Isolation and Characterization of Phytoconstituents from Myanmar Medicinal Plants

Dissertation

zur Erlangung des akademischen Grades
doctor rerum naturalium (Dr. rer. nat.)

vorgelegt der

Mathematisch-Naturwissenschaftlich-Technischen Fakultät
(mathematisch-naturwissenschaftlicher Bereich)
der Martin-Luther-Universität Halle-Wittenberg

von M. Sc. Myint Myint Khine
geboren am 24. September 1964 in Yangon (Myanmar)

Gutachter:

1. Prof. Dr. Ludger Wessjohann
2. Prof. Dr. Karsten Krohn

Halle (Saale), den 03.03.2006

DECLARATION

I hereby declare that I have carried out the analyses and written the thesis myself and that I did not use any devices or received relevant help from any persons other than those mentioned in the text. This dissertation has not been submitted before.

…31.01.06…… ……..

Date… Signature

Acknowledgements

The present study was carried out at the Leibniz Institute for Plant Biochemistry [Halle (Saale)]. Financial support was provided by the Gottlieb Daimler- und Karl Benz-Stiftung. This study would not have succeeded without the permission of the Ministry for Education from Myanmar. In addition, I wish to express my appreciation and gratitude to the many people who have in one way or another helped me over the course of study. In particular I would like to thank the following:

Prof. Ludger Wessjohann, my main supervisor, for inviting me to do my Ph. D. work at IPB and for the encouragements, allowing me to develop at my own pace.

Dr. Norbert Arnold and **Dr. Katrin Franke**, my supervisors, for the advice and encouragement as well as supporting my ideas.

Dr. A. Porzel for the discussions and NMR-spectra measurement.

Mrs. M. Süsse for the NMR-, IR- and UV- spectra measurement.

Mrs. C. Kuhnt, **Mrs. M. Lerbs** and **Dr. J. Schmidt** for mass-spectra measurement.

Mrs. M. Kummer for antifungal test.

Mrs. G. Hahn for HPLC measurement.

Prof. Dr. Aung Aung Min, Department of Botany, Yangon University, for identification of plant material.

Associate Prof. Dr. Daw Hla Ngwe, Department of Chemistry, Yangon University, for collection of plant material.

Prof. K. Merzweiler and **Dr. Ch. Wagner**, University of Halle-Wittenberg**,** for X-ray analysis.

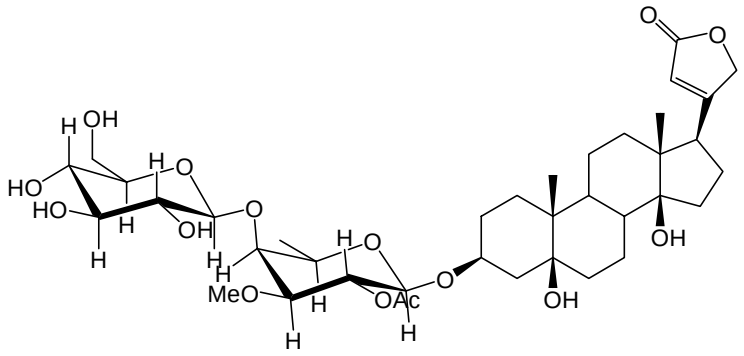
Dr. W. Richter and **S. Hess**, R&D Biopharmaceuticals and Morphochem AG, for anti-proliferative test.

Dr. Hass, Medizin, Hochschule Hannover, for cellular viability test and cell cycle analysis.

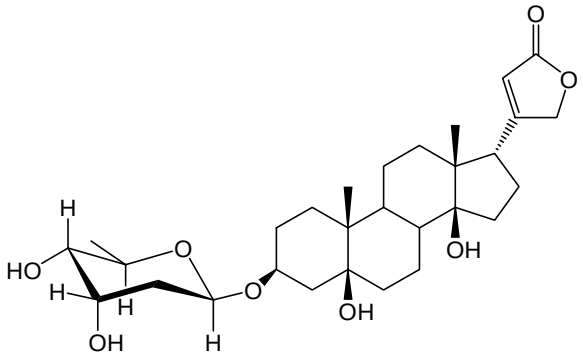
Contents

Summary

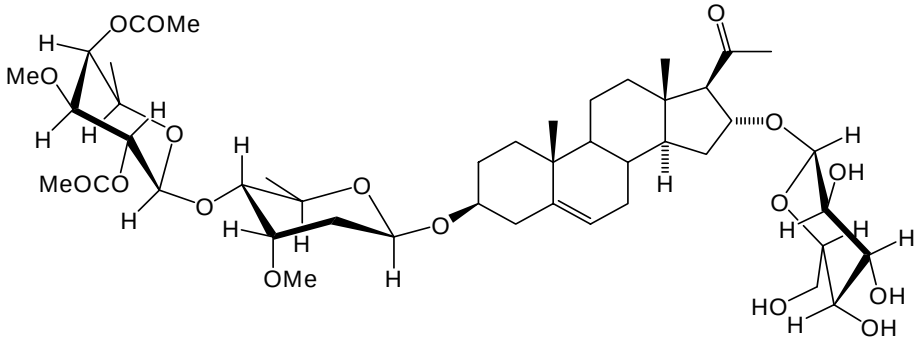
- This thesis describes the isolation, the characterization and pharmacological activities of phytoconstituents from the Myanmar medicinal plants *Streptocaulon tomentosum* Wight & Arnott (Asclepiadaceae), *Curcuma comosa* Roxb. (Zingiberaceae) and *Vitis repens* Wight & Arm. (Vitaceae).
- Triterpenoids, cardenolides, lignanes, and steroidal saponines (compounds **1-20**) including three new substances (**13, 15, 19**) were isolated from the roots of *Streptocaulon tomentosum*.



17α-H-periplogenin-β-glucosyl-(1-4)-2-*O*-acetyl-digitalose (**13**)

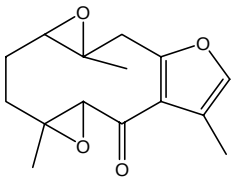


17β-H-periplogenin-β-*D*-digitoxoside (**15**)

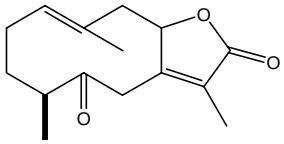


Δ^5-pregnene-3β,16α-diol-3-*O*-[2,4-*O*-diacetyl-β-digitalopyranosyl-(1-4)-β-*D*-cymaropyranoside]-16-*O*- [β-glucopyranoside] (**19**)

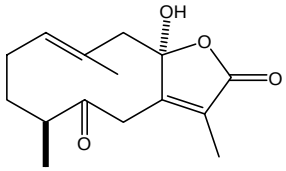
- Curcuminoids and sesquiterpenoids (compounds **21-49**) including nine new sesquiterpenes (**23, 25, 26, 33, 35, 36, 39, 41, 45**) were characterized from the rhizome of *Curcuma comosa*.



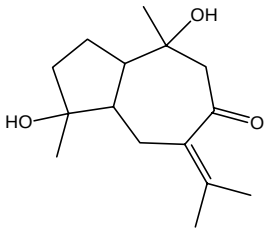
23



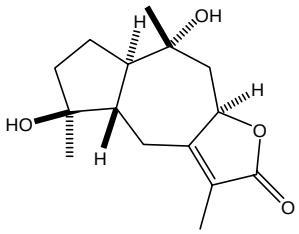
25



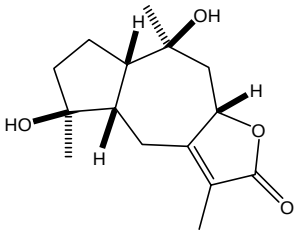
26



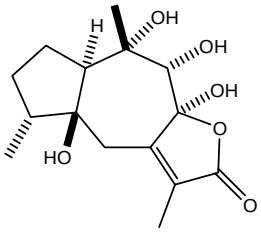
33



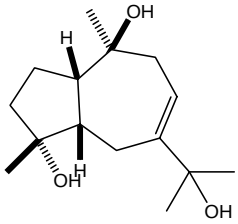
35



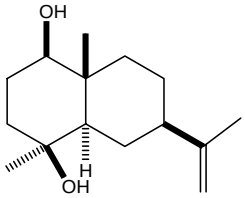
36



39



41



45

- Polyphenols, fatty acids, and lignanes (compounds **50-58**) were obtained from the rhizome of *Vitis repens*.
- Four extracts of each plant were tested for their antifungal properties against *Cladosporium cucumerinum* Ell. & Arth. according to Gottstein *et al*. (1982).
- Six cardenolides isolated from *Streptocaulon tomentosum* were tested for their antiproliferative activity *in vitro* against MCF-7 (human breast cancer cell line) and L 929 (mouse fibroblast cell line). Among six cardenolides, 17α-H-periplogenin-3-*O*-β-*D*-digitoxoside, and 17α-H-periplogenin-3-*O*-β-*D*-cymaroside exhibit significant antiproliferative activity (IC_{50} values, < 1μM) against MCF-7. Four cardenolides were examined for their cellular viability in the tumor cell and U 937 (human leukemic cell line) at concentrations 100 μM, 10 μM, and 1 μM. All these four cardenolides show the induction of apoptosis at 100 μM and 10 μM in both cell lines.

I. INTRODUCTION

Plants have formed the basis for traditional medicinal systems for thousands of years, with the first records dating from about 2600 BC in Mesopotamia. Peoples used oils from cedar and cypress, licorice, myrrh, and poppy juice, among other things, substances that are still in use today for the treatment of a variety of illnesses and infections. Ancient Egyptian, Chinese, and Indian documents show that medicine in these societies included numerous plant-based remedies and preventives. The Greeks and Arabs both contributed substantially to the assimilation, codification, and development of plant-based medicines. The isolation of the active principles from the plants and herbs such as strychnine, morphine, and colchicine began in the early 1800s [Newman *et al*., 2000; Dev, 1999; Fallarino, 1994].

Today approximately 80% of the world's population relies on traditional plant based medicines for primary health care. The remaining 20% of the world's population also depends on plant products for health care [Arvigo & Balick, 1993; Farnsworth *et al*., 1985]. About 25% of prescription drugs dispensed in the United States contain plant extracts or active ingredients derived from plants. Out of a total of 520 new drugs approved for commercial use between 1983 and 1994, 30 were new natural products and 127 were chemically modified natural products.

Despite the great successes already achieved in natural products chemistry and drug development, we have barely begun to tap the potential of our molecular diversity. Only an estimated 5% to 15% of the 250,000 species of higher terrestrial plants in existence have been chemically and pharmacologically investigated in systematic fashion. The percentage of insects, marine organisms, and microbes investigated is far lower still. In the case of microbes, it is estimated that 95% to 99% of existing species are currently not even known, never mind analyzed. There is currently great interest in exploring extreme habitats for useful enzymes from microbes, including acidophiles (from acidic sulfurous hot springs), alkalophiles (from alkaline lakes), halophiles (from salt lakes), thermophiles (from deep sea vents), and psychrophiles (from extremely cold waters) [http://www.aaas.org/ international/africa/gbdi/mod1b.html, Nnadozi *et al*., 2000].

Others have been designed based around the natural ligands of known drug targets. For example, albuterol is based on the hormone adrenaline and binds to the same receptor.

Today, more systematic approaches are used. High-throughput screening is used to test thousands of potential targets with thousands of diverse chemical compounds in order to identify promising lead compounds (chemical entities that interact with targets and therefore have potential as drugs). The alternative method of rational drug design involves the design

and synthesis of compounds based on the known structure of either a specific target or one of its natural ligands. The results of the Human Genome Project and Human Pathogen Genome projects provide many new potential drug targets. For this reason, target identification must be followed by target validation, which confirms the likelihood that interfering with the target protein will impact on the disease.

The development of a new therapeutic drug is a complex, lengthy and expensive process. It can take from 10-15 years and over 500 000 000 $ to bring a drug from concept to market. This includes 2-4 years of pre-clinical development, 3-6 years of clinical development and additional time for dealing with the regulatory authorities (fig. 1) [http://www.http://www.wellcome.ac.uk /en/genome/tacklingdisease/hg09b005; Abrantes-Metz *et al*., 2003].

.

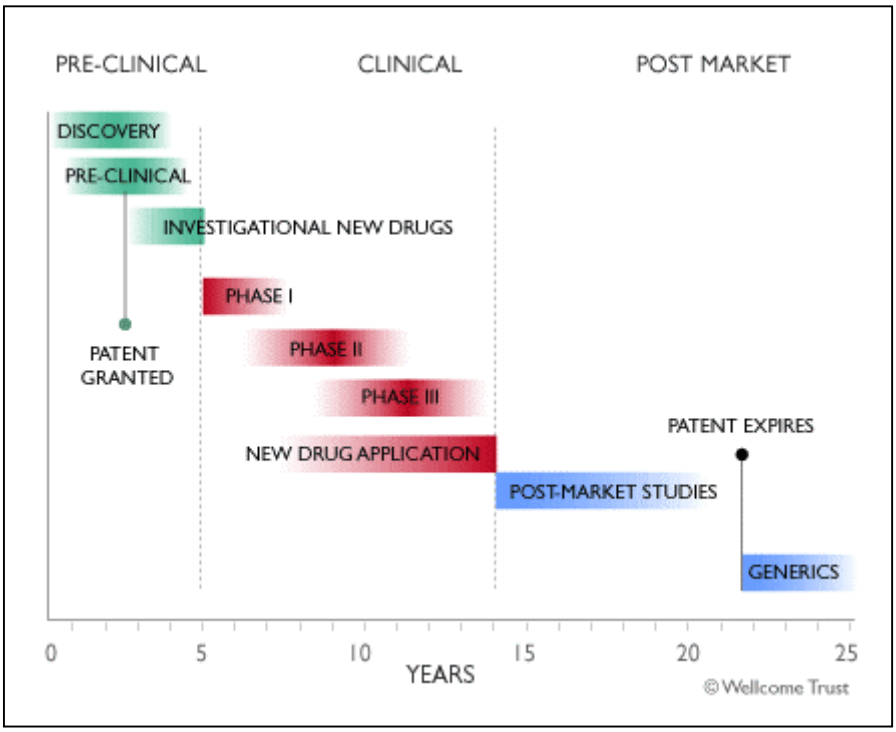


Figure 1. Phases in drug development [http://www.wellcome.ac.uk/en/genome/tacklingdisease/hg09b005.html].

II. GENERAL SECTION

1. Aim of research

- To isolate and characterize the phytoconstituents from Myanmar medicinal plants:

Species	Part used	Traditional medicinal use
Streptocaulon tomentosum Wight & Arn. (Asclepiadaceae)	Root (as powder)	anticancer, snake bite
Curcuma comosa Roxb. (Zingiberaceae)	Rhizome (as powder)	malaria fever
Vitis repens Wight & Arm. (Vitaceae)	Rhizome (as powder)	anticancer

- To test bioactivity of isolated compounds

2. Literature review

2.1. *Streptocaulon tomentosum*

2.1.1. Botanical description of *S. tomentosum* in the genus *Streptocaulon*

Figure 2. *Streptocaulon tomentosum*: plant (left) and roots (right)

The genus *Streptocaulon* belongs to the family Asclepiadaceae and includes five species. Two species, *S. tomentosum* (fig. 2) and *S. griffithii* J. D. Hooker grow in Myanmar. The botanical description of the genus *Streptocaulon* is as follows [Ping *et al*., 2005]:

Lianas to 8 m, densely tawny pilose except for corolla. Petiole 3–7 mm; leaf blade obovate or broadly elliptic, 7–15 × 3–9.5 cm, leathery or thick papery, base rounded to cordate, apex acute or rounded and apiculate; lateral veins 14–20 pairs, subparallel. Inflorescences 4–20 cm,

sometimes thyrsoid; sessile or with peduncle to 8 cm; flowers densely clustered in young inflorescences. Flower buds subglobose to ovoid, ca. 3 × 3 mm. Sepals ovate, ca. 1.3 × 1 mm, acute. Corolla yellow-green outside, yellow-brown inside, glabrous; tube short; lobes ovate, ca. 3 × 1.5 mm. Corona lobes longer than anthers. Ovaries densely pubescent. Follicles oblong or oblong-lanceolate in outline, 7–13 cm × 5–10 mm, horizontal. Seeds oblong, 6–9 × 2–3 mm; coma 3–3.5 cm.

2.1.2. Biological activity of *Streptocaulon* species

The roots of *Streptocaulon tomentosum* are used in Myanmar in traditional medicine for the treatment of anticancer, dysentery and stomachache, and the leaves are used externally for the treatment of snake poisoning and abscesses. In previous studies, nobody reported about the isolation of bioactive substances from *Streptocaulon tomentosum*. However the isolation of cardenolides from the root of *Streptocaulon juventas* (Lour.) Merr. and antiproliferative activity of cardenolides isolated from *S. juventas* have been reported while this study was in progress [Ueda *et al*., 2003a; 2003b]. A methanol extract of the roots of *S. juventas* showed potent antiproliferative activity against the human HT-1080 fibrosarcoma cell line. The activity-guided separation of the MeOH extract resulted in the isolation of sixteen cardenolides, two hemiterpenoids, two phenylpropanoids and a phenylethanoid by means of silica gel column chromatography, MPLC, and preparative TLC. Their antiproliferative activities were examined against the HT-1080 cell line. The isolated cardenolides strongly inhibited the proliferation of the HT-1080 cell line (IC_{50} values, 54-1600 nM) [Ueda *et al.*, 2003b].

2.1.3. Phytochemical constituents of *Streptocaulon* species

Acovenosigenin A digitoxoside (**59**), acovenosigenin A (**60**), 17α-H-digitoxigenin (**17**), digitoxigenin gentiobioside (**61**), digitoxigenin-3-*O*-[*O*-β-glucopyranosyl-(1→6)-*O*-β-glucopyranosyl-(1→4)-3-*O*-acetyl-β-digitoxopyranoside] (**62**), digitoxigenin-3-*O*-[*O*-β-glucopyranosyl-(1→6)-*O*-β-glucopyranosyl-(1→4)-*O*-β-digitalopyranosyl-(1→4)-β-cymaropyranoside] (**63**), digitoxigenin-3-*O*-[*O*-β-glucopyranosyl-(1→6)-*O*-β-glucopyranosyl-(1→4)-β-digitoxopyranoside] (**64**), digitoxigenin sophoroside (**65**), echujin (**66**), 17α-H-periplogenin (**10**), periplogenin-3-*O*-[4-*O*-β-glucopyranosyl-β-digitalopyranoside] (**67**), 17α-H-periplogenin-3-*O*-β-*D*-digitoxoside (**11**), 17α-H-periplogenin-3-*O*-β-*D*-cymaroside (**12**), periplogenin glucoside (**68**), corchorusoside C (**69**), subalpinoside (**70**), (4*R*)-4-hydroxy-3-isopropyl pentyl-β-rutinoside (**71**), (*R*)-2-ethyl-3-

methyl-butyl rutinoside (**72**), caffeic acid (**73**), 4,5-di-*O*-caffeoylquinic acid (**74**), and 2-phenylethyl rutinoside (**75**) were isolated from roots of *Streptocaulon juventas* [Ueda *et al*., 2003a].

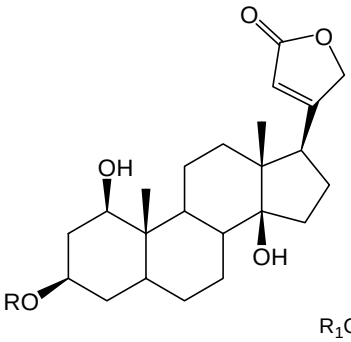
Lupeol acetate (**4**), lupeol (**76**) and 3β-acetyloxy urs-12-ene (**2**) were also obtained from roots of *Streptocaulon juventas* [Tam *et al*., 2002].

2.2. Bioactivities of cardenolides and triterpenes

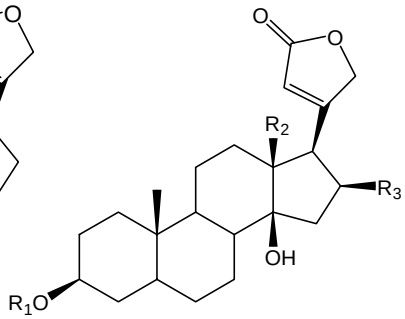
2.2.1. Pharmacological activities of cardenolides

Cardenolides are C_{23} steroid derivatives which are of special interest because of their cardiac activity; they are sometimes called cardiac glycosides. All cardenolides have a 3β-oxygen function, a 14 β-hydroxyl group and an α,β-unsaturated γ-lactone attached at 17β. The AB-ring junction is often *cis* as in digitoxigenin (**17**), but can also be *trans* as in uzarigenin (**77**). Cardenolides occur in several plant families including the Asclepiadaceae, the Apocynaceae, the Scrophulariaceae, the Celastraceae, and the Tiliaceae. The cardiac glycosides digitoxin (**78**) and digoxin (**79**) have been used for the treatment of heart failure for hundreds of years. These compounds are specific inhibitors of the plasma membrane bound Na^+/K^+ ATPase, and there is much evidence which suggests that this inhibition of the enzyme activity is responsible for their cardiotonic as well as toxic effects [Akera & Brody, 1978; Repke, 1963; Skou, 1965].

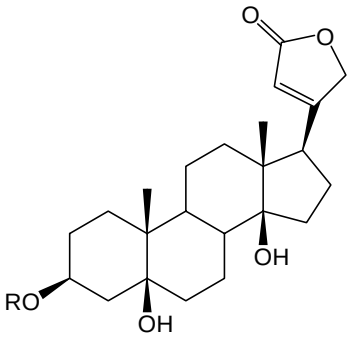
Much of the earlier work on the pharmacological activities, especially anticancer activity, of cardiac glycosides has been carried out in either animals or in isolated organs derived from various species. In the 1960s clear inhibition of malignant cells of cardiac glycosides *in vitro* was reported [Shiratori, 1967]. In 1979, it was observed that breast cancer cells from women on digitalis had more benign characteristics than cancer cells from control patients not on these drugs [Stenkvist *et al*., 1979; 1980] and that five years after a mastectomy, the recurrence among patients not taking digitalis was 9.6 times higher than in patients on these drugs [Stenkvist *et al*., 1982]. In 1999, with this background, a 22-year follow-up of 175 patients with breast carcinoma, of which 32 were on digitalis treatment, when they acquired their breast carcinoma, have been made. It was observed that there was a lower death rate (6%) from breast carcinoma among the patients on digitalis, when compared with patients not on digitalis (34%) [Stenkvist, 1999].



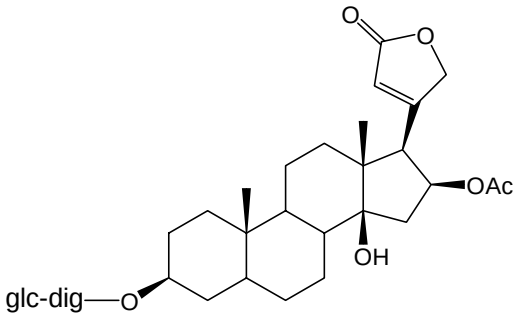
59 R = dig
60 R = H



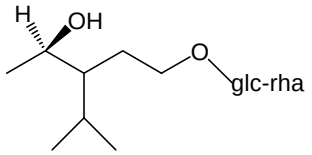
17 R_1 = H, R_2 = CH_3, R_3 = H
61 R_1 = glc-glc, R_2 = CH_3, R_3 = H
62 R_1 = (3-*O*-Ac-dig)-glc-glc, R_2 = CH_3, R_3 = H
63 R_1 = cym-dtl-glc-glc, R_2 = CH_3, R_3 = H
64 R_1 = dig-glc-glc, R_2 = CH_3, R_3 = H
65 R_1 = glc-glc, R_2 = CH_3, R_3 = H
66 R_1 = cym-glc-glc, R_2 = CH_3, R_3 = H
78 R_1 = dig-dig-dig, R_2 = CH_3, R_3 = H
79 R_1 = dig-dig-dig, R_2 = CH_3, R_3 = H
80 R_1 = dig-dig-dig, R_2 = CH_3, R_3 = OH



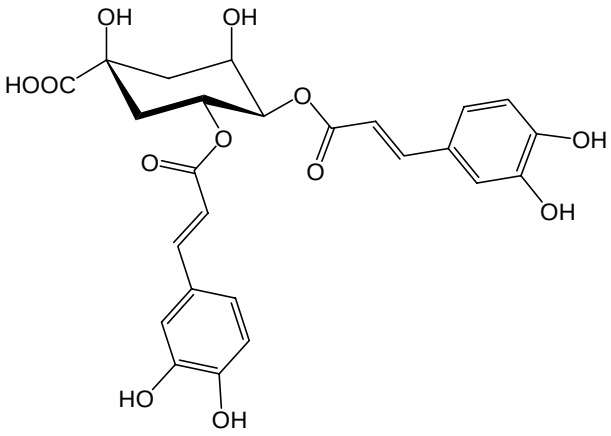
10 R = H
67 R = dtl-glc
11 R = dig
12 R = cym
68 R = glc
69 R = dig-glc



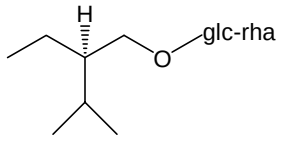
70



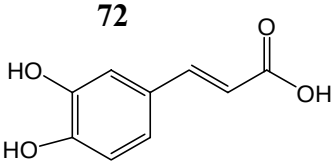
71



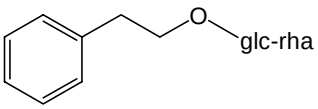
74



72



73



75

In 2003, sixteen cardenolides (**10, 11, 12, 17, 59-70**) isolated from the roots of *S. juventas* were examined for their antiproliferative activity toward three human-derived (HT-1080 fibrosarcoma, lung A549 adenocarcinoma, cervix Hela adenocarcinoma) and three murine-derived (colon 26-L5 carcinoma, Lewis lung carcinoma, B16-BL6 melanoma) cell lines. They selectively and strongly inhibited proliferation of the HT-1080 (IC_{50} 0.054-1.6 μM) and A549 (IC_{50} 0.016-0.65 μM) cell lines [Ueda *et al*., 2003a; Ueda *et al*., 2003b]. In 2005, digitoxin (**78**), digoxin (**79**), gitoxin (**80**) and their corresponding aglycones were evaluated for growth inhibition activity in three human cancer cell lines TK-10 (renal), MCF-7 (breast), and UACC-62 (melanoma) at concentrations commonly found in cardiac patients. Digitoxin (**78**) (IC_{50} 3.2-33.5 nM) and digoxin (**79**) (IC_{50}14.6-29.5 nM) showed the highest level of growth inhibition in the three cell lines investigated [Lázaro *et al*., 2005]. The above-mentioned reports suggest that digitalis may have an anticancer utilization.

2.2.2. Possible effector mechanism for the anticancer effects of digitalis

Na^+/K^+ ATPase or Na pump is a carrier enzyme present in almost every animal cell. Its physiological function is to maintain the Na^+ and K^+ electrochemical gradients through the cell membrane, keeping low Na^+ and high K^+ intracellular concentrations (fig. 3).

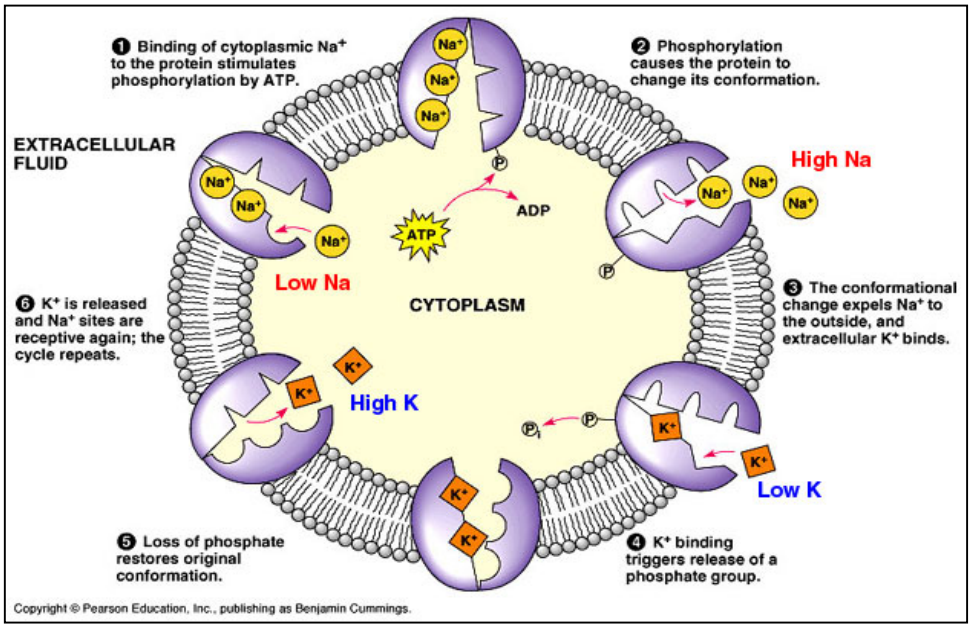


Figure 3. Physiological function of Na pump [http://fajerpc.magnet.fsu.edu/Education/2010/Lectures/12_Membrane_Transport_files/image036.jpg].

In 1988, Repke summarized the knowledge on the role of the Na^+/K^+ ATPase in normal and cancer cell proliferation [Repke *et al*., 1988]. The main pharmacological effect of the cardiac glycosides is Na^+/K^+ ATPase inhibition. Inhibition of the Na pump by digitalis (generic name for cardenolides, bufadienolides and their glycosides) leads to an inhibition of Na^+ ion efflux causing an increase in the intracellular Na^+ ion concentration. This alters the activity of the Na^+/Ca^{++} exchanger causing a transient rise in the intracellular Ca^{++} ion concentration. The increased availability of Ca^{++} ions augments contractility of the cardiac muscle cells and hence results in positive inotropy. Ca^{++} has a pivotal role in the apoptotic process. Increased intracellular Ca^{++} concentration may start apoptosis by itself and are a step in several cascades leading to apoptosis after receptor interaction. Even in the cascade of events triggered by the ligation of the Fas receptor, Ca^{++} plays a crucial role in several steps of the apoptotic pathway [Sen *et al.*, 1999, Chien *et al*., 1999; Hughes *et al*., 1998, Haux, 1999].

2.2.3. Basic principles for bioactivity tests of cardenolides

2.2.3.1. Cell viability and proliferation

Cell viability measurements assess healthy cells in a sample. This can be accomplished either by directly counting the number of healthy cells or by measuring an indicator for healthy cells in cell populations (e.g. in a microplate assay). Whether the cells are actively dividing or quiescent is not distinguished. An increase in cell viability indicates cell growth, while a decrease in viability can be interpreted as the result of either toxic effects of compounds/ agents or suboptimal culture conditions.

In contrast to cell viability analysis, cell proliferation assessment is defined as the measurement of actively dividing cells in a sample. It can be expressed either as the actual number or proportion of proliferating cells in cell culture, tissues, or as relative values in assays for cell populations. Quiescent nongrowing healthy cells are not detected by cell proliferation assays [http://www.roche-applied-science.com/PROD_INF/BIOCHEMI/no3_03/ PDF/p26_28.pdf, 01. 10. 2005].

2.2.3.2. The cell cycle

During development from stem cells to fully differentiated cells, cells in the body alternately divide (mitosis) and "appear" to be resting (interphase). This sequence of activities exhibited by cells is called the cell cycle (fig. 4).

Interphase, which appears to the eye to be a resting stage between cell divisions, is actually a period of diverse activities. Those interphase activities are indispensable in making the next mitosis possible.

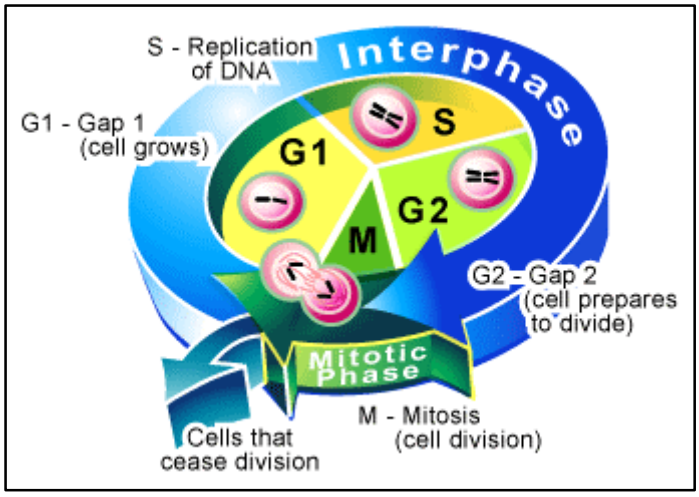


Figure 4. Cell cycle [http://www.cellsalive.com/toc.htm#microbial].

