pumps coupled with a Varian Dynamax semipreparative C_{18} column

(250 x 10.0 mm). Both systems employed a Shimadzu SPD-M10A diode array detector. Optical rotation data was obtained on a PerkinElmer 241 polarimeter. UV spectra were measured on a Shimadzu UV-1201 spectrophotometer. Mass spectra were obtained on a JEOL JMS-HX-110 instrument. NMR spectra were obtained on a JEOL Eclipse (at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) spectrometer. Chemical shifts are given in δ (ppm) and coupling constants (J) are reported in Hz.

Plant Material. The stems of a *Cordia* species (Boraginaceae) were collected by ethnobotanists from Conservation International on July 28, 1994 in Suriname. Duplicate voucher specimens have been deposited in the National Herbarium of Suriname, Paramaribo, Suriname.

Extract Preparation. The stems of a *Cordia* species were dried, ground and extracted with ethyl acetate in Suriname. This yielded an extract labeled E 940273 (1-2 g).

Cytotoxicity Bioassay. The A2780 ovarian cancer cell line cytotoxicity assay was performed at Virginia Polytechnic Institute and State University as previously reported.[22]

Bioassay-guided Fractionation and Isolation of Isodiospyrin and Betulin. The crude bioactive extract E 940273 (IC_{50} = 13 μg/mL, 437 mg) was dissolved in hexanes and fractionated with Sephadex LH-20 through an open column. Eleven fractions were collected, including the three most active (Fraction 3: 9 mg, IC_{50} = 11 μg/mL; Fraction 4: 19 mg, IC_{50} = 3.0 μg/mL; and Fraction 5: 22 mg, IC_{50} = 6.4 μg/mL). Fractions 3 and 4 were combined, and 10 mg of this sample were subjected to RP-C18 flash chromatography, eluting with MeOH-H_2O (7:3). The two most cytotoxic fractions lost activity (IC_{50} = 8.4 μg/mL) and consisted of a total of only 3 mg. In a parallel separation, Fraction 5 was subjected to NP-Si flash chromatography, eluting with hexanes-ethyl acetate (4:1). The most cytotoxic fraction (7 mg, IC_{50} = 5.2 μg/mL) was combined with 9 mg remaining from Fraction 3/4. Preparative RP-C18 HPLC with MeOH:H_2O (4:1) led to nine new fractions. Fraction 5 was purified to yield **5.1** (7.9 mg, IC_{50} = 3.8 μg/mL). Fraction 7 was purified to yield **5.2** (4.2 mg, IC_{50} = 14 μg/mL).

Isodiospyrin (5.1): reddish-orange solid; $[\alpha]^{23}_{D}$ –141° (*c* 0.02, $CHCl_3$), Lit. $[\alpha]^{22}_{D}$ –150° ($CHCl_3$);[17] UV λ_{max} 431, 252, 219 nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.43 (1H, s, 5'-OH), 12.05 (1H, s, 5-OH), 7.61 (1H, s, H-8), 7.30 (1H, s, H-6'), 6.95 (1H, d, *J* = 10.3, H-3), 6.93 (1H, d, *J* = 10.4, H-2), 6.91 (1H, d, *J* = 10.1, H-3'), 6.72, (1H, d, *J* = 10.1, H-2'), 2.03 (3H, s, 7'-CH_3), 2.01 (3H, s, 7-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.4 (C-4'), 190.1 (C-4), 185.0 (C-1'), 184.5 (C-1), 162.0 (C-5'), 158.7 (C-5), 148.2 (C-7'), 145.5 (C-7), 140.2 (C-2'), 139.6 (C-2), 138.8 (C-3'), 137.7 (C-3), 135.2 (C-6), 130.3 (C-8'), 128.9 (C-9'), 128.6 (C-9), 125.8 (C-6'), 121.4 (C-8), 114.3 (C-10'), 113.2 (C-10), 20.7 (C-11'), 20.5 (C-11); EIMS *m/z* 374 $[M]^+$ (100), 359 (76), 345 (14), 331 (20), 319 (13), 189 (19); HRFABMS *m/z* 375.0878 $[M+H]^+$ (calcd for $C_{22}H_{15}O_6$, 375.0869).

Betulin (5.2): white solid; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.70 (1H, d, H-29b), 4.58 (1H, d, H-29a), 3.79 (1H, d, *J* = 10.8, H-28b), 3.33 (1H, d, *J* = 10.8, H-28a), 3.18 (1H, dd, *J* = 5.3, H-3α), 1.67 (3H, s, H-30), 0.99 (3H, s, H-27), 0.97 (3H, s, H-26), 0.96 (3H, s, H-23), 0.80 (3H, s, H-25), 0.75 (3H, s, H-24); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 150.6 (C-20), 109.8 (C-29), 79.2 (C-3), 60.6 (C-28), 55.4 (C-5), 50.5 (C-9), 48.8 (C-19), 47.9 (C-17), 47.9 (C-18), 42.8 (C-14), 41.0 (C-8), 38.9 (C-1), 38.8 (C-4), 37.4 (C-10), 37.2 (C-13), 34.3 (C-7), 34.1 (C-22), 29.8 (C-21), 29.2 (C-16), 28.1 (C-23), 27.5 (C-2), 27.1 (C-15), 25.3 (C-12), 20.9 (C-11), 19.2 (C-30), 18.4 (C-6), 16.2 (C-25), 16.1 (C-26), 15.4 (C-24), 14.8 (C-27); HRFABMS *m/z* 464.3645 $[M-H+Na]^+$ (calcd for $C_{30}H_{49}O_2Na$, 464.3630).

References

(1) Moir, M.; Thomson, R. H. Naturally Occurring Quinones. Part XXII. Terpenoid Quinones in *Cordia* Spp. *J. Chem. Soc. Perkin. 1*. **1973**, 1352-1357.

(2) Manners, G. D.; Jurd, L. The Hydroquinone Terpenoids of *Cordia alliodora*. *J. Chem. Soc. Perkin. 1*. **1977**, 405-410.

(3) Chen, T. K.; Ales, D. C.; Baenzinger, N. C.; Wiemer, D. F. Ant-Repellent Triterpenoids from *Cordia alliodora*. *J. Org. Chem.* **1983,** *48*, 3525-3531.

(4) Bieber, L. W.; Messana, I.; Lins, S. C. N.; Filho, A. A. D. S.; Chiappeta, A. A.; Mello, J. F. D. Meroterpenoid Naphthoquinones from *Cordia corymbosa*. *Phytochemistry* **1990,** *29*, 1955-1959.

(5) Bieber, L. W.; Krebs, H. C.; Schaefer, W. Further Meroterpenoid Naphthoquinones from *Cordia corymbosa*. *Phytochemistry* **1994,** *35*, 1027-1028.

(6) Manners, G. D. The Hydroquinone Terpenoids of *Cordia elaeagnoides*. *J. Chem. Soc. Perkin. 1.* **1983**, 39-43.

(7) Srivastava, S. K.; Srivastava, S. D.; Nigam, S. S. Lupa-20(29)-ene-3-O-*α*-L-rhamnopyranoside from the Roots of *Cordia obliqua*. *J. Indian Chem. Soc.* **1983,** *60*, 202.

(8) Nakamura, N.; Kojima, S.; Lim, Y. A.; Meselhy, M. R.; Hattori, M.; Gupta, M. P.; Correa, M. Dammarane-Type Triterpenes from *Cordia spinescens*. *Phytochemistry* **1997,** *46*, 1139-1141.

(9) Velde, V. V.; Lavie, D.; Zelnik, R.; Matida, A. K.; Panizza, S. Cordialin A and B, Two New Triterpenes from *Cordia verbenacea* DC. *J. Chem. Soc. Perkin. 1.* **1982**, 2697-2700.

(10) Sertie, J. A. A.; Basile, A. C.; Panizza, S.; Matida, A. K.; Zelnik, R. Pharmacological Assay of Cordia verbenacea; Part 1. Anti-Inflammatory Activity and Toxicity of the Crude Extract of the Leaves. *Planta Med.* **1988,** *54*, 7-10.

(11) Ioset, J.-R.; Marston, A.; Gupta, M. P.; Hostettmann, K. Antifungal and Larvicidal Cordiaquinones from the Roots of *Cordia curassavica*. *Phytochemistry* **2000,** *53*, 613-617.

(12) Ioset, J.-R.; Marston, A.; Gupta, M. P.; Hostettmann, K. Antifungal and Larvicidal Meroterpenoid Naphthoquinones and a Naphthoxirene from the Roots of *Cordia linnaei*. *Phytochemistry* **1998,** *47*, 729-734.

(13) Marston, A.; Zagorski, M. G.; Hostettmann, K. Antifungal Polyphenols from *Cordia goetzei* Guerke. *Helv. Chim. Acta* **1988,** *71*, 1210-1219.

(14) Sankaram, A. V. B.; Reddy, V. V. N.; Marthandamurthi, M. ^{13}C NMR Spectra of Some Naturally Occurring Binapthoquinone and Related Compounds. *Phytochemistry* **1986,** *25*, 2867-2871.

(15) Sidhu, G. S.; Prasad, K. K. (-) Isodiospyrin - A Novel Binapthoquinone Showing Atropisomerism and Other Extractives from *Diospyros chloroxylon*. *Tetrahedron Lett.* **1967,** *30*, 2905-2910.

(16) Thomson, R. H. *Naturally Occurring Quinones III - Recent Advances*; Chapman and Hall: London, NY, 1987.

(17) Thomson, R. H. *Naturally Occurring Quinones*. 2nd ed.; Academic Press: London, NY, 1971.

(18) Baker, R. W.; Liu, S.; Sargent, M. V. Synthesis and Absolute Configuration of Axially Chiral Binapthoquinones. *Aust. J. Chem.* **1998,** *51*, 255-266.

(19) Reynolds, W. F.; McLean, S.; Poplawski, J.; Enriquez, R. G.; Escobar, L. I.; Leon, I. Total Assignment of ^{13}C and ^{1}H Spectra of Three Isomeric Triterpenol Derivatives by 2D NMR: An Investigation of the Potential Utility of ^{1}H Chemical Shifts in Structural Investigations of Complex Natural Products. *Tetrahedron* **1986,** *42*, 3419-3428.

(20) Siddiqui, S.; Hafeez, F.; Begum, S.; Siddiqui, B. S. Oleanderol, a New Pentacyclic Triterpene from the Leaves of *Nerium oleander*. *J. Nat. Prod.* **1988,** *51*, 229-233.

(21) Mahato, S. B.; Kundu, A. P. ^{13}C NMR Spectra of Pentacyclic Triterpenoids - A Compilation and Some Salient Features. *Phytochemistry* **1994,** *37*, 1517-1575.

(22) Louie, K. G.; Behrens, B. C.; Kinsella, T. J.; Hamilton, T. C.; Grotzinger, K. R.; McKoy, W. M.; Winker, M. A.; Ozols, R. F. Radiation Survival Parameters of Antineoplastic Drug-sensitive and -resistant Human Ovarian Cancer Cell Lines and Their Modification by Buthionine Sulfoximine. *Cancer Res.* **1985,** *45*, 2110-2115.

VI. SAKURASO-SAPONIN, A KNOWN TRITERPENOID SAPONIN ISOLATED FROM A *MONOPORUS* SPECIES (MYRSINACEAE) FROM MADAGASCAR

6.1 Introduction

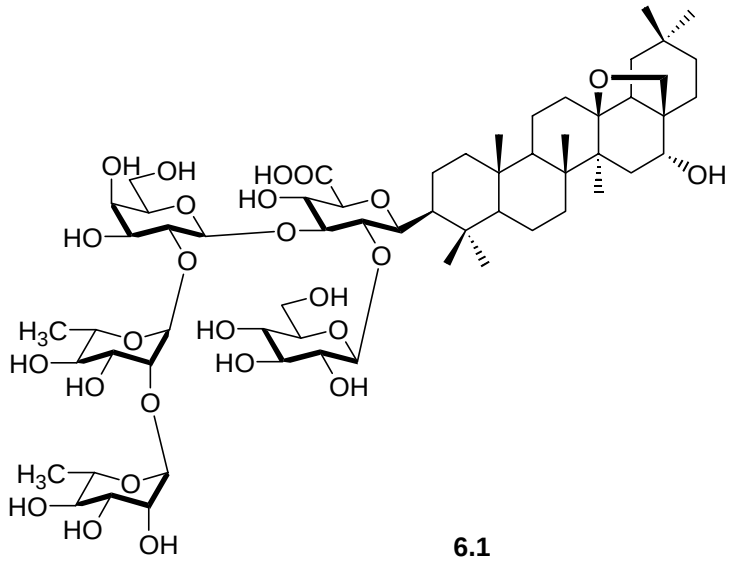
The root extract of a species of *Monoporus* from Madagascar displayed moderate cytotoxicity in the A2780 human ovarian cancer cell line assay, and it was therefore fractionated and examined for potential anticancer compounds. From this extract, a known triterpenoid saponin was isolated by liquid/liquid partition and column chromatography, and it was characterized using liquid-chromatography-mass spectrometry and various NMR techniques. The compound, sakuraso-saponin, was both the major and the most bioactive component observed.

6.1.1 *Previous Investigations of Monoporus Species*

Monoporus is a genus of the plant family Myrsinaceae. About ten species are known to be members of this genus. Although plants of Mysinaceae have been investigated phytochemically, no official record exists of compounds previously isolated from a species of *Monoporus*.

6.1.2 *Chemical Investigation of a Monoporus Species*

Through an ongoing investigation of bioactive compounds from plant collections in the Madagascar rainforest, as part of an ICBG program, the ethanol extract (MG 594) of an unknown species of *Monoporus* was investigated by bioassay-guided fractionation. The dry, crude root material yielded an IC_{50} of 16 μg/mL in the A2780 human ovarian cancer cell line bioassay. Fractionation (liquid-liquid partitioning and reversed-phase column chromatography) afforded the isolation of a known triterpene pentaglycoside, sakuraso-saponin (**6.1**) (Figure 6-1), whose structure was deduced from NMR and MS data by Dr. Shugeng Cao. This chapter reports the isolation and dereplication of this compound.



