ANTIPROLIFERATIVE NATURAL PRODUCTS FROM THE MADAGASCAR RAINFOREST

by

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In

Chemistry

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ABSTRACT

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Yanpeng Hou

As part of an International Cooperative Biodiversity Groups (ICBG) program and a continuing search for anticancer natural products from the Madagascar rainforest, twenty extracts from Madagascar were selected for investigation based on their antiproliferative activity. Bioassay-guided fractionation of five of the extracts yielded sixteen new compounds, and their structures were determined using a combination of 1D and 2D NMR experiments, including COSY, HSQC/HMQC, HMBC, and ROESY/NOESY sequences, mass spectrometry, and chemical conversion. In addition, ten known compounds were obtained from five of the extracts. Studies on the remaining extracts were suspended due to various reasons. A multi-step synthesis of the sesquiterpenoid, (7*R**)-opposite-4(15)-ene-1β,7-diol, was also described.

The first chapter of this dissertation reviews the new compounds isolated from Malagasy plants and marine organism in the last two decades. Chapters II to VI discuss the isolation, structure elucidation and bioactivities of new compounds from *Scutia myrtina*, *Cordyla madagascariensis* ssp. *madagascariensis*, *Elaeodendron alluaudianum*, *Cassipourea lanceolata*, and *Sclerocarya birrea* subsp. *caffra*. Chapter VII describes the synthesis and bioactivity of the sesquiterpenoid, (7*R*∗)-opposite-4(15)-ene-1β,7-diol. The isolation of known compounds is discussed briefly in the last chapter.

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I. New Natural Products Isolated from Madagascar Plants and Marine Organisms*

1.1. Introduction

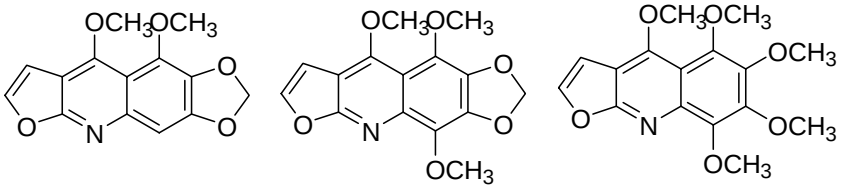
Madagascar's flora is one of the world's richest, with tremendous numbers of species found nowhere else. Of its 10,000 to 12,000 plant species, 80% are endemic. Nine plant families are found nowhere else in the world.[1] Because of this unique biodiversity, scientists from all over the world have studied the flora of Madagascar, in the hope that the scientific investigation of its plants can contribute to drug discovery and conservation of its biodiversity.[1]

In this chapter, work published in the last two decades (1991–2009) on new natural products isolated from Madagascar plants is reviewed. This summary excludes published by the author; this will be discussed in the following chapters.

1.2. Alkaloids

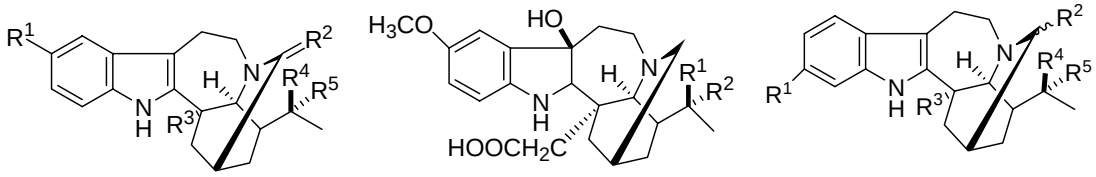
Three new furoquinoline alkaloids, 5-methoxymaculine (**1.1**), 5, 8-dimethoxymaculine (**1.2**), and 4,5,6,7,8-pentamethoxyfuroquinoline (**1.3**), along with four known alkaloids, flindersiamine, kokusaginine, maculine, and skimmianine, were isolated from a CH_2Cl_2/MeOH extract of the wood of *Vepris punctata* using bioassay-directed fractionation. All these compounds were tested against the A2780 human ovarian cell line, and all seven compounds showed IC_{50} values in the range 2.8– 4.2 μg/mL.[2]

* Manuscript in preparation for submission to *Curr. Med. Chem.* Hou, Y.; Harinantenaina, L. New and bioactive natural products isolated from Madagascar plants and marine organisms.



1.1 **1.2** **1.3**

Fifteen cytotoxic alkaloids, including three new indoles and 12 known ones, were isolated from a CH_2Cl_2-MeOH extract of *Tabernaemontana calcarea* using bioassay-directed fractionation. The three new alkaloids, namely 19-*epi*-3-oxovoacristine (**1.4**)**,** 19-*epi*-voacristine hydroxyindolenine (**1.5**) and 3*R/S*-hydroxytabernanthine (**1.6**), were investigated against the A2780 ovarian cancer cell line. They showed mild cytotoxicity with IC_{50} values of 6.8, 10.8 and 7.9 μg/mL respectively. The other 12 known alkaloids, namely voacangine, isovoacangine, coronaridine, 11-hydroxycoronaridine, voacristine, 19-*epi*-voacristine, isovoacristine, ibogamine, 10-methoxyibogamine, 11-methoxyibogamine, heyneanine, and 19-*epi*-heyneanine, also showed mild cytotoxicity with IC_{50} values between 3.5 and 11 μg/mL respectively.[3]

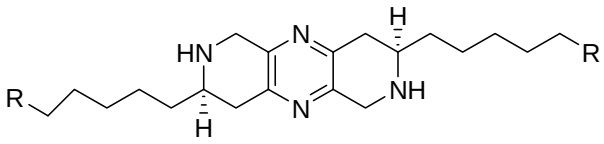


1.4 $R^1 = OCH_3$ $R^2 = O$ $R^3 = COOCH_3$ $R^4 = H$ $R^5 = OH$

1.5 $R^1 = H$ $R^2 = OH$

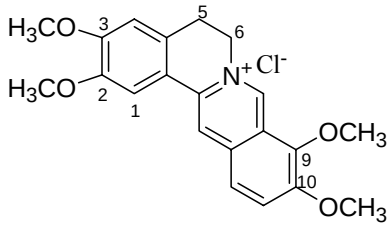
1.6 $R^1 = OCH_3$ $R^2 = OH$ $R^3 = H$ $R^4 = H$ $R^5 = H$

Two novel compounds, barrenazine A (**1.7**) and B (**1.8**), were isolated from an unidentified tunicate collected at Barren Islands, Madagascar. The two compounds possessed an unprecedented heterocyclic skeleton, and were investigated for cytotoxicity against LOVO-DOX colon carcinoma, LN-caP prostate carcinoma and K-52 Leukemia carcinoma cell lines. The results showed that barrenazine A exhibited cytotoxicity with GI_{50} values of 0.900 g/mL, 0.594 g/mL and 0.180 g/mL repectively.[4]

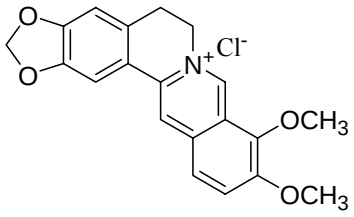


1.7 R = CH_2CH_3
1.8 R = $CH{=}CH_2$

Burasaine (**1.9**), a major alkaloid isolated from the roots of several species of the *Burasaia* genus endemic to Madagascar, was investigated for its cytotoxicity and DNA binding properties. Due to the structural similarity between berberine and burasaine, the hypothesis was raised that DNA could be a receptor for burasaine, and this turned out to be correct. The data in this report strongly supported that both berberine and burasaine form similar interaction complexes with double stranded DNA under the experimental conditions used in the paper. It was also found that neither burasaine nor berberine affected the DNA cleavage/religation reaction catalyzed by topoisomerases. That was possibly due to the presence of a methoxy group at the C-9-position which could prevent hydrogen bonding interactions with guanine residues. Thus based on the mechanism of action of burasaine, the compound is an attractive lead for future synthetic research despite the poor cytotoxicity of the compound itself.[5]

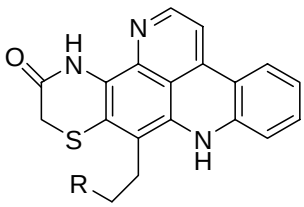


1.9

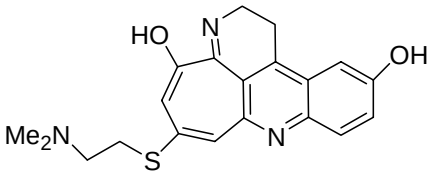


berberine

Three novel alkaloids, two pyridoacridines, namely shermilamine D (**1.10**) and shermilamine E (**1.11**), and one alkaloid, namely tintamine (**1.12**), bearing a novel heterocyclic system, were isolated from the Indian Ocean tunicate *Cystodytes violatinctus* collected at the northwest of Madagascar. It was suggested and synthetically demonstrated that the biosynthesis of pyridoacridines involves a reaction between kynuramine, an oxidation metabolite of tryptophan, and benzoquinone or its precursor. In the similar way, it was also suggested that the pathway of biosynthesis of tintamine involved the reaction between kynuramine and tropolone. But the assumption needed to be proved further.[6]

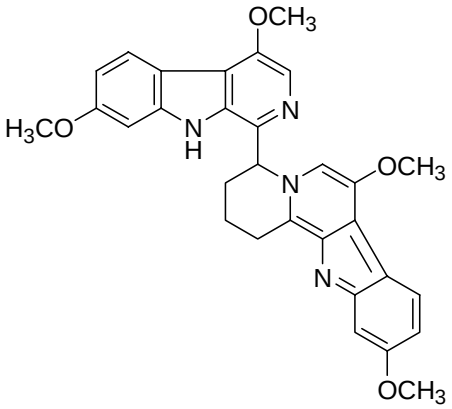


1.10 R = NMe_2
1.11 R = $N(O)Me_2$



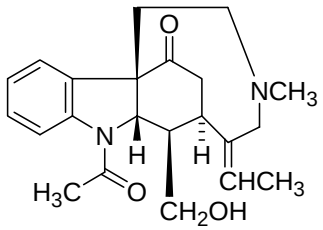
1.12

Two major alkaloids were isolated from the root bark of *Perriera madagascariensis*. One of them was new, named as kirondrine (**1.13**). The other one was isolated previously from the stems of the same plants, identified as 4, 7-dimethoxy-1-vinyl-β-carboline.[7]

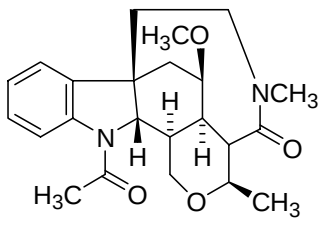


1.13

Eight alkaloids were isolated from the root bark of *Strychnos mostueoides.* Two of them proved to be new compounds. They were named as malagashine (**1.14**) and malagashanine (**1.15**). The other six alkaloids were the Wieland–Gumlich aldehyde, strychnofendlerine, normacusine B, strychnobrasiline, deacetylstrychnobrasiline and spermostrychnine.[8]

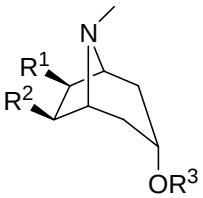


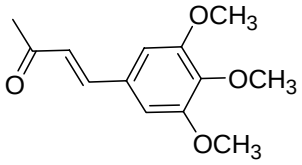
1.14

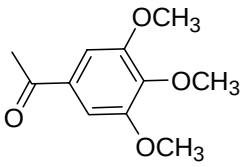


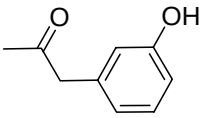
1.15

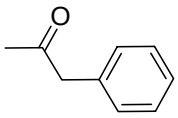
A series of new tropane alkaloid aromatic esters, namely pervilleine A (**1.16**), N–oxide pervilleine A (**1.17**), pervilleine B–H (**1.18**–**1.24**), *cis*-pervilleine B (**1.25**) and *cis*-pervilleine F (**1.26**) were isolated from the stem bark and roots of *Erythroxylum pervillei*.[9, 10] Pervelleine A-F (**1.16**, **1.18**–**1.22**) showed potential cytotoxicity and substantial selectivity against a mutidrug-resistance human oral epidermoid (KB-V1) cell line in the presence of vinblastine. Pervelleine B (**1.18**) and C (**1.19**) also potently reversed the drug resistance of a MDR human ovarian adenocarcinoma (SKVLB) to adriamycin. In addition, pervelleine A-C (**1.16**, **1.18** and **1.19**) and pervelleine F (**1.22**) were found to be effective MDR inhibitory agents mechanistically by inhibiting P–glycoprotein–mediated drug efflux. After further biological tests, pervelleine A (**1.16**) was then chosen as the best candidate for development as a potential MDR inhibitor.



Tmc = 

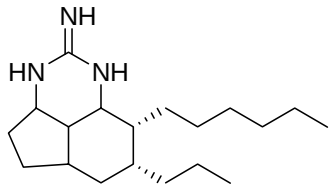
Tmb = 

3-OHPhCH$_2$CO = 

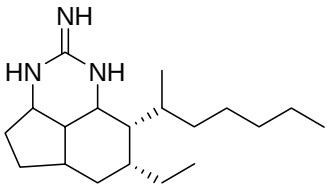
PhCH$_2$CO = 

1.16 R^1 = OH R^2 = OTmc R^3 = Tmb
1.17 R^1 = OH R^2 = OTmc R^3 = Tmb N→O
1.18 R^1 = H R^2 = OTmc R^3 = Tmb
1.19 R^1 = H R^2 = OTmc R^3 = Tmc
1.20 R^1 = OH R^2 = OTmc R^3 = Tmc
1.21 R^1 = H R^2 = OTmc R^3 = 3-OHPhCH$_2$CO
1.22 R^1 = H R^2 = OTmc R^3 = PhCH$_2$CO
1.23 R^1 = H R^2 = OH R^3 = Tmc
1.24 R^1 = OH R^2 = OH R^3 = Tmc
1.25 R^1 = H R^2 = *cis*-OTmc R^3 = Tmb
1.26 R^1 = H R^2 = *cis*-OTmc R^3 = PhCH$_2$CO

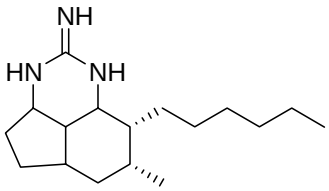
Seven tricyclic guanidine alkaloids, netamines A–G (**1.27**–**1.33**), were isolated from the Malagasy marine sponge, *Biemna laboutei*. Netamines C (**1.29**) and D (**1.30**) showed mild cytotoxicity against three tumor cell lines, NSCL (A549), colon (HT29) and breast (MDA-MB-231), with GI_{50} range of 2.4–6.6 μM, while the other isolates demonstrated less activity against those cell lines.[11]



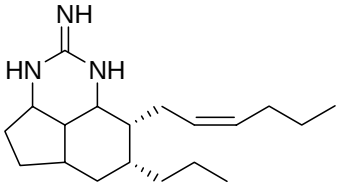
1.27



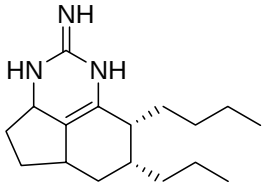
1.28



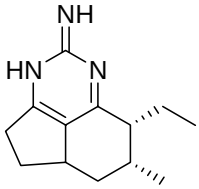
1.29



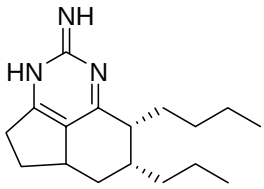
1.30



1.31

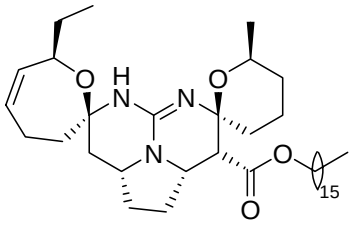


1.32



1.33

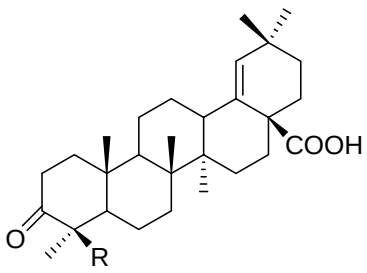
A new polycyclic guanidine alkaloid, ptilomycalin D (**1.34**), was isolated along with the known crambescidic acid from the marine sponge *Monanchora dianchora* collected in the northwest of Madagascar. It is proposed that ptilomycalin D was probably a biogenetic precursor of crambescidic acid, which is the precursor of ptilomycalin A.[12]



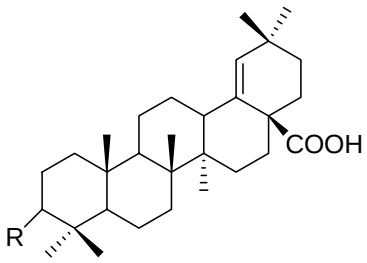
1.34

1.3. Terpenoids

Five new triterpenoids, designated as acridocarpusic acids A-E (**1.35–1.39**), were obtained from a methanol extract of the leaves and flowers of *Acridocarpus vivy*, along with several known compounds using bioassay-guided fractionation. All the isolated compounds were tested for antiproliferative activity against the A2780 human ovarian cell lines. Aridocarpusic C (**1.37**) showed significant activity against the A2780 cells, with an IC_{50} value of 0.7 μg/mL, while other compounds showed moderate activity or no activity.[13]

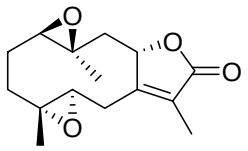


1.35 R = CH_2OH
1.36 R = CH_3OAc
1.37 R = CHO

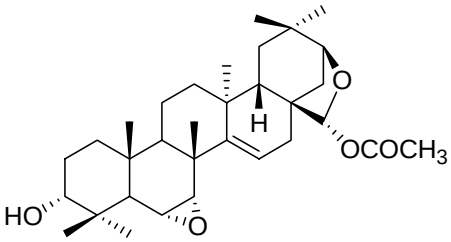


1.38 R = OH
1.39 R = OAc

Continued bioassay-guided fractionation of the wood of *Vepris punctata* led to the isolation of one new sesquiterpenoid, assigned as 1β,10β:4α,5α-diepoxy-7(11)-enegermacr-8α,12-olide (**1.40**), and a new triterpenoid, designated as 28-acetyloxy-6α,7α:21β,28-diepoxytaraxer-3α-ol (**1.41**), along with three known sesquiterpenoids, glechomanolide, isogermafurenolide, (E,E)-germacra-1(10),4,7(11)-triene, and five known triterpenoids. All the isolated compounds were tested for antiproliferative activity against the A2780 human ovarian cancer cell line. The four sesquiterpenoids including **1.40**, glechomanolide, isogermafurenolide and (E,E)-germacra-1(10),4,7(11)-triene displayed moderate cytotoxic activity with IC_{50} values in the range 3.8-6.4 μg/mL, while none of the triterpenoids showed biological activity against the A2780 cells.[14]



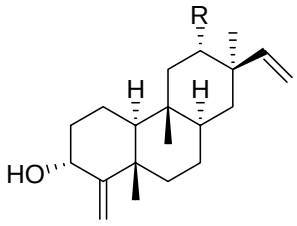
1.40



1.41

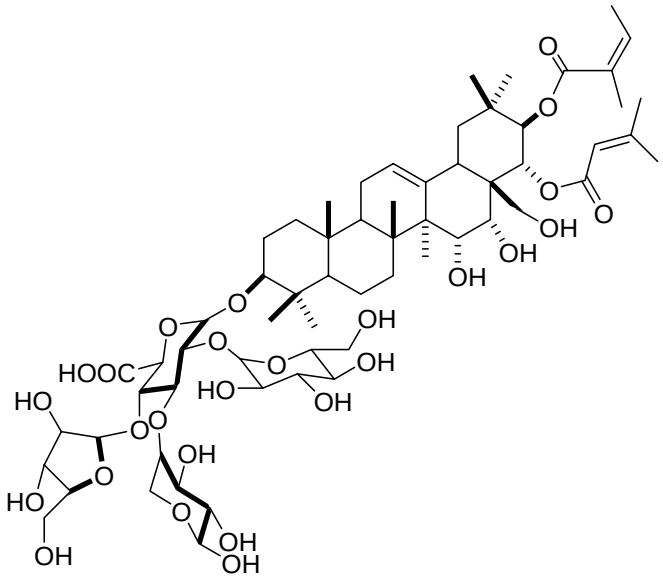
Fractionation of an extract of the bark of *Givotia madagascariensis* resulted in two new erythroxylane diterpenes, givotin A (**1.42**) and givotin B (**1.43**), together with six known compounds. The structures of givotin A (**1.42**) and B (**1.43**) have been established as 3α, 12α-dihydroxy-4(19), 15-erythroxyladiene and 3α-hydroxy-4(19),15-erythroxyladiene, respectively. The isolated compounds were tested for cytotoxicity against the HM02 gastric carcimoma, HEP G2 liver carcinoma, and MCF 7 breast carcinoma cells. As a result, the known compounds, cleistanthol, spruceanol and 1, 2-

dihydroheudelotinol were found to show significant antitumor activities against the three tumor cell lines, although the two new compounds did not display interesting cytotoxicity.[15]



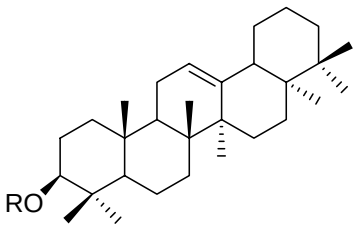
1.42 R = OH
1.43 R = H

A methanol extract of *Pittosporum viridiflorum* was subjected to bioassay-guided fractionation using a mechanism-based bioassay involving genetically engineered mutants of the yeast *Saccharomyces cerevisiae*, specifically engineered yeast strains 1138, 1140 and 1353 in this study. The investigation led to a novel triterpenoid saponin, pittoviridoside (**1.44**), which had a sugar moiety with an unusual 2,3,4,-trisubstituted glycosidic linkage. The compound was tested against the 1138, 1140, 1353 and Sc7 yeast strains, but it only showed weak activity with IC_{12} values of 85, 80, 140 and 100 μg/mL. It was also tested against the A2780 human ovarian cancer cell line, and a weak cytotoxicity was exhibited with an IC_{50} value of 10.1 μg/mL.[16]

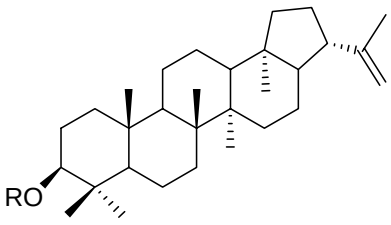


1.44

Two new triterpenoid esters, namely kairatenyl palmitate (**1.45**) and hopenyl palmitate (**1.46**), along with five known triterpenoids, were isolated from an ethanol extract of the small twigs of *Brachylaena ramiflora* using bioassay-guided fractionation. All seven compounds were tested for cytotoxicity against the A2780 human ovarian cell lines. All showed moderate cytotoxicities with IC_{50} values in range 10.0–16.2 μg/mL.[17]

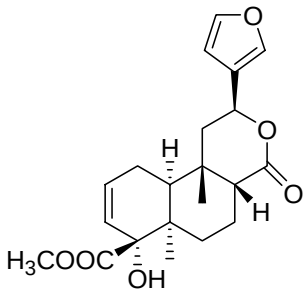


1.45 R = $CH_3(CH_2)_{14}CO$



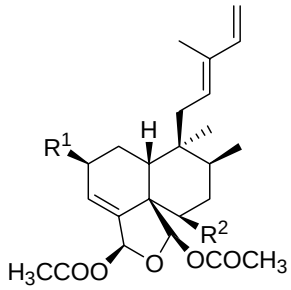
1.46 R = $CH_3(CH_2)_{14}CO$

Two clerodane-type diterpenes, including one new compound, epicordatine (**1.47**), along with the known *N*-acetylnornuciferine were isolated from an ethanol extract of the stems of *Burasaia madagascarensis*. The three compounds were tested against chloroquine-resistant *Plasmodium falciparum* strain K1 for antiplasmodial activity. As a result, the isolated compounds exhibited weak antimalarial activity with IC_{50} values in the range of 29-31 μg/mL, while chloroquine showed an IC_{50} value of 0.12 μg/mL under the same condition.[18]

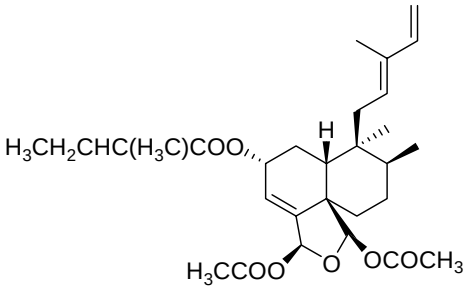


1.47

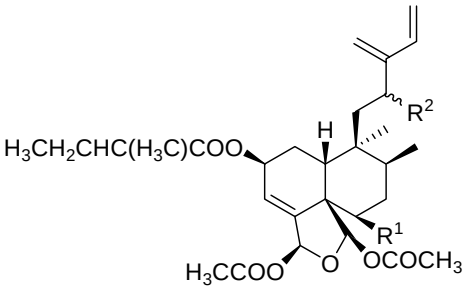
Bioassay-guided isolation of a CH_2Cl_2/MeOH extract of the bark of *Casearia lucida* afforded 11 new clerodane diterpenes, namely, casearlucins A-K (**1.48–1.58**) along with several known compounds. All the isolated compounds exhibited cytotoxicity against the A2780 ovarian cancer cell line with IC_{50} values ranged from 2.7–3.1 μg/mL. Furthermore, six compounds were selected to be investigated against a panel of mammalian cell lines, but none of these compounds showed significant selectivity. These results suggested that these compounds functioned as general cytotoxic agents. In addition, the biological activity profile of these compounds implied their cytotoxicity may depend on the basic skeleton of clerodane diterpenes rather than any specific functional groups.[19]



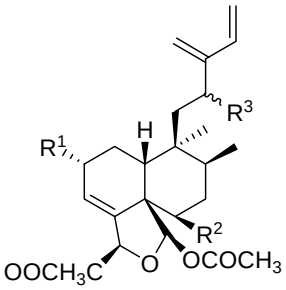
1.48 $R^1 = CH_3CH_2CH(CH_3)COO$ $R^2 = OH$
1.50 $R^1 = CH_3CH_2CH(CH_3)COO$ $R^2 = OCOCH_3$
1.53 $R^1 = CH_3CH_2CH(CH_3)COO$ $R^2 = H$



1.54

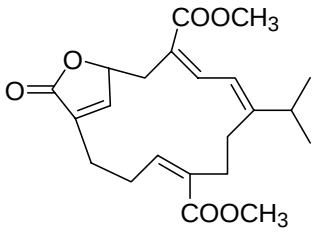


1.51 $R^1 = OCOCH_3$ $R^2 = H$
1.55 $R^1 = OH$ $R^2 =$ ''''OH
1.56 $R^1 = OH$ $R^2 =$ ━OH

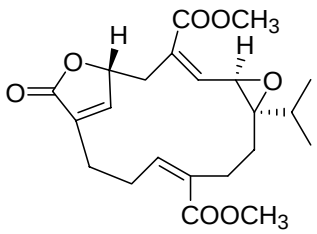


1.49 $R^1 = CH_3COO$ $R^2 = OCH_3$ $R^3 = H$
1.52 $R^1 = CH_3CH_2CH(CH_3)COO$ $R^2 = OCH_3$ $R^3 = H$
1.57 $R^1 = CH_3CH_2CH(CH_3)COO$ $R^2 = OH$ $R^3 =$ ''''OH
1.58 $R^1 = CH_3CH_2CH(CH_3)COO$ $R^2 = OH$ $R^3 =$ ━OH

Two new cembrane diterpenes (**1.59, 1.60**) have been isolated from an unidentified species of soft coral of genus *Sarcophyton*. Compounds **1.59** and **1.60** proved to be inactive against antibiotic assays even under doses up to 250 μg/disc.[20]

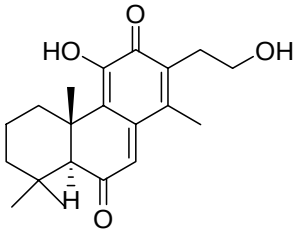


1.59

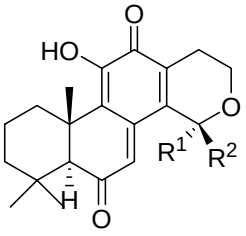


1.60

Three new quinone methide diterpenes (**1.61–1.63**) with the cassane skeleton were isolated from the root bark of *Bobgunnia madagascariensis*. Their structures were established by spectroscopic methods including single-crystal X-ray analysis. Noticeably, **1.62** and **1.63** were diastereomers, and they could interconvert into each other over a two-hour period. Compound **1.61** showed strong antifungal properties towards human pathogenic fungi, particularly against the yeasts *Candida* spp., with evidence of lower minimum inhibitory concentrations (MIC) values than those of the reference compounds amphotericin B and fluconazole. On the other hand, compounds **1.62** and **1.63** only exhibited moderate antifungal properties towards human pathogenic fungi, in particular the yeast *Candida albicans*. Comparing the structural difference among the three compounds, it was suggested that the presence of a hemiacetal function at position C-17 may significantly lower the activity of compounds of this type.[21,22]

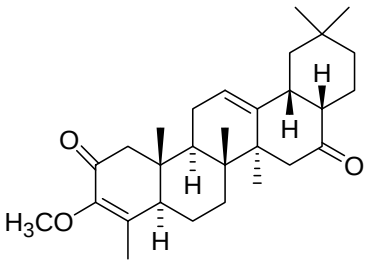


1.61

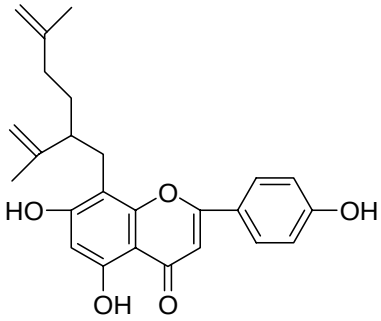


1.62 R^1 = H R^2 = OH
1.63 R^1 = OH R^2 = H

A new bisnoroleanane triterpene, remangilone D (**1.64**)**,** and a new flavone (**1.65**) were isolated from the leaves of *Physena madagascariensis*,[23] but no bioactivity data were reported in the article.

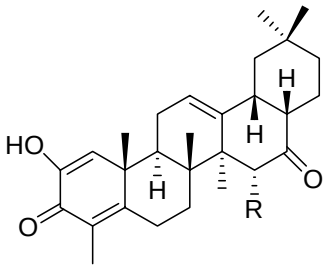


1.64

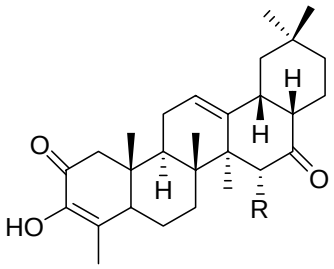


1.65

An earlier publication from the same group described the bioassay-guided fractionation of an extract of the dried leaves of *Physena madagascariensis*. This study led to isolation of three new cytotoxic triterpenes, which were 24, 28-dinorolean-3-one derivatives and designated as remangilones A-C (**1.66–1.68**), respectively. Remangilones A and C were investigated for cytotoxicity against the two established breast carcinoma cell lines MDA-MB-231 and MDA-MA-435. It was found that the two compounds showed IC_{50} values of 8.5 and 2.0 μM, respectively, against the MDA-MB-231 cells, and displayed similar IC_{50} values of 6.6 and 1.6 μM, respectively, against MDA-MA-435 cells. It was also indicated that the two compounds showed prolonged and irreversible cytotoxicity to MDA-MB-231 cells, and that the induced apoptosis (programmed cell death) was observed at concentrations of 2.3 μM. In addition, the fact that the two compounds showed significantly less toxicity towards normal mammary epithelial cells 70N in the same assay suggested that they may be selectively cytotoxic to tumor cells rather than normal cells. Although the explanation of this phenomenon required further understanding of the molecular mechanism of cytotoxicity of remangilones A and C, one possibility considered was the different p53 status among the three cell lines, MDA-MB-231, MDA-MB-435 and 70N.[24]

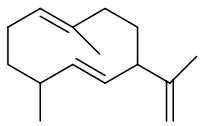


1.66 R = OH
1.67 R = H

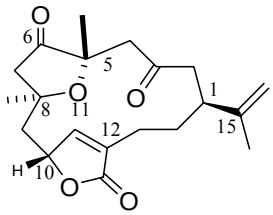


1.68

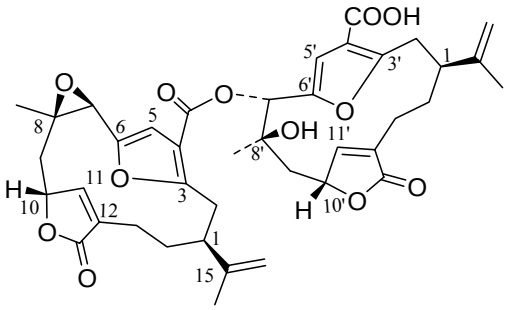
Four new compounds, namely germacrene E (**1.69**), epi-norcembrene (**1.70**) and two bis-pukalide diterpenes, mayotolides A and B (**1.71, 1.72**), were isolated from the Indo-Pacific soft coral *Sinularia erecta*. In addition to the new compounds, the three known diterpenoids, nephthenol, decaryiol and norcembrene, and one known sesquiterpenoid, germacrene D, were obtained from this marine organism. Their structures were determinated through careful analysis of 1D and 2D NMR and MS spectroscopy and additional chemical transformations. No activity data were reported for the isolates in the article.[25]



1.69

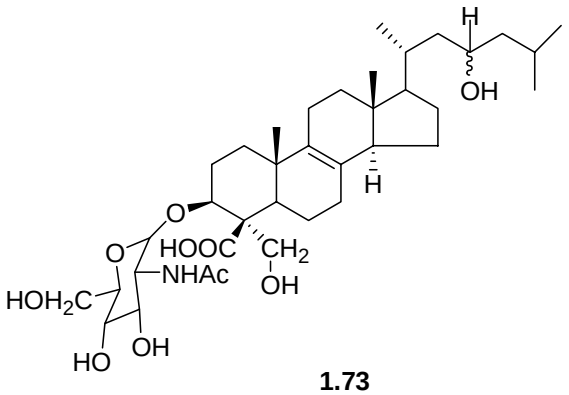


1.70



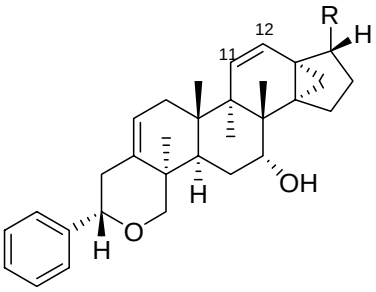
1.71
1.72 8'-epi

Fractionation of an extract of a marine sponge of an *Ulosa* sp. led to a new triterpene glycoside, ulososide B (**1.73**). Its structure was determined based on analysis of its spectroscopic data and comparison between the aglycone upon hydrolysis and an authentic sample. It was found that the compound possessed different aglycon and sugar moieties from all previously reported sponge glycosides.[26]

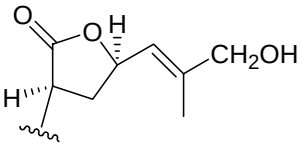


1.73

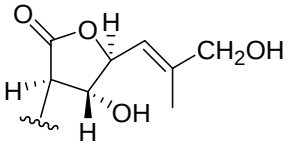
A novel class of triterpenoids, namely dichapetalins A-H (**1.74–1.81**), was isolated from the roots of *Dichapetalum madagascariense.* The isolated compounds were tested for cytotoxicity against brine shrimp and various tumor cell lines. Dichapetalin A (**1.74**) exhibited pronounced cytotoxicity against brine shrimp, which exceeded that of podophyllotoxin by seven-fold, while dichapetalins C (**1.76**) was less active in this bioassay. Dichapetalins A (**1.74**) was also found to inhibit cell growth in a variety of cell systems, but different cell lines varied widely in their responses. This indicated that dichapetalin A displayed a strong and selective cytotoxicity against cultured cancer cells.[27,28] A later article reported the absolute configuration of dichapetalin A (**1.74**) as shown.[29]



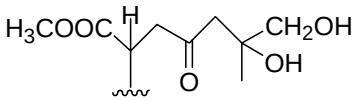
1.74 R =



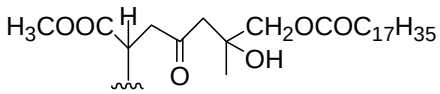
1.75 R =



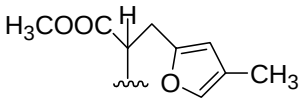
1.76 R =



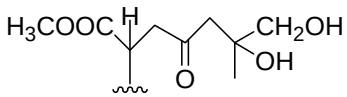
1.77 R =



1.78 R =



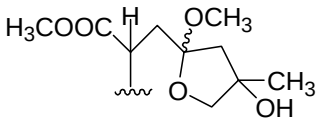
1.79 R =



11, 12-dihydro

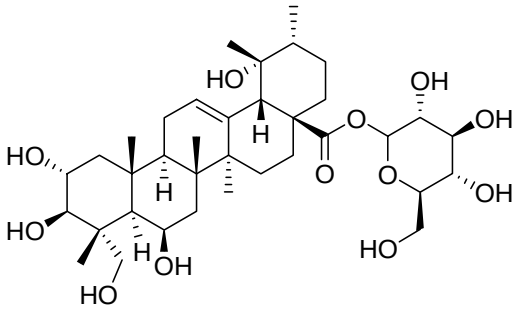
1.80

1.81 R =



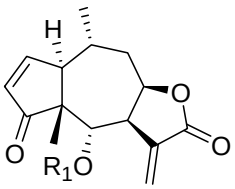
11,12-dihydro

One new triterpene saponin, designated as 28-O-β-D-glucopyranosyl 6β, 23-dihydroxytormentic acid (**1.82**), was isolated from the leaves of *Aphloia madagascariensis* along with the known triterpene saponin, 28-O-β-D-glucopyranosyl 6β-hydroxytormentic acid. No activity data were reported for the isolated compounds in this article.[30]

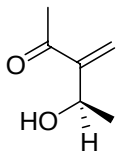


1.82

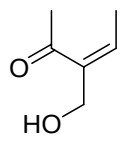
Three sesquiterpenoids were isolated from an ethyl acetate extract of leaves of *Vernoniopsis caudate*. Two of the three compounds were new sesquiterpene lactones (**1.83**, **1.84**), while the other one was identified as a related structure 11α, 13-dihydrohelenalin-[2-(1-hydroxyethyl)acrylate]. The three compounds exhibited strong antiplasmodial activity with IC_{50} values of 1, 0.19, and 0.41 μM, individually against the chloroquine-resistant *Plasmodium falciparum* strain FcB1. But the compounds were also found to be cytotoxic towards KB cells with IC_{50} values less than 1 μM. It was believed that α, β-unsaturated ketone units in the structures might contribute to the antiplasmodial activity and the cytotoxicity of those compounds.[31]



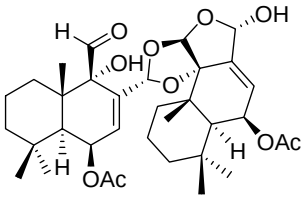
1.83 R_1 =



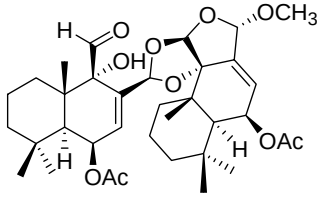
1.84 R_1 =



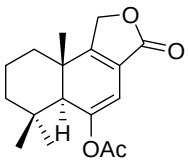
Fractionation of an extract of the Malagasy medicinal plant, *Cinnamosma fragrans* led to two new dimeric drimane sesquiterpenoids, cinnafragrins A (**1.85**) and B (**1.86**), and one new trimeric drimane sesquiterpenoid, cinnafragrin C (**1.87**) along with cinnamodial, D-mannitol, capsicodendrin and delta-tocotrienol.[32] The same group later isolated two new monomeric drimane sesquiterpenes, cinnamacrins A and B (**1.88**–**1.89**), and two new dimeric drimane sesquiterpenes, cinnamacrin C (**1.90**) and cinnafragrin D (**1.91**) from the other Malagasy plant of the same genus, *C. macrocarpa*. In the meantime, the known compounds, bemadienolide, capsicodendrin, cinnamodial, cinnamolide, isopolygodial, and delta-tocotrienol, were isolated and identified.[33] The isolates were tested for α-glucosidase inhibition activities. It turned out that the known compounds capsicodendrin and cinnamodial showed strong inhibition activities and aforementioned cinnafragrin B (**1.86**) displayed mild activity, while the others were inactive. From the structure activity relationship point of view, it seemed that a strong activity required the presence of the C-9 aldehyde group along with the 12-*R* configuration. The known compounds capsicodendrin and cinnamodial were also chosen to evaluate for cytostatic effects on the proliferation of murine leukemia L1210/0 and human T-lymphocyte Molt4/C8 and CEM/0 cells, and they were claimed to be strongly active with IC_{50} values from 0.56 to 1.61 μM. Further tests of those two compounds against multiple viruses did not however show any activity.



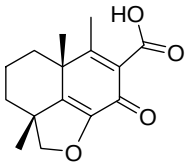
1.85



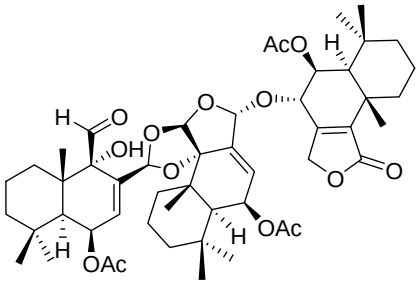
1.86



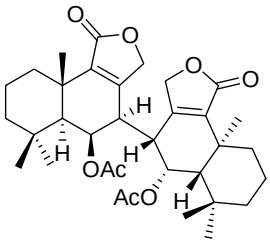
1.88



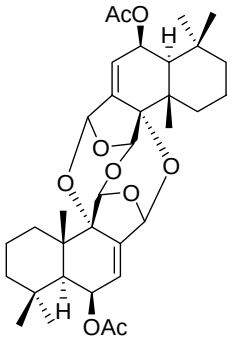
1.89



1.87

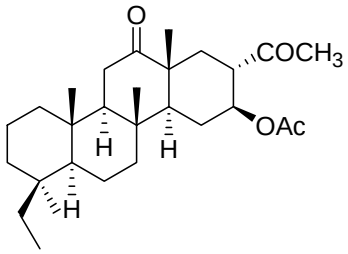


1.90

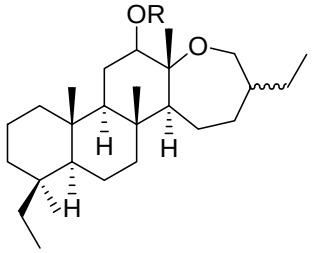


1.91

Fractionation of an extract of the Malagasy sponge *Phyllospongia madagascarensis* resulted in the isolation of two new scalaranes, designated as 16β-acetoxy-20,24-dimethyl-12,24-dioxo-25-norscalarane (**1.92**) and 12β-hydroxy-20,24-dimethyl-13,18-oxa-25-norscalarane (**1.93**), along with the new ethyl ether of **1.93** (**1.94**), which was believed to be an artificial natural product, and the known phyllofolactones F, G, and B. The seven-membered oxacycle in **1.93** made its skeleton unique.[34]

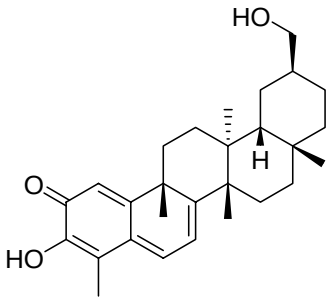


1.92

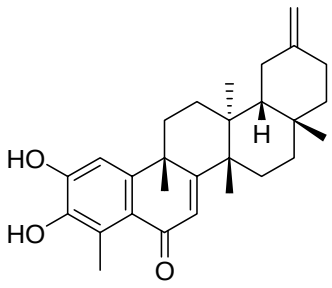


1.93 R = H
1.94 R = CH_2CH_3

Fractionation of an petroleum ether extract of the roots of *Salacia madagascariensis* led to two new compounds, a new bisnortriterpene quinone methide named as 20-epi-isoiguesterinol (**1.95**) and a new 6-oxophenolic triterpene named as 6-oxoisoiguesterin (**1.96**). The two compounds were assayed against *Leishmania donovani*, which is known to be the cause of visceral leishmaniasis. The results showed the two compounds were significantly more active than amphotericin B, which is used to treat the disease. That indicated the potential of the two compounds to be developed into drug candidates/leads against *Leishmania*. In addition, the two compounds were tested for antimicrobial activity against multiple microorganisms and for antiplasmodial activity against the D6 and W2 clones of *Plasmodium falciparum.* The results indicated that both of them showed moderate or weak antimicrobial and antiplasmodial activities.[35]

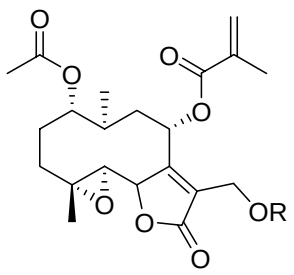


1.95

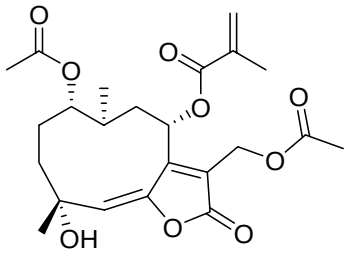


1.96

Three new sesquiterpene lactones, glaucolides K–M (**1.97**–**1.99**) were isolated from leaves of *Vernonia pachyclada* from the Madagascar rainforest using bioassay-guided fractionation. The isolates were tested against the A2780 human ovarian cancer cell line, and glaucolide M (**1.99**) displayed mild cytotoxicity.[36]

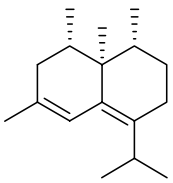


1.97 R = Ac
1.98 R = H

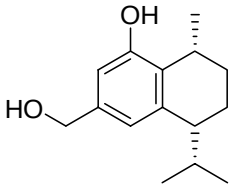


1.99

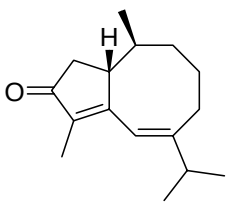
Fractionation of an extract of the wood of *Enterospermum madagascariensis* collected in Madagascar led to three new cadinane- and guaiane-type sesquiterpenoids, 2-hydroxy-10-epi-zonarene (**1.100**), 2,15-dihydroxycalamenene (**1.101**) and guaia-4,6-dien-3-one (**1.102**). Their structures were mainly determined on the basis of a thorough analysis of NMR spectroscopic data.[37]



1.100

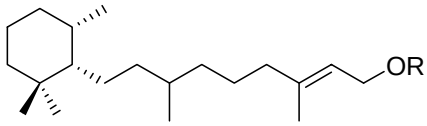


1.101



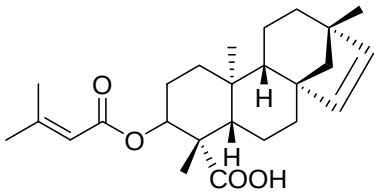
1.102

Two new terpenoids, cassipourol (**1.103**) and cassipouryl acetate (**1.104**) along with three known compounds, 3β,30-dihydroxylup-20(29)-ene, 30-hydroxylup-20(29)-en-3-one, and combretol, were isolated from the roots and leaves of *Cassipourea madagascariensis* from Madagascar rainforest. Cassipourol (**1.103**) and cassipouryl acetate (**1.104**) showed moderate antiproliferative activity against the A2780 human ovarian cancer cell line with IC_{50} values of 2.4 and 2.8 μM, respectively, while the other compounds were weakly active against this assay.[38]



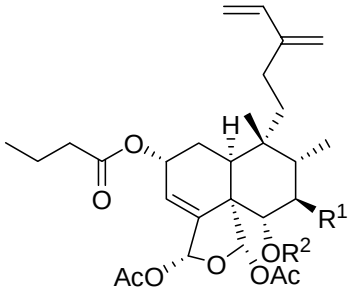
1.103 R = H
1.104 R = $COCH_3$

A new beyerane diterpenoid (**1.105**) was isolated from an acetone extract of the leaves of *Plectranthus saccatus* Benth. using bioassay-guided fractionation. This isolate showed weak insect antifeedant activity against *Spodoptera littoralis*.[39]



1.105

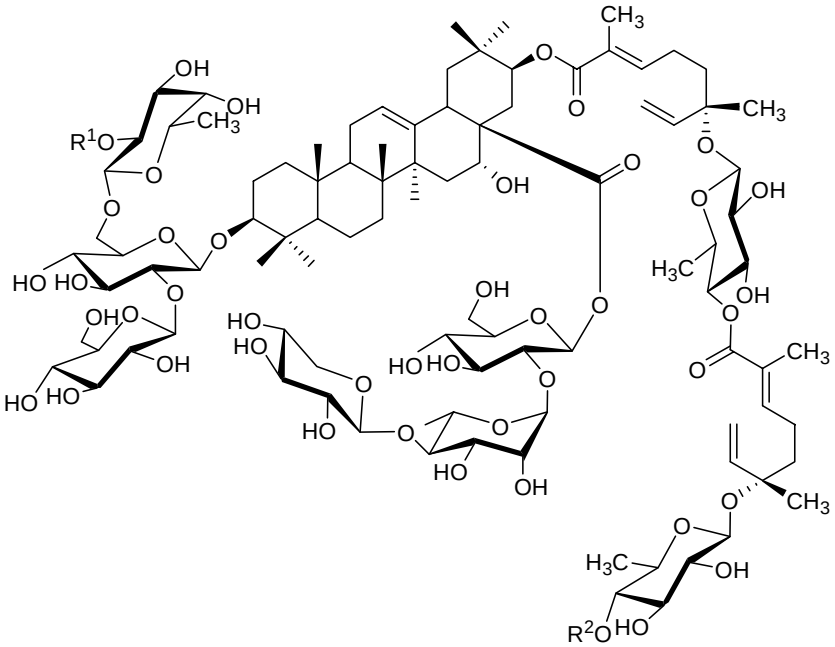
Bioassay-guided fractionation of an extract of the leaves and flowers of *Casearia nigrescens* afforded four new clerodane diterpenoids, caseanigrescens A–D (**1.106**–**1.109**). All four compounds were tested against the A2780 human ovarian cancer cell line and exhibited moderate cytotoxicity with IC_{50} values from 0.83 to 1.4 μM.[40]

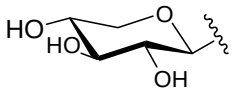


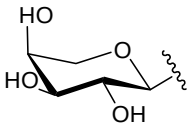
1.106 R^1 = OAc, R^2 = H
1.107 R^1 = OH, R^2 = H
1.108 R^1 = OH, R^2 = Ac
1.109 R^1 = H, R^2 = H

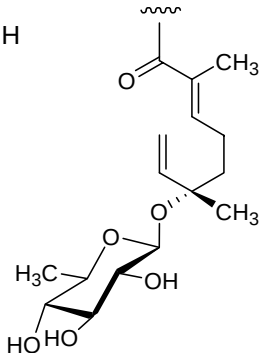
Three new oleanane-type triterpenoid saponins, gummiferaosides A–C (**1.110**–**1.112**) were isolated from an ethanol extract of the roots of *Albizia gummifera* from the Madagascar rainforest. Gummiferaosides A–C were tested for antiproliferative activity against the A2780 human ovarian cancer cell line. The three compounds exhibited moderate activities with IC_{50} values of 0.8, 1.5, and 0.6 μg/mL, respectively.[41]

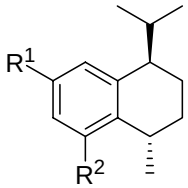
Fractionation of a CH_2Cl_2 extract of the Malagasy shrub *Tarenna madagascariensis* (Rubiaceae) led to the isolation of four new calamenene sesquiterpenes, (+)-(7*R*,10*S*)-2,15-dihydroxycalamenene (**1.113**), (+)-(7*R*,10*S*)-2-hydroxy-15-calamenal (**1.114**), (+)-(7*R*,10*S*)-15-hydroxycalamenene (**1.115**), (+)-(7*R*,10*S*)-15-calamenal (**1.116**), in addition to the new amorphane sesquiterpene (+)-(1*S*,6*R*,7*R*,10*S*)-1-hydroxy-3-oxoamorph-4-ene (**1.117**). Those new compounds did not show any antitumor activity against six human cancer cell lines, indicating that the anticancer activity of crude extracts was not due to these isolates.[42]



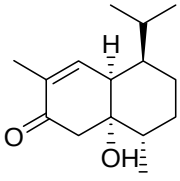
1.110 R^1 =  R^2 = H

1.111 R^1 =  R^2 = H

1.112 R^1 = R^2 = 

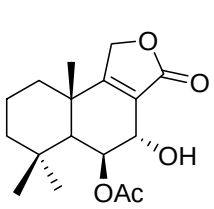


1.113 $R^1 = CH_2OH$, R^2 = OH
1.114 R^1 = CHO, R^2 = OH
1.115 $R^1 = CH_2OH$, R^2 = H
1.116 R^1 = CHO, R^2 = H

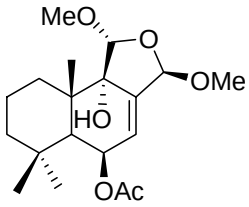


1.117

Fractionation of an EtOAc extract of the barks of *Cinnamosma madagascariensis* resulted in isolation of two new drimane-type sesquiterpenes, cinnamadin (**1.118**) and cinnamodial 11α,12β-dimethyl acetal (**1.119**) along with known compounds, pereniporin B, ugandensolide, polygodial, capsicodendrin, cinnamodial, β-sitosterol, stigmasterol, lignoceric acid, cinnamosmolide, D-mannitol, and δ-tocotrienol. The isolates were evaluated for inhibitory activities against α-glucosidase. Cinnamodial showed the most potent activity with 83.7% inhibition, while cinnamadin (**1.118**) and ugandensolide exhibited moderate activities with 14.9% and 46.2% inhibition, respectively.[43]