Interphase: Interphase generally lasts at least 12 to 24 hours in mammalian tissue. During this period, the cell is constantly synthesizing RNA, producing protein and growing in size. Interphase can be divided into 4 steps: Gap 0 (G0), Gap 1 (G1), S (synthesis) phase, Gap 2 (G2).

Gap 0 (G0): There are times when a cell will leave the cycle and quit dividing. This may be a temporary resting period or more permanent. An example of the latter is a cell that has reached an end stage of development and will no longer divide (e.g. neuron).

Gap 1 (G1): Cells increase in size in Gap 1, produce RNA and synthesize protein. An important cell cycle control mechanism activated during this period (G1 Checkpoint) ensures that everything is ready for DNA synthesis.

S Phase: To produce two similar daughter cells, the complete DNA instructions in the cell must be duplicated. DNA replication occurs during this S (synthesis) phase.

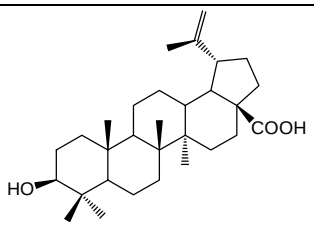
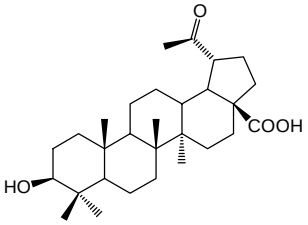
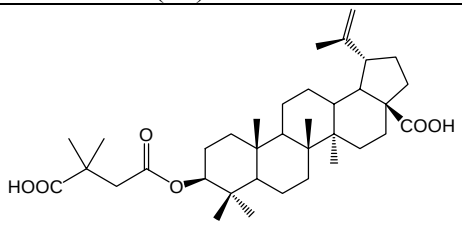
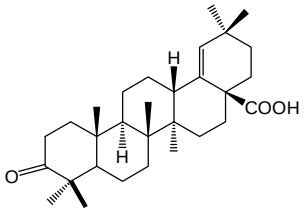
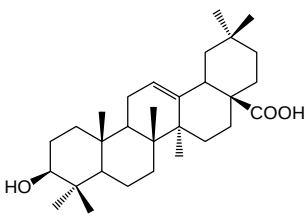
Gap 2 (G2): During the gap between DNA synthesis and mitosis, the cell will continue to grow and produce new proteins. At the end of this gap is another control checkpoint (G2 Checkpoint) to determine if the cell can now proceed to enter M (mitosis) and divide.

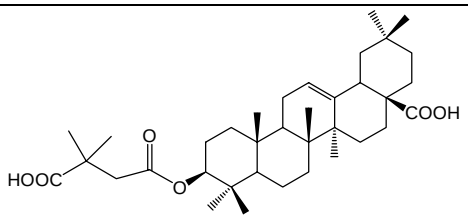
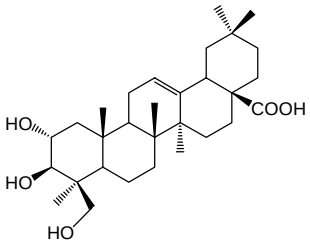
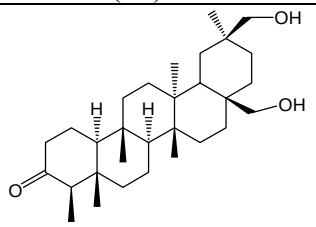
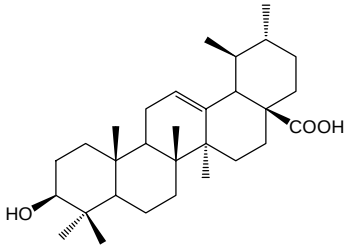
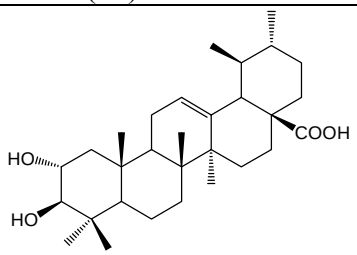
Mitosis or M Phase: Cell growth and protein production stop at this stage in the cell cycle. All of the cell's energy is focused on the complex and orderly division into two similar daughter cells. Mitosis is much shorter than interphase, lasting perhaps only one to two hours.

As in both G1 and G2, there is a checkpoint in the middle of mitosis (metaphase checkpoint) that ensures the cell is ready to complete cell division [http://www.cellsalive.com/toc.htm# microbial, 01. 10. 2005].

2.2.4. Pharmacological activities of triterpenoids

Table 1. Some triterpenoids and their bioactivities.

structure	bioactivity
 betulinic acid (**82**)	**anti-HIV** EC_{50} 1.4 μM, TI 9.3 [Fujioka *et al*., 1994]
 platanic acid (**84**)	**anti-HIV** EC_{50} 6.5 μM, TI 13 [Fujioka *et al*., 1994]
 3-*O*-(3′,3′-dimethylsuccinyl)betulinic acid (**85**)	**anti-HIV** $EC_{50} < 3.5 \times 10^{-4}$ μM, TI > 20000 [Kashiwada *et al*., 1996]
 moronic acid (**86**)	**anti-HIV** EC_{50} < 0.1 μg/ml, TI > 186 [Sun *et al*., 2003]
 oleanolic acid (**81**)	**anti-HIV** EC_{50} 1.7 μg/ml, TI 12.8 [Zhu *et al*., 2001]

 oleanolic ester (**87**)	**anti-HIV** EC_{50} 0.0039 μg/ml, TI 3.750 [Zhu *et al*., 2001]
 hyptatic acid A (**88**)	**anti-cancer** A-549: ED_{50} 5.9 μg/ml HCT-8: ED_{50} 4.2 μg/ml P-388: ED_{50} 6.7 μg/ml [Pettit *et al*., 1994]
 maytenfoliol (**89**)	**anti-cancer** P-388: T/C 120 (10 mg/kg/day) KB:ED_{50} 4.56 μg/ml [Pettit *et al*., 1994]
 ursolic acid (**83**)	**anti-cancer** P-388: ED_{50} 3.15 μg/ml L-1210: ED_{50} 4.00 μg/ml A-549: ED_{50} 4.00 μg/ml [Pettit *et al*., 1994]
 2α-hydroxyursolic acid (**90**)	**anti-cancer** A-549: ED_{50} 4.9 μg/ml HCT-8: ED_{50} 2.7 μg/ml P-388: ED_{50} 6.1 μg/ml [Pettit *et al*., 1994]

The triterpenoids, having a C_{30} skeleton, constitute a large, diverse group of natural products derived from squalene or, in the case of 3β-hydroxytriterpenoids, the 3*S*-isomer of squalene 2,3-epoxide. There is a variety of skeletal types such as lanostanes, cycloartanes, dammaranes, euphanes, tirucallanes, tetranortriterpenoids, quassinoids, lupanes, oleananes, friedelanes, ursanes, hopanes, isomalabicanes and saponins. In excess of 4000 triterpenoids

have been isolated so far and more than 40 skeletal types have been identified. Oleananes and ursanes often occur together and, in the past decade, have been reported from a wide range of families including the Araliaceae, Asclepiadaceae, Bignononiaceae, Cactaceae, Campanulaceae, Celastraceae, Compositae, Ericaceae, Fagaceae, Labiateae, Leguminosae, Phytolaccaceae, Primulaceae, Rosaceae, Rubiaceae, Sapotaceae, Theaceae, Umbelliferae and Urticaceae [Dey & Harborne, 1991]. The biological activities of triterpenoids and triterpenoid saponins are immunostimulation [Press *et al*., 2000], anti-tumor-promoting activity [Konoshima & Takasaki, 2000; Yasmuawa & Akihisa, 2000a; 2000b; Jozova & Novotny, 2000a; 2000b], anti-inflammatory activity [Rios *et al*., 2000] and anti-insect activity [Connolly & Hill, 2002]. Recent research on triterpenoids has been focused on oleanolic acid (**81**), betulinic acid (**82**), ursolic acid (**83**), and their derivatives. All three triterpenoids inhibited HIV-1 protease activity *in vitro* [Mengoni *et al*., 2002; Ma *et al*., 1998].

2.3. *Curcuma comosa* Roxb.

2.3.1. Phylogeny and a new classification of Zingiberaceae

The pantropical Zingiberaceae is the largest family in the order Zingiberales with 53 genera and over 1200 species. Classifications of the family, first proposed in 1889 [Petersen, 1889] and refined by others since that time, recognize four tribes (Globbeae, Hedychieae, Alpinieae, and Zingibereae) based on morphological features.

New phylogenetic analyses based on DNA sequences of the nuclear internal transcribed spacer (ITS) and plastid *matK* regions suggest that at least some of these morphological traits are homoplasious and three of the tribes are paraphyletic. The African genus *Siphonochilus* and Bornean genus *Tamijia* are basal clades. The former Alpinieae and Hedychieae for the most part are monophyletic taxa with the Globbeae and Zingibereae included within the latter. The results of these phylogenetic investigations are used to propose a new classification of the Zingiberaceae that recognizes four subfamilies and four tribes: Siphonochiloideae (Siphonochileae), Tamijioideae (Tamijieae), Alpinioideae (Alpinieae, Riedelieae), and Zingiberoideae (Zingibereae, Globbeae) [Kress *et al*., 2002].

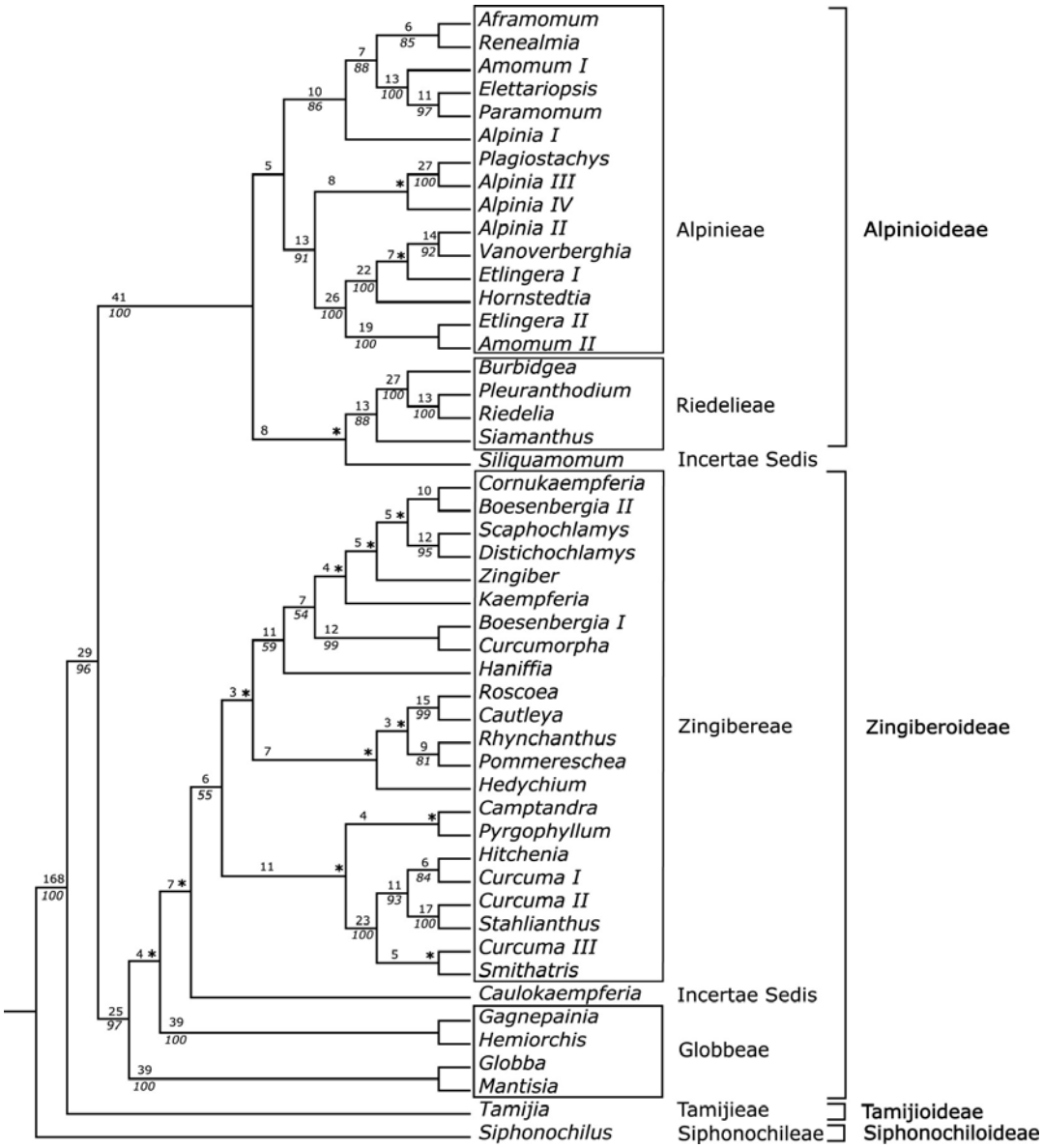


Figure 5. Phylogenetic tree of Zingiberaceae [Kress *et al*., 2002].

2.3.2. Botanical description of *Curcuma comosa* Roxb.

Curcuma comosa Roxb. belongs to the subfamily Zingiberoidae in Zingiberaceae. The genus *Curcuma* has 80 species. More than 50 species have been found in Thailand and 24 species are widely spread in Myanmar [http://www.iupac.org/symposia/proceedings/phuket 97/sirirugsa.pdf; Kress *et al*., 1964]. The botanical description of *Curcuma* species is as follows:

Rootstock large of palmately branched sessile annulate tuber, aromatic with light yellow circling deeper yellow inside when young; colour changing to bright orange on becoming older. Leaves large, lanceolate to oblong-elliptic, leaf-stalk as long as the blade, plain green

except in the earliest, which are clouded with faint brown down the centre above, glabrous on both sides. Flowering spike arising from the centre of the tuft of leaves. Appearing after the leaves are developed, flowers fragrant, pinkish-yellow, longer than the flowering bracts; flower bracts greenish tipped with purplish-red streak, those of the coma tinged with purplish-red at the tip and with white base below. Family Zingiberaceae. Flowering in late August to September [http://www.tuninst.net/MyanMedPlants/DMB-USG/hypoten/hypo.htm#Curcuma-Comosa].

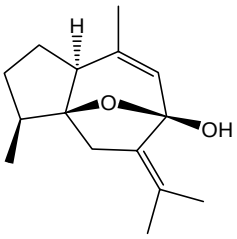
Figure 6. *Curcuma comosa*: plant (left) and rhizome (right)

2.3.3. Previous studies of isolation of secondary metabolites from *Curcuma* species

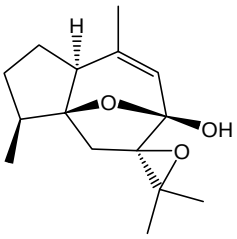
***Curcuma comosa*.**

The rhizomes of *Curcuma comosa* has been used extensively in indigenous medicine in Thailand as an anti-inflammatory agent. It has also been used to reduce malaria fever by combining them with *Artemisia annua* L. and *Aristolochia tagala* Cham. by Myanmar practitioners and as an aromatic stomachic. In 1994, five diphenylheptenoids (**91-95**) were tested their inhibition of mortility of the nematode against *Caenorhabditis elegans* Maupas. It was clear that compound **93** was the most potent inhibitor of nematode motility, with an EC_{95} of 0.7 μg/ml. Compound **95** (EC_{95} of 1 μg/ml) was slightly less active, followed by **91** and **92** (EC_{95} of each 9 μg/ml) [Jurgens *et al*., 1994]. In 1997, three known diarylheptanoids, 1,7-diphenyl-5-hydroxy-(1*E*)-1-heptene (**96**), 5-hydroxy-7-(4-hydroxyphenyl)-1-phenyl-(1*E*)-1-heptene (**97**) and 7-(3,4-dihydroxyphenyl)-5-hydroxy-1-phenyl-(1*E*)-1-heptene (**98**), and a phloracetophenone glucoside (**99**) were also isolated [Suksamrarn *et al*., 1997] (see structures in p. 19)

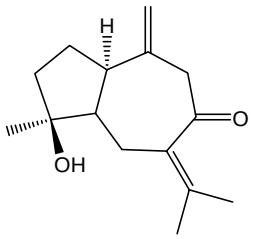
Guaiane type



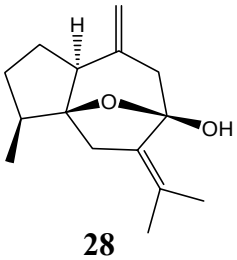
27



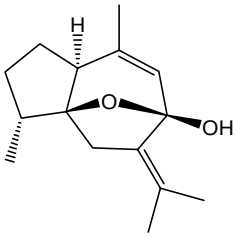
103



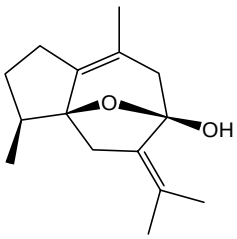
30



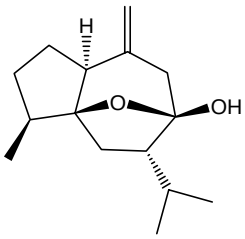
28



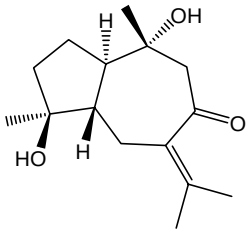
122



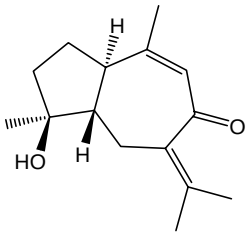
123



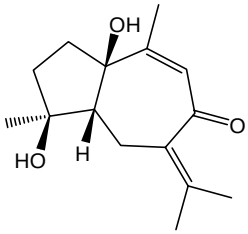
107



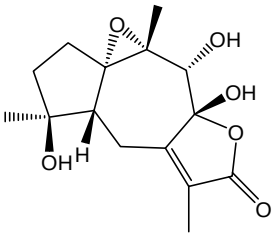
32



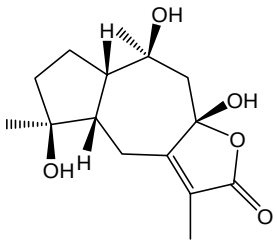
29



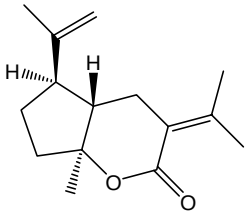
135



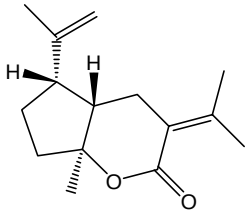
125



38

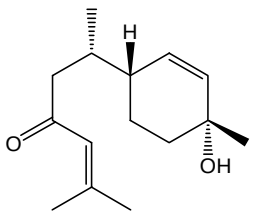


124

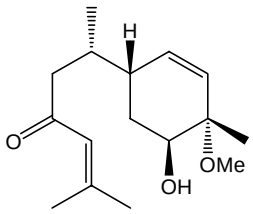


42

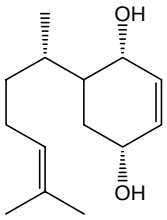
Bisaborane type



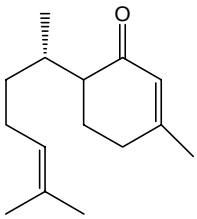
110



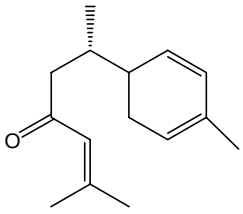
111



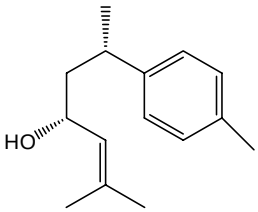
112



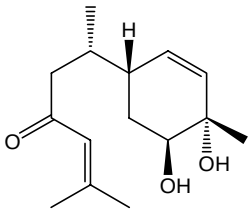
114



115

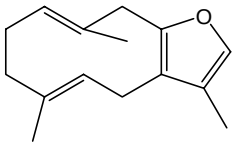


43

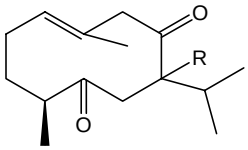


116

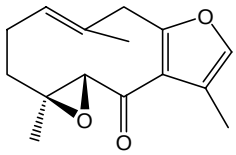
Germacrane type



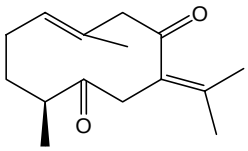
129



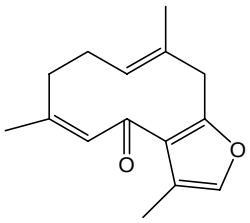
21 R = β-H
133 R = α-H



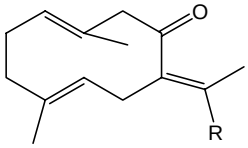
22



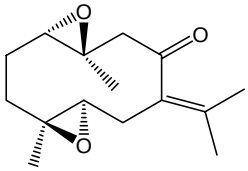
102



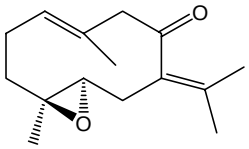
130



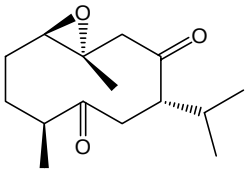
100 R = H
131 R = CH_2OH



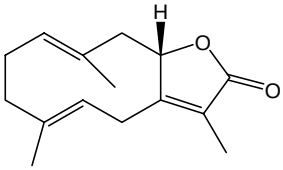
24



101

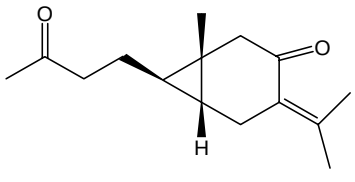


109

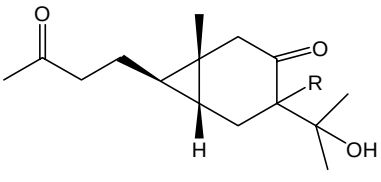


132

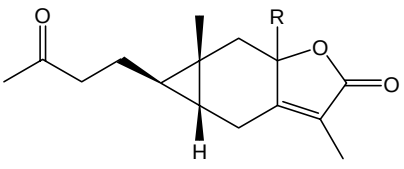
Carabrane type



44

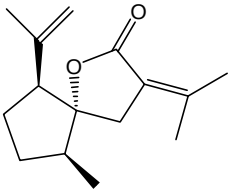


117 R = *β*-H
118 R = *α*-H

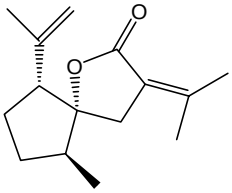


119 R = *β*-H
120 R = *α*-H
121 R = *α*-OH

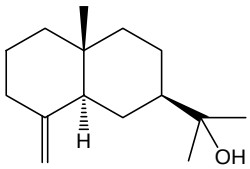
Eudesmane type



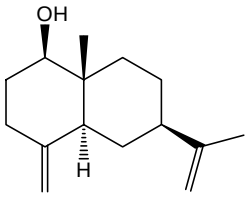
104



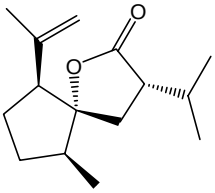
105



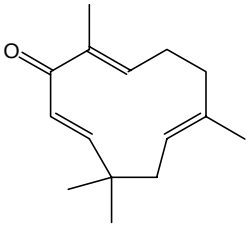
127



128

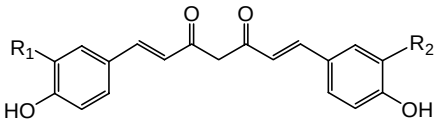


108

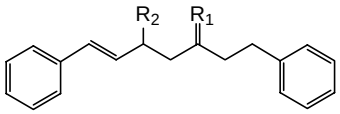


106

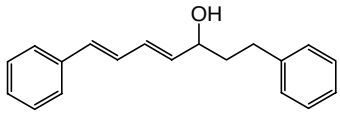
Diarylheptanoids



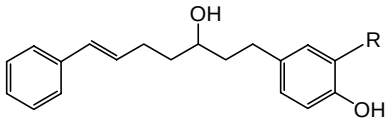
46 $R_1 = R_2 = OMe$
47 $R_1 = H$, $R_2 = OMe$
48 $R_1 = H$, $R_2 = H$



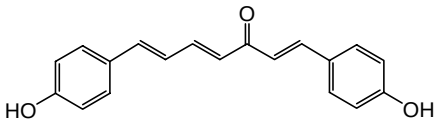
91 $R_1 = H, OCOCH_3$; $R_2 = H$
92 $R_1 = O$; $R_2 = H$
93 $R_1 = H, OH$; $R_2 = H$
95 $R_1 = O$; $R_2 = OH$



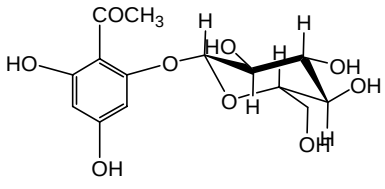
96



97 R = H
98 R = OH



126



99

***Curcuma aromatica* Salisb.**

Rhizomes of *C. aromatica* Salisb. are used as oriental traditional medicines in China, Japan and Southeast Asia. From these plants, many kinds of sesquiterpenes have been isolated. In 1987, three new sesquiterpenes, isozedoarondiol, methylzedoarondiol and neocurdione, were isolated along with 7 known sesquiterpenes, germacrone (**100**), curdione (**21**), (4*S*, 5*S*)-germacrone 4,5-epoxide (**101**), dehydrocurdione (**102**), procurcumenol (**29**), zedoarondiol (**32**) and curcumenone (**44**) from rhizomes of *C. aromatica* [Kuroyanagi *et al*., 1987]. In 1990, further study on the sesquiterpenes has been carried out to give eleven minor sesquiterpenes, having guaiane, seco-guaiane and germacrane skeletons [Kuroyanagi *et al*., 1990] (see structures in p. 17, 18).

***Curcuma heyneana* Val. & V. Zijp.**

C. *heyneana* Val. & V. Zijp. is one of the zingiberaceous plants indigenous to Java Island, Indonesia. The rhizome of this plant is of wide medicinal value in Indonesia, and is considered to be useful for the treatment of skin diseases, abrasions and injuries. A new guaiane sesquiterpene, oxycurcumenol (**103**), together with the known sesquiterpenes germacrone (**100**), dehydrocurdione (**102**), isocurcumenol (**28**), curcumenol (**27**), curcumanolide A (**104**), B (**105**) and zerumbone (**106**) were isolated [Firman *et al*., 1988] (see structures in p. 17-19).

***Curcuma wenyujin* Y.H. Cheng & C. Ling.**

Curcuma wenyujin Y.H. Cheng & C. Ling. is currently used as a clinical remedy for uterus cancer in China. Sesquiterpenes possessing a 7α-isopropyl group, such as curcumol (**107**), curdione (**21**), curcumalactone (**108**), and a new epoxy germacrane, (1*R*, 10*R*)-epoxy-(–)-1,10-dihydrocurdione (**109**), were isolated from the essential oil. Other sesquiterpenes, neocurdione and (1*S*, 10*S*),(4*S*, 5*S*)-germacrone-1(10),4-diepoxide (**24**) were also isolated from this plant [Harimaya *et al*., 1991; Inayama *et al*., 1991] (see structures in p. 17-19).

***Curcuma longa* Salisb.**

The rhizome of *C. longa* Salisb. is also used as a yellow colouring food additive, because it contains curcuminoids. From the rhizomes of this plant, curcuminoids and five new sesquiterpenes, 4-hydroxybisabola-2,10-diene-9-one (**110**), 4-methoxy-5-hydroxy-bisabola-2,10-diene-9-one (**111**), 2,5-dihydroxybisabola-3,10-diene (**112**), and procurcumadiol (**113**) were isolated along with curcumenone (**44**), dehydrocurdione (**102**), (4*S*,5*S*)-germacrone-4,5-epoxide (**101**), bisabola-3,10-diene-2-one (**114**), *α*-turmerone (**115**), bisacumol (**43**), bisacurone (**116**), curcumenol (**27**), isoprocurcumenol (**30**), zedoarondiol (**32**), and procurcumenol (**29**) [Ohshiro *et al*., 1990] (see structures in p. 17-19).

***Curcuma zedoaria* Roscoe. (= *C. aeruginosa* Roxb.)**

The crude drug zedoary, the dried and ground rhizome of *C. zedoaria* Roscoe., has been used medicinally in China. In Japan, it has also been used medicinally, chiefly as an aromatic stomachic. The rhizome of *C. zedoaria* is also widely used as a stimulant, stomachic, carminative, diuretic, anti-diarrheal, anti-emetic, anti-pyretic, anti-inflammatory and depurator in India and Southeast Asia. As it contains bioactive principles, the constituents of zedoary have been investigated extensively and it is recognized to be a rich source of terpenoids. Until now, 3 major curcuminoids and over 40 sesquiterpenes, belonging to eudesmane type, guaiane type, carabrane type, germacrane type, bisaborane type, elemane type and xanthane type, have been isolated from this plant. Some sesquiterpenoids obtained from *C. zedoaria* are cyclopropasesquiterpenes like curcumenone (**44**), curcarabranols A (**117**) and B (**118**), curcumenolactones A (**119**), B (**120**) and C (**121**), 4-epicurcumenol (**122**), neocurcumenol (**123**), gajutsulactones A (**124**) and B (**42**), and zedoarolides A (**125**) and B (**38**) [Matsuda *et al*., 2001a; 2001b; Jang *et al*., 2001; Shiobara *et al*., 1985; Takano *et al*., 1995; Shibuya *et al*., 1987; Hikino *et al*., 1966; 1968; 1971; Kouno & Kawano, 1985].

2.3.4. Pharmacological activities of principal constituents from *Curcuma* species

Sesquiterpenoids and phenolic diarylheptanoids are major constituents in turmeric (*Curcuma*). Curcumin (**46**) and its analogues show various biological activities, including cytotoxicity [Aggarwal *et al*., 2003], nematocidal activity [Kiuchi *et al*., 1993], anticancer activity [Simon *et al*., 1998], topoisomerase inhibition [Roth *et al*., 1998], antioxidant activity [Soudamini *et al.*, 1992], protection against alcohol induced liver toxicity [Rajakrishnan *et al*., 1998], antimalaria activity against *Plasmodium falciparum* Welch. and *Leishmania major* Friedlin. [Rasmussen *et al*., 2000] (see structures in p. 17-19).

Several synthetic curcumin analogues also showed potent antiandrogenic activities against two human prostate cancer cell lines, PC-3 and DU-145, and were superior to hydroxyl flutamide, which is the currently available antiandrogen for the treatment of prostate cancer [Ohtsu *et al*., 2002]. This new class of antiandrogen agents could be developed into clinical trial candidates to control steroid hormone influenced prostate cancer growth [Lee, 2004].

1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (**126**), and procurcumenol (**29**) inhibit the production of TNF-α by lipopolysaccharide (LPS) activated macrophages (IC_{50} 12.3 and 310.5 μM) [Jang *et al*., 2001]. The 80 % acetone extract of Zedoariae Rhizome showed vasorelaxant [Yoshikawa *et al*., 1998], hepatoprotective [Matsuda *et al*., 1998, 2001c], and

nitric oxide production inhibitory activities [Matsuda *et al*., 2001d]. Germacrone (**100**) (IC_{50} 19 μM), isocurcumenol (**28**) (26 μM), *β*-eudesmol (**127**) (16 μM), and *β*-dictyopterol (**128**) (9 μM) show potent vasorelaxant effects [Matsuda *et al*., 2001a]. The effect of isolated constituents from Zedoariae rhizome on NO production from LPS-activated macrophages was examined by Matsuda and coworkers [Matsuda *et al*., 2001b]. Gajustulactones A (**124**), Gajustulactones B (**42**), curcumenone (**44**), furanodiene (**129**), isofuranodienone (**130**), 13-hydroxygermacrone (**131**), glechomanolide (**132**), neocurdione (**133**), curcumenol (**27**), isocurcumenol (**28**), procurcumenol (**29**), curcumin (**46,**) and bis(4-hydroxycinnamoyl)methane (**134**) were found to inhibit NO production (IC_{50} 13-93 μM) [Matsuda *et al*., 2001b]. Principal sesquiterpenes, furanodiene (**129**), germacrone (**100**), curdione (**21**), neocurdione (**133**), curcumenol (**27**), isocurcumenol (**28**), aerugidiol (**135**), zedoarondiol (**32**), curcumenone (**44**) and curcumin (**46**) also show potent protective effect on D-galactosamine/lipopolysaccharide-induced acute liver injury in mice [Matsuda *et al*., 1998].