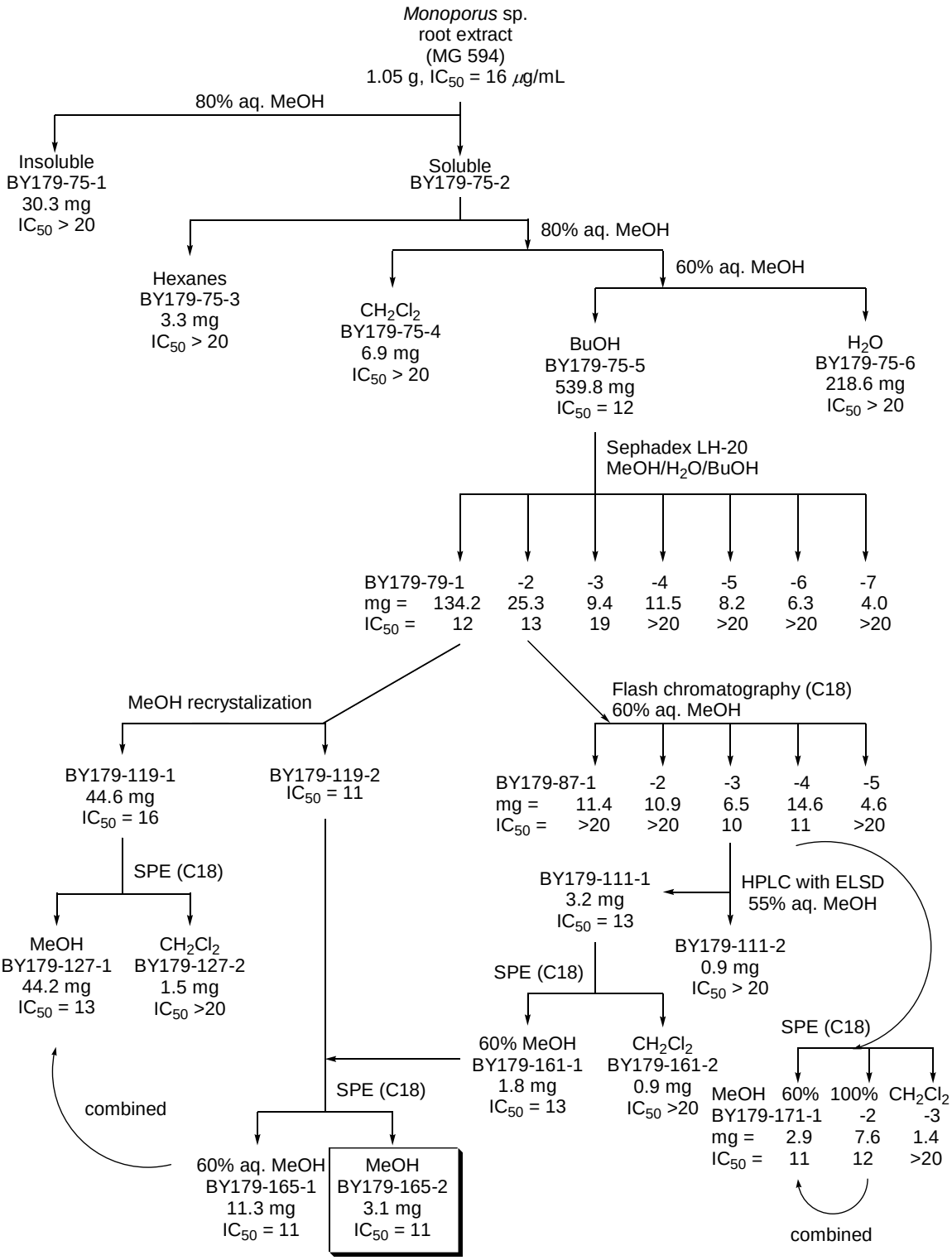
6.1

Figure 6.1. Sakuraso-Saponin from a *Monoporus* Species.

6.2 Results and Discussion

6.2.1 *Isolation of a Known Triterpenoid Saponin from a Monoporus Species*

Sakuraso-saponin (**6.1**) was isolated as indicated in Scheme 6.1. From the dry root extract (MG 594), 1.05 g of crude material was attempted to dissolve in 80% MeOH-H_2O. Approximately 30 mg were found to be insoluble and were subsequently removed. The remainder was taken for liquid-liquid partitioning and extracted with hexanes. After removal of the non-polar layer, the aqueous layer was diluted to yield a 60% MeOH-H_2O solution and then further partitioned with CH_2Cl_2. The MeOH-H_2O layer was evaporated and partitioned between BuOH and H_2O. All five fractions were subjected to solvent removal by rotary evaporation. Testing of the samples in the A2780 cytotoxicity assay indicated that only the BuOH fraction was more active than the crude material; this was also the sample that contained the majority of the dry weight. The BuOH fraction was subjected to separation by Sephadex LH-20 in an open column, but approximately one-third of the initial fraction became irreversibly bound to the solid-phase during this process. Two fractions eluted with MeOH, and totaling approximately 160 mg, retained the original bioactivity, however. The first fraction was subjected to a series of attempts at MeOH recrystallization and solid-phase extraction with a RP-C_{18} cartridge, while the second fraction was subjected to flash chromatography, HPLC, and solid-phase extraction with RP-C_{18}. Ultimately, a pure sample of **6.1** was obtained, totaling 3.1 mg.



Scheme 6.1. Fractionation of a *Monoporus* Species (Myrsinaceae).

6.2.2 *Characterization of a Known Triterpenoid Saponin from a Monoporus Species*

6.2.2.1 Structure of Sakuraso-Saponin (**6.1**)

Sakuraso-saponin (**6.1**) was isolated as a white solid. The structure elucidation of this compound was performed by Dr. Shugeng Cao, who identified the primulagenin A triterpene moiety and the five components of the saccharide moiety (glucuronic acid, glucose, galactose and two rhamnose sugars), based upon the LC-MS spectrum and ^{1}H, ^{13}C, COSY, HMBC, HSQC, TOCSY and ROESY NMR data. **6.1** was first isolated from the leaves of *Rapanea melanophloeos* in 1993,[1] and it was re-isolated from the leaves and stem-bark of *Tapeinosperma clethroides* in 1999.[2] Both plants are members of the Myrsinaceae family.

6.2.3 *Biological Evaluation of a Known Triterpenoid Saponin*

Compound **6.1** was tested in the A2780 assay, and it was moderately active with an IC_{50} value of 11 μg/mL, using actinomycin D as a positive control (IC_{50} = 1-3 ng/mL).

6.3 Experimental Section.

General Experimental Procedures. Solid phase extraction was performed with Supelco Discovery DSC-C_{18} tubes. A mass spectrum was obtained on a Finnigan LC-MS instrument coupled with an Agilent Zorbax C_{18} column (5 cm x 2.5 mm, 3 μ). NMR spectra were obtained on either a JEOL Eclipse (at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) or Varian Inova (at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) spectrometer. Chemical shifts are given in δ (ppm) and coupling constants (J) are reported in Hz.

Plant Material. The roots and wood of a *Monoporus* species (Myrsinaceae) were collected by Stephan Raktonandrasana on October 20, 2000. The specimens were collected around the Natural Reserve of Zahamena in the province of Toamasina, Madagascar. Duplicate voucher specimens have been deposited at the Centre National d'Application des Recherches Pharmaceutiques (CNARP) and the Direction des Recherches Forestieres et Piscicoles Herbarium (TEF) in Antananarivo, Madagascar; the

Missouri Botanical Garden in St. Louis, Missouri (MO); and the Museum National d'Histoire Naturelle in Paris, France (P).

Extract Preparation. The roots and wood of a *Monoporus* species were dried, ground and extracted with ethanol in Madagascar. This yielded extracts labeled MG 594 (9 g) and MG 596 (8.5 g), respectively.

Cytotoxicity Bioassay. The A2780 ovarian cancer cell line cytotoxicity assay was performed at Virginia Polytechnic Institute and State University as previously reported.[3]

Bioassay-guided Fractionation and Isolation of Sakuraso-Saponin. The crude bioactive extract MG 594 (IC_{50} = 16 μg/mL, 1.05 g) was dissolved in MeOH-H_2O (4:1, 200 mL) and extracted with hexanes (200 mL). Water was added to the MeOH-H_2O fraction to yield a MeOH-H_2O solution (3:2) that was subsequently partitioned with CH_2Cl_2. The aqueous fraction was evaporated and partitioned between BuOH (200 mL) and H_2O (200 mL). Evaporation of all solvents yielded a bioactive (IC_{50} = 12 μg/mL) BuOH fraction of 540 mg, which was subjected to further fractionation through a open column of Sephadex LH-20 with MeOH, followed by H_2O. Of the seven fractions collected, the first two (134 mg, IC_{50} = 12 μg/mL; 25 mg, IC_{50} = 13 μg/mL) displayed the greatest cytotoxicty. These two fractions were treated separately, although they ultimately yielded the same compound. Fraction 1 was used to attempt recrystallization in evaporating MeOH, followed by RP-C_{18} solid-phase extraction (eluting with 3:2 MeOH-H_2O and then MeOH). Fraction 2 was subjected to RP-C_{18} flash chromatography with 3:2 MeOH-H_2O, and five fractions were collected. The third of those fractions (6.5 mg, IC_{50} = 10 μg/mL) was also extracted with a RP-C_{18} SPE cartridge (eluting with 3:2 MeOH-H_2O and then CH_2Cl_2). The MeOH-H_2O eluent (1.8 mg, IC_{50} = 13.5 μg/mL) was combined with the purified material from the Fraction 1 route to yield 3.1 mg of **6.1** (IC_{50} = 11 μg/mL).

Sakuraso-saponin (6.1): white solid; ^{1}H NMR (CD_3OD, 500 MHz) δ 5.40 (1H, s, rha-1), 5.16 (1H, d, J = 7.5, gal-1), 4.96 (1H, s, rha'-1), 4.49 (1H, d, J = 8, gluA-1), 4.05 (1H, m,

rha-5), 3.97 (1H, dd, *J* = 3, 2, rha'-2), 3.95 (1H, m, rha-2), 3.93 (1H, s, gal-4), 3.91 (1H, dd, gluA-2), 3.88 (1H, m, H-16), 3.84 (1H, d, glu-6), 3.80 (1H, dd, rha-3), 3.78 (1H, dd, gal-6), 3.71 (1H, s, gal-2), 3.70 (1H, m, rha'-5), 3.68 (1H, dd, *J* = 3.5, rha'-3), 3.66, (1H, dd, *J* = 3.5, gal-6), 3.62 (1H, d, *J* = 3.5, gluA-5), 3.61 (1H, m, gluA-4), 3.52 (1H, dd, *J* = 8, 3.5, glu-6), 3.49 (1H, d, *J* = 7.5, H-28), 3.38 (1H, m, glu-5), 3.36 (3H, m, rha-4, rha'-4, glu-3), 3.22 (1H, t, *J* = 9, glu-2), 3.11 (1H, d, *J* = 4, H-28), 3.05 (1H, t, *J* = 9.5, glu-4), 2.37 (1H, t, *J* = 12.5, H-19), 2.06 (1H, m, H-15), 1.75 (2H, m, H-12), 1.48 (3H, m, H-6, -18), 1.28 (3H, s, rha'-6), 1.25 (3H, d, *J* = 6, rha-6), 1.22 (3H, s, H-27), 1.14 (3H, s, H-26), 1.05 (3H, s, H-23), 0.94 (3H, s, H-29), 0.90 (3H, s, H-30), 0.89 (3H, s, H-25), 0.86 (3H, s, H-24), 0.72 (1H, d, *J* = 11, H-5); ^{13}C NMR (CD_3OD, 125 MHz) δ 106.0 (gluA-1), 104.1 (rha'-1), 102.9 (glu-1), 101.1 (rha-1), 101.1 (gal-1), 92.5 (C-3), 88.6 (C-13), 80.0 (rha-2), 79.2 (gluA-3), 78.9 (C-28), 78.4 (glu-3), 78.3 (glu-5), 78.1 (C-16), 77.1 (gal-5), 76.3 (gluA-5), 76.2 (glu-2), 76.1 (gal-2), 74.3 (rha-4), 74.3 (rha'-4), 72.7 (gluA-4), 72.5 (glu-4), 72.3 (rha-3), 72.2 (rha'-2), 71.9 (rha'-3), 70.5 (rha-5, rha'-5), 70.4 (gal-4), 63.7 (glu-6), 63.0 (gal-6), 56.9 (C-5), 52.6 (C-18), 51.5 (C-9), 45.6 (C-17), 45.5 (C-14), 43.5 (C-8), 40.9 (C-4), 40.4 (C-1), 40.0 (C-19), 38.0 (C-10), 37.6 (C-21), 37.3 (C-15), 35.4 (C-7), 34.1 (C-29), 33.5 (C-22), 32.6 (C-20), 32.4 (C-12), 28.5 (C-23), 27.4 (C-2), 25.1 (C-30), 20.1 (C-27), 20.1 (C-11), 19.0 (C-26), 18.9 (C-6), 18.2 (rha-6), 18.1 (rha'-6), 16.9 (C-24, -25); LC-MS *m*/*z* 1251.8545.

References

(1) Ohtani, K.; Mavi, S.; Hostettmann, K. Molluscicidal and Anitfungal Triterpenoid Saponins from *Rapanea melanophloeos* Leaves. *Phytochemistry* **1993,** *33*, 83-86.

(2) Lavaud, C.; Pichelin, O.; Massiot, G.; Men-Olivier, L. L.; Sevenet, T.; Cosson, J.-P. Sakuraso-Saponin from *Tapeinosperma clethroides*. *Fitoterapia* **1999,** *70*, 116-118.

(3) Louie, K. G.; Behrens, B. C.; Kinsella, T. J.; Hamilton, T. C.; Grotzinger, K. R.; McKoy, W. M.; Winker, M. A.; Ozols, R. F. Radiation Survival Parameters of Antineoplastic Drug-sensitive and -resistant Human Ovarian Cancer Cell Lines and Their Modification by Buthionine Sulfoximine. *Cancer Res.* **1985,** *45*, 2110-2115.

VII. MISCELLANEOUS PLANTS STUDIED

7.1 Introduction

Over the past four years, many plants have been investigated for cytotoxic metabolites through the ICBG program at Virginia Tech. Unfortunately, not all of these plants yielded compounds of interest. Here we report, for record keeping purposes, a list of the plants studied and reasons for the lack of positive results.

7.1.1 *Investigation of Lecythis charteracea and Lecythis corrugata*

Lecythis is a genus of the Lecythidaceae family of plants. Two plant extracts were obtained from Suriname and examined for compounds with cytotoxicity towards the A2780 line of ovarian cancer cells.

The extract of *Lecythis charteracea* (M 940659) had an initial IC_{50} value of 23 μg/mL. Liquid/liquid partitioning of 238 mg of crude material, followed by both normal- and reversed-phase flash chromatography, led to numerous fractions with IC_{50} values of more than 20 μg/mL. Only a single fraction demonstrated a moderately significant improvement in cytotoxicity (IC_{50} = 15 μg/mL), and it contained only 2.3 mg. Due to the dim prospect of isolating a bioactive compound of interest, this extract was subsequently dropped.

The extract of *Lecythis corrugata* (M 960064) had an initial IC_{50} value of 43 μg/mL. Liquid/liquid partitioning of 6.219 g of wet crude material led to a single fraction of 75 mg with an IC_{50} = 25 μg/mL. Parallel fractionation of 1.335 g of wet crude material (by chromatography with MCI gel) led to a single fraction of 79 mg with an IC_{50} = 21 μg/mL. These two most promising fractions bore no resemblance to one another, with the former soluble in hexanes while the latter was soluble in 40% MeOH-H_2O. Subsequent normal-phase flash chromatography led to a loss of activity for all fractions (IC_{50} > 30 μg/mL). Due to this loss of bioactivity and the dim prospect of isolating a bioactive compound of interest, the extract was ultimately dropped.

7.1.2 *Investigation of a Dracaena Species*

Dracaena is a genus of the Convallariaceae family of plants. From Madagascar, two plant extracts were obtained and examined for compounds with cytotoxicity towards the A2780 line of ovarian cancer cells.