1.118

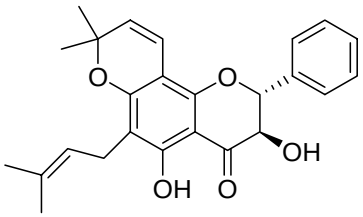


1.119

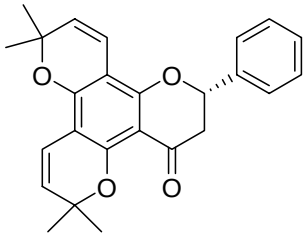
1.4. Flavonoids

Two new flavonoids, isomundulinol (**1.120**) and 3-deoxy-MS-II (**1.121**), and eight known flavonoids, 8-(3,3-dimethylallyl)-5,7-dimethoxyflavanone, MS-II, mundulone, munetone, rotenolone, rotenone, and tephrosin were isolated from the MeOH extracts of *Mundulea chapelieri.* The structures of the two new flavonoids were determined by 1D and 2D experiments. The absolute configuration of isomundulinol was determined by a CD experiment and $J_{2,3}$ value (11.2Hz) between H-2 and H-3. Finally, the data suggested that the absolute configuration of isomundulinol should be 2*R*, 3*R*. Similarly, the absolute configuration of 3-deoxy-MS-II was suggested as 2*R*. All the isolated compounds were

tested against the A2780 human ovarian cancer cell line. Most compounds showed a weak activity with IC_{50} value in the range 20-33 μg/mL, except for rotenolone and rotenone, with IC_{50} values of 0.5 and 0.7 μg/mL respectively.[44]



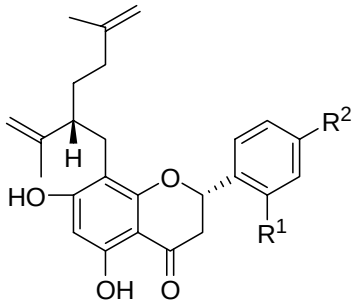
1.120

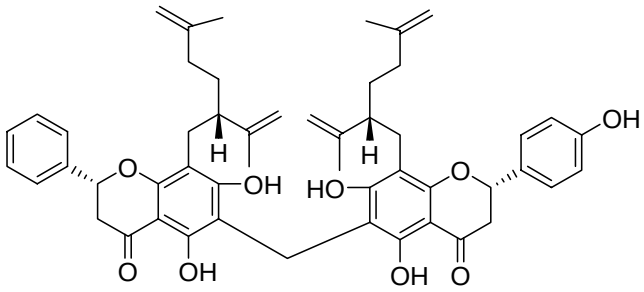


1.121

Antibacterial bioassay-guided fractionation of the MeOH extracts of the dried leaves of *Physena madagascariensis* led to the isolation of two new flavanones, namely remangiflavanones A and B (**1.122**, **1.123**), and a third dimeric flavonone, remangiflavanone C (**1.124**). The three isolated flavonoids were tested against several Gram-positive and Gram-negative bacteria. Remangiflavanone A and B displayed activity at concentrations as low as 4 μM, and remangiflavanone B was slightly more potent than remangiflavanone A in general. Remangiflavanone C showed no activity against all tested organisms.[45]

Another investigation of an extract of the twigs, leaves and flowers of same species, *P. madagascariensis*, using antiproliferative bioassay-guided fractionation, led to two new flavanones, remangiflavanones D and E (**1.125** and **1.126**), in addition to the aforementioned remangiflavanones A-C. All of the isolates were tested against the A2780 human ovarian cancer cell line, and it was found out that remangiflavanone C showed the best activity with IC_{50} value of 2.5 μg/mL.[46]

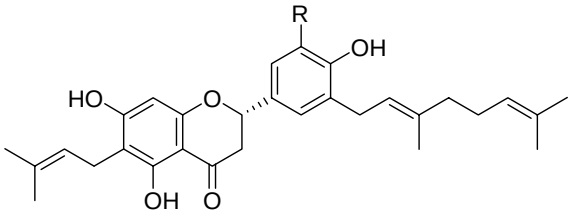


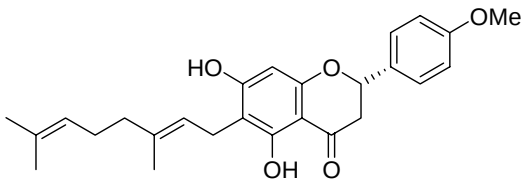


1.122 R^1 = H, R^2 = OH
1.123 R^1 = OH, R^2 = OH
1.125 R^1 = H, R^2 = H
1.126 R^1 = H, R^2 = OH

1.124

Three new flavanones, designated as schizolaenone A (**1.127**), schizolaenone B (**1.128**), and 4'-O-methylbonannione A (**1.129**), as well as three known flavonoids, nymphaeol A, bonannione A, macarangaflavanone B, and bonanniol A were isolated from an ethanol extract of the fruits of *Schizolaena hystrix* using bioassay-guided fractionation. All of the isolates were evaluated for antiproliferative activity against the A2780 human ovarian cancer cell line. It was found that all tested compounds except nymphaeol A were weakly active against A2780 cells, and that nymphaeol displayed moderate antiproliferative activity with IC_{50} value of 5.5 μg/mL.[47]





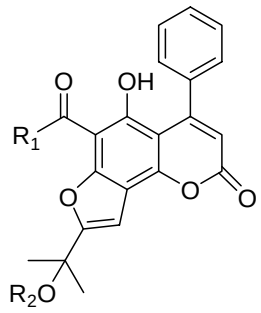
1.127 R = H
1.128 R = OH

1.129

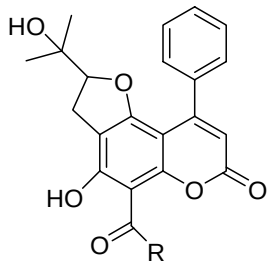
1.5. Coumarins

Seven new coumarins, ochrocarpins A–G (**1.130–1.136**), and three new benzophenone derivatives, designated as ochrocarpinones A-C (**1.137**–**1.139**), were isolated from a CH_2Cl_2-MeOH extract of the bark of *Ochrocarpos punctatus* using bioassay-directed fractionation, along with five known coumarins, mammea A/AC cyclo F, mammea A/AD cyclo D, mammea A/AB cyclo F, mammea A/AA cyclo F and mammea A/AB cyclo D and the other known compound, 15,16-dihydroperoxyplukenentione. All the isolated compounds were tested for antiproliferative activity against the A2780 human ovarian cancer cells. All the compounds showed weak antiproliferative activity with IC_{50} values ranging from 3.2 to 11.4 μg/mL.[48]

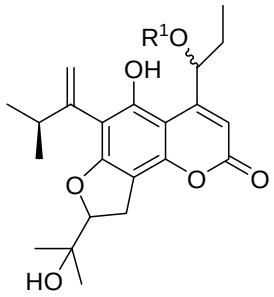
Fractionation of the dried stem bark of *Cedrelopsis microfoliata* led to three new compounds, a prenylated chalcone, designated as microfolian (**1.140**), and a prenylated flavanone, named as microfolione (**1.141**), and a new coumarin, assigned as microfolicoumarin (**1.142**) along with the known compounds, agrandol, cedrecoumarin A, obliquin and sesquichamaenol. Compounds **1.141**, agrandol, and **1.142** were found to exhibit weak agonistic activity against both α- and β-estrogenic receptors within ranges from 10 to 100 μg/mL. On the other hand, compounds **1.140**, agrandol, and **1.142** showed anti-inflammatory activity by inhibiting the luminol-induced chemiluminescence of reactive oxygen metabolites generated by human polymorphonuclear leucocytes activated with opsonized zymosan, with IC_{50} values of 4.0, 3.2 and 13 μg/mL, repectively, and demonstrating superoxide scavenging activity with IC_{50} values of 3.0, 3.0 and 0.2 μg/mL, respectively.[49]



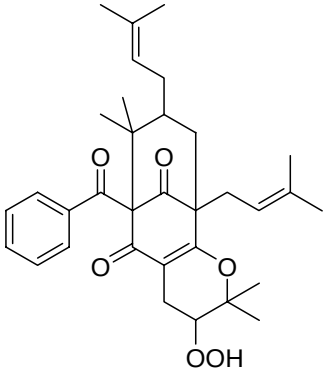
1.130 $R^1 = CH(CH_3)CH_2CH_3$ $R^2 = H$
1.131 $R^1 = CH_2CH(CH_3)_2$ $R^2 = H$
1.132 $R^1 = CH(CH_3)_2$ $R^2 = H$
1.133 $R^1 = CH(CH_3)CH_2CH_3$ $R^2 = CH3$



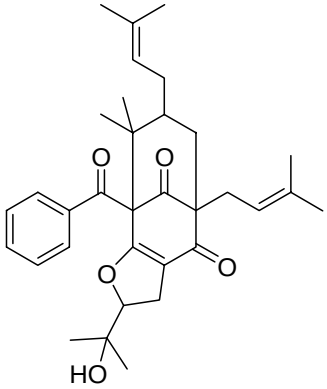
1.134 $R = CH(CH_3)_2$



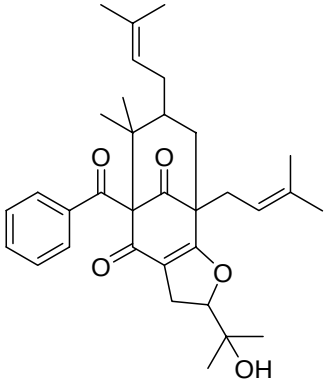
1.135 R^1=⋯$COCH_3$
1.136 R^1=—$COCH_3$



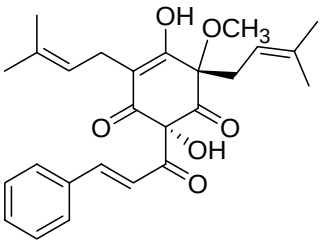
1.137



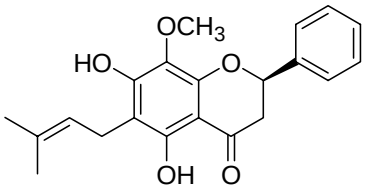
1.138



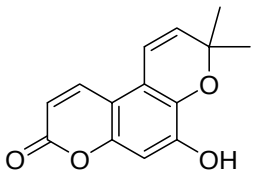
1.139



1.140



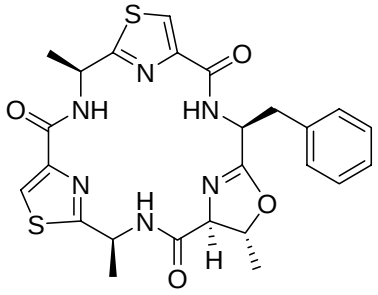
1.141



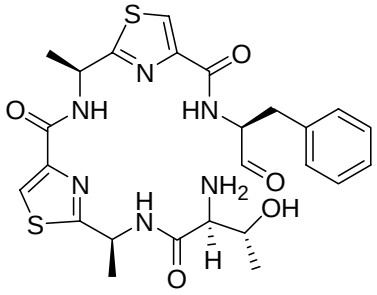
1.142

1.6. Miscellaneous Compounds

Two new cyclic hexapeptides, didmolamides A (**1.143**) and B (**1.144**), were isolated from the marine ascidian *Didemnum molle* collected in Madagascar. Both didmolamides were tested for cytotoxicity against several cultured tumor cell lines (A549, HT29, and MEL28). The compounds showed weak cytotoxicity with IC_{50} values ranging from 10 to 20 μg/mL.[50]

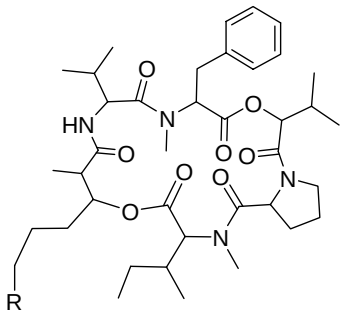


1.143



1.144

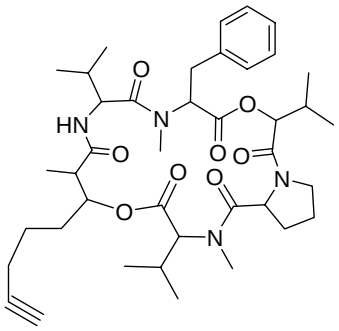
Four new cyclic depsipeptides, designated as antanapeptins A-D (**1.145–1.148**), were isolated from a collection of *Lyngbya majuscule* from Madagascar, along with the promising antineoplastic depsipeptide, dolastatin 16 (**1.149**). The new antanapeptins were tested for biological activities in brine shrimp toxicity and sodium channel modulation, and for antimicrobial activity. However, the tested compounds did not show any activities in those tests. It was noticeable that this type of compound reported previously showed either biological activities or no activity at all in related bioassays. Thus the relationships between the structure and function of this type of compound needs to be clarified.[51]



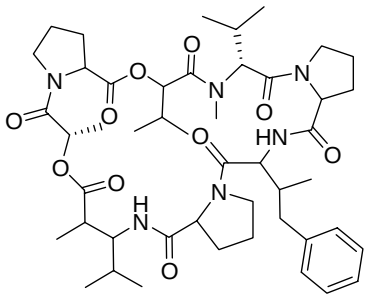
1.145 R =

1.146 R =

1.147 R =

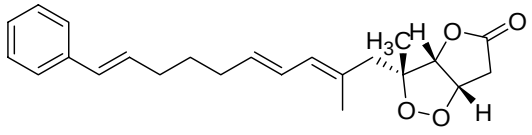


1.148

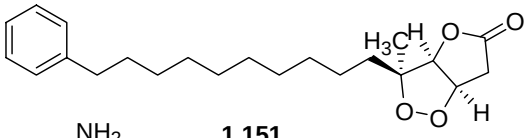


1.149

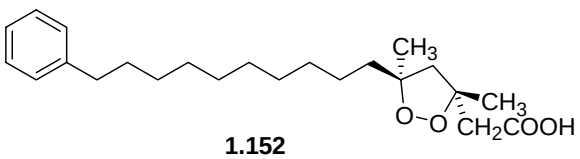
Cytotoxic bioassay-guided fractionation of an MeOH-EtOAc (1:1) extract of the marine sponge *Plakortis aff. simplex* led to the isolation of three new cyclic peroxides including two new 1,2-dioxane peroxylactones, designated as plakortolides H (**1.150**) and I (**1.151**), and one new 1,2-dioxolane, assigned as andavadoic acid (**1.152**), along with the known N(3)-methyladenine (**1.153**). The isolates were tested for cytotoxicity against multiple cultured cancer cell lines. The results indicated that the cytotoxicity of the crude extract was due to the andavadoic acid (**1.152**), and it exhibited good activities against 13 human cancer cell lines with GI_{50} values in the submicromolar range. But the compound did not show any significant selectivity against these cells.[52]



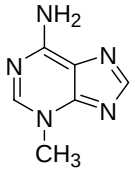
1.150



1.151

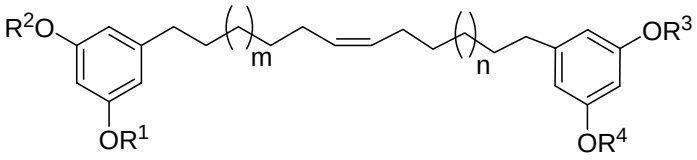


1.152

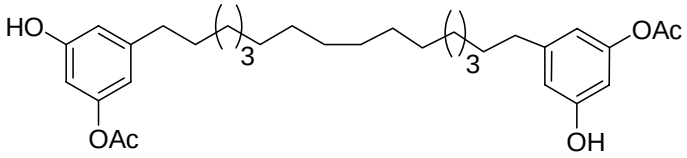


1.153

Eight new *bis*-5-alkylresorcinols, designated as oncostemonols A–H (**1.154–1.161**), were obtained from a CH_2Cl_2–MeOH extract of the leaves of *Oncostemon bojerianum* using bioassay-directed fractionation. All the isolated compounds were subjected to the A2780 human ovarian cancer cell line for antiproliferative activity. All the compounds showed similarly weak cytotoxic activities with IC_{50} values between 9.4 and 11.4 μg/mL. It was then argued that probably the basic skeleton of this type of compounds rather than any specific subtituents led to their antiproliferative activity.[53]



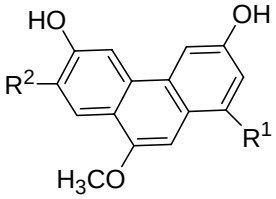
1.154 $R^1 = R^4 = Ac$ $R^2 = R^3 = H$ m = n = 3
1.155 $R^1 = Ac$ $R^2 = R^3 = R^4 = H$ m = n = 3
1.156 $R^1 = R^4 = H$ $R^2 = R^3 = Me$ m = n = 3
1.157 $R^2 = Me$ $R^1 = R^3 = R^4 = H$ m = n = 3
1.158 $R^1 = H$ $R^2 = R^3 = R^4 = Me$ m = n = 3
1.160 $R^1 = R^2 = R^3 = R^4 = H$ m = n = 5
1.160 $R^1 = R^2 = R^3 = R^4 = H$ m = 5 n = 3

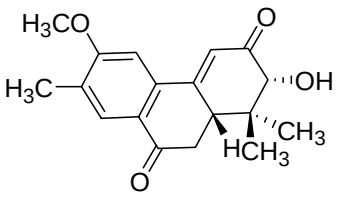


1.159

Five novel phenanthrene and hexahydrophenanthrene derivatives, assigned as **1.160**–**1.164**, were obtained from *Domohinea perrieri Lcandri* (Euphorbiaceae) collected in Madagascar. The isolated compounds were screened for cytotoxicity against a series of human cancer cell lines, including A-431, BC1, Col2, HT, KB, KB-V, Mel2, LNCaP, Lul, U373 and ZR-75-1. Compounds **1.160** and **1.161** showed significant bioactivities against several cell lines and also displayed some selectivity, while no significant activity was

detected when compounds **1.162** and **1.163** were tested against these cells. In addition, the five compounds were also tested in a DNA strand-scission assay, and it was found that compounds **1.160**–**1.162** exhibited the best activity.[54]

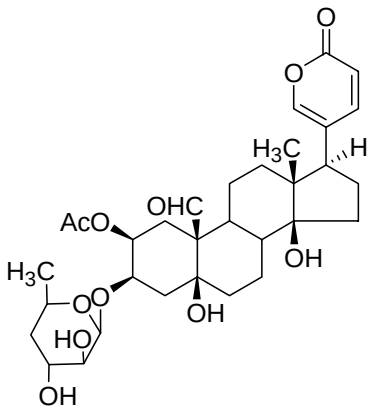




1.160 $R^1 = CH_3$ $R^2 = CH_3$
1.161 $R^1 = CH_2OH$ $R^2 = CH_3$
1.162 $R^1 = CH_3$ $R^2 = CH_2OH$
1.163 $R^1 = CH_2OH$ $R^2 = CH_2OH$

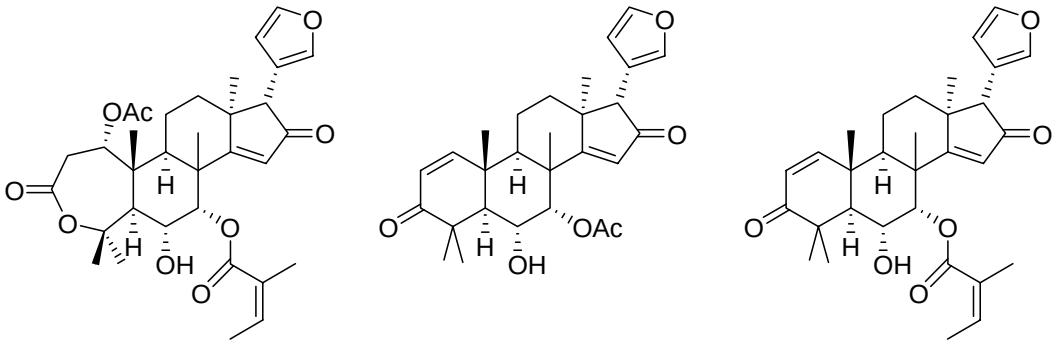
1.164

Bioassay-guided fractionation of an extract of the leaves of *Kalanchoe tomentosa* collected in Madagascar afforded the potent cytotoxic bufadienolidic glycoside, kalanchoside (**1.165**). The structure of **1.165** was determined based on extensive analysis of its NMR and mass spectroscopic data.[55]



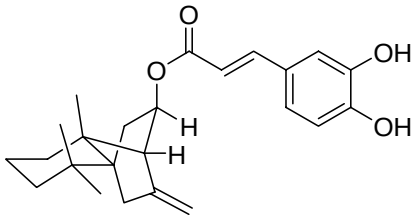
1.165

Fractionation of an extract of the seeds of the Malagasy plant *Quivisia papinae* led to three new compounds including one novel evodulone limonoid, quivisianthone (**1.166**), and two new azadiradione derivatives, 6α-hydroxyazadiradione (**1.167**) and 7-deacetyl-7-angeloyl-6α-hydroxyazadiradione (**1.168**), along with the known azadiradione. The structure of quivisianthone was unique, and it was the first reported evodulone limonoid possessing both a lactone ring A and an azadiradione-type ring D.[56]



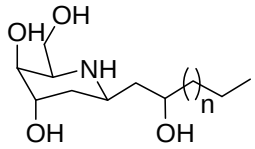
1.166 **1.167** **1.168**

A new cyclomyltaylane sesquiterpenoid, myltayl-4(12)-ene-2-caffeate (**1.169**), was isolated from the Malagasy liverwort, *Bazzania nitida*, along with the known (+)-(l*S*,4*R*)-7-hydroxycalamenene. Compound **1.169** was tested against LPS-induced RAW 264.7 cells for activity of inhibition of nitric oxide, and potent activity with an IC_{50} value of 6.3 μM was exhibited.[57]



1.169

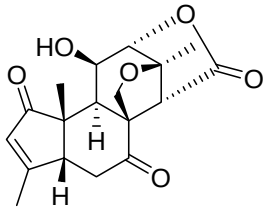
An investigation on a Malagasy marine sponge of a *Batzella* sp. led to the isolation of three new C-alkylated iminosugars, batzellasides A–C (**1.170**–**1.172**), together with the known halitoxin polymer. Compounds **1.170**–**1.172** were evaluated for their antibacterial activity against *Staphylococcus epidermidis*, and all of them showed MIC values less than 6.3 μg/mL. This study was claimed to show the first examples of azasugars/iminosugars from marine organisms rather than terrestrial sources.[58]



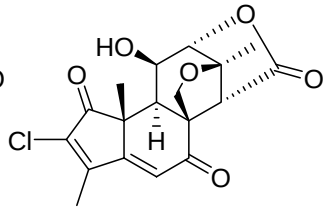
1.170 n = 8
1.171 n = 9
1.172 n = 10

Three novel C18 quassinoids, 5β,6-dihydrosamaderine A (**1.173**), 2-chlorosamaderine A (**1.174**), and samaderolactone A (**1.175**), and a novel C19 quassinoid, 3,4β-dihydrosamaderine C (**1.176**) were isolated from the MeOH and EtOAC extracts of the leaves of a Malagasy plant, *Samadera madagascariensis*, along with the known quassinoids samaderine A, samaderine B, and cedronin. All the new compounds except samaderolactone A (**1.175**) were tested against the NCI 60 cancer cell line panel, but

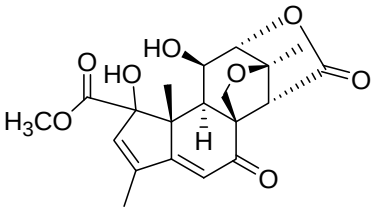
unfortunately the compounds were either inactive or only weakly active against those cell lines.[59]



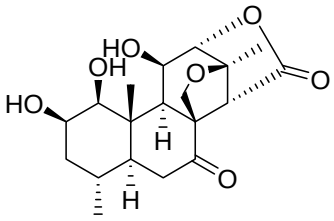
1.173



1.174

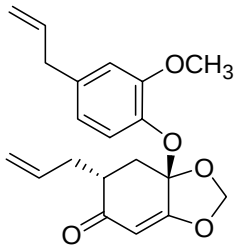


1.175

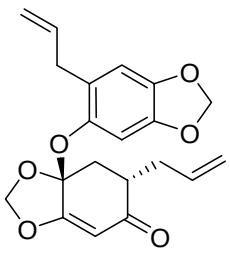


1.176

The two new lignans, didymochlaenone A (**1.177**) and B (**1.178**), were isolated from an ethanol extract of *Didymochlaena truncatula* collected in Madagascar, but were found not to be active towards the A2780 human ovarian cancer cell line.[60]

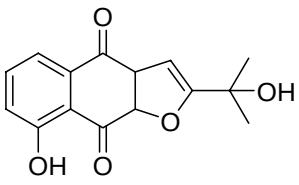


1.177

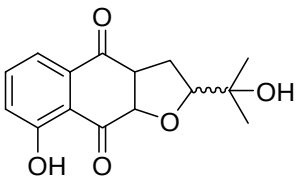


1.178

Two new naphthoquinones, avicequinones D and E (**1.179** and **1.180**), along with two known naphthoquinones, avicequinone C and stenocarpoquinone-B, were isolated from ethanol extracts of the roots and stems of *Mendonica cowanii* collected from the Madagascar rainforest. All the isolates were tested against the A2780 human ovarian cancer cell line and the kinase Akt. The new compounds, **1.179** and **1.180**, and the known compounds, avicequinone C and stenocarpoquinone-B, exhibited IC_{50} values of 7.4, 8.8, 9.8 and 50 μM respectively against A2780 cells, but were inactive or only weakly active against the kinase Akt.[61]

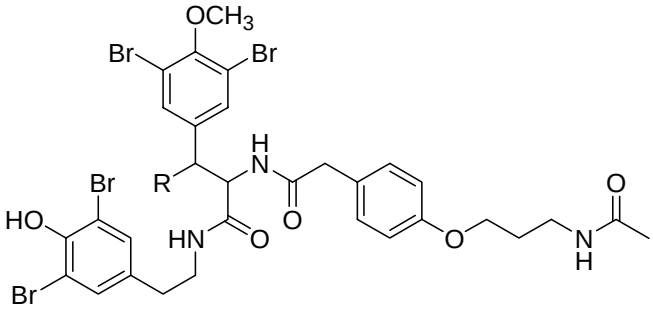


1.179



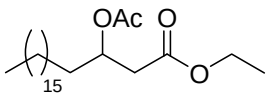
1.180

Fractionation of the Malagasy marine sponge, *Iotrochota purpurea*, afforded two new metabolites, itampolins A (**1.181**) and itampolins B (**1.182**). The structures of the two compounds, which were determined on the basis of analysis of their spectroscopic data and chemical conversion, contained the dibromotyrosine building blocks. No bioactivity data were reported for those compounds. [62]

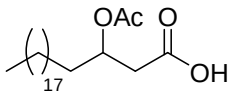


1.181 R = H
1.182 R = OH

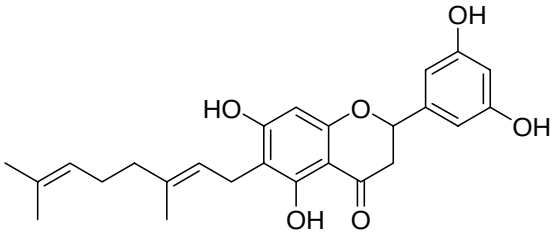
A continued effort to isolate antiproliferative compounds applying bioassay-guided approach from an ethanol extract of the Malagasy plant *Schizolaena hystrix* collected in the Madagascar rainforest led to the isolation of two new long long-chain compounds, 3*S*-acetoxyeicosanoic acid ethyl ester (**1.183**) and 3*S*-acetoxydoeicosanoic acid (**1.184**), and one new flavonoid schizolaenone C (**1.185**). The new compounds were tested against the A2780 human ovarian cancer cell line for antiproliferative activity, and all of them turned out to be weakly active against A2780 cells. [63]



1.183



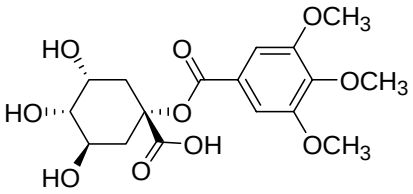
1.184



1.185

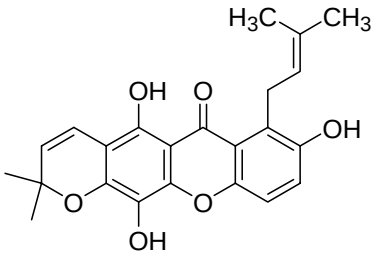
Antiplasmodial bioassay-guided fractionation of an extract of *Sloanea rhodantha* (Baker) Capuron *var. rhodantha* collected from Madagascar rainforest afforded several compounds including a new compound, identified as 1-O-eudesmoylquinic acid (**1.186**). All the isolates were tested against the drug-sensitive HB3 and drug-resistant FCM29

strains of *Plasmodium falciparum*, and the A2780 human ovarian cancer cell line. Weak activities against the antiplasmodial assays were displayed by some of those compounds. The new compound (**1.186**) did not show significant activities against either the antiplasmodial or the antiproliferative assays.[64]

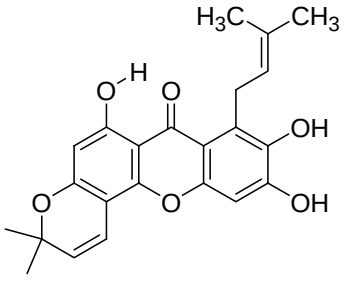


1.186

Antiproliferative bioassay-guided fractionation of an ethanol extract of the leaves of *Terminalia calcicola*, which were collected from the Madagascar rainforest, afforded two new xanthones, termicalcicolanone A (**1.187**) and termicalcicolanone B (**1.188**). Both compounds were evaluated for antiproliferative activity against the A2780 human ovarian cancer cell line and showed weak activity with IC_{50} values of 40.6 and 8.1 μM, respectively.[65]

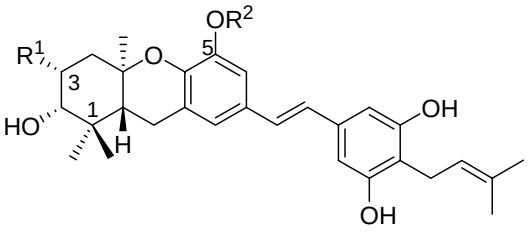


1.187

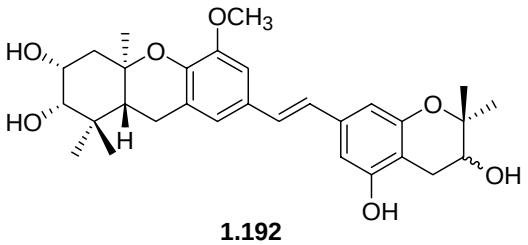


1.188

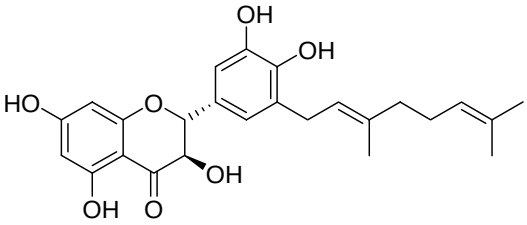
Antiproliferative bioassay-guided isolation of the fruits of *Macaranga alnifolia* collected from the Madagascar rainforest afforded four new prenylated stilbenes, named as schweinfurthins E–H (**1.189**–**1.192**), and one new geranylated dihydroflavonol, named as alnifoliol (**1.193**) along with the known compounds including vedelianin, bonanniol A, diplacol, bonannione A and diplacone. All isolates were tested against the A2780 ovarian cancer cell line for antiproliferative activity. The known compound, vedelianin, showed the best activity (IC_{50} = 0.13 μM), while the new compounds schweinfurthins E–H (**1.189**–**1.193**) exhibited activities with IC_{50} values of 0.26, 5.0, 0.39, 4.5 and 27.3 μM, repectively. Schweinfurthin E (**1.189**) was also tested against the 60 human cancer cell line panel at the National Cancer Institute (NCI), and this compound showed a mean panel GI_{50} value of 0.19 μM, with the leukemia subpanel showing the greatest sensitivity and the ovarian subpanel showing the least sensitivity to this compound. The best sensitivity was observed when this compound was tested against leukemia (MOLT-4), CNS (SF-295), and renal (A498 and CAKI-1) cells, with all of the GI_{50} and TGI values for these cells being less than 10 nM. Structure and anticancer activity relationships of several prenylated stilbenes including schweinfurthins E–H (**1.189**–**1.193**), the previously reported schweinfurthins A–D, and other compounds with similar structures, were also discussed in the article. It was argued that a free hydroxyl group at C-5 could reduce their activities, while a free C-3-OH group might enhance the activity. In the meantime, it was pointed out that the chain length and cyclization of the geranyl side chain could play an important role in mediating anticancer activities of those compounds. It was also found that the more polar the compound was, the better the activity would be. That phenomenon was proposed to be related to the solubility of these compounds.[66]



1.189 R^1 = OH R^2 = CH_3
1.190 R^1 = H R^2 = CH_3
1.191 R^1 = R^2 = H
vedelianin R^1 = OH R^2 = H

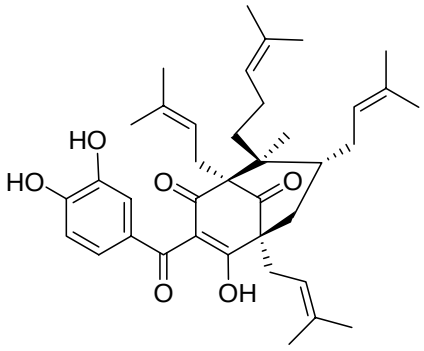


1.192

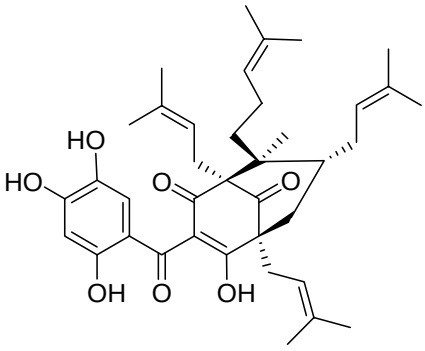


1.193

Two new guttiferones, named guttiferone K (**1.194**) and guttiferone L (**1.195**), were isolated from an ethanol extract of the fruits of *Rheedia calcicola* collected from the Madagascar rainforest. The two compounds were evaluated against the A2780 human ovarian cancer cell line for their antiproliferative activity, and both of them were weakly active against the A2780 cells. Given the fact that previously reported compounds of this type also showed weak activities against certain tumor cells, this investigation was consistent with previous studies of guttiferones.[67]

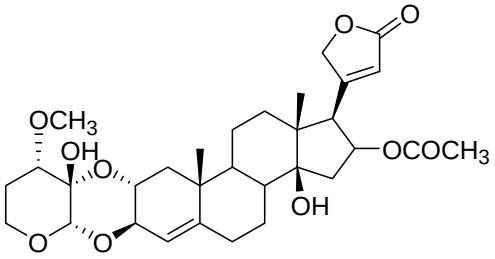


1.194

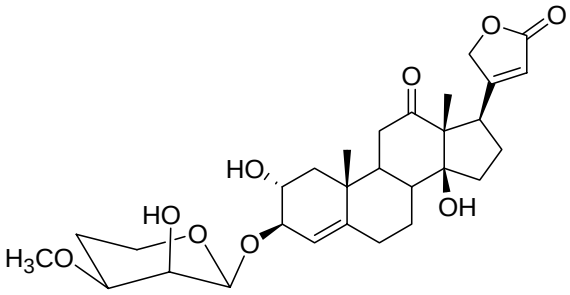


1.195

Antiproliferative bioassay-guided fractionation of an ethanol extract of the wood of an *Elaeodendron* species afforded two new cardenolides, elaeodendrosides T (**1.196**) and U (**1.197**), along with the known cardenolides, elaeodendroside B, elaeodendroside F, elaeodendroside G, and (2α,3β,14β)-trihydroxy-3-*O*-(4-deoxy-3-*O*-methyl-α-L-erythropentopyranosyl)card-4,20(22)-dienolide. All the isolated compounds were tested against the A2780 human ovarian cancer cell line to evaluate their antiproliferative activity. It was found that elaeodendrosides T (**1.196**) and B showed the strongest activities with IC_{50} values of 0.085 and 0.019 μM, repectively, and the other compounds showed IC_{50} values from 0.19-30 μM. In addition, elaeodendrosides T (**1.196**) and B were also tested against four other cultured human cancer cell lines, MDA-MB-435 breast cancer, HT-29 colon cancer, H522-T1 non-small-cell lung cancer, and U937 histiocytic lymphoma, and both of them showed strong activities. On the basis of the activity profile, it was proposed that the 1,4-dioxane rings between rings A and A′ in this type of compound, as indicated for elaeodendroside T (**1.196**), B, F and G, were important for their antiproliferative activity.[68]

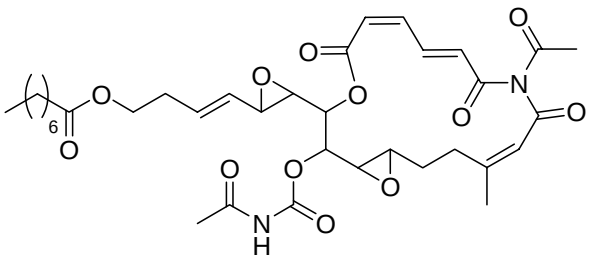


1.196

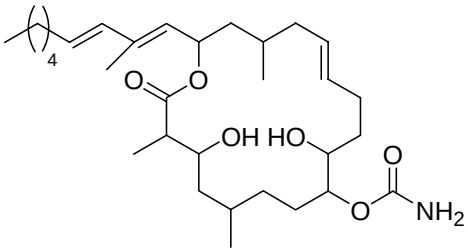


1.197

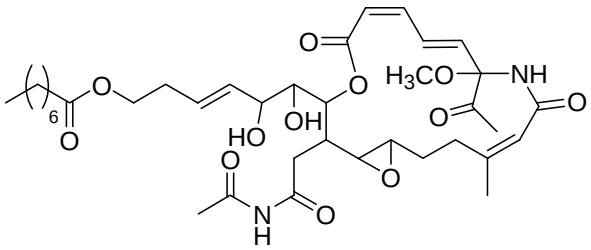
Fractionation of an extract of the marine sponge of a *Fascaplysinopsis* sp. led to three novel nitrogenous macrolides, named salarin A (**1.198**) and B (**1.199**) with unique chemical structures, and tulearin A (**1.200**). Salarin A and tulearin A were tested against the two human leukemia cell lines, K562 and UT7, for antiproliferative activity. Tulearin A was found to exhibit inhibitory activity to both cell lines in a dose- and time-dependent manner, while salarin A showed less activity against the UT7 cells than tulearin A and was inactive against K562 cells. Recollection of the sponges was underway to determine the stereochemistry of these unique compounds and obtain their further biological properties.[69]



1.198

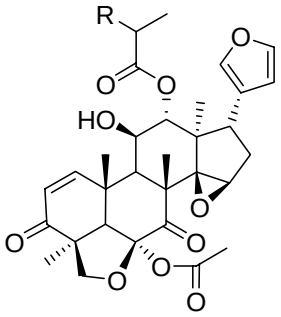


1.200

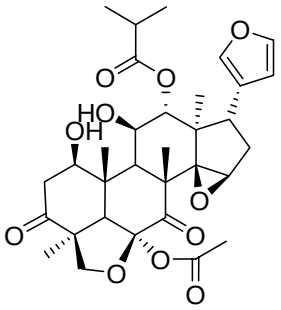


1.199

Bioassay-guided fractionation of an ethanol extract of a *Malleastrum* sp. collected from the humid forest in Madagascar led to the isolation of three new limonoids, named as malleastrones A–C (**1.201**–**1.203**), each of which contained the rare hexacyclic tetranortriterpenoid skeleton. Structure determination of those compounds was carried out by thorough analysis of 1D and 2D NMR, mass spectroscopic, and X-ray crystallographic data. Malleastrones A-C were tested against the A2780 human ovarian cancer cell line, and also against four additional human tumor cell lines, MDA-MB-435 breast cancer, HT-29 colon cancer, H522-T1 non-small-cell lung cancer, and U937 histiocytic lymphoma. It turned out that malleastrones A and B showed significant antiproliferative activity against all the test tumor cell lines with IC_{50} values in the range of 0.19-0.63 μM.[70]

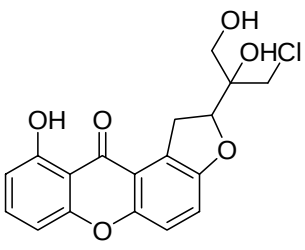


1.201 R = CH_3
1.202 R = CH_2CH_3

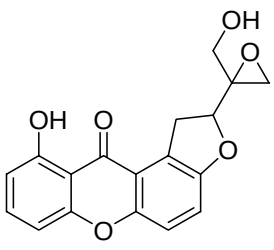


1.203

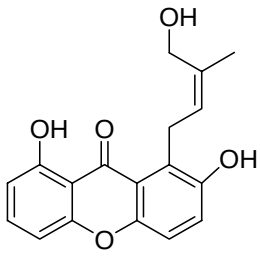
Bioassay-guided fractionation of an extract of the wood stems and roots of *Psorospermum* cf. *molluscum* led to two new dihydrofuranoxanthones, designated 3',4'-deoxy-4'-chloropsoroxanthin-(3',5'-diol) (**1.204**) and psoroxanthin (**1.205**), and one new hydroxyprenylated xanthone (**1.206**). Compounds **1.204** and **1.205** were found to be active against the *Escherichia coli* SOS PQ37 chromotest assay, and they were also tested against various cancer cell lines, including the A2780 human ovarian cancer cell line, HCT116 human colon cancer cell line and ABAE bovine endothelial cell line. Compound **1.204** showed potent activity and good selectivity against the ABAE bovine endothelial cell line with an IC_{50} value of 4 nM. Compound **1.205** showed less activity against these cells.[71]



1.204

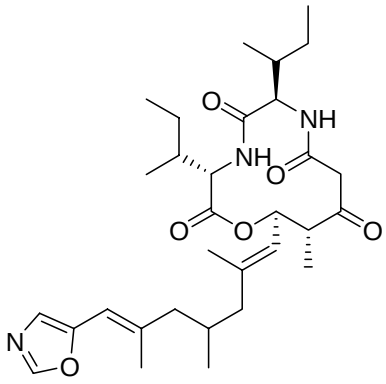


1.205

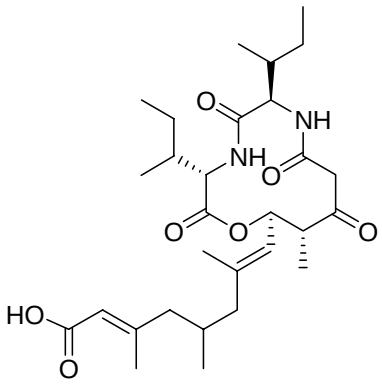


1.206

Bioassay-guided fractionation of a MeOH/CH_2Cl_2 extract of the marine sponge *Fascaplysinopsis* sp. led to the isolation of two lipodepsipeptides, designated as taumycins A and B (**1.207** and **1.208**). Although both of the two compounds showed toxicity to brine shrimp larvae with IC_{50} values of 10 μg/mL, only taumycin A (**1.207**) exhibited inhibitory activity against the human UT-7 leukemic cell line with an IC_{50} value of 1 μM.[72]



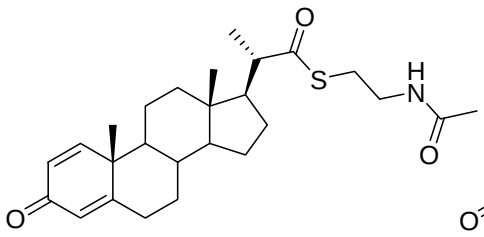
1.207



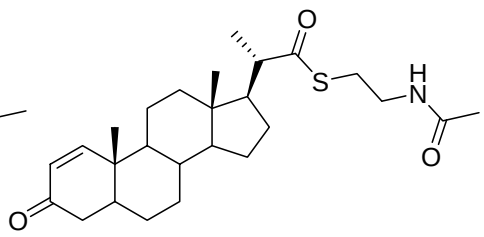
1.208

Bioassay-guided fractionation of an isopropanol extract of the soft coral *Paragorgia* sp. afforded three new steroid thioesters, designated as parathiosteroids A–C (**1.209**–**1.211**). Those compounds were considered to be the first example of isolation of steroids with a C-22 thioester in their side chain in nature. The three compounds were tested against the A-549 lung carcinoma, the HT-29 colorectal carcinoma, and MDA-MB 231 breast adenocarcinoma cell lines. The three compounds exhibited cytotoxicity at the micromolar level. In addition, the synthesis of analogs of steroids was carried out, leading to information on their structure activity relationships. For example, the $XCH_2CH_2NHCOCH_3$ (X = S, O, NH) moiety in the side chain and a low degree of

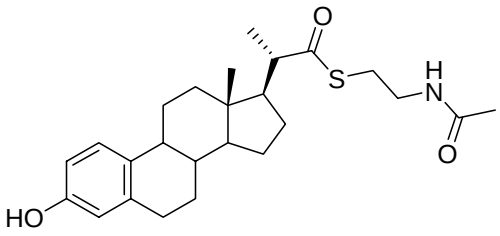
oxidation in the A-ring are required for those type compounds to display high antiproliferative activity.[73]



1.209



1.210



1.211

1.7. Conclusions

Plants and marine organisms in Madagascar are very rich resources for natural products. The isolated compounds reviewed in this chapter cover various types of natural products including terpenoids, alkaloids, flavonoids and many others, and possess all kinds of skeletons, including some novel ones first isolated from Nature. The reason for the chemical diversity isolated from the plants in Madagascar may be due to the great biodiversity of the flora and fauna of Madagascar.

Plants and marine organisms in Madagascar can also be rich resources for the discovery of biologically active natural products. Almost all the isolated compounds reviewed in this chapter have been scanned for biological activity, with a very broad

range of assays including anticancer, antimalarial, antibiotic, anti-inflammatory, antifungal, and antidiabetes bioassays among others. A number of compounds showed significant biological activities against the respective bioassays. Furthermore, those biologically active natural products can provide clues and even candidates/leads for new drug discovery and development. In addition, it is worth noting that a large portion of the reviewed compounds were investigated for cytotoxicity or antiproliferative activity against variable cancer cell lines, and it is not uncommon to find compounds with good activities against those cultured cells. Thus it is concluded that plants in Madagascar can be a wonderful source for discovery of novel drugs, particularly novel anticancer drugs.

Based on the conclusions above, my research has mainly focused on the isolation and structure elucidation of bioactive natural products from Malagasy plants and marine organisms applying bioassay-guided fractionation and modern spectroscopy in addition to chemical conversions. The most frequently used bioassay in my research has been the A2780 human ovarian cancer cell line in addition to the occasional use of a few other cancer cell lines and several antimalarial bioassays. The other aspect of my research has been synthesis of bioactive natural products, specifically a sesquiterpenoid identified from a Malagasy plant. All my research will be discussed in detail in the following chapters.

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II. Antiproliferative and Antimalarial Anthraquinones of *Scutia myrtina* from the Madagascar Forest

This chapter is a modified and slightly expanded version of the published article.[1] Contributions from co-authors of the article are described as follows in the order of the names listed. The author of this dissertation (Mr. Yanpeng Hou) conducted the isolation and structure elucidation of the title anthraquinones, and drafted the manuscript. Dr. Shugeng Cao provided invaluable advice and hints for structure elucidation of the compounds, and he also proofread the manuscript before submission. Ms. Peggy Brodie performed the A2780 bioassay on the isolated fractions and compounds. Dr. Fidisoa Ratovoson, and Dr. Martin W. Callmander from Missouri Botanical Garden made the plant collections and identification. Dr. Etienne Rakotobe and Dr. Vincent E. Rasamison from Madagascar carried out the initial plant extraction. Dr. Michael Ratsimbason from Madagascar performed the *Plasmodium falciparum* FCM29 bioassay on the title compounds. Mr. John N. Alumasa and Dr. Paul D. Roepe from Georgetown University performed the *Plasmodium falciparum* Dd2 bioassay on the title compounds. Dr. David G. I. Kingston was a mentor for this work and the corresponding author for the published article. He provided critical suggestions for this work and crucial revisions to the manuscript.

2.1. Introduction

In a continuing search for biologically active natural products from tropical rainforests as part of an International Cooperative Biodiversity Groups (ICBG) program, an extract from the bark of *Scutia myrtina* collected in Madagascar was evaluated for antiproliferative activity against the A2780 human ovarian cancer cell line. The extract was selected for bioassay−guided fractionation based on its initial activity against this assay with an IC_{50} value of 12 μg/mL.

2.1.1. *Previous Investigations of Scutia*

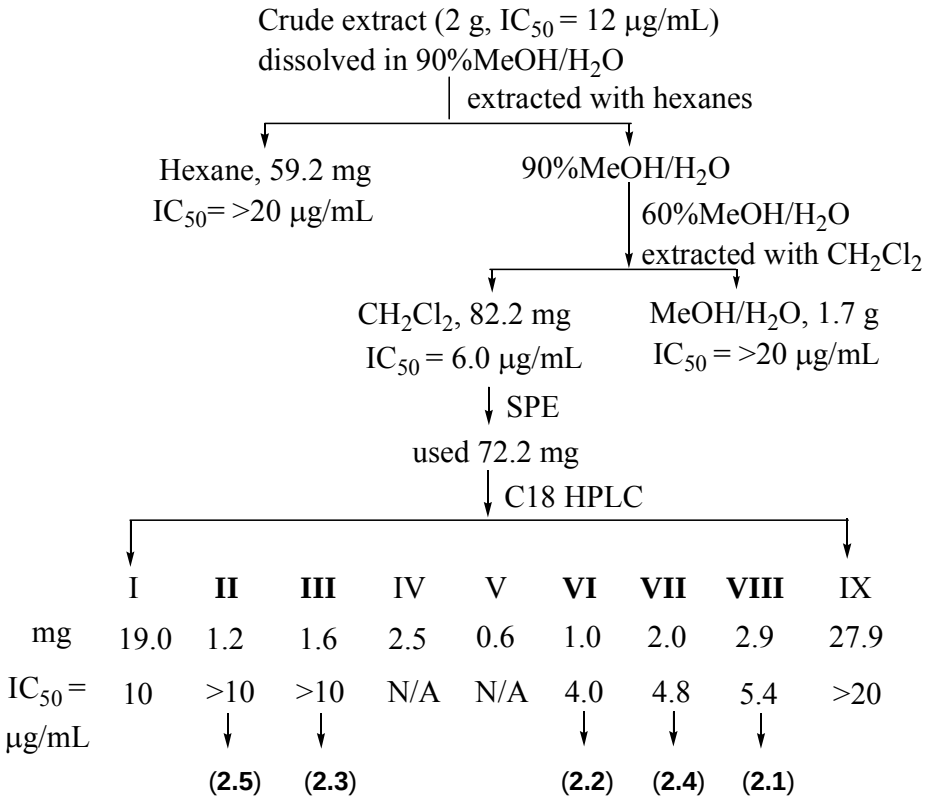
The genus *Scutia* belongs to the Rhamnaceae family, and includes 23 species. The roots of *Scutia myrtina* have been investigated previously, and afforded two perylenequinones with in vitro anthelmintic activity but no in vivo activity.[2] In addition, several cyclopeptide alkaloids have been isolated from other *Scutia* species,[3-6,7] some of which showed moderate antimicrobial activity.[3]

2.2. Results and Discussion

2.2.1. *Isolation of Compounds from Scutia myrtina*

The crude ethanol extract of *Scutia myrtina* was suspended in MeOH–H_2O (9:1) and extracted using hexanes. The aqueous methanol was then diluted to MeOH–H_2O (6:4) and extracted by CH_2Cl_2. Both organic extracts and the residual aqueous methanol solution were evaporated to generate three fractions. The antiproliferative activities of those fractions against the A2780 ovarian cancer cell line were obtained. It was found that the CH_2Cl_2 fraction showed the best activity (6 μg/mL). The CH_2Cl_2 fraction was

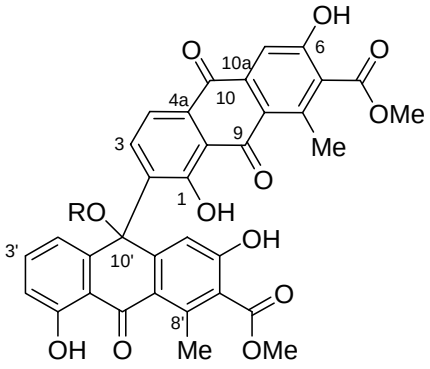
then subjected to reversed-phased C18 HPLC eluted using MeOH–H_2O (75:25) to yield scutianthraquinones A-D (**2.1–2.4**) and aloesaponarin I (**2.5**). The fractionation tree is shown in Scheme 2.1 and a detailed description of the isolation procedure is given in the Experimental Section.