2.4. *Vitis repens* Wight & Arm.

2.4.1. Botanical describtion of *Vitis repens* Wight & Arm.

Vitis repens Wight & Arm. (syn. *Cissus repens* Lan.) belongs to the family Vitaceae. There are 26 genera and 350 species in Vitaceae (order Vitales). The botanical description of *Vitis repens* Wight & Arm. is as follows [http://persoon.si.edu/myanmar/]:

Trendrillar climber, slender, scandent, glabrous, glaucous with fusiform to tuberculous rhizome. The tendrils borne at the nodes, opposed to the leaf, forked at the tips. Stems 6-angled with ridges and furrows. Leaves alternate, simple, the tips acuminate, margin serrulate, base truncate to cordate. White patch in pale green coloured leaf blade. Inflorescence axillary, compound umbellate cymes, flowers small yellowish green, bracteate. Fruits berries, globose, dark red, shining black in fully ripe [http://persoon.si.edu/myanmar/].

Figure 7. *Vitis repens*: plant (left) and rhizome (right).

2.4.2. Phytochemical constituents from *Vitis* species

***Vitis repens* Wight & Arm. (= *Cissus repens* Lan.)**

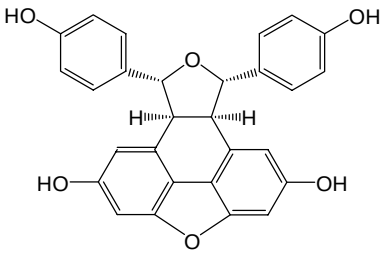
The rhizome of *Vitis repens* Wight & Arm. is used for the treatment of sore, carbuncles, ulcers, hepatitis and jaundice, peptic ulcer, tumors and hypertension in Myanmar traditional medicine. There is no reports about the investigation of phytochemical constituents from it.

***Vitis thunbergii* Sieb. & Zucc.**

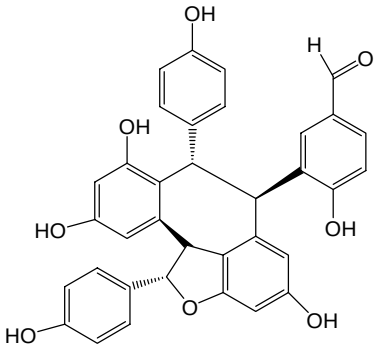
The roots of *V. thunbergii* Sieb. & Zucc. are traditionally used for the treatment of diarrhea, fracture and injury, jaundice, and hepatitis in Taiwan. Plants in the genus *Vitis* commonly contain oligomers of resveratrol. Several polyphenols were isolated from the aerial parts of *V. thunbergii* [Dou *et al*., 2003]. Four new resveratrol derivatives, vitisinols A-D (**136-139**), together with (+)-ε-viniferin (**140**), (−)-viniferal (**141**), ampelopsin C (**142**), miyabenol A (**143**), (+)-vitisin A (**144**), and vitisin C (**145**) were also isolated from the roots [Huang *et al*., 2005].

***Vitis vinifera 'Kyohou'*(Wine/Grapes)**

Two new hydroxystilbenoids named vitisin C (**145**) and viniferal together with (+)-vitisin A (**144**) and (+)-cis-vitisin A (**146**) were isolated from the corks of *V. viniferal* [Ito *et al*., 1996; 1998].



136

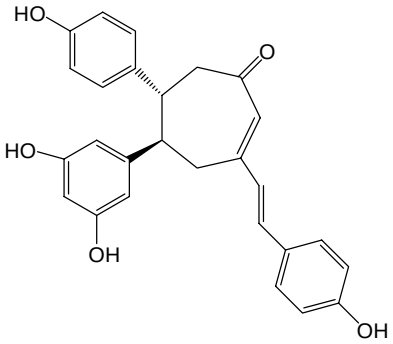


137

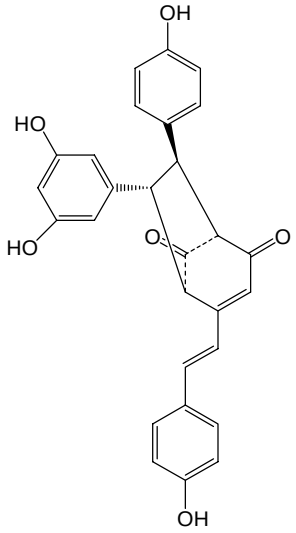
2.4.3. Bioactivities of some phytochemical constituents isolated from *Vitis* species

Some reseveratrol derivatives were tested for their antiplatelet and antioxidative activities by Huang and coworkers (Huang *et al*., 2005). The inhibitory effects on AA- (arachidonic acid) and U46619- (9,11-dideoxy-11α,9α-epoxy-methanoprostaglandin $F_{2\alpha}$) induced platelet aggregation are shown in tab. 2. Aspirin was used as positive control against AA (32.7 μM) and U46619 (no effect). All compounds, with the exception of vitisinol B (**137**) and (+)-ε-viniferin (**140**), showed potent activity. The free radical scavenging activity of all tested

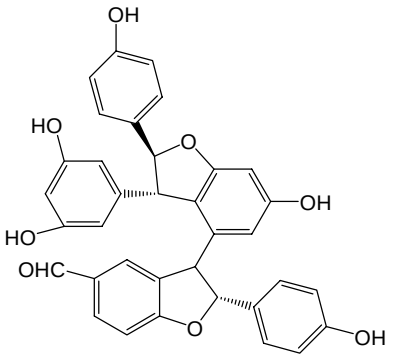
compounds was more potent than that of the standard antioxidant Trolox, $C_{14}H_{18}O_4$ (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid rac-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) (see in tab. 2, p. 25).



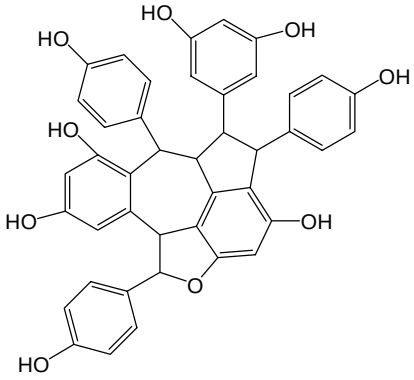
138



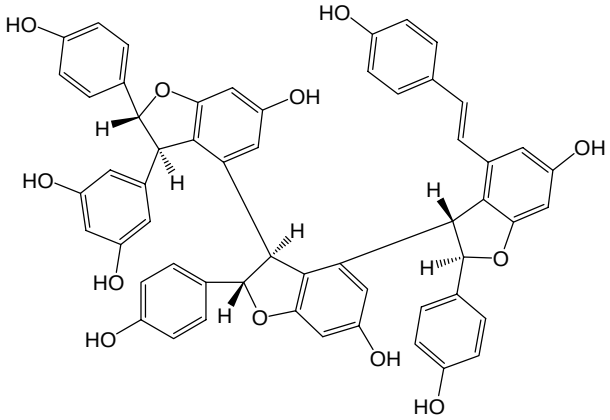
139



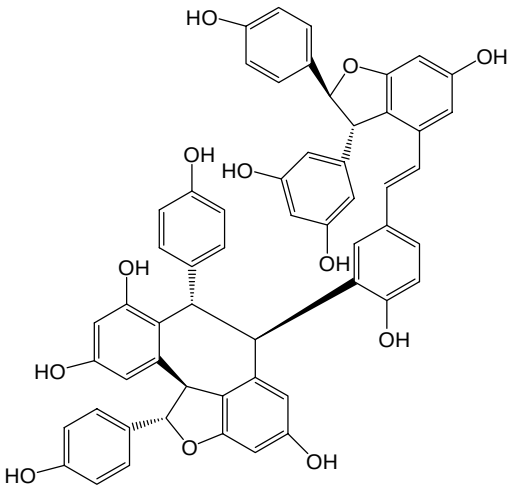
141



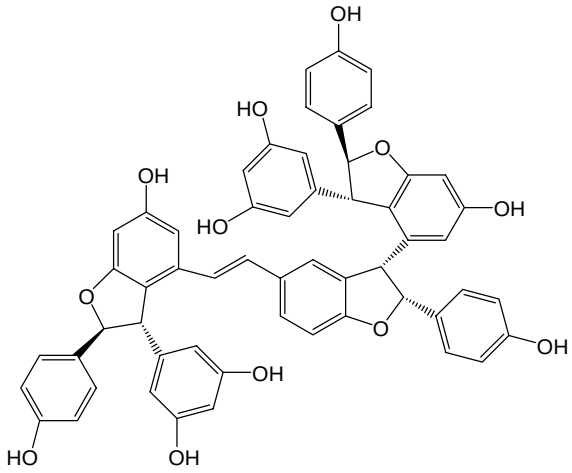
142



143



144



145

Table 2. Effect of compounds isolated from *Vitis* species on the platelet aggregation induced by arachidonic acid (AA) and 9, 11-dideoxy-11α, 9 α-epoxy-methanoprostaglandin $F_{2\alpha}$ (U46619, TXA_2 analogous), and on $ABTS^{\bullet+}$ (2,2′-azinobis (3- ethylbenzo thiazoline- 6-sulfonic acid).

Structure	**IC_{50} (μM)**		**Free radical scavenging activity on $ABTS^{\bullet+}$**
	AA	**U46619**	**EC_{50} (μM)**
vitisinol B (**137**)	>100	7.8 ± 2.2	3.6 ± 0.1
vitisinol C (**138**)	13.4 ± 2.2	10.5 ± 3.4	4.5 ± 0.1
vitisinol D (**139**)	15.0 ± 4.8	5.7 ± 1.4	4.1 ± 0.1
(+)-ε-viniferin (**140**)	>100	>100	2.8 ± 0.1
(−)-viniferal (**141**)	7.0 ± 2.9	3.1 ± 2.5	4.4 ± 0.1
ampelopsin C (**142**)	8.1 ± 1.1	5.9 ± 0.9	5.4 ± 1.2
miyabenol A (**143**)	9.0 ± 1.6	7.5 ± 2.0	6.6 ± 1.2
(+)-vitisin A (**144**)	10.3 ± 1.2	13.3 ± 2.1	13.8 ± 2.7
(+)-vitisin C (**145**)	5.7 ± 1.3	3.9 ± 0.7	4.8 ± 0.1
Aspirin	32.7 ± 6.4	n.d	
Trolox			28.4 ± 5.2

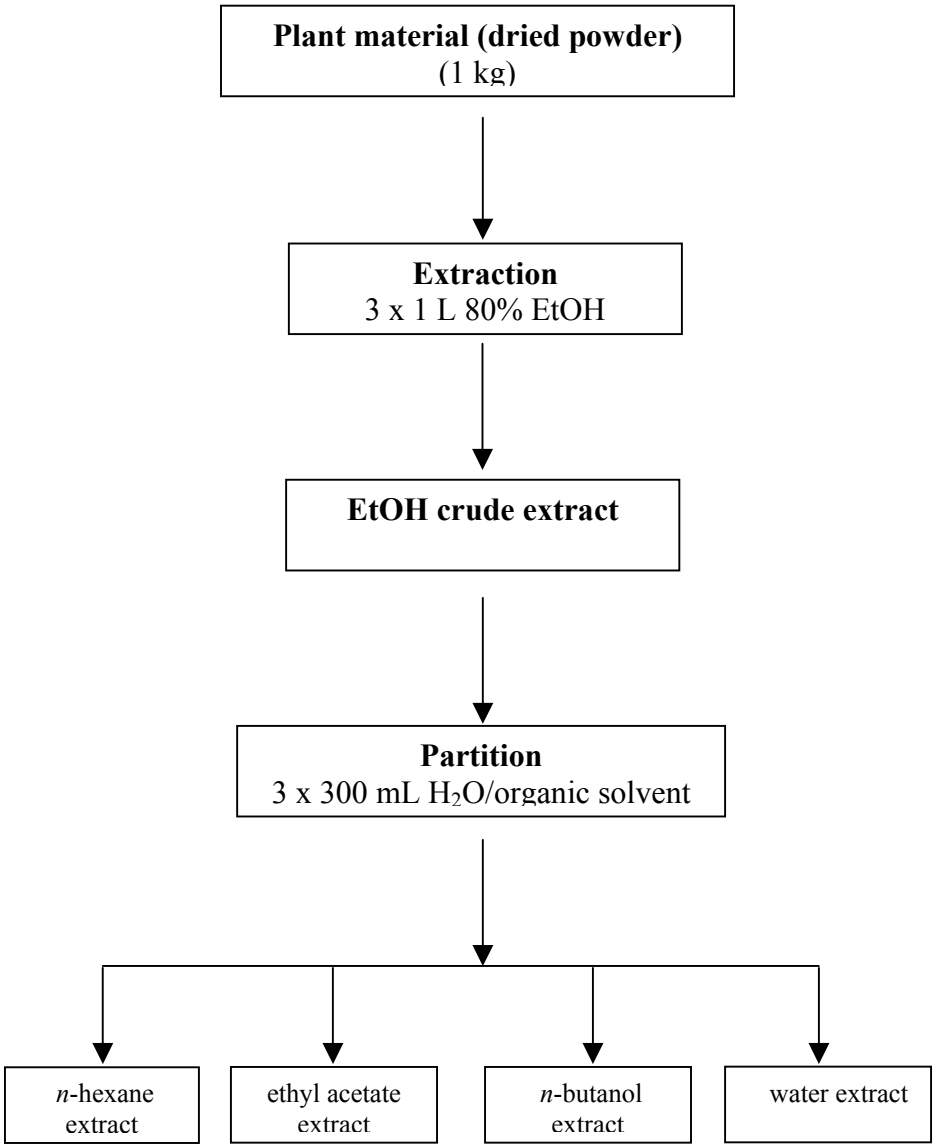
III. RESULTS AND DISCUSSION

3. Investigation of bioconstituents from *Streptocaulon tomentosum* root

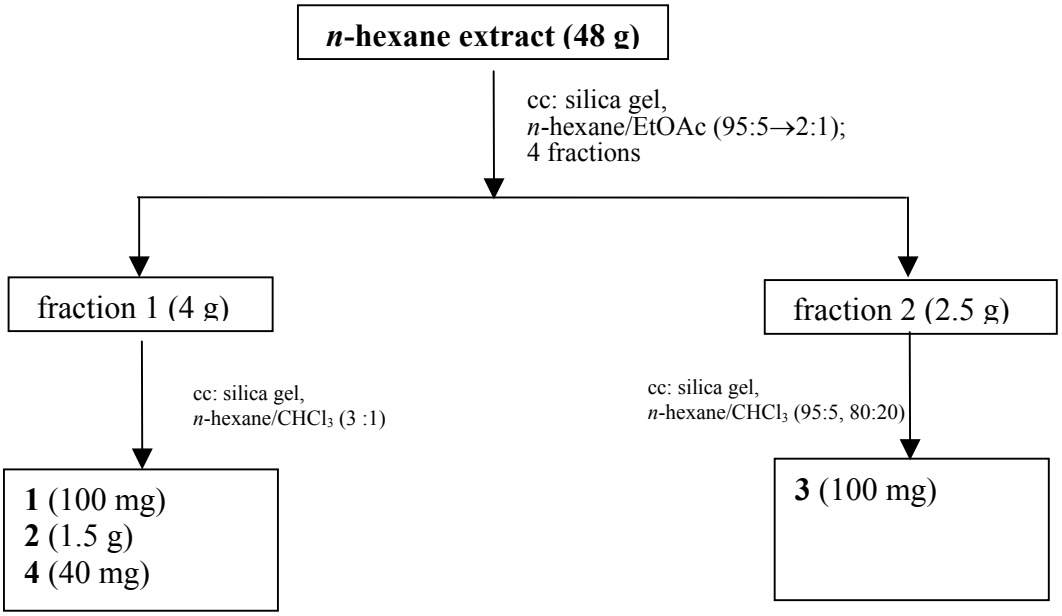
3.1. Extraction and isolation of phytoconstituents

The extraction of the plant material follows Scheme 1 - 4.

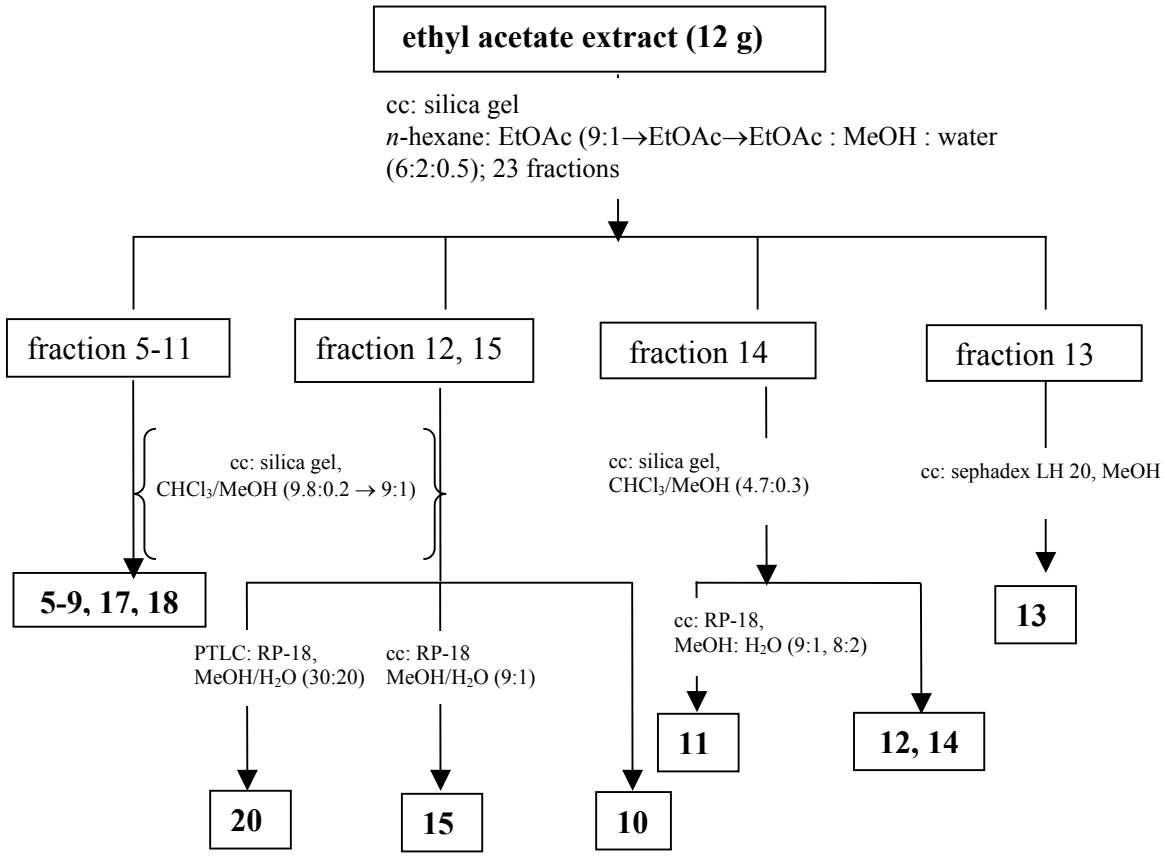
Scheme 1. General extraction scheme for plant materials



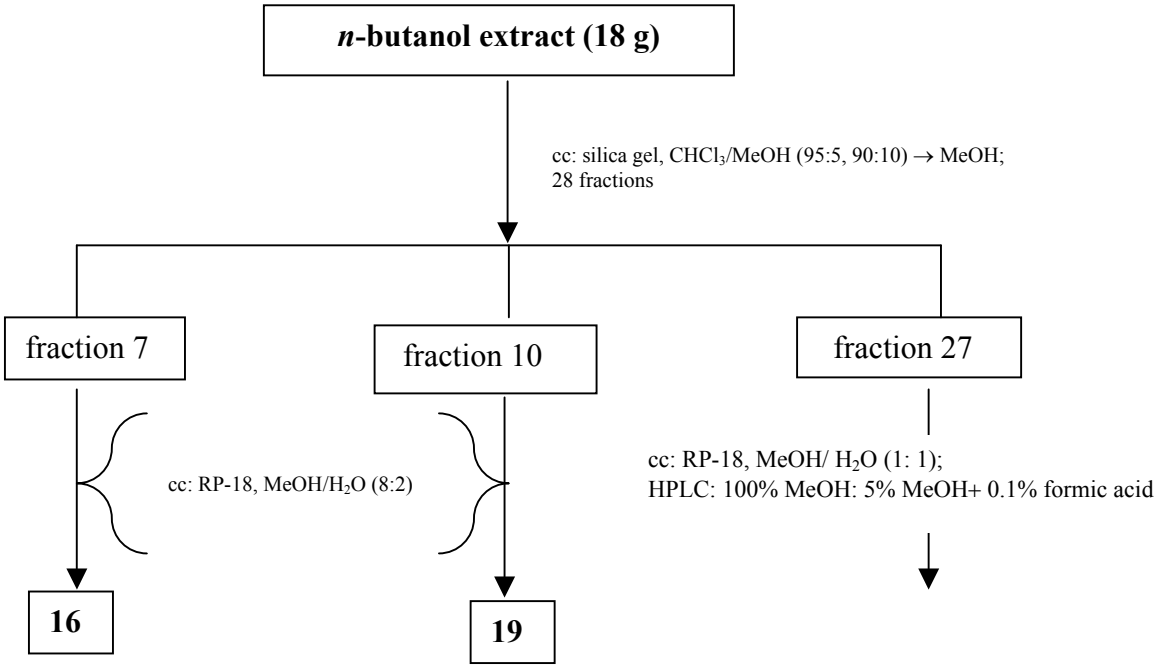
Scheme 2. Isolation of triterpenoids from *n*-hexane fraction



Scheme 3. Isolation of bioconstituents from ethyl acetate fraction

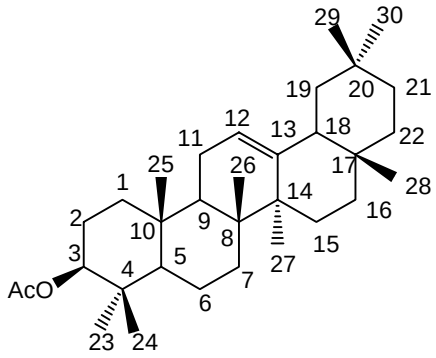


Scheme 4. Isolation of phytoconstituents from *n*-butanol fraction

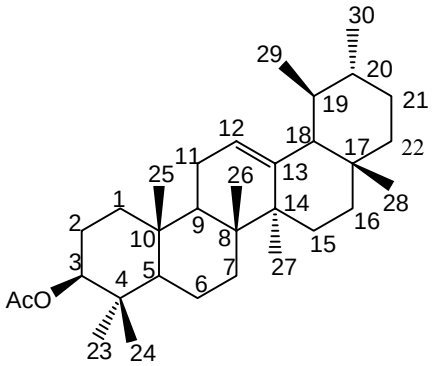


3.2. Structure elucidation of triterpenes

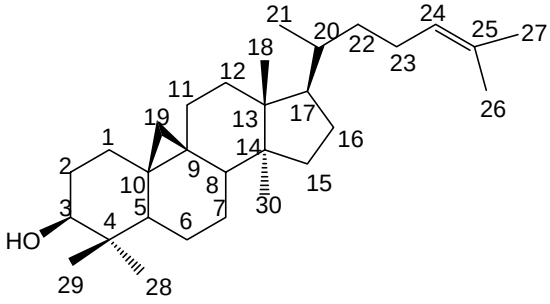
3.2.1. *β*-Amyrin acetate (1), *α*-amyrin acetate (2), cycloartenol (3), lupeol acetate (4)



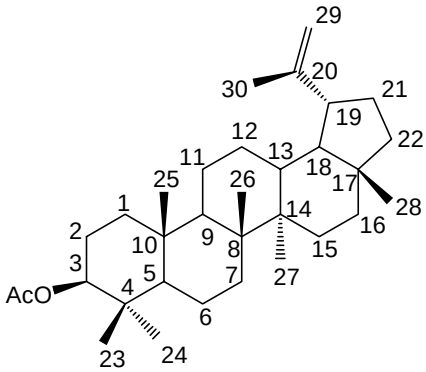
1



2



3

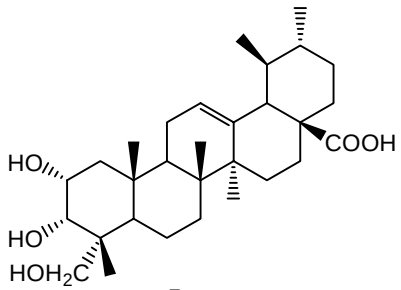


4

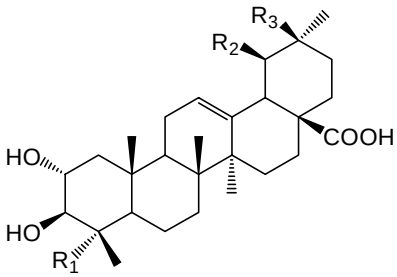
The *n*-hexane fraction was repeatedly chromatographed on a silicagel column and β-amyrin acetate (**1**), α-amyrin acetate (**2**), cycloartenol (**3**), and lupeol acetate (**4**) were obtained (scheme 1, 2). The structures elucidations were determined by ^{1}H NMR, ^{13}C NMR, ESI-MS, GC-MS and confirmed by comparison with the literature values [Matsunaga *et al*., 1988; Chen *et al*., 1993; Hisham *et al*., 1993; De Pascual Teresa *et al*., 1987].

3.2.2. 2α,3α,23-Trihydroxy-urs-12-en-28-oic-acid (5), 2α,3β-dihydroxy-urs-12-en-28-oic-acid (6), 2α,3β-dihydroxy-olean-12-en-28-oic-acid (7), 2α,3β,23-trihydroxy-urs-12-en-28-oic-acid (8), 2α,3β,23-trihydroxy-olean-12-en-28-oic-acid (9)

Compound **5-9** were isolated from fraction 5-11 of the ethyl acetate extract after repeated column chromatography on silica gel (scheme 1, 3). Identification of these known compounds was based on 1D and 2D NMR, MS and comparison of their spectroscopic data with literature values [Sashida *et al*., 1992; Kojima & Ogura, 1986; Yaguchi, 1988].



5



6 $R_1 = CH_3$, $R_2 = CH_3$, $R_3 = H$,
7 $R_1 = CH_3$, $R_2 = H$, $R_3 = CH_3$,
8 $R_1 = CH_2OH$, $R_2 = CH_3$, $R_3 = H$,
9 $R_1 = CH_2OH$, $R_2 = H$, $R_3 = CH_3$

In the compounds **5, 8** and **9** the molecular formula is $C_{30}H_{48}O_5$ by means of HR-ESI-MS. In compound **6** and **7** the molecular formula is $C_{30}H_{48}O_4$. Their mass spectra present ions at *m/z* 248 resulting from the retro-Diels-Alder fragmentation characteristic of the ursane and oleane skeletons. Furthermore they possess an ion at *m/z* 203 characteristic of Δ^{12}- triterpenoids [Budzikiewicz *et al*., 1963]. In the ^{1}H NMR (C_5D_5N) spectra of these compounds, the signal of H-18 permitted the distinction between the oleane and ursane skeletons. The H-18 signal appears at δ 2.6 ppm in the ursane skeleton and at δ 3.3 ppm in the oleane skeleton. The proton signals of H-29 and H-30 in the ursane skeletons appears as a doublet, but in oleane as a singlet. The chemical shifts of C-12 and C-13 (δ 125 and 139 ppm in ursane, δ 122 and 144 ppm in oleane) and H-12 (δ 5.20-5.4 ppm) suggests that these compounds are Δ^{12}-unsaturated triterpenoids. The ^{13}C NMR spectra (tab. 3) clearly exhibited the difference in the chemical shifts of C-12, C-13, C-17, C-18, C-19, C-20, C-22, C-27, C-29 and C-30 between the ursane

group (**5, 6, 8**) and the oleane group (**7, 9**). The coupling constant of H-3 (*J* 2.3 Hz) in **5** suggested that two OH groups at C-2 and 3 were at the *cis* position. Besides, the ROESY correlation between H-3 and H-2, H-23, H-24 also confirmed the *β*-configuration of H-2 and H-3. However, the coupling constant of H-3 (*J* 9.4 Hz) in **6-9** showed that the two OH groups at C-2 and C-3 were in *trans* position and there was no ROESY correlation between H-3 and H-2 (see HMBC, COSY, ROESY in tab. 4).

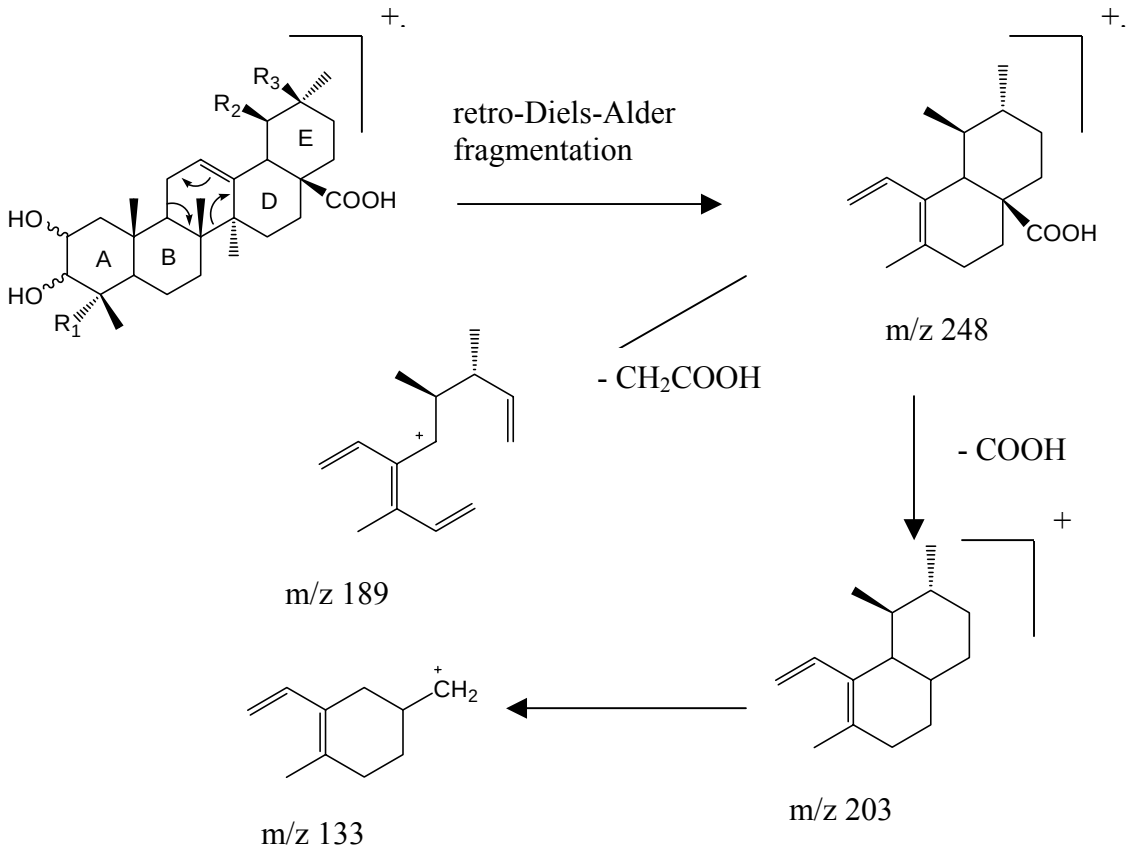


Figure 8. Proposed EI mass spectral fragmentation of triterpenoids **1-9**.

Table 3. ^{13}C NMR spectral data of triterpenoid **2-9**
(300, 500 MHz, **2-4** in $CDCl_3$; **5-7** in C_5D_5N; **8-9** in CD_3OD).