The root extract of an unknown species of *Dracaena* (MG 1894) had an initial IC_{50} value of 20 μg/mL. Liquid/liquid partitioning of 215 mg of crude material, followed by RP-C18 solid phase extraction, led to two fractions totaling 10.1 mg with an IC_{50} average of 3.6 μg/mL. Subsequent HPLC led to a loss of activity; all fractions had an IC_{50} value > 20 μg/mL. Repetition of the fractionation process with an additional 216 mg of crude extract led to similar problems with loss of bioactivity. Due to this and the fact that the genus has been well studied (and its components thoroughly documented), this extract was ultimately dropped.

The stem extract of an unknown species of *Dracaena* (MG 1895) had an initial IC_{50} value of 22 μg/mL. Liquid/liquid partitioning of 116 mg of crude material led to a single fraction of 4.7 mg with an IC_{50} = 4.7 μg/mL. Subsequent RP-C18 solid phase extraction led to a loss of activity; the most active fraction had an IC_{50} value of 7.6 μg/mL. Recombining all fractions and resubmitting the sum total for bioassay led to an IC_{50} of only 17 μg/mL, which is more than three times less active than the original CH_2Cl_2 fraction from the liquid/liquid partition. Repetition of the fractionation process with an additional 108 mg of crude extract failed to reveal any similar fractions with promising cytotoxicity. As discussed with the root extract of this *Dracaena* species, the genus has been well studied and its components have been thoroughly documented. Due to the loss of bioactivity and the fact that only 640 mg crude extract was ultimately available, this extract was dropped.

7.1.3 *Investigation of Apodytes thouarsiana and Another Apodytes Species*

Apodytes is a genus of the Icacinaceae family of plants. From Madagascar, two plant extracts were obtained and examined for compounds with cytotoxicity towards the A2780 line of ovarian cancer cells.

The bark extract of an unknown species of *Apodytes* (MG 1485) had an initial IC_{50} value of 40 μg/mL. Liquid/liquid partitioning of 217 mg of crude material led to

four fractions with similar masses and bioactivities. The most active fraction had an IC_{50} = 20 μg/mL. Due to the lack of a single promising fraction and an overall lack of cytotoxicity, this extract was subsequently dropped.

The leaf extract of *Apodytes thouarsiana* (MG 2223) had an initial IC_{50} value of 20 μg/mL. Liquid/liquid partitioning of 208 mg of crude material, followed by NP-Diol and RP-C18 solid phase extraction, led to a single fraction of 5.7 mg with an IC_{50} = 7.9 μg/mL. This fraction contained approximately 10 different components, all of similar percentage. Isolation of any of them would not have yielded enough material for structure elucidation. An additional attempt at fractionation was made, beginning with twice as much crude extract (400 mg). A fraction of 9.2 mg (also containing approximately 10 different components) was obtained with an IC_{50} = 4.0 μg/mL. Further HPLC separation led to either pure fractions of < 1 mg or impure fractions. Due to the lack of any major isolable component(s), this extract was ultimately dropped.

7.1.4 *Investigation of a Boswellia Species*

Boswellia is a genus of the Burseraceae family of plants. From Madagascar, one plant extract was obtained and examined for compounds with activity towards Akt.

The leaf extract of a species of *Boswellia* (MG 2172) was reported to have an initial IC_{50} value of 6.6 μg/mL. Liquid/liquid partitioning of 208 mg of crude material led to four fractions, all with IC_{50} values equal to or greater than 49 μg/mL. Repeated bioassay testing of all fractions, including crude and detanninized crude samples, yielded similar results, with the exception of the H_2O fraction. Although the IC_{50} of this fraction was 26 μg/mL, the material proved to be insoluble in all solvents except 100% H_2O. Due to the solubility problems and an overall lack of Akt activity, this extract was subsequently dropped.

VIII. GENERAL CONCLUSIONS

Of the five known compounds isolated from *Cerbera manghas* and the *Cordia* and *Monoporus* plants, three have a basic polycyclic triterpene skeleton, although the cardiac glycoside and the saponin also have attached sugar units. These are very common metabolites in the plant kingdom, and history has shown that they hold little promise as anticancer agents. Cerbinal and isodiospyrin, while interesting because of their size, shape and color, also do not appear to good drug candidates or leads. However, it would be intriguing to determine the cause of the moderate cytotoxicity of each.

Of the eleven compounds isolated from *Macaranga alnifolia* and the *Tambourissa* plant, the schweinfurthins are by far the most interesting. Although vedelianin is a known compound, the level of its cytotoxicity in the A2780 assay is comparable to that of Taxol®. Schweinfurthins E and G have remarkably similar biological activities, while schweinfurthins F and H are both more than an order of magnitude less cytotoxic. There appear to be no skeletal reasons for these differences, so it would be beneficial to examine these structure-activity relationships in more detail. The schweinfurthin analogues that are being synthesized by David Wiemer's group at the University of Iowa will surely provide insight into the data presented here.

The flavonoids from *Macaranga alnifolia*, on the other hand, appear to have more obvious structure-activity relationships. Diplacol and diplacone are approximately twice as cytotoxic in the A2780 assay as bonnaniol A and bonannione A. Apparently, two hydroxyl groups (at the meta and para positions) on the B-ring lead to an improvement in activity. An alkyl substituent at the other meta position, however, may counteract any substantial gains, as evidenced by the fact that alnifoliol is the least active of the isolated flavonoids. Similar to the triterpenes, this compound class has been well studied, and it is unlikely to produce any clinical pharmaceuticals.

Tambouranolide is a molecule that requires further study before its full potential as a drug is known. Hydroxybutanolides are a relatively new class of compounds, and their biological activity is briefly documented and poorly understood. The mechanism of action may depend upon their structural resemblance to surfactants, with both polar and

non-polar moieties. The absolute configuration of tambouranolide was determined based upon the literature of related compounds, but it would be beneficial to confirm the stereochemistry of the molecule through circular dichroism or x-ray studies. Schweinfurthin H also would benefit from additional structural studies, as the relative stereochemistry of the 2"-OH remains unassigned.

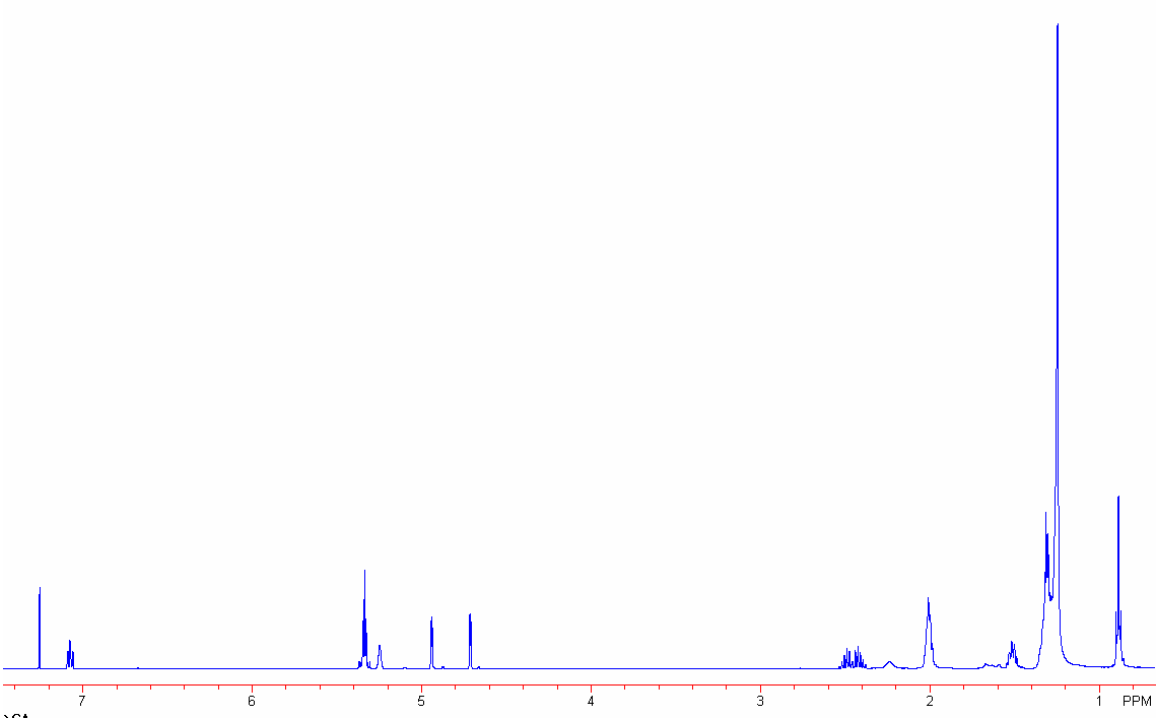
A total of sixteen cytotoxic compounds have been isolated from plants of the rainforests of Madagascar and Suriname. The results of this project are summarized in Table 8.1.

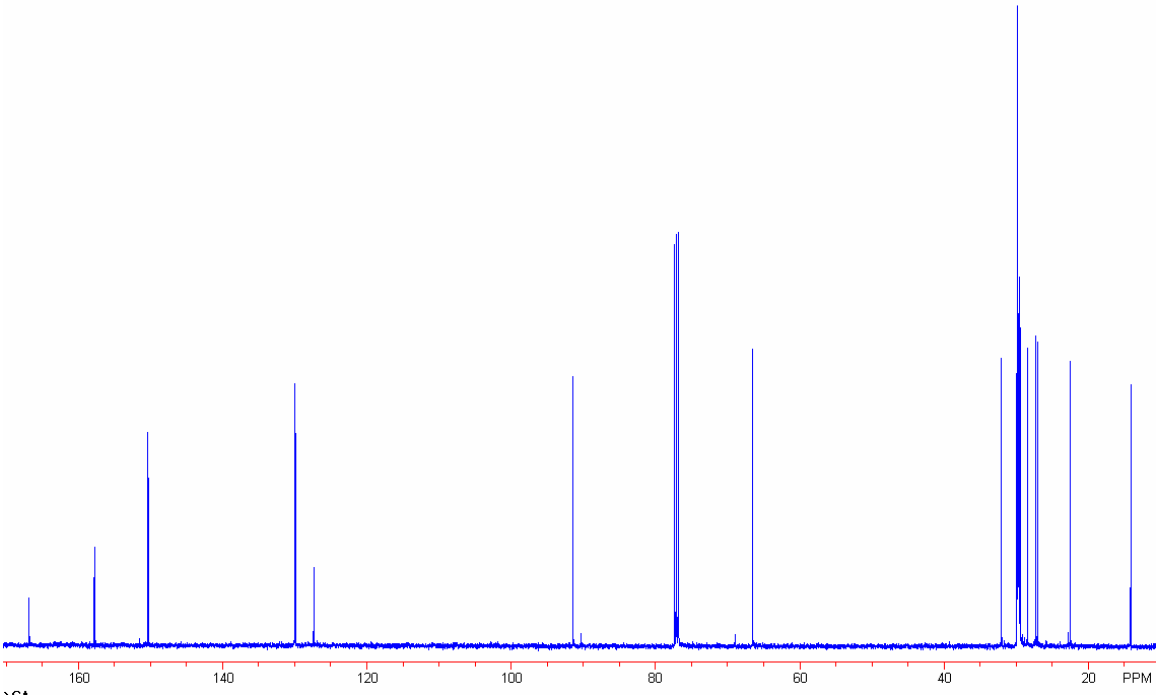
Table 8.1. Summary of Compounds Isolated.

Compound	Natural Product Class	Plant	IC_{50} (μg/mL)	New / Known
Alnifoliol	Dihydroflavanol	*Macaranga alnifolia*	12	New
Betulin	Triterpene glycoside	*Cordia* sp.	14	Known
Bonanniol A	Dihydroflavanol	*Macaranga alnifolia*	10	Known
Bonannione A	Flavanone	*Macaranga alnifolia*	10	Known
Cerbinal	Iridoid	*Cerbera manghas*	1.0	Known
Diplacol	Dihydroflavanol	*Macaranga alnifolia*	4.9	Known
Diplacone	Flavanone	*Macaranga alnifolia*	4.7	Known
Isodiospyrin	Napthoquinone dimer	*Cordia* sp.	3.8	Known
Neriifolin	Cardiac glycoside	*Cerbera manghas*	0.01	Known
Sakuraso-saponin	Triterpenoid saponin	*Monoporus* sp.	11	Known
Schweinfurthin E	Prenylated stilbene	*Macaranga alnifolia*	0.13	New
Schweinfurthin F	Prenylated stilbene	*Macaranga alnifolia*	2.4	New
Schweinfurthin G	Prenylated stilbene	*Macaranga alnifolia*	0.18	New
Schweinfurthin H	Prenylated stilbene	*Macaranga alnifolia*	2.3	New
Tambouranolide	Hydroxybutanolide	*Tambourissa* sp.	8	New
Vedelianin	Prenylated stilbene	*Macaranga alnifolia*	0.06	Known

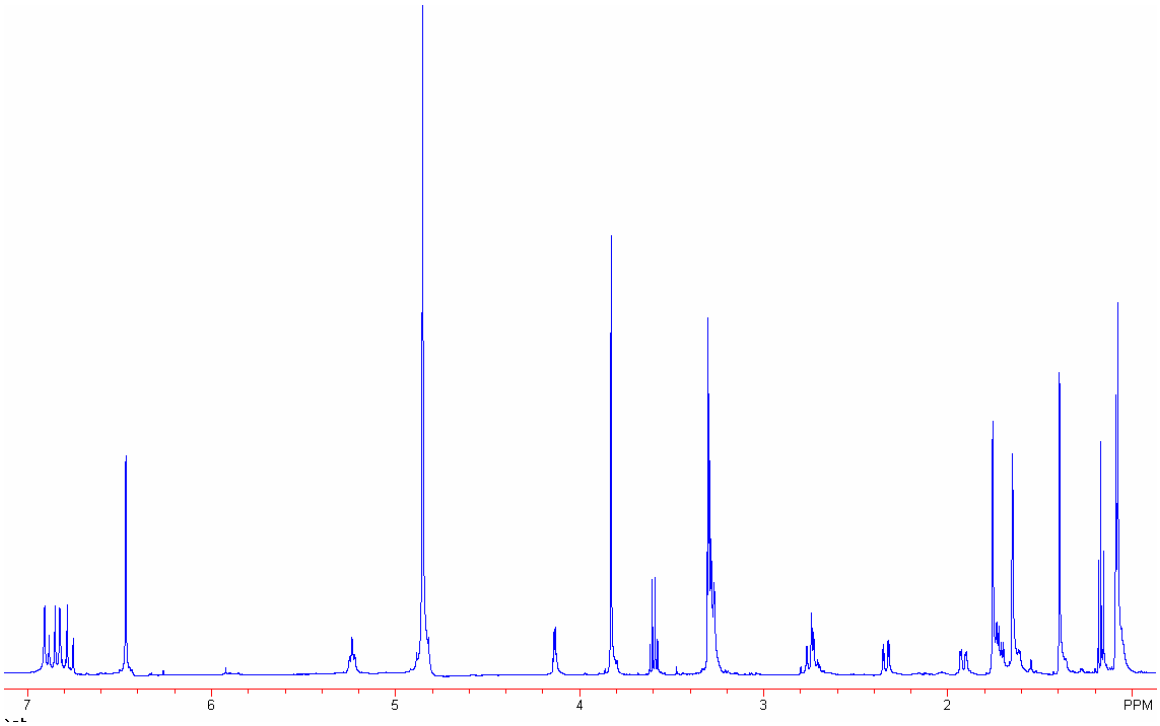
APPENDIX
(^{1}H and ^{13}C NMR Spectra)