Scheme 2.1. Separation of *Scutia myrtina* extract

2.2.2. *Structure Elucidation of Scutianthraquinone A*

Scutianthraquinone A (**2.1)** was obtained as a light−brown amorphous solid. Its molecular formula was established as $C_{39}H_{32}O_{13}$ on the basis of its molecular ion peak in its negative ion HRFAB mass spectrum. Its UV−vis spectra showed characteristic absorptions of anthraquinones at 248, 272, 310 and 475 nm.[8] The ^{1}H NMR spectrum of **1** (Table 2.1) in CD_3OD showed signals for an AB system at δ_H 8.64 (d, J = 7.8 Hz, H-3) and δ_H 7.86 (d, J = 7.8 Hz, H-4), an ABC system at δ_H 6.86 (br d, J = 7.7 Hz, H-2'), δ_H 7.33 (t, J = 7.7 Hz, H-3') and δ_H 6.71 (br d, J = 7.7 Hz, H-4'), two aromatic proton singlets at δ_H 7.52 (s, H-5) and δ_H 6.66 (s, H-5'), two methoxyl groups at δ_H 3.89 (s, COO*CH*$_3$-7) and δ_H 3.88 (s, COO*CH*$_3$-7'), and two methyl groups at δ_H 2.49 (s, CH_3-8) and δ_H 2.77 (s, CH_3-8'). The ^{1}H NMR spectrum in DMSO-d_6 also showed resonances for four hydroxyl protons at δ_H 13.68, 13.24, 11.80 and 10.99, indicating that each was intramolecularly hydrogen bonded. In the ^{13}C NMR spectrum six carbonyl carbons, 24 aromatic carbons, one oxygenated quaternary carbon, two methoxyl carbons, one methine carbon, one methylene carbon and four methyl carbons were identified. On the basis of its UV, ^{1}H NMR, and ^{13}C NMR spectra and its molecular formula, the structure of **2.1** was assigned as an anthrone−substituted anthraquinone. (Figure 2.1)



2.1 R = $COCH(CH_3)CH_2CH_3$

Figure 2.1. Structure of scutianthraquinone A (**2.1**)

Table 2.1. ^{1}H NMR Data of Compounds **2.1-2.4**[c]

no.	**2.1**[a]	**2.2**[a]	**2.3**[a]	**2.4**[a]
3	8.64 d (7.8)	8.65 d (8.1)	8.66 d (8.0)	8.67 d (7.8)
4	7.86 d (7.8)	7.92 d (8.1)	7.89 d (8.0)	7.95 d (7.8)
5	7.52 s	7.54 s	7.57 s	7.53 s
7-COOCH_3	3.89 s	3.89 s	3.89 s	3.90 s[e]
8-CH_3	2.49 s	2.54 s	2.55 s	2.53 s[f]
2′	6.86 br d (7.7)	6.86 br d (8.3)	6.83 br d (8.0)	
3′	7.33/7.33 t (7.7, 7.7)[d]	7.33 t (8.3, 8.3)	7.33 t (8.0, 8.0)	8.32/8.32 d (8.5)[d]
4′	6.71 br d (7.7)	6.71 br d (8.3)	6.76 d (8.0)	6.92 d (8.5)
5′	6.67/6.66 s[d]	6.65 s	6.75 s	6.60/6.59 s[d]
7′-COOCH_3	3.88 s	3.88 s	3.89 s	3.84 s[e]
8′-CH_3	2.77 s	2.76 s	2.74 s	2.60 s[f]
10′-OCO$C\underline{H}(CH_3)CH_2CH_3$	2.53 m			2.41 m[g]
10′-OCOCH$(CH_3)C\underline{H_2}CH_3$	1.69 m 1.53 m			1.62 m[g]
10′-OCOCH$(C\underline{H_3})CH_2CH_3$	1.15/1.12 d (7.2)[d]			1.05/1.03 d (6.8)[d,g]
10′-OCOCH$(CH_3)CH_2C\underline{H_3}$	0.85/0.85 t (7.4)[d]			0.85/0.84 t (7.1)[d,g]
10′-OCO$C\underline{H}(CH_3)_2$		2.68 m		
10′-OCOCH$(C\underline{H_3})_2$		1.17/1.15 d (7.2)[d]		
2″				6.85 br d (8.1)
3″				7.30 t (8.1)
4″				6.62 br d (8.1)
5″				6.58/6.57 s[d]
7″-COOCH_3				3.89 s[e]
8″-CH_3				2.78 s
10″- OCO$C\underline{H}(CH_3)CH_2CH_3$				2.58 m[g]
10″- OCOCH$(CH_3)C\underline{H_2}CH_3$				1.69 m[g]
10″- OCOCH$(C\underline{H_3})CH_2CH_3$				1.17/1.16 d (6.3)[d,g]
10″- OCOCH$(CH_3)CH_2C\underline{H_3}$				0.76 t (7.1)[g]
OH[b]	13.68 br s 13.24 s 11.80 br s 10.99 br s	14.89, 13.25, 11.92 10.79 (all br s)	13.23, 11.93, 11.73, 10.90 (all br s)	14.92, 13.66, 13.23, 11.91, 10.77, 9.71 (all br s)

[a]in CD_3OD. [b]in DMSO-d_6. [c]δ (ppm) 500 & 600 MHz, 25 °C. [d]splits of signals due to atropisomers. [e,f]resonances may be interchanged. [g]resonances for 10′-$OCO(CH_3)CHCH_2CH_3$ and 10″-$OCO(CH_3)CHCH_2CH_3$ may be interchanged.

The complete ^{1}H NMR and ^{13}C NMR assignments and connectivities of **2.1** were determined from a combination of COSY, HSQC, HMBC and ROESY data. The COSY spectrum showed correlations that confirmed the connectivity of H-3 and H-4, and of H-2', H-3' and H-4'. (Figure 2.2) In the HMBC spectrum, the correlations of H-4 and H-5 to C-10 (δ_C 183.6) and H-4' and H-5' to C-10' (δ_C 78.2) indicated the positions of these protons. The position of CH_3-8 was established by three–bond HMBC correlations from the protons of CH_3-8 and H-5 to C-7 and C-8a (δ_C 131.8, 123.5) along with a two–bond correlation from the protons of CH_3-8 to C-8 (δ_C 143.5). The position of CH_3-8' was established by three–bond HMBC correlations from protons of CH_3-8' and H-5' to C-7' and C-8'a (δ_C 126.6, 124.1) along with two–bond correlations from protons of CH_3-8' to C-8' (δ_C 141.9) (Figure 2.2). The presence of two carbomethoxy groups ($COOCH_3$-7, $COOCH_3$-7') was indicated by HMBC correlations from their methyl protons to the corresponding carbonyl carbons (*CO*OCH_3-7, 7', δ_C 170.6, 169.9), and their positions were established by ROESY correlations (Figure 2.3) and by comparison of ^{13}C NMR data for C-5/6/7/8/8a/10a and C-5′/6′/7′/8′/8′a/10′a of **2.1** and the corresponding carbons of the known anthraquinone aloesaponarin I (**2.5**).[9] A ROESY correlation from the protons of COO*CH*$_3$ -7' to those of CH_3-8' unambiguously located the carbomethoxy group at C-7' instead of C-6'. A corresponding ROESY correlation from the protons of COO*CH*$_3$-7 to CH_3-8 was not observed, but the almost identical chemical shifts between C-5/6/7/8/8a/10a and C-5'/6'/7'/8'/8'a/10'a in the ^{13}C NMR of **2.1** indicated that COO*CH*$_3$-7 must reside on C-7 instead of C-6. Finally, the chemical shifts of C-5/6/7/8/8a/10a showed the same pattern as those of C-5/6/7/8/8a/10a of **2.5,** offering further confirmation of the assigned positions of the carbomethoxy groups.

The anthrone and anthraquinone moieties were connected through the bond between C-2 and C-10', as evidenced by the key HMBC correlation of H-3 to C-10' (δ_C 78.2) (Figure 2.2). The presence of a 2-methylbutanoyl group was established by COSY correlations between a methine proton (δ_H 2.53, m) and methyl (δ_H 1.15/1.12, d, J = 7.2 Hz) and methylene protons (δ_H 1.69, m; δ_H 1.53, m). The methylene protons were part of an ethyl group, as shown by the COSY correlations to methyl protons at δ_H 0.85 (t, J = 7.4 Hz) (Figure 2.2).

Supporting evidence for the 2-methylbutanoyl group was provided by HMBC correlations from its methylene protons (δ_H 1.69, m; δ_H 1.53, m) and methyl protons (δ_H 1.15/1.12, d, J = 7.2 Hz) to a carbonyl group (δ_C 174.9) (Figure 2.2). The group was assigned to the C-10' position by ROESY correlations from CH_3-2" (δ_H 1.15/1.12, d, J = 7.2 Hz) to H-3 and H-4' and from H_3-4" (δ_H 0.85, t, J = 7.4 Hz) to H-4 and H-5' (Figure 2.3). The negative ion LC–ESI–MS/MS of **2.1** gave a series of fragment ions which were entirely consistent with the assigned structure (Scheme 2.2). The structure of **2.1,** except for the absolute configurations of C-10' and the 2-methylbutanoyl group, was thus established.

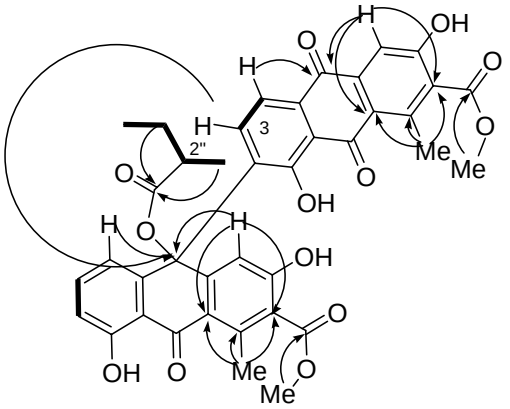
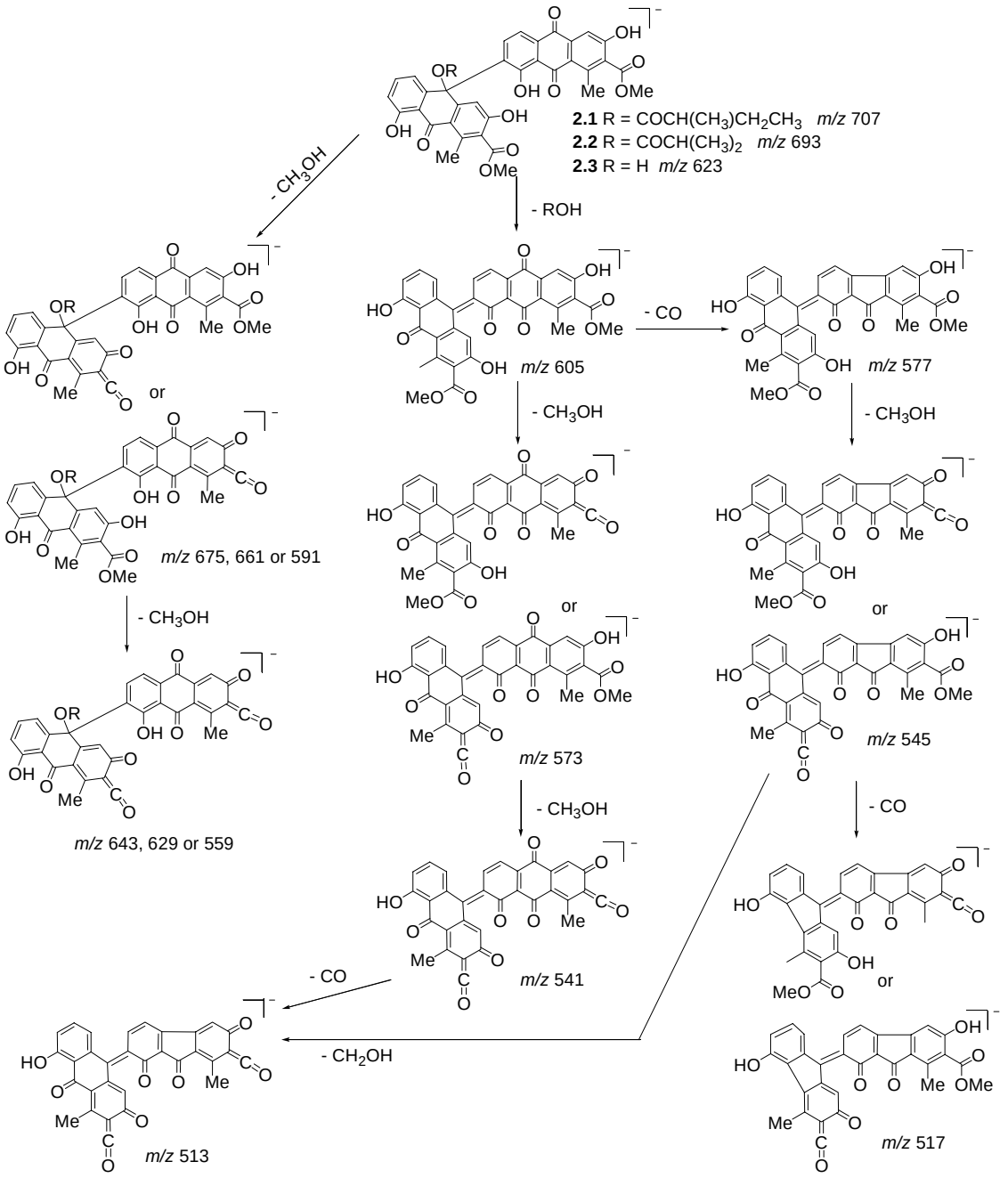


Figure 2.2. Key COSY (bold) and HMBC (arrows) correlations of **2.1**



Scheme 2.2. Proposed (-)ESI-MS/MS fragmentation pathways of **2.1**–**2.3**

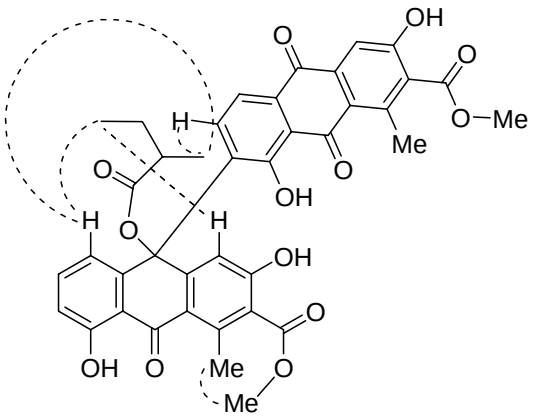


Figure 2.3. Key ROESY correlations of **2.1**

Analysis of the ^{1}H and ^{13}C NMR spectra of **2.1** indicated the existence of two atropisomers. The signals for H-3' in its ^{1}H NMR spectrum in CD_3OD appeared as two overlapping triplets, while the signal for H-5' appeared as two singlets; each signal integrated for only one proton. In addition, the signal of CH_3-2" of the 2-methylbutanoyl group was split into two overlapping doublets, and that of H_3-4" appeared as two overlapping triplets. The splittings of the signals for C-8, C-4′, C-5, and the carbons of the 2-methylbutanoyl group were also observed in the ^{13}C NMR spectrum. A series of ^{1}H NMR spectra of **2.1** were obtained in DMSO-d_6 at a series of elevated temperatures, and the split signals of the above protons either merged completely (H-3′ and H-5′) or partially (the protons of the two methyl groups in the 2-methylbutanoyl group) as the temperature increased. An example is shown in Table 2.2. The atropisomerism of **2.1** presumably arises because the rotation of the C-2–C-10′ bond is restricted by steric effects.

Table 2.2. Dependence of the separation between the two resonances of the 2′′-CH_3 group with temperature in the variable temperature ^{1}H NMR spectra of **2.1**.[a]

Temperature (^{o}C)	25	50	80	100	120
Δδ (Hz)	20.0	16.2	12.4	10.2	8.5

[a] ^{1}H NMR data was obtained in DMSO-d_6 using a 600 MHz NMR spectrometer.

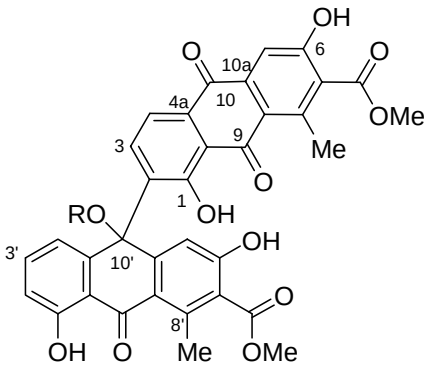
2.2.3. *Structure Elucidation of Scutianthraquinone B*

Scutianthraquinone B (**2.2**) was obtained as a light–brown amorphous solid. Its molecular formula was established as $C_{38}H_{30}O_{13}$, differing from that of **2.1** by a CH_2 group, on the basis of its $[M–H]^-$ peak in its negative ion HRESI mass spectrum. The UV–vis spectra showed characteristic features of anthraquinones at 248, 272, 310 and 475 nm. The ^{1}H NMR spectrum of **2.2** was almost identical to that of **2.1**, except that the signals from the C-10' substituent lacked signals for one methylene group compared with **2.1** (Table 2.1). This observation indicated that an isobutanoyl group in **2.2** had replaced the 2-methylbutanoyl group in **2.1**. HMBC correlations of the H_3-3a" and H_3-3b" methyl protons (δ_H 1.17/1.15, d, J = 7.2 Hz) to C-1" (δ_C 174.0) and C-2" (δ_C 35.0) and TOCSY correlations between the H_3-3a"/H_3-3b" methyl protons and H-2" (δ_H 2.68, m) were in agreement with the above assignment. The other HMBC and TOCSY correlations of **2.2** showed the same patterns as those of **2.1**, and confirmed the similarity between **2.2** and **2.1**. An attempt to determine if the isobutanoyl group was located at C-10' by ROESY correlations by the same strategy applied to **2.1** was not successful, due to the small quantity of **2.2** and a corresponding weak signal. The substituent was thus assigned to C-10' based on the close similarity of the ^{1}H NMR chemical shifts of **2.1** and **2.2** If the isobutanoyl group were located at any other position significant differences in these shifts would be expected, as observed for the monomeric anthraquinone aloesaponarin I and its

acetylated form.[10] In addition, the four intramolecularly hydrogen bonded hydroxyl protons at δ_H 14.89, 13.25, 11.92 and 10.79 in the ^{1}H NMR spectrum of **2.2** in DMSO-d_6 indicated that the isobutanoyl group was not acylating any of the four phenolic hydroxyl groups, and thus indirectly supported its assignment to C-10'. The LC–ESI–MS/MS profile of **2.2** was essentially identical to that of **2.1**, after allowing for the molecular weight differences, and thus also supported the proposed structure of **2.2**. The proposed fragmentation pattern is shown in Scheme 2.2. Based on the arguments above, the structure of **2.2** was assigned as shown in Figure 2.4.

2.2.4. *Structure Elucidation of Scutianthraquinone C*

Scutianthraquinone C (**2.3**) was obtained as a light–brown amorphous solid. Its molecular formula was established as $C_{34}H_{24}O_{12}$ on the basis of its molecular ion peak in its negative ion HRFAB mass spectrum. The UV–vis spectra showed characteristic features of anthraquinones at 248, 272, 310 and 475 nm. The ^{1}H NMR spectrum of **2.3** was almost identical to that of **2.1** except for the absence of signals for the 2-methylbutanoyl group in **2.1**, indicating the presence of a free hydroxyl group at C-10′. The HMBC and COSY spectra and LC–ESI–MS/MS profile (see proposed fragmentation pattern in Scheme 2.2) of **2.3** supported the above deduction. Thus, the structure of **2.3** was established as shown in Figure 2.4.



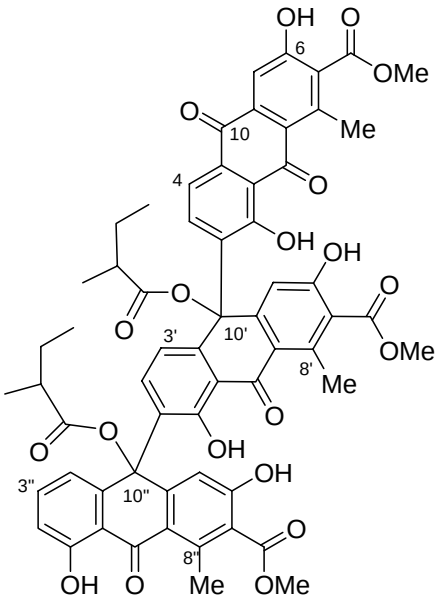
2.2 R = $COCH(CH_3)_2$
2.3 R = H

Figure 2.4. Structures of scutianthraquinone B (**2.2**) and scutianthraquinone C (**2.3**)

2.2.5. *Structure Elucidation of Scutianthraquinone D*

Scutianthraquinone D (**2.4)** was obtained as a light–brown amorphous solid. Its molecular formula was established as $C_{61}H_{52}O_{20}$ on the basis of its $[M–H]^-$ peak in its negative ion HRESI mass spectrum. The UV–vis spectra showed characteristic features of anthraquinones at 248, 272, 310, 353 and 475 nm. In the ^{1}H NMR spectrum of **2.4** in CD_3OD, two AB systems (δ_H 8.67, d, J = 7.8 Hz, H-3 and δ_H 7.95, d, J = 7.8 Hz, H-4, and δ_H 8.32, d, J = 8.5 Hz, H-3′ and δ_H 6.92, d, J = 8.5 Hz, H-4′) and one ABC system (δ_H 6.85, br, d, J = 8.1 Hz, H-2″, δ_H 7.30, t, J = 8.1 Hz, H-3″ and δ_H 6.62, br, d, J = 8.1 Hz, H-4″) were observed, and three aromatic proton singlets (δ_H 7.53, H-5 and δ_H 6.60/6.59, 6.58/6.57, H-5′, H-5″) also appeared (Table 2.1). Singlets for three methoxyl protons (δ_H 3.90, 3.89, 3.84) and three methyl protons (δ_H 2.53, 2.60, 2.78) were also observed. In the ^{1}H NMR spectrum of **2.4** in DMSO-d_6, signals for six intramolecularly hydrogen bonded hydroxyl protons (δ_H 14.92, 13.66, 13.23, 11.91, 10.77 and 9.71) were also observed (Table 2.1). The COSY spectrum of **2.4** confirmed the aforementioned AB

and ABC systems, and COSY correlations of the protons from two 2-methylbutanoyl groups also were observed. Careful comparison of ^{1}H NMR and COSY data of **2.4** to those of **2.1** indicated the presence of an additional monomeric anthrone unit in **2.4**, indicating a trimeric structure. The existence of six intramolecularly hydrogen bonded hydroxyl protons and comparison of the chemical shifts and coupling patterns in the ^{1}H NMR of **2.4** to those of **2.1** indicated that the two 2-methylbutanoyl groups must be located at C-10 and C-10′, and established the structure of **2.4** as the bisanthrone-anthraquinone shown in Figure 2.5. The LC–ESI–MS/MS spectrum of **4** fully supported the proposed structure, and the proposed fragmentation pathways of **4** are shown in Scheme 2.3.



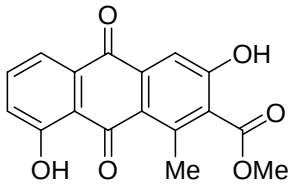
2.4

Figure 2.5. Structures of scutianthraquinone D (**2.4**)

Only a few anthrone–anthraquinones with the two moieties connected by a bond between the C-2 position of the anthraquinone unit and C-10′ of the anthrone unit have been reported,[11-17] and compounds **2.1**–**2.3** are novel examples of this small structural type. Compound **2.4** is the first reported bisanthrone–anthraquinone isolated from Nature.

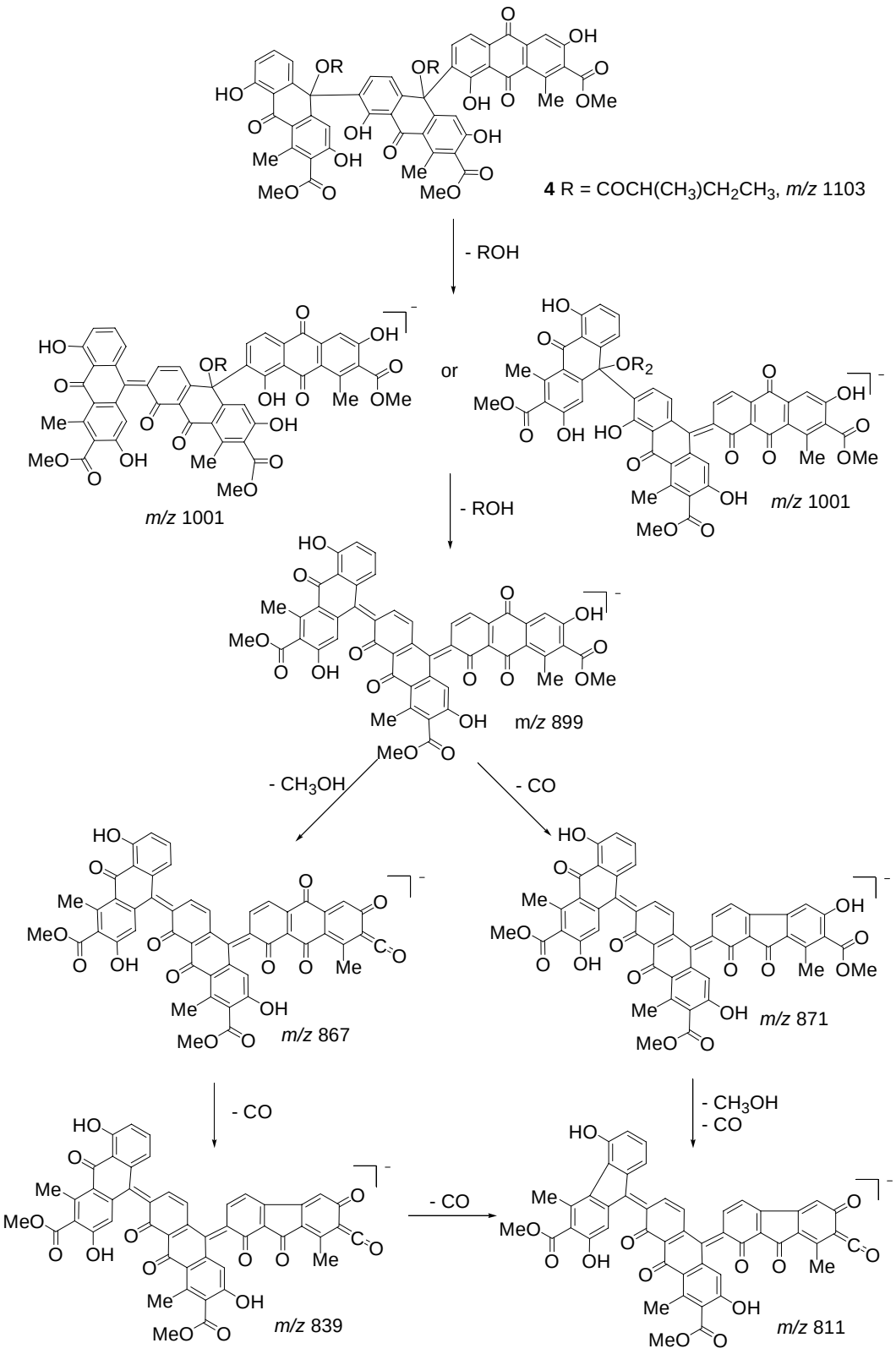
2.2.6. *Identification of the Known Aloesaponarin I*

The structure of aloesaponarin I (**2.5**) (Figure 2.6) was assigned by comparison of its spectroscopic data with literature data.[9]



2.5

Figure 2.6. Structure of aloesaponarin I (**2.5**)



Scheme 2.3. Proposed (-)ESI-MS/MS fragmentation pathways of **2.4**

2.2.7. *Antiproliferative and Antiplasmodial Activities of Isolated Compounds*

The isolated compounds were tested against the A2780 human ovarian cancer cell line and were also tested against chloroquine-resistant strains of *Plasmodium falciparum* Dd2 and FCM29. The results are shown in Table 2.3. Compounds **2.1**–**2.5** exhibited weak antiproliferative activities against the A2780 human ovarian cancer cell line, with the trimer **2.4** being slightly more active than dimers **2.1**, **2.2** and **2.3**, and the monomer **2.5** showed the weakest activity. Compounds **2.1**–**2.5** showed moderate antiplasmodial activities against the chloroquine–resistant *P. falciparum* Dd2, with IC_{50} values in the range of 1–6 μM; the dimers **2.1**–**2.3** showed slightly better activities than the trimer **2.4**, and the monomer **2.5** displayed the least activity. The dimers **2.1** and **2.2** with C-10′ substituents exhibited slightly better activities than the dimer **2.3** with a free hydroxyl group at C-10′. With respect to the activities against *P. falciparum* FCM29, **2.1**, **2.2**, and **2.4** showed moderate activities with IC_{50} values from 1.2 to 5.6 μM compared to chloroquine (IC_{50} = 0.41 μM). The selectivities of **2.1**–**2.5** for antiplasmodial as opposed to antiproliferative activities, as determined by the quotient of antiproliferative activity and antiplasmodial activity, vary between 0.8 and 6 (Table 2.3).

Table 2.3. IC_{50} (μM) values of **2.1**–**2.5** against Human A2780[a] and *Plasmodium falciparum* strains FCM29 and Dd2

Compound	A2780 IC_{50} (μM)	*P. falciparum* Dd2 IC_{50} (μM)	Selectivity Index (Dd2 vs A2780)	*P. falciparum* FCM29 IC_{50} (μM)	Selectivity Index (FCM29 vs A2780)
CQ	NT	0.147		0.41	
Quinine	NT	NT		0.22	
2.1	7.6 ± 0.8	1.23	6.1	1.2	6.3
2.2	5.8 ± 2.5	1.14	5.1	5.4	1.1
2.3	>16	3.14	>5	15.4	>1
2.4	4.3 ± 0.9	3.68	1.2	5.6	0.8
2.5	>32	5.58	>5.7	77.6	NM

[a] Paclitaxel (IC_{50} 0.014 μM) was the positive control.

These selectivities are lower than would be desirable for future drug development, and this fact, coupled with the chemical complexity of the compounds, makes them challenging compounds to develop. Some related anthrone-anthraquinones had selectivities of up to 400 between the chloroquine-sensitive 3D7 strain of *P. falciparum* and the KB cell line,[18] so it is possible that compounds of this class might ultimately be developable as antimalarial agents. This conclusion is supported by the fact that some phenylanthraquinones, including monomeric[19,20] and dimeric examples,[21] have promising antimalarial bioactivities.

2.2.8. *Examples of Bioactive Anthraquinones*

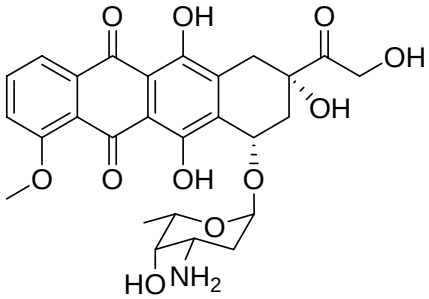
Anthraquinones and anthraquinone-related compounds have shown anticancer,[22,23,24] antimalarial,[25] and anti-infective[26] activities. Herein some selected examples are discussed.

Doxorubicin (**2.6**) belongs to family of anthracycline antibiotics, the aglycone of which has an anthraquinone chromophore and a tetracyclic system. It has been one of most widely used drugs for cancer chemotherapy. In addition to doxorubicin, several of its analogs have also been developed into drugs in clinical medical usage, and efforts are still being carried out to discover and develop new anthracycline drugs.[22]

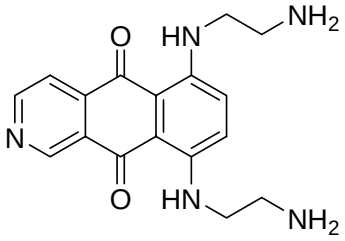
Pixantrone (**2.7**) is a novel anthraquinone-based immunosuppressant drug used in treatment of non-Hodgkin's lymphomas. It is an analogue of mitoxantrone but with a lower cardiotoxicity. Several phase III clinical studies of pixantrone are underway.[23]

Emodin (**2.8**), aloe-emodin (**2.9**) and rhein **(2.10**) are major anthraquinones isolated from a well known traditional Chinese medicine, rhubarb. These components showed promising anticancer activities. Emodin was found to be able to inhibit cellular proliferation, induce apoptosis and prevent metastasis by modulating several molecular targets related to tumor cell growth, apoptosis and metastasis. Aloe-emodin and rhein showed capabilities of disruption of cell cycle and induction of apoptosis, respectively, although relatively few studies have been carried out on those compounds. All those studies suggested that those anthraquinones could have potential therapeutical values.[24]

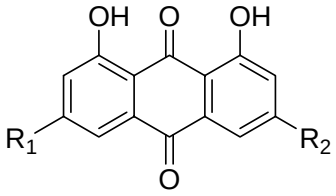
Rufigallol (**2.11**) is a synthetic poly-hydroxyl anthraquinone, and it exihibited potent antiplasmodial activity against *Plasmodium falciparum* with IC_{50} value of 10.5 ng/mL.[27]



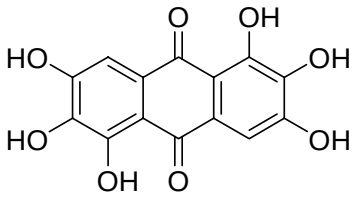
2.6. Doxorubicin



2.7. Pixantrone



2.8. Emodin: R_1 = OH, R_2 = CH_3
2.9. Aloe-emodin: R_1 = H, R_2 = CH_2OH
2.10. Rhein: R_1 = H, R_2 = COOH



2.11 Rufigallol

Figure 2.7. Examples of bioactive anthraquinone-related compounds

2.3. Experimental Section

2.3.1. *General Experimental Procedures*

Optical rotations were recorded on a JASCO P-2000 polarimeter. UV spectra were obtained on a Shimadzu UV-1201 spectrophotometer. NMR spectra were obtained on Bruker Avance 600, JEOL Eclipse 500, Varian Inova 400, and Varian Unity 400 spectrometers. HRFAB mass spectra were obtained on a JEOL-JMS-HX-110 instrument. HRESI mass spectra were obtained on an Agilent 6220 TOF LC/MS. LC-ESIMS was performed on Agilent 1100 and Thermo TSQ Quantum instruments. Chemical shifts are given in δ (ppm), and coupling constants (*J*) are reported in Hz. HPLC was performed using Shimadzu LC-10A pumps coupled with a Varian Dynamax semipreparative

column (250 × 10 mm). The HPLC instrument employed a Shimadzu SPD-M10A diode array detector.

2.3.2. *Antiproliferative Bioassay*

The A2780 ovarian cancer cell line assay was performed by Ms. Peggy Brodie at Virginia Polytechnic Institute and State University. In brief, "Human ovarian cancer cells (A2780) grown to 95% confluency were harvested and resuspended in growth medium (RPMI1640 supplemented with 10% fetal bovine serum and 2 mM L-glutamine). Cells were counted using a hemacytometer and a solution containing $2.5x10^5$ cells per ml was prepared in growth media. Eleven columns of a 96 well microtitre plate were seeded with 199 µl of cell suspension per well, and the remaining column contained media only (one hundred percent inhibition control). The plate was incubated for 3 hours at 37ºC/5%CO_2 to allow the cells to adhere to the wells. Following this incubation, potential cytotoxic agents, prepared in DMSO, were added to the wells in an appropriate series of concentrations, 1 µl per well. One column of wells was left with no inhibitor (zero percent inhibition control), and 4 dilutions of a known compound (taxol or actinomycin) was included as a positive control. The plate was incubated for 2 days at 37ºC/5%CO_2, then the media gently shaken from the wells and replaced with reaction media (supplemented growth medium containing 1% alamarBlue), and incubated for another 3 hours. The level of alamarBlue converted to a fluorescent compound by living cells was then analyzed using a Cytofluor Series 4000 plate reader (Perseptive Biosystems) with an excitation wavelength of 530 nm, an emission wavelength of 590 nm, and gain of 45. The percent inhibition of cell growth was calculated using the zero

percent and one hundred percent controls present on the plate, and an IC_{50} value (concentration of cytotoxic agent which produces 50% inhibition) was calculated using a linear extrapolation of the data which lie either side of the 50% inhibition level. Samples were analyzed in triplicate on at least two separate occasions to produce a reliable IC_{50} value." (Provided by Ms. Peggy Brodie) The A2780 cell line is a drug sensitive ovarian cancer cell line.[28]

2.3.3. *Antimalarial Bioassay*

Antiplasmodial assays with the chloroquine-resistant strain *Plasmodium falciparum* FCM29 were performed at the Centre National d'Application des Recherches Pharmaceutiques. Assays with the chloroquine-resistant *Plasmodium falciparum* Dd2 were performed at Georgetown University. Both assays used the previously reported SYBR green method.[29]

2.3.4. *Plant Material Collection and Extraction*

Bark of the climbing shrub *Scutia myrtina* (Burm. f.) Kurz (Rhamnaceae) was collected in the Montagne des Français region, a dry forest on limestone, Antsiranana, Madagascar, at an elevation of 280 m, at 12.24.41 S, 49.22.17 E, on February 14, 2005. Its assigned collection number is Randrianasolo.S (SSR) et al. 517. The collection was made from a woody liana, with one pair of spines in each node, green fruit becoming black when mature, brown seeds, growing on slope. Its vernacular name is *Roiavotra*. *Scutia myrtina* has a large distribution from Africa to south east Asia. This species is somewhat variable but the variation is chaotic and nearly coextensive throughout the vast range, so that the

recognition of infraspecific taxa is not practicable. Voucher specimens have been deposited at herbaria of the Centre National d'Application des Recherches Pharmaceutiques, Madagascar (CNARP); the Parc Botanique et Zoologique de Tsimbazaza, Madagascar (TAN); the Missouri Botanical Garden, St. Louis, Missouri (MO); and the Muséum National d'Histoires Naturelles, Paris, France (P).

The dried and powdered bark of *Scutia myrtina* (254 g) was extracted at room temperature with EtOH (1.2 L) for 24 h. After filtration, the solvent was evaporated to dryness under reduced pressure, affording a crude ethanolic extract (7.86 g).

2.3.5. *Extract Fractionation*

A total of 2.5 g of extract was supplied to VPISU (MG 3219), and this had an IC_{50} value of 12 μg/mL against A2780 cells. This extract (2.0 g) was suspended in aqueous MeOH (90%MeOH-H_2O, 60 mL) and extracted with hexanes (3 × 60 mL). The aqueous layer was then diluted to 60% MeOH (v/v) with H_2O and extracted with CH_2Cl_2 (3 × 90 mL). The CH_2Cl_2 extract (82.2 mg) was found to be the most active against A2780 cells (IC_{50} = 6 μg/mL) and was separated via semipreparative HPLC over a C18 column using MeOH-H_2O (75:25) to afford 9 fractions (I-IX). Fractions II, III, VI, VII and VIII afforded aloesaponarin I (**2.5**, 1.2 mg, t_R 19.6 min), scutianthraquinone C (**2.3**, 1.6 mg, t_R 21.4 min), scutianthraquinone B (**2.2**, 1.0 mg, t_R 31.6 min), scutianthraquinone D (**2.4**, 2.0 mg, t_R 34.6 min) and scutianthraquinone A (**2.1**, 2.9 mg, t_R 38.5 min), respectively.

2.3.6. *Scutianthraquinone A* (**2.1**)

Light-brown amorphous solid; $[\alpha]_D^{25}$ +60.8 (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε): 203 (4.35), 248 (4.09), 272 (4.01), 310 (4.03), 351 sh (3.83) and 475 sh (3.36); HRFABMS *m/z*: 708.1790 $[M]^-$ (Calcd for $C_{39}H_{32}O_{13}$: 708.1843); LC-ESIMS *m/z* (rel. int.): $[M-H]^-$ 707 (1), 675 (21), 643 (1), 605 (4), 577 (100), 573 (14), 545 (40), 541 (2), 517 (3); ^{1}H NMR: see Table 2.1; ^{13}C NMR (CD_3OD): 192.2 (C-9/C-9′), 190.9 (C-9/C-9′), 183.6 (C-10), 174.9 (C-10′-O*C*OCH(CH_3)CH_2CH_3), 170.6 (C-7-*C*OOCH_3), 169.9 (C-7′-*C*OOCH_3), 163.2 (C-1′), 159.8 (C-1), 149.1 (C-6), 149.1 (C-6′), 143.6 (C-4′a), 143.5 (C-8), 141.9 (C-8′), 140.6 (C-2), 138.6 (C-10a/c-10′a), 136.0 (C-10a/C-10′a), 135.9 (C-3′), 133.9 (C-4a), 131.8 (C-7/8a), 131.7 (C-3), 126.6 (C-7′/8a′), 124.1 (C-7′/8a′), 123.5 (C-7/8a), 119.2 (C-4), 119.1 (C-9′a), 118.6 (C-9a), 118.4 (C-2′), 118.1/118.0 (C-4′), 113.9 (C-5), 112.7/112.6 (C-5′), 78.2 (C-10′), 53.0 (C-7-COO*C*H_3), 53.0 (C-7′-COO*C*H_3), 43.0 (C-10′-OCO*C*H(CH_3)CH_2CH_3), 28.0/27.9 (C-10′-OCOCH(CH_3)*C*H_2CH_3), 21.2 (8′-CH_3), 20.6 (8-CH_3), 17.0/16.9 (C-10′-OCOCH(*C*H_3)CH_2CH_3), 12.0 (C-10′-OCOCH(CH_3)CH_2*C*H_3).

2.3.7. *Scutianthraquinone B* (**2.2**)

Light-brown amorphous solid; $[\alpha]_D^{25}$ +134.8 (*c* 0.04, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε): 203 (4.13), 248 (3.85), 272 (3.78), 310 (3.74), 353 sh (3.54) and 475 (3.08); HRESIMS *m/z*: 693.1614 $[M-H]^-$ (Calcd for $C_{38}H_{30}O_{13}$: 693.1608); LC-ESIMS *m/z* (rel. int.): 693 (1) $[M-H]^-$, 661 (48), 629 (4), 605 (12), 577 (100), 573 (22), 545 (80), 541 (2), 517 (8); ^{1}H NMR: see Table 2.1.

2.3.8. *Scutianthraquinone C* (**2.3**)

Light-brown amorphous solid; $[\alpha]_D^{25}$ +122.2 (*c* 0.04, MeOH); UV (MeOH) λ_{max} nm (log ε): 203 (4.24), 248 (3.98), 272 (3.92), 310 (3.90), and 475 sh (3.29); HRFABMS *m/z* 624.1296 $[M]^-$ (Calcd for $C_{34}H_{24}O_{12}$: 624.1268); LCESIMS *m/z* (rel. int.): 623 (12) $[M-H]^-$, 605 (4), 591 (100), 577 (88), 573 (28), 545 (84), 541 (64), 517 (12), 513 (10), 487 (8), 459 (4), 311 (5); ^{1}H NMR: see Table 2.1.

2.3.9. *Scutianthraquinone D* (**2.4**)

Light-brown amorphous solid; $[\alpha]_D^{25}$ -34.7 (*c* 0.04, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε): 203 (4.64), 248 (4.40), 272 (4.34), 310 (4.25), 353 (4.12) and 475 sh (3.51); HRESIMS *m/z*: 1103.2977 $[M-H]^-$ (Calcd for $C_{61}H_{52}O_{20}$: 1103.2979); LC-ESIMS *m/z* (rel. int.): 1103 (20) $[M-H]^-$, 1071 (1), 1001 (6), 899 (40), 871 (100), 867 (14), 839 (4), 811(1); ^{1}H NMR: see Table 2.1.

2.3.10. *Aloesaponarin I* (**2.5**)

Light-brown amorphous solid; UV (MeOH) λ_{max} nm (log ε): 215 (4.00), 270 (3.87), 280 (3.86), 307 sh (3.53), 408 (3.27), 430 (3.26) and 475 sh (2.98); HRFABMS *m/z*: 312.0609 $[M]^-$ (Calcd for $C_{17}H_{12}O_6$: 312.0634); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (1H, s, H-5), 7.78 (1H, br, d, *J* = 8.0 Hz, H-4), 7.63 (1H, t, *J* = 8.0 Hz, H-3), 7.32 (1H, br, d, *J* = 8.0 Hz, H-2), 4.07 (3H, s, 7-COO<u>*CH*$_3$</u>), 2.98 (3H, s, 8-<u>*CH*$_3$</u>); ^{1}H NMR (500 MHz, CD_3OD) δ 7.70 (1H, br, d, *J* = 7.8 Hz, H-4), 7.65 (1H, t, *J* = 7.8 Hz, H-3), 7.61 (1H, s, H-5), 7.28 (1H, br, d, *J* = 7.8 Hz, H-2), 3.94 (3H, s, 7-COO<u>*CH*$_3$</u>), 2.69 (3H, s, 8-<u>*CH*$_3$</u>); ^{13}C NMR (500 MHz, CD_3OD) δ 191.1 (C-9), 184.0 (C-10), 169.9 (7-<u>*C*</u>$OOCH_3$), 163.7 (C-1),

161.7 (C-6), 143.3 (C-8), 138.8 (C-10a), 137.0 (C-3), 134.3 (C-4a), 131.7 (C-8a/7), 125.7 (C-2), 124.0 (C-7/8a), 119.7 (C-4), 118.4 (C-9a), 113.7 (C-5), 53.2 (7-COO<u>C</u>H_3), 20.6 (8-CH_3).

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III. Antiproliferative Cassane Diterpenoids of *Cordyla madagascariensis* ssp. *madagascariensis* from the Madagascar Rainforest

This chapter is a modified and slightly expanded version of the published article.[1] Contributions from co-authors of the article are described as follows in the order of the names listed. The author of this dissertation (Mr. Yanpeng Hou) conducted the isolation and structure elucidation of the title diterpenoids, and drafted the manuscript. Dr. Shugeng Cao was a mentor for this work, and in particular, he provided invaluable advice and hints for structure elucidation of the compounds, and he also proofread the manuscript before submission. Ms. Peggy Brodie performed the A2780 bioassay on the isolated fractions and compounds. Dr. James S. Miller, Dr. Chris Birkinshaw, Dr. Fidisoa Ratovoson, and Dr. Roland Rakotondrajaona from Missouri Botanical Garden made the plant collections and identification. Dr. Rabodo Andriantsiferana and Dr. Vincent E. Rasamison from Madagascar carried out the initial plant extraction. Dr. David G. I. Kingston was a mentor for this work and the corresponding author for the published article. He provided critical suggestions for this work and crucial revisions to the manuscript.

3.1. Introduction

In a continuing search for biologically active natural products from tropical rainforests as part of an International Cooperative Biodiversity Group (ICBG) program,[2] an extract was obtained from the fruit of a plant originally identified as a *Dalbergia* sp. but later re-identified as *Cordyla madagascariensis* ssp. *madagascariensis*; both the *Cordyla* and

Dalbergia genera belong to the Fabaceae family. The plant was collected in Madagascar, and its extract showed moderate antiproliferative activity against the A2780 human ovarian cancer cell line with an IC_{50} value of 21 μg/mL. The extract was selected for investigation on the basis of its antiproliferative activity.

3.1.1. *Previous Investigations of Cordyla*

The genus *Cordyla* belongs to the Fabaceae family, and ten species in addition to four subspecies and two varieties have been classified in the genus. No previous work has been reported on the chemistry of *Cordyla* sp. except for the isolation of some flavonoids from the heartwood of *Cordyla africana*,[3,4] and some recent research on distribution patterns of flavonoids in multiple species of the genus.[5]

3.2. Results and Discussion

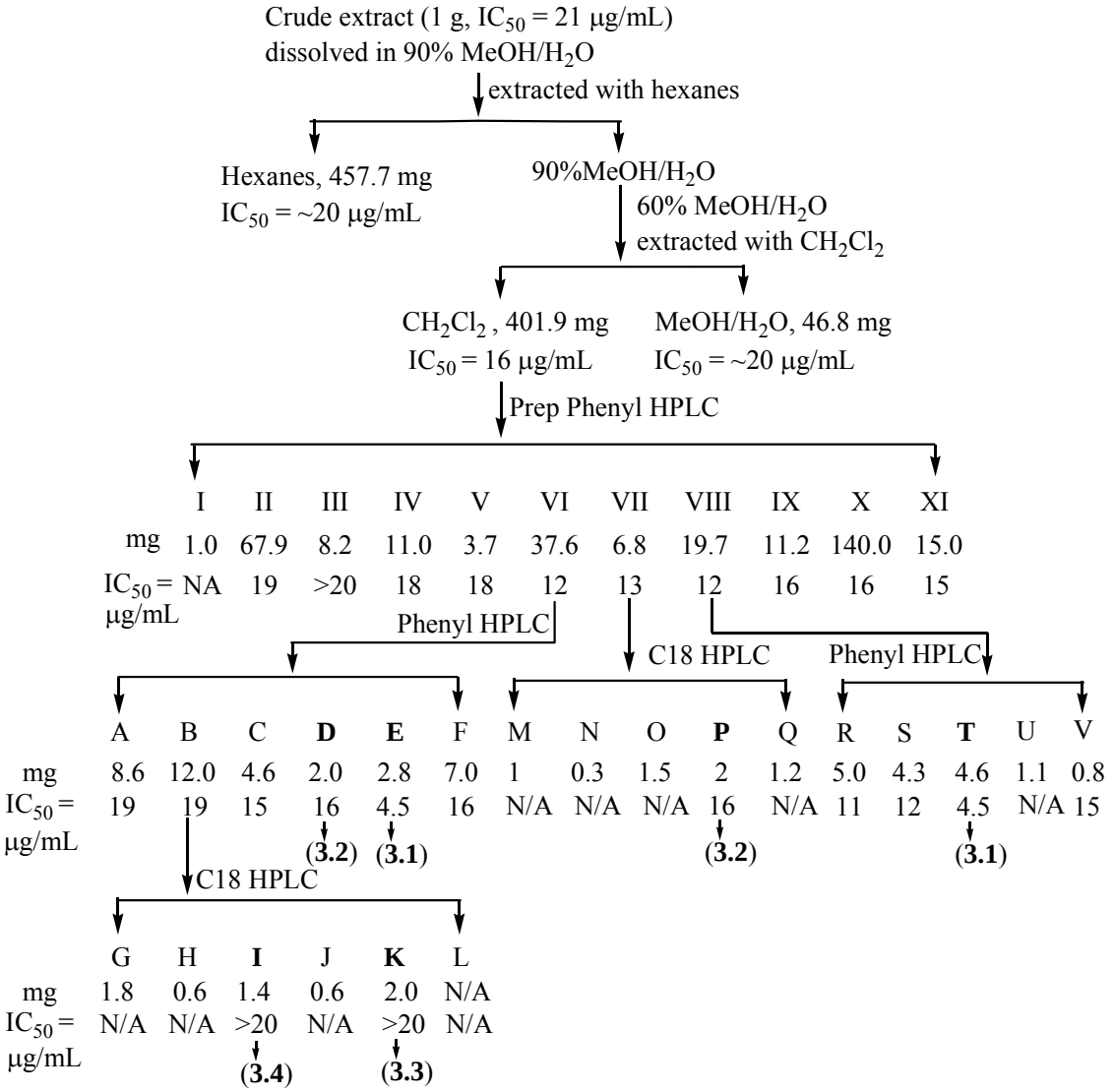
3.2.1. *Isolation of Compounds from Cordyla madagascariensis* ssp. *madagascariensis*

The crude extract of *Cordyla madagascariensis* ssp. *madagascariensis* was suspended in 90% aqueous methanol and extracted using hexanes, and then the aqueous methanol was adjusted to methanol-water (6:4) and extracted with CH_2Cl_2. As a result, three fractions were generated, and their antiproliferative activities against the A2780 ovarian cancer cell line were obtained. It was found that the CH_2Cl_2 fraction showed the best activity (16 μg/mL). This fraction was thus subjected to a multiple-step separation using reversed-phased phenyl and C18 HPLC to yield cordylanes A–D (**3.1**–**3.4**). The fractionation tree is shown in Scheme 3.1 and the detailed description of the isolation procedure is given in the Experimental Section.

3.2.2. *Structure Elucidation of Cordylane A*

Cordylane A (**3.1**) was obtained as a white powder. Its molecular formula was established as $C_{24}H_{34}O_6$ on the basis of a molecular ion peak at *m/z* 418.2331 in its HRFAB mass spectrum. Its ^{1}H NMR spectrum in C_6D_6 (Table 3.1) showed signals for one secondary methyl group (δ_H 0.83, d, *J* = 6.8 Hz, H-17), one tertiary methyl group (δ_H 1.02, s, H-20) and two acetoxy methyl groups (δ_H 1.82, s; 1.64, s). The signals of two oxymethylene groups could also be observed (δ_H 4.72, d, *J* = 11.4 Hz, H-18a; δ_H 4.27, d, *J* = 11.4 Hz, H-18b) and (δ_H 3.47, d, *J* = 10.8 Hz, H-19a; δ_H 3.24, d, *J* = 10.8Hz, H-19b). In addition, the signal of one oxymethine proton was observed in the low field region of the spectrum (δ_H 5.71, ddd, *J* = 3.0, 3.0, 2.0 Hz, H-6). A disubstituted furan ring was also present, as evidenced by characteristic resonances (δ_H 7.16, d, *J* = 2.0 Hz, H-16; δ_H 6.04, d, *J* = 2.0 Hz, H-15). In the ^{13}C NMR spectrum, the carbons of the furan ring (δ_C 149.6, C-12; 122.6, C-13; 109.7, C-15; 140.8, C-16), a tertiary carbon (δ_C 69.9, C-6), two secondary carbons (δ_C 64.7, C-18; 67.9, C-19) and two ester carbonyl carbons (δ_C 169.8, C-6-$OCOCH_3$; 170.4, C-18-$OCOCH_3$) were identified (Table 3.2). The complete ^{1}H and ^{13}C NMR assignments and connectivities were determined from a combination of COSY, HSQC, and HMBC data. The COSY spectrum showed correlations that indicated the connectivity of H-5, H-6, H_2-7, H-8, H-9 and H_2-11, in addition to demonstrating the connectivity of H-8, H-14 and H_3-17. These data allowed the assembly of the B and C rings. In the HMBC spectrum, H-18a and H-18b showed correlations to the quaternary C-4 (δ_C 42.5) and to one ester carbonyl carbon at C-18, while H-19a and H-19b also showed correlations to C-4. These correlations indicated that the two oxymethylene groups were

connected to C-4, and that one *O*-acetyl group was positioned at C-18. The key COSY and HMBC correlations are shown in Figure 3.1. Furthermore, it was apparent that the other acetyl group was connected to the oxygenated C-6. Analysis of the coupling constants and ROESY correlations enabled determination of the relative configuration at C-4, C-6, C-10 and C-14. The key ROESY correlations are shown in Figure 3.2. The ROESY correlations from H_3-20 to H-2_{ax} (δ_H 1.33, m), H-8 (δ_H 1.84, m), H-11_{ax} (δ_H 2.38, dd, J = 17.6, 10 Hz) and H_2-18, from H-5 (δ_H 1.56, d, J = 2.0 Hz) to H-3_{ax} (δ_H 1.16, m) and H-9 (δ_H 1.40, m), from H-9 to H-7_{ax} (δ_H 1.88, m) and H_3-17, from H_2-19 to H-5, H-6, and from H-19b to H-3_{ax} indicated that rings A and B had chair conformations with *trans*-fused ring junctions,[6] and thus confirmed the relative configuration at C-4, C-6, C-10 and C-14. The configuration of C-6 was also indicated by the coupling constants of H-6 (J = 3.0, 3.0, 2.0 Hz), which showed that H-6 was in the equatorial position of the chair conformation of ring B, and thus that the *O*-acetyl group at C-6 existed in the β-axial orientation. Therefore, **3.1** was assigned as the new compound 6β-acetoxy-cassa-18β-acetoxy-12,15-dien-19α-ol (Figure 3.3).