C-Atom	δ_C [ppm]							
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	38.5	32.0	37.8	42.8	47.9	47.7	48.0	47.9
2	23.4	30.4	21.0	66.2	68.6	68.6	69.7	69.7
3	80.9	78.8	80.9	78.9	83.8	83.8	78.1	78.1
4	37.7	40.5	43.0	41.9	40.0	40.0	44.1	44.1
5	55.3	47.1	55.4	43.5	55.9	55.9	48.1	48.1
6	18.3	21.2	18.3	18.3	18.8	18.8	19.1	19.1
7	32.9	28.2	37.1	33.2	33.2	33.2	33.6	33.6
8	39.7	48.0	38.4	40.1	39.8	39.8	40.8	40.5
9	47.7	20.1	50.3	47.9	48.1	48.1	48.2	48.2
10	36.8	26.5	34.2	38.3	38.4	38.5	39.0	39.0
11	22.8	26.1	18.3	23.7	23.7	23.7	24.5	24.5
12	124.2	37.2	23.8	125.5	125.5	122.4	126.6	123.4
13	139.5	45.3	38.1	139.3	139.3	144.7	139.8	145.4
14	42.1	48.8	42.8	42.5	42.5	42.2	43.4	43.0
15	28.2	32.9	27.5	28.6	28.6	28.2	29.1	28.8
16	26.7	26.7	35.6	24.9	24.9	23.9	24.6	24.0
17	33.8	52.3	40.9	48.0	48.0	46.6	48.9	47.6
18	59.0	18.1	48.3	53.5	53.5	41.9	54.3	42.7
19	39.7	29.9	48.0	39.4	39.4	46.4	40.4	47.2
20	39.7	35.9	150.8	39.4	39.3	30.9	40.4	31.8
21	31.3	18.3	29.9	31.0	31.0	33.5	31.6	33.3
22	41.6	36.4	40.0	37.4	37.4	34.2	38.1	34.9
23	28.1	25.0	25.1	71.2	29.4	29.3	66.2	66.2
24	15.8	125.1	16.6	17.8	17.7	17.7	13.9	13.9
25	14.2	130.8	16.3	17.1	16.9	16.9	17.5	17.5
26	16.8	17.6	16.1	17.5	17.5	17.7	17.8	17.7
27	17.6	25.8	14.6	23.8	23.9	26.1	24.1	26.5
28	28.8	19.4	18.1	179.9	179.9	179.9	181.7	181.5
29	23.3	14.1	109.3	17.5	17.5	33.2	17.7	33.6
30	21.4	25.5	19.3	21.3	21.4	23.7	21.6	24.0
CO<u>Me</u>	21.5		21.4					
CO	170.8		170.8					

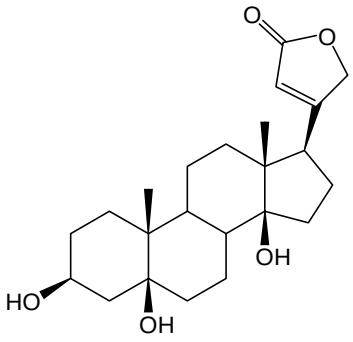
Table 4. NMR data of triterpenoids **5-9** (500 MHz, **5-7** in C_5D_5N; **8-9** in CD_3OD).

H-Atom	δ_H [ppm]					HMBC	COSY	ROESY
	5	**6**	**7**	**8**	**9**	**(5-9)**	**(5-9)**	**(5-9)**
1	1.82, 1.94 (*m*)	1.28, 2.26 (*m*)	1.28, 2.26 (*m*)	0.88, 1.96 (*m*)	0.88, 1.96 (*m*)	C-2, 3, 25	H-25, 2	
2	4.289 (*m*)	4.115 (*ddd*, 11/9.4/4.4)	4.115 (*ddd,* 11/9.4/4.4)	3.687 (*m*)	3.687 (*m*)	C-3	H-1, 3	H-3 (in **5**), 25, 24
3	4.168 (*d*, 2.3)	3.420 (*d*, 9.4)	3.420 (*d*, 9.4)	3.350 (*dd,*9.7/2.4)	3.350 (*dd,*9.7/2.4)	C-24, 4, 1	H-2	H-2 (in **5**), 23, 24
5	2.02-2.08 (*m*)	1.04 (*m*)	1.04 (*m*)	1.28 (*m*)	1.28 (*m)*	C-25, 10, 4	H-6	
6	1.34, 1.60 (*m*)	1.36, 1.54 (*m*)	1.36, 1.54 (*m*)	1.38 (*m*)	1.38 (*m*)	C-25, 5	H-5, 7	
7	1.34, 1.72 (*m*)	1.84, 2.04 (*m*)	1.84, 2.04 (*m*)	1.54, 1.74 (*m*)	1.54, 1.74 (*m*)	C-26, 8, 14	H-6	
9	1.94 (*m*)	1.76 (*m*)	1.76 (*m*)	1.66 (*m*)	1.66 (*m*)	C-25, 11, 10, 8, 5,	H-11	H-25, 26
11	1.96-2.08 (*m*)	1.98 (*m*)	1.98 (*m*)	1.94 (*m*)	1.94 (*m*)	C-12, 13, 9, 8, 10	H-12, 9	
12	5.480 (*brs*)	5.476 (*m*)	5.476 (*m*)	5.242 (*m*)	5.242 (*m*)	C-11, 14, 9, 18, 13	H-11	H-18, 29
15	1.14-2.36 (*m*)	1.18, 2.36 (*m*)	1.18, 2.36 (*m*)	1.08 (*m*)	1.08 (*m*)	C-27, 26, 16, 8, 14	H-16	
16	1.98-2.06 (*m*)	2.00, 2.12 (*m*)	2.00, 2.12 (*m*)	1.94 (*m*)	1.94 (*m*)	C-15, 17	H-15	
18	2.626 (*br d,* 11.3)	2.641 (*br d,* 11.4)	3.314 (*dd,* 13.9, 4.0)	2.202 (*d,* 11.2)	2.849 (*dd*, 13.6, 3.9)	C-9, 16, 19, 20, 14, 17, 12, 13, 28	H-19	H-29, 20 (in ursane type), 12
19	1.42 (*m*)	1.46 (*m*)	1.28, 1.80 (*m*)	1.38 (*m*)	1.14, 1.70 (*m*)	C-29, 30, 22, 20, 17, 18	H-29 (ursane type)	
20	1.00 (*m*)	1.04 (*m*)		0.98 (*m*)		C-30, 21 (in ursane type)		
21	1.34, 1.44 (*m*)	1.40 (*m*)	1.36, 1.56 (*m*)	1.36, 1.52 (*m*)	1.28, 1.66 (*m*)	C-29, 30, 22, 20, 17,	H-22	
22	1.96 (*m*)	1.98 (*m*)	1.18, 1.46 (*m*)	1.66 (*m*)	1.20, 1.40 (*m*)	C-17, 28	H-21	
23	3.77, 3.94 (*d*, 10.8)	1.291 (*s*)	1.291 (*s*)	3.261 (*d,* 11.0)	3.261 (*d,* 11.0)	C-24, 2, 3, 4, 5		H-3
24	0.87 (*s*)	1.092 (*s*)	1.092 (*s*)	0.692 (*s*)	0.690 (*s*)	C-25, 4, 5, 23, 3		H-25, 2, 3, 23
25	1.00 (*s*)	0.991 (*s*)	0.991 (*s*)	1.042 (*s*)	1.028 (*s*)	C-24, 11, 10, 4, 5, 9, 1		H-24, 26, 3
26	1.07 (*s*)	1.060 (*s*)	1.032 (*s*)	0.846 (*s*)	0.813 (*s*)	C-25, 7, 8, 14, 9		
27	1.14 (*s*)	1.220 (*s*)	1.275 (*s*)	1.132 (*s*)	1.175 (*s*)	C-15, 8,14, 18, 12, 13	H-15	
29	0.965 (*d*, 6.4)	0.995 (*d*, 4.9)	0.954 (*s*)	0.865 (*d,* 6.4)	0.907 (*s*)	C-19, 20	H-19	
30	0.925 (*d,* 6.2)	0.960 (*d*, 5.9)	1.014 (*s*)	0.967 (*d,* 6.0)	0.941 (*s*)	C-21, 19, 20	H-20	

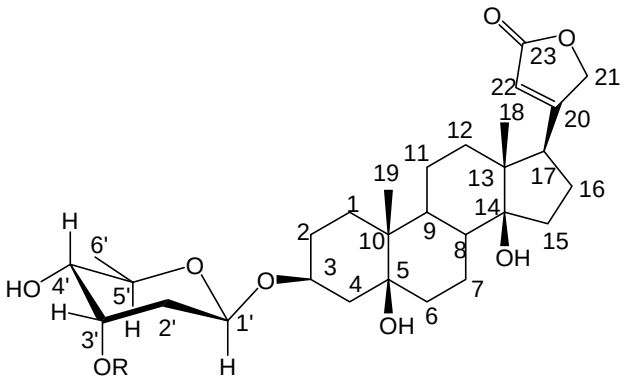
3.3. Structure elucidation of cardenolides

3.3.1. 17α-H-Periplogenin (10), 17α-H-periplogenin-β-*D* digitoxose (11), 17α-H-periplogenin-β-*D* cymarose (12)

Fraction 12 of the ethyl acetate extract gave compound **10**. Compound **11** and **12** were obtained from fraction 14 (see in scheme 1 and 3) [Kawaguchi *et al*., 1988].



10



11 R = H
12 R = CH_3

The ^{1}H NMR (C_5D_5N) data of compound **10** (HR-ESI-MS: 413.23098 $[M+Na]^+$, calc. for $C_{23}H_{34}O_5Na$ 413.22984) agreed with the characteristic peaks of cardenolides. The signal of H-21 a and b in the butenolide ring showed at δ 5.36 and 5.08 ppm (*dd*, *J* 18.1/1.4 Hz). The H-22 was observed as a singlet at δ 6.17 ppm. The H-3 signal appeared as a broad singlet at δ 4.46 ppm. The H-17 signal appeared at δ 2.84 as *dd* (*J* 9/3 Hz) and the H_3-18 and H_3-19 signals at 0.88 and 0.94 as singlet. According to EI-MS, the fragments at *m/z* 391 and 373 indicated the presence of a cardenolide aglycone.

Compound **11** (HR-ESI-MS: 543.2924 $[M+Na]^+$, calc. for $C_{29}H_{44}O_8Na$ 543.2928) and compound **12** (557.3088 $[M+Na]^+$, calc. for $C_{30}H_{46}O_8Na$ 557.3084) exhibited a mass difference of 131 and 145 in comparison to compound **10**. Because this difference was derived from the sugar moiety, the molecular formula of these sugars was deduced to be $C_6H_{11}O_3$ and $C_7H_{13}O_3$, identified a digitoxose and cymarose by acid hydrolysis and GC-MS analysis required derivatization MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoroacetamide) with authentic sugars sample. The data agree with 17α-H-periplogenin-β-*D*-digitoxose (**11**), and 17α-H-periplogenin-β-*D*-cymarose (**12**) [Ueda *et al*., 2003].

Compound **12** was crystallized in MeOH and its structure and relative configuration was determined by X-ray analysis (^{1}H data and ^{13}C data in tab. 9, 10).

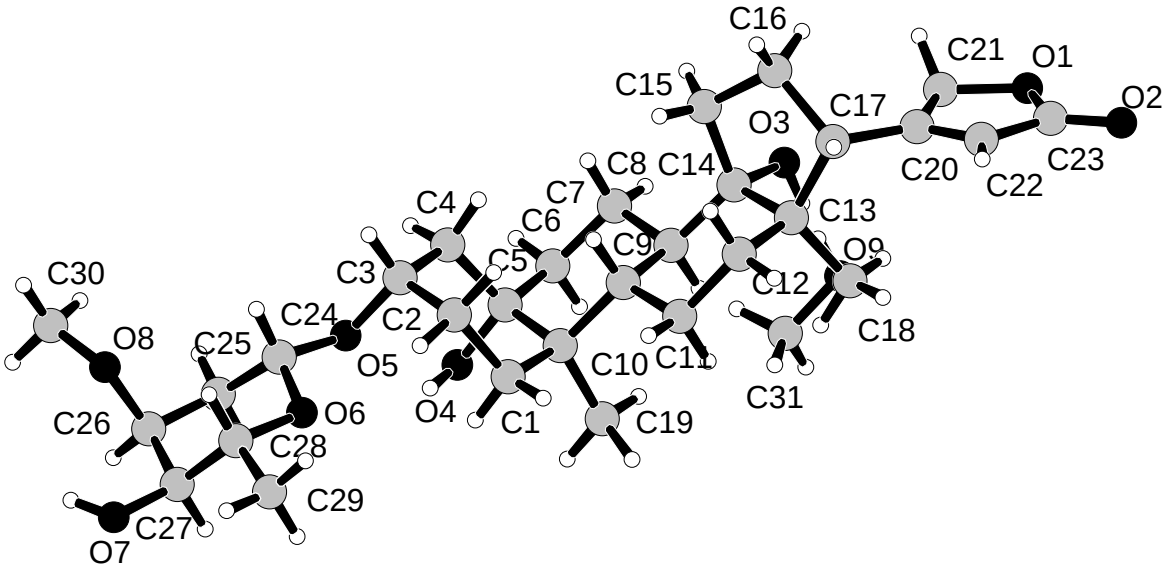


Figure 9. X-ray crystal structure of **12.**

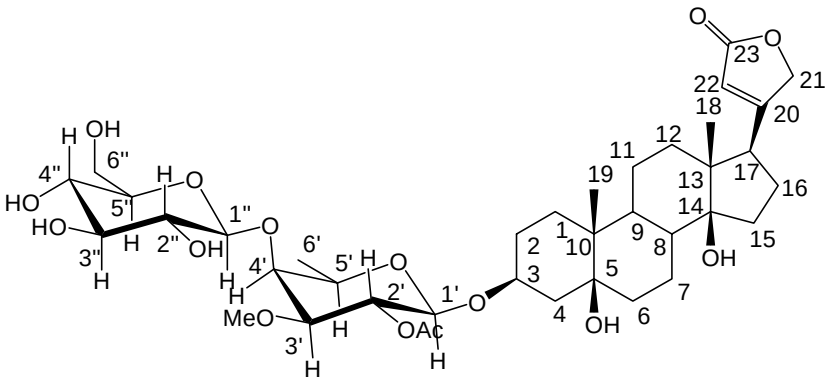
3.3.2. 17α-H-Periplogenin-β-glucosyl-(1-4)-2-*O*-acetyl-digitalose (13)

Fraction 13 of the ethyl acetate extract afforded the new compound **13** after column chromatography on sephadex LH 20 with MeOH.

The ESI-MS spectrum exhibited a $[M+Na]^+$ ion at *m/z* 777. Its molecular formula, $C_{38}H_{58}O_{15}$, (calcd. for $C_{38}H_{58}O_{15}Na$ 777.36679) was obtained through a combined application of ESI-MS, EI-MS, FT-ICR, ^1H NMR, and ^{13}C NMR. The ^1H NMR spectrum (in CD_3OD) of the aglycone was similar to compound **10**. The signal of H-21a and b also showed a pair of double doublets at δ 5.09 ppm and δ 4.91 ppm. The H-22 was observed as a singlet at δ 5.89 ppm. The H-3 signal appeared as a broad singlet at δ 4.12 ppm, revealing its α-configuration. The ^1H NMR and ^{13}C NMR data (in CD_3OD) demonstrated two molecules of sugar by two anomeric protons at δ 4.44 ppm for H 1′ (*dd*, *J* = 8, 3.8 Hz) and δ 4.57 ppm for H 1″ (*dd*, *J* = 8, 3.5 Hz). They were connected to the anomeric carbons at δ 102.1 and 104.6 ppm in the HSQC spectrum respectively. Their chemical shifts and coupling constants suggested β-linkage of the sugars.

The structural assignment was confirmed by carrying out 2D NMR techniques such as HSQC and H-H COSY. The HMBC spectral analysis displayed correlation peaks between H-3 and C-1′of the digitalosyl (3-*O*-methyl-β-fucopyranosyl) unit, the anomeric proton of the glucosyl residue and C-4′ of the digitalosyl unit, H-2′ of digitalosyl and OAc. The connectivity between H-H and H-C in the NOESY and HMBC were also presented in tab. 5. According to

these spectral data, the structure of compound **13** was assigned as 17*α*-H-periplogenin-3-*O*-*β*-glucopyranosyl-(1-4)-2-*O*-acetyl-3-*O*-methyl-*β*-fucopyranoside. It is a new combination of the known aglycone and sugar moieties (see 2 D spectra in fig. 12-15).



13

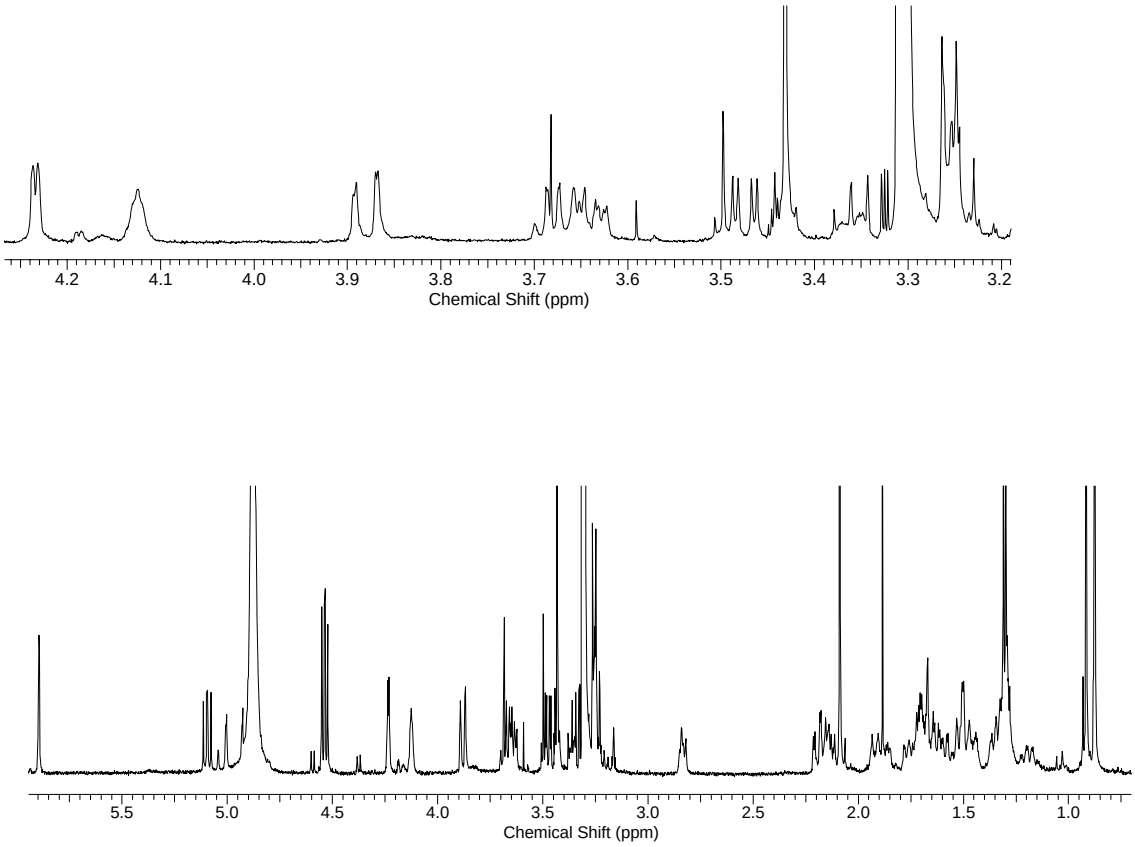


Figure 10. ^{1}H NMR spectrum of the new cardenolide **13** in CD_3OD (500 MHz).

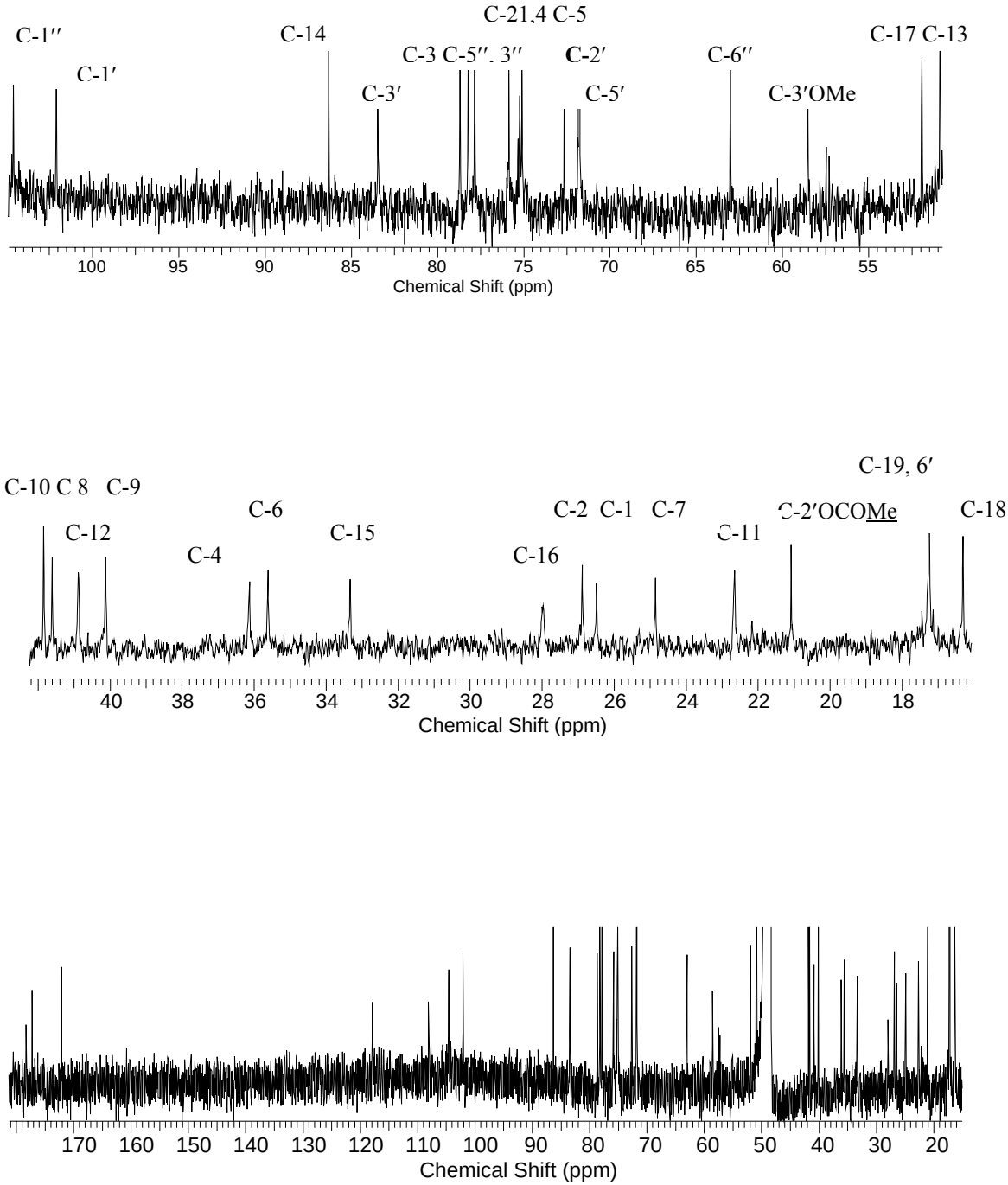


Figure 11. ^{13}C NMR spectrum of the new cardenolide **13** in CD_3OD (500 MHz).

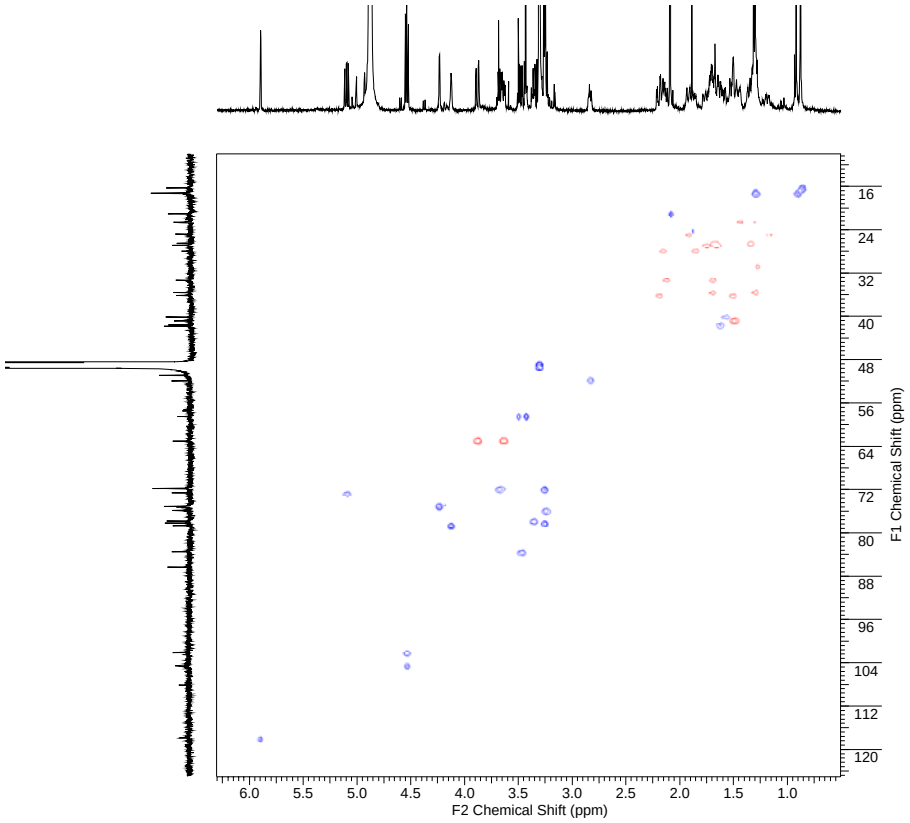


Figure 12. HSQC NMR spectrum of the new cardenolide **13** in CD_3OD (500 MHz).

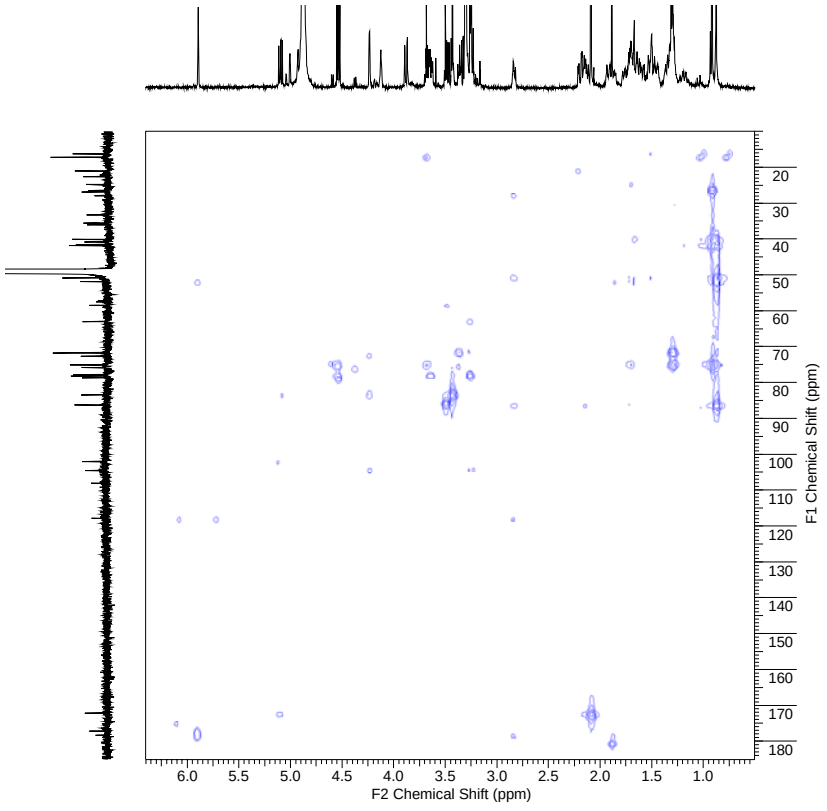


Figure 13. HMBC NMR spectrum of the new cardenolide **13** in CD_3OD (500 MHz).

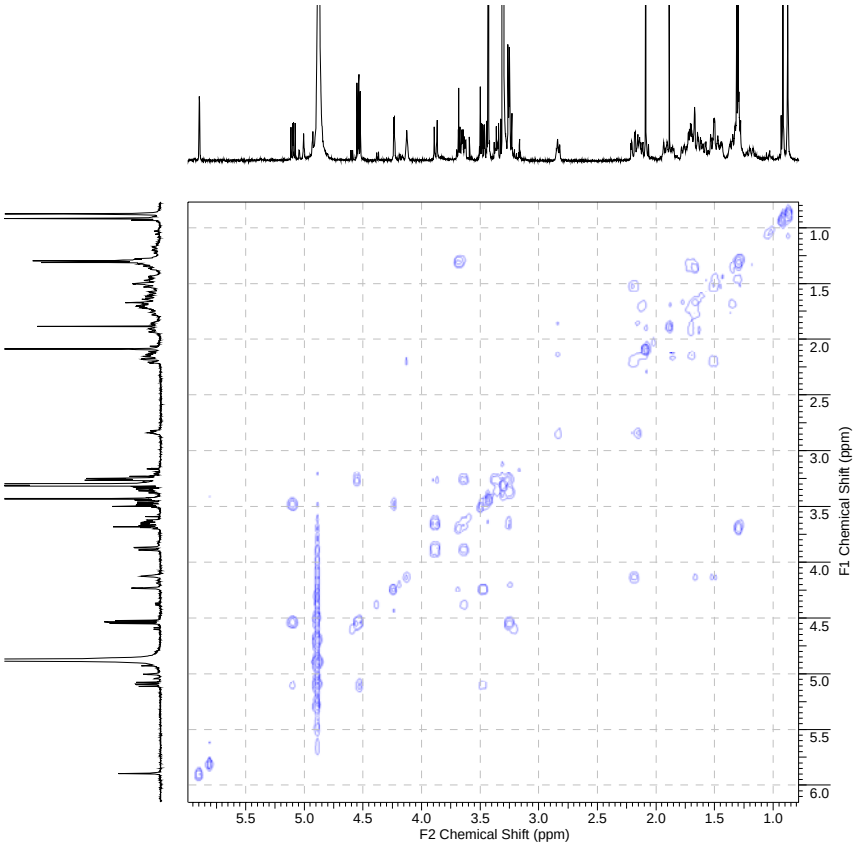


Figure 14. COSY NMR spectrum of the new cardenolide **13** in CD_3OD (500 MHz).

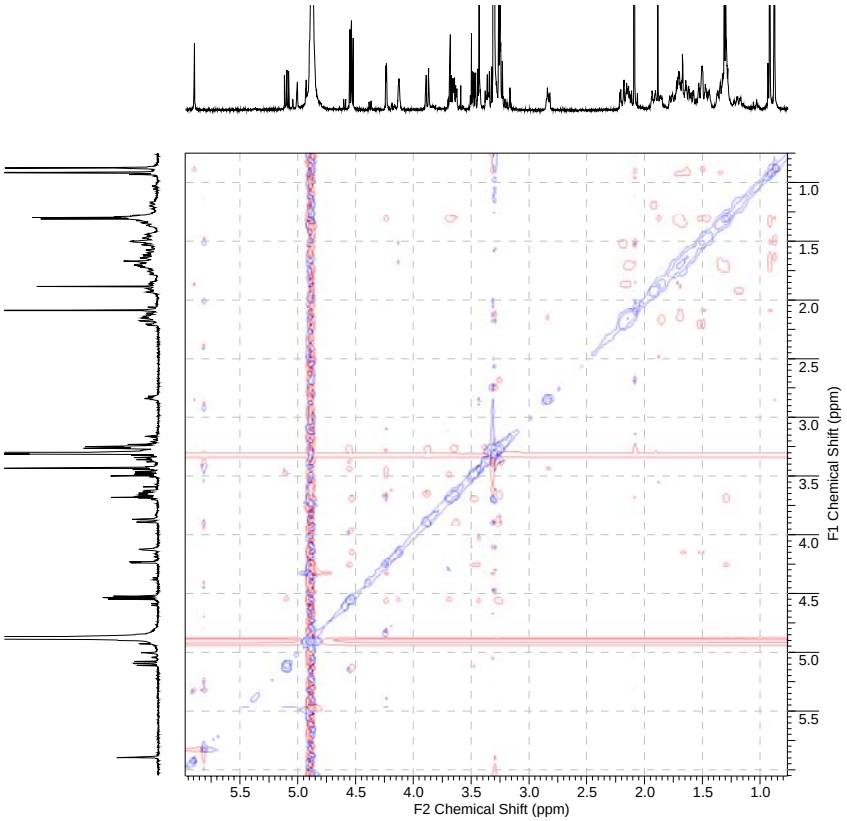


Figure 15. ROESY NMR spectrum of the new cardenolide **13** in CD_3OD (500 MHz).

Table 5. NMR data of compound **13** (500 MHz, CD_3OD).