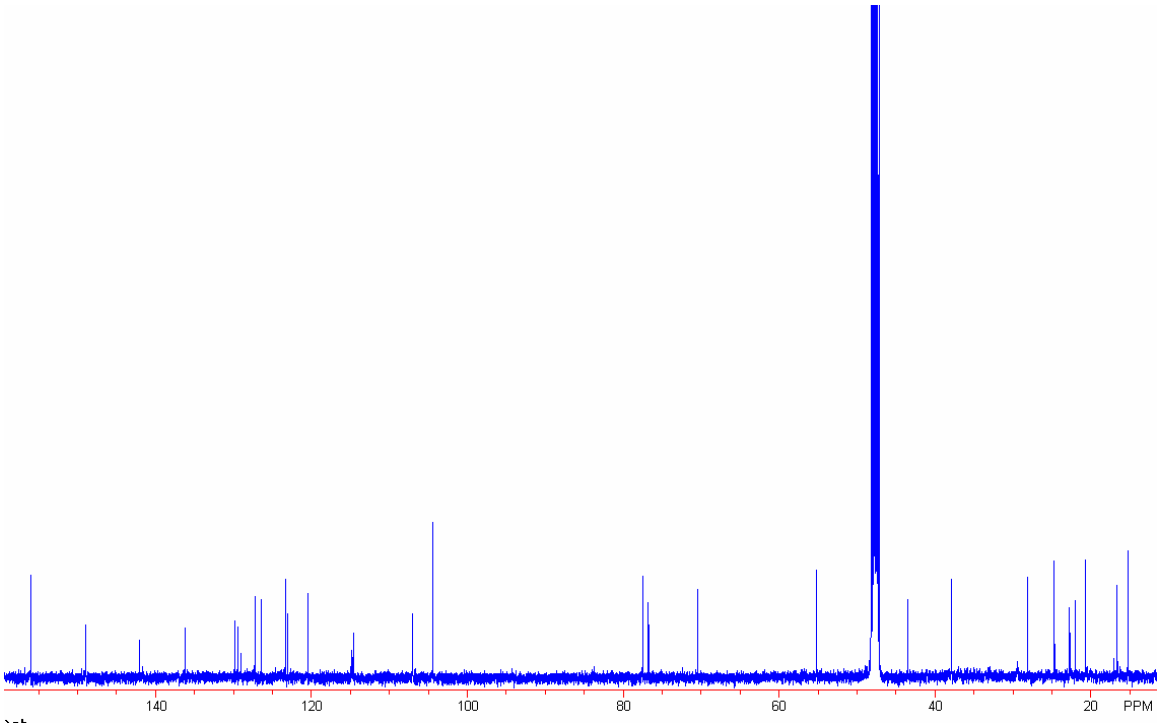
BY179-249-6 (**2.1)** Tambouranolide



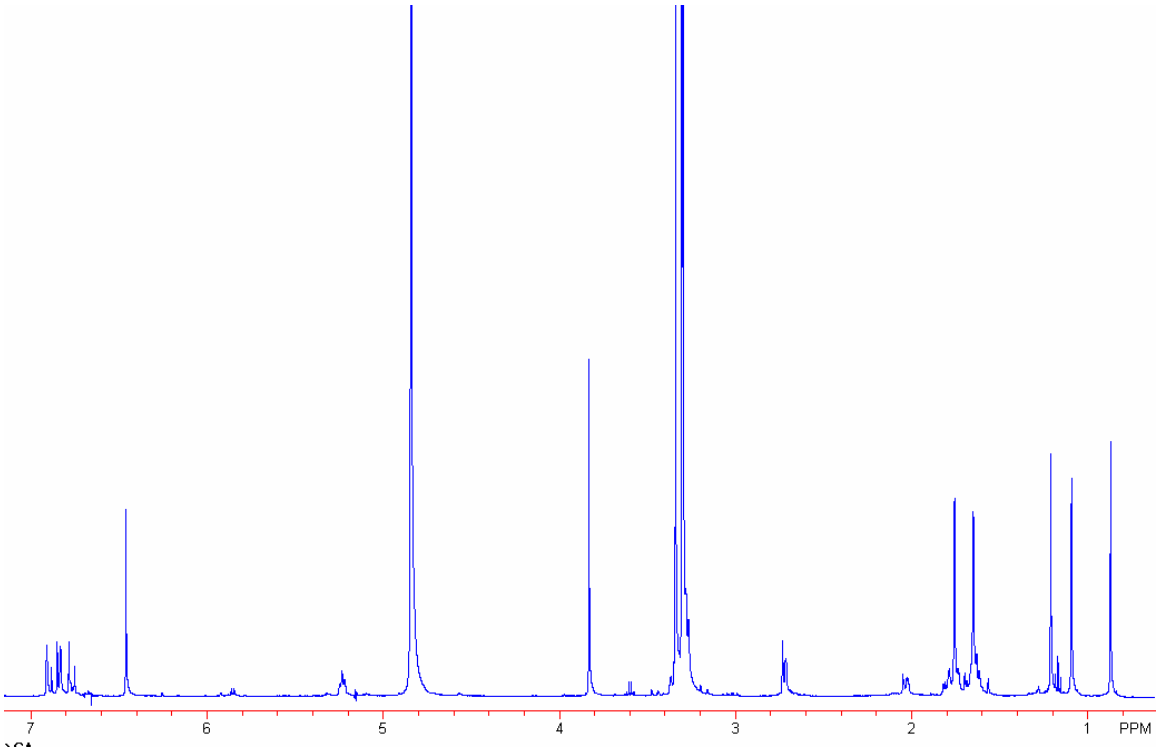


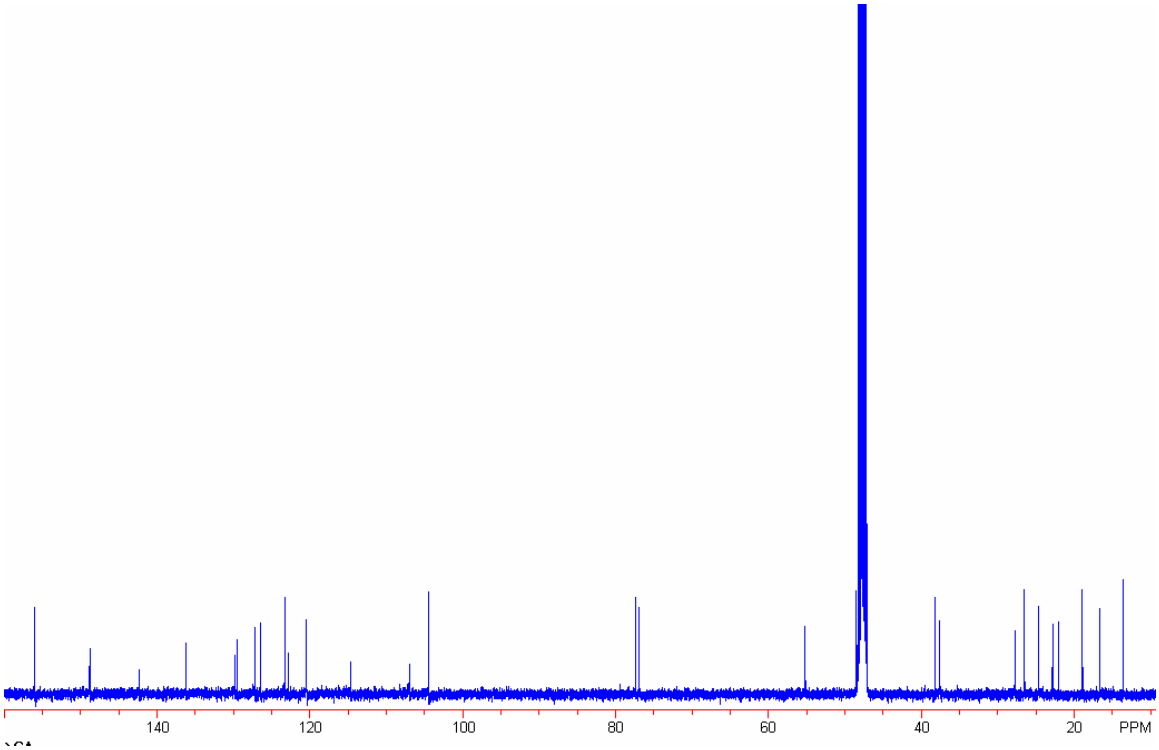
BY179-243-4 (**3.1**) Schweinfurthin E



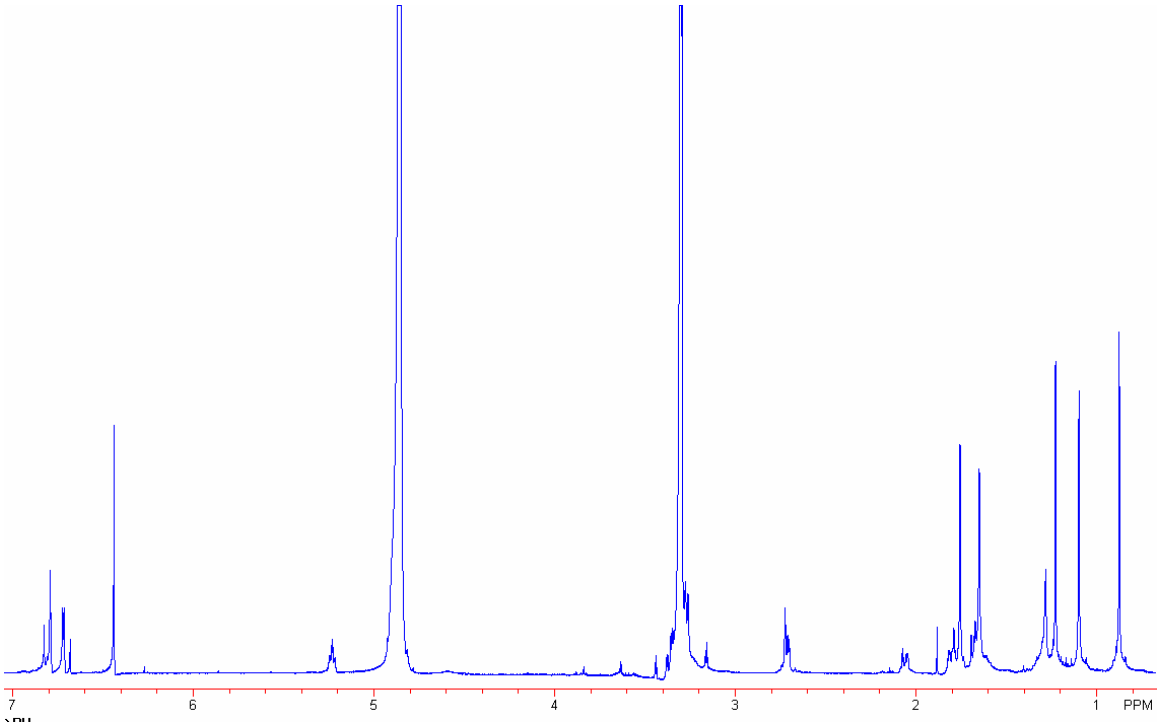


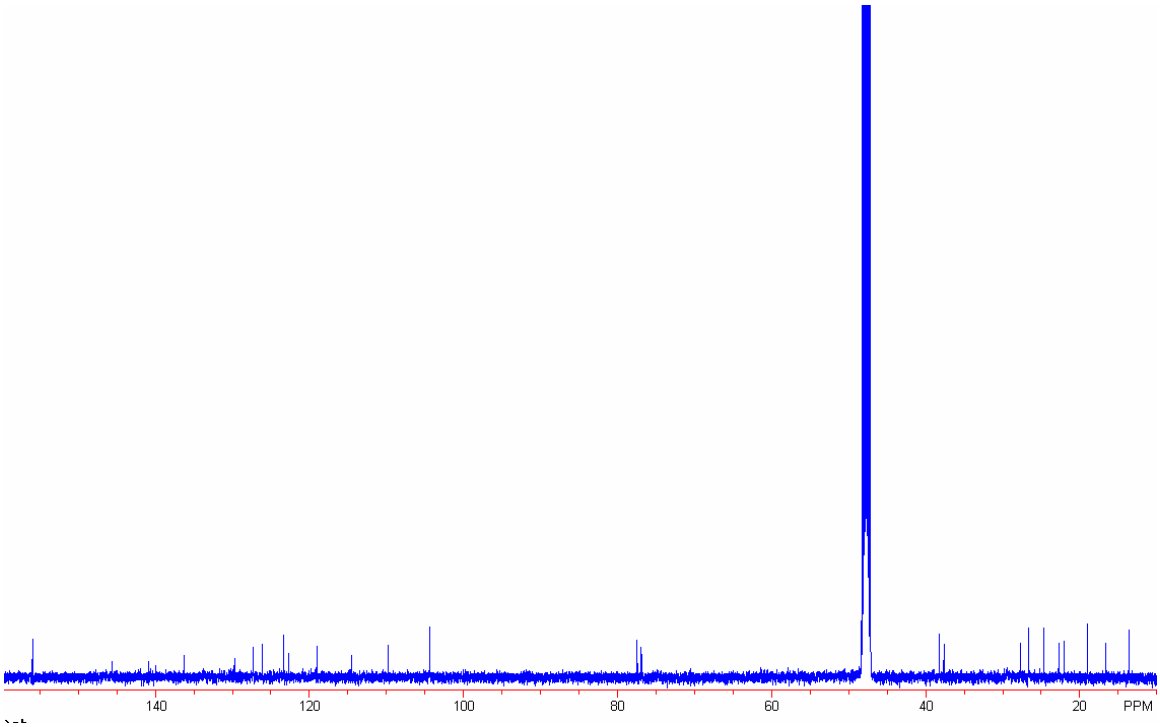
BY179-243-6 (**3.2**) Schweinfurthin F



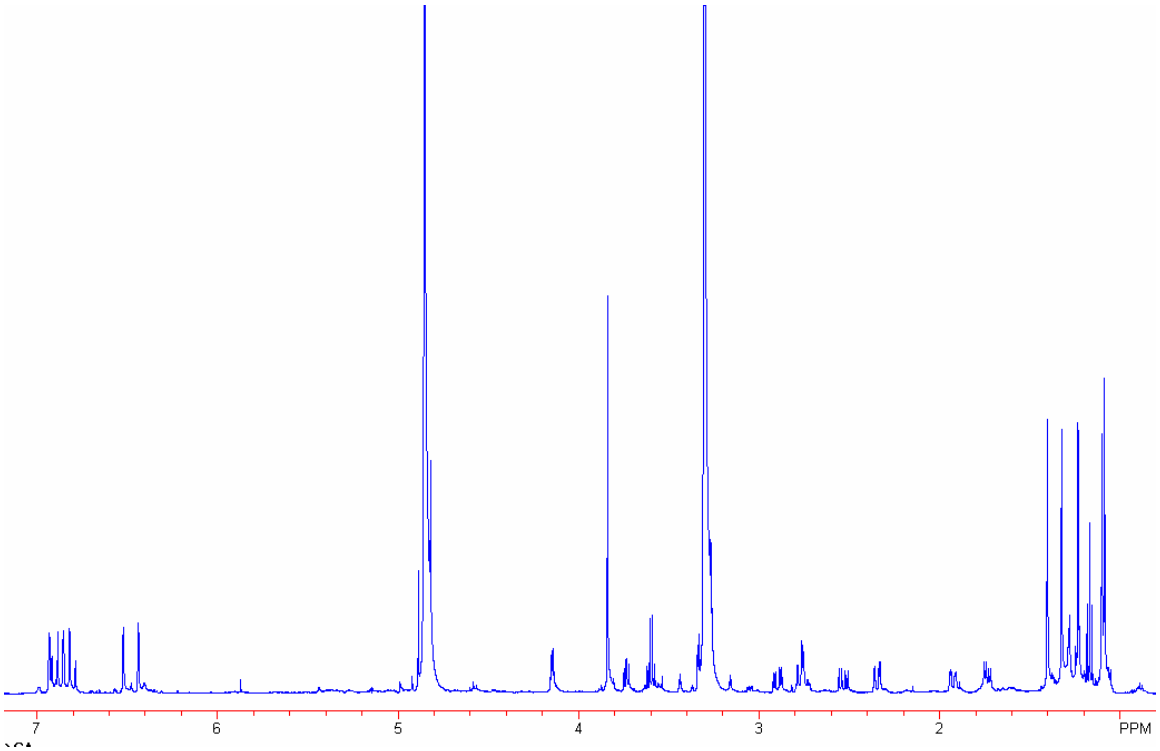


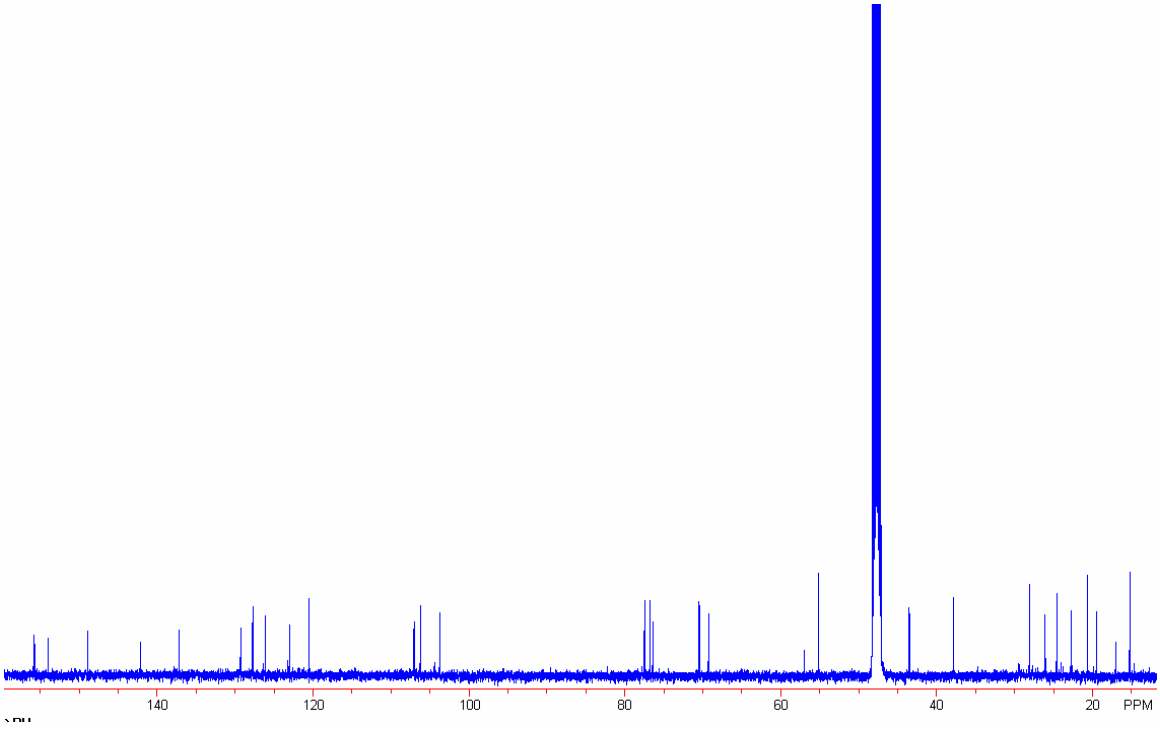
BY179-243-2 (**3.3**) Schweinfurthin G



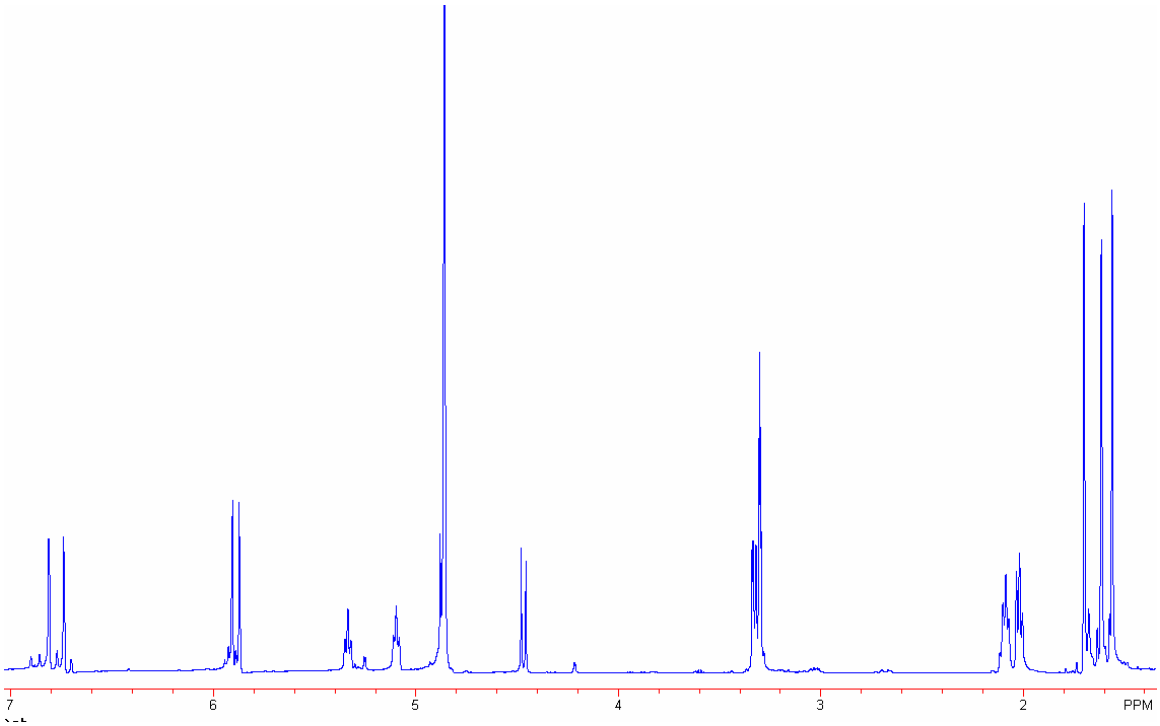


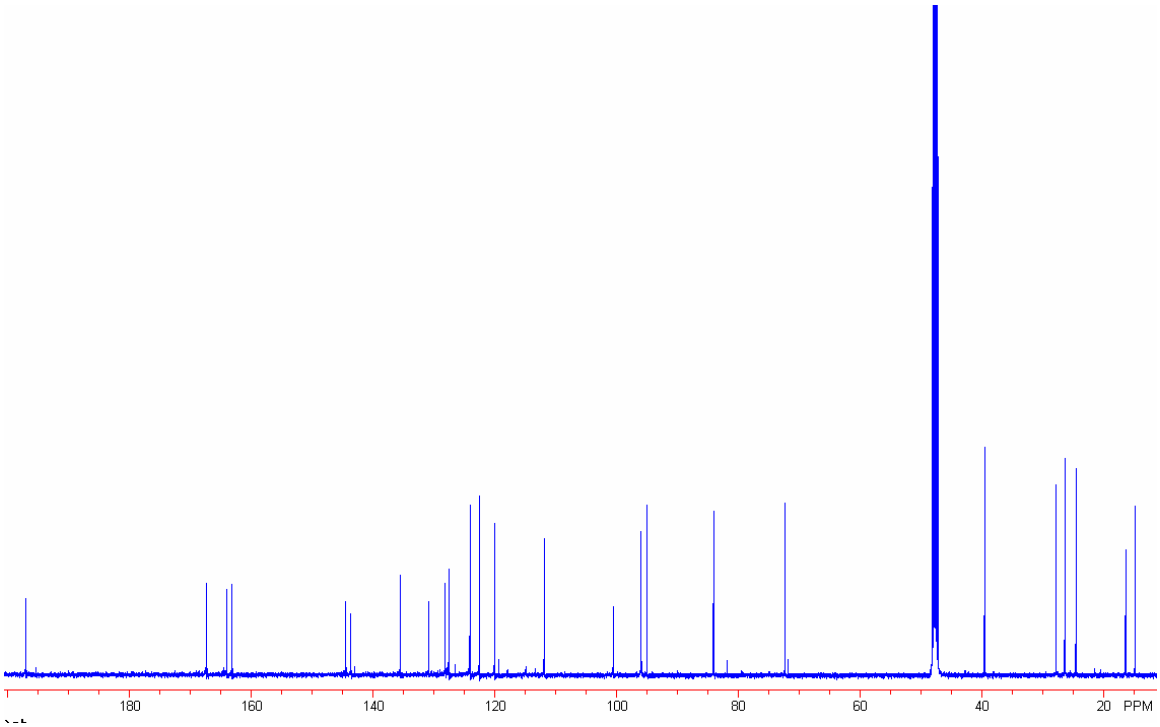
BY179-243-3 (**3.4**) Schweinfurthin H



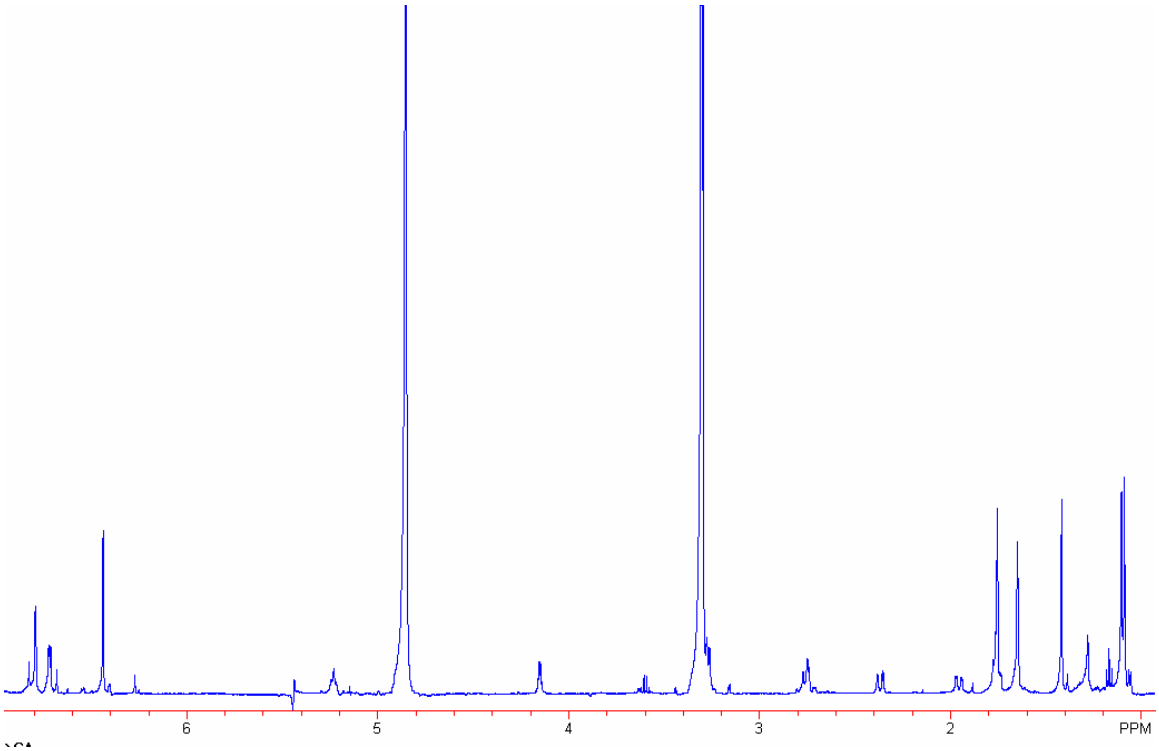


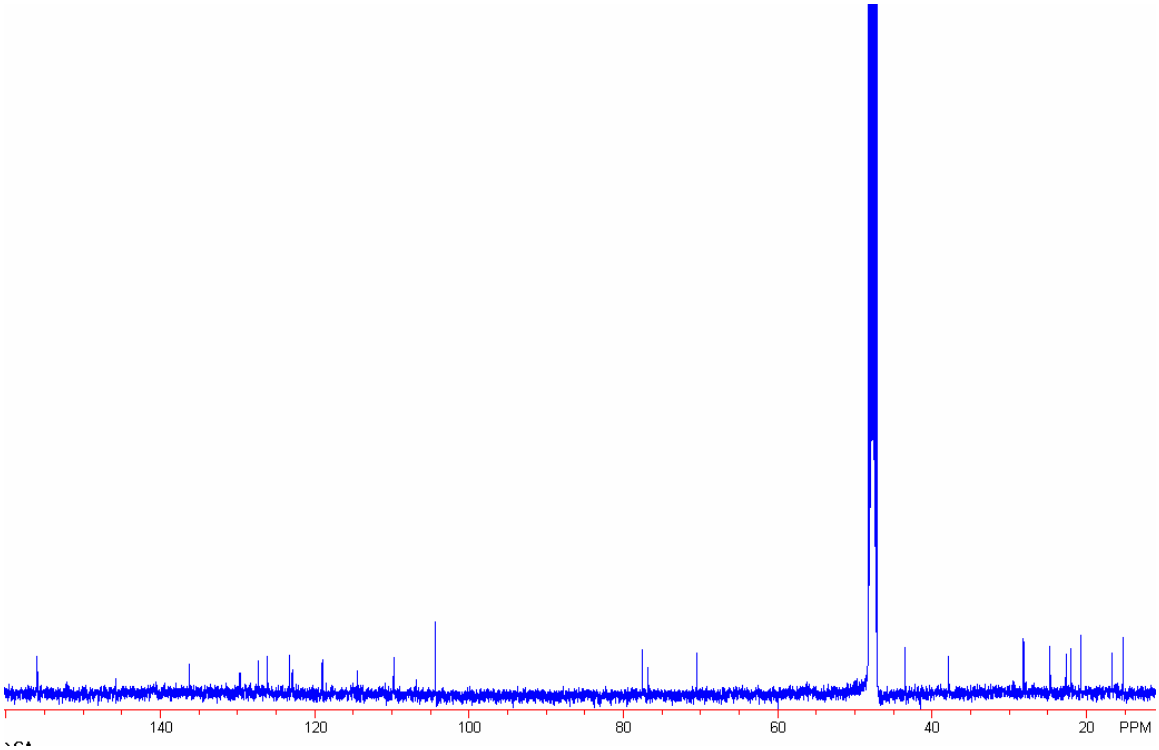
BY179-253-2 (**3.5**) Alnifoliol



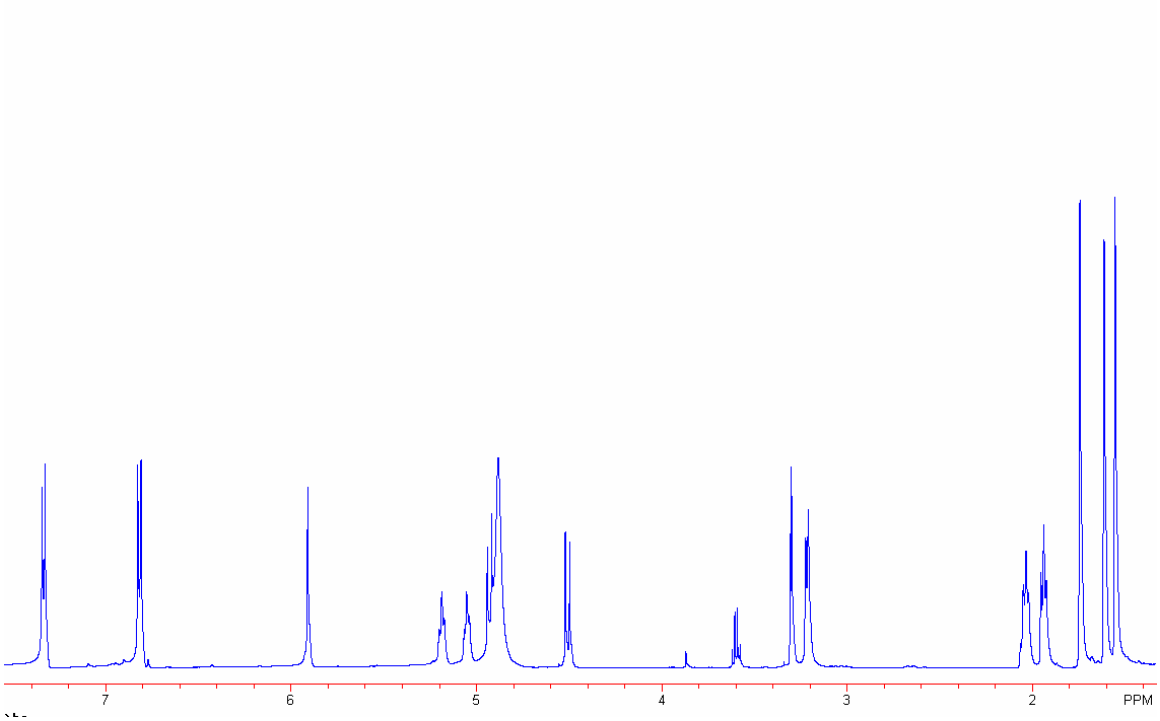