Scheme 3.1. Separation of *Cordyla madagascariensis* ssp. *madagascariensis* extract

Table 3.1. [1]H NMR Data of Compounds **3.1–3.4**[c]

no.	**3.1**[a]	**3.2**[b]	**3.3**[b]	**3.4**[b]
1	1.34 m	1.76 m	1.82 m	1.82 m
	0.72 ddd (15.0, 15.0, 5.0)	1.17 m	1.11 m	1.06 m
2	1.49 m	1.69 m	1.97 m	1.97 m
	1.33 m	1.56 m	1.56 m	1.56 m
3	1.67 m	1.93 m	2.77 m	2.26 m
	1.16 m	1.80 m	1.22 m	1.19 m
5	1.56 d (2.0)	1.62 d (2.0)	1.36 dd (12.4, 2.4)	1.37 m
6	5.71 ddd (3.0, 3.0, 2.0)	5.46 ddd (2.8, 2.8, 2.0)	1.88 m	1.90 m
			1.72 m	1.80 m
7	1.88 m	1.86 m	1.71 m	2.34 m
	1.44 m	1.59 m	1.41 m	1.30 m
8	1.84 m	1.97 m	1.75 m	2.20 m
9	1.40 m	1.64 m	1.52 m	1.39 m
11	2.49 dd (17.6, 8.0)	2.62 dd (16.8, 7.2)	2.57 dd (16.4, 6.4)	2.73 dd (16.8, 5.2)
	2.38 dd (17.6, 10.0)	2.44 dd (16.8, 10.0)	2.32 m	2.43 br d (16.8)
14	2.34 m	2.55 m	2.59 m	
15	6.04 d (2.0)	6.18 d (1.6)	6.16 d (1.2)	6.45 d (2.0)
16	7.16 d (2.0)	7.23 d (1.6)	7.21 d (1.2)	7.26 d (2.0)
17	0.83 d (6.8)	0.96 d (7.2)	0.97 d (7.2)	5.07 d (1.6)
				4.88 d (1.6)
18	4.72 d (11.4)	4.07 d (11.2)		
	4.27 d (11.4)	3.69 d (11.2)		
19	3.47 d (10.8)	4.11 d (11.6)	3.83 d (10.6)	3.83 d (10.4)
	3.24 d (10.8)	3.89 d (11.6)	3.46 d (10.6)	3.47 d (10.4)
20	1.02 s	1.20 s	0.88 s	0.93 s
6-OCO*CH*$_3$	1.82 s	2.05 s		
18/19-OCO*CH*$_3$	1.64 s	2.05 s		

[a] in C_6D_6. [b]in CD_3OD. [c]δ (ppm) 400 MHz.

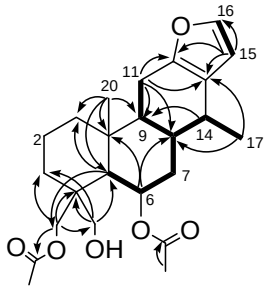


Figure 3.1. Key COSY (bold) and HMBC (arrows) correlations of **3.1**

Table 3.2. ^{13}C NMR Data of Compounds **3.1–3.4**

no.	**3.1**[a,c]	**3.2**[a,c]	**3.2**[b,d]	**3.3**[b,d]	**3.4**[b,d]
1	41.1	41.3	42.5	40.8	40.4
2	18.2	18.1	18.8	20.1	20.1
3	30.6	30.4	31.2	33.2	33.1
4	42.5	43.6	44.1	51.0	51.0
5	50.3	54.3	53.7	51.2	50.6
6	69.9	71.6	72.0	24.4	24.1
7	36.4	36.5	37.0	32.7	31.8
8	31.1	31.1	32.3	37.2	37.6
9	46.2	46.1	47.2	40.4	53.3
10	37.8	38.1	39.0	38.7	38.8
11	22.3	22.3	22.8	23.4	23.8
12	149.6	149.5	150.3	150.6	153.5
13	122.6	122.6	123.4	123.5	120.0
14	31.4	31.4	32.4	32.9	144.3
15	109.7	109.7	110.3	110.3	107.2
16	140.8	140.9	141.6	141.5	142.6
17	17.8	17.8	17.9	17.8	104.0
18	64.7	60.8	61.4	179.7	179.6
19	67.9	70.5	71.4	70.9	70.8
20	17.9	17.9	18.6	14.2	14.0
6-O*CO*CH_3	169.8	171.2	172.9		
6-OCO*CH_3*	21.4	21.3	21.8		
18/19-O*CO*CH_3	170.4	169.4	172.0		
18/19-OCO*CH_3*	20.4	20.7	20.9		

[a] in C_6D_6. [b] in CD_3OD. [c] δ (ppm) 100 MHz. [d] δ (ppm) 125 MHz.

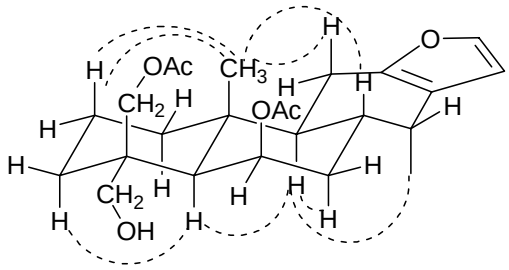


Figure 3.2. Key ROESY correlations of **3.1**

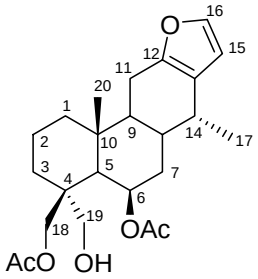


Figure 3.3. Structure of cordylane A (**3.1**)

3.2.3. *Structure Elucidation of Cordylane B*

Cordylane B (**3.2**) was obtained as a white powder. Its molecular formula was established as $C_{24}H_{34}O_6$, identical to that of **3.1,** on the basis of a molecular ion peak at *m/z* 418.2366 in its HRFAB mass spectrum. Nearly identical resonances were observed in the ^{1}H NMR spectra of **3.2** and **3.1,** with the exception of the difference in chemical shift of two oxymethylene groups H_2-18 and H_2-19. To avoid the overlapping of the ^{1}H NMR signals of H-16 in C_6D_6, further one and two-dimensional NMR data of **3.2** were also obtained in CD_3OD, and the following discussion is based on these data. The additional 2D-NMR experiments indicated that H_2-19 (δ_H 4.11, d, J = 11.6 Hz and δ_H 3.89, d, J = 11.6 Hz) had an HMBC correlation to an acetoxy carbonyl group (δ_C 172.0). In addition, H-18a (δ_H 4.07, d, J = 11.2 Hz) showed a ROESY correlation to H_3-20 (δ_H 1.20, s). These data indicated that **3.2** differed from **3.1** only in the configuration at C-4. By similar arguments to those used above for **3.1**, **3.2** were determined as the new compound, 6β-acetoxy-cassa-19α-acetoxy-12,15-dien-18β-ol (Figure 3.4).

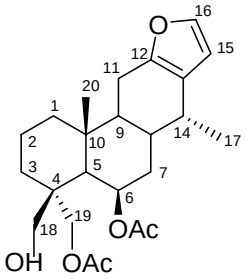


Figure 3.4. Structure of cordylane B (**3.2**)

3.2.4. *Structure Elucidation of Cordylane C*

Cordylane C (**3.3**) was obtained as a white powder. Its molecular formula was established as $C_{20}H_{28}O_4$ on the basis of a molecular ion peak at *m/z* 332.1988 in its HRFAB mass spectrum. Comparison of the ^{1}H NMR spectra of **3.1** and **3.3** showed that the two compounds were very similar, but that the oxymethine (H-6), oxymethylene (H_2-18), and both acetoxy methyl groups that appeared in the ^{1}H NMR spectrum of **3.1** were absent in the ^{1}H NMR spectrum of **3.3**. This suggested that **3.3** had a similar structure to **3.1** with the exception of the aforementioned functional groups. After further analysis of 2D NMR data, it was found that the oxymethylene protons H_2-19 (δ_H 3.83, d, J = 10.6 Hz and δ_H 3.46, d, J = 10.6 Hz) of **3.3** showed an HMBC correlation with the carbonyl carbon (δ_C 179.7, C-18) of a carboxylic acid. This indicated that the oxymethylene and carbonyl groups must be connected to the same carbon (C-4). ROESY correlations from H_2-19 to H-6_{eq} (δ_H 1.88, m) and from H-19b to H-3_{ax} (δ_H 1.22, m) and H-5 (δ_H 1.36, dd, J = 12.4, 2.4 Hz) indicated that the C-19 oxymethylene group was in the α-orientation. As in the case of **3.1**, the full structure and configuration of **3.3** was determined by a combination of one and two-dimensional NMR and mass spectroscopic methods. **3.3** was

thus determined as the new compound cassa-12,15-dien-19α-hydroxyl-18β-oic acid (Figure 3.5).

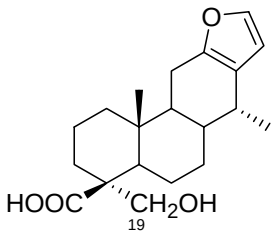


Figure 3.5. Structure of cordylane C (**3.3**)

3.2.5. *Structure Elucidation of Cordylane D*

Cordylane D (**3.4)** was obtained as a white powder. Its molecular formula was established as $C_{20}H_{26}O_4$ on the basis of its molecular ion peak at *m/z* 330.1841 in its HRFAB mass spectrum. Compound **3.4** had a similar ^{1}H NMR spectrum to that of **3.3**, except that the resonance of the methyl group (δ_H 0.97, d, J = 7.2 Hz, H_3-17) in the ^{1}H NMR spectrum of **3.3** was replaced by signals for an olefinic methylene group (δ_H 5.07, d, J = 1.6 Hz and δ_H 4.88, d, J = 1.6 Hz, H_2-17). These protons were connected to an olefinic carbon at C-17 (δ_C 104.0) via an HSQC correlation, indicating that the C-17 methyl group in **3.3** was converted to a vinylic methylene in **3.4**. This was confirmed by HMBC correlations between both of the methylene hydrogens to an olefinic carbon (δ_C 120.0, C-13), and a methine carbon (δ_C 37.6) assigned as C-8. The structure of **3.4** was assigned by a combination of one and two-dimensional NMR and MS methods and by comparison with the data of compound **3.3**. Its structure was assigned as the new compound cassa-12,14(17),15-trien-19α-hydroxyl-18β-oic acid (Figure 3.6).

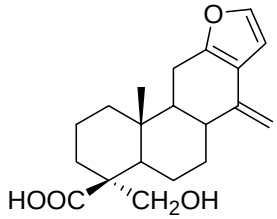
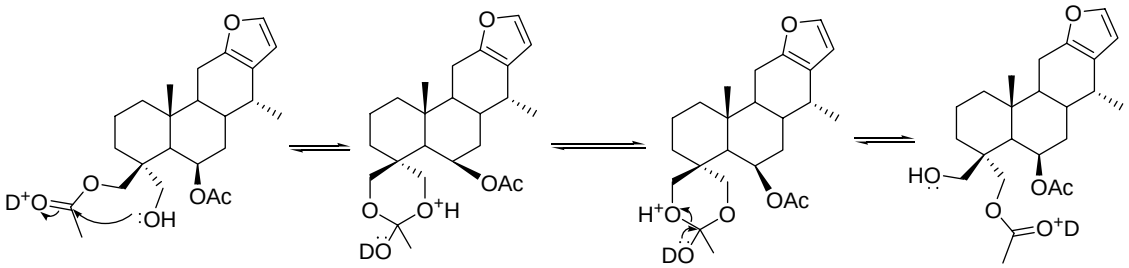


Figure 3.6. Structure of cordylane D (**3.4**)

3.2.6. *Possible Acid Catalyzed Transesterification of Cordylane A and Cordylane B*

An interesting phenomenon observed in this study was that compound **3.1** was unstable under acidic conditions. Although compound **3.1** was stable in CD_3OD and C_6D_6, when it was dissolved in an old sample of $CDCl_3$ for a ^1H NMR experiment, the spectrum showed the presence of a mixture of **3.1** and **3.2** even after a short period of time. Presumably the trace amounts of DCl formed on storage of $CDCl_3$ catalyzed the transesterification reaction of **3.1**. The proposed mechanism is shown in Scheme 3.2. Given this observation, it is not possible to state unambiguously that both **3.1** and **3.2** are natural products, since conceivably one could have been converted to the other during extraction in Madagascar or during shipment to the USA and storage.

Scheme 3.2. Mechanism of transesterification of cordylane A and B



protonated **3.1** protonated **3.2**

3.2.7. *Antiproliferative Activities of Isolated Compounds*

All the isolated compounds were tested for antiproliferative activity against the A2780 human ovarian cancer cell line. It was found that **3.1** and **3.2** showed mild antiproliferative activity with IC_{50} values equal to 10 and 36 μM, respectively. Compounds **3.3** and **3.4** did not show any significant antiproliferative activity with IC_{50} values greater than 60 μM.

3.2.8 *Bioactivities of Cassane-type Diterpenoids*

Cassane diterpenoids have been reported primarily from the Fabaceae family, except for two investigations from the Asteraceae and Poaceae families, respectively. The reported cassane diterpenoids were predominantly distributed in the genus *Caesalpinia* with a few compounds reported from other genera. The isolated cassane diterpenoids exhibited anticancer[7-15] antimalarial[16-18], antiviral[19,20] and antifungal [21,22] activities. However, none of the compounds identified to date are potent enough to serve as lead compounds for drug development.

3.3. Experimental Section

3.3.1. *General Experimental Procedures*

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. IR and UV spectra were performed on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. NMR spectra were obtained on JEOL Eclipse 500, Varian Inova 400, and Varian Unity 400 spectrometers. Mass spectra were obtained on a JEOL-JMS-HX-110 instrument. Chemical shifts are given in δ (ppm), and coupling constants (*J*) are reported

in Hz. HPLC was performed using Shimadzu LC-8A pumps coupled with a Varian Dynamax preparative phenyl column (250 × 21.4 mm) and Shimadzu LC-10A pumps coupled with a Varian Dynamax semipreparative column (250 × 10 mm). Both HPLC instruments employed a Shimadzu SPD-M10A diode array detector.

3.3.2. *Antiproliferative Bioassay*

The A2780 ovarian cancer cell line assay was performed by Ms. Peggy Brodie at Virginia Polytechnic Institute and State University as previously reported[23] and described in Chapter II. The A2780 cell line is a drug sensitive ovarian cancer cell line.[24]

3.3.3. *Plant Material Collection*

The plant sample of *Cordyla madagascariensis* R. Vig. subsp. *madagascariensis* was collected on March 30, 2004 by Roland Rakotondrajaona et al. in Antsiranana, Madagascar, in the vicinity of Mahavanoma (12°23'10"S 49°20'19"E). It was assigned collector number R. Rakotondrajaona 319, and it was determined by R. Rakotondrajaona. The specimens were from a tree 15 m high and diameter at breast height of 40 cm. Voucher specimens have been deposited at herbaria of the Centre National d'Application des Recherches Pharmaceutiques, Madagascar (CNARP), the Parc Botanique et Zoologique de Tsimbazaza, Madagascar (TAN), the Missouri Botanical Garden, St. Louis, Missouri (MO), and the Muséum National d'Histoires Naturelles, Paris, France (P).

3.3.4. *Plant Material Extraction and Extract Fractionation*

Dried fruits from the tree described above (245 g) were extracted with EtOH to give 24.1 g of extract, which was assigned the number MG2180. A total of 1.64 g of extract was supplied to VPISU, and this had an IC_{50} value of 21 μg/mL against A2780 cells. A portion of this extract (1.0 g) was suspended in aqueous MeOH (90% MeOH/H_2O, 40 mL), and extracted with hexanes (3 × 40 mL). The aqueous layer was then diluted to 60% MeOH (v/v) with H_2O and extracted with CH_2Cl_2 (3 × 60 mL). The CH_2Cl_2 extract (401.9 mg) was found to be the most active (IC_{50} = 16 μg/mL) and was separated via preparative HPLC over a phenyl column using MeOH-H_2O (70:30) to afford 11 fractions (I-XI), of which fractions VI-VIII were found to display the highest antiproliferative activity (IC_{50} = 12, 13 and 12 μg/mL, respectively). Fraction VI was further separated via semi-preparative HPLC on a phenyl column using MeOH-H_2O (70:30). Six fractions (A-F) were collected. Fraction D afforded cordylane B (**3.2**, 2.0 mg, t_R 26.7 min), while fraction E afforded cordylane A (**3.1**, 2.8 mg, t_R 28.6 min). Fraction B was then subjected to semipreparative HPLC on an RP-C18 column using MeOH-H_2O (70:30) to afford six fractions (G-L). Fraction I afforded cordylane D (**3.4**, 1.4 mg, t_R 25.5 min) and fraction K afforded cordylane C (**3.3**, 2.0 mg, t_R 28.6 min). Fraction VII was separated using semi-preparative RP-C18 HPLC using MeOH-H_2O (75:25). Five fractions (M-Q) were collected. Fraction P afforded cordylane B (**3.2**, 2.0 mg, t_R 27.3 min). Fraction VIII was also separated using semi-preparative phenyl HPLC using MeOH-H_2O (70:30) to obtain five fractions (R-V), of which fraction T afforded cordylane A (**3.1**, 4.6 mg, t_R 24.9 min).

3.3.5. *Cordylane A* (**3.1**)

white powder; $[\alpha]_D^{23}$ +17.7 (c 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 217 (3.63) nm**;** IR: ν_{max} 3372, 2945, 2831, 1738, 1461, 1370, 1239, 1026 cm^{-1}; ^1H and ^{13}C NMR see Tables 3.1 and 3.2; HRFABMS *m/z* 418.2331 $[M]^+$ (calcd for $C_{24}H_{34}O_6$, 418.2355).

3.3.6. *Cordylane B* (**3.2**)

white powder; $[\alpha]_D^{23}$ +15.0 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.28) nm**;** IR: ν_{max} 3339, 2945, 2831, 1729, 1461, 1370, 1239, 1029 cm^{-1}; ^1H and ^{13}C NMR see Tables 3.1 and 3.2; HRFABMS *m/z* 418.2366 $[M]^+$ (calcd for $C_{24}H_{34}O_6$, 418.2355).

3.3.7. *Cordylane C* (**3.3**)

white powder; $[\alpha]_D^{23}$ +68.0 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 219 (3.58) nm**;** IR: ν_{max} 3320, 2951, 2834, 1710, 1468, 1029 cm^{-1}; ^1H and ^{13}C NMR see Tables 3.1 and 3.2; HRFABMS *m/z* 332.1988 $[M]^+$ (calcd for $C_{20}H_{28}O_4$, 332.1988).

3.3.8. *Cordylane D* (**3.4**)

white powder; $[\alpha]_D^{23}$ +40.0 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 209 (3.35) nm; IR: ν_{max} 3300, 2940, 1710, 1475, 2833, 1030 cm^{-1}; ^1H and ^{13}C NMR see Tables 3.1 and 3.2; HRFABMS *m/z* 330.1841 $[M]^+$ (calcd for, $C_{20}H_{26}O_4$, 330.1831).

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IV. Antiproliferative Cardenolide Glycosides of *Elaeodendron alluaudianum* from the Madagascar Rainforest

This chapter is a modified and slightly expanded version of the published article.[1] Contributions from co-authors of the article are described as follows in the order of the names listed. The author of this dissertation (Mr. Yanpeng Hou) conducted the isolation and structure elucidation of the title cardenolide glycosides, and drafted the manuscript. Dr. Shugeng Cao provided advice and hints for structure elucidation of the compounds, and he also proofread the manuscript before submission. Ms. Peggy Brodie performed the A2780 bioassay on the isolated fractions and compounds. Dr. Martin Callmander, Dr. Fidisoa Ratovoson, and Dr. Richard Randrianaivoand from Missouri Botanical Garden made the plant collections and identification. Dr. Etienne Rakotobe, Dr. Vincent E. Rasamison, and Dr. Stephan Rakotonandrasana from Madagascar carried out the initial plant extraction. Dr. Karen Tendyke and Dr. Edward M. Suh from Eisai Research Institute performed the U937 bioassay on the title compounds. Dr. David G. I. Kingston was a mentor for this work and the corresponding author for the published article. He provided critical suggestions for this work and crucial revisions to the manuscript.

4.1. Introduction

In a continuing search for biologically active natural products from tropical rainforests as part of an International Cooperative Biodiversity Groups (ICBG) program,[2] an extract of the stems of a plant which was initially identified as a *Hippocratea* sp. but was later revised to *Elaeodendron alluaudianum* H. Perrier (Celastraceae), was obtained from

Madagascar. The extract had significant antiproliferative activity against the A2780 ovarian cancer cell line with an IC_{50} value of 3.3 μg/mL, and it was thus selected for bioassay-guided fractionation.

4.1.1. *Previous Investigations of Elaeodendron*

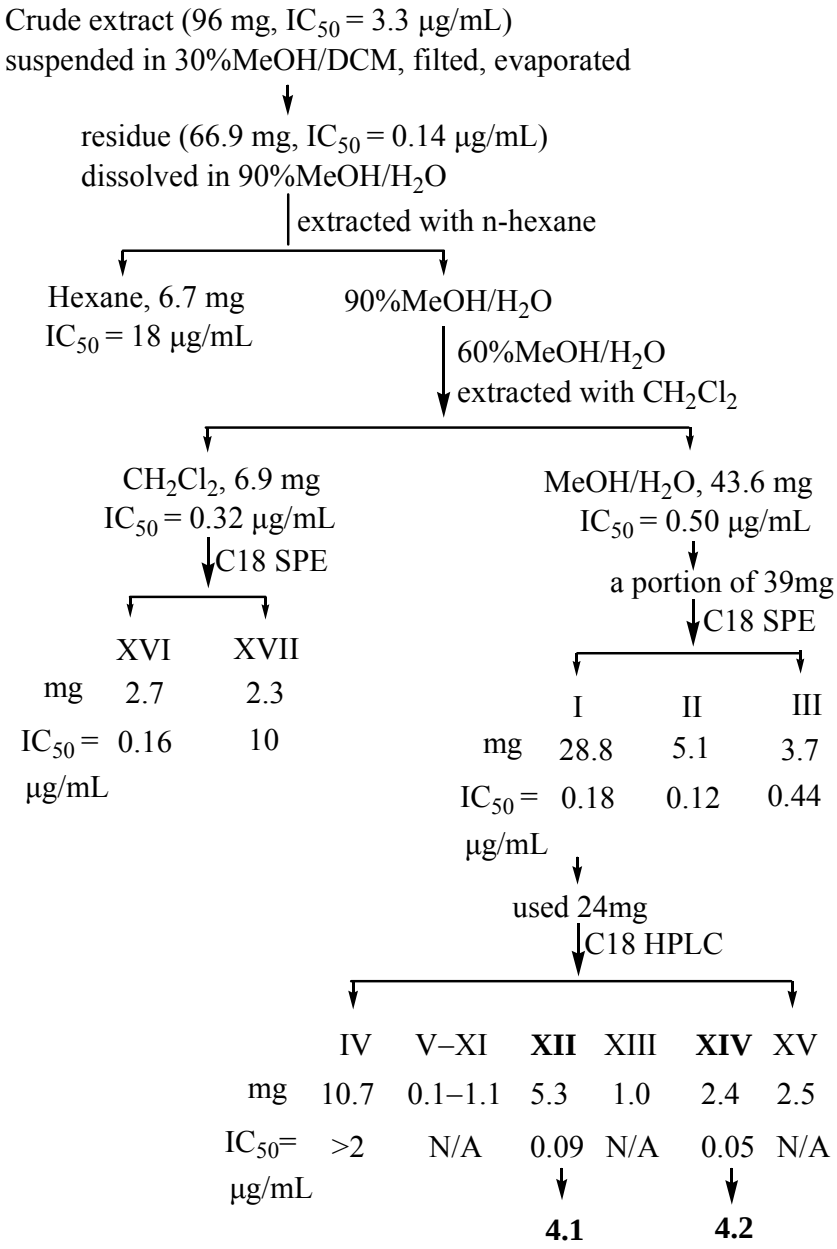
As noted previously,[3] there are about 40 species in the genus *Elaeodendron* from the Mexican coast, Bermuda, Africa, Madagascar (incl. the Mascarenes), India, Melanesia, and Australia.[4,5] The plants in this genus are usually glabrous trees or shrubs,[4,5] and flavonoids,[6,7] terpenoids,[8-14] and cardenolides[15-23] have been isolated from them. The cytotoxicities and cardiac activities of cardenolides have been widely studied.[24-39]

4.2. Results and Discussion

4.2.1. *Isolation of Compounds from Elaeodendron alluaudianum*

A portion of the crude ethanol extract of *Elaeodendron alluaudianum* (96 mg) was suspended in 20 mL of 30% MeOH/CH_2Cl_2 and filtered. The filtrate was evaporated to afford 67 mg residue (IC_{50} = 0.14 μg/mL). The residue was suspended in MeOH–H_2O (9:1) and extracted using hexanes. The aqueous methanol fraction was then diluted to MeOH–H_2O (6:4) and extracted with CH_2Cl_2. Both organic extracts and the residual aqueous methanol solution were evaporated to generate three fractions, and the antiproliferative activities of those fractions against the A2780 ovarian cancer cell line were obtained. It was found that the aqueous methanol and CH_2Cl_2 fractions (IC_{50} = 0.50 and 0.32 μg/mL) showed the best activities. The aqueous methanol fraction was then selected for fractionation due to its relatively larger amount of materials, and it was

separated firstly by a step-gradient elution from a reversed-phase C18 SPE cartridge. The most active fraction was eluted with MeOH–H_2O (3:7), and this fraction was then separated on a semi-preparative reversed-phased C18 HPLC column with elution by MeOH–H_2O (75:25) to yield two major significantly antiproliferative compounds, designated as elaeodendroside V (**4.1**) (IC_{50} = 0.09 μg/mL) and W (**4.2**) (IC_{50} = 0.05 μg/mL). The CH_2Cl_2 fraction was also subjected to a reversed-phased C18 SPE to afford two subfractions. The fractionation tree is shown in Scheme 4.1, and a detailed description of the isolation procedure is given in the Experimental Section. It is noticeable that fraction II, III and XVI also exhibited good antiproliferative activity, and the preliminary spectroscopic data indicated the existence of relatively significant amounts of cardenolide glycosides in those fractions. Thus, it is not surprising that these fractions exhibit significant biological activities because cardenolide glycosides are known to show antiproliferative activity and cytotoxicity. However, no further fractionation on those fractions was carried out since cardenolide glycosides are known to have unfavorable toxicity profiles, which reduce the potential for them to become anticancer agents. In conclusion, all the results indicated that the antiproliferative activity of this extract was probably due to the presence of cardenolide glycoside–type compounds.



Scheme 4.1. Separation of *Elaeodendron alluaudianum* extract

4.2.2. *Structure Elucidation of Elaeodendroside V*

Elaeodendroside V (**4.1**) was obtained as a white amorphous solid. Its molecular formula was established as $C_{35}H_{54}O_{16}$ on the basis of a protonated molecular ion peak at *m/z* 731.3496 in its HRFAB mass spectrum. Its ^{1}H NMR spectrum in CD_3OD showed

characteristic signals of a α,β-unsaturated γ-lactone (δ_H 5.01, dd, J = 18.4, 1.6 Hz, H-21a; δ_H 4.92, dd, J = 18.4, 1.6 Hz, H-21b; and δ_H 5.91, s, H-22) (Table 4.1). Its ^{13}C NMR spectrum contained 35 signals (Table 4.2), which were assigned as two methyls, 11 methylenes (including three oxymethylenes), 16 methines (including twelve oxymethines and one olefinic carbon), and six quaternary carbons (including two oxyquaternary carbons, one olefinic carbon and one carbonyl carbon) based on ^{13}C NMR (Table 4.2) and HSQC spectra.

The complete ^{1}H and ^{13}C NMR signal assignments and connectivity were determined from a combination of COSY, TOCSY, HSQC, and HMBC data and comparison with the spectra of known cardenolides.[40] COSY and TOCSY correlations established three spin systems, which were H_2-1–H_2-2–H-3–H_2-4 in ring A, H_2-6–H_2-7–H-8–H–9–H-11–H_2-12 in rings B and C, and H_2-15–H_2-16–H-17 in ring D (Figure 4.1). Further assembly of rings A–D and the α,β-unsaturated γ-lactone of the aglycone was determined on the basis of HMBC correlations. HMBC correlations of H_2-19 to C-1, C-5, and C-9, H_2-1 to C-9, and H_2-6 to C-5 established the connectivity of rings A and B. Correlations of H_2-12, H_2-16, H-17, and H_3-18 to C-13 and of H_2-16 and H_3-18 to C-14 indicated the connectivity of rings C and D. In the meantime, an HMBC correlation of H_2-16 to C-20 suggested the location of the lactone ring at C-17 (Figure 4.1). These correlations established the flat structure of the aglycone of **4.1**.

Table 4.1. ^{1}H NMR Data of Compounds **4.1** and **4.2**[c]

no.	**4.1**[a]	**4.1**[b]	**4.2**[b]
1	3.03 m	2.24 m	2.47 m
	2.85 td (13.8, 2.5)	2.19 m	2.24 m
2	2.45 m	1.94 m	1.92 m
	2.25 m	1.82 m	1.85 m
3	4.48 m	4.15 m	4.19 br s
4	2.21 m	2.10 dd (15.4, 3.0)	2.09 m
	1.84 m	1.64 m	1.72 m
6	2.34 m	1.87 m	1.89 m
	1.67 br d (12.9)	1.47 m	1.68 m
7	2.44 m	1.99 m	2.06 m
	1,49 m	1.25 m	1.28 m
8	2.40 m	1.81 m	2.00 m
9	2.19 m	1.78 m	1.73 m
11	4.52 m	3.93 td (9.8, 4.2)	3.94 dd (9.8, 4.7)
12	1.97 m	1.66 m	1.67 m
	1.89 m	1.54 dd (13.2, 9.8)	1.49 dd (13.2, 10.8)
15	2.31 m	2.17 m	2.15 m
	1.97 m	1.72 m	1.68 m
16	2.09 m	2.17 m	2.14 m
	2.00 m	1.91 m	1.89 m
17	3.00 m	2.93 t (7.2)	2.94 t (7.2)
18	1.13 s	0.91 s	0.90 s
19	4.66 d (10.7)	4.18 d (11.2)	9.97 s
	4.38 m	3.80 m	
21	5.28 br d (18.2)	5.01 dd (18.4, 1.6)	5.00 br d (18.4)
	5.03 m	4.92 dd (18.4, 1.6)	4.92 br d (18.4)
22	6.11 s	5.91 s	5.91 s
1'	5.37 d (8.0)	4.72 d (8.0)	4.73 d (8.0)
2'	3.90~4.60[d]	3.35 m	3.35 m
3'	3.90~4.60[d]	4.33 t (3.0)	4.33 t (2.8)
4'	3.90~4.60[d]	3.28 dd (9.6, 3.0)	3.28 m
5'	3.90~4.60[d]	3.85 m	3.85 m
6'	1.60 d (6.0)	1.30 d (6.0)	1.30 d (6.0)
1"	5.49 d (7.7)	4.73 d (7.6)	4.73 d (8.0)
2"	3.90~4.60[d]	3.33 m	3.33 m
3"	3.90~4.60[d]	4.05 t (2.9)	4.05 t (2.8)
4"	3.90~4.60[d]	3.53 dd (9.2, 2.9)	3.52 dd (9.2, 2.9)
5"	3.90~4.60[d]	3.67 m	3.67 m
6"	4.37 m	3.84 m	3.84 m
	4.30 m	3.68 m	3.69 m
5-OH	5.88 s		
11-OH	5.77 d (4.7)		
14-OH	5.53 s		

[a] in pyridine-d_5. [b] in CD_3OD. [c] δ (ppm) 500 MHz.
[d] resonances not assignedbecause of overlapping resonances.

Table 4.2. ^{13}C NMR Data of Compounds **4.1** and **4.2**[c]

no.	**4.1**[a]	**4.1**[b]	**4.2**[b]
1	22.7	22.4	21.3
2	27.6	27.7	27.1
3	74.8	76.2	75.5
4	35.3	35.9	35.7
5	76.3	77.5	75.5
6	37.3	37.0	38.7
7	25.0	25.1	25.5
8	40.8	41.1	42.2
9	45.1	45.2	46.1
10	45.4	45.7	56.7
11	68.7	69.1	68.5
12	50.7	50.6	50.1
13	51.0	51.8	51.0
14	85.0	85.9	85.4
15	33.5	33.5	33.1
16	28.0	28.0	28.0
17	51.4	51.1	51.5
18	18.2	17.8	17.6
19	65.6	65.9	211.1
20	174.8	177.2	177.2
21	74.0	75.5	75.5
22	118.1	118.1	118.1
23	175.8	177.7	177.5
1'	99.3	99.5	99.5
2'	65~80[d]	72.3	72.4
3'	65~80[d]	72.2	72.2
4'	83.8	84.0	84.0
5'	65~80[d]	69.9	69.9
6'	18.7	18.3	18.3
1"	104.3	103.7	103.7
2"	65~80[d]	72.4	72.4
3"	65~80[d]	73.3	73.3
4"	65~80[d]	68.6	68.6
5"	65~80[d]	75.4	75.4
6"	63.0	62.8	62.8

[a] in pyridine-d_5. [b] in CD_3OD. [c] δ (ppm) 100 MHz.
[d] resonances not assigned because of overlapping ^{1}H NMR resonances in the HMBC spectrum.

The structures of the sugar moieties of **4.1** were determined by 1D TOCSY, COSY, and HMBC data. Two sugar units were clearly shown by COSY and TOCSY correlations of two spin systems, H-1′–H-2′ –H-3′ –H-4′ –H-5′ –H_3-6′ and H-1″–H-2″ –H-3″ –H-4″ –H-5″ –H_2-6″ (Figure 1). An HMBC correlation of H-3 to C-1′ indicated the sugars were connected to the aglycone at C-3. In the meantime, HMBC correlations of H-1″ to C-4′ and of H-4′ to C-1″ established that the two sugars were connected from C-1″ to C-4′ (Figure 4.1).

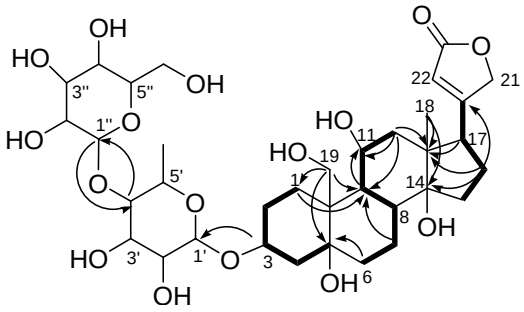


Figure 4.1. Key COSY (bold) and HMBC (arrows) correlations of **4.1**

The relative configuration of the aglycone of **4.1** was established by analysis of its ROESY correlations and calculation of coupling constants (Figure 4.2 and Table 4.1). The ROESY correlations of H_3-18 to H-22, H_2-21, H-8, and H-11, and of H_2-19 to H-8 and H-11 indicated that the C-17 side chain, H-8, H-11, and the oxymethylene at C-10 were all β-oriented. Calculation of coupling constants of H-11 (td, J = 9.8, 4.2 Hz) suggested that the adjacent H-9 occupied the α-orientation. The above assignments indicated that the B/C ring junction was *trans*-fused. ROESY spectra of **4.1** obtained in pyridine-d_5 showed a correlation of C-14-OH to H_3-18 that suggested a *cis*-fused ring junction of rings C and D. Determination of the relative configuration of the remaining

portions of **4.1** was carried out by comparison with literature data.[40,32] These comparisons indicated that rings A and B were connected by *cis*-fused ring junctions and that the C-3 side chain (sugar moiety) existed in the β-orientation. The relative configuration of the sugar moiety was established mainly by calculation of coupling constants and was further proved by ROESY correlations (Table 4.1 and Figure 4.2). Coupling constants of H-1′ (d, J = 8.0 Hz), H-3′ (t, J = 3.0 Hz), and H-4′ (dd, J = 9.6, 3.0 Hz) indicated that H-1′, H-2′, H-4′, and H-5′ were in the axial orientation, and that H-3′ was in the equatorial orientation. Coupling constants of H-1″ (d, J = 7.6 Hz), H-3″ (t, J = 2.9 Hz), and H-4″ (dd, J = 9.2, 2.9 Hz) suggested that H-1″, H-2″, H-4″, and H-5″ were in the axial orientation, and that H-3″ was in the equatorial orientation. Those assignments were proved by ROESY correlations of H-1′ to H-5′, H-1″ to H-5″, and H-2″ to H-4″. In addition, structure of the sugar moiety of **4.1** could also be confirmed by comparison with compounds sarmentogenin-3β-O-[β-allosyl-(1→4)-β-6-deoxyalloside and securigenin-3β-O-[β-allosyl-(1→4)-β-6-deoxyalloside,[35] and **4.1** had identical ^{1}H and ^{13}C NMR data to those of the sugar moieties of reported compounds. Therefore, the structure and configuration of **4.1** was determined as the new compound sarmentologenin-3β-*O*-[β-allosyl-(1→4)-β-6-deoxyalloside] (Figure 4.3).

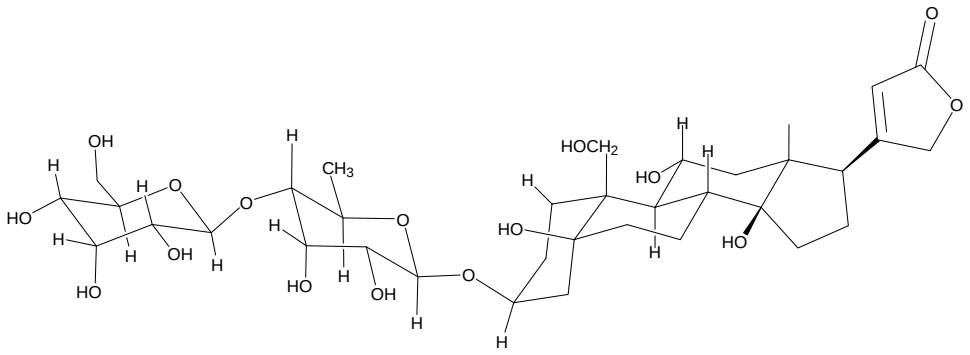


Figure 4.2. Key ROESY correlations of **4.1**

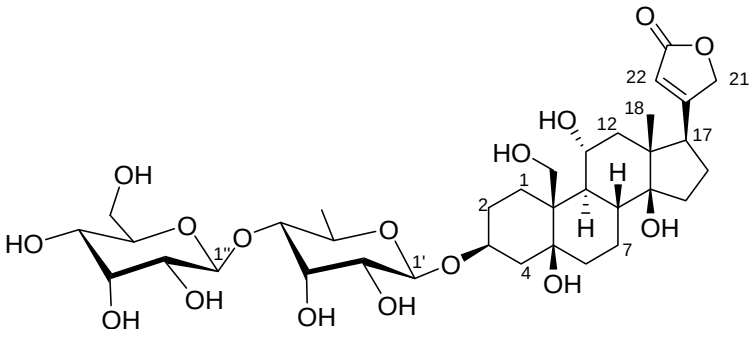


Figure 4.3. Structure of elaeodendroside V (**4.1**)

4.2.3. *Structure Elucidation of Elaeodendroside W*

Elaeodendroside W (**4.2**) was obtained as a white amorphous solid. Its molecular formula was established as $C_{35}H_{52}O_{16}$, which was two units less than that of **4.1**, on the basis of a sodiated molecular ion peak at *m/z* 751.311 in its MALDI-TOF/TOF mass spectrum. The ^{1}H NMR spectrum of **4.2** showed characteristic signals of a α,β-unsaturated γ-lactone (δ_H 5.00, br d, J = 18.4 Hz, H-21a, δ_H 4.92, br d, J = 18.4 Hz, H-21b, and δ_H 5.91, s, H-22) (Table 4.1). Comparison of the ^{1}H NMR and ^{13}C NMR spectra

of compounds **4.1** and **4.2** showed that they were very similar, but that the oxymethylene resonances (δ_H 4.18, d, J = 11.2 Hz, H-19a, δ_H 3.80, m, H-19b and δ_C 65.9, C-19) that appeared in the spectra of **4.1** were absent in the spectra of **4.2**, and that the aldehyde resonances (δ_H 9.97, s, H-19 and δ_C 211.1, C-19) that appeared in the spectra of **4.2** were absent in the spectra of **4.1** (Tables 4.1 and 4.2). Those data indicated that **4.2** had a similar structure to **4.1** except that **4.2** has an aldehyde instead of a hydroxymethyl group at C-19. Further comparison of the ^{1}H and ^{13}C NMR spectra of **4.2** and the known cardenolide glycoside, sarmentosigenin-3β-*O*-β-6-deoxyguloside,[32] confirmed the assignments, since the ^{1}H NMR and ^{13}C NMR data of the aglycone of **4.2** were identical to the literature data for sarmentosigenin. The structure of **4.2** was further confirmed by analysis of 2D NMR spectra including COSY, HMQC, HMBC, and ROESY spectra. Therefore, the structure and configuration of **4.2** was determined as the new compound sarmentosigenin-3β-*O*-[β-allosyl-(1→4)-β-6-deoxyalloside] (Figure 4.4).

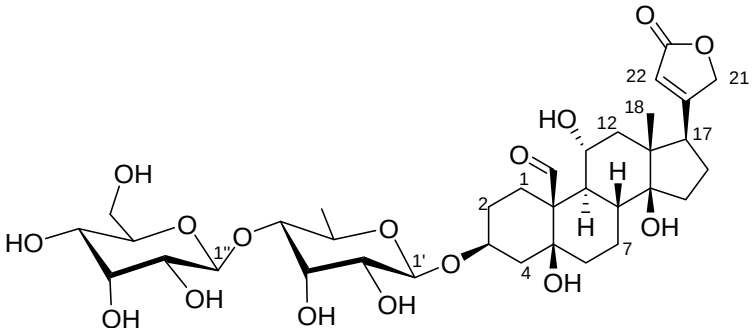


Figure 4.4. Structure of elaeodendroside W (**4.2**)

4.2.4. *Antiproliferative Activities of Isolated Compounds*

Elaeodendroside V (**4.1**) and elaeodendroside W (**4.2**) were tested for antiproliferative activity against the A2780 human ovarian cancer cell line and the U937 human histiocytic lymphoma cell line. It was found that both **4.1** and **4.2** showed significant antiproliferative activity, with IC_{50} values of 0.12 and 0.07 μM against the A2780 human ovarian cancer cell line and 0.15 and 0.08 μM against the U937 human histiocytic lymphoma cell line, respectively. The antiproliferative activities of **4.1** and **4.2** do not appear to be correlated with the oxidation status of C-19 because their IC_{50} values were very close to each other. The known cardenolide glycoside, sarmentosigenin-3β-*O*-β-6-deoxyguloside, which possesses the same aglycone as **4.2** but contains a 6-deoxygulose, also showed significant cytotoxicity with IC_{50} value of 0.074 μM against a KB cell line, while another cardenolide with an identical aglycone to **4.2** but glycosylated with rhamnose instead of allose also showed significant cytotoxicity with an IC_{50} value of 0.049 μM (0.028 μg/mL) against the HSG cell line.[41] Those data suggested that compounds with the same skeletons as **4.1** and **4.2** might show significant activities against cultured cancer cells. The cytotoxicity and antiproliferative activities of many structurally diverse cardenolide glycosides against cultured tumor cells have been widely investigated. Some recent reports are cited.[3,36,37] This class of compounds has not however found any clinical applications, in part because of unfavorable toxicity profiles.

4.3. Experimental Section

4.3.1. *General Experimental Procedures*

Optical rotations were recorded on a JASCO P-2000 polarimeter. IR and UV spectra were performed on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. NMR spectra were obtained on JEOL Eclipse 500, Varian Inova 400, and Varian Unity 400 spectrometers. Mass spectra were obtained on a JEOL-JMS-HX-110 and an Applied Biosystems 4800 MALDI-TOF/TOF instruments. Chemical shifts are given in δ (ppm), and coupling constants (*J*) are reported in Hz. HPLC was performed with Shimadzu LC-10A pumps coupled with a Varian Dynamax semi-preparative C18 column (250 × 10 mm). Both HPLC instruments employed a Shimadzu SPD-M10A diode array detector.

4.3.2. *Antiproliferative Bioassay*

The A2780 ovarian cancer cell line assay was performed by Ms. Peggy Brodie at Virginia Polytechnic Institute and State University as previously reported[42] and described in Chapter II. The A2780 cell line is a drug-sensitive ovarian cancer cell line.[43]

The U937 human histiocytic lymphoma cell line assay was performed at Eisai Research Institute. The cells were cultured in 96-well plates in the absence or continuous presence of 0.005 to 10 μg/ml extract for 96 h. Cell growth was assessed using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega) according to the manufacturer's recommendations. Luminescence was read on the EnVision 2102 Multilabel Reader (Perkin-Elmer). IC_{50} values were determined as the concentration of an extract at which

cell growth was inhibited by 50% compared to untreated cell population. Two independent repeating experiments were preformed.

4.3.3. *Plant Material Collection*

Root, stem, and leaf samples of *Elaeodendron alluaudianum* H. Perrier (Celastraceae) were collected in the forest of Bemosa, a dense humid forest, in northern Madagascar, at an elevation 200 m, at 13.14.17 S, 49.37.50 E, on November 2, 2005. The tree was 10 m high with diameter at breast height of 12 cm and white flowers. It was identified by R. H. Archer (South African National Biodiversity Institute); its assigned collector number is Randrianaivo et al. 1281.

4.3.4. *Plant Material Extraction and Extract Fractionation*

The stems of the dried plant sample described above (270 g) were extracted with EtOH to give 5.78 g of extract designated MG 3593. A total of 1.63 g of extract was supplied to VPISU, and this had an IC_{50} value of 3.3 μg/mL against A2780 cells. A portion of this extract (96 mg) was suspended in 20 mL of 30% MeOH/CH_2Cl_2 and filtered. The filtrate was evaporated to afford 67 mg residue (IC_{50} = 0.14 μg/mL). The residue was suspended in aqueous MeOH (90% MeOH/H_2O, 10 mL), and extracted with hexanes (3 × 10 mL). The aqueous layer was then diluted to 60% MeOH (v/v) with H_2O and extracted with CH_2Cl_2 (3 × 15 mL). It was found that the aqueous methanol and CH_2Cl_2 fractions (IC_{50} = 0.50 and 0.32 μg/mL) showed the best activity. A portion of the aqueous methanol fraction (39 mg) was then loaded on a C18 SPE cartridge and eluted with solvent systems

of 30% MeOH/H_2O, 70% MeOH/H_2O and MeOH to obtain three subfractions (I–III). The most active subfraction was Fraction I (IC_{50} = 0.14 μg/mL), and this was separated via semi-preparative HPLC over a C18 column using MeOH/H_2O (25:75) to afford 12 fractions (IV–XV). Fraction XII afforded elaeodendroside V (**4.1**, 5.3 mg, t_R 35.7 min), and fraction XIV afforded elaeodendroside W (**4.2**, 2.4 mg, t_R 44.1 min). The CH_2Cl_2 fraction was also subjected to a reversed-phased C18 SPE to afford two subfractions (XVI–XVII) eluted using the MeOH/H_2O solvent system.

4.3.5. *Elaeodendroside V (**4.1**)*

White amorphous solid; $[\alpha]_D^{23}$ +1.6 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 215 (4.31) nm; IR: ν_{max} 3382, 2944, 2828, 1732, 1028 cm^{-1}; ^1H and ^{13}C NMR spectra, see Tables 4.1 and 4.2; HRFABMS *m/z* 731.3496 $[M+H]^+$ (calcd for $C_{35}H_{55}O_{16}$, 731.3490).

4.3.6. *Elaeodendroside W (**4.2**)*

White amorphous solid; $[\alpha]_D^{23}$ +1.6 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.34) nm; IR: ν_{max} 3382, 2945, 2833, 1731, 1026 cm^{-1}; ^1H and ^{13}C NMR spectra, see Tables 4.1 and 4.2; MALDI-TOF/TOF-MS *m/z* 751.311 $[M+Na]^+$ (calcd for $C_{35}H_{52}O_{16}Na$, 751.315).

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V. Euphane Triterpenoids of *Cassipourea lanceolata* from The Madagascar Rainforest

This chapter is a modified and slightly expanded version of a prepared manuscript.[1] Contributions from co-authors of the article are described as follows in the order of the names listed. The author of this dissertation (Mr. Yanpeng Hou) conducted the isolation and structure elucidation of the title triterpenoids, and drafted the manuscript. Dr. Shugeng Cao was a mentor for this work, and in particular, he provided invaluable advice and hints for structure elucidation of the compounds, and he also proofread the manuscript before submission. Ms. Peggy Brodie performed the A2780 bioassay on the isolated fractions and compounds. Dr. James S. Miller, Dr. Chris Birkinshaw, and Mr. N. Mamisoa Andrianjafy from Missouri Botanical Garden made the plant collections and identification. Dr. Rabodo Andriantsiferana and Dr. Vincent E. Rasamison from Madagascar carried out the initial plant extraction. Dr. David G. I. Kingston was a mentor for this work and the corresponding author for the article. He provided critical suggestions for this work and crucial revisions to the manuscript.

5.1. Introduction

In a continuing search for biologically active natural products from tropical rainforests as part of an International Cooperative Biodiversity Group (ICBG) program,[2] an extract from the leaves and fruit of *Cassipourea lanceolata* (Rhizophoraceae) was obtained. This extract showed weak antiproliferative activity against the A2780 human ovarian cancer

cell line with an IC_{50} value of 17 μg/mL. The extract was selected for study on the basis of its antiproliferative activity and unknown phytochemistry.

5.1.1. *Previous Investigations of Cassipourea*

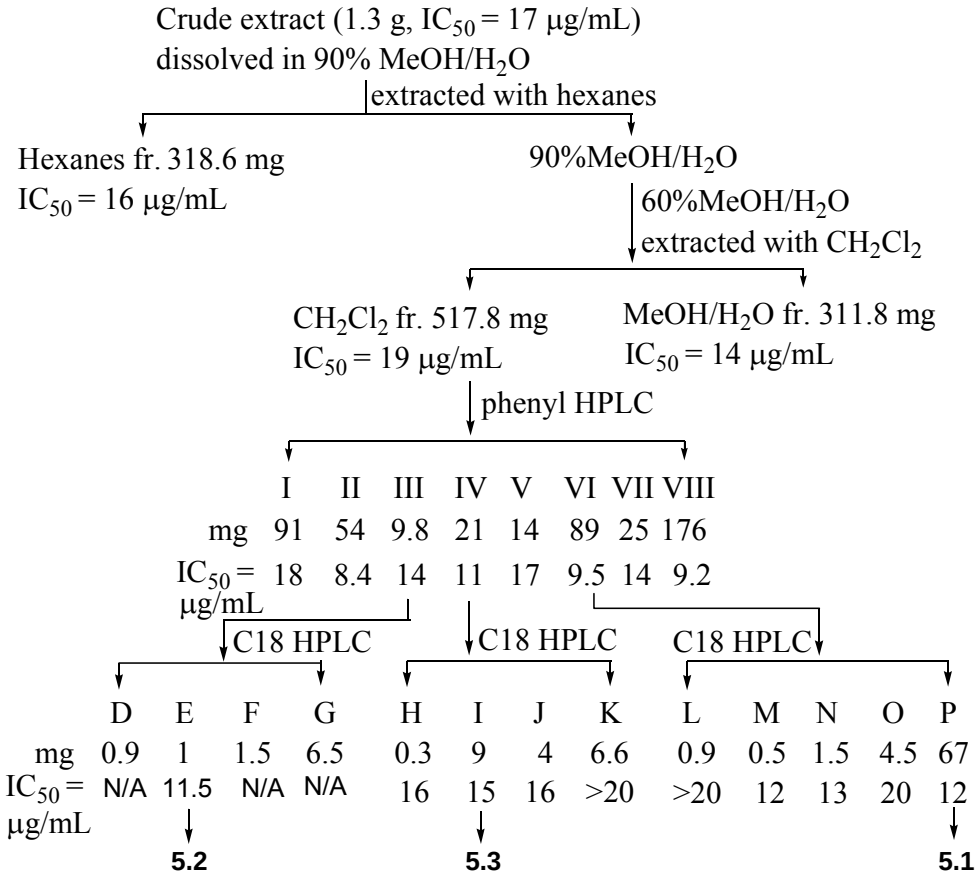
The genus *Cassipourea* belongs to the Rhizophoraceae family, and 79 species in addition to one subspecies and seven varieties have been classified in the genus. No previous work has been reported on the chemistry of *C. lanceolata*, but studies of *C. guianensis* gave sulfur-containing alkaloids and amides,[3-5] work on *C. gerrardii* yielded a flavonol glycoside[6] and proanthocyanidins,[7] and an earlier study in our group yielded bioactive diterpenes from *Cassipourea madagascariensis.*[8]

5.2. Results and Discussion

5.2.1. *Isolation of Compounds from Cassipourea lanceolata*

The crude extract of *Cassipourea lanceolata* was suspended in 90% aqueous methanol and extracted using hexanes, and then the aqueous methanol was adjusted to methanol-water (6:4) and extracted with CH_2Cl_2. As a result, three fractions were generated, and their antiproliferative activities against the A2780 ovarian cancer cell line were obtained. It was found that the three fractions showed nearly equal IC_{50} values (16, 19, and 14 μg/mL), and they were all subjected to further fractionations. The CH_2Cl_2 fraction was subjected to a preparative phenyl HPLC column to yield eight fractions (I-VIII). Fraction II, III, IV, VI, and VIII were among the most active fractions and subjected to further fractionations using reversed-phased C18 HPLC to yield a series of fractions. But no activity enhancement was achieved for those fractions. However, three new compounds

(**5.1**–**5.3**) were obtained, and their structures were elucidated as described in the following section. The hexanes and the aqueous methanol fractions were fractionated by Si gel column chromatography and preparative C18 HPLC, respectively. Several fractions were obtained, but none of them showed increased activities against A2780 cells, so no further fractionation was performed for those fractions. The fractionation tree is shown in Scheme 5.1 and the detailed description of the isolation procedure is given in the Experimental Section.