Atom	δ_H [ppm]	Significant COSY	Significant NOESY	δ_C [ppm]	$^nJ_{CH}$ coupling. δ_H [ppm], HMBC
1	1.36, 1.70 (*m*)			26.5	1.02 (19)
2	1.75, 1.64 (*m*)			26.9	
3	4.37 (*br s*)	2.20, 1.65 (4), 4.84 (1′)		78.7	4.84 (1′)
4	2.20, 1.65 (*m*)			36.1	1.70 (6A)
5				75.1	1.65 (4B), 1.70 (6A)
6	1.70, 1.50 (*m*)			35.6	1.02 (19)
7	2.30, 1.35 (*m*)			24.9	1.70 (6A), 1.85 (8)
8	1.85 (*m*)			41.6	2.30 (7A)
9	1.60 (*m*)			40.1	1.85 (8), 1.02 (19)
10				41.8	1.02 (19)
11	1.35, 1.25 (*m*)			22.7	1.60 (9)
12	1.36 (*m*)			40.9	1.00 (18), 2.81 (17)
13				50.9	1.00 (18), 2.81 (17)
14				86.3	1.00 (18), 2.81 (17), 2.10, 1.85 (15)
15	2.10, 1.85 (*m*)			33.3	
16	2.10, 2.00 (*m*)			28.0	2.81 (17)
17	2.818 (*m*)	2.10 (16A)	2.10 (16A)	51.9	2.00 (16B), 1.00(18)
18	1.001 (*s*)			16.3	1.36 (12)
19	1.029 (*s*)			17.3	
20				178.3	5.10 (21B), 6.16 (22), 2.81 (17)
21	5.10, 5.34 (*dd*, 8.4/1.7)			75.3	2.81 (17)
22	6.161 (*br s*)			117.9	2.81 (17)
23				177.2	6.16 (22)
1′	4.841 (*d*, 8.0)	5.82 (2′)	3.60 (3′) 3.73 (5′)	102.1	3.73 (5′), 5.82 (2′)
2′	5.828 (*dd*, 10.2/8.0)	3.60 (3′)	3.60 (3′)	72.7	3.60 (3′), 4.44 (4′)
3′	3.608 (*dd*, 10.1/3.0)	4.44 (4′)	3.45 (OMe)	83.5	5.82 (2′), 4.44 (4′), 3.45 (OMe)
4′	4.448 (*br d*, 3.0)	3.60 (3′)	3.60 (3′)	75.2	3.73 (5′)
5′	3.738 (*m*)	1.55 (6′)	4.44 (4′)	71.8	1.55 (6′)
6′	1.557 (*d*, 6.4)	3.73 (5′)	3.73 (5′)	17.3	3.73 (5′)
3′-OMe	3.457 (*s*)			58.5	3.60 (3′)
2′-OAc	2.225 (*s*)			21.1	
2′-OCOMe				172.2	5.82 (2′), 2.22 (COMe)
1″	5.149 (*d*, 7.7)	3.96 (5″)	4.24 (3″) 3.96 (5″)	104.6	4.44 (4′), 3.99 (2″)
2″	3.99	4.24 (3″)		75.9	5.14 (1″)
3″	4.248 (*dd*, 8.8/8.8)	3.99 (2″)	5.14 (1″)	77.9	4.19 (4″)
4″	4.190 (*dd*, 9.4/8.8)	3.96 (5″)	3.99 (2″)	71.8	3.96 (5″)
5″	3.96	4.60 (6″A)	4.60 (6″A)	78.2	4.36 (6″B), 4.19 (4″)
6″	4.36, 4.60 (*br d,* 11.5)	3.96 (5″)	3.96 (5″)	63.0	3.96 (5″)

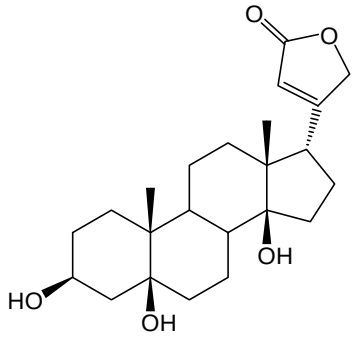
3.3.3. 17*β*-H-Periplogenin (14), 17*β*-H-periplogenin-*β*-*D*-digitoxose (15), 17*β*-H-periplogenin-*β*-*D*-cymarose (16)

The subfractions 14 and 15 of the ethyl acetate extract provided, after silica gel and RP-18 column chromatography, the known compound **14** and the unknown compound **15**. Compound **16** was isolated from the *n*-butanol fraction. The compound **14** and **16** were identified as 17*β*-H-periplogenin **(14)** and 17*β*-H-periplogenin-*β*-*D*-cymarose **(16)** by different MS and 2D NMR experiments and by comparison with the spectral data from literature [Furuya *et al*., 1988].

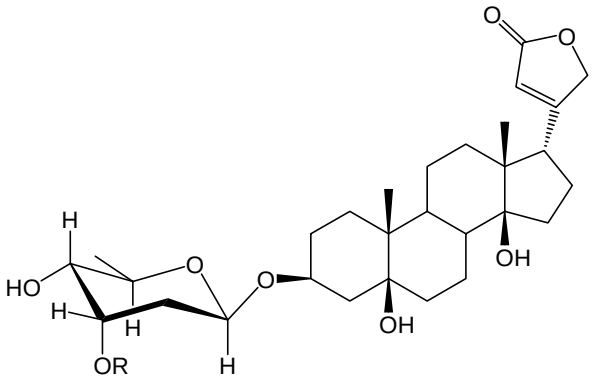
Compound **14** had the composition $C_{23}H_{34}O_5$ on the basis of HR-MS. In the ^1H NMR (C_5D_5N) spectrum of **14**, the methyl proton signals H_3-18 and H_3-19 were shifted downfield to δ 1.227 and 1.186 ppm (each 3H, *s*) by comparison with that of compound **10**. The methine proton signal of H-17, coupling with the methylene protons of C-16, was also shifted downfield to δ 3.463 (1H, *t*, *J* 9.5 Hz). In the ROESY spectrum, a correlation peak between H-17 and H_3-18 was found. In the ^{13}C NMR spectrum (tab. 9), the carbon C-12 and C-17 signals were shifted upfield to δ 31.1 and 48.8 ppm. Thereby the configuration of H-17 was revealed to be β [Furuya *et al*., 1988].

Compound **15** was isolated as a white powder. The molecular formula $C_{29}H_{44}O_8$ was deduced from positive ion ESI-FT-ICR-MS (*m/z* 543.29240 $[M+Na]^+$, calcd. for $C_{29}H_{44}O_8Na$ 543.29283). The ^1H and ^{13}C NMR data of the aglycone agree with the characteristic peaks of 17*β*-H-periplogenin [Furuya *et al*., 1988]. The HMBC correlation between C-3 and the anomeric proton H-1′ proved the presence of the sugar moiety at C-3. This is further supported by the NOE of H-3 with H-1′. According to the EI-MS data, the fragments at *m/z* 391 and 373 indicated a aglycone moiety and *m/z* 131 and 113 fragments of a 2,6-deoxy sugar moiety. The 2D NMR correlation peaks of the sugar moiety and the vicinal ^1H, ^1H coupling constants confirmed the presence of digitoxose (shown in tab. 8). After hydrolyses with HCl, the hydrolysed sugar moiety could be also identified as digitoxose by GC analysis as its trimethylsilylether.

According to the spectral data, compound **15** was identified as a new combination of the known aglycone and known sugar 17*β*-H-periplogenin-*β*-*D*-digitoxoside.



14

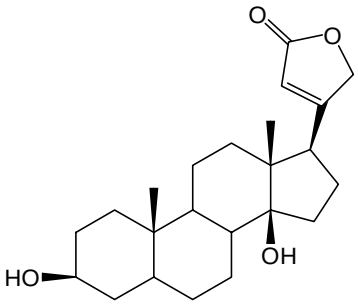


15 R = H
16 R = Me

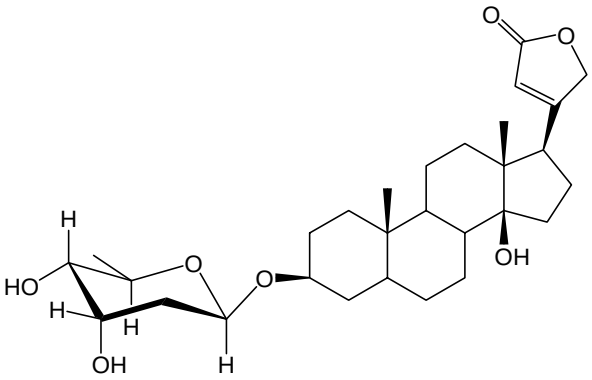
3.3.4. 17α-H-Digitoxigenin (17), 17α-H-digitoxigenin-β-*D*-digitoxoside (18)

Compounds **17** and **18** were isolated from fractions 10 and 11 of the ethyl acetate extract after silica gel chromatography (see in scheme 1 and 3). Their structures were confirmed by 2D NMR (tab. 8, 11) and comparison to reported data [Danieli *et al*., 1966; Habermehl *et al*., 1985].

.



17



18

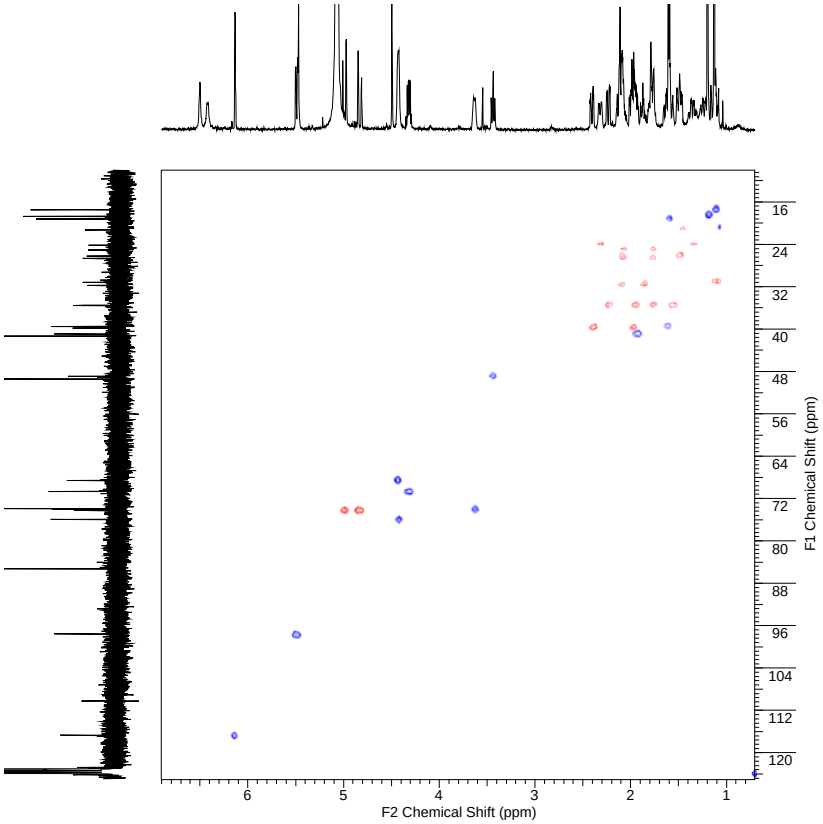


Figure 16. HSQC NMR spectrum of the new cardenolide **15** in C_5D_5N (500 MHz).

Table 6. ^{1}J-^{13}C-^{1}H correlation of **15**.

δ_C [ppm] Aglycone	δ_H [ppm] Aglycone	δ_C [ppm] Sugar	δ_H [ppm] Sugar
26.2	1.48, 2.12	97.5	5.489
26.6	1.76, 2.12	39.8	1.99, 2.41
75.9	4.426	68.6	4.439
35.5	1.76, 2.24	74.1	3.633
35.5	1.56, 1.94	70.6	4.319
24.1	1.32, 2.34	19.2	1.602
40.9	1.94		
39.5	1.62		
21.3	1.42		
31.1	1.12, 1.18		
31.7	1.86, 2.12		
25.0	1.78, 2.06		
48.9	3.438		
18.7	1.198		
17.5	1.127		
74.2	4.83, 4.99		
116.6	6.133		

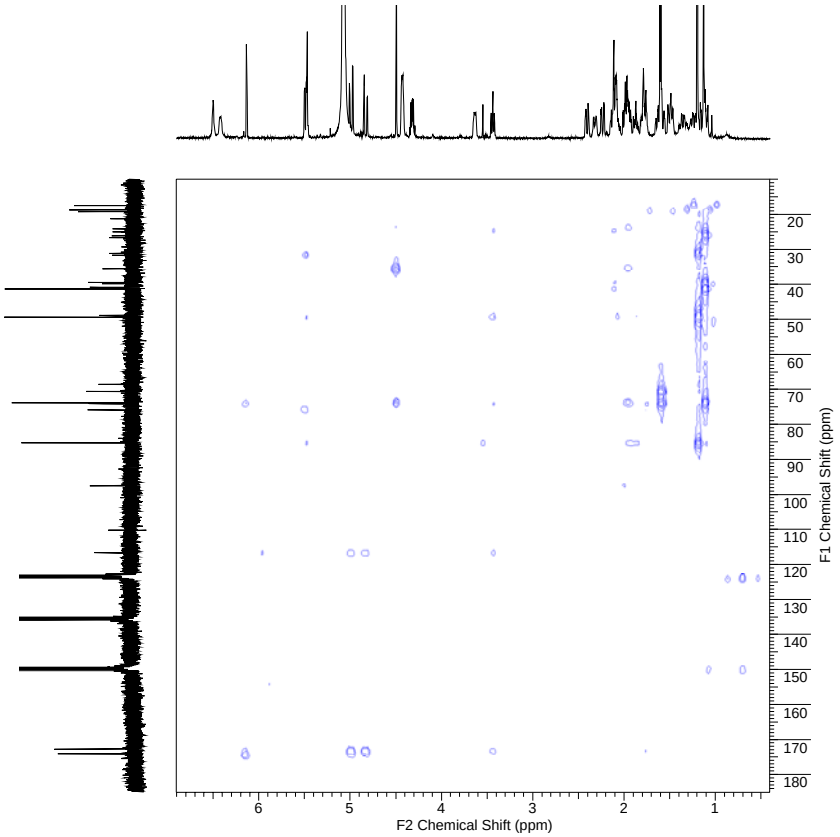


Figure 17. HMBC NMR spectrum of the new cardenolide **15** in C_5D_5N (500 MHz).

Table 7. Long-rang ^{13}C-^{1}H correlation of **15**.

δ_C [ppm]	δ_H [ppm]	δ_C [ppm]	δ_H [ppm]
26.2	1.12	25.0	2.12, 3.43
26.6	1.12	48.9	1.19, 2.06, 1.86
75.9	5.48	18.7	1.12
35.5	4.48	17.5	1.62
73.9	1.12, 1.56	172.8	6.13, 3.43
35.5	1.94	74.2	6.13, 3.43
24.1	1.94	116.6	3.43, 4.83, 4.99
40.9	1.62	174.2	3.43, 4.83, 4.99
39.5	1.12, 2.12	97.5	1.99
41.3	1.12, 2.12	39.8	5.48
21.3	1.12	68.6	2.41
31.1	1.19, 3.43	74.1	4.32, 4.43
49.4	1.19, 3.43	70.6	1.60
85.3	1.19, 3.43	19.2	4.32
31.7	1.78		

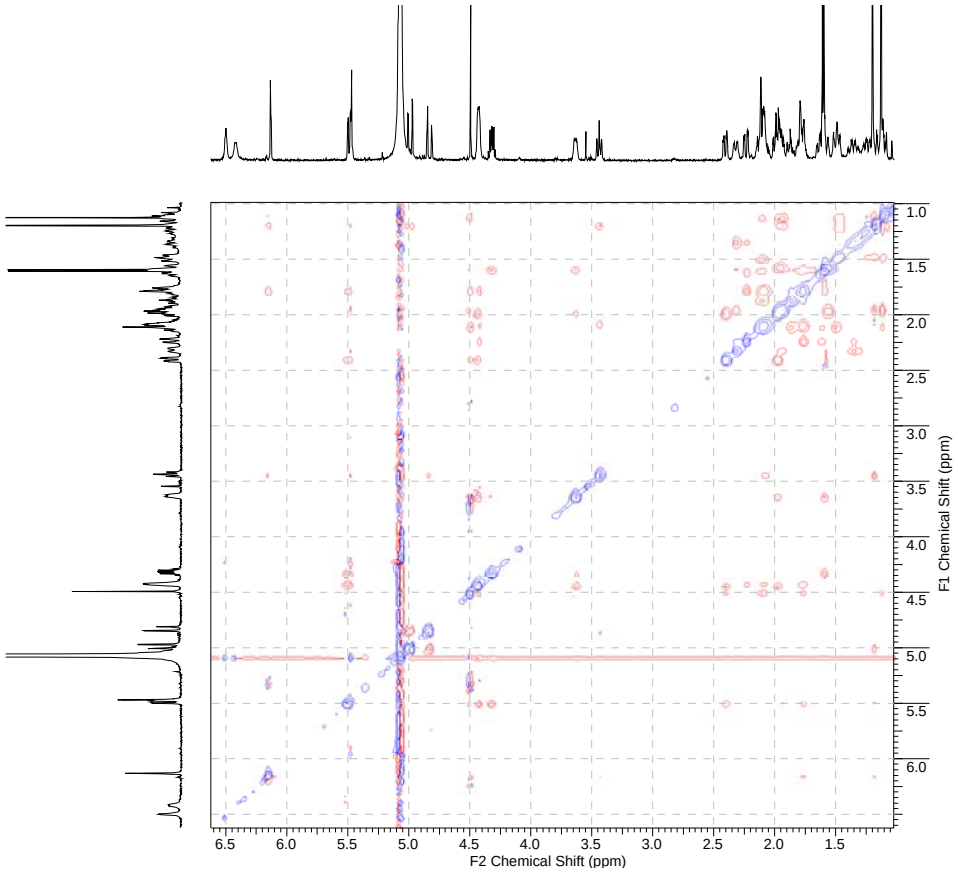


Figure 18. ROESY NMR spectrum of the new cardenolide **15** in C_5D_5N (500 MHz).

Table 8. Significant ROESY correletion of cardenolides **11, 12, 14-16, 18** (500 MHz, C_5D_5N).

H-Atom	$^{n}J_{HH}$ coupling. δ_H [ppm] ROESY					
	11	**12**	**14**	**15**	**16**	**18**
3	5.46 (1′)	5.17 (1′)		5.49 (1′)	5.18 (1′)	
17	2.10 (16A)		1.19 (18)	1.19 (18)	1.20 (18)	1.02 (18) 2.12 (16A) 2.12 (15A) 6.15 (22)
19				4.48 (5-OH)		1.76 (1A)
1′	4.30 (5′) 1.96 (2′A)	4.23 (5′) 2.30 (2′A)		4.31 (5′) 2.41 (2′A)	4.13 (5′) 2.30 (2′A)	4.36 (5′) 2.47 (2′A)
3′	3.62 (4′) 1.96 (2′B)	3.41 (OMe) 3.53 (4′)		3.63 (4′) 2.41 (2′A) 1.99 (2′B)	3.42 (OMe) 3.56 (4′)	3.66 (4′) 2.47 (2′A) 2.14 (2′B)
4′	1.59 (6′)	1.54 (6′)		1.60 (6′)	1.54 (6′)	1.63 (6′)
5′	1.59 (6′)	1.54 (6′)		1.60 (6′)	1.54 (6′)	1.63 (6′)

Table 9. ^{13}C NMR spectral data of cardenolides **10-12, 14-18** (300, 500 MHz, C_5D_5N).

C-Atom	δ_C [ppm]							
	10	**11**	**12**	**14**	**15**	**16**	**17**	**18**
1	25.95	26.3	26.2	25.7	26.2	26.0	27.5	30.8
2	28.7	26.6	26.6	28.6	26.6	26.4	28.9	27.1
3	67.9	75.9	75.9	67.8	75.9	75.9	66.1	73.1
4	36.1	35.6	35.6	37.9	35.5	35.4	34.5	30.4
5	74.5	73.8	73.8	74.5	73.9	73.8	36.9	37.0
6	37.9	35.6	35.6	35.9	35.5	35.4	27.5	27.1
7	24.6	24.5	24.5	24.0	24.1	24.0	22.3	21.9
8	41.5	41.1	41.1	40.8	40.9	40.7	42.1	41.8
9	40.1	39.4	39.4	39.3	39.5	39.4	36.0	35.8
10	41.1	41.3	41.3	41.3	41.3	41.1	35.9	35.5
11	22.3	22.2	22.2	21.2	21.3	21.1	21.8	21.5
12	39.3	40.1	40.0	31.1	31.1	31.0	33.4	39.8
13	50.1	50.1	50.1	49.3	49.4	49.2	50.3	50.1
14	84.7	84.7	84.7	85.3	85.3	85.2	84.8	84.6
15	33.3	33.3	33.3	31.6	31.7	31.6	30.6	33.1
16	27.4	27.4	27.4	24.9	25.0	24.9	27.5	27.3
17	51.4	51.4	51.4	48.8	48.9	48.8	51.6	51.4
18	16.4	16.4	16.4	18.6	18.7	18.5	16.4	16.2
19	17.6	17.4	17.4	17.4	17.5	17.3	24.3	23.9
20	175.9	175.9	175.8	172.9	172.8	172.9	174.5	174.6
21	73.8	73.8	73.8	74.2	74.2	73.8	73.8	73.7
22	117.7	117.7	117.7	116.7	116.6	116.6	117.7	117.6
23	174.5	174.5	174.4	174.3	174.2	174.3	175.9	176.1
1′		97.6	97.4		97.5	97.4		96.7
2′		39.9	35.9		39.8	35.8		40.1
3′		68.6	78.8		68.6	78.7		68.7
4′		74.1	74.1		74.1	74.0		74.2
5′		70.7	71.2		70.6	71.1		70.3
6′		19.2	19.2		19.2	19.0		19.1
3′-OMe			58.1			58.0		

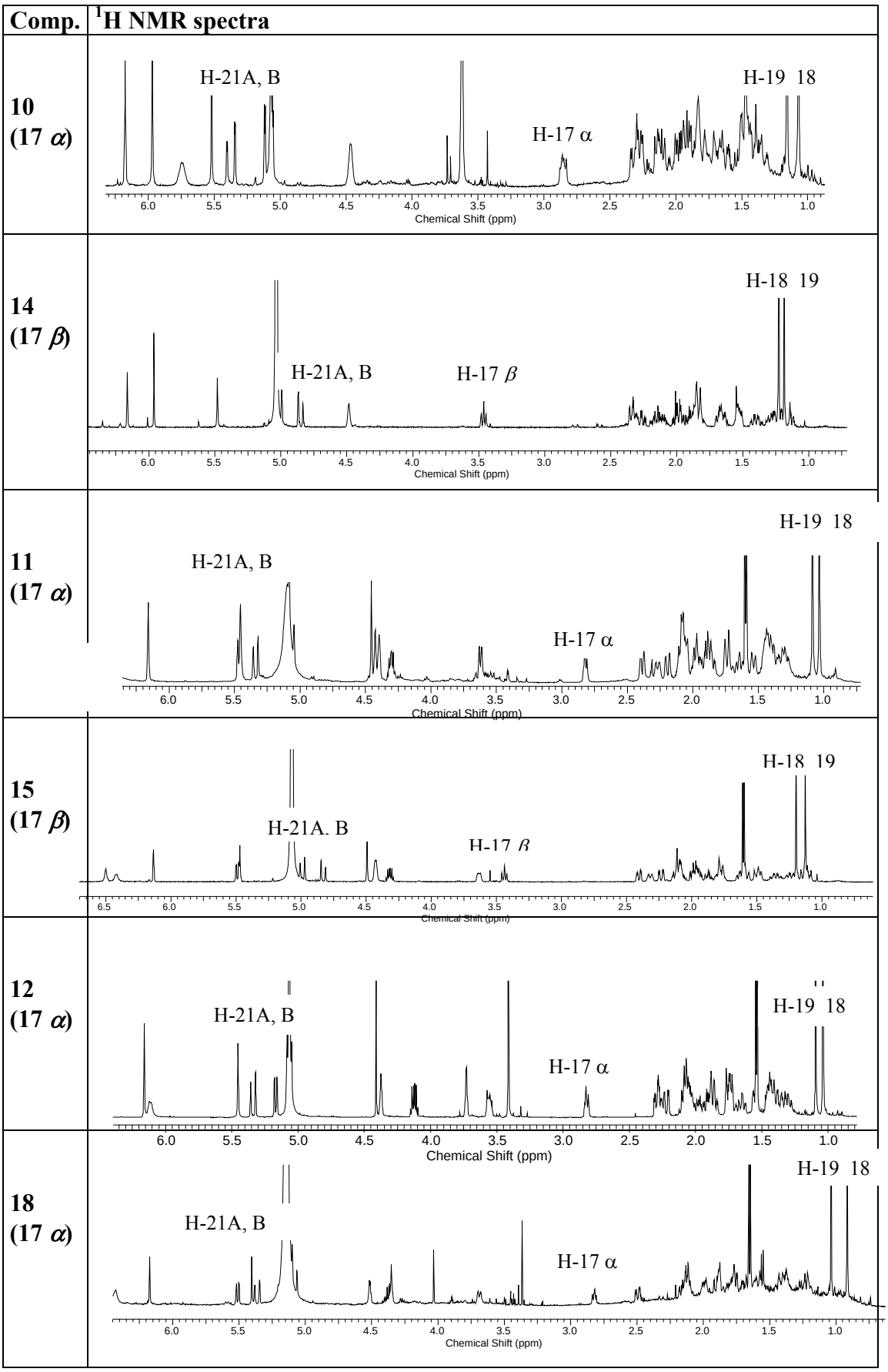


Figure 19. Examples for ^{1}H NMR spectra of 17α H and 17β H cardenolides in C_5D_5N (500 MHz).

Table 10. ^{1}H NMR spectral data of cardenolides **10-12, 14-18** (300, 500 MHz, C_5D_5N).

H-Atom	δ_H [ppm]							
	10	**11**	**12**	**14**	**15**	**16**	**17**	**18**
1		1.44, 2.08 (*m*)	1.42, 2.04 (*m*)	1.50, 2.28 (*m*)	1.48, 2.12 (*m*)	1.48, 2.08 (*m*)		1.56, 1.76 (*m*)
2		1.72, 2.08 (*m*)	1.72, 2.04 (*m*)	1.84 (*m*)	1.76, 2.12 (*m*)	1.78, 2.08 (*m*)		1.22, 1.82 (*m*)
3	4.46 (*br s*)	4.397 (*br s*)	4.373 (*br s*)	4.483 (*br s*)	4.426 (*br s*)	4.424 (*br s*)	4.42 (*br s*)	4.335 (*br s*)
4		1.72, 2.18 (*m*)	1.74, 2.22 (*m*)	1.82, 2.34 (*m*)	1.76, 2.24 (*m*)	1.76, 2.24 (*m*)		1.60, 1.87 (*m*)
5								1.86 (*m*)
6		1.52, 1.90 (*m*)	1.52, 2.22 (*m*)	1.64, 1.98 (*m*)	1.56, 1.94 (*m*)	1.56, 1.96 (*m*)		1.82 (*m*)
7		1.30, 2.28 (*m*)	1.30, 2.28 (*m*)	1.34, 2.34 (*m*)	1.32, 2.34 (*m*)	1.34, 2.31 (*m*)		2.12 (*m*)
8		1.84 (*m*)	1.84 (*m*)	1.96 (*m*)	1.94 (*m*)	1.94 (*m*)		1.78 (*m*)
9		1.64(*m*)	1.64 (*m*)	1.66 (*m*)	1.62 (*m*)	1.62 (*m*)		1.76 (*m*)
11		1.24, 1.38 (*m*)	1.26, 1.40 (*m*)	1.24, 1.50 (*m*)	1.42 (*m*)	1.24, 1.48 (*m*)		1.38 (*m*)
12		1.40 (*m*)	1.40 (*m*)	1.12, 1.18 (*m*)	1.12, 1.18 (*m*)	1.12, 1.18 (*m*)		1.40 (*m*)
15		1.86, 2.08 (*m*)	1.86, 2.08 (*m*)	1.88, 2.18 (*m*)	1.86, 2.12 (*m*)	1.86, 2.10 (*m*)		1.90, 2.12 (*m*)
16		1.96, 2.10 (*m*)	1.96, 2.10 (*m*)	1.84, 2.13 (*m*)	1.78, 2.06 (*m*)	1.78, 2.08 (*m*)		1.98, 2.12 (*m*)
17	2.84 (*dd*, 9/3)	2.817 (*m*)	2.818 (*d*, 8.0)	3.463 (*t*, 9.5)	3.438 (*br dd*, 9.6/9.6)	3.447 (*br dd*, 9.6/9.6)	2.84 (*m*)	2.805 (*m*)
18	0.88 (*s*)	1.036 (*s*)	1.038 (*s*)	1.227 (*s*)	1.198 (*s*)	1.201 (*s*)	1.05 (*s*)	1.021 (*s*)
19	0.94 (*s*)	1.087 (*s*)	1.093 (*s*)	1.186 (*s*)	1.127 (*s*)	1.133 (*s*)	0.99 (*s*)	0.899 (*s*)
21	5.08, 5.36 (*dd*, 18.1/1.4)	5.06, 5.34 (*dd*, 18.1/1.4)	5.06, 5.34 (*dd*, 18.1/1.4)	4.85, 4.99 (*dd*, 17.5/1.4)	4.83, 4.99 (*dd*, 17.6/1.8)	4.83, 4.99 (*dd*, 17.6/1.8)	5.06, 5.36 (*dd*, 18.1/1.4)	5.06, 5.34 (*dd*, 18.1/1.4)
22	6.17 (*br s*)	6.158 (*br s*)	6.159 (*br s*)	6.162 (*br s*)	6.133 (*br s*)	6.131 (*br s*)	6.15 (*br s*)	6.156 (*br s*)
1′		5.465 (*dd*, 9.7/1.4)	5.177 (*dd*, 9.7/1.8)		5.489 (*dd*, 9.6/1.9)	5.186 (*dd*, 9.6/1.9)		5.494 (*dd*, 9.7/1.7)
2′		1.96 (*m*), 2.391 (*br d*, 13.2)	1.74, 2.30 (*m*)		1.99 (*ddd*, 3.2 /9.6/2.6) 2.41 (13.6 /3.6/1.9)	1.92-2.00 2.26-2.34 (*m*)		2.472
3′		4.426 (*d*, 2.7)	3.554 (*d*, 2.9)		4.439 (*d*, 2.6)	3.734 (*d*, 2.9)		4.498 (*d*, 2.9)
4′		3.623 (*dd*, 2.4/9.3)	3.536 (*dd*, 2.4/9.3)		3.633 (*m*)	3.562 (*m*)		3.669 (*dd*, 2.4/9.3)
5′		4.305 (*m*)	4.233 (*m*)		4.319 (*dq*, 9.4/6.2)	4.135 (*dq*, 9.4/6.2)		4.368 (*m*)
6′		1.597 (*d*, 6.1)	1.540 (*d*, 6.1)		1.602 (*d*, 6.2)	1.544 (*d*, 6.2)		1.634 (*d*, 6.3)
3′-OMe			3.417 (*s*)			3.417 (*s*)		

Table 11. Long range HMBC correletion of cardenolides **11, 12, 14-18** (500 MHz, C_5D_5N).