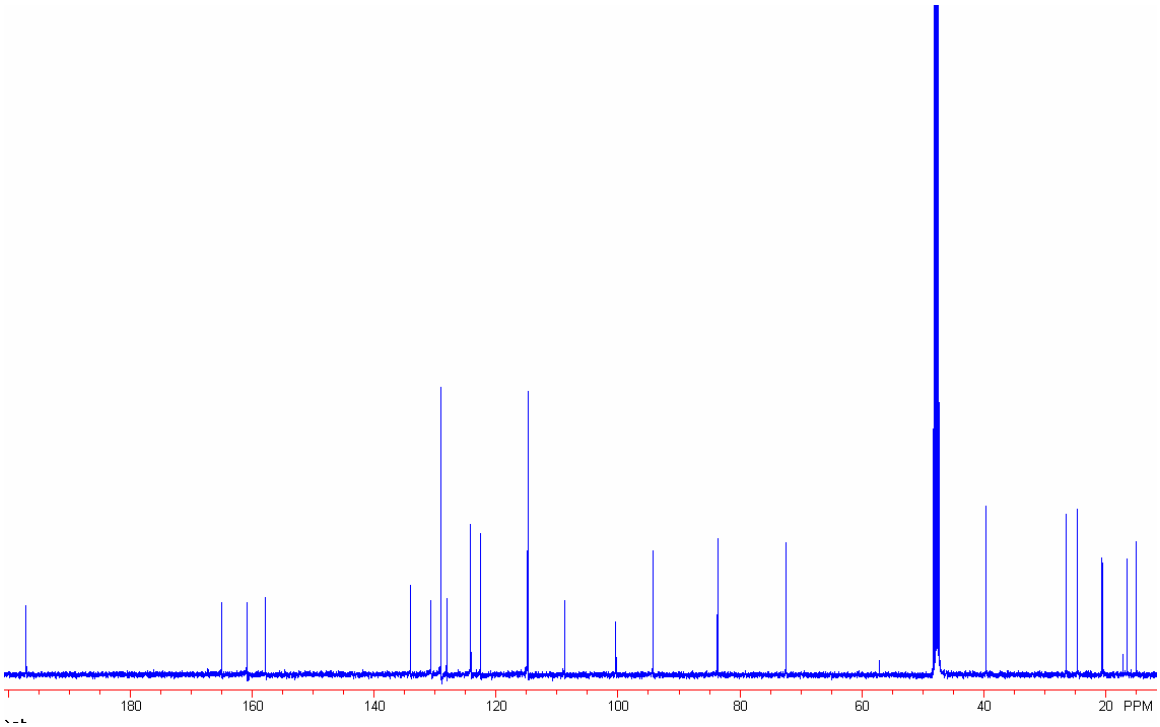
BY179-243-1 (**3.6**) Vedelianin



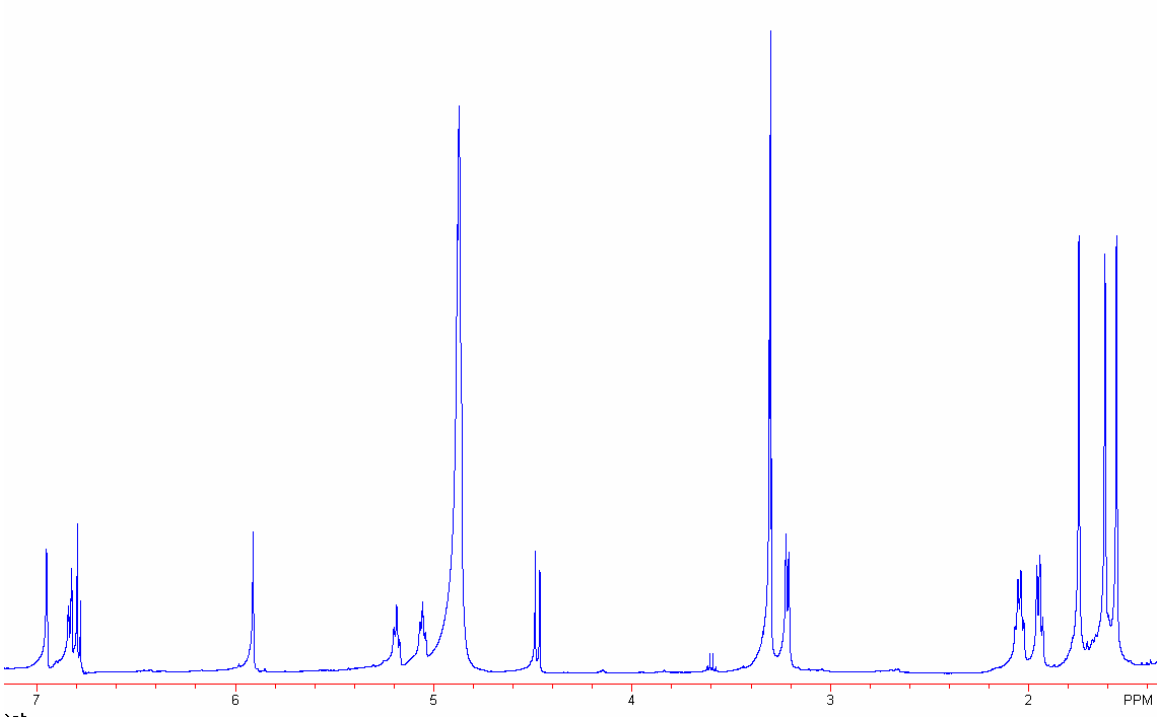


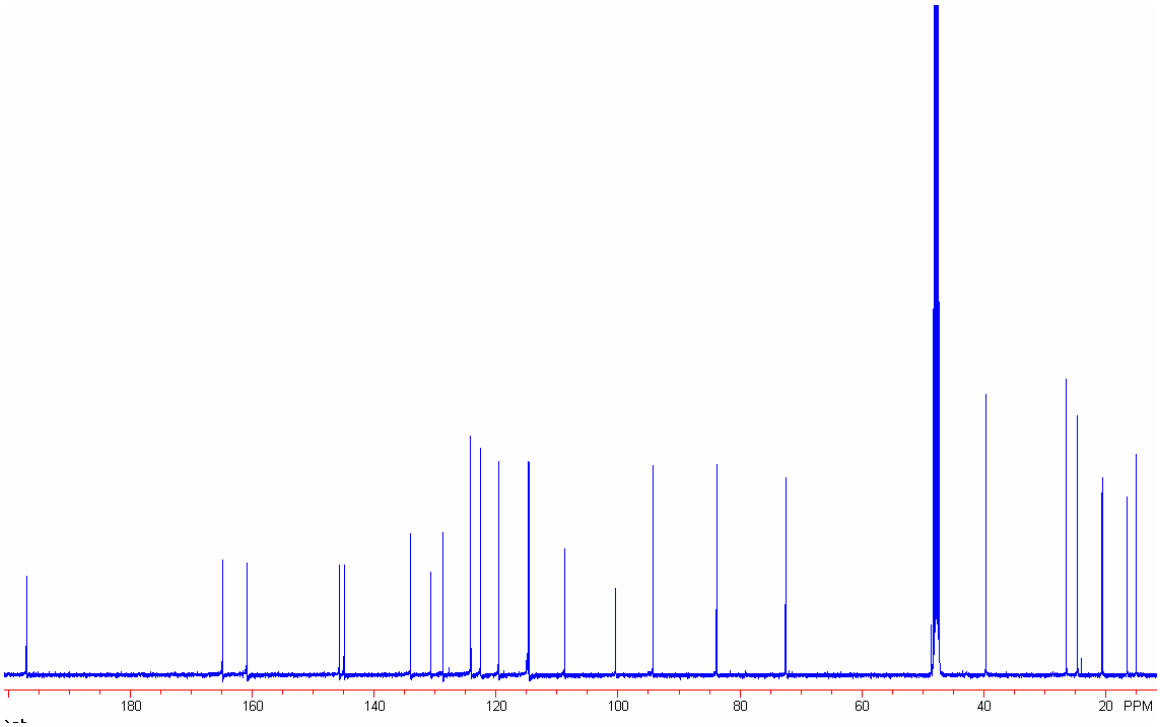
BY179-233-3 (**3.7**) Bonanniol A



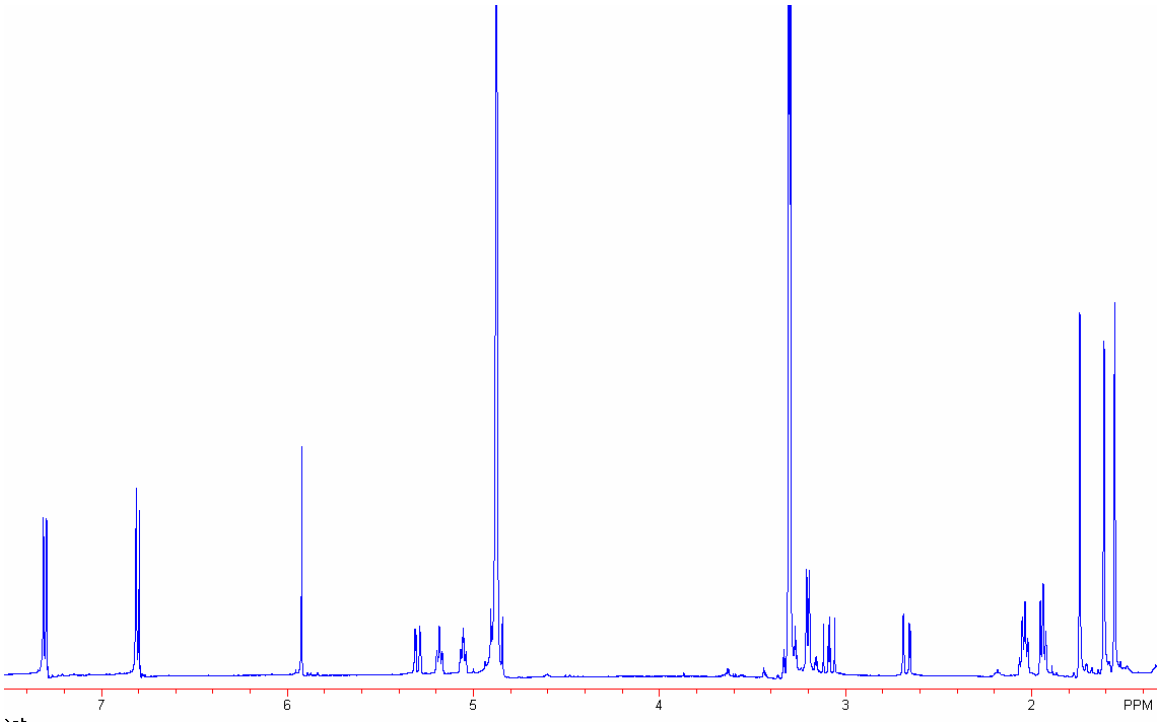


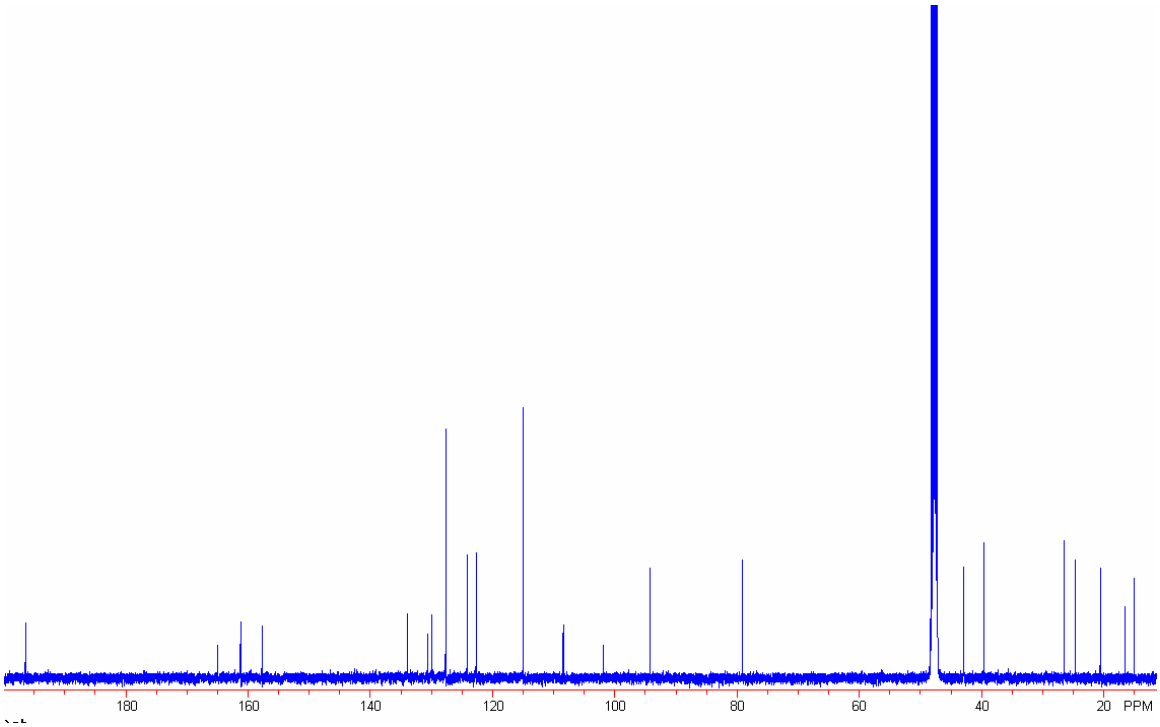
BY179-233-2 (**3.8**) Diplacol



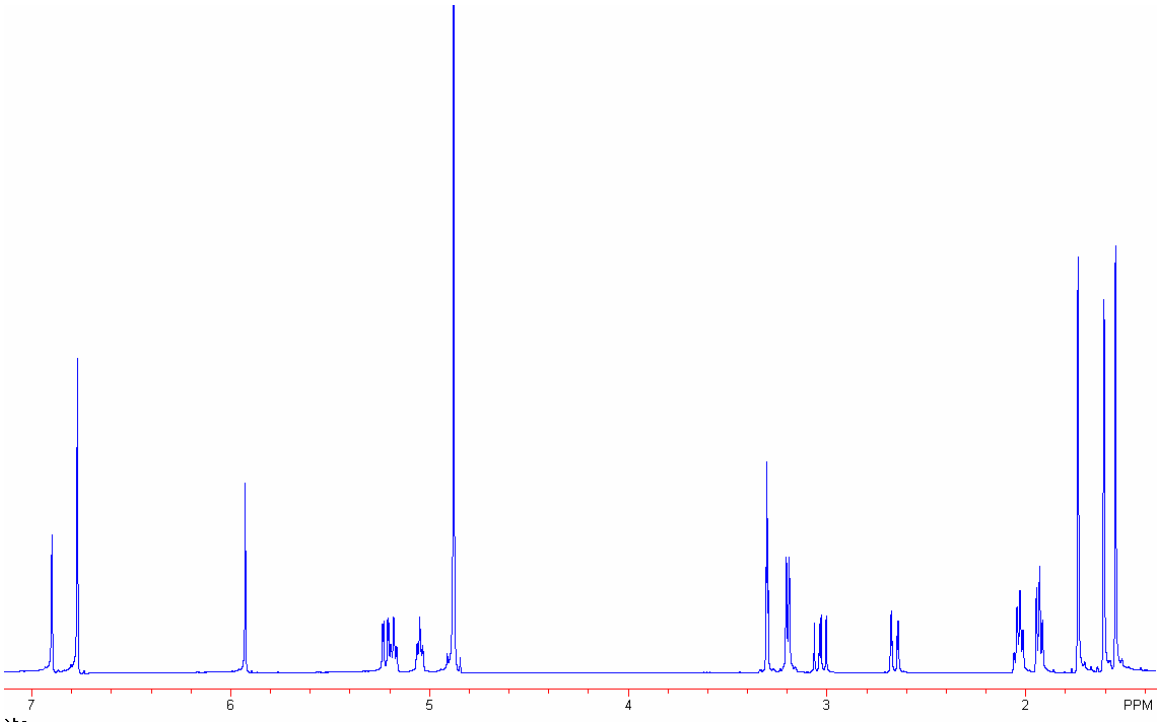


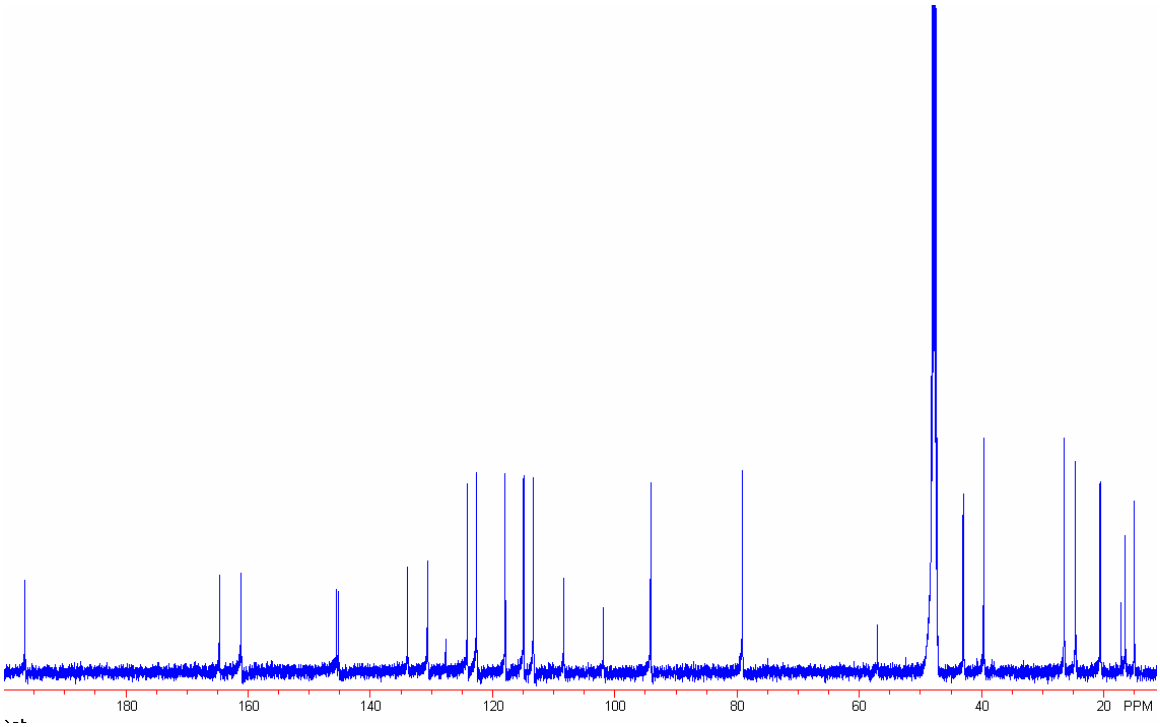
BY179-255-2 (**3.9**) Bonannione A



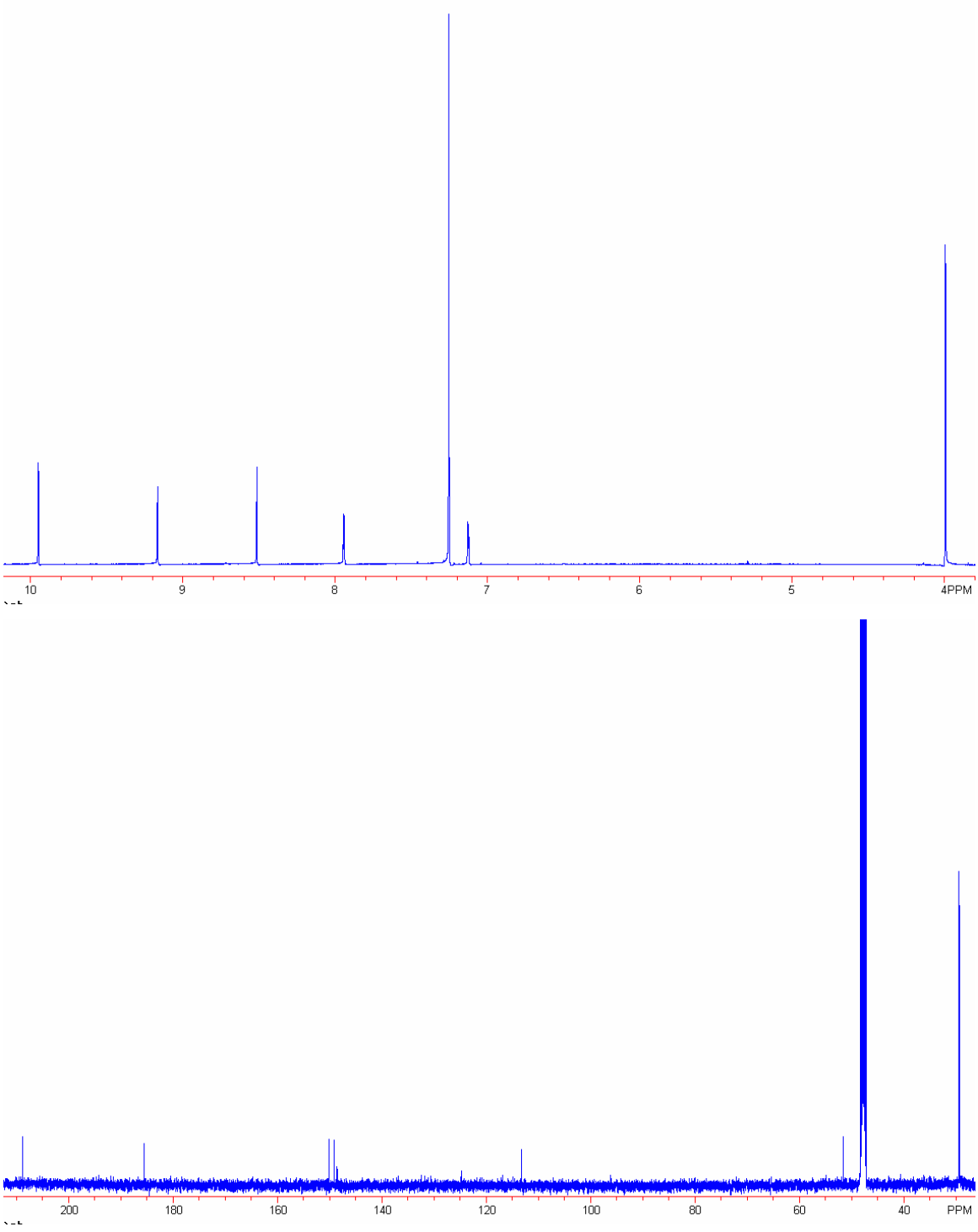


BY179-253-3 (**3.10**) Diplacone

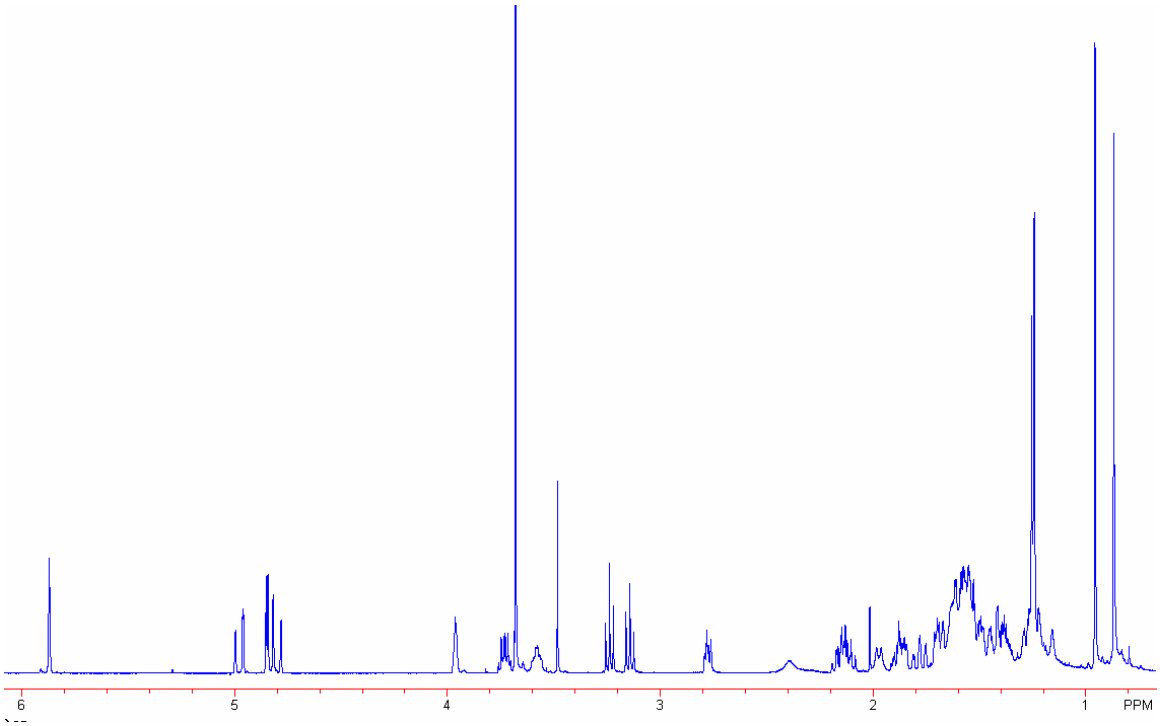


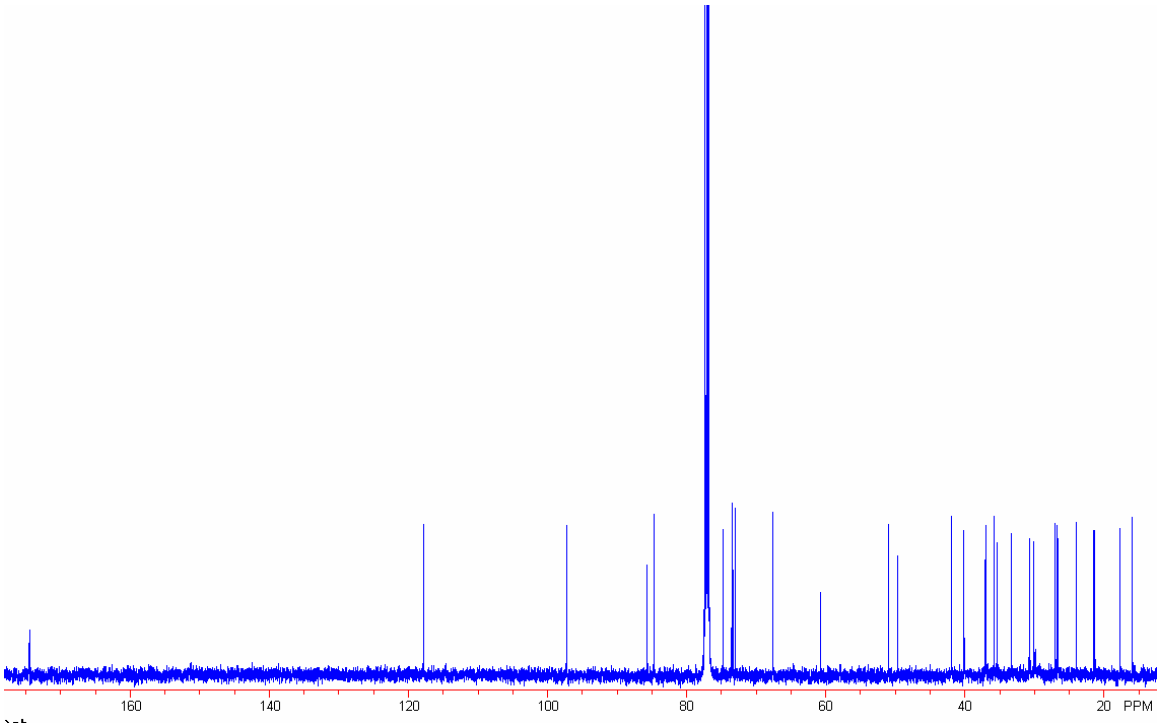


BY179-137-2 (**4.1**) Cerbinal

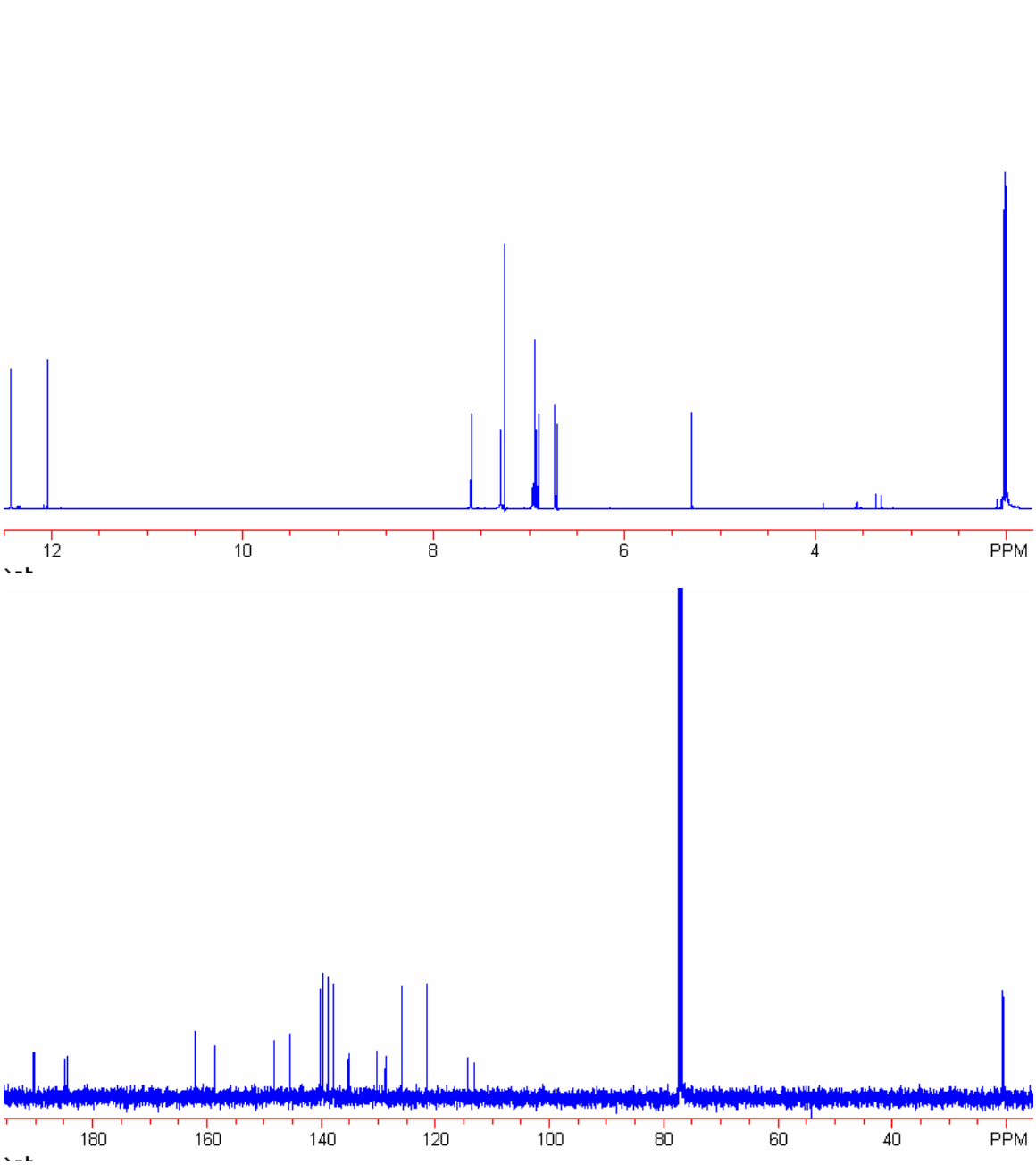


BY179-159-1 (**4.2**) Neriifolin

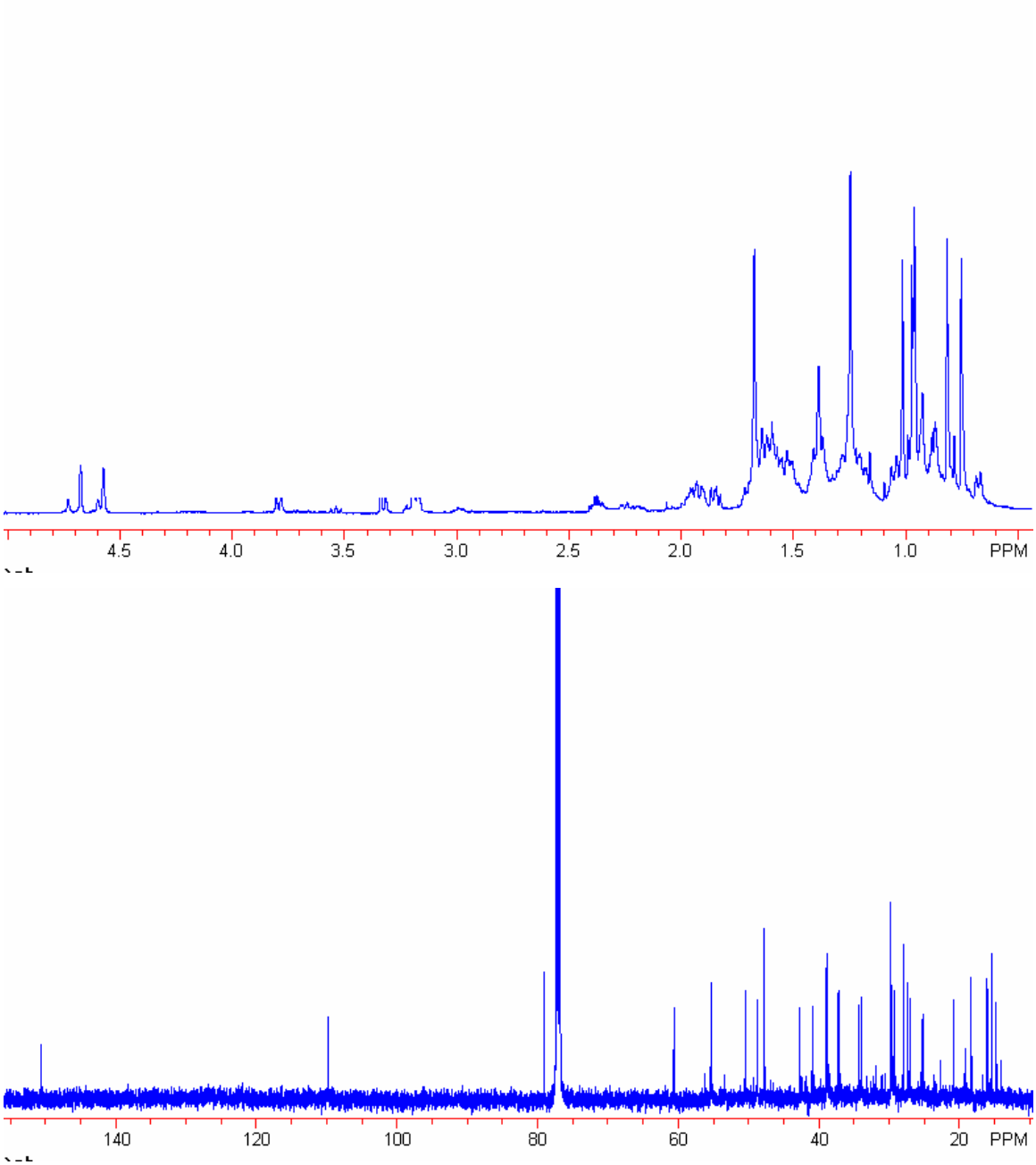




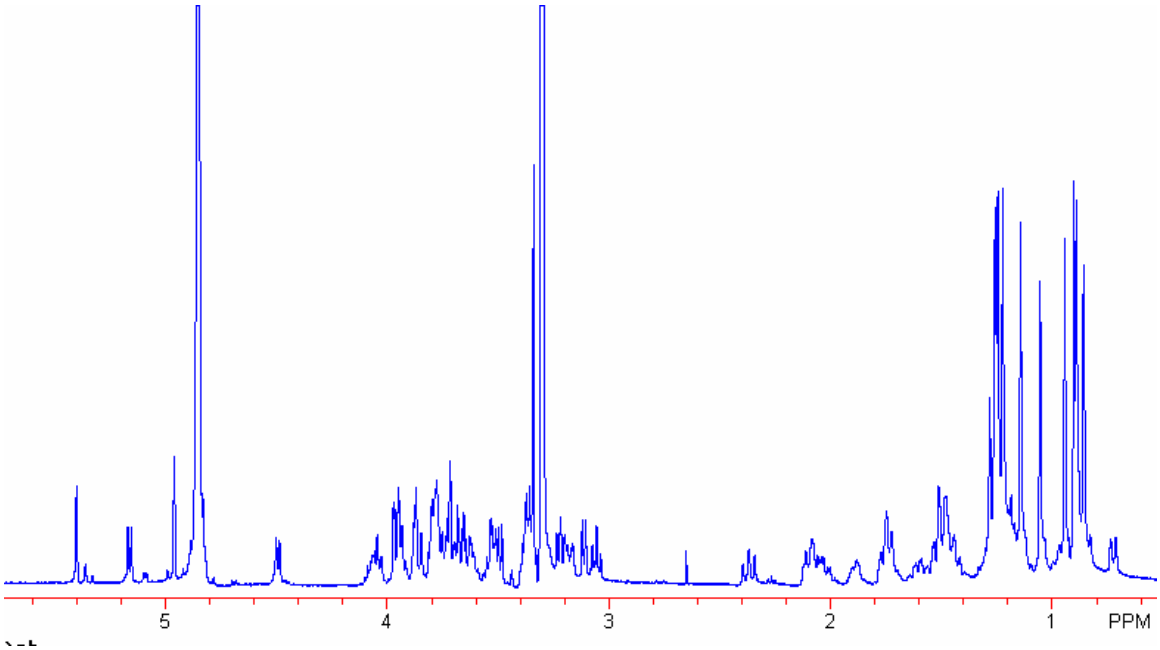