Scheme 5.1. Separation of *Cassipourea lanceolata* extract

5.2.2. *Structure Elucidation of (24E)-Eupha-7,24-diene-1β,3β,11α,26-tetraol (**5.1**)*

(24*E*)-Eupha-7,24-diene-1β,3β,11α,26-tetraol (**5.1**) was obtained as a white amorphous solid. Its molecular formula was established as $C_{30}H_{50}O_4$ on the basis of an $[M+H]^+$ ion peak at *m/z* 475.3783 in its HRFAB mass spectrum. Its ^{1}H NMR spectrum showed two olefinic protons (δ_H 5.34, br d, *J* = 2.8 Hz, H-7; δ_H 5.42, br t, *J* = 6.8 Hz, H-24), one oxymethylene (δ_H 3.91, s, H_2-26), three oxymethines (δ_H 3.64, dd, *J* = 11.6, 4.8 Hz, H-1; δ_H 3.25, dd, *J* = 11.8, 4.2 Hz, H-3; δ_H 4.20, ddd, *J* = 10.4, 10.0, 5.2 Hz, H-11) and seven methyls (δ_H 0.89, s, H_3-18; δ_H 0.84, s, H_3-19; δ_H 0.90, d, H_3-21; δ_H 1.66, s, H_3-27; δ_H 0.94, s, H_3-28; δ_H 0.84, s, H_3-29; δ_H 0.91, s, H_3-30;) (Table 5.1). Its ^{13}C NMR and HSQC spectra indicated 30 carbons, and those were composed of four olefinic carbons including two tertiary carbons (δ_C 121.1, C-7; δ_C 127.2, C-24) and two quaternary carbons (δ_C 144.0, C-8; δ_C 135.8, C-25), four oxygenated carbons including one secondary carbon (δ_C 69.2, C-26) and three tertiary carbons (δ_C 75.7, C-1; δ_C 76.6, C-3; δ_C 66.9, C-11), seven methylenes, four methines and seven methyls (Table 5.2). Based on the above information, **5.1** was deduced to be a tetracyclic triterpenoid. (Figure 5.1) The complete ^{1}H and ^{13}C NMR assignments were made by a combination of COSY, TOCSY, HSQC and HMBC data, and the key COSY and HMBC correlations are shown in Figure 5.2. COSY and TOCSY correlations indicated coupling systems of H-1–H_2-2–H-3, H-5–H_2-6–H-7, H-9–H-11–H_2-12, H_2-15–H_2-16–H-17–H-20–H_3-21, and H-20–H_2-22–H_2-23–H-24 (Figure 5.2). A series of HMBC correlations indicated assignments of the aforementioned seven methyls. The 28-CH_3 and 29-CH_3 were determined at C-4 through the HMBC correlations from H_3-28 and H_3-29 to quaternary C-4, from H_3-28 to C-29, and from H_3-29 to C-28. The 18-CH_3 and 30-CH_3 were elucidated at C-13 and C-14,

respectively, on the basis of HMBC correlations from both H_3-18 and H_3-30 to both C-13 and C-14. The 18-CH_3 and 30-CH_3 were differentiated through the HMBC correlation from H_3-18 to the tertiary C-17, which was the carbon in the cyclopentane ring connecting to the side chain. The assignment of the 19-CH_3 was assisted by the HMBC correlations from H_3-19 to the quaternary C-10, the tertiary C-5 and the tertiary C-9. The 21-CH_3 was assigned based on its doublet signal in the ^{1}H NMR spectrum of **5.1** in addition to the HMBC correlations from H_3-21 to C-17 and C-20. The 27-CH_3 was assigned on the basis of its chemical shift in the ^{1}H NMR of **5.1** together with the HMBC correlations from H_3-27 to the olefinic C-24 and C-25, and the oxygenated secondary C-26, and these correlations also established the fragment from C-24 to C-27. The other three hydroxyl groups were determined to reside at C-1, C-3 and C-11 after further analysis of the HMBC spectrum. The HMBC correlations from H-1 to C-19 and from H_3-19 to C-1 secured a hydroxyl group at C-1. The C-3–OH was assigned according to the HMBC correlations from H-3 to C-28 and C-29 and from H_3-28 to C-3. The C-11–OH was determined through the HMBC correlations from H_2-12 to C-18 in addition to the COSY correlations from H-11 to H_2-12. The position of the other double bond was assigned on the basis of an HMBC correlation from H_3-30 to C-8 in addition to the coupling system of H-5–H_2-6–H-7. Analysis of ROESY correlations and calculation of coupling constants enabled determination of the relative configuration at C-1, C-3, C-5, C-9, C-10, C-11, C-13, C-14 and C-17, and the configuration of $\Delta^{24(25)}$. The key ROESY correlations were shown in Figure 5.3. The configuration of C-1 was indicated by the coupling constants of H-1 (dd, J = 11.6, 4.8 Hz), which showed that H-1 was in the axial position of ring A, and which also implied H-1–OH existed in the β-equatorial

orientation. The ROESY correlations from H-1 to H-3, H-5 and H-9, from H-3 to H-5, and from H-5 to H-9 established that H-3, H-5 and H-9 were also α-axial orientated, and that both ring A and ring B existed as chair conformation except partial distortion of ring B due to the double bond of $\Delta^{7(8)}$. The coupling constants of H-11 (ddd, J = 10.4, 10.0, 5.2 Hz) indicated H-11 occupied a β-axial position, which meant the α-orientation of H-11–OH given the fact that the adjacent H-9 was α-axial orientated. The ROESY correlations from H-9 to H_3-18, from H-11 to H_3-30 in addition to those from H_3-30 to H-17 established that 18-CH_3 resided in the α-orientation, that 30-CH_3 existed in the β-orientation, that the side chain at C-17 was α- orientated, and that ring C adapted a boat conformation. The double bond $\Delta^{24(25)}$ was determined as *E*-configuration on the basis of ROESY correlations from H-24 to H_2-26 in addition to those from H_3-27 to H_2-23 and H-22a. The planar structure of **5.1** was thus established as shown, excluding stereochemistry.

Table 5.1. [1]H NMR Data of Compounds **5.1**–**5.3**[a]

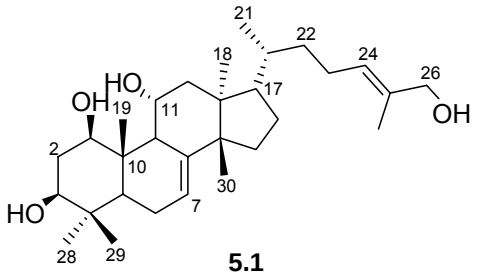
no.	**5.1**[b]	**5.2**[c]	**5.3**[b]
1	3.64 dd (11.6, 4.8)	2.55 m	3.84 dd (11.6, 5.2)
		0.96 m	
2	1.85 m	1.71 m	1.86 m
	1.80 m	1.63 m	1.80 m
3	3.25 dd (11.8, 4.2)	3.18 dd (11.3, 4.6)	3.23 dd (11.6, 4.0)
5	1.29 dd (11.6, 6.0)	1.05 m	1.21 dd (10.4, 5.6)
6	2.27 m	1.81 m	2.23 m
	2.10 m	1.48 m	2.22 m
7	5.34 br d (2.8)	2.42 br dd (19.5, 5.6)	5.33 br s
		2.23 m	
9	2.32 m		
11	4.20 ddd (10.4, 10.0, 5.2)		5.81 br s
12	2.43 dd (10.4, 9.2)	2.55 d (18.8)	2.26 m
	1.58 m	2.33 d (18.8)	2.26 m
15	1.58 m	1.80 m	1.70 m
	1.47 m	1.43 m	1.30 m
16	1.99 m	2.07 m	1.99 m
	1.34 m	1.41 m	1.36 m
17	1.54 m	1.77 m	1.66 m
18	0.89 s	0.93 s	0.67 s
19	0.84 s	1.20 s	0.94 s
20	1.47 m	1.44 m	1.47 m
21	0.90 [d]	0.92 [d]	0.90 d (6.8)
22	1.70 m	1.52 m	1.76 m
	1.07 m	1.12 m	1.33 m
23	2.13 m	2.13 m	2.00 m
	1.97 m	1.98 m	1.06 m
24	5.42 br t (6.8)	5.39 br t (7.0)	3.18 dd (10.0, 1.6)
26	3.91 s	3.91 s	1.17 s
27	1.66 s	1.65 s	1.14 s
28	0.94 s	1.02 s	0.94 s
29	0.84 s	0.82 s	0.86 s
30	0.91 s	1.03 s	0.89 s

[a] in CD_3OD [b] δ (ppm) 400 MHz. [c]δ (ppm) 500 MHz. [d] coupling constants not calculated due to overlapping

Table 5.2. ^{13}C NMR Data of Compounds **5.1**–**5.3**[a]

no.	**5.1**[b]	**5.2**[c]	**5.3**[b]
1	75.7	35.6	74.7
2	37.0	28.4	38.2
3	76.6	79.7	76.7
4	40.5	40.2	40.4
5	50.0	53.3	48.6
6	25.6	19.3	25.0
7	121.1	31.1	119.1
8	144.0	164.8	143.1
9	58.4	141.0	144.4
10	43.8	38.5	43.8
11	66.9	201.7	119.0
12	47.6	52.4	39.9
13	45.2	45.9	45.3
14	52.5	52.5	50.9
15	35.8	36.9	31.0
16	29.4	28.6	29.4
17	54.8	51.8	52.2
18	22.1	18.0	17.1
19	9.6	20.4	14.3
20	37.3	37.3	38.0
21	19.1	18.8	19.7
22	36.3	36.9	29.2
23	26.1	25.5	34.0
24	127.2	127.2	80.7
25	135.8	135.9	74.0
26	69.2	69.1	25.8
27	13.9	13.8	25.1
28	28.0	29.0	28.1
29	15.0	16.6	15.6
30	27.6	24.6	23.6

[a] in CD_3OD [b] δ (ppm) 100 MHz. [c]δ (ppm) 125 MHz.



5.1

Figure 5.1. Structure of **5.1**

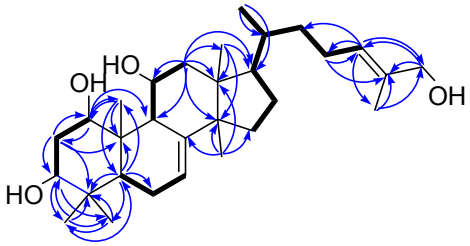


Figure 5.2. Key COSY (bold) and HMBC (arrows) correlations of **5.1**

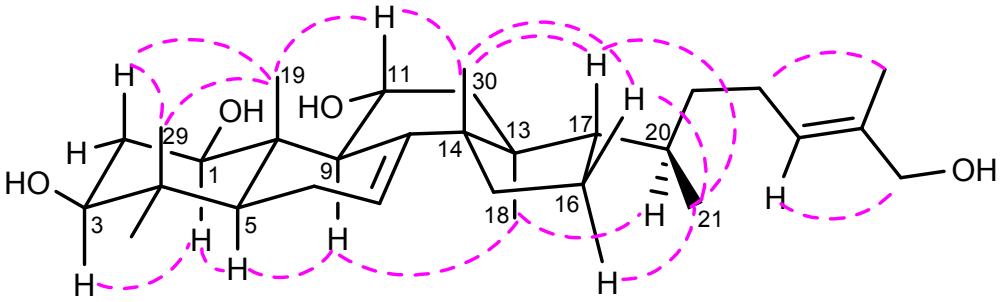


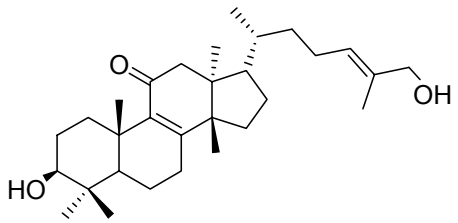
Figure 5.3. Key ROESY correlations of **5.1**

5.2.3. *Structure Elucidation of* (24*E*)*-Eupha-8-ene-3β,26-diol-11-one* (***5.2***)

(24*E*)-Eupha-8-ene-3β,26-diol-11-one (**5.2**), was obtained as white amorphous solid. Its molecular formula was established as $C_{30}H_{46}O_3$ on the basis of an $[M+H]^+$ ion peak at *m/z* 457.3693 in its HRAPCI mass spectrum. Comparison of the ^{1}H NMR spectra of **5.1** and **5.2** showed that the two compounds were very similar except that the olefinic methine (H-7) and the oxygenated methines (H-11 and H-1) that appeared in the ^{1}H NMR spectrum of **5.1** were absent in the ^{1}H NMR spectrum of **5.2**. In addition, although seven methyls could also be observed in ^{1}H NMR of **5.2** similarly as **5.1**, the resonances of several methyls showed at different chemical shifts (Table 5.1). The ^{13}C NMR of **5.2** showed 30 carbons as same as **5.1**. But comparison of the ^{13}C NMR spectra of **5.1** and **5.2** demonstrated that the resonances of the olefinic methine (C-7) and two oxygenated methines (C-1 and C-11) in ^{13}C NMR of **5.1** were absent in the ^{13}C NMR spectra of **5.2**, but alternatively signals of one quaternary olefinc carbon (δ_C 141.0, C-9), one ketone

carbonyl carbon (δ_C 201.7, C-11), and one methelene (δ_C 35.6, C-1) were observed in the ^{13}C NMR of **5.2** (Table 5.2). The above discussion suggested that **5.2** had a similar structure as **5.1** except the difference in aforementioned groups. The complete ^{1}H and ^{13}C NMR assignments were determined from a combination of COSY, TOCSY, HMQC and HMBC data. COSY and TOCSY correlations indicated coupling systems of H_2-1–H_2-2–H-3 and H-5–H_2-6–H_2–7 in addition to the coupling systems identical to **5.1**. On the basis of its HMBC data, the seven methyls were assigned firstly using the same deductions as described in **5.1**. The α, β- unsaturated ketone unit from C-8 to C-10 was indicated by the HMBC correlations from H_3-30, H-6a and H_2-7 to C-8, and from H_3-19 and H_2-7 to C-9, and from H_2-12 to C-10. The other functional groups were determined to locate at respective positions using similar methods as described previously in **5.1**. The relative configuration of **5.2** was determined on the basis of its ROESY correlations and calculations of coupling constants (Table 5.1). The configuration of ring A and B was determined as the same manner as **5.1**. As a result, C-3–OH was determined to be β-equatorially oriented, and ring A/B adapted the chair/twisted chair conformation. 14-CH_3 was positioned in the β- orientation based on the ROESY correlation from β-equatorially oriented H-7a (br dd, J = 19.5, 5.6 Hz) to H_3-30, and H-7a was determined to adapt the β-orientation due to its coupling constants and the ROESY correlation from α-axial H-5 to H-7b. Furthermore, 13-CH_3 had to be α-oriented from the biosynthetic point of view.[9] The ROESY correlation from H_3-30 to H-17 established that the side chain was orientated in the α-orientation. In addition, the aforementioned assignment of the configuration of ring C/D was consistent with biogenetically co-concurred **5.1**. The *E*-configuration of $\Delta^{24(25)}$ was established in the same way as discussed in **5.1**. The absolute

configuration of C-20 in **5.2** will be discussed later as mentioned in **5.1**. Thus, the structure of **5.2** was established as shown. (Figure 5.4)



5.2

Figure 5.4. Structure of **5.2**

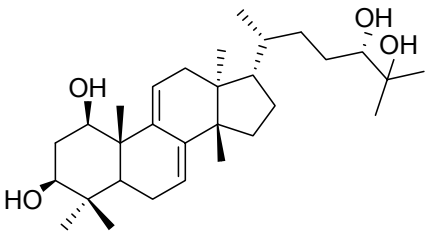
5.2.4. *Structure Elucidation of (24S)-eupha-7,9(11)-diene-1β,3β,24,25-tetraol (**5.3**)*

(24*S*)-eupha-7,9(11)-diene-1β,3β,24,25-tetraol (**5.3**), was obtained as a white amorphous solid. Its molecular formula was established as $C_{30}H_{50}O_4$ on the basis of an $[M+H-H_2O]^+$ ion peak at *m/z* 457.3693 in its HRAPCI mass spectrum and an $[M+H]^+$ ion peak at *m/z* 475.3 in its ESIMS spectrum. In its ^1H NMR spectrum, two olefinic protons (δ_H 5.33, br s, H-7; δ_H 5.81, br s, H-11), three oxymethines (δ_H 3.84, dd, *J* = 11.6, 5.2 Hz, H-1; δ_H 3.23, dd, *J* = 11.6, 4.0 Hz, H-3; δ_H 3.18, dd, *J* = 10.0, 1.6 Hz, H-24) and eight methyls (δ_H 0.67, s, H_3-18; δ_H 0.94, s, H_3-19; δ_H 0.90, d, *J* = 6.8 Hz, H_3-21; δ_H 1.17, s, H_3-26; δ_H 1.14, s, H_3-27; δ_H 0.94, s, H_3-28; δ_H 0.86, s, H_3-29; δ_H 0.89, s, H_3-30) were observed (Table 5.1). Its ^{13}C NMR and HSQC spectra indicated 30 carbons that were composed of four olefinic carbons including two tertiary carbons (δ_C 119.1, C-7 and δ_C 119.0, C-11) and two quaternary carbons (δ_C 143.1, C-8; δ_C 144.4, C-9), four oxygenated carbons including three tertiary carbons (δ_C 74.7, C-1; δ_C 76.7, C-3; δ_C 80.7, C-24) and one quaternary carbon (δ_C 74.0, C-25), eight methyls, seven methylenes, three methines

and four additional quaternary carbons (Table 2). On the basis of aforementioned information and comparison to **5.1** and **5.2**, compound **5.3** was deduced to be another tetracyclic triterpenoid. The complete assignments of its ^{1}H and ^{13}C NMR were determined from a combination of COSY, TOCSY, HSQC and HMBC data. COSY correlations indicated several coupling systems that were H-1–H_2-2–H-3, H-5–H_2-6–H-7, H-11–H_2-12, H_2-15–H_2-16–H-17–H-20–H_3-21, and H-20–H_2-22–H_2-23–H-24. The positions of eight methyls were assigned on the basis of the HMBC correlations that showed similar patterns as **5.1** and **5.2**. The diene $\Delta^{7(8),\ 9(11)}$ unit was established through the HMBC correlations from H_2-12 to C-11 and C-9 in addition to those from H_2-12 to C-18, C-13 and C-14, the HMBC correlation from H_3-19 to C-9, and the HMBC correlation from H_3-30 to C-8, together with the aforementioned related COSY correlations. The C-24–C-25–C-26/27 unit including hydroxyl groups at C-24 and C-25 was indicated on the basis of the HMBC correlations from both H_3-26 and H_3-27 to both C-24 and C-25 in addition to those from H_3-26 to C-27 and from H_3-27 to C-26. C-1–OH and C-3–OH were located in the same manners as described previously in **5.1** and **5.2**. The relative configuration of **5.3** was established through analysis of its ROESY spectrum, calculation of coupling constants and consideration of the biosynthetic pathway of tetracyclic triterpenoids[9] in a similar way as **5.1** and **5.2**. As a result, the relative configuration of **5.3** was determined as shown (Figure 5.5) and was consistent with the biogenetic co-concurrence of **5.1** and **5.2**.

The absolute configuration of C-24 in **5.3** were determined by converting **5.3** to the two Mosher esters **5.4** and **5.5** with (*R*)- and (*S*)- methoxyphenylacetic acid (MPA), using the EDCI/DMAP coupling conditions.[10,11] The structures of **5.4** and **5.5** were confirmed as

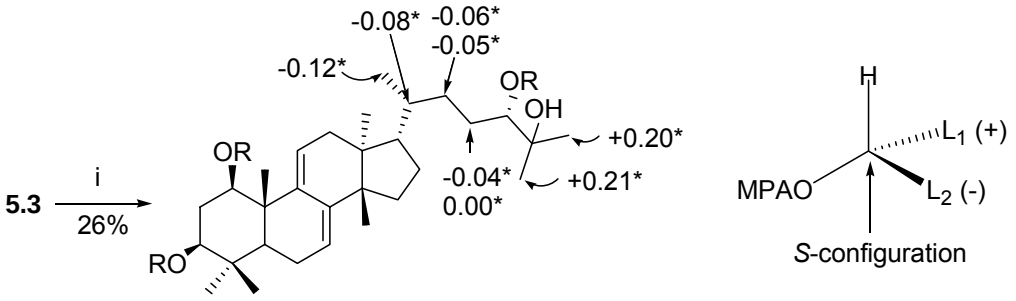
shown in Scheme 5.2 after analysis of both their HRESI mass and LC-ESIMS/MS spectra, and their 1D and 2D NMR data (see Experimental Section). The chemical shifts from H-20 to H_3-27 between the two esters were significantly different, and these differences were recorded by Δ^{RS}, which were values of the chemical shifts in ^{1}H NMR spectrum of *R*-MPA (**5.4**) subtracted those in *S*-MPA (**5.5**) at corresponding protons (Scheme 5.2). A model was established based on these differences as shown in Scheme 5.2, in which L_1 represented the moiety from C20 to C23, while L_2 represented the moiety from C25 to C27. That led to the conclusion that **5.3** had the *S*- configuration at C-24.



5.3

Figure 5.5. Structure of **5.3**

Scheme 5.2. Determination of absolute configuration at C-24



5.4 R = (*R*)-MPA
5.5 R = (*S*)-MPA

*$\Delta^{RS} = \delta^R$ (**5.4**) - δ^S (**5.5**)

(i) (*R*)-MPA or (*S*)-MPA, EDCI, DMAP, CH_2Cl_2, rt, 36 hr.

5.2.5. *Determination of absolute configuration at C-20 of **5.1**, **5.2**, and **5.3***

The absolute configuration of C-20 in compounds **5.1**, **5.2** and **5.3** was determined mainly on the basis of ROESY correlations using a method discussed in the literature.[12] In the literature, it is argued that the stereochemistry of a euphane [C-20(*R*)] triterpenoid could be determined through the ROESY correlation from H_3-21 to H-16α in addition to the absence of a ROESY correlation from H_3-21 to H_3-18, and that a tirucallane [C-20(*S*)] triterpenoid could be determined by the ROESY correlation from H_3-21 to H_3-18 in addition to that from H_3-21 to H-12. This argument was derived from careful analysis of the ROESY spectra of the two diastereomers, kansenone [C-20 (*R*)] and epi-kansenone [C-20 (*S*)] and by comparison with previously reported compounds. The determined configuration for the five compounds discussed in the literature, which were kansenone, epi-kansenone, kansenonol, 11-oxo-kansenonol and kansenol, turned out to be consistent with the X-ray crystallographic studies on euphane and tirucallane triterpenoids.[12,13] In the ROESY spectra of **5.1**, correlations of H_3-21 to H_2-16, H-17, and H_2-23, those of H_3-18 to H-16α (δ_H 1.34, m) and H-20, and that of H-22a (δ_H 1.70, m) to H-12β (δ_H 1.58, m) were observed in addition to the absence of correlations between H_3-21 and H_3-18 and between H_3-21 and H_2-12. (Figure 5.3) Those ROESY correlations showed a nearly identical pattern to those of kansenone and kansenonol[12] and indicated that **5.1** is a euphane triterpenoid with a (*R*)-configuration at C-20 instead of a triucallane triterpenoid with an (*S*)-configuration at C-20. Compound **5.2** was also determined as a euphane triterpenoid based on similar key ROESY correlations to those of **5.1**. Compound **5.3** had almost identical ROESY correlation patterns in ring C/D and H-20–H-22 to those of kansenol.[12] In particular, a correlation from H_3-21 to H-16α (δ_H 1.36, m) in addition to

the absence of one from H_3-21 to H_3-18, indicated that **5.3** was a euphane triterpenoid with an (*R*)-configuration at C-20.

Based on these considerations, the structures of **5.1**, **5.2** and **5.3** were assigned as (24*E*)-eupha-7,24-diene-1β,3β,11α,26-tetraol, (24*E*)-eupha-8-ene-3β,26-diol-11-one and (24*S*)-eupha-7,9(11)-diene-1β,3β,24,25-tetraol, respectively.

5.2.6. *Antiproliferative Activities of Isolated Compounds*

All the isolates were tested against the A2780 ovarian cancer cell line. Compounds **5.1**, **5.2** and **5.3** showed very weak antiproliferative activities with IC_{50} values of 25, 25, and 32 μM, respectively.

5.2.7. *Bioactivities of Euphane Triterpenoids*

Only a few articles have reported the biological activities of euphane triterpenoids. Several compounds of this type have been shown to significantly arrest the division of cultured individual *Xenopus laevis* cells at the blastular stage.[12] Several others showed moderate inhibitory activities towards the P388 lymphocytic leukemia cell line.[14] In addition, (24*E*)-3β-hydroxy-7,24-euphadien-26-oic acid was the first euphane triterpenoid found to inhibit DNA polymerase β.[15]

5.3. Experimental Section

5.3.1. *General Experimental Procedures*

Optical rotations were recorded on a JASCO P-2000 polarimeter. UV spectra were performed on a Shimadzu UV-1201 spectrophotometer. NMR spectra were obtained on Bruker Avance 600 equipped with a 1.7 mm CapProbe, JEOL Eclipse 500, Varian Inova 400, and Varian Unity 400 spectrometers. HRFAB mass spectra were obtained on a JEOL-JMS-HX-110 instrument. HRAPCI mass spectra were obtained on an Agilent 6220 TOF LC/MS. LC-ESIMS was performed on Agilent 1100 and Thermo TSQ Quantum instruments. Chemical shifts are given in δ (ppm), and coupling constants (*J*) are reported in Hz. HPLC was performed using Shimadzu LC-8A pumps coupled with a Varian Dynamax preparative phenyl column (250 × 21.4 mm) and a Varian Dynamax preparative C18 column (250 × 21.4 mm), and Shimadzu LC-10A pumps coupled with a Varian Dynamax semipreparative C18 column (250 × 10 mm). Both HPLC instruments employed a Shimadzu SPD-M10A diode array detector and the Shimadzu LC-10A pumps were also coupled with a Sedex Model 75 evaporative light scattering detector.

5.3.2. *Antiproliferative Bioassay*

The A2780 ovarian cancer cell line assay was performed by Ms. Peggy Brodie at Virginia Polytechnic Institute and State University as previously reported[16] and described in Chapter II. The A2780 cell line is a drug sensitive ovarian cancer cell line.[17]

5.3.3. *Plant Material Collection*

Leaves and fruit of *Cassipourea lanceolata* Tul. were collected in October 2001 as collection NMA 213. The collection was made by N. M. Andrianjafy et al. from a plant growing in a dense humid forest adjacent on the edge of the Lac Alaotra Biologic Reserve of the Zahamena National Park, Toamasina Province, Madagascar (17 37 39S; 048 56 48E, elevation 600 m). The specimen accessed was a small tree 8 m in height, with interpetiolar stipules and green fruits. The vernacular name is Moara fotsy, and the tree is used in Madagascar for house construction. Duplicate voucher specimens were deposited at Centre National d'Application des Recherches Pharmaceutiques (CNARP), the Departement des Recherches Forestieres et Piscicoles Herbarium in Antananarivo, Madagascar (TEF), the Missouri Botanical Garden, St. Louis, Missouri (MO), and the Museum National d'Histoire Naturelle in Paris, France (P).

5.3.4. *Plant Material Extraction and Extract Fractionation*

Dried leaves and fruit (317 g) were extracted with EtOH to give 12.9 g of extract, which was assigned the number MG1002. A total of 2.8 g of extract was supplied to VPISU, and this had an IC_{50} value of 17 μg/mL against A2780 cells. A portion of this extract (1.3 g) was suspended in aqueous MeOH (90% MeOH/H_2O, 40 mL), and extracted with *n*-hexane (3 × 40 mL). The aqueous layer was then diluted to 60% MeOH (v/v) with H_2O and extracted with CH_2Cl_2 (3 × 60 mL). The CH_2Cl_2 extract (517.8 mg, IC_{50} = 19 μg/mL) was separated via preparative HPLC over a Phenyl column using MeOH-H_2O (85:15) to afford 8 fractions (I-VIII), of which fractions II (54 mg), III (9.8 mg), IV (21 mg), VI (89 mg) and VIII (176 mg) were found to display the highest

antiproliferative activity (IC_{50} = 8.4, 14, 11, 9.5 and 9.2 μg/mL, respectively), and these fractions were subjected to the further separation. Fraction II was subjected to preparative HPLC on an RP-C18 column using MeOH-H_2O (75:25) to afford three fractions (A-C). Fraction III was further separated via semi-preparative HPLC on an RP-C18 column using MeOH-H_2O (80:20). Four fractions (D-G) were collected. Fraction E afforded **5.2** (1.0 mg, t_R 29.0 min). Fraction IV was subjected to semipreparative HPLC on an RP-C18 column using MeOH-H_2O (75:25) to afford four fractions (H-K). Fraction I afforded **5.3** (9.0 mg, t_R 32.7 min). Fraction VI was separated using preparative RP-C18 HPLC using MeOH-H_2O (80:20). Five fractions (L-P) were collected. Fraction P afforded **5.1** (67 mg, t_R 51.1 min).

5.3.5 *(24E)-Eupha-7,24-diene-1β,3β,11α,26-tetraol (**5.1**)*

white amorphous solid; $[\alpha]_D^{25}$ -4.8 (c 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 222 (3.49) nm**;** ^1H and ^{13}C NMR see Tables 5.1 and 5.2; HRFABMS *m/z* 475.3782 $[M+H]^+$ (calcd for $C_{30}H_{51}O_4$, 475.3787).

5.3.6 *(24E)-Eupha-8-ene-3β,26-diol-11-one (**5.2**)*

white powder; $[\alpha]_D^{25}$ +70.4 (c 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.36) nm, 255 (3.32) nm, 323 (2.88) nm**;** ^1H and ^{13}C NMR see Tables 5.1 and 5.2; HRAPCIMS *m/z* 457.3693 $[M+H]^+$ (calcd for $C_{30}H_{49}O_3$, 457.3682).

5.3.7. *(24S)-Eupha-7,9(11)-diene-1β,3β,24,25-tetraol (**5.3**)*

white powder; $[\alpha]_D^{25}$ -30.2 (c 0.50, MeOH); UV (MeOH) λ_{max} (log ε) 209 (3.38) nm, 232 (3.49) nm**;** ^{1}H and ^{13}C NMR see Tables 5.1 and 5.2; HRAPCIMS *m/z* 457.3693 $[M+H-H_2O]^+$ (calcd for $C_{30}H_{49}O_3$, 457.3682). ESIMS *m/z* (rel. int.): 475.3 (30) $[M+H]^+$, 457.3 (100) $[M+H-H_2O]^+$, 439.3 (60) $[M+H-2H_2O]^+$, 421.3 (10) $[M+H-3H_2O]^+$.

5.3.8. *Mosher Esters **5.4** and **5.5***

(*R*)-MPA (6 mg) was added in a stirred solution of **5.3** (1 mg), DMAP (4 mg) and EDCI (18 mg) in 0.3 mL CH_2Cl_2. After 36 hrs, EtOAc was added and then the solution was filtrated and evaporated under reduced pressure. The residue was subject to RP-C18 HPLC using gradient MeOH/H_2O condition (50%~100% MeOH/H_2O from 0~30 min followed by MeOH from 30~50 min). Analysis of LC/MS/MS spectra indicated a product of **5.4** that derived from esterification of all three secondary hydroxyl groups in **5.3.** Then the peak at t_R = 39.0 mins was collected to generate **5.4** (0.5 mg). **5.5** (0.5 mg, t_R = 42.5 mins) was obtained from a reaction between **5.3** (1 mg) and (*S*)-MPA using the same procedure.

5.3.8. *(R)-Methoxyphenylacetic acid Mosher ester of **5.3** (**5.4**)*

white powder; $[\alpha]_D^{25}$ -44.0 (c 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 245 (3.74) nm; ^{1}H NMR [$CDCl_3$, 600 MHz (CapProbe)]: δ 5.18 (1H, br s, H-7), 5.01 (1H, dd, *J* = 12.1, 5.2 Hz, H-1), 4.77 (1H, dd, *J* = 7.2, 2.5 Hz, H-24), 4.72 (1H, dd, *J* = 12.4, 4.1 Hz, H-3), 4.17 (1H, br s, H-11), 1.80 (1H, m, H-22a), 1.68 (1H, m, H-23a), 1.32 (1H, m, H-23b), 1.18 (1H, m, H-23b), 1.18 (1H, m, H-20), 1.15 (3H, s, H-26), 1.13 (3H, s, H-27), 0.91

(3H, s, H-29), 0.89 (3H, s, H-19), 0.74 (3H, s, H-28), 0.70 (3H, d, *J* = 6.6 Hz, H-21), 0.54 (3H, s, H-30), 0.32 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 600 MHz (CapProbe), data extracted from its HSQC spectrum): δ 116.9 (C-11), 116.8 (C-7), 81.2 (C-24), 76.7 (C-3), 75.7 (C-1), 35.4 (C-20), 27.6 (C-22), 26.9 (C-28), 26.7 (C-23), 26.4 (C-27), 25.0 (C-26), 22.2 (C-30), 18.7 (C-21), 16.2 (C-18), 15.8 (C-29), 14.6 (C-19); HRESIMS *m/z* 941.5199 $[M+Na]^+$ (calcd for, $C_{57}H_{74}O_{10}Na$, 941.5180). ESIMS/MS *m/z* (rel. int.): 941.5 (34) $[M+Na]^+$, 775.4 (25) $[M+Na\text{-}MPA]^+$, 609.3 (100) $[M+Na\text{-}2MPA]^+$, 443.2 (4) $[M+Na\text{-}3MPA]^+$, 189.0 (100) $[MPA+Na]^+$.

5.3.9. *(S)-Methoxyphenylacetic acid Mosher ester of **5.3** (**5.5**)*

white powder; $[\alpha]_D^{25}$ +10 (c 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 248 (3.78) nm; ^{1}H NMR [$CDCl_3$, 600 MHz (CapProbe)]: δ 5.16 (1H, br s, H-7), 5.02 (1H, dd, *J* = 11.7, 4.2 Hz, H-1), 4.76 (1H, dd, overlapping, H-24), 4.70 (1H, dd, *J* = overlapping, H-3), 4.16 (1H, br s, H-11), 1.86 (1H, m, H-22a), 1.72 (1H, m, H-23a), 1.32 (1H, m, H-23b), 1.27 (1H, m, H-20), 1.23 (1H, m, H-23b), 0.95 (3H, s, H-26), 0.92 (3H, s, H-27), 0.90 (3H, s, H-19), 0.72 (3H, s, H-29), 0.82 (3H, d, *J* = 6.6 Hz, H-21), 0.56 (3H, s, H-30), 0.39 (3H, s, H-28), 0.39 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 600 MHz (CapProbe), data extracted from its HSQC spectrum): δ 116.9 (C-7), 116.6 (C-11), 81.2 (C-24), 76.6 (C-3), 75.7 (C-1), 36.1 (C-20), 27.9 (C-22), 26.8 (C-23), 26.4 (C-28), 25.8 (C-27), 24.8 (C-26), 22.2 (C-30), 18.9 (C-21), 16.4 (C-18), 15.4 (C-29), 14.5 (C-19); HRESIMS *m/z* 941.5198 $[M+Na]^+$ (calcd for, $C_{57}H_{74}O_{10}Na$, 941.5180). ESIMS/MS *m/z* (rel. int.): 941.5 (21) $[M+Na]^+$, 775.4 (28) $[M+Na\text{-}MPA]^+$, 609.3 (88) $[M+Na\text{-}2MPA]^+$, 443.2 (3) $[M+Na\text{-}3MPA]^+$, 189.0 (100) $[MPA+Na]^+$.

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VI. Antiproliferative Alkylated Hydroquinones and Benzoquinones of *Sclerocarya birrea* subsp. *caffra* from the Madagascar Rainforest*

6.1. Introduction

In a continuing search for biologically active natural products from tropical rainforests as part of an International Cooperative Biodiversity Group (ICBG) program, an extract from the fruits of *Sclerocarya birrea* subsp. *caffra* collected in Madagascar was evaluated for antiproliferative activity against the A2780 human ovarian cancer cell line. The extract was selected for bioassay−guided fractionation based on its initial activity against this assay with an IC_{50} value of 6.5 μg/mL.

6.1.1. *Previous Investigations of Sclerocarya*

The genus *Sclerocarya* belongs to the Anacardiceae family, and includes 11 species.[1] The only previous chemical investigations on *Sclerocarya* resulted in the isolation of several phenolic components and flavonoids from *Sclerocarya birrea*[2-5] and 2-hydroxy-4-methoxybenzaldehyde from *S. caffra*.[6]

6.2. Results and Discussion

6.2.1. *Isolation of Compounds from Sclerocarya birrea* subsp. *caffra*

The crude ethanol extract of *Sclerocarya birrea* subsp. *caffra* was suspended in MeOH–H_2O (9:1) and extracted using hexanes. The aqueous methanol layer was then

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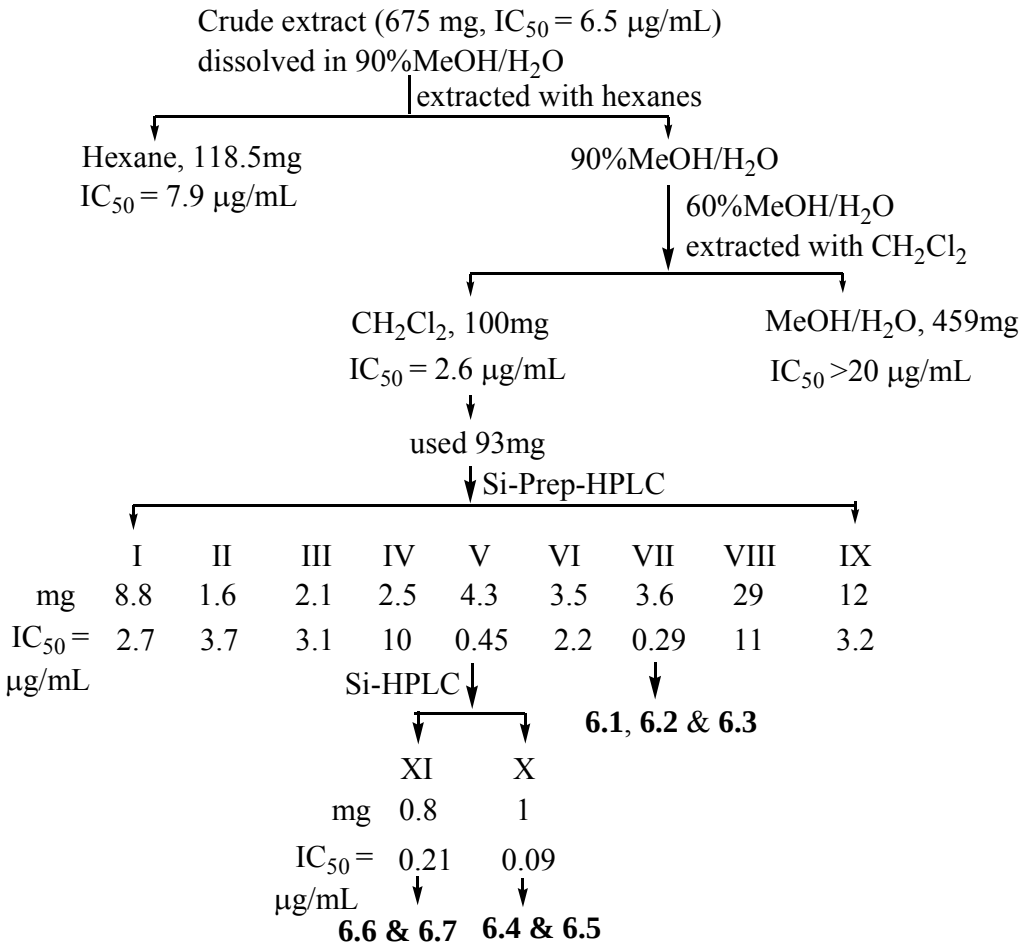
diluted to MeOH–H_2O (6:4) and extracted with CH_2Cl_2. Both organic extracts and the residual aqueous methanol solution were evaporated to generate three fractions. The antiproliferative activities of those fractions against the A2780 ovarian cancer cell line were obtained. It was found that the CH_2Cl_2 fraction showed the best activity (IC_{50} = 2.6 μg/mL). The CH_2Cl_2 fraction was then subjected to HPLC on Si gel with elution by $CHCl_3$ to yield two active fractions V (IC_{50} = 0.45 μg/mL) and VII (IC_{50} = 0.29 μg/mL). Compounds **6.1**, **6.2**, and **6.3** were identified as being present in fraction VII, although no separation of these compounds could be achieved on Si and Diol HPLC columns. Fraction V was subjected to HPLC on Si gel to yield fraction X (IC_{50} = 0.21 μg/mL), which led to the identification of compounds **6.4** and **6.5**, and fraction XI (IC_{50} = 0.09 μg/mL), which resulted in the identification of compounds **6.6** and **6.7**. The fractionation tree is shown in Scheme 6.1, and a detailed description of the isolation procedure is given in the Experimental Section.



Scheme 6.1. Separation of *Sclerocarya birrea* subsp. *caffra* extract

6.2.2. *Structure Elucidation of Fraction VII (**6.1**, **6.2**, and **6.3**)*

The ^{1}H NMR and HSQC spectra of fraction VII showed signals for three aromatic protons in an ABX system at δ_H 6.78 (1H, d, J = 8.5 Hz, H-6), δ_H 6.62 (1H, dd, J = 8.5, 3.0 Hz, H-5), and δ_H 6.54 (1H, d, J = 3.0 Hz, H-3), olefinic methines at δ_H 5.34 (m) with an integration equal to 0.6 proton, an oxymethine at δ_H 3.98 (1H, m, H-2′), a methylene at δ_H 2.75 (2H, m, H-1′), a methylene δ_H 2.00 (m) with an integration equal to 2.4 protons, and δ_H 1.51 (m, H-3′), several other methylenes at δ_H 1.20–1.40, and a methyl group at δ_H 0.87 (3H, t, J = 6.9 Hz, H-17′) (Table 6.1). The integrations of these signals suggested that fraction VII was a mixture of at least two compounds. In addition, signals for six

aromatic carbons, two olefinic carbons, an oxymethine carbon, a methyl carbon and several methylenes were indicated in its ^{13}C NMR spectra (Table 6.2). The HMBC correlations of H-1′ to C-1 (δ_C 149.5), C-2 (δ_C 126.6), and C-3 (δ_C 118.0), that of H-3 to C-1, and those of H-6 to C-2 and C-4 (δ_C 148.9) established that the ABX coupling system resulted from a 2-alkylated 1,4-disubtituted aromatic ring. The COSY correlations of of H-1′ to H-2′ and H-2′ to H-3′ along with the HMBC correlations of H-1′ to C-2′ (δ_C 74.7) and C-3′ (δ_C 37.0) demonstrated the oxymethine was positioned at C-2′. Key COSY and HMBC correlations are shown in Figure 6.2. The full assignment of the structure of fraction VII was assisted by its GC-MS spectra and other NMR data.

The GC-MS chromatogram of this fraction consisted of two incompletely separated peaks. One peak (t_R 13.63 min) showed a molecular ion at *m/z* 364, which implied the structure **6.1** (Figure 6.1) with a heptadecyl side chain along with the aforementioned interpretations of NMR data, while the other peak (t_R 13.49 min) displayed a molecular ion at *m/z* 362 that suggested a similar structure as **6.1** but a heptadecenyl side chain. This deduction agrees with the aforementioned suggestion that fraction VII was composed of at least two compounds which exhibited almost identical NMR spectra except for the olefinic methines at δ_H 5.34 (m) and the methylenes at δ_H 2.00 (m) in the heptadecenyl side chain.

Table 6.1. ^{1}H NMR Data of Fraction VII (**6.1**, **6.2** & **6.3**), X (**6.4** & **6.5**), and XI (**6.6** & **6.7**)[a,c]

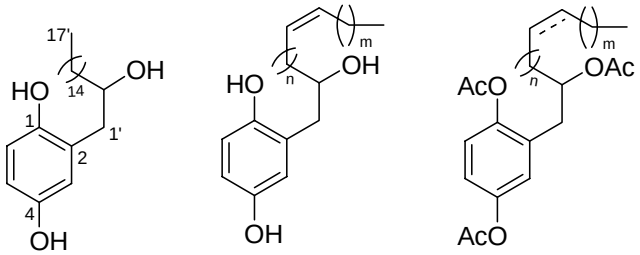
no.	**Fraction VII**	**Fraction X**	**Fraction XI**
3	6.54 d (3.0)	6.54 d (3.0)	6.55 d (2.5)
5	6.62 dd (8.5, 3.0)	6.61 dd (8.5, 3.0)	6.70 dd (9.8, 2.5)
6	6.78 d (8.5)	6.63 d (8.5)	6.75 d (9.8)
1′	2.78 dd (14.5, 2.8)	2.53 t (7.8)	2.41 t (7.6)
	2.73 dd (14.5, 7.2)		
2′	3.98 m	1.58 m	1.49 m
3′	1.51 m	1.2-1.4	1.2-1.4
-CH=CH-[b]	5.34 m	5.34 m	5.34 m
-C*H*$_2$CH=CHC*H*$_2$-[b]	2.00 m	2.00 m	2.00 m
17′	0.87 t (6.9)	0.88 t (6.6)	0.87 t (6.7)
Other methylenes	1.2-1.4	1.2-1.4	1.2-1.4

[a] in $CDCl_3$. [b]not applicable to **6.1**, **6.4**, and **6.6**. [c]δ (ppm) 500 MHz.

Table 6.2. ^{13}C NMR Data of Fraction VII (**6.1**, **6.2** & **6.3**), X (**6.4** & **6.5**), and XI (**6.6** & **6.7**)[a,c]

no.	**Fraction VII**[d]	**Fraction X**	**Fraction XI**
1	149.5	147.3	187.5
2	126.6	130.0	149.8
3	118.0	116.8	132.5
4	148.9	149.2	187.6
5	114.8	113.3	136.3
6	118.0	116.0	136.9
1′	38.9	30.1	29.1
2′	74.7	29-30	27.9
3′	37.0	29-30	29-30
-CH=CH-[b]	130.1, 129.8	130.1, 130.0	130.0, 129.9
-*C*H$_2$CH=CH*C*H$_2$-[b]	27.2, 27.2	27.3, 27.3	27.3, 27.3
15′	31.9	31.9	31.9
16′	22.7	22.7	22.7
17′	14.2	14.2	14.2
Other methylenes	28-30	29-30	29-30

[a] in $CDCl_3$. [b]not applicable to **6.1**, **6.4**, and **6.6**. [c]δ (ppm) 125 MHz. [d]δ (ppm) 100 MHz.



6.1 **6.2** m = 5, n = 7 **6.3** m = 7, n = 5 **6.8** m+n = 12

Figure 6.1. Structures of **6.1**, **6.2, 6.3** & **6.8**

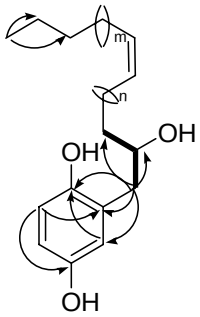


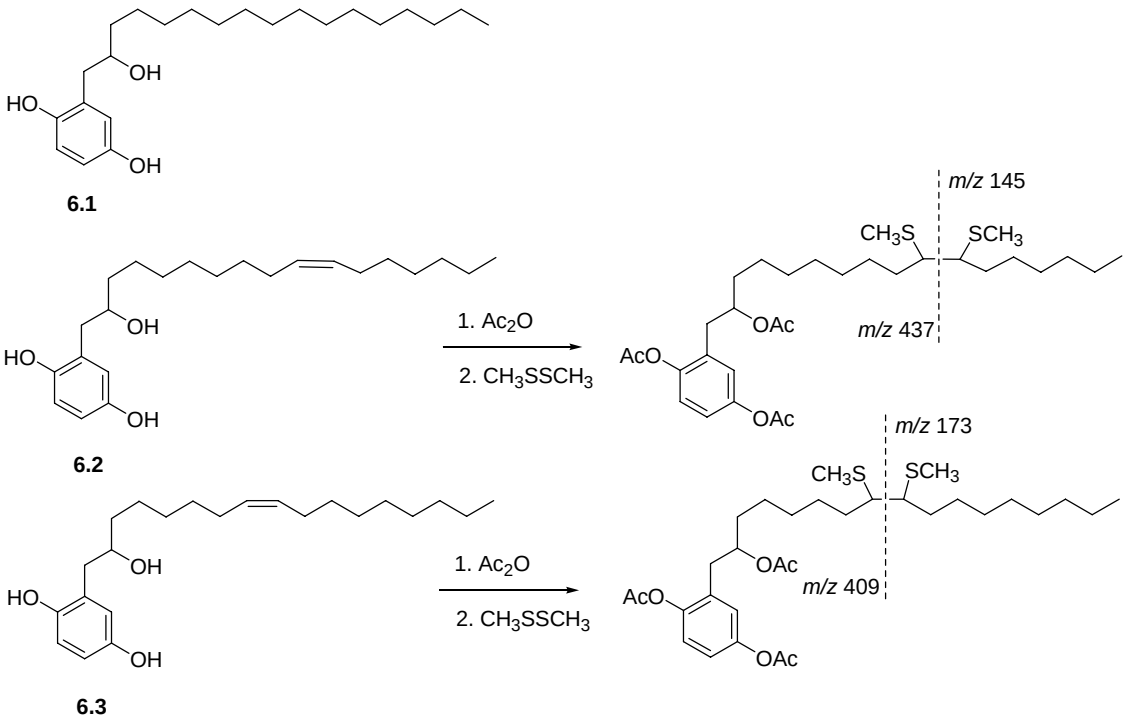
Figure 6.2. Key COSY (bold) and HMBC (arrows) correlations of **6.1**, **6.2**, and **6.3**

The position of the double bond in the heptadecenyl side chain was determined by applying a previously reported approach.[7,8] Firstly, acetylation of fraction VII by acetic anhydride yielded the acetate derivatives (**6.8**), which were then reacted with dimethyl disulfide (DMDS) to afford the DMDS adducts (Scheme 6.2). The crude products were then subjected to GC-MS without further purification and two peaks (t_R 24.97 and 24.37 min, respectively) with close retention times displayed the expected molecular ions of *m/z* 582. Fragmentation analysis of the two molecular ions indicated an ion pair at *m/z* 173/409 ($C_{10}H_{21}S$ and M-$C_{10}H_{21}S$) and a second ion pair at *m/z* 145/437 ($C_8H_{17}S$ and M-$C_8H_{17}S$), respectively (Scheme 6.2). These results indicated that the structures of **6.2** and **6.3** were as shown (Figure 6.1). The geometry of the double bonds in these compounds was determined to be *Z*-configuration by comparison of the ^{13}C NMR chemical shifts of their allylic carbons to those of the known monoenic pheromones (*Z*)- and (*E*)-9-teradecen-1-yl acetates.[9] It has been indicated that the ^{13}C NMR chemical shifts of the allylic carbons of (*Z*)-double bonds were shifted upfield, and that those of (*E*)-double bonds were shifted downfield comparing with their corresponding saturated compounds.[9]

Compound **6.3** was identical to the known compound, 2′(*R*)-hydroxylanneaquinol,[10] which was isolated from *Lannea welwitschii* and has also recently been synthesized,[11]

except for the unassigned absolute configuration at C-2′, and their NMR data were also identical. In summary, two new compounds **6.1** and **6.2** in addition to the compound **6.3** were identified from fraction VII.

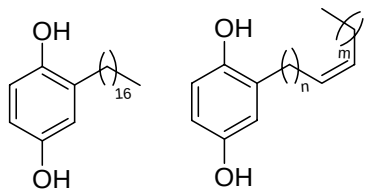
Scheme 6.2. Determination of double bond positions of **6.2** and **6.3** by GC-MS on the dimethyl disulfide adducts of their acetates



6.2.2. *Structure Elucidation of Fraction X*

The ^{1}H NMR, ^{13}C NMR, and HSQC spectra of fraction X displayed similar patterns to those of fraction VII except for absence of the oxymethine signal at δ_H 3.98 (m, H-2′) and δ_C (74.7, C-2′). Its ^{1}H NMR spectrum showed signals for three aromatic protons in an ABX system at δ_H 6.63 (d, J = 8.5 Hz, H-6), δ_H 6.61 (dd, J = 8.5, 3.0 Hz, H-5), and δ_H 6.54 (d, J = 3.0 Hz, H-3), olefinic methines at δ_H 5.34 (m), methylenes at δ_H 2.53 (t, J =

7.8 Hz H-1′), δ_H 2.00 (m), several other methylenes at δ_H 1.20–1.40, and a methyl group at δ_H 0.88 (t, J = 6.6 Hz, H-17′) (Table 6.1). Signal for six aromatic carbons, two olefinic carbons, a methyl carbon and several methylenes were indicated in its ^{13}C NMR spectra (Table 6.2). GC-MS chromatogram of this fraction indicated a major peak with a molecular ion at *m/z* 346 and a minor peak with a molecular ion at *m/z* 348. Overall analysis of the above information and comparison with the known compounds, lanneaquinol[10] and 2-[10(*Z*)-haptadecenyl]-1,4-hydroquinone,[8] suggested that fraction X contained two compounds with the structures of **6.4**, with a heptadecyl side chain, and **6.5**, with a heptadecenyl side chain (Figure 6.3). Those components however were inseparable under the conditions used in this study, and their NMR spectra almost completely overlapped. Due to the limited quantity of materials, the position of the double bond in compound 6.5 was not determined. The geometry of the double bond of **6.5** was determined to be *Z* in the same way as **6.2** and **6.3**. Compound **6.4** has been synthesized previously,[12] and the aforementioned lanneaquinol[10] and 2-[10(*Z*)-heptadecenyl]-1,4-hydroquinone[8] had identical skeletons to that of **6.5**.