C-Atom	$^nJ_{CH}$ coupling. δ_H [ppm] HMBC					
	11	**12**	**14**	**15**	**16**	**18**
1	1.08 (19), 1.72 (2B)	1.09 (19), 1.72 (2B)	1.18 (19)	1.12 (19)	2.08 (2A), 1.13 (19)	0.89 (19), 1.82 (2A)
2	2.08 (1A)	2.04 (1A)	2.28 (1A), 1.18 (19)	1.12 (19)	2.08 (1A), 1.13 (19)	1.56 (1B), 0.89 (19)
3	5.46 (1′), 1.72 (2B)	5.17 (1′), 1.72 (2B)	1.84 (2)	5.48 (1′)	5.18 (1′)	5.49 (1′), 1.60 (4B), 1.56 (1B)
4	4.45 (5OH) 1.90 (6A)	4.41 (5OH) 1.90 (6A)	5.96 (5OH), 1.98 (6A)	4.48 (5OH)	4.42 (3)	1.86 (5)
5	1.08 (19)	1.09 (19)	1.18 (19), 1.98 (6A)	1.12 (19), 1.56 (6B)		0.89 (19), 1.82 (6)
6	1.84 (8), 1.30 (7B)	1.84 (8), 1.30 (7B)	5.96 (5OH)	1.94 (8)	1.94 (8), 1.34 (7B)	2.12 (7)
7	1.84 (8), 1.52 (6B)	1.84 (8), 1.52 (6B)	1.96 (8), 1.98 (6A)	1.94 (8), 1.94 (6A)	1.94 (8), 1.96 (6A)	1.78 (8), 1.82 (6)
8	1.30 (7B)	1.64 (9), 1.30 (7B)	1.66 (9)	1.62 (9)	1.62 (9), 1.34 (7B)	1.38 (11)
9	1.08 (19), 1.24 (11B), 1.38 (11A)	1.26 (11B), 1.40 (11A), 1.09 (19)	1.50 (1B), 1.50 (11)	1.12 (19), 2.12 (1A)	2.08 (1A), 1.94 (8), 1.13 (19)	0.89 (19), 1.78 (8)
11	1.40 (12)	1. 40 (12)			1.12 (12)	1.40 (12)
12, 13	1.03 (18),	1.03 (18),	1.22 (18),	1.19 (18),	1.20 (18),	1.02 (18),
14	2.81 (17)	2.81 (17)	3.46 (17)	3.43 (17)	3.44 (17)	2.80 (17)
15	1.96 (16B), 2.81 (17)	1.96 (16B), 2.81 (17)	1.84 (16B)	1.78 (16B)	1.78 (16B), 2.08 (16A)	1.98 (16B)
16	2.81 (17), 1.86 (15B)	2.81 (17), 1.86 (15B)	2.18 (15A), 3.46 (17)	2.12 (15A), 3.43 (17)	1.86, 2.10 (15), 3.44 (17)	2.80 (17), 1.90 (15B)
17	1.03 (18), 6.15 (22), 1.96 (16B)	1.03 (18), 6.15 (22), 1.96 (16B),	1.22 (18), 2.18 (15A), 1.84 (16B)	1.19 (18), 2.06 (16A), 1.86 (15B)	2.08 (16A), 1.86 (15B), 1.20 (18)	1.02 (18), 1.98 (16B), 6.15 (22)
18	1.40 (12)	1. 40 (12)	3.46 (17), 1.12 (12)	1.12 (12)	1.12 (12)	1.38 (11), 1.40 (12)
19	2.08 (1A), 1.38 (11A)	2.04 (1A), 1.64 (9)	1.66 (9), 1.50 (11)	1.62 (9)	2.08 (1A), 1.62 (9), 1.25 (11B)	1.56 (1B), 1.76 (9)
21	6.15 (22), 2.81 (17)	6.15 (22), 2.81 (17)	3.46 (17), 6.16 (22)	6.13 (22), 3.43 (17)	6.13 (22), 3.44 (17)	6.15 (22), 2.80 (17)
22	2.81 (17), 5.06 (21B), 5.34 (21A)	2.81 (17), 5.06 (21B), 5.34 (21A)	3.46 (17), 4.84 (21B), 4.99 (21A)	3.43 (17), 4.83 (21B), 4.99 (21A)	3.44 (17), 4.82 (21B), 4.98 (21A)	2.80 (17), 5.06 (21B), 5.34 (21A)
1′	4.30 (5′), 1.96 (2′B)	1.74 (2′B), 2.30 (2′A), 4.23 (5′)		1.99 (2′B)	1.96 (2′B), 3.73 (3′), 4.13 (5′)	4.33 (3), 4.36 (5′), 2.14 (2′B)
2′	5.46 (1′)	5.17 (1′)		5.48 (1′)	5.18 (1′)	5.49 (1′)
3′	3.62 (4′)	3.41 (OMe)		2.41 (2′A)	2.30 (2′A)	2.47 (2′A)
4′	4.30 (5′), 4.42 (3′)	4.23 (5′)		4.32 (5′), 4.43 (3′)	3.73 (3′), 4.13 (5′), 1.54 (6′)	4.36 (5′), 4.49 (3′), 1.63 (6′)
5′	1.59 (6′)	1.54 (6′)		1.60 (6′)	1.54 (6′)	1.63 (6′)
6′	4.30 (5′)			4.32 (5′)	4.13 (5′)	4.36 (5′)
3′-OMe		3.55 (3′)			3.73 (3′)	

3.4. Structure elucidation of the pregnane glycoside Δ^5-pregnene-3β,16α-diol-3-*O*-[2,4-*O*-diacetyl-β-digitalopyranosyl-(1-4)-β-*D*-cymaropyranoside]-16-*O*-[β-glucopyranoside] (19)

The new compound **19** was isolated as a white amorphous solid from the initial *n*-butanol/ water partition (18 g) by repeated chromatography on silicagel 60 (solvent $CHCl_3$/MeOH, 9/1 v/v) and RP-18 (solvent MeOH/H_2O, 8/2 v/v). The molecular formula $C_{45}H_{70}O_{17}$ was deduced from ESI-FT-ICR-MS (*m/z* 905.45077 $[M+Na]^+$, calcd. for $C_{45}H_{70}O_{17}Na$ 905.45052). The spectral data (^{13}C, ^1H) were close to reported data for known pregnane glycosides [Dong *et al*., 2001]. The structure was also confirmed by 2D NMR experiments.

Table 12. ^1H NMR data of compound **19** (500 MHz, C_5D_5N).

H-Atom (aglycone)	δ_H [ppm]	J_{HH} [Hz]	H-Atom (sugar)	δ_H [ppm]	J_{HH} [Hz]
1	0.98, 1.68	(*m*)	1′	5.271	(*d*, 10.0)
2	1.72, 2.06	(*m*)	2′A	1.886	(*br d*, 12.8)
			2′B	2.366	(*br dd*, 12.8, 10.0)
3	3.798	(*m*)	3′	4.040	(*m*)
4	2.54, 2.38	(*m*)	4′	3.554	(*dd*, 9.2, 2.6)
5			5′	4.233	(*m*)
6	5.337	(*d*, 4.9)	6′	1.480	(*d*, 6.2)
7	1.46, 1.82	(*m*)	3′OMe	3.526	(*s*)
8	1.30	(*m*)	1″	4.806	(*d*, 8.0)
9	0.88	(*m*)	2″	5.612	(*m*)
11	1.24, 1.44	(*m*)	3″	3.707	(*dd*, 10.2, 3.4)
12	1.228, 1.834	(*m*)	4″	5.586	(*m*)
14	1.414	(*m*)	5″	3.966	(*m*)
15	1.66, 1.96	(*m*)	6″	1.329	(*d*, 6.5)
16	5.200		3″OMe	3.433	(*s*)
17	3.025	(*br d*, 6.1)	2″COMe	2.160	(*s*)
18	0.626	(*s*)	4″COMe	1.934	(*s*)
19	0.918	(*s*)	1‴	4.923	(*d*, 7.9)
21	2.322	(*s*)	2‴	4.052	(*dd*, 8.6, 7.9)
			3‴	4.254	(*dd*, 9.2, 8.6)
			4‴	4.308	(*dd*, 9.2, 9.2)
			5‴	3.861	(*m*)
			6‴A	4.487	(*dd*, 11.7, 4.6)
			6‴B	4.395	(*dd*, 9.5, 2.2)

The ^1H NMR spectrum showed three methyl groups on the aglycone [δ 0.626 ppm (3H, *s*, CH_3-18), 0.918 ppm (3H, *s*, CH_3-19), 2.322 ppm (3H, *s*, CH_3-21)], one olefinic methine group [δ 5.337 ppm (1H, *d*, *J* = 4.9, CH-6)], seven methylenes (δ 2.600-1.00 ppm) and five methine groups [δ 1.271 ppm (1H, *m*, CH-8), 1.414 ppm (1H, *m*, CH-14), 0.888 ppm (1H, *m*, CH-9),

3.798 ppm (1H, *m*, CH-3), 5.200 ppm (1H, *m*, CH-16)]. From a 2D ROESY experiment the α-configuration of H-17, H-14 and the β-configuration of H-16 and H_3-18 could be deduced.

Table 13. ^{13}C NMR data of compound **19** (500 MHz, C_5D_5N).

C -Atom (aglycone)	δ_C [ppm]	Type	C-Atom (sugar moiety)	δ_C [ppm]	Type
1	37.3	CH_2	1′	96.2	CH
2	30.2	CH_2	2′	36.6	CH_2
3	77.3	CH	3′	77.2	CH
4	39.2	CH_2	4′	84.2	CH
5	140.8	C_q	5′	68.7	CH
6	121.6	CH	6′	18.5	CH_3
7	31.9	CH_2	3′-OMe	58.2	CH_3
8	31.4	CH	1″	103.1	CH
9	50.1	CH	2″	71.4	CH
10	36.8	C_q	3″	80.1	CH
11	20.9	CH_2	4″	69.2	CH
12	38.7	CH_2	5″	69.5	CH
13	44.9	C_q	6″	16.6	CH_3
14	54.4	CH	3″-OMe	57.6	CH_3
15	33.7	CH_2	2″-COMe	21.0	CH_3
16	81.0	CH	4″-COMe	20.4	CH_3
17	72.1	CH	2″-CO	169.7	C_q
18	14.6	CH_3	4″-CO	170.6	C_q
19	19.3	CH_3	1‴	105.0	CH
20	208.2	C_q	2‴	75.3	CH
21	32.2	CH_3	3‴	78.5	CH
			4‴	71.4	CH
			5‴	78.2	CH
			6‴	62.5	CH_2

In the ^{13}C spectrum the signals at δ 96.2 ppm, δ 103.1 ppm and δ 105.1 ppm were assigned to three anomeric sugar signals. The proton signals and coupling constant values of the three anomeric protons at δ 5.271 ppm (1H, *d*, J = 10.0 Hz), δ 4.806 ppm (1H, *d*, J = 8.0 Hz) and δ 4.923 ppm (1H, *d*, J = 7.9 Hz) also indicated the presence of three sugar moieties with β-anomeric configurations. The types of sugar were confirmed by their coupling constants (shown in tab. 12) and by NOE, COSY, and HMBC experiments.

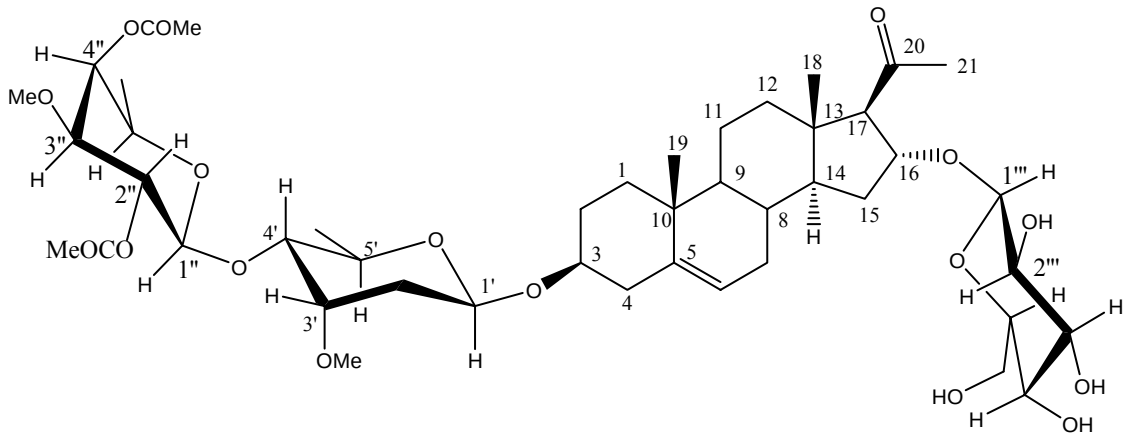
Table 14. Long-range ^{13}C-^{1}H-correlation of compound **19** (500 MHz, C_5D_5N).

C-Atom (aglycone)	$^{n}J_{CH}$ coupling δ_H [ppm] HMBC	C-Atom (sugar)	$^{n}J_{CH}$ coupling δ_H [ppm] HMBC
1	0.91 (19)	1′	4.23 (5′)
2		2′	5.27 (1′)
3	5.27 (1′), 2.54 (4)	3′	3.52 (OMe)
4		4′	4.80 (1″), 1.48 (6′)
5	0.91 (19)	5′	1.48 (6′)
6	1.46 (7B), 1.30 (8)	6′	
7		3′OMe	4.04 (3′)
8		1″	5.61 (2″), 3.96 (5″)
9	0.91 (19)	2″	5.58 (4″), 3.70 (3″)
10	0.91 (19)	3″	5.61 (2″), 5.58 (4″), 3.433 (OMe)
11	1.22 (12A)	4″	3.70 (3″), 3.96 (5″)
12	3.02 (17), 0.62 (18)	5″	1.32 (6″)
13	3.02 (17), 0.62 (18) 1.96 (15A), 1.41 (14)	6″	3.96 (5″)
14	0.62 (18), 1.96 (15A)	3″OMe	3.70 (3″)
15		2″COMe	5.61 (2″), 2.16 (2″COMe)
16	4.92 (1‴), 3.02 (17), 1.96 (15A)	4″COMe	1.93 (4″COMe), 4.30 (4‴)
17	0.62 (18), 2.32 (21)	1‴	5.20 (16), 3.86 (5‴)
18	3.02 (17)	2‴	4.25 (3‴)
19	0.98 (1B)	3‴	4.30 (4‴), 4.05 (2‴)
		4‴	4.25 (3‴)
		5‴	4.30 (4‴)
		6‴	4.30 (4‴)

In the HMBC spectrum, the anomeric proton signals at δ 4.923 (H-1‴ of glucose) and 4.806 ppm (H-1″ of digitalose) showed cross-peaks with the carbon signals at δ 81.0 (C-16 of the aglycone) and 84.2 ppm (C-4′ of cymarose). The H-H ROESY correlations between the anomeric proton H-1′ of the cymarose and H-3 of the agylcone were also observed. According to the above correlations, the glucose and the cymarose, which is connected to terminal digitalose, are attached to C-16 and C-3 of the aglycone, respectively. The signals at δ 3.433 ppm and δ 3.526 ppm belong to two methoxy groups and the two methyl singlet proton signals of acetyl groups at 1.934, 2.160 ppm were also connected with the ^{13}C signals at 57.6 (C-3″of the terminal sugar), 58.2 (C-3′ of the inner sugar), 170.6 (C-4″ CO) and 169.7 ppm (C-2″ CO) respectively in the HMBC spectrum.

These also provided the evidence of two acetyl groups attached to C-2″ and C-4″ of the digitalose. From all data, the structure of compound **19** was established as the new glycoside

Δ^5-pregnene-3β,16α-diol-3-*O*-[2,4-*O*-diacetyl-β-digitalopyranosyl(1-4)-β-*D*-cymaropyranoside]-16-*O*-[β-glucopyranoside] of a known aglycone and known sugars.



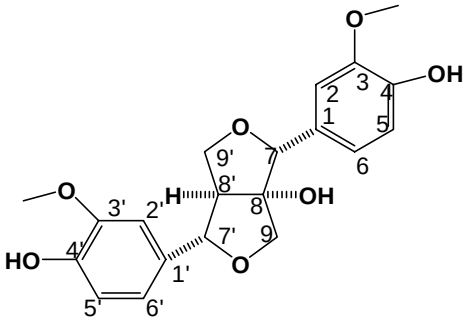
19

Table 15. ^{1}H-^{1}H-correlation of compound **19** (500 MHz, C_5D_5N).

H-Atom	$^nJ_{HH}$ coupling δ_H [ppm]	H-Atom	$^nJ_{HH}$ coupling δ_H [ppm]
	COSY		**NOESY**
1 (A)	0.91 (19)	14	3.02 (17)
3	2.54 (4A)	16	4.92 (1‴)
4 (A)	2.38 (4B)	1′	3.79 (3), 4.23 (5′)
7	1.30 (8)	3′	3.55 (4′)
8	0.88 (9)	4′	4.04 (3')
14	1.96 (15A)	5′	3.55 (4′)
16	3.02 (17)	6'	4.23 (5′)
1′	1.88, 2.36 (2′)	1″	3.55 (4′), 3.70 (3″), 3.96 (5″)
3′	2.36 (2′A), 3.52 (OMe)	2″	3.70 (3″)
4′	4.23 (5′)	3″	3.43 (OMe)
5′	1.48 (6′)	4″	3.96 (5″), 3.43 (OMe)
1″	5.61 (2″)	6″	3.96 (5")
2″	3.70 (3″)	1‴	4.05 (2‴), 4.25 (3‴), 3.86 (5‴)
4″	3.70 (3″)	3‴	3.86 (5‴)
5″	1.32 (6″)	4‴	4.05 (2‴)
1‴	4.05 (2‴)	5‴	4.39 (6‴B), 4.48 (6‴A)
2‴	4.25 (3‴)		
5‴	4.39 (6‴B)		

3.5. Structure elucidation of the lignan 8-hydroxy pinoresinol (20)

The known compound **20** was isolated from the ethylacetate extract by repeated chromatography on silicagel 60 and PTLC on RP-18 (scheme 1 and 3). The spectral data (^{13}C, ^{1}H) were close to reported data [Cowan *et al*., 2001]. The structure elucidation was made by 1D and 2D NMR (tab. 16, 17).



20

Table 16. NMR data of compound **20** (500 MHz, $CDCl_3$: CD_3OD, 3:1).

H-Atom	δ_H [ppm]	$^{n}J_{HH}$ coupling δ_H [ppm]	
		COSY	**NOESY**
2,	7.012 (*s*)	6.871 (6)	4.764 (7)
2′	7.012 (*s*)	6.871 (6′)	4.857 (7′), 3.115 (8′)
3 (OMe)	3.912 (*s*)		7.012 (2)
3′(OMe)	3.903 (*s*)		7.012 (2′)
5, 5′	6.871 (*m*)		
6, 6′	6.871 (*m*)		
7	4.764 (*br s*)	4.046 (9A)	6.871 (6)
7′	4.857 (*br s*)	3.115 (8′)	6.871 (6′)
8′	3.115 (*m*)		4.511 (9′A)
9B	3.907 (*d*, 4.7) ,	4.046 (9A)	
9A	4.046 (*d*, 9.2)		4.764 (7)
9′B	3.820 (*dd*, 6/9)	3.115 (8′)	4.764 (7), 4.857 (7′)
9′A	4.511 (*t*, 8.7)	3.115 (8′)	

Table 17. ^{13}C NMR data and long range ^{13}C-^{1}H-correlation of compound **20** (500 MHz, $CDCl_3$: CD_3OD, 3:1).

C-Atom	δ_C [ppm]	$^{n}J_{CH}$ coupling δ_H [ppm] HMBC
1	126.9	6.87 (5,6), 4.76 (7)
2	110.4	4.76 (7)
3	147.1	7.01 (2), 3.91 (3-OMe), 6.87 (5,6)
4	145.8	7.01 (2), 6.87 (5,6)
5	114.5	
6	119.7	7.01 (2), 4.76 (7)
7	87.6	7.01 (2), 4.04 (9A), 3.90 (9B), 4.51 (9′A)
8	91.0	4.76 (7), 4.85 (7′), 4.51 (9′A), 3.90 (9B), 3.11 (8′)
9	74.5	4.76 (7), 4.85 (7′)
1′	131.7	6.87 (5′,6′), 4.85 (7′)
2′	109.5	4.85 (7′)
3′	147.1	7.01 (2′), 3.903 (3′-OMe), 6.87 (5′,6′)
4′	145.6	7.01 (2′), 6.87 (5′,6′)
5′	114.4	
6′	119.2	7.01 (2′), 4.85 (7′)
7′	86.2	7.01 (2′), 4.51 (9′A), 3.82 (9′B), 4.04 (9A), 3.90 (9B)
8′	60.1	4.85 (7′), 4.04 (9A), 3.82 (9′B)
9′	70.9	4.85 (7′)
3 OMe	55.5	
3′OMe	55.5	

3.6. Chemotaxonomic significance of the isolated phytoconstituents for the genus *Streptocaulon*

S. tomentosum and *S. juventas* belong to the family Asclepiadaceae. The chemotaxonomic position of *S. tomentosum* is confused as it is considered by some authors to be synonymous with *S. juventas* [Ping *et al*., 2005]. Recently, however, we found the chemical differences between these two species. According to literature [Ueda *et al*., 2003a], sixteen cardenolides, two hemiterpenoids, (4*R*)-4-hydroxy-3-(1-methyl ethyl)pentyl rutinoside, (*R*)-2-ethyl-3-methyl-butyl rutinoside, two phenyl propanoids and a phenylethanoid were reported from the air-dried roots of *S. juventas*.

The chemical constituents isolated from the air-dried roots of *S. tomentosum* are nine cardenolides, eight triterpenes, one lignan and one pregnane glycoside (compound **1-20**).

According to the above chemical investigation of the two species, chemical differences are found between *S. tomentosum* and *S. juventas*. Firstly, major cardenolides (digitoxigenin-3-*O*-[*O*-*β*-glucopyranosyl-(1→6)-*O*-*β*-glucopyranosyl-(1→4)-3-*O*-acetyl-*β*-digitoxopyranoside] (**62**), digitoxigenin-3-*O*-[*O*-*β*-glucopyranosyl-(1→6)-*O*-*β*-glucopyranosyl-(1→4)-*O*-*β*-digitalopyranosyl-(1→4)-*β*-cymaropyranoside] (**63**), digitoxigenin-3-*O*-[*O*-*β*-glucopyranosyl-(1→6)-*O*-*β*-glucopyranosyl-(1→4)-*β*-digitoxopyranoside] (**64**), digitoxigenin sophoroside (**65**), echujin (**66**), periplogenin glucoside (**68**), corchorusoside C (**69**)), and minor cardenolides (acovenosigenin A digitoxoside (**59**), acovenosigenin A (**60**)**,** digitoxigenin gentiobioside (**61**)) of *S. juventas* are absent in *S. tomentosum*. Secondly, major triterpenoids (**5, 8, 9**) and minor triterpenoids (**6, 7**) of *S. tomentosum* are not found in *S. juventas*. They were replaced in *S. juventas* by the compounds (4*R*)-4-hydroxy-3-(1-methyl ethyl)pentyl rutinoside (**71**), (*R*)-2-ethyl-3-methyl-butyl rutinoside (**72**), two phenyl propanoids (**73, 74**) and a phenylethanoid (**75**). Finally, the lignan and pregnane glycoside found in *S. tomentosum* were not detected in *S. juventas*. Therefore, the above chemical results from two species put in doubt *S. tomentosum* as a synonym of *S. juventas*, at least, based on secondary metabolites they might be considered as separate subspecies.

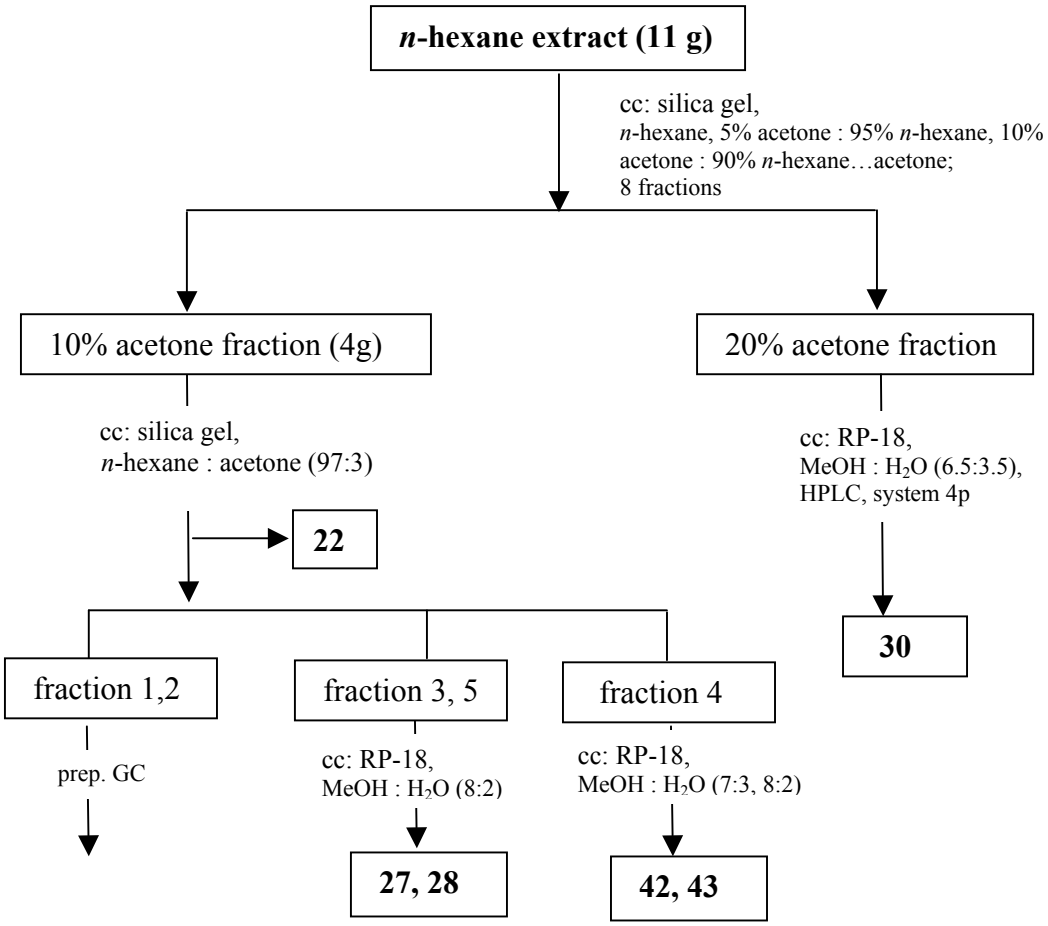
Table18. Chemical difference between *Streptocaulon juventas* and *Streptocaulon tomentosum*

	S. juventas	***S. tomentosum***
cardenolides		
17β-H-periplogenin-3-*O*-β-*D*-digitoxoside (**15**)	–	+
17α-H-periplogenin-3-*O*-β-*D*-digitoxoside (**11**)	++	+
17α-H-periplogenin-3-*O*-β-*D*-cymaroside (**12**)	+	+
17α-H-periplogenin (**10**)	+	+
17β-H-periplogenin (**14**)	–	+
17α-H-digitoxigenin (**17**)	+	+
17α-H-digitoxigenin-3-*O*-β-D-digitoxoside (**18**)	–	+
17β-H-periplogenin-3-*O*-β-*D*-cymaroside (**16**)	–	+
17α-H-periplogenin-3-*O*-β-glucopyranosyl-(1→4)-2-*O*-acetyl-β-digitalopyranoside (**13**)	–	+
Acovenosigenin A digitoxoside (**59**)	+	–
Acovenosigenin A (**60**)	+	–
digitoxigenin genitiobioside (**61**)	+	–
Digitoxigenin-3-*O*-[*O*-β-glucopyranosyl-(1→6)-*O*-β-glucopyranosyl-(1→4)-3-*O*-acetyl-β-digitoxopyranoside] (**62**)	+	–
Digitoxigenin-3-*O*-[*O*-β-glucopyranosyl-(1→6)-*O*-β-glucopyranosyl-(1→4)-*O*-β-digitalopyranosyl-(1→4)-β-cymaropyranoside] (**63**)	++	–
Digitoxigenin-3-*O*-[*O*-β-glucopyranosyl-(1→6)-*O*-β-glucopyranosyl-(1→4)-β-digitoxopyranoside] (**64**)	+	–
digitoxigenin sophoroside (**65**)	+	–
echujin (**66**)	++	–
Periplogenin-3-*O*-[4-*O*-β-glucopyranosyl-β-digitalopyranoside] (**67**)	+	–
periplogenin glucoside (**68**)	+	–
corchorusoside C (**69**)	+++	–
subalpinoside (**70**)	+++	–
Hemiterpenoids		
(4*R*)-4-hydroxy-3-isopropyl pentyl β-rutinoside (**71**)	+	–
(*R*)-2-ethyl-3-methyl-butyl rutinoside (**72**)	+	–
Two phenyl propanoids (73, 74)	+	–
Phenylethanoid (**75**)	+	–
Terpenoids		
2α,3β-dihydroxyolean-12-en-28-oic acid (**7**)	–	+
2α,3β-dihydroxyurs-12-en-28-oic acid (**6**)	–	+
2α,3β-23-trihydroxyolean-12-en-28-oic-acid (**9**)	–	+
2α,3β-23-trihydroxy-urs-12-en-28-oic-acid (**8**)	–	+
Esculentic acid (**5**)	–	+
Lignan		
8-hydroxy pinoresinol (**20**)	–	+
Pregnane glycoside		
Δ^5-pregnene-3β,16α-diol 3-*O*-[2,4-*O*-diacetyl-β-digitalopyranosyl(1-4)-β-*D*-cymaro-pyranoside]-16-*O*-[β-*D*-glucopyranoside] (**19**)	–	+

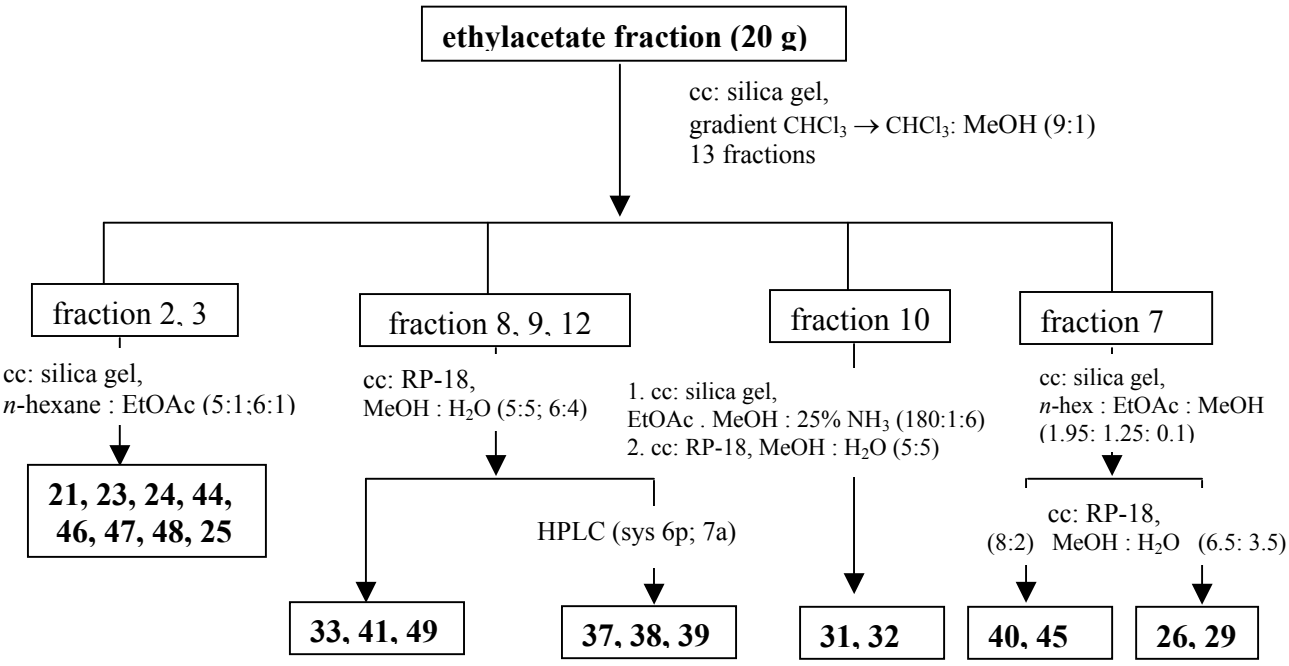
4. Investigation of bioactive constituents from *Curcuma comosa* rhizome

4.1. Extraction and isolation of phytoconstituents

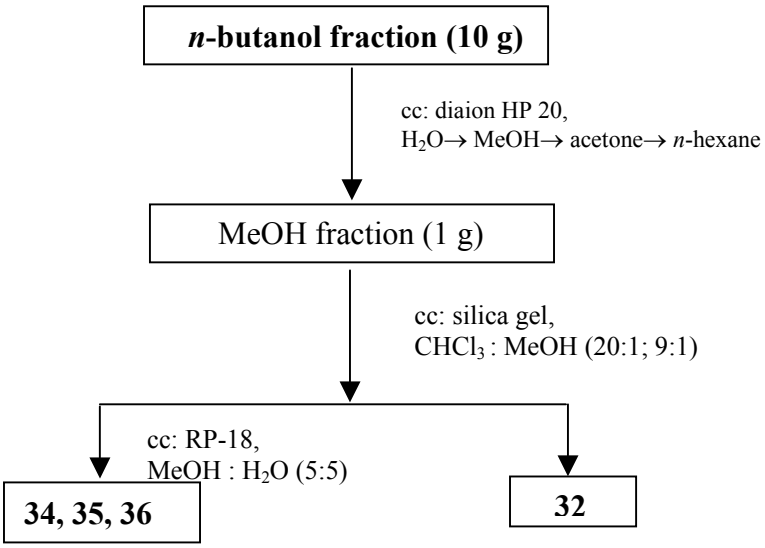
Scheme 5. Isolation of phytoconstituents from *n*-hexane fraction



Scheme 6. Isolation of phytoconstituents from ethylacetate fraction



Scheme 7. Isolation of phytoconstituents from *n*-butanol fraction

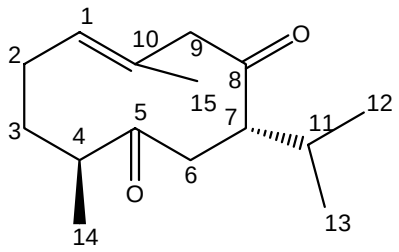


4.2. Structure elucidation of sesquiterpenes

4.2.1. Germacrane type sesquiterpenes

4.2.1.1. Curdione (21)

The ethyl acetate portion partitioned from the 80% ethanol extract of the dried rhizomes of *C. comosa* was individually subjected to silica gel chromatography to give the known compound curdione (**21**) (see in scheme 1 and 6). The spectral data of **21**, mp 47-49 °C, indicated that it was curdione [Kuroyanagi *et al*., 1990]. The structure was confirmed by 2D NMR experiments. The NMR data are shown in tab. 19.