BY179-73-3 (**5.1**) Isodiospyrin

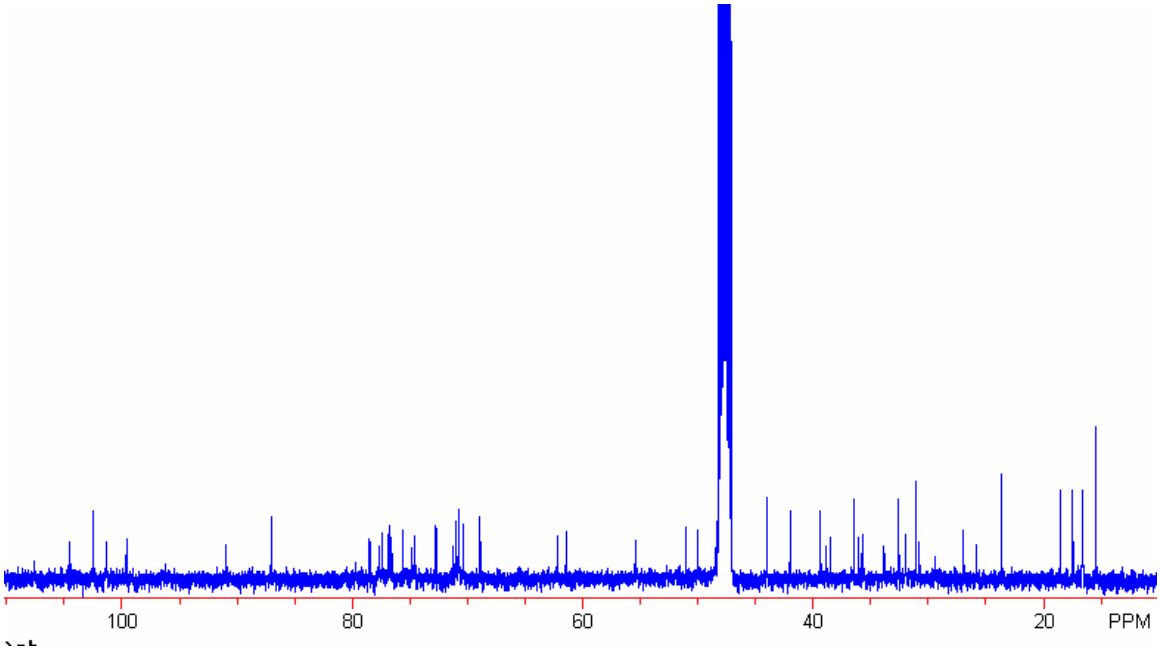


BY179-63-7b (**5.2**) Betulin



BY179-165-2 (**6.1**) Sakuraso-saponin





VITA

Brent J. Yoder was born on October 24, 1977 in Phoenix, AZ. He also spent his childhood years in Lancaster, CA before moving to Fort Wayne, IN and graduating from Carroll High School in 1996. He enrolled at Hesston College in Hesston, KS that fall as a pre-pharmacy major, but a newfound interest in organic chemistry prompted him to change his major during his sophomore year. In 1998, he took his Associate of Arts degree to Eastern Mennonite University in Harrisonburg, VA to complete a Bachelor of Science degree with a major in biochemistry and a minor in business administration.