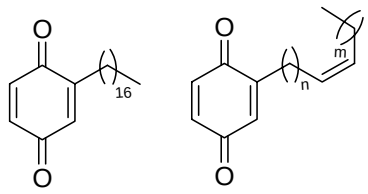
6.4 **6.5** m+n = 14

Figure 6.3. Structures of **6.4** and **6.5**

6.2.3. *Structure Elucidation of Fraction XI*

The ^{1}H NMR of fraction XI displayed a similar pattern to that of fraction X, showing signals for three aromatic protons in an ABX system at δ_H 6.75 (d, J = 9.8 Hz, H-6), δ_H 6.70 (dd, J = 9.8, 2.5 Hz, H-5), and δ_H 6.55 (d, J = 2.5 Hz, H-3), olefinic methines at δ_H 5.34 (m), methylenes at δ_H 2.41 (t, J = 7.6 Hz H-1′), δ_H 2.00 (m), several other methylenes at δ_H 1.20–1.40, and a methyl group at δ_H 0.87 (t, J = 6.7 Hz, H-17′) (Table 6.1). Its ^{13}C NMR spectrum was different from that of fraction X, with signals for two carbonyl carbons (δ_C 187.5, C-1 and δ_C 187.6, C-4) and a distinct pattern in the region from δ_C 110 to δ_C 160. In addition to the aforementioned two carbonyl carbons, six olefinic carbons (δ_C 149.8, 136.9, 136.3, 132.5, 130.0, 129.9), a methyl carbon (δ_C 14.2) and several methylenes were indicated in its ^{13}C NMR spectra (Table 2). The HMBC correlations of H-1′ to C-1, C-2 (δ_C 149.8), and C-3 (δ_C 132.5), H-3 to C-1 and C-5 (δ_C 136.3), H-5 to C-3, and H-6 to C-4, in addition to the ABX coupling system established the presence of a 2-subtituted 1,4-quinone moiety. The observed NMR data of this portion of the molecule were identical to those of the corresponding portion of a known compound, 2-undecyl-1,4-quinone.[13] GC-MS chromatogram of this fraction indicated a major peak with a molecular ion at *m/z* 344 and a minor peak with a molecular ion at *m/z* 346. These data indicated that fraction XI contained two compounds, one having a heptadecyl side chain at C-2, designated as **6.6**, and the other having a heptadecenyl side chain, designated as **6.7** (Figure 6.4). Similarly to **6.1**–**6.3** and **6.4**–**6.5**, those components were inseparable under the conditions used in this study, and their NMR spectra almost completely overlapped. The geometry of the double bond of **6.7** was determined to be *Z* in the same way as **6.2** and **6.3**. The double bond position in the side chain of **6.7** was not

determined due to the relatively small quantity of materials available. Compound **6.6** has been synthesized in a previous investigation but without any NMR data reported.[14] Compound **6.7** has not been reported in the literature to date.



6.6 **6.7** m+n = 14

Figure 6.4. Structures of **6.6** and **6.7**

6.2.4. *Antiproliferative Activities of Isolated Fractions and Compounds*

Fraction VII (**6.1**, **6.2** & **6.3**), X (**6.4** & **6.5**), and XI (**6.6** & **6.7**) were tested for antiproliferative activity against the A2780 human ovarian cancer cell line. It was found that these fractions showed strong antiproliferative activities, with IC_{50} values of 0.29, 0.09 and 0.21 μg/mL, repectively. The cytotoxicity of the 2-alkylated 1,4-benzoquinones is reported for the first time.

Alkylated hydroquinones have been reported to be cytotoxic.[8,10] Regarding the 2-alkyl-1,4-hydroquinones, it has been suggested that their 2-alkyl dihydroxybenzene moieties played a more important role than the length of the side chains and the presence of double bonds.[10] Another investigation indicated that 5-alkyl-l,3-dihydroxybenzenes (5-alkylresorcinols) and 6-alky1-1,2,4-trihydroxybenzenes showed activities of Cu(II)-dependent DNA cleavage, in addition to the fact that the latter compounds were 50-100 times more potent than the corresponding former ones and that their potency increased with increasing of the length of side chains.[15]

6.3. Experimental Section

6.3.1. General Experimental Procedures

Optical rotations were recorded on a JASCO P-2000 polarimeter. UV spectra were obtained on a Shimadzu UV-1201 spectrophotometer. NMR spectra were obtained on JEOL Eclipse 500 and Varian Inova 400 spectrometers. Chemical shifts are given in δ (ppm), and coupling constants (J) are reported in Hz. GC/MS spectra were recorded on Agilent 6890N Network GC System and 5973 Inert Mass Selective Detector coupled with an Agilent 19091S-433 capillary column (28.6 m × 250 μm). HRESI mass spectra were obtained on an Agilent 6220 TOF LC/MS. HPLC was performed using Shimadzu LC-10A pumps coupled with a Varian Dynamax Si preparative column (250 × 21.4 mm) and a Varian Dynamax Si semipreparative column (250 × 10.0 mm). The HPLC instruments employed the Shimadzu SPD-M10A diode array detectors.

6.3.2. *Antiproliferative Bioassay*

The A2780 ovarian cancer cell line assay was performed by Ms. Peggy Brodie at Virginia Polytechnic Institute and State University as previously reported[16] and described in Chapter II. The A2780 cell line is a drug sensitive ovarian cancer cell line.[17]

6.3.3. *Plant Material Collection and Extraction*

Fruits of *Sclerocarya birrea* subsp. *caffra* (Sond.) Kokwaro (Anacardiaceae) were collected in bay area of Dunes, in Antsiranana, Diana, Madagascar, at an elevation 4 m, at 12.14.30. S, 49.22.31. E, on May 13, 2004. The tree was 7 m high with diameter at breast height of 20 cm, white resin with characteristic odor, grouped leaves at the

extremity of branches, and globular and spherical fruit with white resin. The fruits are comestible. The species was identified by N. M. Andrianjafy; its assigned collector number is Andrianjafy et al. 461.

6.3.4. *Extract Fractionation*

A total of 1.4 g of extract was supplied to VPISU (MG 2257), and this had an IC_{50} value of 6.5 μg/mL against A2780 cells. This extract (675 mg) was suspended in aqueous MeOH (90%MeOH-H_2O, 30 mL) and extracted with hexanes (3 × 30 mL). The aqueous layer was then diluted to 60% MeOH (v/v) with H_2O and extracted with CH_2Cl_2 (3 × 45 mL). The CH_2Cl_2 extract (93 mg) was found to be the most active against A2780 cells (IC_{50} = 2.6 μg/mL) and was separated via preparative HPLC over a Si gel column using 100% $CHCl_3$ to afford 9 fractions (I-IX). Fraction VII (3.6 mg, t_R 29.4 min) afforded **6.1**, **6.2** and **6.3**. Fraction V (t_R 16.20 min) was separated via semipreparative HPLC over a Si gel column using 100% $CHCl_3$ to afforded fraction X (0.8 mg, t_R 7.00 min), which led to **6.4** and **6.5**, and fraction XI (1.0 mg, t_R 16.5 min), which afforded **6.6** and **6.7**.

6.3.5. *Fraction VII (**6.1**, **6.2**, and **6.3**)*

Wax: $[\alpha]_D^{25}$ +20.0 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 231 (3.27), 290 (3.11) nm**;** ^1H and ^{13}C NMR see Tables 6.1 and 6.2; GC-MS *m/z* (rel. Int.): **6.1** (t_R 13.63 min), 364 (16) $[M]^+$, 346 (56), 149 (8), 136 (10), 124 (100), 123 (40); **6.2** & **6.3** (t_R 13.49 min), 362 (21) $[M]^+$, 344 (23), 149 (5), 136 (10), 124 (100), 123 (34).

6.3.6. *Acetylation of Fraction VII*

Fraction VII (1 mg) was added to a stirred solution of acetic anhydride (0.1 mL) and catalytic amount of 4-(dimethylamino) pridine (DMAP) in pridine (0.1 mL) at room temperature. After 24 h, H_2O was added to the reaction mixture, which was extracted with $CHCl_3$. The reaction layer was washed by H_2O, brine, dried by anhydrous Na_2SO_4, and evaporated under reduced pressure to afford the 1,4,2′-triacetyl derivatives (1.8 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.02 (d, J = 9.4 Hz, H-6), 6.93-6.97 (m, H-3 and H-5), 5.30 (m, CH=CH), 4.98 (m, H-2′), 2.78 (m, H-1′a), 2.64 (m, H-1′b), 2.31, 2.25, and 1.96 (s, 3 × $OCOCH_3$), 0.84 (t, J = 6.9 Hz, H-17′).

6.3.7. *Dimethyl Disulfide Adducts of the Acetates* ***6.8***

To the above acetates were added dimethyl disulfide (DMDS, 0.3 mL) and a catalytic trace of iodine, and the mixture was stirred for 24 hrs at room temperature under a nitrogen atmosphere. Et_2O was then added, and the reaction mixture was washed with aqueous KOH solution. The organic layer was evaporated to afford dimethyl disulfide adducts, which were subjected to GC-MS analysis without further purification. GC-MS *m/z* (rel. Int.): **6.2** adduct (t_R 24.97 min), 582 (3) $[M]^+$, 437 (10) (M-$C_8H_{17}S$), 377 (100), 335 (16), 207 (12), 165 (20), 145 (24) ($C_8H_{17}S$), 123 (32), 87 (10), 61 (22); **6.3** adduct (t_R 24.37 min), 582 (9) $[M]^+$, 409 (M-$C_{10}H_{21}S$), 377 (4), 349 (100), 301 (36), 259 (65), 217 (32), 207 (26), 173 (40) ($C_{10}H_{21}S$), 165 (32), 149 (20), 123 (54), 87 (24), 61 (40).

6.3.8. *Fraction X (**6.4** and **6.5**)*

Wax: UV ($CHCl_3$) λ_{max} (log ε) 230 (3.29), 290 (3.36) nm**;** ^1H and ^{13}C NMR see Tables 6.1 and 6.2; GC-MS *m/z* (rel. Int.): **6.4** (t_R 12.05 min), 348 (100) $[M]^+$, 149 (2), 136 (7), 123 (70); **6.5** (t_R 11.98 min), 346 (100) $[M]^+$, 149 (8), 136 (20), 123 (100).

6.3.9. *Fraction XI (**6.6** and **6.7**)*

Wax: UV ($CHCl_3$) λ_{max} (log ε) 249 (3.66), 290 (3.21) nm**;** ^1H and ^{13}C NMR see Tables 6.1 and 6.2; GC-MS *m/z* (rel. Int.): **6.6** & **6.7** (t_R 10.40 min), 346 (16) $[M]^+$ for **6.6**, 344 (20) [M]+ for **6.7**, 149 (19), 136 (30), 123 (100).

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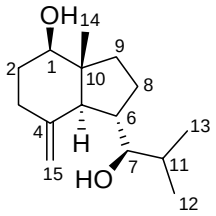
VII. Synthesis of (7*R**)-opposite-4(15)-ene-1β,7-diol

7.1. Introduction

(7*R**)-opposite-4(15)-ene-1β,7-diol (**7.1**) (Figure 7.1) is a sequiterpenoid that was identified as the major component from a mixture of several components isolated from *Cyphostemma laza* by Dr. Shugeng Cao in Dr. Kingston's group. This mixture exhibited a highly potent antiproliferative activity against the A2780 human ovarian cancer cell line with an IC_{50} value of 0.045 μg/mL. However, it was difficult to further purify this mixture to obtain individual compounds due to its limited quantity (~ 0.8 mg). Although **7.1** was isolated[1] and synthesized[2] before, this compound has not been recorded as being tested for anticancer activity. A synthesis of **7.1** was thus needed to determine whether the antiproliferative activity of this mixture was due to this major component or not.



7.1

Figure 7.1. Structure of (7*R**)-opposite-4(15)-ene-1β,7-diol

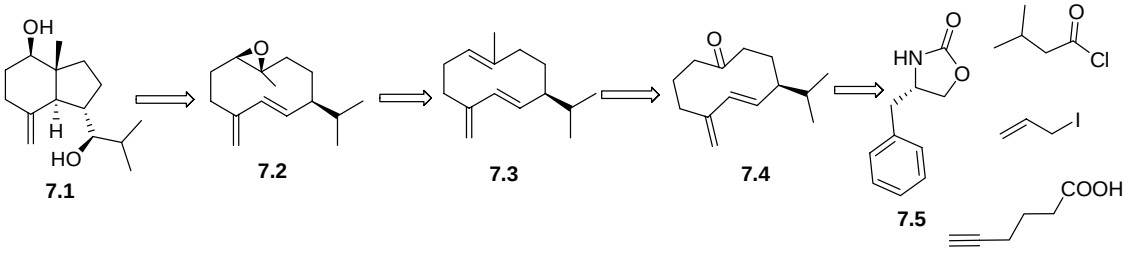
7.2. Results and Discussion

7.2.1. *General Retrosynthetic Analysis*

Compound **7.1** was synthesized previously through acid catalyzed rearrangement of epoxygermacrene D (**7.2**) along with several other sesquiterpenoids.[2] Epoxygermacrene D could be obtained by *m*-chloroperbenzoic acid (*m*CPBA) epoxidation of (−)-

germacrene D (**7.3**), which was reported as a potent sex attractant pheromone of the American cockroach.[3] (−)-Germacrene D could be synthesized from the (−)-dienone (**7.4**) applying the McMurry procedure,[4,5] and **7.4** could be produced following a procedure in the literature starting from the commercially available (*S*)-4-benzyl-2-oxazolidinone (**7.5**) and isovaleryl chloride.[6]

Scheme 7.1. Retrosynthetic analysis

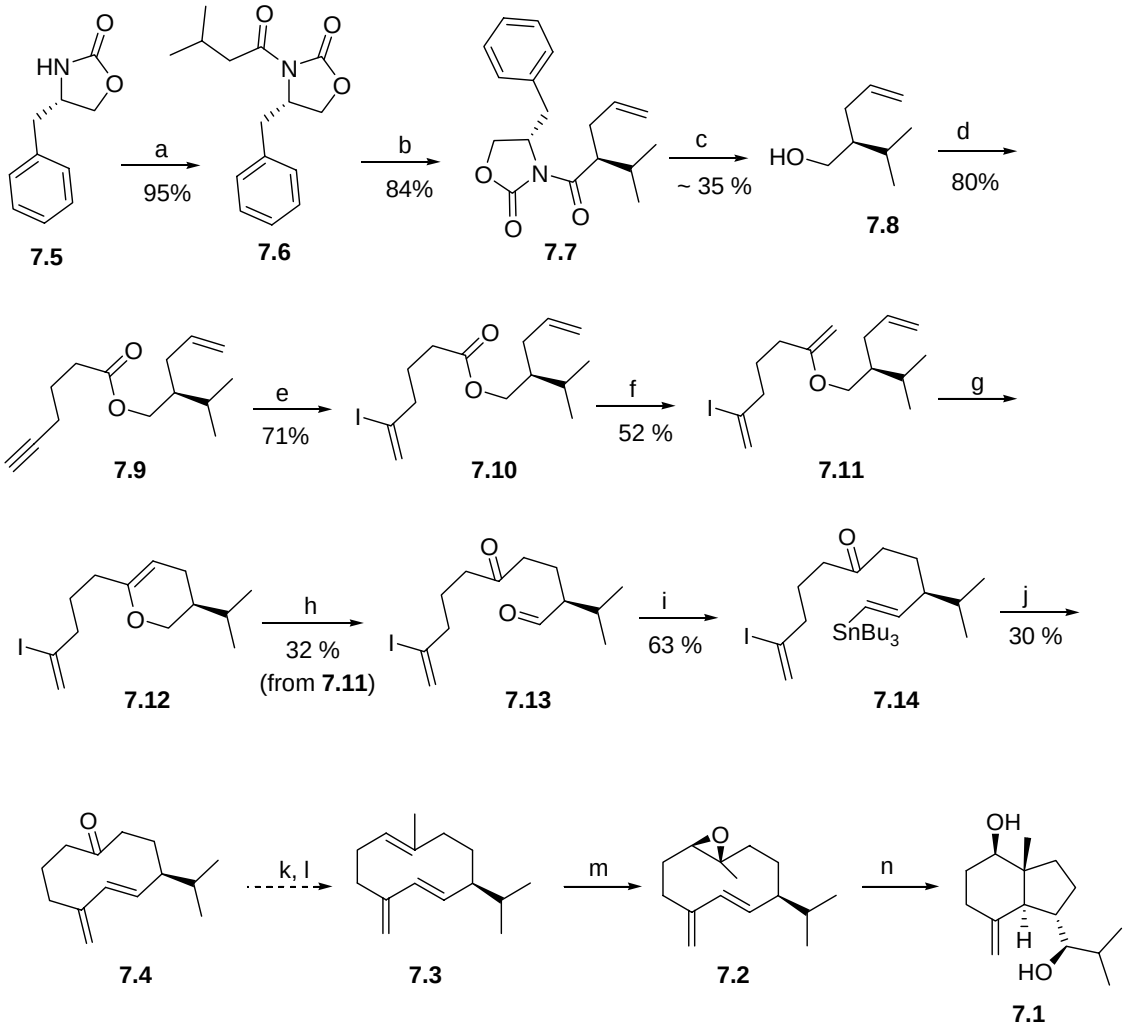


7.2.2. *Synthesis of* ***7.1***

The synthetic route is shown in Scheme 7.2. *N*-acylation of commercially available (*S*)-4-benzyl-2-oxazolidinone (**7.5**) with isovaleryl chloride provided *N*-isovaleryloxazolidinone (**7.6**) (95%). Allylation of **7.6** with allyl iodide yielded a single alkene diastereomer (**7.7**) (84%). Compound **7.7** was then converted to the intermediate crude acid (88%) by reaction with LiOOH-Na_2SO_3 along with the recovery of **7.5** (90%).[7] The crude acid was further reduced by using $LiAlH_4$ to yield alcohol **7.8** (35%). The alkyne ester (**7.9**) was derived from esterification of **7.8** with commercially available hex-5-ynoic acid (80%). Chemoselective iodoboration of **7.9**, followed by protonolysis,[8] yielded the alkenyl iodide (**7.10**) (71%). Ester methylenation[9,10] of **7.10** yielded the triene

7.11 (52%), which was then subjected to a 2nd generation Grubbs catalyst mediated ring closing

Scheme 7.2. Synthesis of (7*R**)-opposite-4(15)-ene-1β,7-diol (**7.1**)



Reagents and conditions: a) BuLi, −78 °C, THF, 1 hr; isovaleryl chloride, −78 to 25 °C; b) LDA, allyl iodide, THF, −78 °C to −20 °C, 3 hr; c) H_2O_2, LiOH, THF, H_2O, 0 to 25 °C, 5 hr, then Na_2SO_3; then $LiAlH_4$, Et_2O, 0 °C, 2 hr; d) hex-5-ynoic acid, EDCI, DMAP, CH_2Cl_2, 25 °C, 4 hr; e) *B*-I-9-BBN, pentane, −25 °C, 2 hr, then AcOH, 1 hr; f) CH_2Br_2, Zn, cat. $PbCl_2$, $TiCl_4$, TMEDA, THF, 25 °C, 3.5 hr;
g) cat. [1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(phenylmethylene)(tricyclohexylphosphine)Ruthenium (20%), Toluene, 70 °C, 10 min; h) 2 M HCl, THF, 25 °C, 2 hr; then PCC, SiO_2, CH_2Cl_2, 25 °C, 3 hr; i) Bu_3SnCHI_2, $CrCl_2$, DMF, 25 °C, 3.5 hr; j) cat. Pd_2dba_3, $AsPh_3$, NMP, 75 °C, 12 hr; k) LHMDS, −78 °C, 30 mins; then HMPA, Tf_2NPh, 1 hr; l) Me_2CuLi, −15 °C, 12 hr; m) *m*-chloroperoxybenzoic acid, CH_2Cl_2, 0 °C; n) 80% aq. AcOH, 0 °C, 1 hr.

metathesis (RCM). Without further purification the crude RCM product, the dihydropyran **7.12,** was directly converted to the aldehyde (**7.13**) (33% from **7.11**). Stannylation of **7.13** yielded the stannane (**7.14**) (53%),[6] which underwent an intramolecular Stille coupling to yield the cyclic sesquiterpenoid **7.4** (30%, overall yield 0.5% in 11 steps).

Given the expected limited quantity of the synthetic germacrene D which could be obtained by these steps, it became clear that the synthesis would need to be scaled up significantly to produce enough material for the final low yield transformations. Although germacrene D is not commercially available, Dr. Kingston was able to locate a sample through his connection with Givaudan Inc.. A sample of essential oil containing 40% germacrene D was eventually obtained from the A. M. Todd Company. The essential oil was purified on a Si gel open column to generate (−)-germacrene D[11] (yield 30%). The structure of the compound was confirmed by comparing its ^{1}H and ^{13}C NMR data with the recorded data in the literature.[12,13] In addition, its optical rotation value (see Experimental Section) was very close to the reported value of natural (−)-germacrene D.[14]

Epoxidation of the isolated (−)-germacrene D with *m*-chloroperbenzoic acid afforded a crude product (96%), which mainly contained epoxygermacrene D (**7.2**) (more than 85% purity judging from its NMR spectra). Interestingly, this reaction showed not only the expected regioselectivity but also stereoselectivity, given the fact that only one major epoxygermacrene D diastereomer was detected by ^{1}H and ^{13}C NMR spectra. Only partial ^{1}H NMR data of epoxygermacrene D have been reported previously.[3] Therefore, complete assignments of the ^{1}H and ^{13}C NMR spectra of epoxygermacrene D were

established using a combination of 1D and 2D NMR experiments, including COSY, HMQC, HMBC, and ROESY sequences. The key COSY and HMBC correlations are shown in Figure 7.2 (a). The complete assignments of NMR data are shown in the Experimental Section. The relative configuration of the epoxygermacrene D (**7.2**) as shown was evidenced by the large coupling constants of H-6 and H-7 (J = 10.4 Hz), H-9b (J = 13.8 Hz), and H-1 (J = 11.0 Hz), which indicated the axial orientations of those protons, and the ROESY correlation of H-9b to H-1 (Figure 7.2 (b) and Experimental Section).

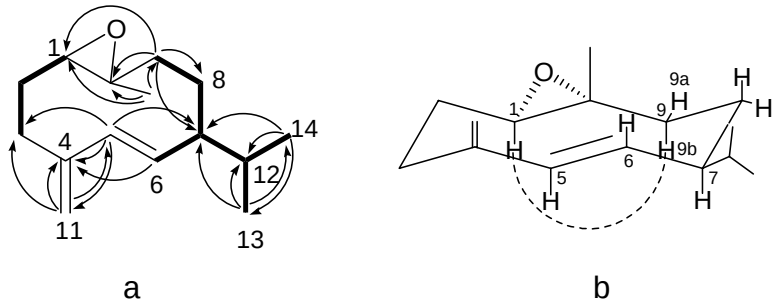


Figure 7.2. (a) Key COSY (bold) and HMBC (arrows) correlations of **7.2**

(b) The relative configuration of **7.2**

(dotted lines indicated ROESY correlations)

Acid catalyzed rearrangement of the crude epoxygermacrene D afforded a mixture of a series of derivatives, which were separated using C18 and Si HPLC chromatography. The target compound, (7*R**)-opposite-4(15)-ene-1β,7-diol (**7.1**), was obtained with an isolated yield of 2.0%. Its structure was confirmed by comparing its spectroscopic data with those of the recorded data for this compound in the literature[1] and the data for the compound obtained by Dr. Shugeng Cao, and those data matched each other very well. At the same

time, several other sesquiterpenoid analogs were also obtained, and their structures have been elucidated as **7.15**–**7.18** (isolated yield 10.8, 2.7, 0.9, 10.6%, respectively) (Figure 7.3). Their structures were elucidated using a combination of 1D and 2D NMR spectroscopy, mass spectroscopy, comparison with the literature data, and chemical conversion; their ^{13}C NMR data are shown in Table 7.1. Compound **7.15** has been reported with unassigned stereochemistry at C-7.[2] In this study, compound **7.15** was hydrolyzed in 1 M NaOH in MeOH/H_2O (1:1) at 45 ^{o}C for 12 hr to produce compound **7.1**, in which the configuration at C-7 was *R**. Thus the stereochemistry at C-7 of compound **7.15** was determined as *R**. Compounds **7.16** and **7.18** were also reported along with compound **7.15**, but no complete NMR data were given for them.[2] In this study, complete assignments of their ^{1}H and ^{13}C NMR data are provided as shown in Table 7.1 and the Experimental Section. Compound **7.17** has been reported and belongs to the relatively rare class of isodaucane sesquiterpenoids, and its NMR data were identical to those in the literature.[15,16] Thus epoxygermacrene D was converted to a series of sesquiterpenoids with diverse skeletons. Proposed mechanisms for these conversions are given in Scheme 7.3.

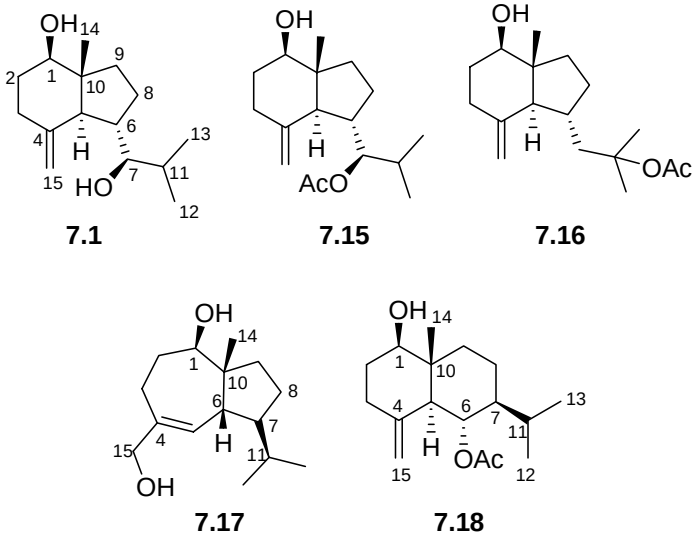


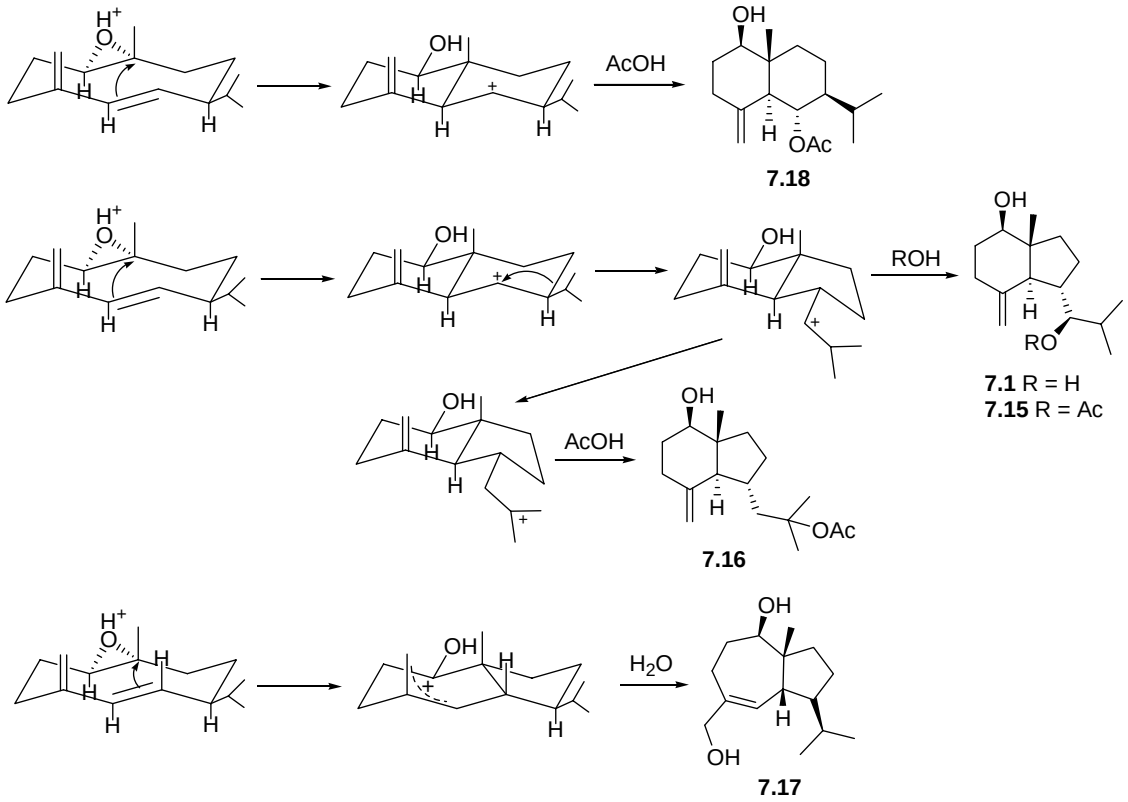
Figure 7.3. Structures of sesquiterpenoid analogs

Table 7.1. ^{13}C NMR Data of Compounds **7.1** and **7.15–7.18**[a,b]

no.	**7.1**	**7.15**	**7.16**	**7.17**	**7.18**
1	79.1	79.0	79.3	76.7	79.2
2	31.9	31.9	31.9	28.0	32.2
3	35.0	34.7	34.6	25.1	35.0
4	149.0	146.3	145.9	138.1	144.5
5	56.5	55.8	57.7	131.0	53.5
6	39.5	38.2	32.4	51.4	70.8
7	82.8	82.1	46.9	55.8	49.0
8	26.1	25.9	29.9	27.1	18.1
9	37.4	37.0	37.3	39.7	36.1
10	49.6	49.4	47.5	46.3	42.2
11	31.4	30.3	82.8	33.0	26.2
12	14.8	15.4	26.3	20.0	16.0
13	20.6	20.2	26.3	22.2	21.3
14	12.4	12.2	12.0	20.0	11.6
15	107.8	107.5	106.5	67.4	107.8
$CH_3\underline{C}(O)O$-		171.5	170.6		171.1
$\underline{C}H_3C(O)O$-		21.2	22.7		21.2

[a] in $CDCl_3$. [b] δ (ppm) 150 MHz.

Scheme 7.3. Proposed mechanisms of epoxygermacrene D rearrangement



7.2.3. *Antiproliferative activity of **7.1**, **7.15-7.18***

All the collected fractions obtained from separation of the products of the rearrangement of epoxygermacrene D were tested against the A2780 human ovarian cancer cell line. These fractions were found to be either weakly active or inactive. In particular, compounds **7.1** and **7.17** did not show activity against A2780 cells (IC_{50} > 20 μg/mL), and compounds **7.15**, **7.16** and **7.18** showed only weak activity against the A2780 cells with IC_{50} values from 23 to 30 μM.

These results indicated that potent activity of the mixture, which was isolated by Dr. Cao and mentioned in the Introduction of this chapter, was not due to the major component **7.1** but other minor components with presumably highly potent antiproliferative activities.

7.3. Experimental Section

7.3.1. *General Experimental Procedures*

All reactions requiring anhydrous conditions were performed in oven-dried glassware under nitrogen or argon. Tetrahydrofuran (THF) was distilled with sodium/benzophenone, and dichloromethane (DCM) was distilled with calcium hydride. The other solvents were purchased accordingly and of either HPLC or reagent grade. All reactions were monitored using analytical TLC plates (silica gel 60 GF, Merck), which were supported on aluminum back, unless stated otherwise, and these TLC plates were visualized with either UV light at 254 nm or potassium permanganate (K_2MnO_4) solution or vanillin/sulfuric acid spray. Preparative TLC plates were either silica gel 60 GF, 1000 or 500 microns, 20 cm ×20 cm from Analtech, or aluminium oxide 60 F_{254}, 20 cm ×20 cm from EM Science, or C18 silica gel, with 254 nm fluorescent indicator, 20 cm ×20 cm from J. T. Baker. Optical rotations were recorded on a JASCO P-2000 polarimeter. NMR spectra were obtained on Bruker Avance 600 equipped with a 1.7 mm CapProbe, JEOL Eclipse 500, Varian Inova 400, and Varian Unity 400 spectrometers. HRESI mass spectra were obtained on an Agilent 6220 TOF LC/MS. Chemical shifts are given in δ (ppm), and coupling constants (J) are reported in Hz. HPLC was performed using Shimadzu LC-10A pumps coupled with a Varian Dynamax C18 semipreparative column (250 × 10 mm) and a Varian Dynamax Si semipreparative column (250 × 10 mm). The HPLC instrument employed a Shimadzu SPD-M10A diode array detector.

7.3.2. *Antiproliferative Bioassay*

The A2780 ovarian cancer cell line assay was performed by Ms. Peggy Brodie at Virginia Polytechnic Institute and State University as previously reported[17] and described in chapter II. The A2780 cell line is a drug sensitive ovarian cancer cell line.[18]

7.3.3. *Synthetic Procedures*

_N_-isovaleryloxazolidinone 7.6

n-BuLi (2.5 M in hexanes; 3.6 ml, 9.00 mmol) was added dropwise to a stirred solution of (*S*)-(−)-4-benzyl-2-oxazolidinone (**7.5**, 1.085 g, 6.13 mmol) in THF 30 ml at -78 ^{o}C, the cooling bath was then adjusted to room temperature in 2 h. Saturated aq. NH_4Cl (40 ml) was added cautiously to the reaction mixture, and the resulting solution was extracted with EtOAc (3 × 100 ml). The combined organic layers were washed with H_2O (10 ml) and brine (10 ml), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was subjected to SiO_2 gel column chromatography for purification, and a colorless oil, the *N*-isovaleryloxazolidinone **7.6** (1.50 g, 95%) was obtained. ^{1}H NMR data were identical to the reported data for this compound.[19]

(4*S*)-3-[(2*S*)-2-(1-Methylethyl)pent-4-enoyl]-4-(phenylmethyl)-oxazolidin-2-one 7.7

n-BuLi (2.5 M in hexanes; 12.4 ml, 31.0 mmol) was added dropwise to a stirred solution of diisopropylamine (4.5 ml, 33.7 mmol) in 15 ml THF at -78 ^{o}C. The cooling bath was then adjusted to 0 ^{o}C in about 1 h, and the reaction mixture was kept at 0 ^{o}C for 15 min. The cooling bath was cooled to -78 ^{o}C, *N*-isovaleryloxazolidinone **7.6** (6.5 g, 24.8 mmol) in THF 10 ml was added dropwise. After 1 h at -78 ^{o}C, allyl iodide (3.4 ml, 39.6 mmol)

in THF (3 ml) was added dropwise and the cooling bath was adjust to -20 ^{o}C in about 1 h. The reaction continued at -20 ^{o}C for 2.5 h, and saturated aq. NH_4Cl (3 ml) was then added cautiously to quench the reaction. The reaction mixture was extracted with Et_2O (3 × 50 ml), and the combined organic layers were washed using H_2O (5 ml) and brine (5 ml), dried over Na_2SO_4, and evaporated under reduced pressure. Purification of the residue by SiO_2 gel column chromatography eluted using EtOAc:Hexanes (1:9) to yield a light yellow oil, the alkene **7.7** (6.44 g, 86%). The ^{1}H and ^{13}C NMR data were identical to the reported values for this compound.[6]

(2*S*)-2-(1-Methylethyl)pent-4-en-1-ol 7.8

H_2O_2 (35% w/w in H_2O, 16.6 ml, 171 mmol) was added dropwise to a stirred solution of the alkene **7.7** (6.44 g) in THF (200 ml) and H_2O (62.5 ml) at 0 ^{o}C. LiOH monohydrate (1.8 g, 41.5 mmol) in H_2O (40 ml) was added portionwise to the reaction mixture at 0 ^{o}C, and the cooling bath was then removed to allow the reaction mixture to warm to room temperature. The reacting mixture was stirred for 5 h before it was recooled to 0 ^{o}C, and aq. Na_2SO_3 (1.5 M, 130 ml, 195 mmol) was then added. The resultion mixture was concentrated under reduced pressure, and the resulting concentrate was adjust to pH 12–13 using aq. 1.5 M NaOH and extracted with CH_2Cl_2 (5 × 100 ml), and the combined organic layers were washed using H_2O (100 ml), which was later combined with the aqueous basic layer. The CH_2Cl_2 solution was further washed with brine (20 ml), dried over Na_2SO_4, and evaporated under reduced pressure to yield a white solid, the chiral auxiliary, (*S*)-(−)-4-benzyl-2-oxazolidinone **7.5** in an nearly quantative yield. The aqueous basic layer was adjusted to pH 1 at 0 ^{o}C using aq. HCl (6 M) and extracted with

EtOAc (5 × 100 ml). The combined EtOAc layers were washed with brine (20 ml), dried over Na_2SO_4, and evaporated under the reduced pressure to yield a light yellow oil, (2*S*)-2-(1-methylethyl)pent-4 enoic acid (2.79 g, 88%), which was directly used in the next step without further purification. ^1H and ^{13}C NMR data was identical to the reported values for this compound.[6]

(2*S*)-2-(1-methylethyl)pent-4 enoic acid (2.79 g, 19.6 mmol) was dissolved in Et_2O (150 ml), and $LiAlH_4$ (1.44g, 37.9 mmol) was then added portionwise in this solution at 0 ^oC. After 2 h, H_2O (1.44 ml), aq. NaOH (15% w/w, 1.44 ml), and H_2O (4.32 ml) were very slowly added successively to the reaction mixture,[20] which was then stirred for 0.5 h at 0 ^oC. The resulting very thick white mixture was filtered on a sintered glass funnel and washed with Et_2O (100 ml), and the filtrates were evaporated under reduced pressure to yield a colorless oil, the alcohol **7.8** (0.8 g, 33%), which was directly used in the next step without further purification. The ^1H and ^{13}C NMR data were identical to the reported values for this compound.[6]

(2*S*)-2-(1-Methylethyl)pent-4-yl hex-5-ynoate 7.9

Hex-5-yonic acid (0.74 ml, 0.752 g, 6.70 mmol) was added dropwise in a stirred solution of the alcohol **7.8** (0.37 g, 2.89 mmol), EDCI (1.22 g, 6.36 mmol), and DMAP (0.36 g, 2.94 mmol) in CH_2Cl_2 (30 ml) at room temperature. After 5 h, Et_2O (30 ml) was added in the reaction mixture which was then filtrated through a short pad of SiO_2 gel washed using Et_2O. The filtrates were evaporated under reduced pressure, and the residue was subjected to SiO_2 gel column chromatography eluted using Et_2O:petroether (0.5:9.5) to yield a colorless oil, the alkyne ester **7.9** (0.51 g, 80%). The ^1H and ^{13}C NMR data were identical to the reported values for this compound.[6]

(2*S*)-2-(1-methylethyl)pent-4-en-1-yl 5-iodohex-5-enoate 7.10

The alkyne **7.9** (0.96 g, 4.32 mmol) in pentane (2 ml) was slowly added dropwise to a stirred solution of *B*-I-9-BBN (1 M in hexanes, 5.15 ml, 5.15 mmol) in pentane (14 ml) at -25 ^{o}C. After 2 h, glacial acetic acid (4.6 ml) was added dropwise to the reaction mixture, and the cooling bath was then adjusted to 0 ^{o}C. After 1 h, aq. NaOH (3 M, 50 ml) and H_2O_2 (35% w/w in H_2O, 8 ml) were added slowly in the reaction mixture successively, and the cooling bath was removed to allow the reaction to continue at room temperature. After 30 min, the organic layer was separated and the aqueous layer was further extracted with petroether (5 × 50 ml) and Et_2O (50 ml). The organic layers were then combined, washed using H_2O (2ml), saturated aq. $NaHCO_3$ (2ml), saturated aq. Na_2SO_3 (2ml), H_2O (2 ml) and brine (2 ml), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was subjected to SiO_2 gel column chromatography eluted using Et_2O:petrother (0.5:9.5) to yield a light yellow oil, the alkenyl iodide ester **7.10** (1.05 g, 71%). The ^{1}H and ^{13}C NMR data were identical to the reported values for this compound.[6]

(2*S*)-[2-(1-Methylethyl)pent-4-en-1-yloxy]-6-iodohepta-1,6-diene 7.11

$TiCl_4$ (1 M in CH_2Cl_2, 5.3 ml, 5.3 mmol) was slowly added dropwise to a stirred solution of THF (2.5 ml) at 0 ^{o}C, and TEMDA (1.6 ml, 10.7 mmol) was then slowly added dropwise, and the reaction mixture was stirred for 20 min at 0 ^{o}C. A mixture of Zn dust (activated, 0.785 g, 12.0 mmol)[21] and catalytic $PbCl_2$ (0.017 g, 0.062 mmol) was then slowly added portionwise to the reaction mixture at 0 ^{o}C, which was then warmed to room termperature. After 30 min, a solution of alkenyl iodide ester **7.10** (0.312 g, 0.89

mmol) and CH_2Br_2 (0.20 ml, 2.85 mmol) in THF (1.6 ml) was added slowly in the stirred reaction mixture over 10 min. After 3.5 hr, the reaction mixture was recooled to 0 ^oC, and Et_3N (1.4 ml) and saturated aq. K_2CO_3 (1.8 ml) was cautiously added in the mixture. After 15 min, the reaction mixture was filtrated through a pad of basic alumina (activity III[22], 40 g) washed using Et_3N:Et_2O (1:99). The filtrate was dried under reduced pressure. Purification of the residue using Al_2O_3 PTLC developed using Et_2O:petroether (10:90) to produce a clear colorless oil, the triene **7.11** (0.16 g, 52%). The ^1H and ^{13}C NMR data were identical to the reported values for this compound.[6]

(2*S*)-9-Iodo-2-(1-methylethyl)-5-oxodec-9-enal 7.13

Grubbs catalyst (2nd generation, 30 mg, 0.035 mmol) was added in a solution of the triene **7.11** (70 mg, 0.199 mmol) in 60 ml toluene. The reaction flask was placed in a preheated oil bath at 70 ^oC, and the reaction mixture was stirred for 10 min. The reaction mixture was then rapidly filtrated through a pad of basic alumina (Activity III, 15 g) eluted using Et_2O:petroether (20:80), and the filtrate was evaporated under reduced pressure to yield a light yellow oil, the crude dihydropyran **7.12**, which was immediately dissolved in THF (1ml). HCl (2 M in H_2O, 0.1 ml) was added to the reaction mixture which was stirred for 2 h. Na_2HCO_3 (0.20 g) was added in the reaction mixture, which was then filtrated. The filtrate was evaporated under reduced pressure to afford a residue which was then dissolved in CH_2Cl_2 (1ml). PCC (0.20 g, 0.93 mmol) and SiO_2 (0.20 g) were added to the stirred reaction mixture, which was diluted by adding Et_2O (1 ml) after 3 h. The resulting solution was subjected to a pad of Florisil® (Aldrich, 6 g) eluted using Et_2O (25 ml). The filtrate was evaporated under reduced pressure. Purification of the

residue on a SiO_2 PTLC plate developed by EtOAc:Hexanes (2:8) to yield an oil, the aldehyde **7.13** (22 mg, 32%). $[\alpha]_D^{25}$ +18.5 (*c* 1.0, $CHCl_3$). The ^1H and ^{13}C NMR data were identical to the reported values for this compound.[6]

Tributyl(dibromomethyl)stannane ($Bu_3SnCHBr_2$) and

Tributyl(diiodomethyl)stannane (Bu_3SnCHI_2)

n-BuLi ((2.5 M in hexanes; 1.77 ml, 4.43 mmol) was added dropwise in a stirred solution of diisopropylamine (0.73 ml, 5.17 mmol) in THF (5 ml) and Et_2O (7.4 ml) at -78 ^oC. The cooling bath was then adjusted to 0 ^oC in about 1 h, and the reaction mixture was stirred at 0 ^oC for 15 min. The cooling bath was then cooled to -95 ^oC ($MeOH/N_2$-bath), and a solution of CH_2Br_2 (0.34 ml, 4.80 mmol) in THF (3.7 ml) was added dropwise to the reaction mixture. The reaction temperature remained between -95 ^oC~-90 ^oC. After 15 min, a solution of Bu_3SnCl (1 ml, 1.2 g, 3.69 mmol) was added dropwise in the reaction mixture, which was then allowed to warm to -63 ^oC. Saturated aq. NH_4Cl (0.5 ml) was added cautiously to quench the reaction, and then the cooling bath was removed to allow the reaction mixture to warm to room termperature. The reaction mixture was then filtrated through a short pad of Si gel eluted using petroether, and the filtrate was then concentrated under reduced pressure. The concentrate was diluted with H_2O and then extracted with petroether. The combined organic layers were evaporated under reduced pressure. Purification of the residue on SiO_2 gel column chromatography eluted using 100% petroether to give $Bu_3SnCHBr_2$ (1.50 g, 90%). The ^1H and ^{13}C NMR data were identical to the reported values for this compound.[23]

Dry NaI (0.62 g, 4.13 mmol) was added in a stirred solution of $Bu_3SnCHBr_2$ (0.48 g, 0.10 mmol) in acetone (6 ml) at room temperature. The reaction flask was then wrapped using aluminum foil film to avoid the light. After 18 h, the reaction mixture was evaporated under reduced pressure, and the residue was treated with hexane (20 ml), filtrated and evaporated under reduced pressure. The residue was further diluted with CH_2Cl_2 (20 ml), filtrated and evaporated under reduced pressure. A yellow oil, Bu_3SnCHI_2 (0.58 g, 100%), was afforded. The ^1H and ^{13}C NMR data were identical to those reported values for this compound.[6]

(9*S*, 10*E*)-11-(Tributylstannyl)-2-iodo-9-(1-methylethyl)undec-1-en-6-one 7.14

Dry and deoxygenated DMF (1.5 ml) was added dropwise in a flask with well stirred $CrCl_2$ (0.09 g, 0.72 mmol) at 0 ^oC, and the reaction mixture was slowly warmed to room temperature over 15 min. The reaction flask was then wrapped using aluminum foil to exclude light. A solution of the aldehyde **7.13** (12 mg, 0.036 mmol) and Bu_3SnCHI_2 (0.08 g, 0.14mmol) in deoxygenated THF (0.5 ml) was added dropwise in the reaction mixture. After 2.5 h, H_2O (1 ml) was added in the reaction mixture which was then extracted with Et_2O (5 × 3 ml). The combined organic layers were washed with H_2O, brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified on a C18 PTLC plate developed using CH_2Cl_2:MeCN (30:70) to yield a colorless oil, the stannane, **7.14** (14 mg, 63%). $[\alpha]_D^{25}$ -9.0 (*c* 1.0, $CHCl_3$). The ^1H and ^{13}C NMR data were identical to the reported values for this compound.[6] HRESIMS *m/z*: 625.1994 $[M+H]^+$ (Calcd for $C_{26}H_{51}IO^{119}Sn$: 625.1928).

(4*S*,5*E*)-7-Methylene-4-(1-methylethyl)cyclodec-5-en-1-one 7.4

Degassed NMP (0.5 ml) was added to dissolve the stannane **7.14** (3 mg, 0.005 mmol) and $AsPh_3$ (0.9 mg, 0.003 mmol). $Pd(dba)_3$ (0.7 mg, 0.001 mmol) was added in the above well stirred solution at room temperature. The reaction flask was wrapped by aluminum foil to exclude light, and the reaction mixture was then heated to 75 ^oC. After 12 h, the reaction mixture was cooled to room temperature, diluted with H_2O (0.5 ml), and extracted with Et_2O (3 × 2 ml). The combined organic layers were washed with aq. $CuSO_4$ (1 M, 2 × 1 ml), brine, dried over Na_2SO_4, and evaporated under reduced pressure to yield a crude oil. The crude product was subjected to SiO_2 PTLC developed using Et_2O:hexanes (10:90) (R_f 0.21) to yield a colorless oil, the dienone **7.4** (0.3 mg, 30%). $[\alpha]_D^{25}$ -237 (*c* 0.03, hexanes). The ^1H NMR data were identical to those reported values for this compound.[6]

(–)-Germacrene D from a Natural Source

A crude oil containing germacrene D (40% purity) from a natural source was obtained from the A.M.TODD company. The crude oil (2.7 g) was subjected to SiO_2 gel column chromatograph with elution by hexanes to yield 3 fractions. The most polar fraction contained (–)-germacrene D (0.64 g, 30%). $[\alpha]_D^{25}$ -208.7 (*c* 1.0, $CHCl_3$). The ^1H and ^{13}C NMR data were identical to the reported values for this compound.[12,13]

Epoxygermacrene D

$NaHCO_3$ (10% w/w in H_2O, 4 ml) was added in a well stirred solution of the previously obtained germacrene D (100 mg) in CH_2Cl_2 (2 ml), and the reaction mixture was cooled to 0 ^oC. *m*-Chloroperbenzoic acid (132 mg) in CH_2Cl_2 (4 ml) was slowly added dropwise to the reaction mixture. After 45 min, the reaction mixture was extracted thoroughly with aq. Na_2CO_3 (10% w/w, 5 × 10 ml). The organic layer was then washed with brine, dried over Na_2SO_4, and evaporated under reduced temperature to yield a colorless oil (104 mg), which mainly contained epoxygermacrene D (more than 85% purity judging from its NMR spectra). This sample was used directly in the next stage without further purification. ^1H NMR (500 MHz, $CDCl_3$): δ 6.04 (1H, d, *J* = 15.8 Hz, H-5), 5.48 (1H, dd, *J* = 15.8, 10.4 Hz, H-6), 4.92 (1H, br, s, H-11a), 4.90 (1H, br, s, H-11b), 2.58 (1H, br, d, *J* = 11.0 Hz, H-1), 2.52 (1H, m, H-3a), 2.28 (1H, m, H-3b), 2.17 (1H, ddd, *J* = 13.8, 6.0, 2.2 Hz, H-9a), 1.98 (1H, m, H-2a), 1.90-1.80 (2H, m, H-7 and H-8a), 1.52-1.38 (3H, m, H-8b, H-12, H-2b), 1.29 (3H, s, H_3-15), 1.03 (1H, td, *J* = 13.8, 1.95 Hz, H-9b), 0.90 (3H, d, *J* = 6.6 Hz, H_3-13), 0.85 (3H, d, *J* = 6.6 Hz, H_3-14). ^{13}C NMR (500 MHz, $CDCl_3$): δ 148.3 (C-4), 136.6 (C-6), 133.1 (C-5), 112.6 (C-11), 69.0 (C-1), 61.4 (C-10), 51.9 (C-7), 38.9 (C-9), 32.5 (C-12), 31.2 (C-3), 30.2 (C-8), 29.2 (C-2), 20.9 (C-13), 19.7 (C-14), 16.6 (C-15).

Synthesis of (7*R)-opposite-4(15)-ene-1β,7-diol 7.1**, **7.15-7.18**

Epoxygermacrene D (83 mg) was dissolved in CH_2Cl_2 (2 ml), and aq. acetic acid (80% v/v, 2 ml) was added dropwise at 0 ^oC. After 1 h, the reaction mixture was adjusted to pH 9 by adding aq, Na_2CO_3. The organic layer was then washed by aq. Na_2CO_3 (10% w/w),

H_2O, brine, dried (anhydrous Na_2SO_4), and evaporated under reduced pressure. The residue was separated by HPLC on a C18 column with elution by a gradient of MeCN/H_2O (0~30 min, 60% MeCN; 30~35min, 60~70% MeCN; 35~60min, 70% MeCN). Collection of a peak (t_R 12.9 min) was evaporated, and the residue (~2 mg) was separated by HPLC on a Si column with elution by isopropanol/hexanes (1:10) to afford the (7*R**)-opposite-4(15)-ene-1β,7-diol **7.1** (t_R 14.4 min, 0.8 mg) and compound **7.17** (t_R 28.1 min, 0.3 mg). Collection of a peak (t_R 19.9 min) and a peak (t_R 21.9 min) from the C18 column afforded compounds **7.18** and **7.15**, respectively. Collection of a peak (t_R 19.3 min) from the C18 column was subjected to a Si HPLC with elution by isopropanol/hexanes (1:10) to yield compounds **7.18** (t_R 10.9 min, 1.0 mg) and **7.16** (t_R 12.0 min, 1.1 mg).

(7*R**)-opposite-4(15)-ene-1β,7-diol (**7.1**): $[\alpha]_D^{25}$ +103 (*c* 0.1, $CHCl_3$). The ^1H and ^{13}C NMR data were identical to the reported values for this compound.[1] ^1H NMR (600 MHz, $CDCl_3$): δ 4.95 (1H, d, *J* = 1.6 Hz, H-15a), 4.80 (1H, d, *J* = 1.6 Hz, H-15b), 3.58 (1H, br, d, *J* = 10.7 Hz, H-1), 3.22 (1H, ddd, *J* = 9.7, 3.0 Hz, H-7), 2.32 (1H, m, H-6), 2.29 (1H, m, H-3a), 2.11 (1H, m, H-3b), 1.90 (1H, m, H-8a), 1.86 (1H, m, H-2a), 1.83 (1H, m, H-5), 1.75 (1H, m, H-11), 1.75 (1H, m, H-9a), 1.50 (1H, m, H-2b), 1.38 (1H, m, H-9b), 1.32 (1H, m, H-8b), 0.99 (3H, d, *J* = 7.2 Hz, H_3-12/H_3-13), 0.90 (3H, d, *J* = 7.2 Hz, H_3-12/H_3-13), 0.66 (3H, s, H-14). ^{13}C NMR (150 MHz, $CDCl_3$): δ 149.0 (C-4), 107.8 (C-15), 82.8 (C-7), 79.1 (C-1), 56.5 (C-5), 49.6 (C-10), 39.5 (C-6), 37.4 (C-9), 35.0 (C-3), 31.9 (C-2), 31.4 (C-11), 26.1 (C-8), 20.6 (C-13), 14.8 (C-12), 12.4 (C-14). HRESIMS *m/z*: 261.1826 $[M+Na]^+$ (Calcd for $C_{15}H_{26}NaO_2$: 261.1830).