21

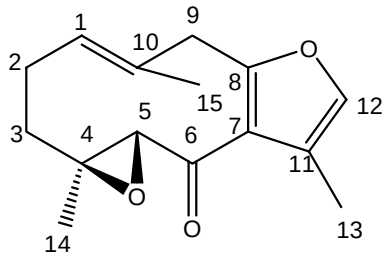
Table 19. NMR data of compound **21** (500 MHz, $CDCl_3$).

Atom	δ_H [ppm]	$^nJ_{HH}$ coupling δ_H [ppm]		C-Atom	δ_C [ppm]	$^nJ_{CH}$ coupling δ_H [ppm]
		COSY	NOESY			HMBC
1	5.163 (*m*)	2.11 (2), 1.65 (15)		1	131.5	2.11 (2), 3.06 (9A) 2.94 (9B)
2	2.11 (*m*)	1.58 (3B)	1.65 (15)	2	26.5	2.12, 1.58 (3)
3	2.12 (*m*) 1.58 (*m*)		0.98 (14) 0.98 (14)	3	34.1	2.11 (2) 0.98 (14)
4	2.34 (*m*)	2.12 (3A), 0.98 (14)	0.98 (14)	4	46.8	2.12 (3A) 1.58 (3B)
				5	214.2	2.85 (7) 2.40 (6B) 2.12, 1.58 (3) 0.98 (14)
6	2.71 (*m*), 2.402 (*dd*, 16.6/2.2)		1.88 (11)	6	44.3	2.85 (7) 1.88 (11)
7	2.851 (*ddd*, 8.8/8.8/2.2)	2.71, 2.402 (6)	0.95 (12) 0.88 (13)	7	53.6	2.40 (6B) 1.88 (11) 0.95 (12) 0.88 (13)
				8	210.9	3.06, 2.94 (9) 2.40 (6B) 2.85 (7) 0.98 (14)
9	3.069 (*d*, 10.7) 2.940 (*d*, 10.7)	2.940 (9B)	1.65 (15)	9	55.9	1.65 (15)
				10	129.8	1.65 (15) 1.88 (11) 3.06, 2.94 (9)
11	1.88 (*m*)	0.95 (12), 0.88 (13)	0.95 (12) 0.88 (13)	11	30.0	0.95 (12) 0.88 (13)
12	0.951 (*d*, 6.7)			12	19.9	2.85 (7) 1.88 (11) 0.88 (13)
13	0.885 (*d*, 6.6)			13	21.2	1.88 (11) 0.95 (12)
14	0.984 (*d*, 7.0)	2.34 (4)		14	18.6	1.58 (3B)
15	1.657 (*s*)			15	16.7	3.06 (9A) 1.58 (3B)

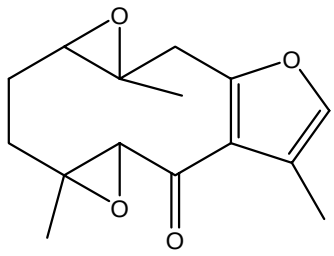
4.2.1.2. Zederone (22), 1a,5,7a-trimethyl-1a,6a,7a,8,9,9a-hexahydrobisoxireno [4,5:8,9]cyclodeca[1,2-*b*]furan-6(2*H*)-one (23)

The *n*-hexane extract was subjected to silica gel chromatography and eluted with a *n*-hexane/acetone gradient under reduced pressure. The known compound zederone (**22**) was obtained from the 10% acetone fraction after further column chromatography on silica gel (*n*-hexane : acetone 97:3). The ethyl acetate extract was individually subjected to silica gel chromatography to give a new natural compound (**23**) (see in scheme 1 and 6).

In an earlier study, the structure of compound **22** was confirmed by ^{1}H NMR and NOE and the absolute configuration was identified based on the derivative 12-bromozederone by X-ray analysis [Shibuya *et al*., 1987]. In our study, the structure elucidation of zederone was done by 1D, 2D NMR and HR-ESI-MS and GC-MS.



22



23

Table 20. ^{1}H and ^{13}C NMR spectrum of the new natural compound **23** (400 MHz**,** $CDCl_3$).

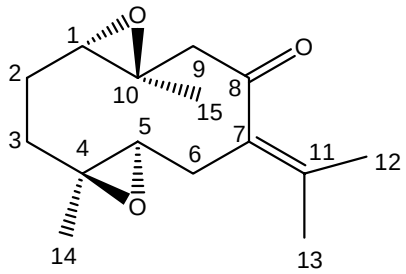
No of H	δ_H [ppm]	Multiplicity	J_{HH} [Hz]	C-Type	δ_C[ppm]
1H	7.10	*m*		CH	63.5
1H	3.78	*s*		CH_2	24.0
1H	3.69	*br d*	16.7	CH_2	36.4
1H	2.94	*dd*	10.5	Cq	64.0
1H	2.83	*br d*	17.2	CH	69.3
1H	2.41	*m*		Cq	190.1
1H	2.22	*m*		Cq	123.7
3H	2.18	*d*	1.3	Cq	156.4
3H	1.51-1.60	*m*		CH_2	39.8
3H	1.34	*s*		Cq	58.2
3H	1.16	*s*		Cq	122.8
				CH	138.7
				CH_3	10.8
				CH_3	15.6
				CH_3	17.1

Table 21. NMR data of compound **22** (500 MHz, $CDCl_3$).

H-Atom	δ_H [ppm]	$^nJ_{HH}$ coupling δ_H [ppm]		C-Atom	δ_C [ppm]	$^nJ_{CH}$ coupling δ_H [ppm]
		COSY	ROESY			HMBC
1	5.485 (*br d*, 12.0)	3.75 (9) 2.52 (2A) 2.23 (2B) 1.60 (15)	3.75 (9) 2.52 (2A) 2.23 (2B) 1.29 (3B)	1	131.1	2.23 (2B) 3.75 (9) 1.60 (15)
2	2.524 (*dddd*, 13.8/13/13/3.6) 2.236 (*m*)	2.30 (3A) 1.29 (3B)	1.60 (15) 1.34 (14)	2	24.7	5.48 (1) 1.29 (3B)
3	2.302 (*ddd*, 13.0/3.6/3.6) 1.293 (*ddd*, 13.8/13.0/4.2)		1.29 (3B)	3	38.0	2.52 (2A) 1.34 (14) 1.60 (15)
4				4	64.0	3.81 (5) 1.34 (14)
5	3.816 (*s*)		1.29 (3B) 5.48 (1)	5	66.5	1.34 (14)
6				6	192.0	3.81 (5)
7				7	123.1	2.11 (13)
				8	156.9	7.09 (12) 3.75 (9)
9	3.757 (*d*, 16.4)	2.11 (13) 1.60 (15)	1.60 (15)	9	41.9	5.48 (1) 1.60 (15)
				10	131.0	3.75 (9) 1.60 (15)
11				11	122.1	3.75 (9) 7.09 (12) 2.11 (13)
12	7.090 (*m*)	3.75 (9)	2.11 (13)	12	137.9	2.11 (13)
13	2.116 (*d*, 1.3)	7.09 (12)		13	10.4	
14	1.345 (*d*, 0.6)	2.30 (3A)		14	15.2	1.29 (3B)
15	1.605 (*br s*)			15	15.8	5.48 (1) 3.75 (9)

4.2.1.3. (1*S*, 10*S*), (4*S*, 5*S*)-Germacrone-1(10), 4(5)-diepoxide (24)

The known compound **24** was isolated according to scheme 6. The structure of **24** was confirmed by spectroscopic analysis such as HR-ESI-MS, 1D and 2D NMR (see in tab. 22) and also by comparison with reported values [Harimaya *et al*., 1991].



24

4.2.1.4. Germacrane type sesquiterpenes 3,6,10-trimethyl-7,8,11,11a-tetrahydrocyclodeca[*b*]furan-2,5(4*H*,6*H*)-dione (25), 11a-hydroxy-3,6,10-trimethyl-7,8,11,11a-tetrahydrocyclodeca[*b*]furan-2,5(4*H*,6*H*)-dione – methane (26)

Fraction 3 of the ethyl acetate extract separated by column chromatography on silica gel gave compound **25** together with 3 known curcuminoids. The unknown compound **25** was repurified by preparative HPLC using an ODS column and the solvent system MeCN-H_2O (20:80). Compound **25** possessed the molecular formula ($C_{15}H_{20}O_3$), $[\alpha]_D$ = + 35.2 °(c = 0.15, MeOH). The ^{1}H NMR spectrum ($CDCl_3$) also showed two methyl signals of H_3-13 and H_3-15 at δ 1.849, 1.822 ppm as singlets and one methyl signal H_3-14 at δ 1.090 ppm (*d*) as a doublet. The proton signals of three methines appeared at δ 4.92 ppm (H-1, H-8) and δ 2.425 ppm (*m*) (H-4) together with 4 methylene signals (H-2, H-3, H-6, H-9). In the ^{13}C NMR spectrum, one carbonyl at δ 208.8 ppm, a trisubstituted olefin (δ 133.4, 128.9 ppm), two methines (C-4, C-8) (48.04, 79.7 ppm), an α,β-unsaturated γ-lactone moiety (δ 173.5, 155.2, 128.9), three methyl groups (δ 16.0, 9.2, 18.6 ppm) together with four methylene groups (δ 46.1, 41.6, 35.9, 27.3 ppm) were also found (NMR data in tab.24).

The new compound **26** was isolated from fraction 7 according to scheme 6. Compound **26** was purified again by preparative HPLC and recrystallized from methanol. Its relative configuration was identified by X-ray crystallography (see in fig. 20). Compound **26** was obtained as colourless platesheets (HR-ESI-MS: 287.12547 ($[M+Na]^+$, calc. for $C_{15}H_{20}O_4Na$ 287.1253802). The ^{1}H NMR spectrum ($CDCl_3$) showed two methyl signals as singlets (H_3-13, 15) at δ 1.855 and 1.933 ppm attached to unsaturated carbon atoms and one methyl signal

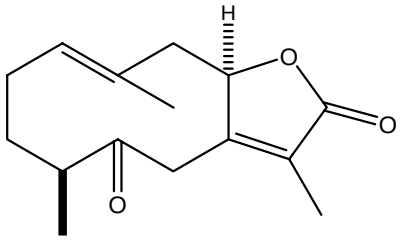
Table 22. NMR data of compound **24** (500 MHz, $CDCl_3$).

H-Atom	δ_H [ppm]	$^nJ_{HH}$ coupling δ_H [ppm]		C-Atom	δ_C [ppm]	$^nJ_{CH}$ coupling δ_H [ppm]
		COSY	ROESY			HMBC
1	2.918 (*d*, 10.8)	2.06 (2A), 1.44 (15)	2.64 (9B)	1	61.3	1.44 (15), 3.00, 2.64 (9), 1.46 (2B)
2	2.06, 1.46 (*m*)	1.28 (3B)	1.46 (2B) 1.14 (14)	2	22.8	2.91 (1), 1.28 (3B)
3	2.21, 1.28 (*m*)	1.46 (2B) 1.14 (14)	1.28 (3B)	3	35.7	2.91 (1), 2.06, 1.46 (2), 1.14 (14)
				4	60.1	2.06, 1.46 (2), 2.21, 1.28 (3), 2.85, 2.26 (6), 1.14 (14)
5	2.652 (*dd*, 10.9/2.2)	2.26 (6B)	2.26 (6B)	5	64.0	2.85, 2.26 (6), 2.21, 1.28 (3), 1.14 (14), 1.79 (12) 1.86 (13),
6	2.855 (*dd*, 14.2/2.2) 2.260 (*dd*, 14.2/10.8)	1.79 (12) 2.65 (5) 1.79 (12) 1.86 (13)	1.86 (13) 2.85 (6A)	6	29.2	2.65 (5), 1.86 (13)
				7	134.3	3.00 (9A), 2.85, 2.26 (6), 1.79 (12), 1.86 (13)
				8	207.2	3.00, 2.64 (9), 2.85, 2.26 (6), 1.79 (12), 1.86 (13)
9	3.007 (*d*, 10.8) 2.644 (*d*, 10.8)	2.64 (9B) 1.44 (15)	1.44 (15) 3.00 (9A)	9	54.5	2.91 (1), 1.44 (15)
				10	58.4	3.00, 2.64 (9), 2.91 (1), 1.44 (15)
				11	137.8	2.85, 2.26 (6), 1.79 (12), 1.86 (13)
12	1.794 (*s*)			12	22.9	1.86 (13)
13	1.862 (*s*)			13	20.8	1.79 (12)
14	1.143 (*s*)			14	15.5	1.28 (3B)
15	1.444 (*s*)			15	17.3	3.00, 2.64 (9)

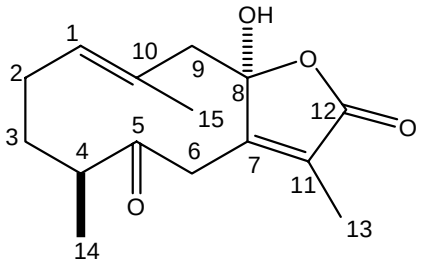
(H_3-14) at δ 1.064 ppm as doublet. The proton signals of two methines (H-1, H-4) appeared at δ 4.878 ppm (*d*, *J* = 10.7) and δ 2.458 ppm (*m*) together with four methylene signals (H-2, H-3, H-6, H-9). The ^{13}C NMR spectrum ($CDCl_3$) showed the signals due to one carbonyl (δ 209.6 ppm), a trisubstituted olefin (δ 133.8, 130.5 ppm), one methine (δ 47.8 ppm), an α,β-unsaturated γ-lactone moiety (δ 172.3, 154.6, 129.9, 106.9 ppm) with one OH-group, two tertiary methyls and one secondary methyl (δ 16.5, 9.2, 18.4 ppm) and four methylene groups (δ 49.7, 40.2, 36.0, 27.2 ppm).

In the HMBC experiment, long-range correlations were observed between the following proton and carbon pairs: H-1 and C-15, C-9; H-4 and C-14, C-2, C-3, C-5; H-6 and C-5, C-7, C-8, C-11, C-12; H-14 and C-3, C-4, C-5; H-13 and C-7, C-8, C-9, C-11, C-12; H-15 and C-8, C-9, C-10.

The relative configuration of **26** was clarified by a ROESY experiment as shown in fig. 23-25. In the ROESY experiment, the NOE correlations between the following proton pairs were observed: H-1 and H-9β, H-2β; H-14 and H-3β, H-6β, H-4; H-13 and H-6β; H-15 and H-9α, H-6α, H-2α.



25



26

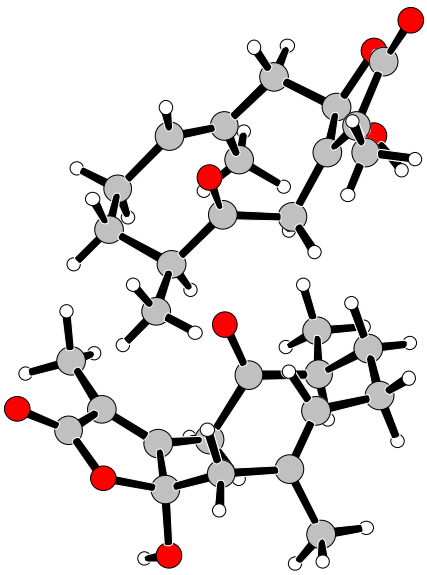


Figure 20. X-ray crystal structure of compound **26.**

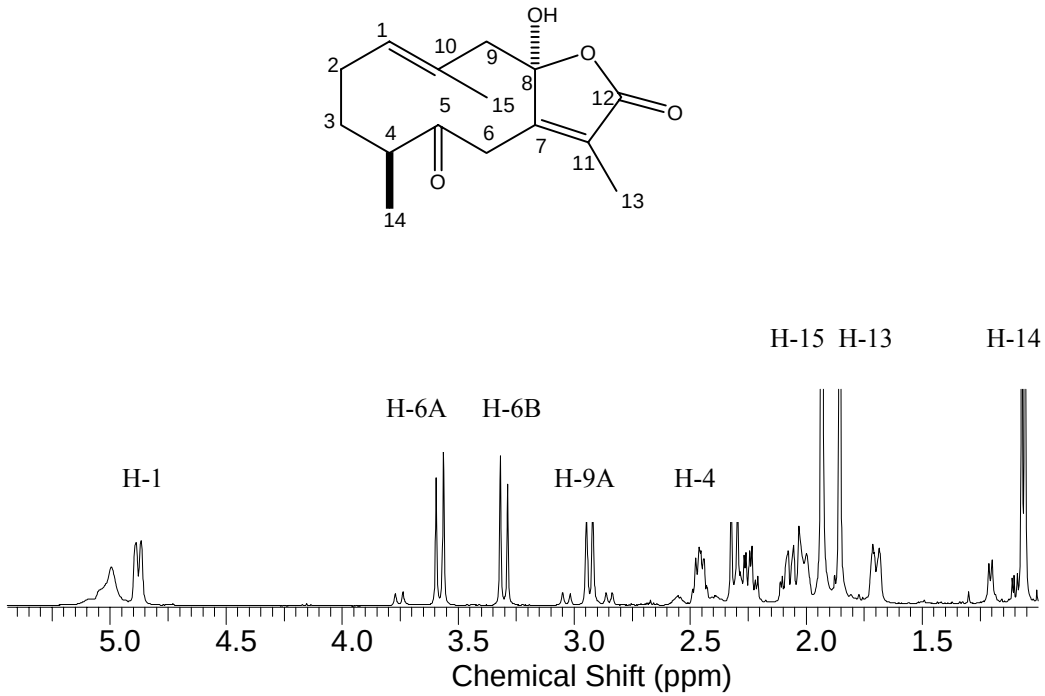


Figure 21. ^{1}H NMR spectrum of compound **26** in $CDCl_3$ (500 MHz).

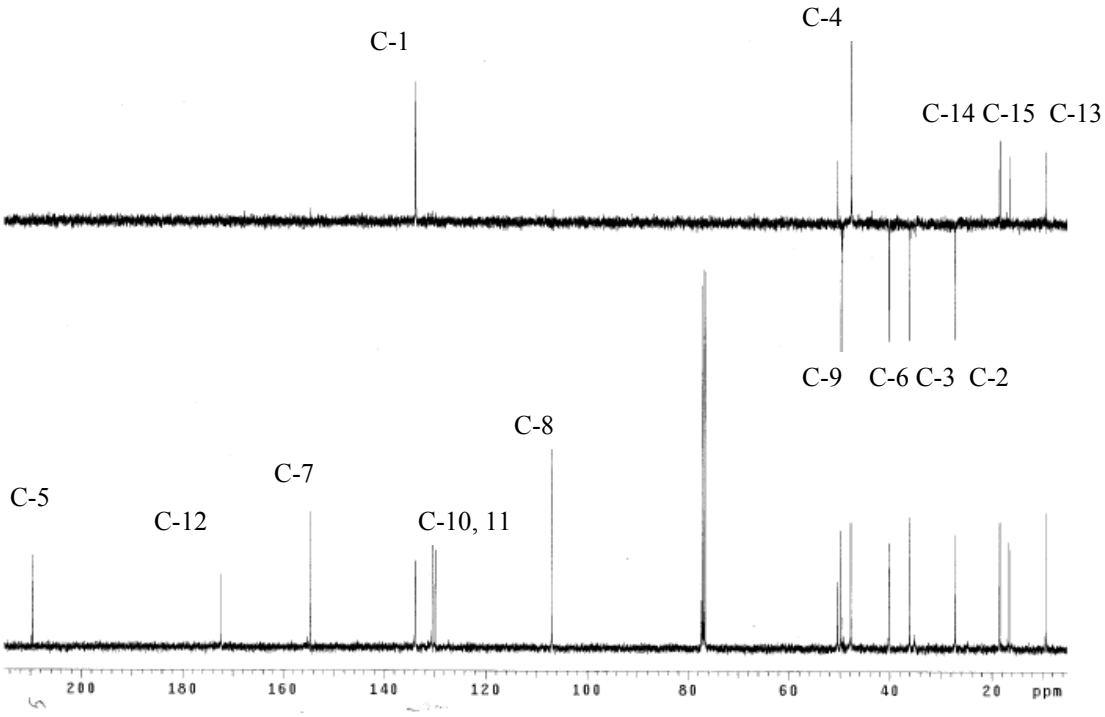


Figure 22. ^{13}C and DEPT NMR spectra of **26** in $CDCl_3$ (500MHz).

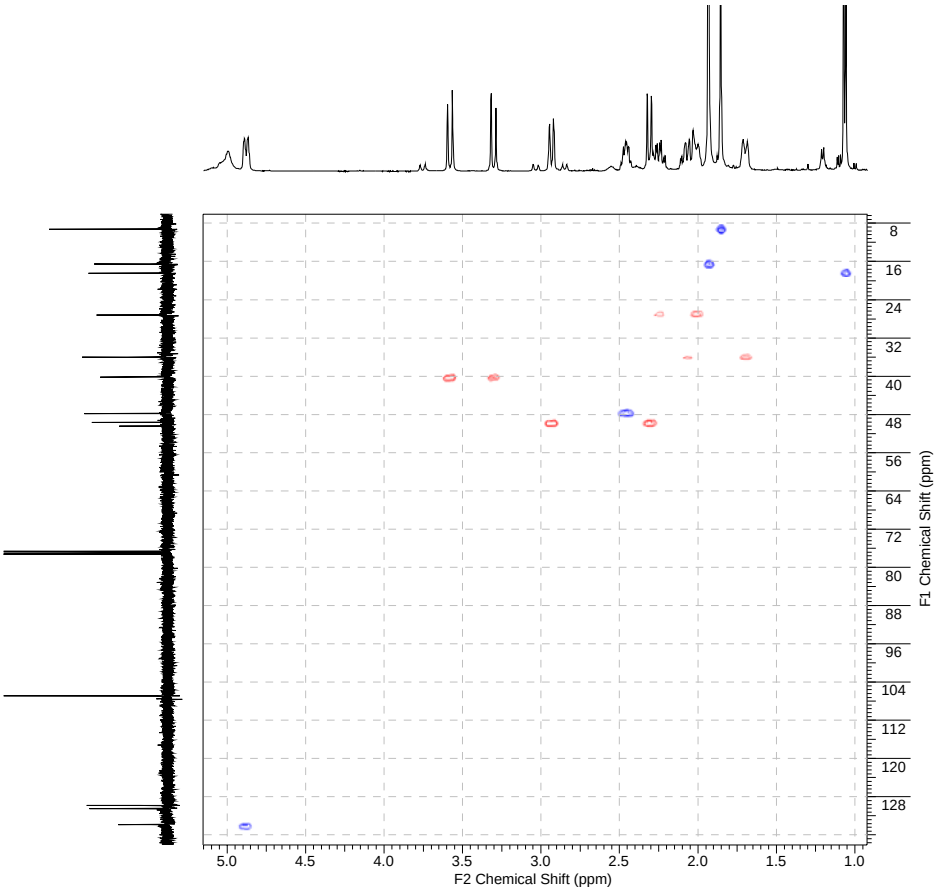


Figure 23. HSQC NMR spectrum of compound **26** in $CDCl_3$ (500MHz).

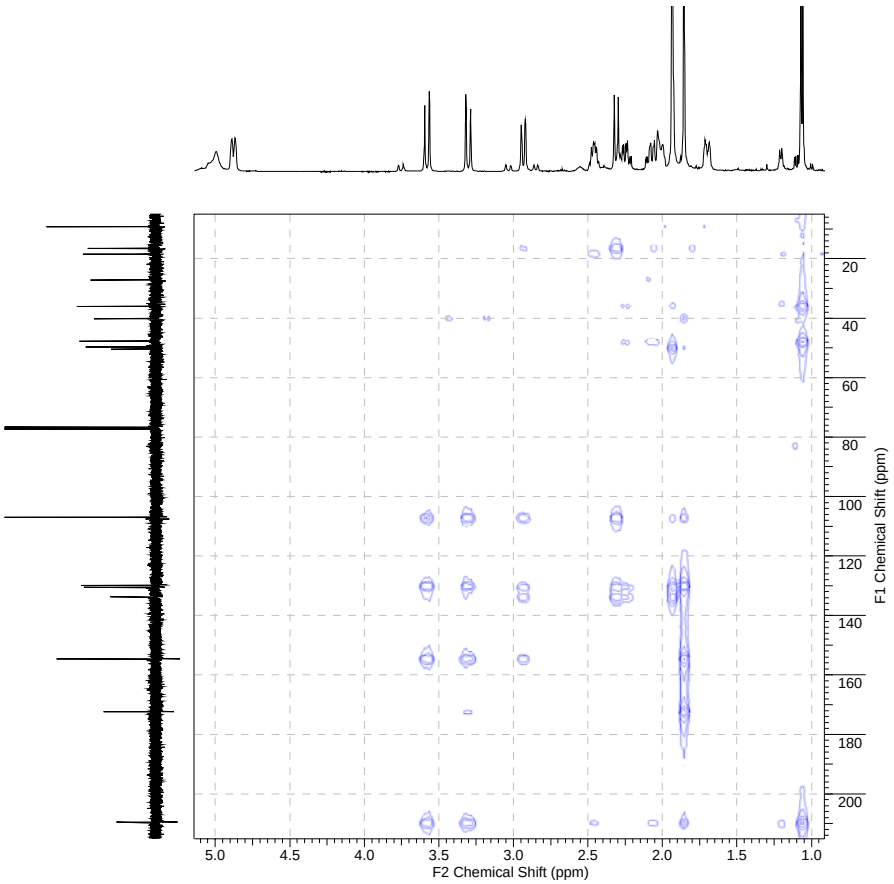


Figure 24. HMBC NMR spectrum of compound **26** in $CDCl_3$ (500MHz).

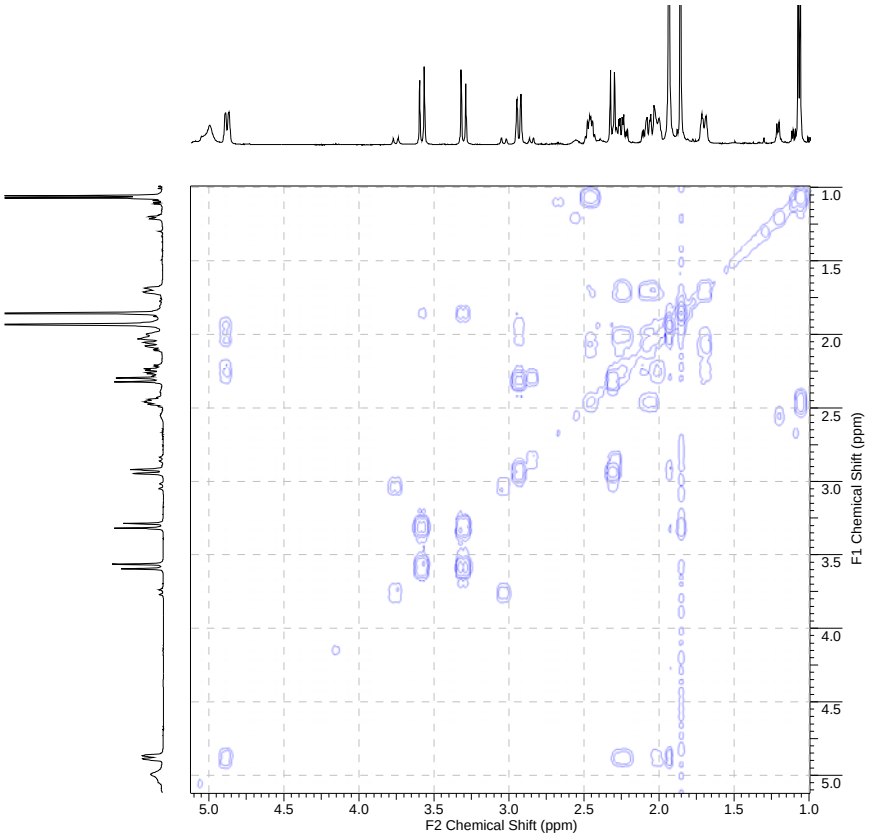


Figure 25. COSY NMR spectrum of compound **26** in $CDCl_3$ (500MHz).

Table 23. NMR data of compound **26** (500 MHz, $CDCl_3$).

H-Atom	δ_H [ppm]	$^nJ_{HH}$ coupling δ_H [ppm]		C-Atom	δ_C [ppm]	$^nJ_{CH}$ coupling δ_H [ppm]
		COSY	**ROESY**			**HMBC**
1	4.877 (*d*, 10.7)	2.00, 2.20 (2)	2.30 (9B) 2.00 (2B)	1	133.8	2.00, 2.20 (2) 2.93, 2.30 (9) 1.65 (3B) 1.93 (15)
2	2.00, 2.20 (*m*)	1.65, 2.10 (3)	1.65, 2.10 (3) 1.93 (15)	2	27.2	2.45 (4), 1.65 (3B) 2.10 (3A)
3	1.65, 2.10 (*m*)	2.10 (3A)	2.45 (4)	3	36.0	2.45 (4)
4	2.45 (*m*)	2.10 (3A) 1.06 (14)	2.20 (2A) 1.06 (14) 1.93 (15)	4	47.8	3.57, 3.30 (6) 2.00, 2.20 (2) 1.65, 2.10 (3) 1.06 (14)
				5	209.6	3.57, 3.30 (6) 1.65, 2.10 (3) 2.45 (4) 1.85 (13) 1.06 (14)
6	3.579 (*d*, 15.4) 3.303 (*d*, 15.7)	3.30 (6B) 1.85 (13)	2.45 (4), 1.93 (15)	6	40.2	1.85 (13)
				7	154.6	3.57, 3.30 (6) 2.93, 2.30 (9) 1.85 (13)
8				8	106.9	3.57, 3.30 (6) 2.93, 2.30 (9) 1.85 (13), 1.93 (15)
9	2.933 (*d*, 13.4) 2.309 (*d*, 13.4)	2.30 (9B) 1.93 (15)	2.30 (9B) 1.93 (15)	9	49.7	4.87 (1), 1.85 (13), 1.93 (15)
				10	130.5	2.93, 2.30 (9) 1.93 (15)
				11	129.9	3.57, 3.30 (6) 1.85 (13)
				12	172.3	3.30 (6B), 1.85 (13)
13	1.855 (*s*)			13	9.2	3.30 (6B)
14	1.064 (*d*, 6.8)	2.45 (4)	2.45 (4) 1.65 (3B)	14	18.4	2.45 (4) 1.65, 2.10 (3)
15	1.933 (*s*)			15	16.5	4.87 (1), 2.93, 2.30 (9)

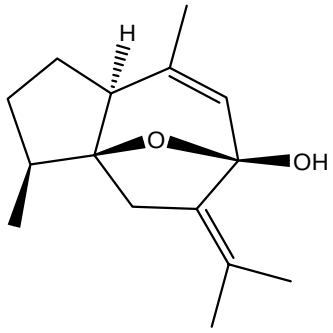
Table 24. NMR data of compound **25** (500 MHz, $CDCl_3$).