While at EMU, he was exposed to research through his work with Dr. Glenn M. Kauffman on the isolation and structure determination of novel bicyclic products from the reaction of 2-methylcyclohexanone with 1,4-dichloro-2-butenes. They had the opportunity to continue their studies down the street at James Madison University through an NSF-REU program in the summer of 1999. At the conclusion of the program, Brent was voted the best presenter at the research symposium and awarded a travel scholarship to present his results at the National Conferences on Undergraduate Research in Missoula, MT. He also found time to work as a pharmacy technician at the Harrisonburg-Rockingham Free Clinic during his junior and senior years.

He entered the graduate program at Virginia Polytechnic Institute and State University in August 2000 and joined the natural products group of Dr. David G. I. Kingston. During the fall of 2003, he had the opportunity to serve as an instructor of organic chemistry at Virginia Tech, and he was given a graduate research award by the chemistry department for the 2004-2005 academic year. In May 2005, he was awarded a Future Professoriate Graduate Certificate, and in December 2005, he was awarded a Doctor of Philosophy degree with a major in organic chemistry.

Brent Yoder is a member of the American Chemical Society, the Chemical Education Division of ACS, the American Society of Pharmacognosy, and Blacksburg Christian Fellowship. He has been a child of God since 1989 and a husband of Rachel since 2004.