(7*R**)-opposite-4(15)-ene-7-acetoxy-1β-ol (**7.15**): $[\alpha]_D^{25}$ +94.3 (*c* 0.4, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.85 (1H, d, *J* = 1.5 Hz, H-15a), 4.70 (1H, dd, *J* = 10.2, 2.4 Hz, H-7), 4.67 (1H, d, *J* = 1.5 Hz, H-15b), 3.53 (1H, ddd, *J* = 11.4, 4.9, 4.8 Hz, H-1), 2.43 (1H, m, H-6), 2.21 (1H, ddd, *J* = 13.6, 5.4, 2.0 Hz, H-3a), 1.96 (1H, m, H-3b), 1.91-1.87 (2H, m, H-11 and H-8a), 1.85 (1H, d, 10.5 Hz, H-5), 1.78 (1H, m, H-2a), 1.72 (1H, *J* = 10.5, 8.8 Hz, H-9a), 1.50-1.35 (4H, m, H-2b, H-8b, H-9b, and 1-OH), 0.93 (3H, d, *J* = 6.6 Hz, H-13), 0.87 (3H, *J* = 6.9 Hz, H-12), 0.62 (3H, s, H-14). ^{13}C NMR (150 MHz, $CDCl_3$): see Table 7.1. HRESIMS *m/z*: 303.1940 $[M+Na]^+$ (Calcd for $C_{17}H_{28}NaO_3$: 303.1936).

Compound **7.16**: $[\alpha]_D^{25}$ +159 (*c* 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.86 (1H, d, *J* = 1.5 Hz, H-15a), 4.58 (1H, d, *J* = 1.5 Hz, H-15b), 3.52 (1H, ddd, *J* = 11.3, 4.9, 4.9 Hz, H-1), 2.29 (1H, ddd, *J* = 17.4, 5.4, 2.0 Hz, H-3a), 2.16 (1H, m, H-6), 2.05 (1H, m, H-3b), 1.94 (1H, br, d, *J* = 13.6 Hz, H-7a), 1.81 (1H, m, H-2a), 1.71 (1H, m, H-9a), 1.62 (1H, dd, *J* = 14.2, 10.5 Hz, H-7b), 1.54 (1H, d, *J* = 12.3 Hz, H-5), 1.50-1.40 (2H, m, H-2b and H-9b), 1.49 (3H, s, H-13), 1.48 (3H, s, H-12), 1.37 (1H, d, *J* = 4.8 Hz, 1-OH), 0.65 (3H, s, H-14). ^{13}C NMR (150 MHz, $CDCl_3$): see Table 7.1.

Compound **7.17**: $[\alpha]_D^{25}$ +30.0 (*c* 0.03, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.39 (1H, d, *J* = 3.7 Hz, H-5), 4.07 (2H, d, *J* = 5.7 Hz, H-15), 3.49 (1H, ddd, *J* = 10.6, 5.4, 5.2 Hz, H-1), 1.00 (3H, s, H-14), 0.92 (6H, t, *J* = 6.5 Hz, H-13 and H-12), 2.30-1.20 (the rest of protons). ^{13}C NMR (150 MHz, $CDCl_3$): see Table 7.1.

Compound **7.18**: $[\alpha]_D^{25}$ +44.0 (*c* 0.1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.11 (1H, t, *J* = 10.2 Hz, H-6), 4.82 (1H, br, s, H-15a), 4.60 (1H, br, s, H-15b), 3.45 (1H, ddd, *J* = 10.9, 5.7, 5.2 Hz, H-1), 2.30 (1H, ddd, *J* = 13.1, 4.9, 2.0 Hz, H-3a), 2.06 (1H, br, dd, *J* = 13.2, 4.7 Hz, H-3b), 1.98 (1H, d, *J* = 12.7 Hz, H-5), 1.98 (1H, m, H-9a), 1.88 (1H, m, H-

2a), 1.67 (1H, d of quintet, J = 7.0, 2.0 Hz, H-11), 1.63-1.49 (2H, m, H-8a and H-2b), 1.43-1.25 (2H, m, H-8b and H-7), 1.35 (1H, d, J = 5.6 Hz, 1-OH), 1.19 (1H, ddd, J = 13.2, 12.9, 3.7 Hz, H-9b), 0.94 (3H, d, J = 7.0 Hz, H-13), 0.89 (3H, d, J = 7.0 Hz, H-12), 0.78 (3H, s, H-14). ^{13}C NMR (150 MHz, $CDCl_3$): see Table 7.1.

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(20) The alternative method to consume the excess $LiAlH_4$ was also used in other batches. An equal volume of saturated aq. potassium sodium tartrate tetrahydrate was added in the resulting reaction mixture after reaction with $LiAlH_4$. The reaction mixture was stirred for 15 h at room temperature. The resulting two clear layers were separated, and the aqueous layer was further extracted with Et_2O. All organic layers were combined and evaporated under reduced pressure to yield the alcohol **7.8** (~30%).

(21) Before usage, commercial Zinc dust needs to be activated washed successively using 5% aq. HCl, H_2O, MeOH and hexanes, and dried under reduced pressure.

(22) Activity III alumina could be prepared from adding 6% H_2O into the commercially available alumina I (Aldrich, basic, Brockmann I).

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VIII. Miscellaneous Extracts

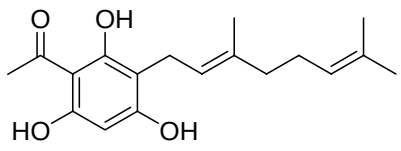
8.1. Introduction

During the course of my Ph.D study, a number of extracts have been studied in addition to the ones mentioned in the previous chapters. Fractionation of these extracts resulted in either the isolation of known compounds, or in the preparation of inactive fractions towards the A2780 human ovarian cancer cell line, or occasionally in the isolation of very small quantities of materials which were not sufficient for further isolation and structure elucidation.

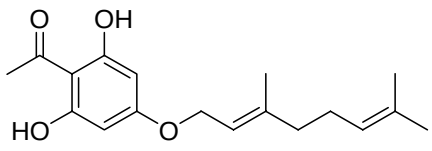
8.2. Known Compounds

8.2.1. Chemical Investigation of Evodia sp.

Bioassay guided fractionation of an ethanol extract of an unidentified species of *Evodia* (Rutaceae) led to the isolation of the two known acetophenones, 2,4,6-trihydroxy-3-geranylacetophenone (**8.1**)[1] and 4-(l'-geranyloxy)-2,6-dihydroxyacetophenone (**8.2**)[2] (Figure 8.1). Their structures were determined based on their 1D and 2D NMR data, and comparison with the literature data. The isolated compounds showed weak antiproliferative activities against the A2780 human ovarian cancer cell line with IC_{50} values of 62.5 and 82 μM, respectively.



8.1

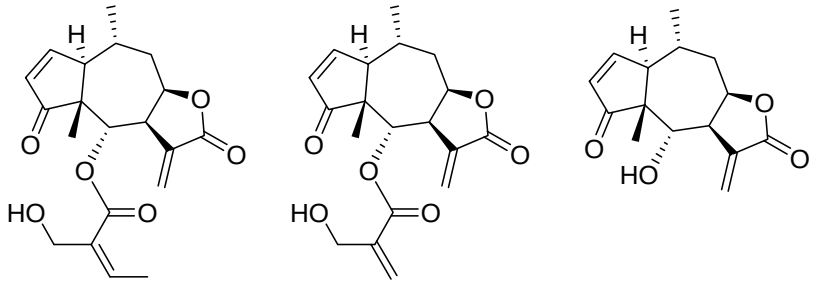


8.2

Figure 8.1. Structures of **8.1** and **8.2**

8.2.2. Chemical Investigation of Anisopappus longipes

Bioassay guided fractionation of an ethanol extract of *Anisopappus longipes* led to the isolation of the three known sesquiterpene lactones, helenalin-[(2-hydroxyethyl-3-methyl)acrylate] (**8.3**)[3], kingiolide (**8.4**)[4] and helenalin (**8.5**)[4] (Figure 8.2). Their structures were determined based on their 1D and 2D NMR data, and comparison with the literature data.



8.3 **8.4** **8.5**

Figure 8.2. Structures of **8.3**, **8.4**, and **8.5**

8.2.3. Chemical Investigation of Carissa obovata

Bioassay guided fractionation of an ethanol extract of *Carissa obovata* led to the isolation of the known cardenolide glycoside, elaeodendroside V (**8.6**) (Figure 8.3).[5] Their structure was determined based on its 1D and 2D NMR data, and comparison with the literature data.

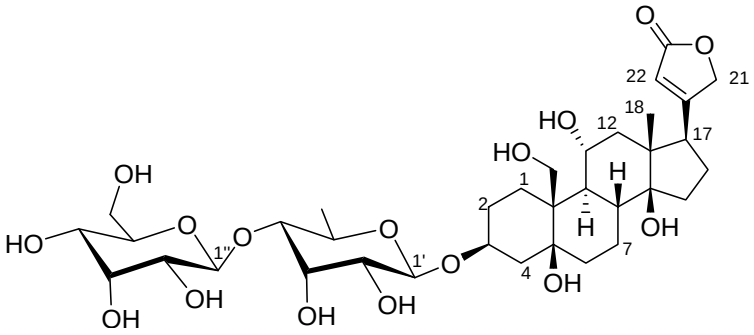


Figure 8.3. Structure of **8.6**

8.3. Suspended Extracts

Bioassay guided fractionation of extracts from the following species resulted in either inactive fractions towards A2780 cells or inadequate materials for further investigation.

- *Bemibicia* sp. MG 1877
- *Bemibicia* sp. MG 1878
- *Dalbergia* sp. MG 2179
- *Evodia* sp. MG 0993
- *Thecacoris horridum* MG 3009
- *Stephanostegia hildebrandtii* MG 3364
- *Helmiopsis pseudo-populus* MG 3258
- *Helmiopsis pseudo-populus* MG 3259
- *Helmiopsis pseudo-populus* MG3261
- *Helmiopsis* sp. MG 4348
- *Carissa septentrionalis* MG 4244
- Brown algae NB 04-05-72

References

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IX. Summary of Natural Products Isolated

Table 9.1. Summary of Natural Products Isolated

Compound	**Plant sources**	**Natural products class**	**IC_{50}[a] μM**	**New /Known**
scutianthraquinones A	*Scutia myrtina*	anthrone-anthraquinone	7.6,[a] 1.23,[b] 1.20[c]	new
scutianthraquinones B	*Scutia myrtina*	anthrone-anthraquinone	5.8,[a] 1.14,[b] 5.4[c]	new
scutianthraquinones C	*Scutia myrtina*	anthrone-anthraquinone	>16,[a] 3.14,[b] 15.4[c]	new
scutianthraquinones D	*Scutia myrtina*	bisanthrone-anthraquinone	4.3,[a] 3.68,[b] 5.6[c]	new
aloesaponarin I	*Scutia myrtina*	anthraquinone	>32,[a] 5.58,[b] 77.6[c]	known
cordylane A	*Cordyla madagascariensis* ssp. *madagascariensis*	cassane diterpenoid	10	new
cordylane B	*Cordyla madagascariensis* ssp. *madagascariensis*	cassane diterpenoid	36	new
cordylane C	*Cordyla madagascariensis* ssp. *madagascariensis*	cassane diterpenoid	>60	new
cordylane D	*Cordyla madagascariensis* ssp. *madagascariensis*	cassane diterpenoid	>60	new
elaeodendroside V	*Elaeodendron alluaudianum*	cardenolide glycoside	0.12,[a] 0.15[d]	new
elaeodendroside W	*Elaeodendron alluaudianum*	cardenolide glycoside	0.07,[a] 0.08[d]	new
(24*E*)-eupha-7,24-diene-1β,3β,11α,26-tetraol	*Cassipourea lanceolata*	euphane triterpenoids	25	new
(24*E*)-eupha-8-ene-3β,26-diol-11-one	*Cassipourea lanceolata*	euphane triterpenoids	25	new
(24*S*)-eupha-7,9(11)-diene-1β,3β,24,25-tetraol	*Cassipourea lanceolata*	euphane triterpenoids	32	new

[a]A2780 human ovarian cancer cell line. [b]chloroquine-resistant *Plasmodium falciparum* strain Dd2. [c]chloroquine-resistant *Plasmodium falciparum* strain FCM29. [d]U937 human histiocytic lymphoma cell line.

Table 9.1. Summary of Natural Products Isolated (*continued*)

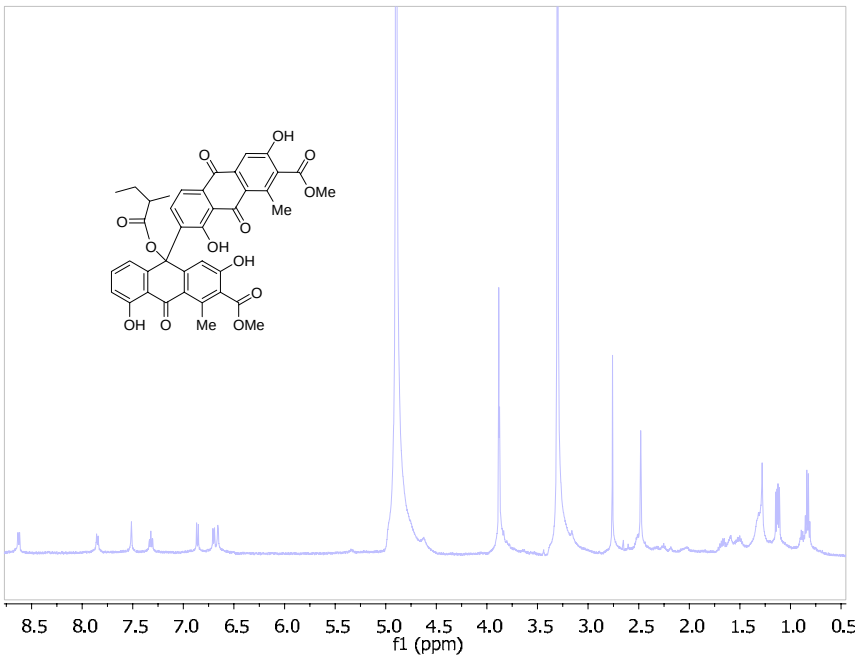
Compound	**Plant sources**	**Natural products class**	**IC_{50}[a] μM**	**New /Known**
2-[2-hydroxyl-hepadecyl]-1,4-hydroquinone	*Sclerocarya birrea* subsp. *caffra*	hydroquinone	0.29[e]	new
2-[2-hydroxyl-8(*Z*)-hepadecenyl]-1.4-hydroquinone	*Sclerocarya birrea* subsp. *caffra*	hydroquinone		new
2-[2-hydroxyl-10(*Z*)-hepadecenyl]-1.4-hydroquinone	*Sclerocarya birrea* subsp. *caffra*	hydroquinone		known
2-hepadecyl-1,4-hydroquinone	*Sclerocarya birrea* subsp. *caffra*	hydroquinone	0.09[e]	known
2-hepadecenyl-1,4-hydroquinone	*Sclerocarya birrea* subsp. *caffra*	hydroquinone		new /known
2-hepadecyl-1,4-benzoquinone	*Sclerocarya birrea* subsp. *caffra*	benzoquinone	0.21[e]	known
2-hepadecenyl-1,4-benzoquinone	*Sclerocarya birrea* subsp. *caffra*	benzoquinone		new
2,4,6-trihydroxy-3-geranylacetophenone	*Evodia* sp.	acetophenone	62.5	known
4-(l'-geranyloxy)-2,6-dihydroxyacetophenone	*Evodia* sp.	acetophenone	82	known
helenalin-[(2-hydroxyethyl-3-methyl)acrylate]	*Anisopappus longipes*	sesquiterpene lactone	n/t	known
kingiolide	*Anisopappus longipes*	sesquiterpene lactone	n/t	known
helenalin	*Anisopappus longipes*	sesquiterpene lactone	n/t	known

[a]A2780 human ovarian cancer cell line.
[e]The activity of the mixture.
n/t = not tested.

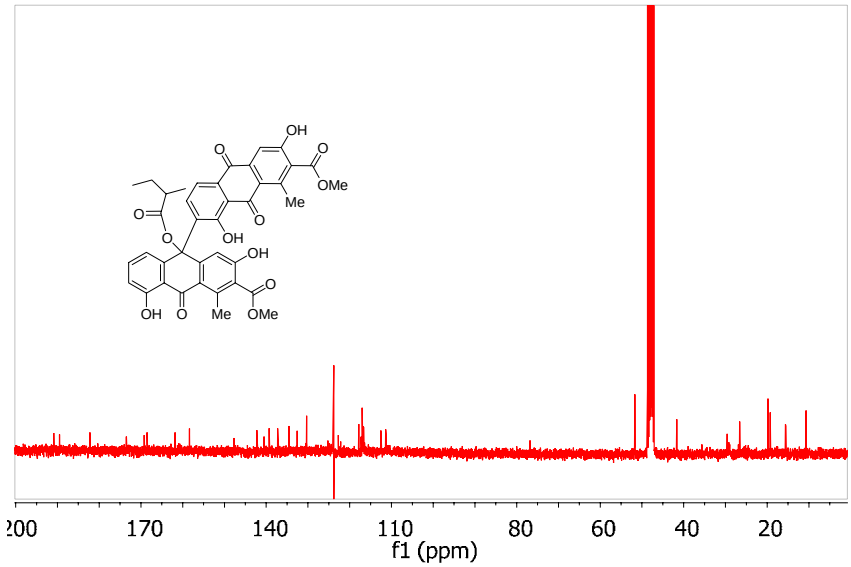
X. Overall Conclusions

The novel anthrone-anthraquinones discovered from *Scutia mytina* showed weak *in vitro* antiproliferative activity. However they displayed moderate antiplasmodial activity, and that provided evidence to make this class of compounds possible candidates for antimalaria drug development. The new cardenolide glycosides isolated from *Elaeodendron alluaudianum* showed potent *in vitro* antiproliferative activity. Their chances to be developed into anticancer drugs, however, were significantly reduced due to the typical unfavorable toxicity profiles exhibited by this class of compounds. The alkylated hydroquinones and benzoquinones obtained from *Sclerocarya birrea* subsp. *caffra* displayed strong *in vitro* antiproliferative activity. However, no *in vivo* anticancer profiles have been reported in the literature for this class of compounds. The new euphane triterpenoids discovered from *Cassipourea lanceolata* and the new cassane diterpenoids isolated from *Cordyla madagascariensis* ssp. *madagascariensis* showed weak antiproliferative activities, which diminished their opportunities to become anticancer drug leads. The fact that the synthesized sesquiterpenoid, (7*R**)-opposite-4(15)-ene-1β,7-diol, was inactive against the A2780 cells indicated that the highly potent antiproliferative activity of the mixture, which was isolated from *Cyphostemma laza* in very small quantity and contained this compound as a major component, was due to some other minor components in the mixture. That provided valuable information for the further investigation on this plant species.

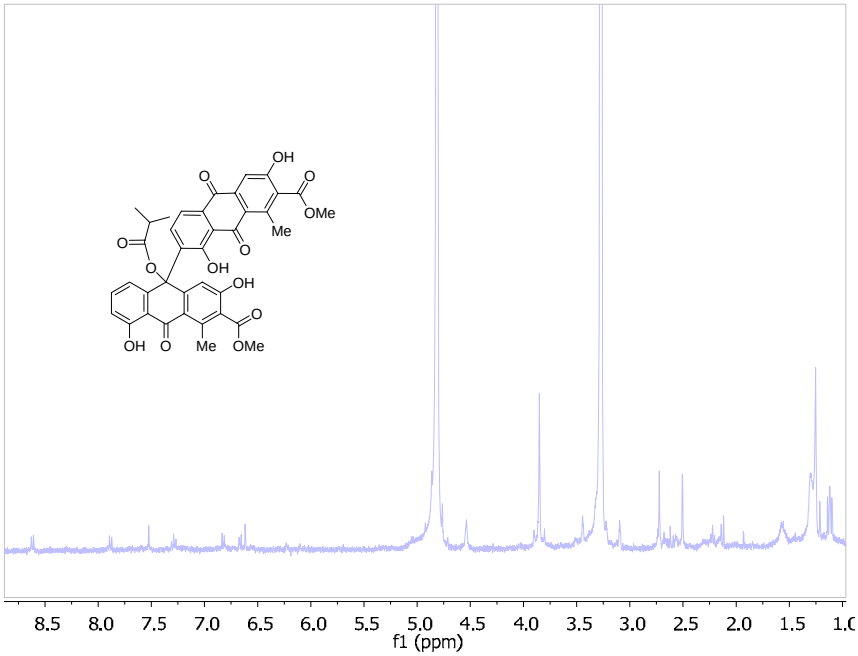
APPENDIX



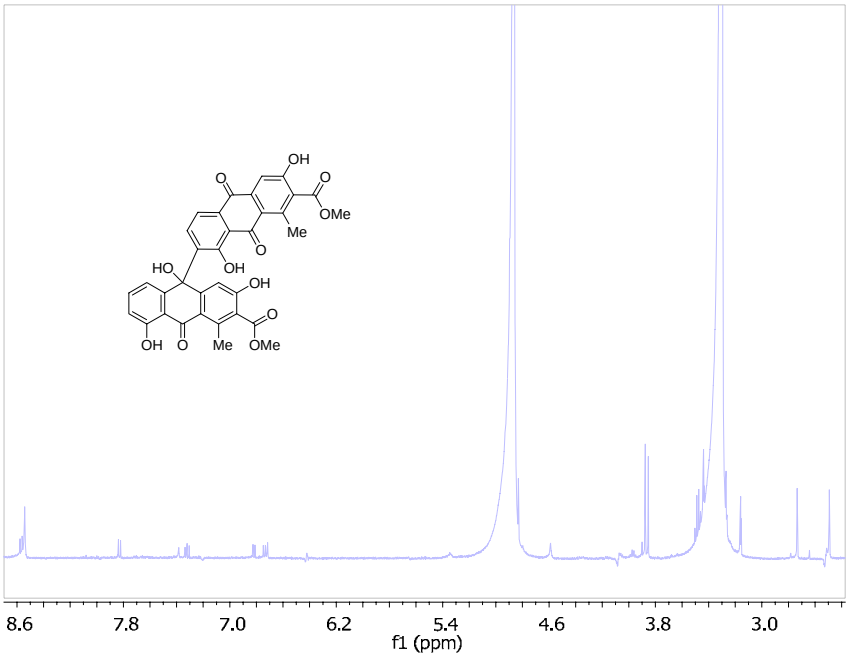
^{1}H NMR of **2.1** (CD_3OD)



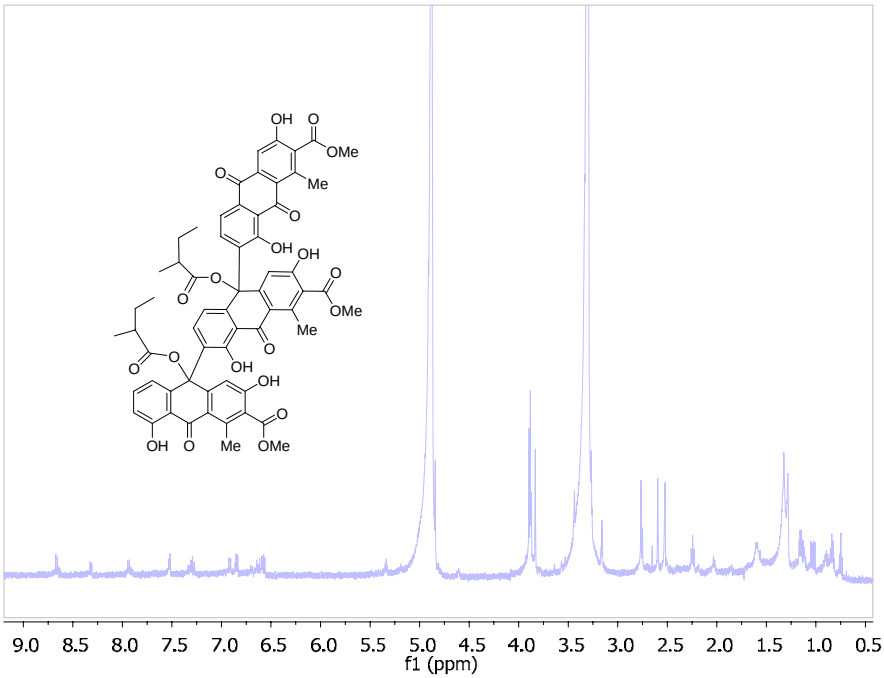
^{13}C NMR of **2.1** (CD_3OD)



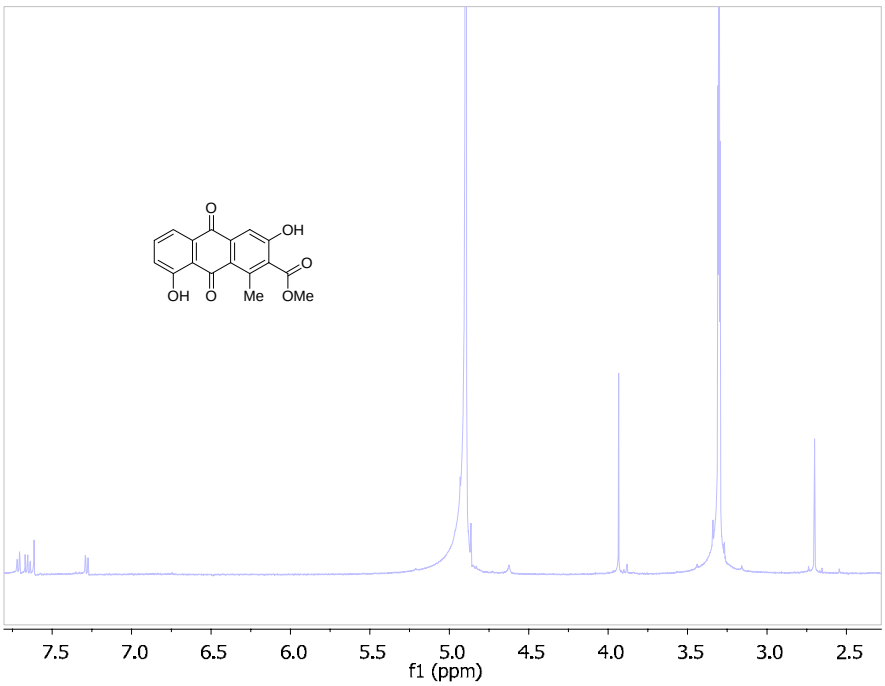
^{1}H NMR of **2.2** (CD_3OD)



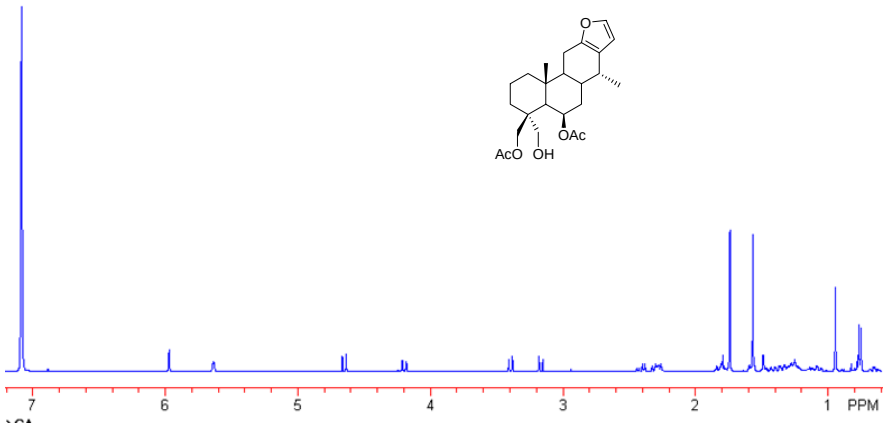
^{1}H NMR of **2.3** (CD_3OD)



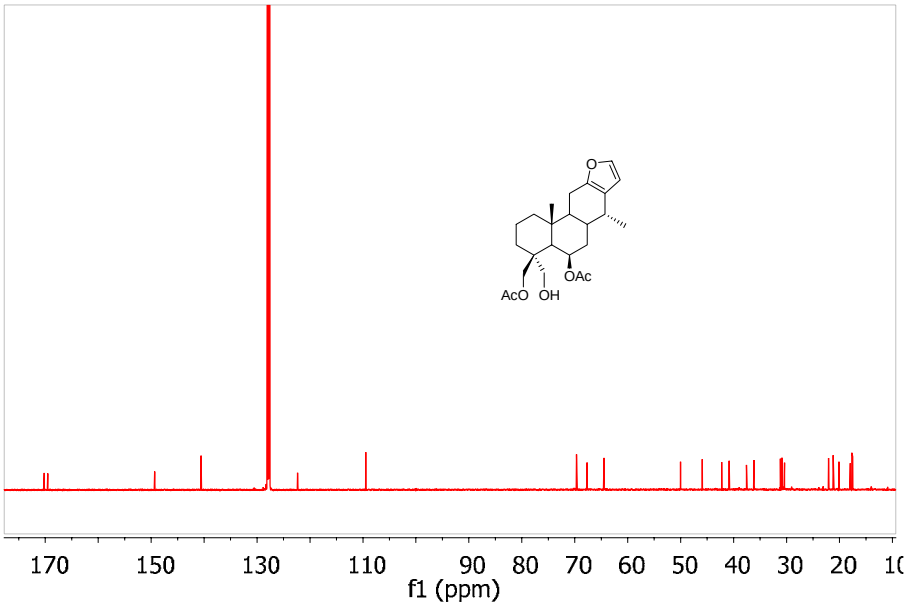
^{1}H NMR of **2.4** (CD_3OD)



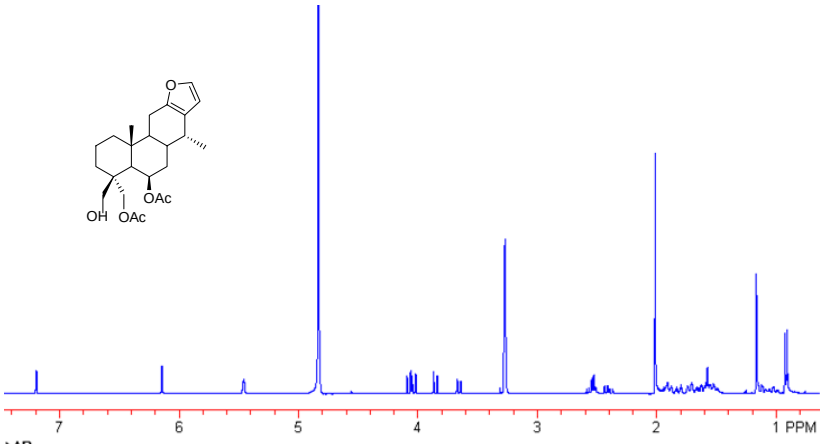
^{1}H NMR of **2.5** (CD_3OD)



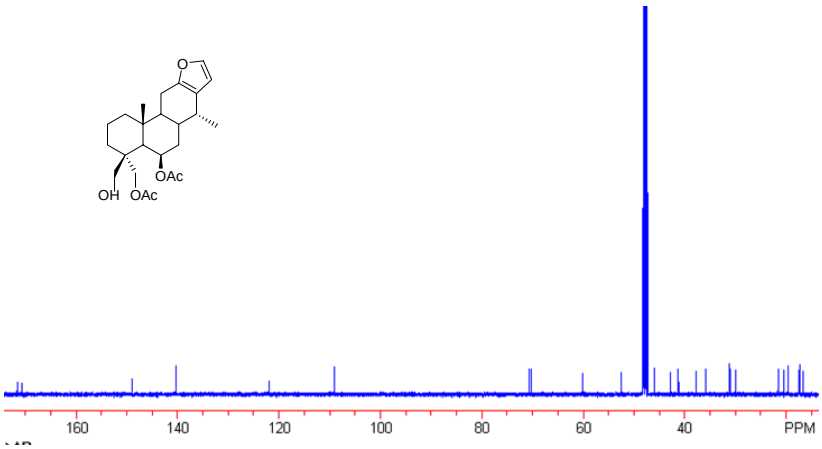
^{1}H NMR of **3.1** (C_6D_6)



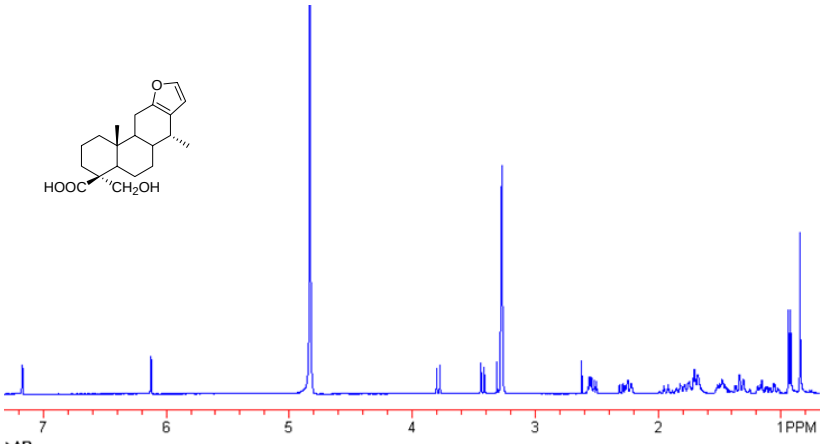
^{13}C NMR of **3.1** (C_6D_6)



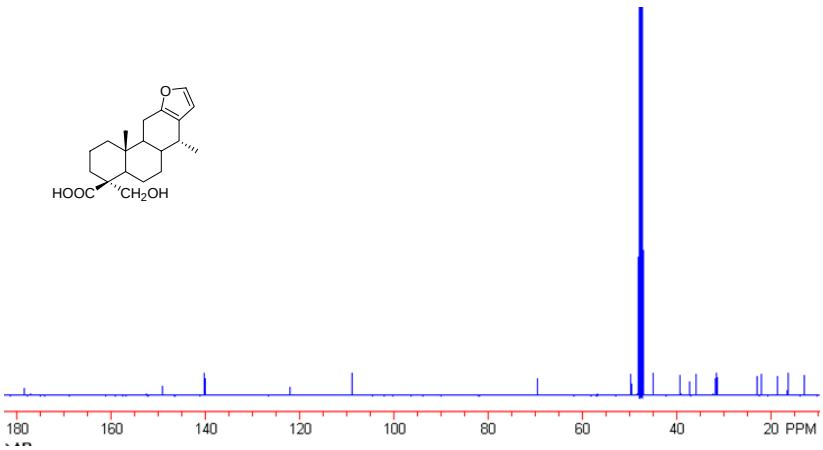
^{1}H NMR of **3.2** (CD_3OD)



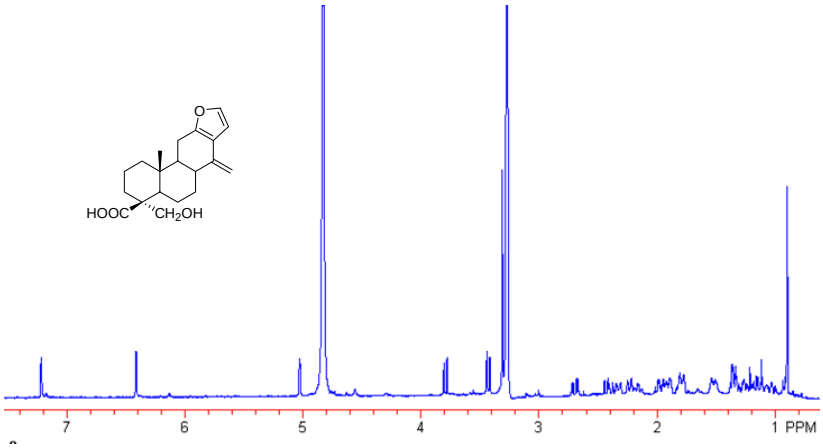
^{13}C NMR of **3.2** (CD_3OD)



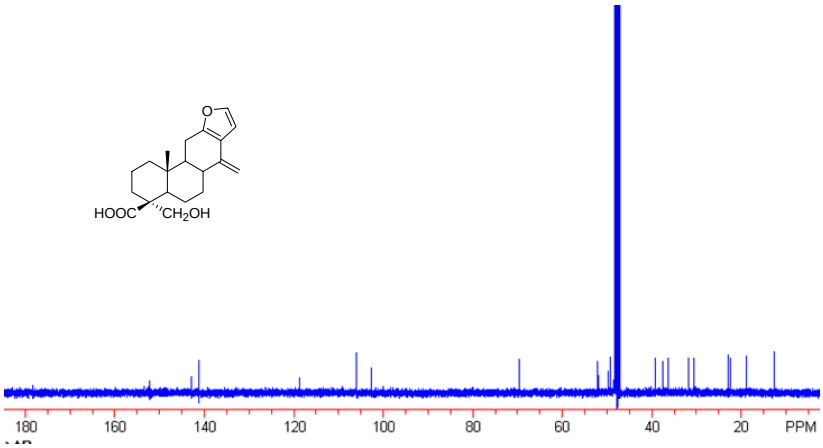
^{1}H NMR of **3.3** (CD_3OD)



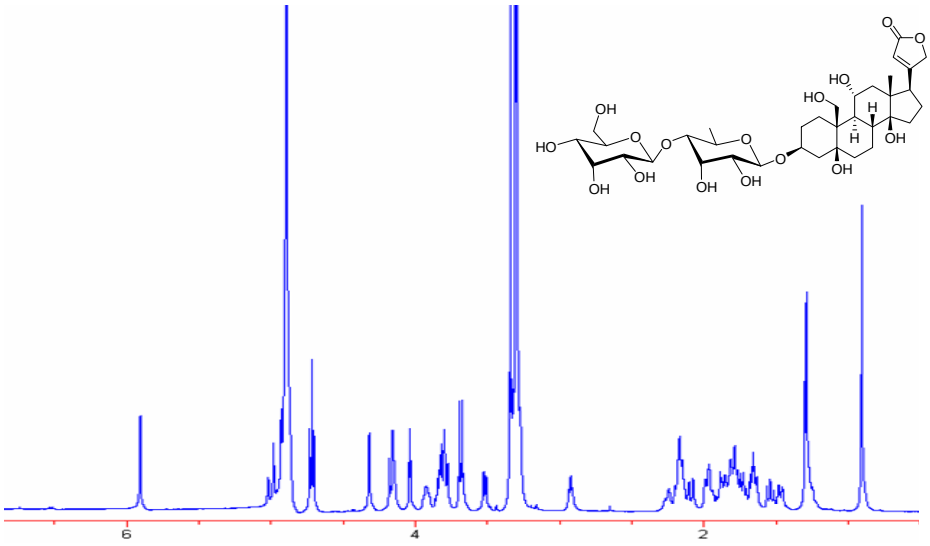
^{13}C NMR of **3.3** (CD_3OD)



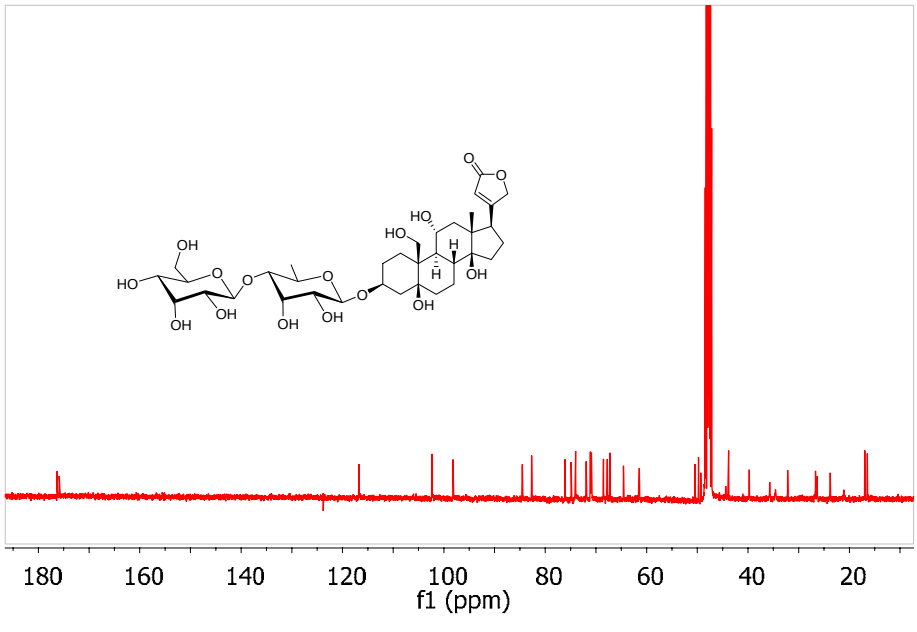
^{1}H NMR of **3.4** (CD_3OD)



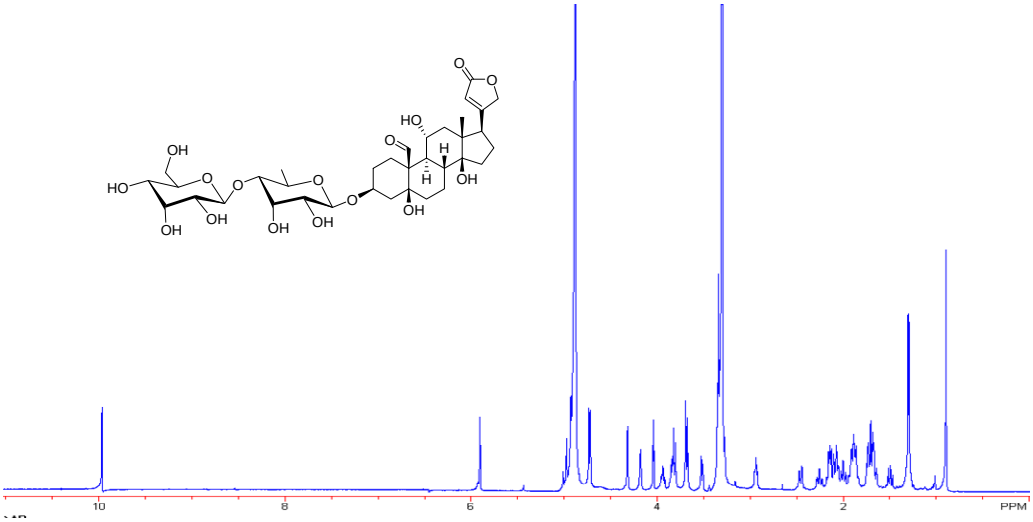
^{13}C NMR of **3.4** (CD_3OD)



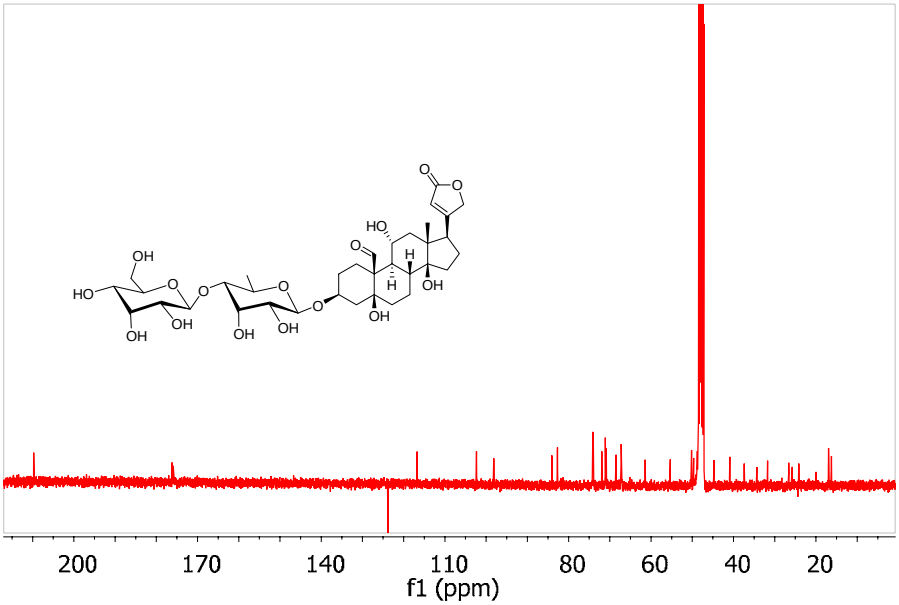
^{1}H NMR of **4.1** (CD_3OD)



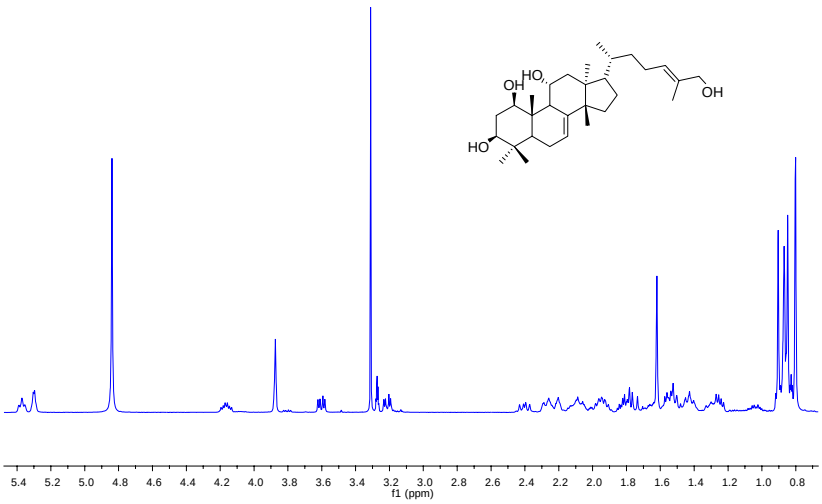
^{13}C NMR of **4.1** (CD_3OD)



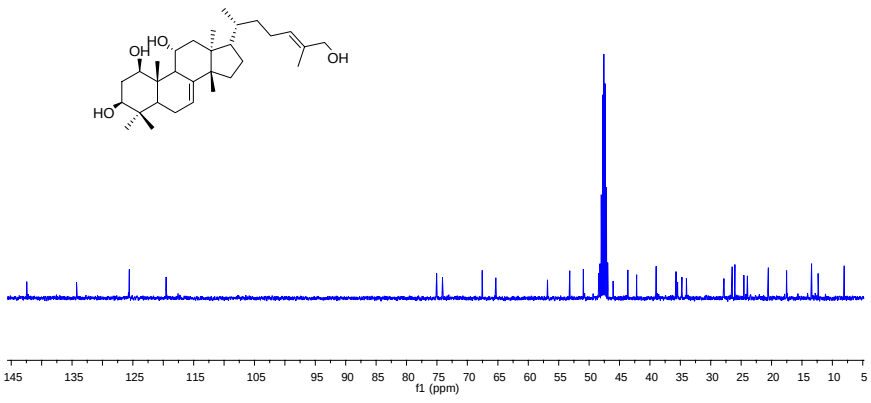
^{1}H NMR of **4.2** (CD_3OD)



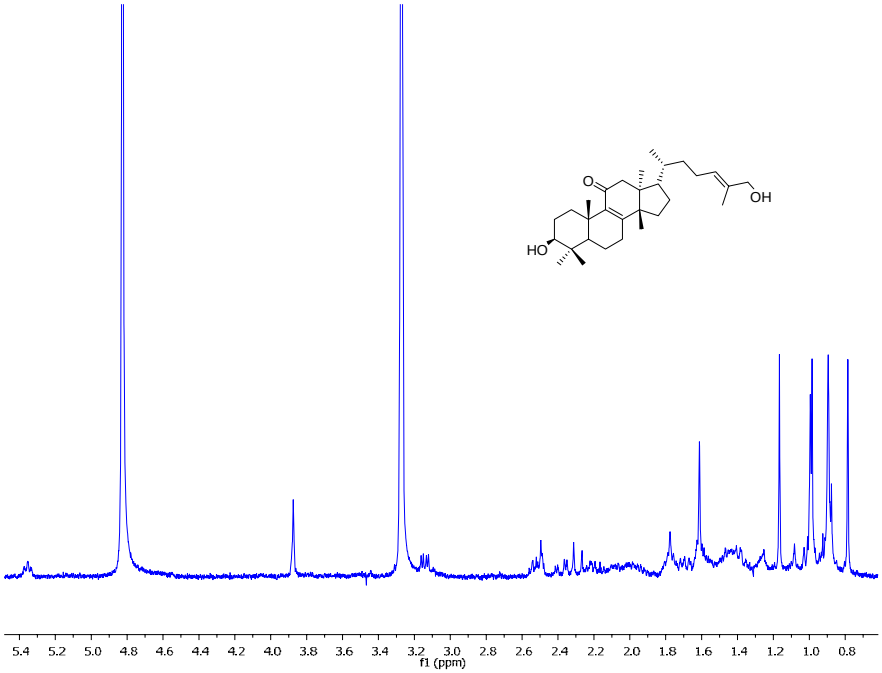
^{13}C NMR of **4.2** (CD_3OD)



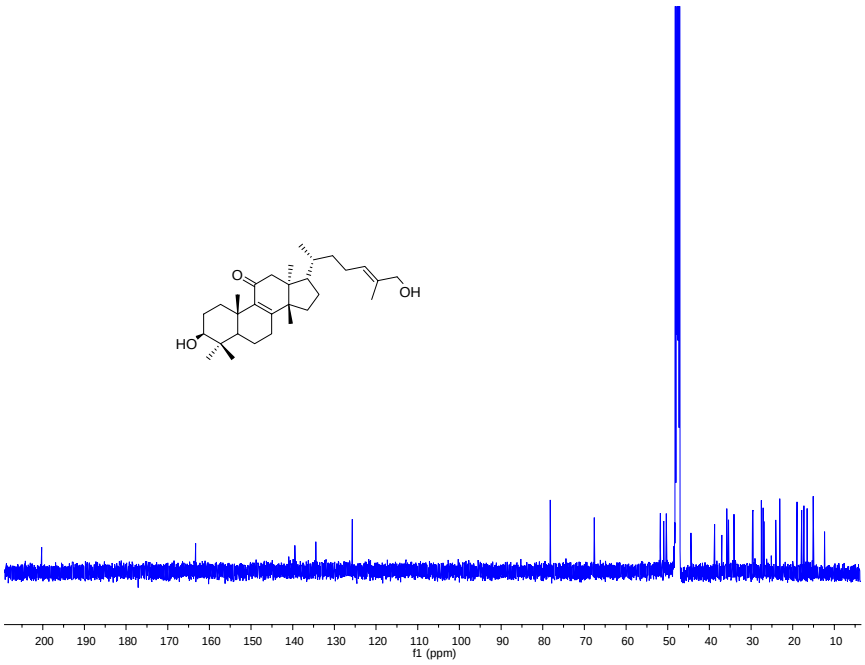
^{1}H NMR of **5.1** (CD_3OD)



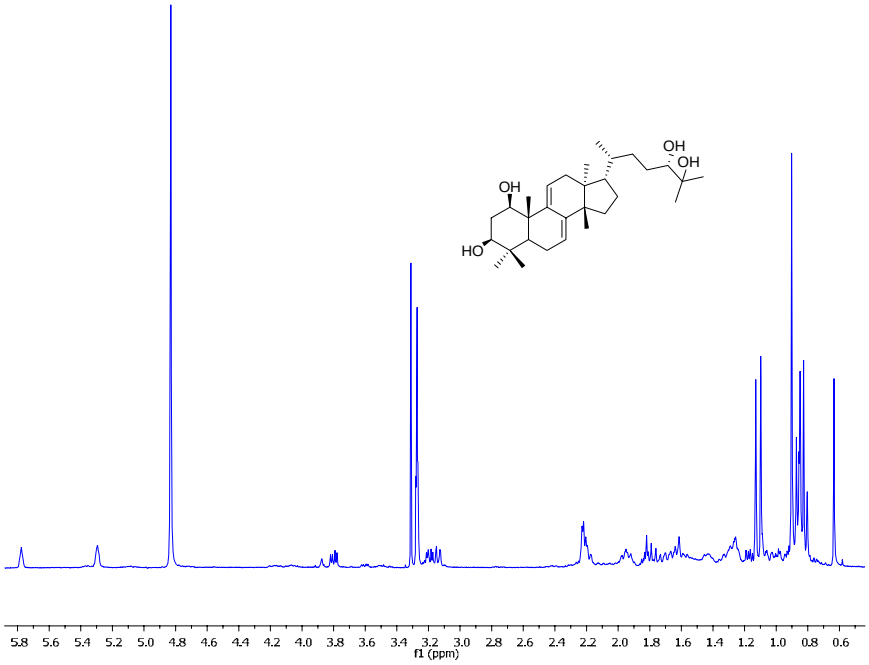
^{13}C NMR of **5.1** (CD_3OD)



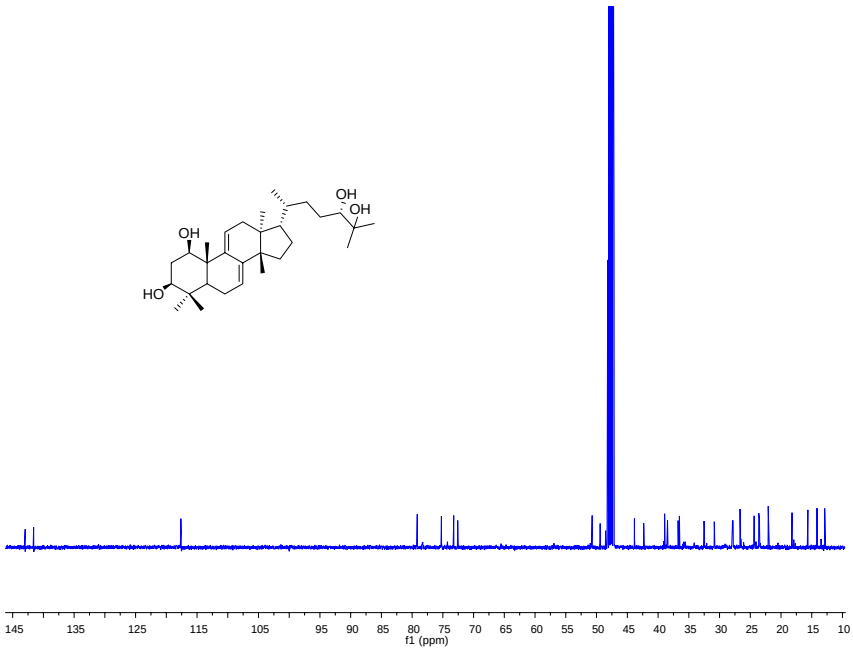
^{1}H NMR of **5.2** (CD_3OD)



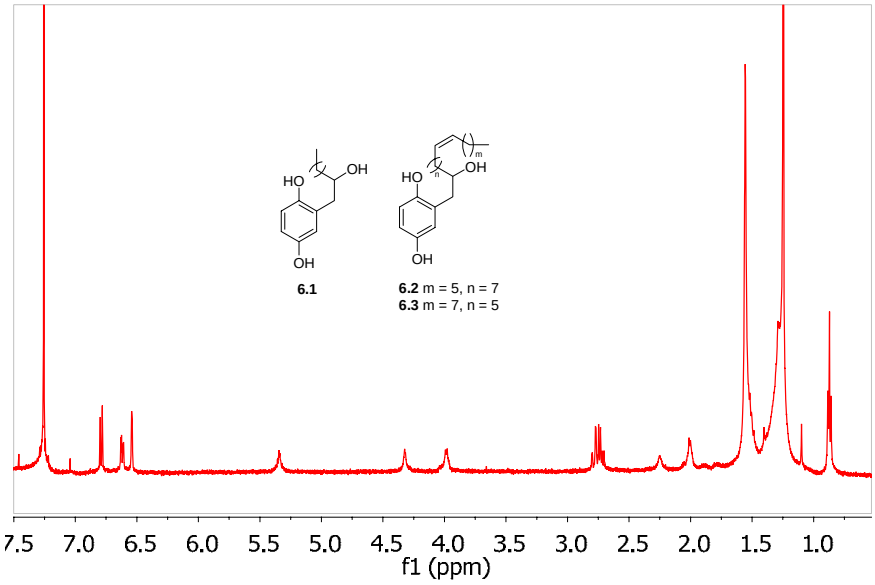
^{13}C NMR of **5.2** (CD_3OD)



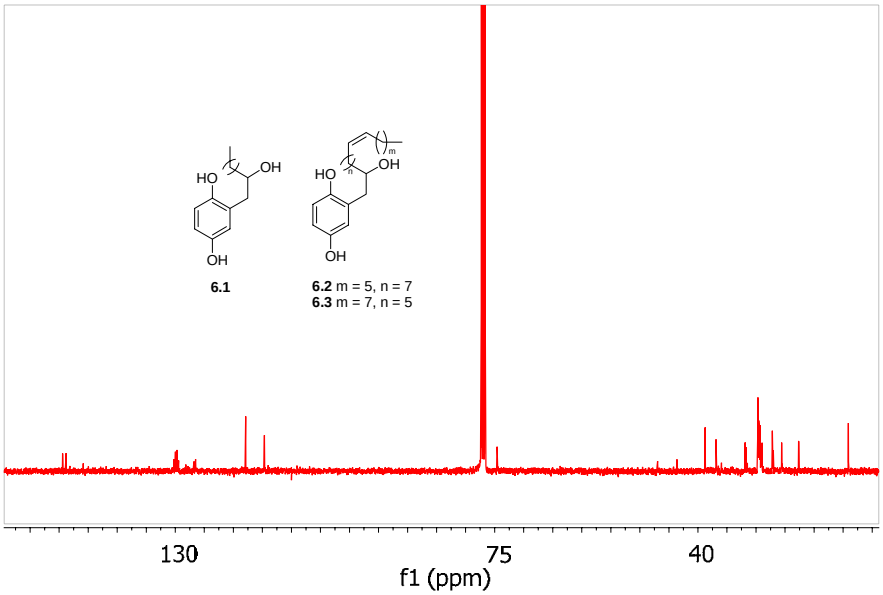
^{1}H NMR of **5.3** (CD_3OD)



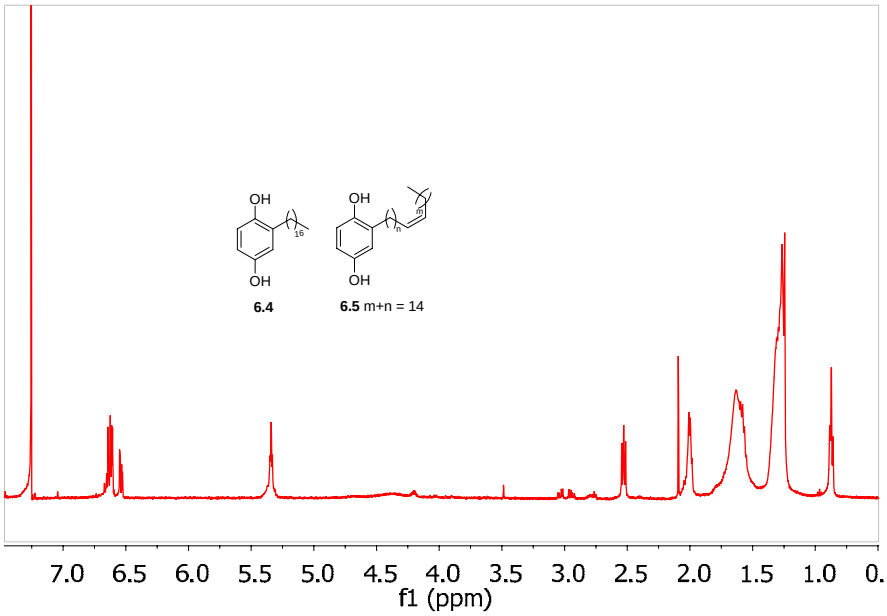
^{13}C NMR of **5.3** (CD_3OD)



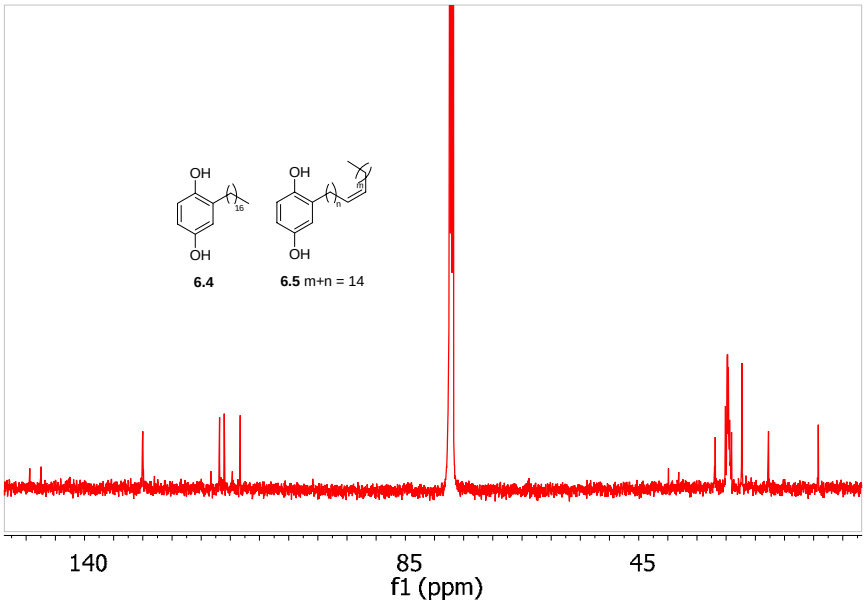
^{1}H NMR of fraction VII (**6.1**, **6.2** and **6.3**) ($CDCl_3$)



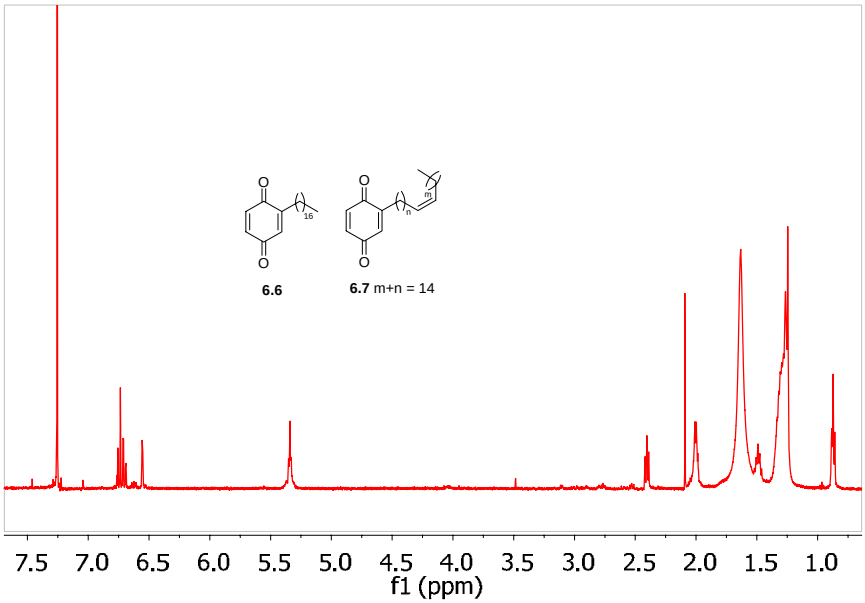
^{13}C NMR of fraction VII (**6.1**, **6.2** and **6.3**) ($CDCl_3$)



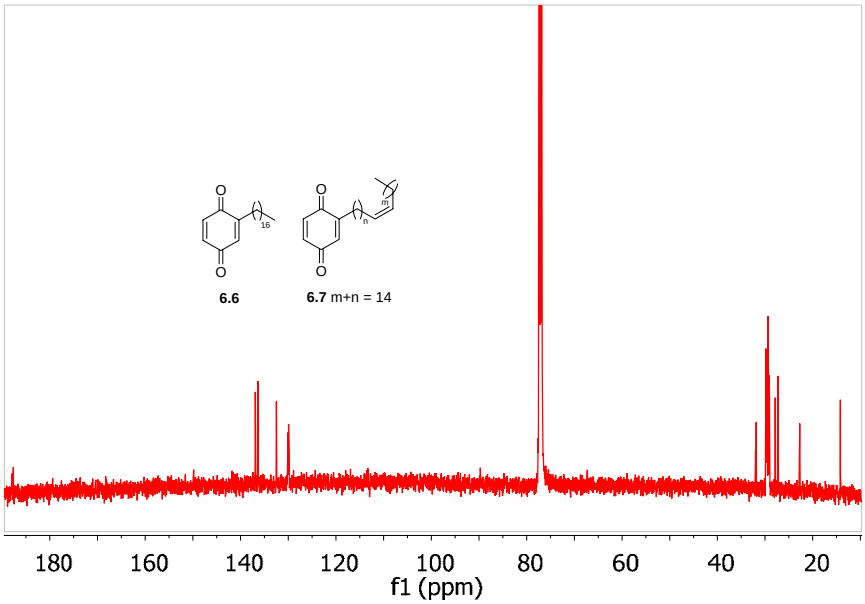
^{1}H NMR of fraction X (**6.4** and **6.5**) ($CDCl_3$)



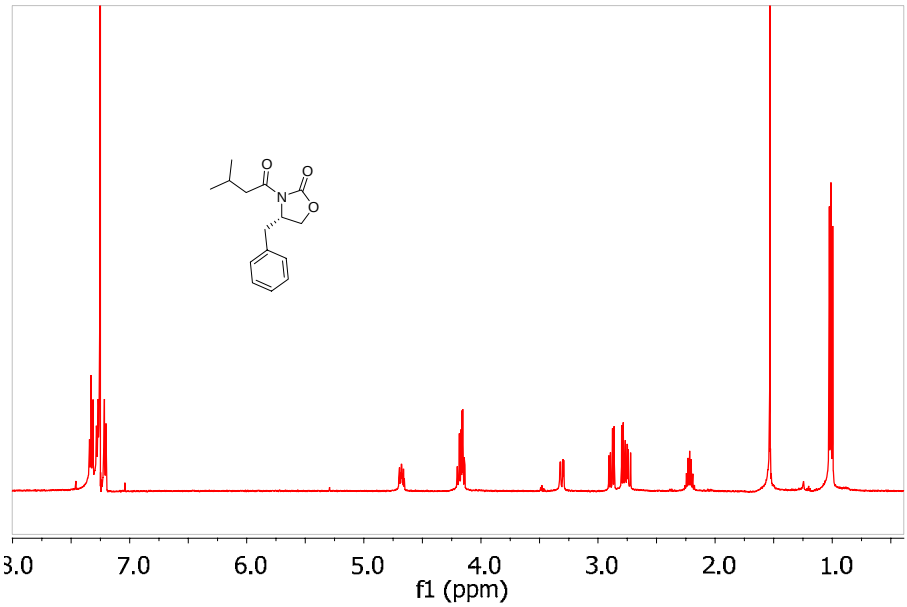
^{13}C NMR of fraction X (**6.4** and **6.5**) ($CDCl_3$)



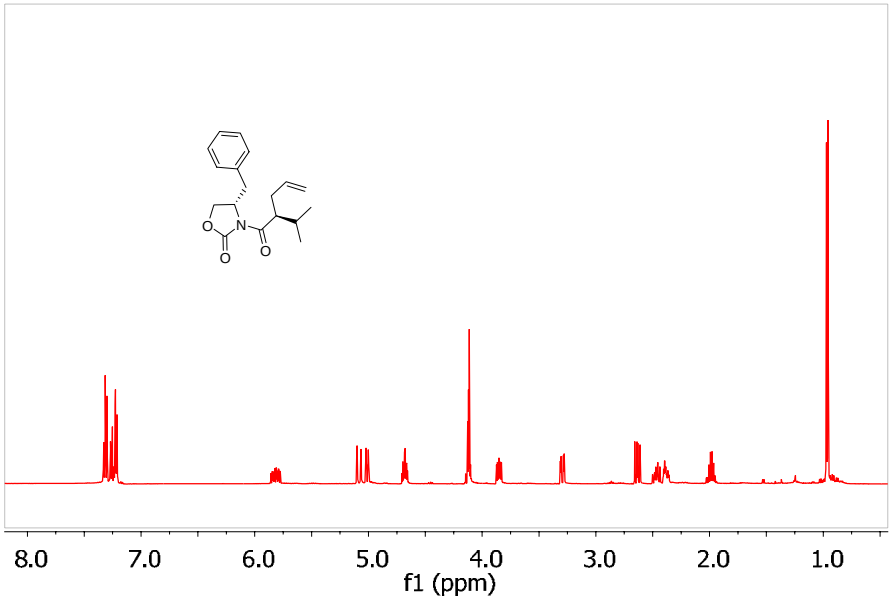
^{1}H NMR of fraction XI (**6.6** and **6.7**) ($CDCl_3$)



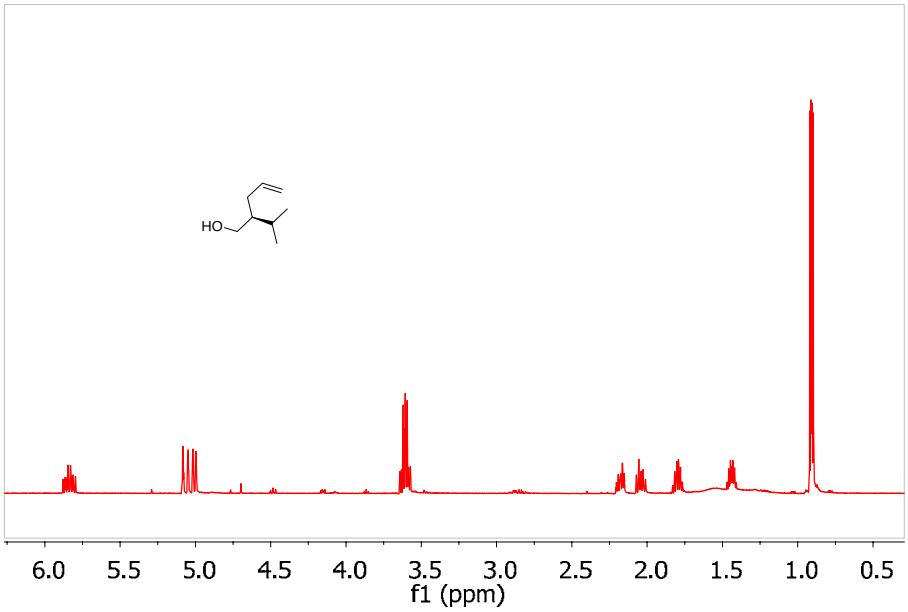
^{13}C NMR of fraction XI (**6.6** and **6.7**) ($CDCl_3$)



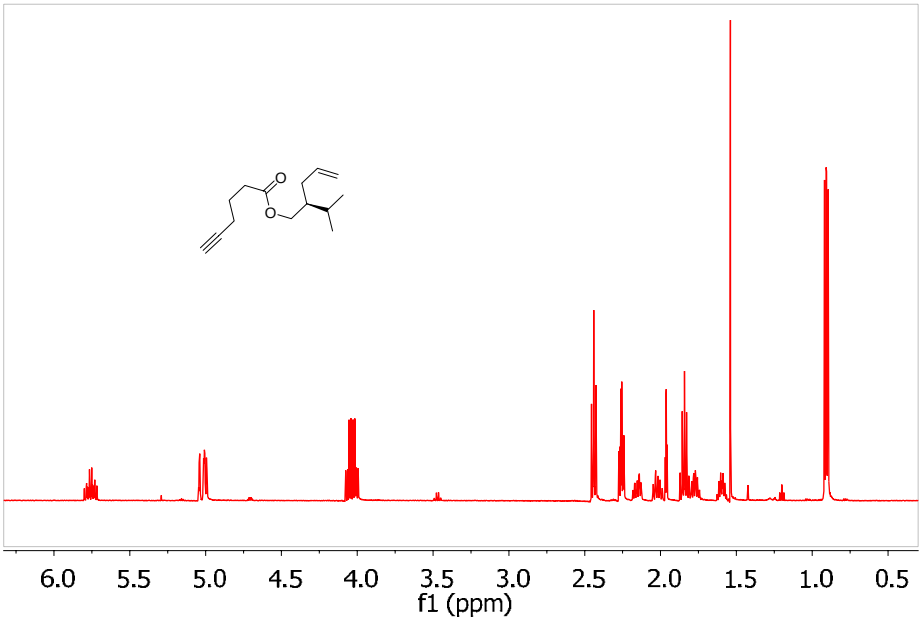
^{1}H NMR of **7.6** ($CDCl_3$)



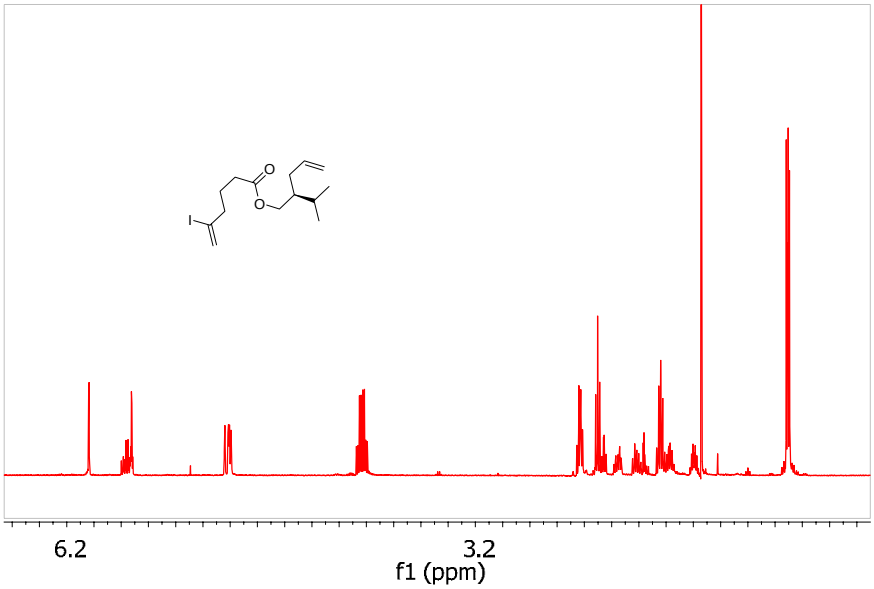
^{1}H NMR of **7.7** ($CDCl_3$)



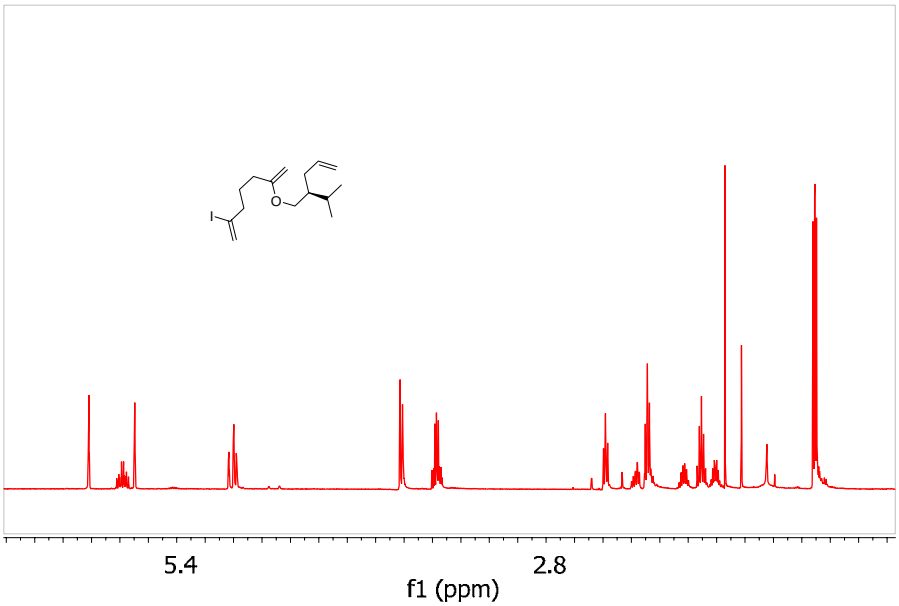
^{1}H NMR of **7.8** ($CDCl_3$)



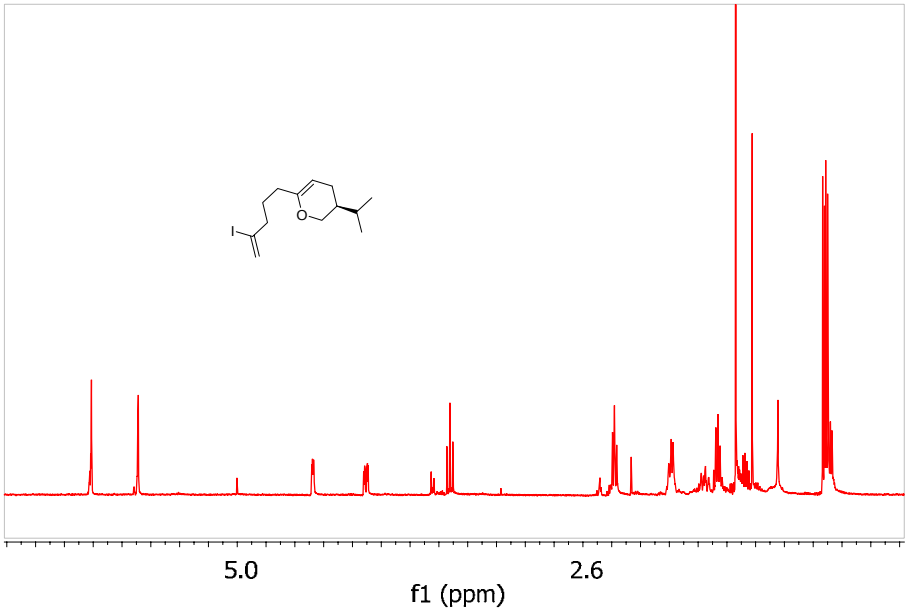
^{1}H NMR of **7.9** ($CDCl_3$)



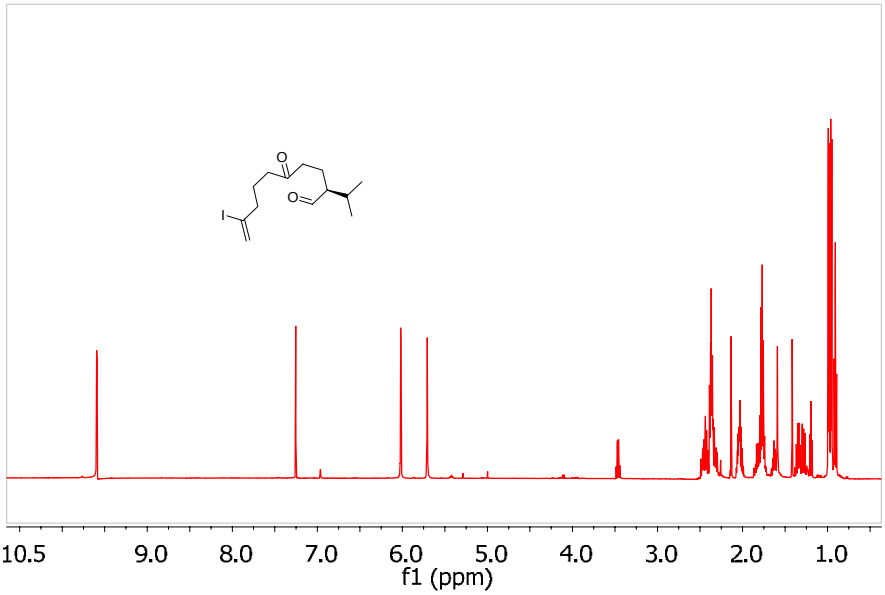
^{1}H NMR of **7.10** ($CDCl_3$)



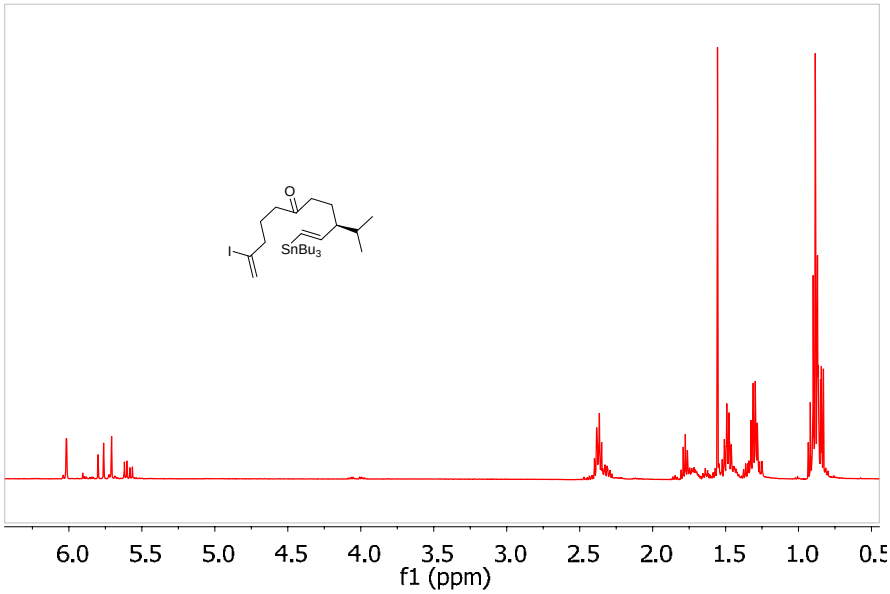
^{1}H NMR of **7.11** ($CDCl_3$)



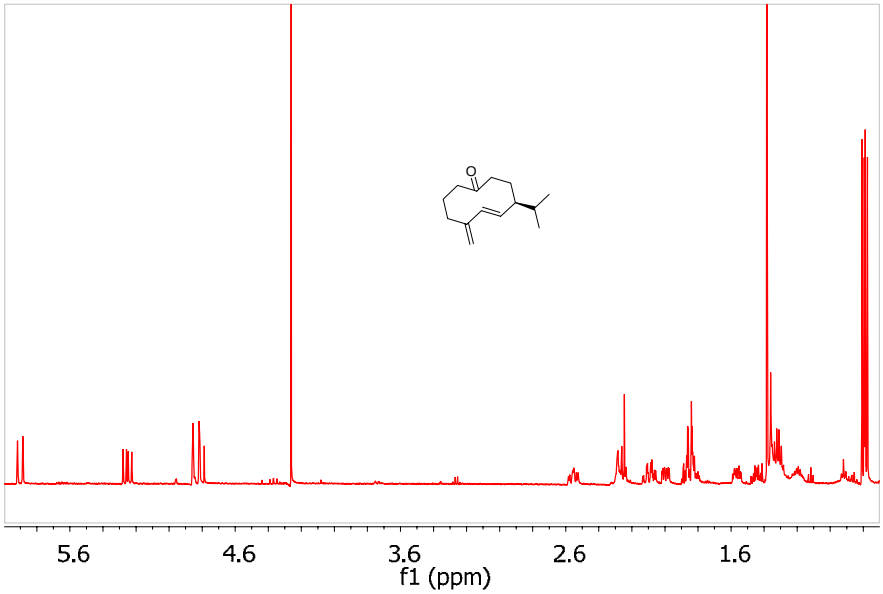
^{1}H NMR of **7.12** ($CDCl_3$)



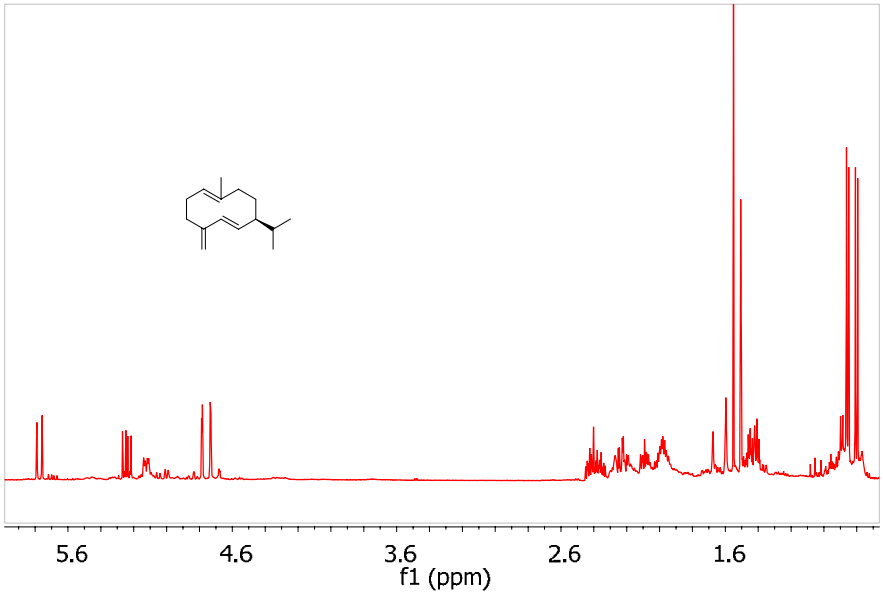
^{1}H NMR of **7.13** ($CDCl_3$)



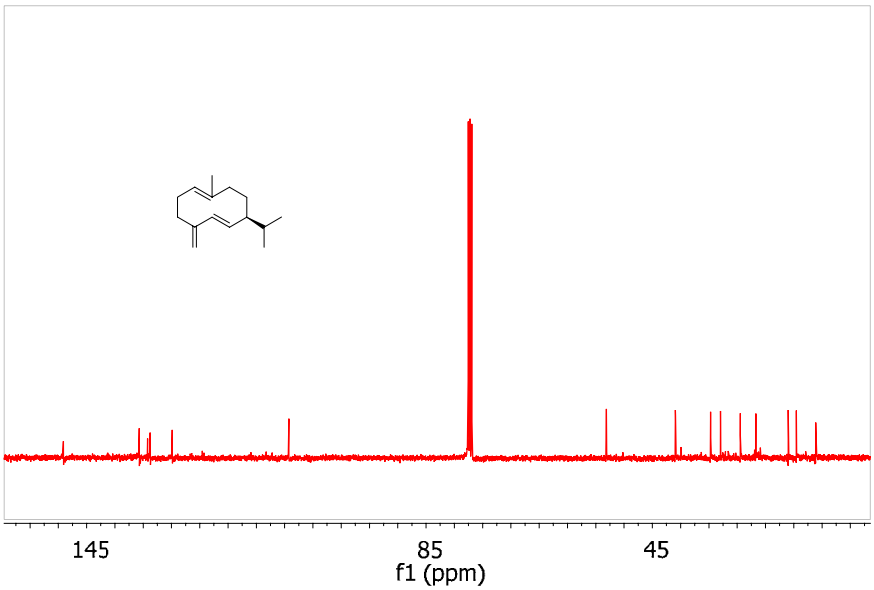
^{1}H NMR of **7.14** ($CDCl_3$)



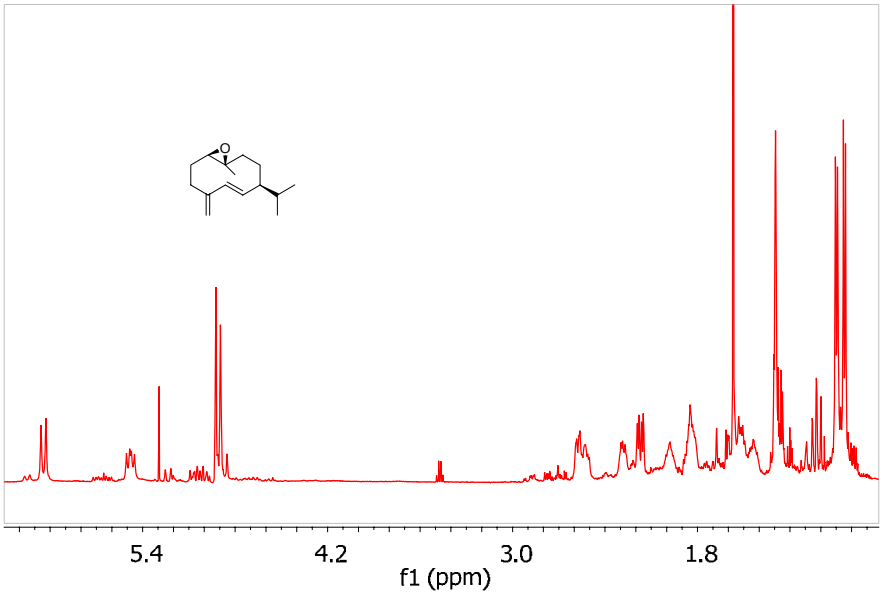
^{1}H NMR of **7.4** (C_6D_6)



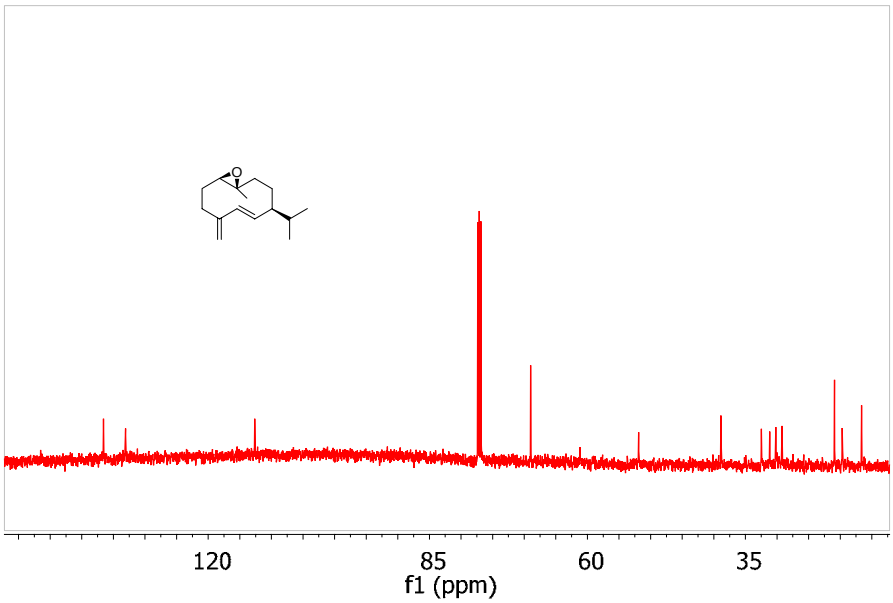
^{1}H NMR of **7.3** ($CDCl_3$)



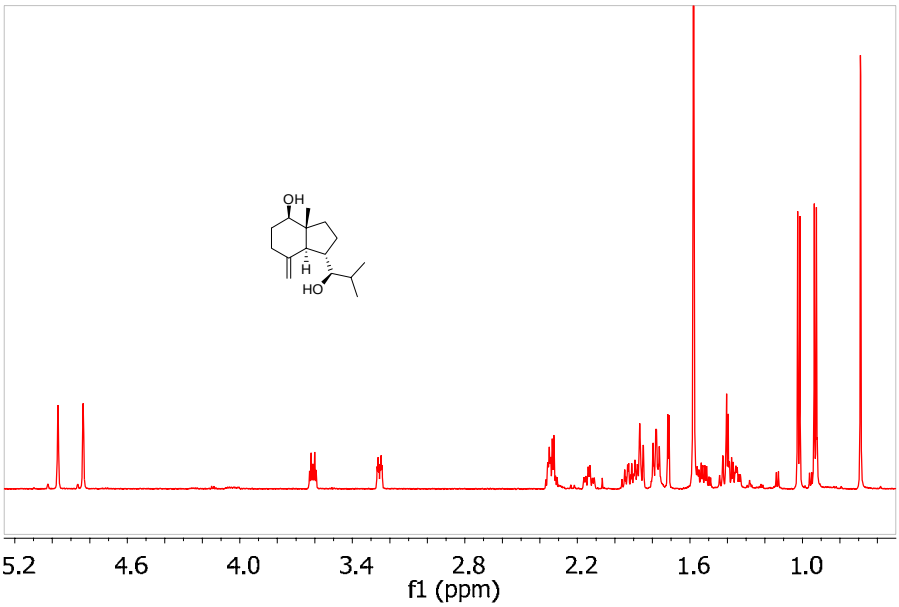
^{13}C NMR of **7.3** ($CDCl_3$)



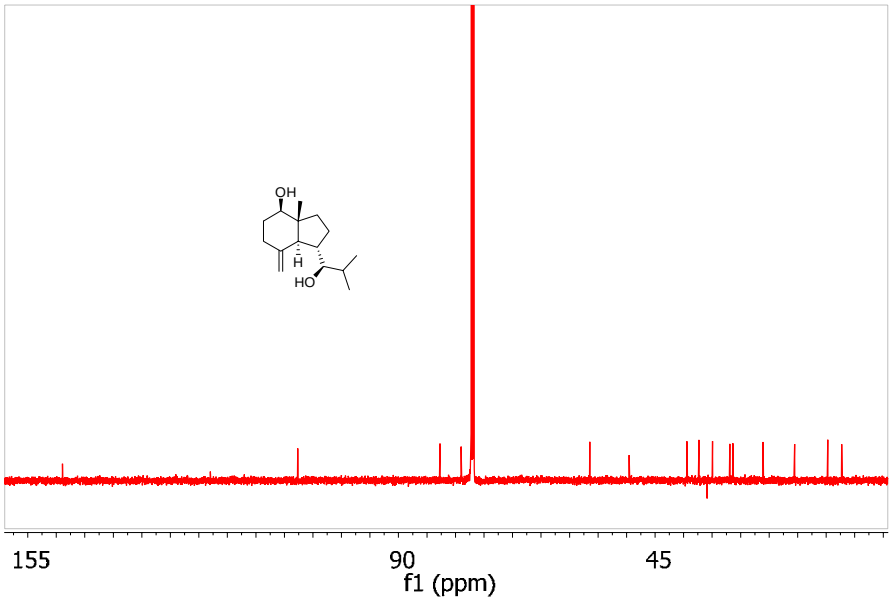
^{1}H NMR of **7.4** ($CDCl_3$)



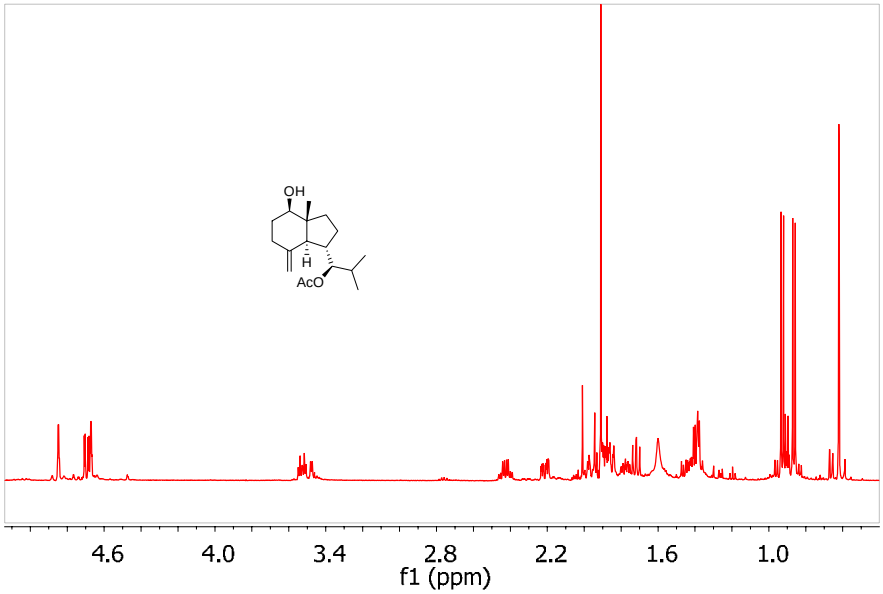
^{13}C NMR of **7.4** ($CDCl_3$)



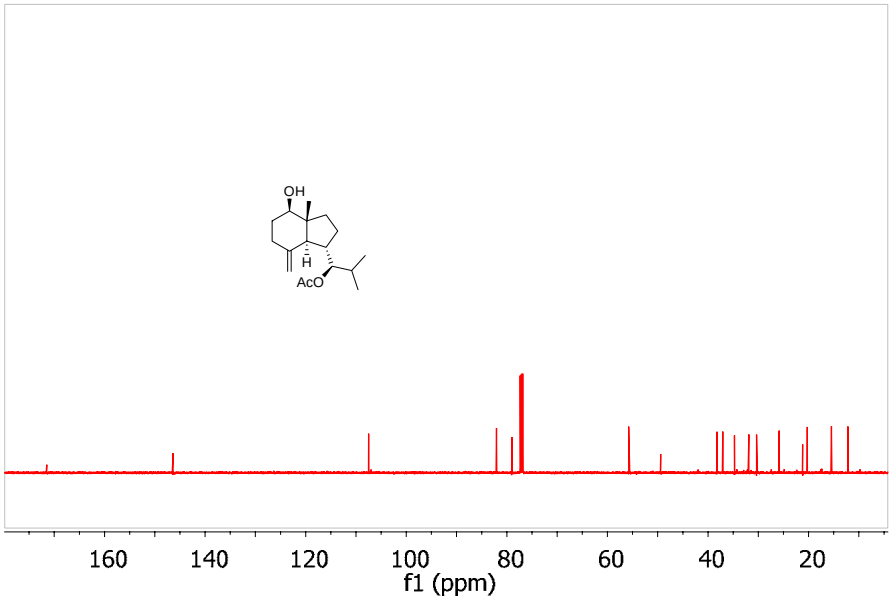
^{1}H NMR of **7.1** ($CDCl_3$)



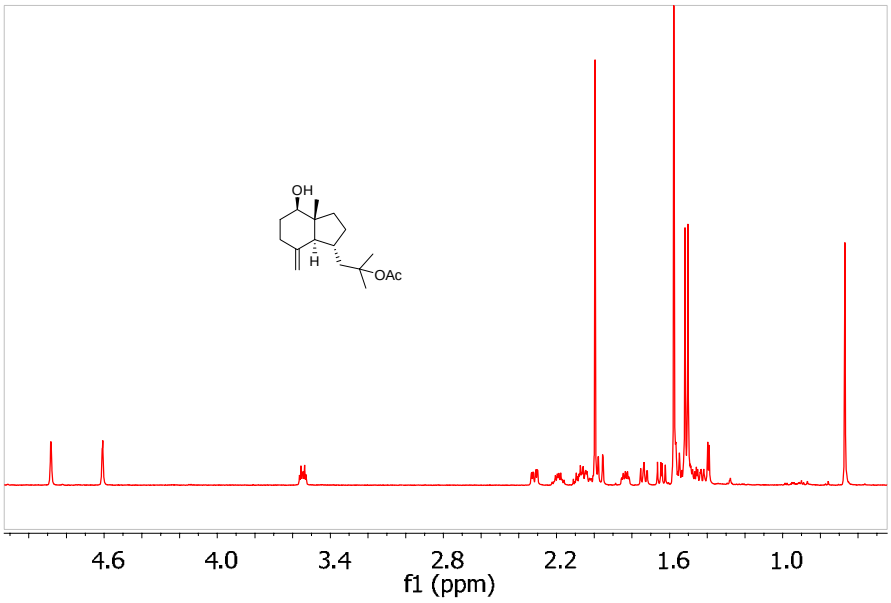
^{13}C NMR of **7.1** ($CDCl_3$)



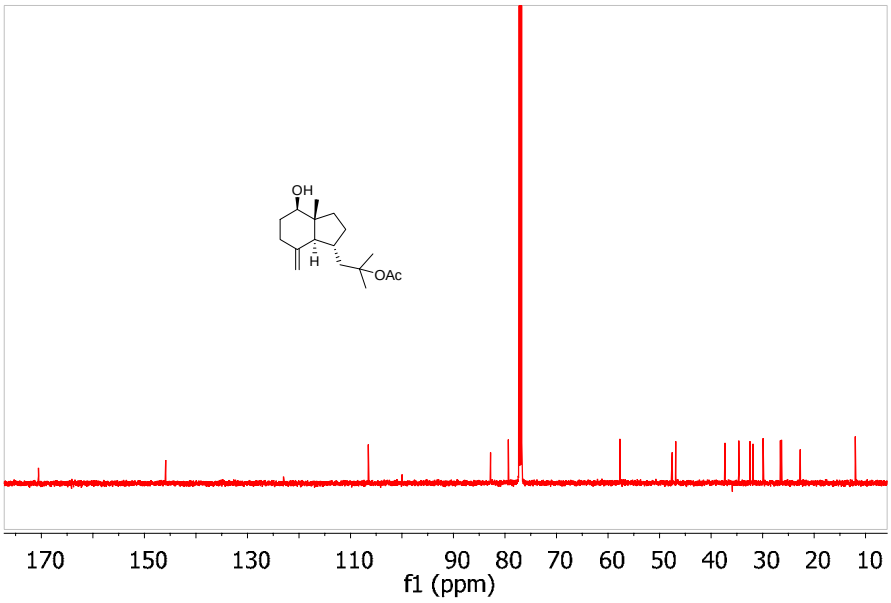
^{1}H NMR of **7.15** ($CDCl_3$)



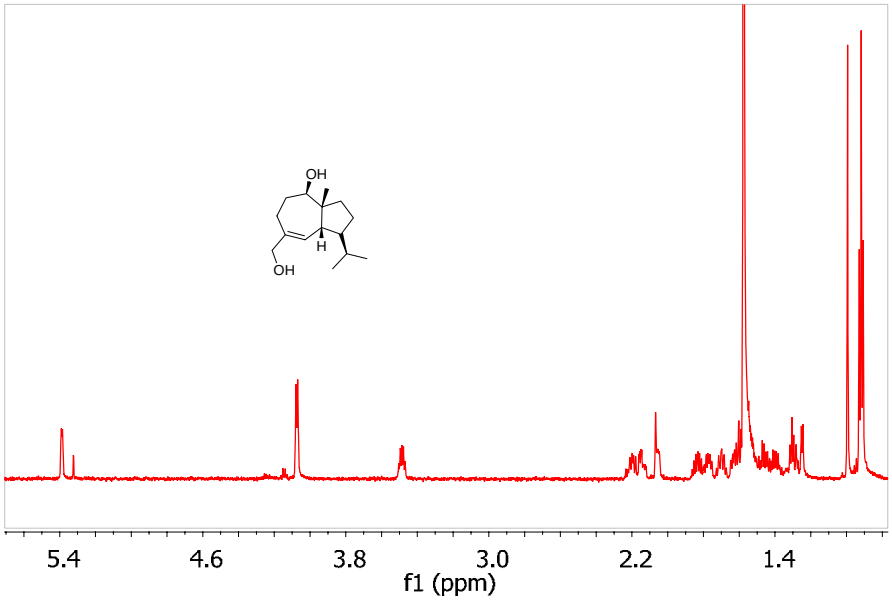
^{13}C NMR of **7.15** ($CDCl_3$)



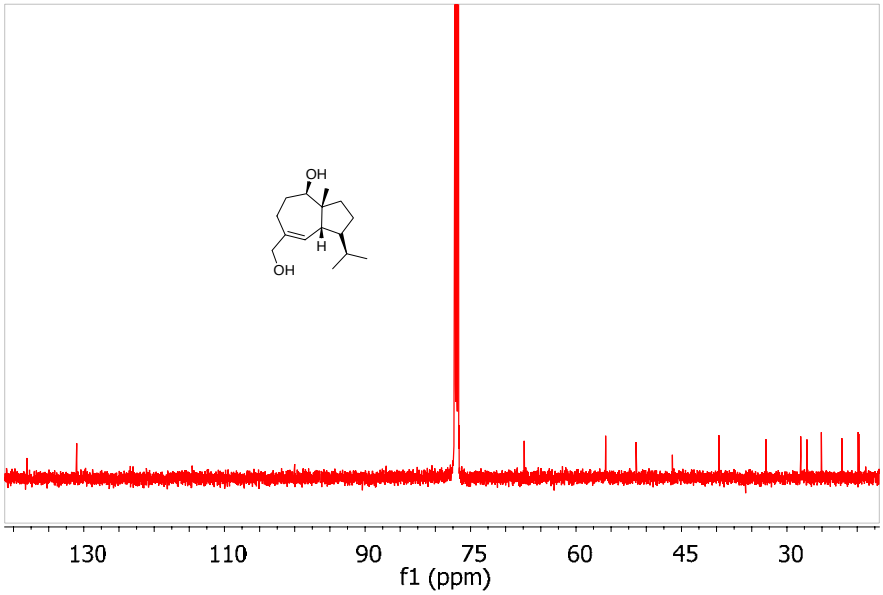
^{1}H NMR of **7.16** ($CDCl_3$)



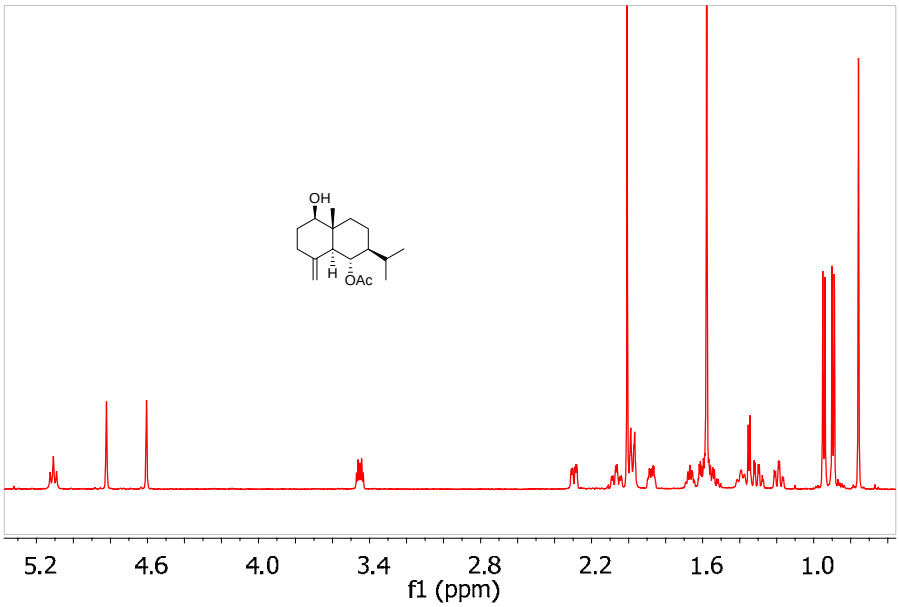
^{13}C NMR of **7.16** ($CDCl_3$)



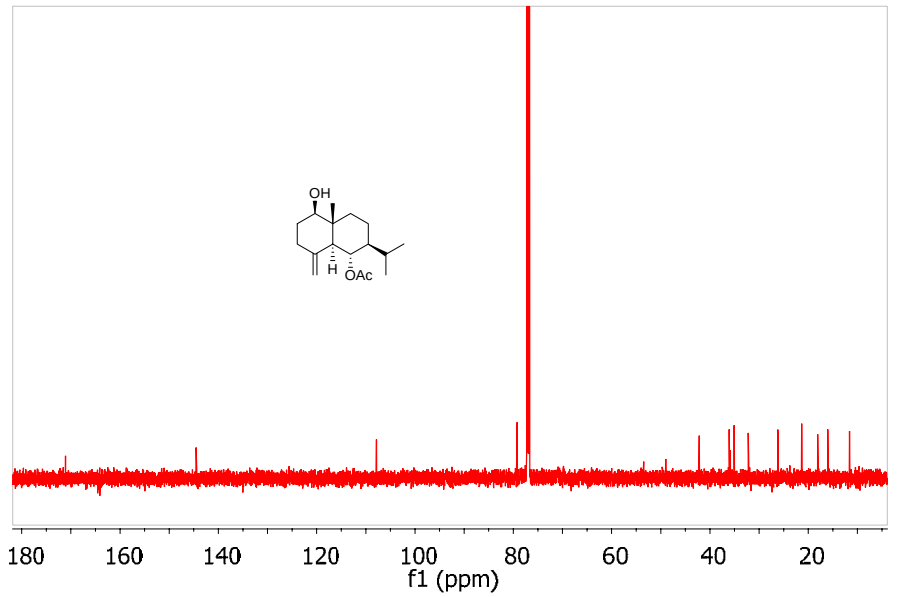
^{1}H NMR of **7.17** ($CDCl_3$)



^{13}C NMR of **7.17** ($CDCl_3$)



^{1}H NMR of **7.18** ($CDCl_3$)



^{13}C NMR of **7.18** ($CDCl_3$)