Atom	δ_H [ppm]	$^nJ_{HH}$ coupling δ_H [ppm]		δ_C	$^nJ_{CH}$ coupling δ_H [ppm]
		COSY	ROESY	[ppm]	HMBC
1	4.92 (*br s*)	2.06, 2.20 (2)	2.06, 2.20 (2)	133.4	2.06, 2.20 (2)
2	2.06, 2.20 (*m*)	2.20 (2A)		27.3	
3	1.72, 2.04 (*m*)	2.04 (3A)		35.9	2.20 (2A), 1.09 (14)
4	2.44 (*m*)	1.09 (14)	1.09 (14), 1.82 (15)(py)	48.0	1.09 (14)
5				208.2	3.36 (6), 2.44 (4), 1.09 (14)
6	3.36 (*m*)		2.44 (4), 1.09 (14)	41.6	
7				155.2	3.36 (6), 2.04 (9B)
8	4.92 (*br s*)		2.94 (9A)(py) 1.82 (15)(py)	79.7	3.36 (6), 2.04 (9B)
9	2.04, 2.94 (*m*)	2.94 (9A)	1.82 (15)(py)	46.1	
10				128.9	2.04 (9B), 1.82 (15)
11				128.9	3.36 (6), 1.85 (13)
12				173.5	1.85 (13)
13	1.85 (*s*)			9.2	
14	1.09 (*d*, 6.7)			18.6	2.44 (4)
15	1.82 (*s*)			16.0	2.04 (9B)

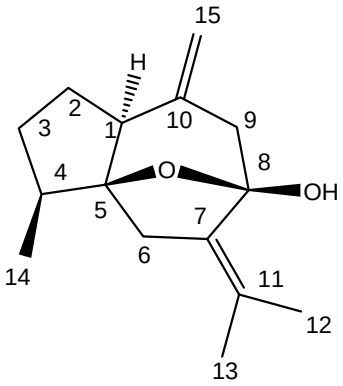
4.2.2. Guaiane type sesquiterpenes

4.2.2.1. Curcumenol (27), isocurcumenol (28), procurcumenol (29), isoprocurcumenol (30)

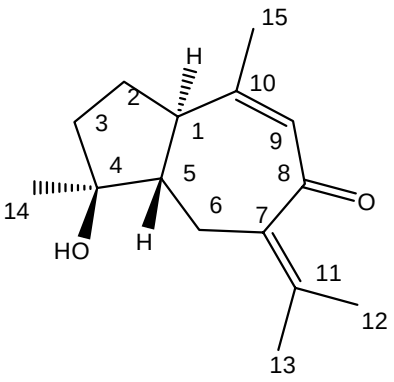
From the *n*-hexane soluble extract the known compounds curcumenol (**27**)**,** isocurcumenol (**28**) and isoprocurcumenol (**30**) were isolated according to scheme 1 and 5. Fraction 7 of the ethylacetate extract gave the known procurcumenol (**29**) [Ohshiro *et al*., 1990; Giang & Son, 2002; Jang *et al*., 2001; Kuroyanagi *et al*., 1990]. The compounds **27**-**30** exhibited the molecular formula $C_{15}H_{22}O_2$ determined from the molecular ion peak at *m/z* 234 (M^+) in the EI-MS and by HR-MS measurements. The compounds were further characterized by IR spectroscopy as well as ^{1}H NMR and ^{13}C NMR (tab. 25). The relative configuration of **27** was determined by X-ray crystallography (fig. 26).



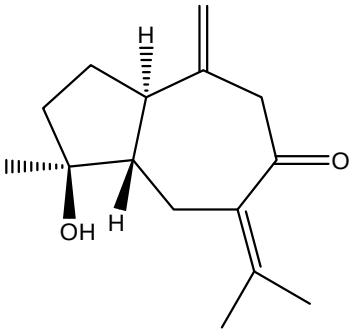
27



28



29



30

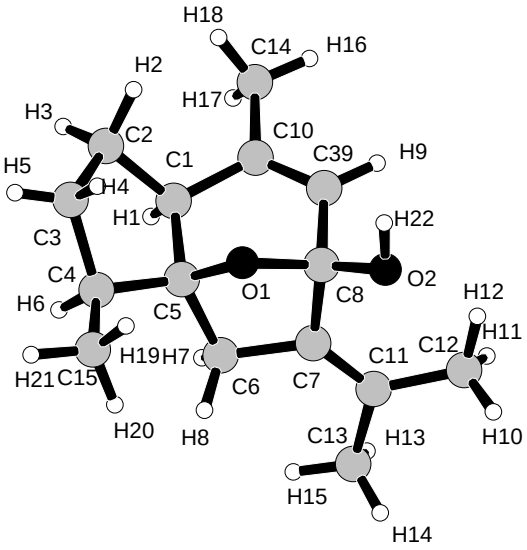


Figure 26. X-ray crystal structure of compound **27.**

Table 25. ^{13}C NMR data of compound **27-30** (400 MHz, $CDCl_3$).

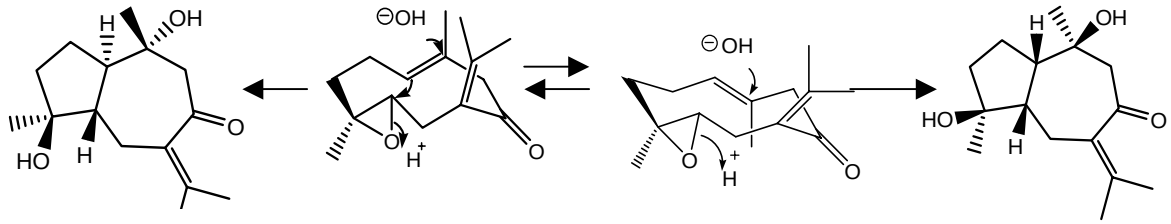
C-Atom	δ_C [ppm]			
	27	**28**	**29**	**30**
1	51.2	53.0	50.4	51.3
2	27.6	28.6	26.8	24.9
3	31.3	31.0	39.8	28.3
4	40.4	41.9	80.2	80.0
5	85.6	87.4	53.7	59.0
6	37.2	39.2	28.6	40.1
7	122.1	134.1	136.7	134.6
8	101.4	104.0	199.2	203.6
9	125.5	36.4	128.9	53.9
10	139.0	145.4	155.2	141.9
11	137.1	127.2	136.2	144.0
12	19.0	22.8	21.2	22.6
13	22.4	19.2	22.3	23.0
14	21.1	12.7	24.2	24.5
15	12.0	112.5	23.3	111.7

4.2.2.2. Isozedoarondiol (31), zedoarondiol (32), 1,4-dihydroxy-1,4-dimethyl-7-(1-methylethylidene)octahydroazulen-6(1*H*)-one-methane (33)

Fraction 10 and 9 of the ethylacetate extract were chromatographed according to scheme 1 and 6 to give the known compounds isozedoarondiol (**31**)**,** zedoarondiol (**32**), and a new isomer of zedoarondiol (**33**). Structural confirmations of **31** and **32** were obtained by MS, optical properties and ^1H and ^{13}C NMR spectroscopy data are in almost full agreement with the corresponding literature data for both [Kuroyanagi *et al*., 1987].

The molecular formula of the new zedoarondiol isomer (**33**) was determined as $C_{15}H_{24}O_3$ by high resolution mass spectrometry. It showed an absorption maximum at 255 nm (3.5) in the UV spectrum and absorptions at 3420 (OH), 1662 (conjucated ketone) and 1603 (double bond) cm^{-1} in the IR spectrum, suggesting the presence of hydroxyl groups and an α,β-unsaturated ketone. The ^1H NMR spectrum (in $CDCl_3$) showed the presence of four methyl groups [δ 1.16 (*s*), 1.26 (*s*), 1.81 (*s*), 1.89 ppm] at quarternary carbons, indicating two methyl groups attached to oxygenated carbon and the other two groups attached to unsaturated carbon. The ^{13}C NMR spectrum showed the signals of one carbonyl (δ 205.6 ppm), a tetrasubstituted double bond (δ 140.0, 135.8 ppm) and two tertiary carbinol carbons (δ 80.4, 71.5 ppm), together with those due to two methine (δ 54.7 and 50.1 ppm), four methylene (δ 57.3, 39.9, 28.0 and 21.4 ppm) and four methyl (δ 30.1, 22.9, 21.7 and 21.7 ppm) carbons. Two proton signals at δ 2.51 ppm (*d*, J = 11.7) and 2.92 ppm (*d*, J = 11.7) were assignable to a methylene group, adjacent to the ketone group. The ^1H and ^{13}C NMR spectra of

zedoarondiol (**32**) (mp. 134 °C, $C_{15}H_{24}O_3$, UV 258 (3.86)) also showed the presence of the same functional groups as in **31** [δ 1.18 (*s*), 1.20 (*s*), 1.83 (*s*), 1.92 (*s*), and 22.5, 20.5, 22.2, 23.0, 79.8, 72.6, 134.5, 142.1, and 203.2 ppm] (NMR data for **31-33** are listed in tab. 26, 27). The ^{1}H and ^{13}C NMR spectral data suggested that **31** and **32** had the same plane structure, having a guaiane-type skeleton, and might be diastereomers. From the transannular cyclization reaction mechanism, **31** and **32** should be formed from as shown in fig. 27.

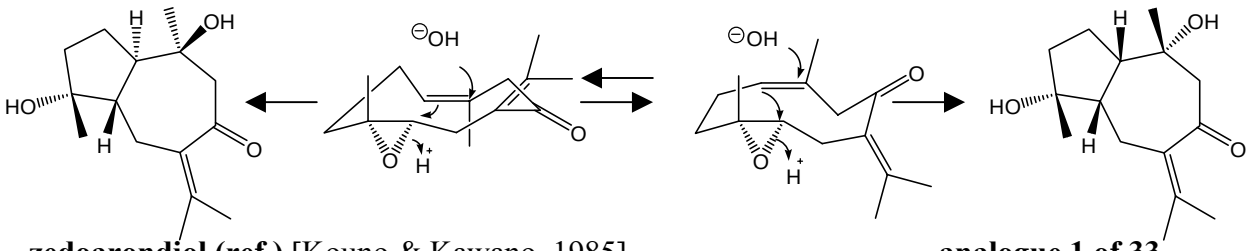


32 **31**

Figure 27. The transannular cyclization reaction mechanism of XY-epoxide to **31** and **32,** depending on conformational prefolding.

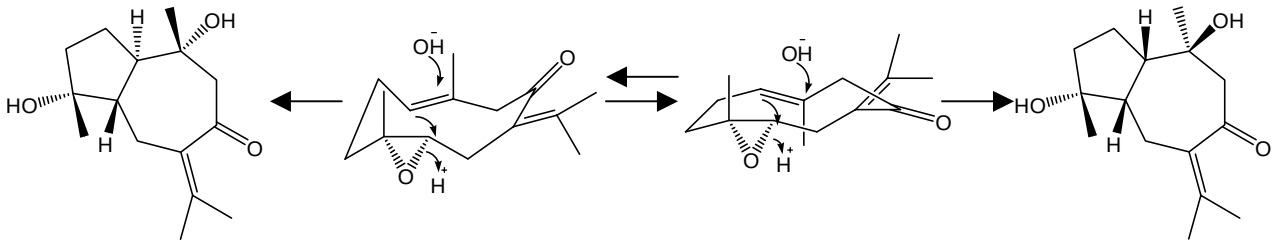
32 **31**

The configuration of the new compound **33** could not be determined by NOE due to the lack of sample. The speculation from (4*S*, 5*R*) or (4*R*, 5*S*)-germacrone 4,5-epoxide for stereochemistry of **33**, based on possible biogenetic pathway is as follows:

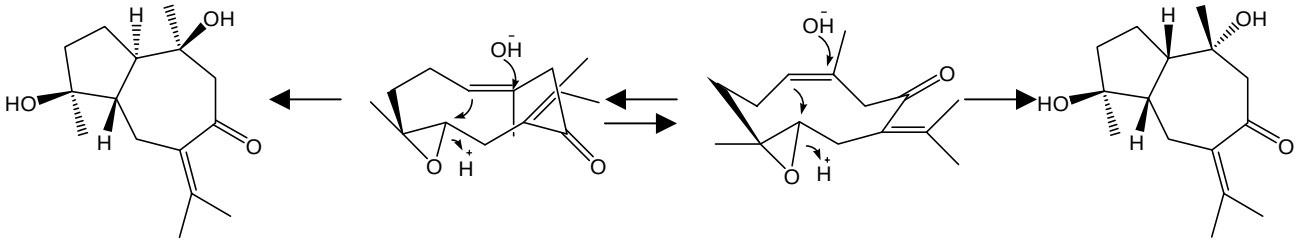


zedoarondiol (ref.) [Kouno & Kawano, 1985] **analogue 1 of 33**

The compound **33** can also be transformed via following transannular cyclization.



analogue 2 of 33 **analogue 3 of 33**



analogue 4 of 33 **analogue 5 of 33**

Table 26. ^{1}H NMR data of compounds **31-33** and data from literature (400 MHz, $CDCl_3$).

δ_H [ppm]			
31	**32**	**33**	**Zedoarondiol (ref.)** [Kouno & Kawano, 1985]
1.22 (*s*)	1.18 (*s*)	1.16 (*s*)	1.19 (*s*)
1.42 (*s*)	1.20 (*s*)	1.26 (*s*)	1.21(*s*)
1.87 (*s*)	1.83 (*s*)	1.81 (*s*)	1.83(*s*)
2.01 (*s*)	1.92 (*s*)	1.89 (*s*)	1.93 (*s*)
1.48-1.84 (*m*)	1.34 (*t*, 11.0) 1.64-1.80 (*m*)	1.50-1.80 (*m*)	1.39 (*td*, 11.5/2.0)
2.83 (*m*)	1.93-2.02 (*m*)		1.94-2.03 (*m*)
2.41 (*d*, 16.0)	2.59 (*d*, 12.6)	2.51 (*d*, 11.7)	2.59 (*d*, 12.5)
2.51 (*d*, 14.0)	2.82 (*d*, 15.1)	2.83 (*d*, 15.6)	2.82 (*dd*, 15.0/2.0)
3.23 (*d*, 16.0)	2.96 (*d*, 12.6)	2.92 (*d*, 11.7)	2.96 (*dd*, 12.5/1.0)

4.2.2.3. Zedoalactone A (34), 5,8-dihydroxy-3,5,8-trimethyl-4a,5,6,7,7a,8,9,9a-octahydroazuleno[6,5-*b*]furan-2(4*H*)-one (35), 5,8-dihydroxy-3,5,8-trimethyl-4a,5,6,7,7a,8,9,9a-octahydroazuleno[6,5-*b*]furan-2(4*H*)-one (36)

The new compounds **35** and **36** together with the known zedoalactone A (**34**) were isolated from the *n*-butanol fraction of *C. comosa* as shown in scheme 1 and 7.

Compounds **34-36** gave the molecular formula $C_{15}H_{22}O_4$ from HR-MS spectrum. The IR spectral data showed a hydroxyl group (3390 cm^{-1}) and an α,β-unsaturated γ-lactone (1730 cm^{-1}). The proton NMR spectra indicated the presence of three methyl groups (δ 1.237 *s*, 1.392 *s*, 1.831 *d* ppm in **34**), (δ 1.245 *s*, 1.281 *s*, 1.813 *dd* ppm in **35**), and (δ 1.317 *s*, 1.396 *s*, 1.790 *d* ppm in **36**). Four sets of methylene protons, two methine protons and a carbinyl proton (δ 4.920 *ddq* in **34**, 5.133 *d* ppm in **35**) were also found. In the ^{13}C NMR ($CDCl_3$) spectra, three methyl groups appeared at δ 8.0, 25.0, 31.8 ppm in **34**, δ 8.7, 23.5, 24.0 ppm in

35 and δ 8.8, 25.6, 32.5 ppm in **36**, respectively. Four methylenes showed at δ 24.5, 37.1, 24.9 and 35.7 ppm in **34**, δ 23.5, 41.2, 29.8 and 46.3 ppm in **35**, δ 24.5, 37.1, 24.6 and 40.3 ppm in

Table 27. ^{13}C NMR data of compounds **31-33** (400 MHz, $CDCl_3$).

C-Atom	δ_C [ppm] in $CDCl_3$			C-Type	δ_C [ppm] in C_5D_5N	
	31	**32**	**33**		**33**	**Zedoarondiol (ref)** [Kouno & Kawano, 1985]
1	53.4	55.7	54.7	CH	54.7	56.7
2	25.4	21.9	21.4	CH_2	21.7	22.8
3	27.7	28.5	28.0	CH_2	28.5	29.1
4	82.7	79.8	80.4	C-OH	79.0	79.2
5	51.7	51.7	50.1	CH	50.3	52.3
6	37.1	39.6	39.9	CH_2	39.9	40.3
7	134.1	134.5	135.8	C=C	136.5	136.1
8	203.6	203.2	205.6	C=O	202.8	203.1
9	50.5	59.7	57.3	CH_2	58.4	61.1
10	73.5	72.6	71.5	C-OH	70.8	71.9
11	144.5	142.1	140.0	C=C	138.1	139.8
12	22.5	22.2	22.0	CH_3	22.6	22.8
13	23.3	23.0	22.9	CH_3	22.6	23.0
14	25.2	22.5	22.0	CH_3	21.7	21.9
15	32.4	20.5	30.0	CH_3	30.0	20.8

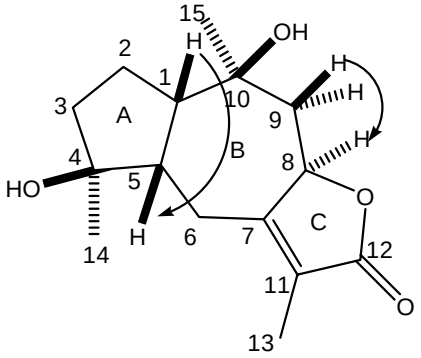
36 and three methines at δ 50.8, 51.5, 80.8 ppm in **34**, δ 48.1, 53.2, 79.0 ppm in **35**, δ 47.7, 52.6, 79.1 ppm in **36,** respectively. The ^{13}C signals at δ 161.4, 122.5, 175.5, 80.8 ppm in **34**, δ 162.4, 122.2, 174.2, 79.0 ppm in **35**, and δ 163.6, 121.8, 174.7, 79.1 ppm in **36** indicated the presence of an α,β-unsaturated γ-lactone moiety at the B ring system. The HMBC spectra revealed a coupled relationship of the H-13 with three quaternary carbons (C-7, C-11, C-12) and also of both H-6 and H-8 with C-1, C-5, C-7, C-8, C-11 on the γ-lactone ring. The H-9 was correlated with C-1, C-10, C-8 and C-7. The methyl proton H_3-14 was also correlated with C-6, C-3, C-5, and C-4, and H-15 with C-9, C-1, C-10 and C-8. These data indicated that **35** and **36** should be new stereoisomers of zedoalactone A as described by Takano *et al*. in 1995.

The relative configuration of **35** was determined by a ROESY experiment in $CDCl_3$, in which correlations were observed between the following protons: H-5 and H-6β *eq* (δ 2.997 ppm, *dd*, *J* = 2.8), H-15, H-9β *ax* (δ 1.755 ppm, *dd*); H-14 and H-6α *ax*, H-6β *eq*, H-1, H-3; H-8 and H-9α *eq* (δ 2.307 ppm, *J* = 2.7), H-1. The coupling constants of H-5 in the axial orientation showed the ABX type vicinal coupling with C-6 methylene protons, H-6α *ax* (δ 2.058 ppm, *J* = 13.3 Hz) and H-6β *eq* (δ 2.997 ppm, *dd*, *J* = 2.8). H-8 *eq* (δ 5.133 ppm, *J* = 11.2) showed the ABX type vicinal coupling with H-9α *eq* (δ 2.307 ppm, *J* = 2.7), and H-9β

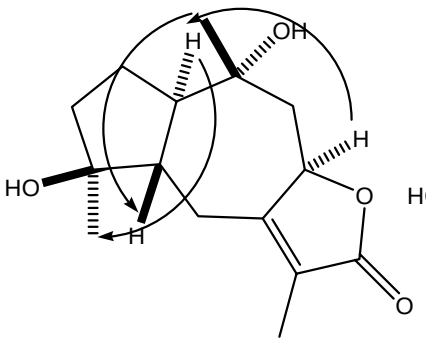
ax (δ 1.755 ppm, J = 11.3) and also showed the NOE enhancement with H-1 and H-6α *ax*. Thus, H-8 had the α-orientation. The stereochemistry of **36** was confirmed by the coupling constants and ROESY experiments (in $CDCl_3$ and C_5D_5N). The signals in C_5D_5N at H-1 (δ 3.55 ppm) and H-5 (δ 2.70 ppm) of the guaiane frame work showed a coupling (J = 7.0) and NOE enhancement, which indicated that the A/B-ring annelation has the *cis*-configuration. There were no ROESY correlations of H-1 to H_3-14 and H-8, H-5 to H_3-15. The H-8 signal correlated with H-5 and H-9A. Therefore, compound **35** and **36** were identified as the new epimers of compound **34** [Takano *et al*., 1995]. The ROESY correlations of three isomers are shown in fig. 28.

Table 28. ^{1}H NMR data of zedoalactones **34-36** (500 MHz, $CDCl_3$).

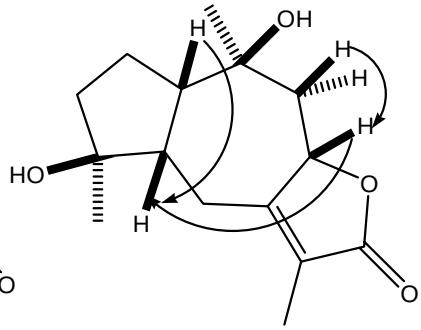
H-Atom	**δ_H [ppm]**		
	34	**35**	**36**
1	2.71 (*m*)	1.97 (*m*)	2.856 (*dddd*, 12.3/7.9/5.1/1.4)
2 (A)	1.85 (*m*)	1.82 (*m*)	1.81 (*m*)
	1.49 (*m*)	1.70 (*m*)	1.34 (*m*)
3	1.80 (*m*)	1.70 (*m*)	1.72 (*m*)
5	2.004 (*ddd*, 13.3/6.6/3.7)	1.575 (*ddd*, 13.0/9.0/2.8)	2.23 (*m*)
6 (A)	2.71 (*m*)	2.997 (*dd*, 15.7/2.8)	2.719 (*m*)
6 (B)	1.85 (*m*)	2.058 (*dd*, 14.7/13.3)	2.23 (*m*)
8	4.920 (*ddq*, 6.9/2.6/2.0)	5.133 (*d*, 11.2)	5.279 (*dqd*, 11.7/1.8/1.7)
9 (A)	2.331 (*dd*, 16.0/6.9)	2.307 (*dd*, 14.7/2.7)	2.281 (*ddd*, 13.7/3.4/1.7)
9 (B)	2.093 (*ddd*, 16.0/2.6/0.7)	1.755 (*dd*, 14.7/11.3)	1.678 (*dd*, 13.7/11.7)
13	1.831(*d*, 2.0)	1.813 (*dd*, 1.7/1.7)	1.790 (*dd*, 1.8/1.4)
14	1.392 (*s*)	1.281 (*s*)	1.396 (*s*)
15	1.237 (*s*)	1.245 (*s*)	1.317 (*s*)



Zedoalactone A (34)



35



36

Figure 28. Significant ROESY correlation of compound **34-36** in $CDCl_3$ (500 MHz).

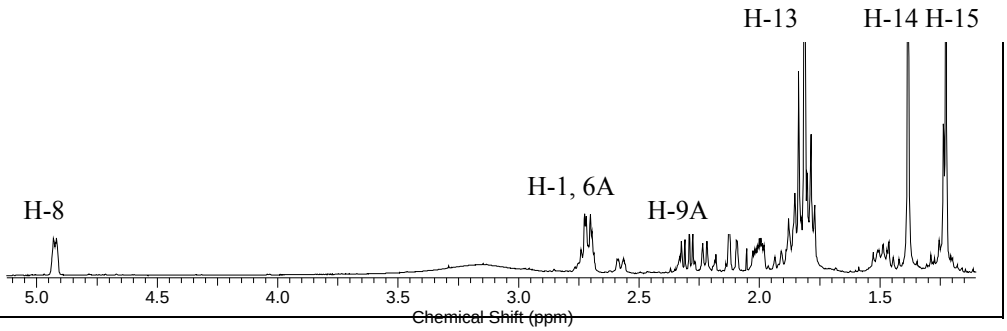
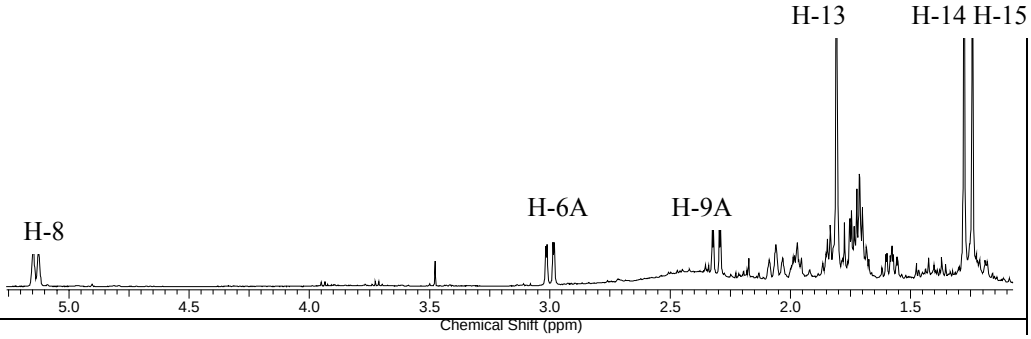
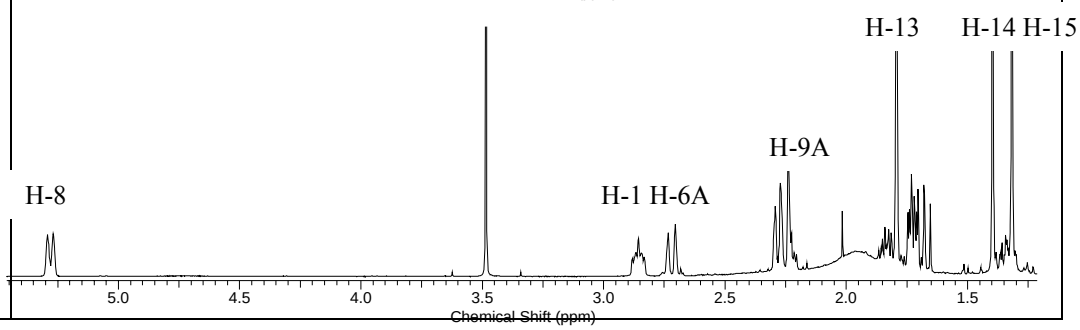
Comp.	^{1}H NMR spectra
34	
35	
36	

Figure 29. ^{1}H NMR spectra of zedoalactones **34-36** in $CDCl_3$ (500 MHz).

Table 29. ^{13}C NMR data of zedoalactones **34-36** (500 MHz, a in $CDCl_3$; b in C_5D_5N).

C-Atom	34[a]	35[a]	36[a]	36[b]
1	51.5	53.2	52.6	53.1
2	24.5	23.5	24.5	24.9
3	37.1	41.2	37.1	37.8
4	81.6	80.4	81.8	80.7
5	50.8	48.1	47.7	48.4
6	24.9	29.8	24.6	24.9
7	161.4	162.4	163.6	165.4
8	80.8	79.0	79.1	79.8
9	35.7	46.3	40.3	41.2
10	73.5	72.6	72.3	71.2
11	122.5	122.2	121.8	121.3
12	175.5	174.2	174.7	174.9
13	8.0	8.7	8.8	8.8
14	25.0	23.5	25.6	25.8
15	31.8	24.0	32.5	32.4

Table 30. Long range HMBC correletion of zedoalactones **34-36** (500 MHz, $CDCl_3$).

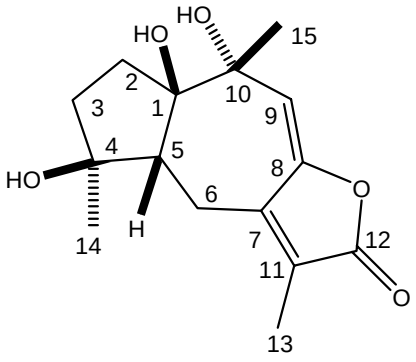
C-Atom	$^{n}J_{CH}$ coupling. δ_H [ppm] HMBC		
	34	**35**	**36**
1	2.00 (5), 1.23 (15) 1.80 (3), 1.85 (2A) 2.33 (9A), 2.09 (9B)	2.99 (6A), 2.30 (9A) 1.75 (9B), 1.57 (5) 1.70 (3), 1.24 (15) 1.82 (2A), 1.70 (2B)	1.31 (15), 2.23 (5) 2.71 (6A), 2.23 (6B) 2.28 (9A), 1.81 (2A) 1.34 (2B)
2	2.71 (1), 1.80 (3)	1.97 (1), 1.70 (3)	2.85 (1), 1.72 (3)
3	1.85 (2A), 1.49 (2B) 1.39 (14), 1.23 (15)	1.82 (2A), 1.70 (2B) 1.28 (14)	2.23 (5), 1.39 (14)
4	2.00 (5), 1.80 (3) 1.39 (14)	1.82 (2A), 1.70 (2B) 1.70 (3), 1.57 (5) 1.28 (14)	2.23 (5), 1.39 (14) 1.72 (3), 1.81 (2A)
5	2.71 (1), 1.39 (14)	2.99 (6A), 2.05 (6B) 1.70 (3), 1.28 (14)	2.85 (1), 2.71 (6A) 2.23 (6B), 1.72 (3) 1.39 (14)
6	2.00 (5), 1.39 (14)	1.57 (5), 1.28 (14)	2.23 (5)
7	1.83 (13), 2.71 (6A) 1.85 (6B), (9B)	2.99 (6A), 2.05 (6B) 2.30 (9A), 1.81 (13)	2.71 (6A), 2.23 (6B) 2.23 (5), 1.79 (13)
8	2.71 (1), 2.33 (9A) 2.09 (9B), 1.83 (13)	2.99 (6A), 2.30 (9A) 1.75 (9B), 1.81 (13)	2.71 (6A), 2.28 (9A) 1.79 (13)
9	1.23 (15)	1.24 (15)	2.85 (1), 1.31 (15)
10	2.71 (1), 2.33 (9A) 2.09 (9B), 1.23 (15)	2.30 (9A) 1.75 (9B) 1.97 (1), 1.57 (5) 1.24 (15)	2.85 (1), 1.31 (15) 2.28 (9A)
11	2.71 (6A), 1.83 (13)	2.99 (6A), 2.05 (6B) 1.81 (13)	2.71 (6A), 2.23 (6B) 1.79 (13)
12	1.83 (13)	2.05 (6B), 1.81 (13)	1.79 (13)
15	2.09 (9B)	1.75 (9B)	

Table 31. ^{1}H-^{1}H correlations of zedoalactones **34-36** (500 MHz, $CDCl_3$).

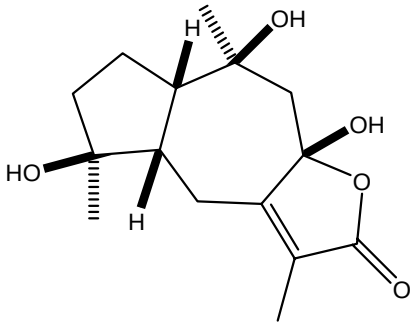
H-Atom	$^{1}J_{HH}$ coupling. δ_H [ppm]			$^{n}J_{HH}$ coupling. δ_H [ppm]		
	COSY			ROESY		
	34	**35**	**36**	**34**	**35**	**36**
1	1.85 (2A) 1.49 (2B)	1.82 (2A) 1.70 (2B)	1.81 (2A) 1.34 (2B)	1.23 (15)	1.28 (14)	1.31 (15)
2 (A)	1.49 (2B)	1.70 (2B)	2.85 (1)		1.70 (2B)	2.85 (1)
2 (B)			2.85 (1)		1.97 (1)	
3	1.49 (2B)			1.49 (2B)		
5	2.71 (1)	1.97 (1)	2.85 (1)	2.71 (1)	1.97 (1) 1.75 (9B)	2.85 (1) 1.39 (14)
6 (A)	2.00 (5)	1.57 (5)	2.23 (6B)	2.00 (5)	1.57 (5)	2.23 (5)
6 (B)	2.00 (5)	1.57 (5)		2.00 (5)		2.23 (5)
8				1.85 (6B)	1.97 (1)	2.23 (5)
9 (A)	2.09 (9B) 4.92 (8)	1.75 (9B) 5.13 (8)	1.67 (9B) 5.27 (8)	2.09 (9B) 4.92 (8)	1.75 (9B) 5.13 (8)	1.67 (9B) 5.27 (8)
9 (B)	4.92 (8)	5.13 (8)	5.27 (8)	4.92 (8)	1.24 (15)	
13	4.92 (8)	5.13 (8)	5.27 (8)	4.92 (8)	2.99 (6A)	5.27 (8)
14				2.00 (5) 2.71 (6A)	2.99 (6A) 2.05 (6B)	2.71 (6A) 2.23 (6B)
15				1.49 (2B)	1.57 (5) 2.30 (9A)	2.28 (9A)

4.2.2.4. Zedoalactone B (37), zedoarolide B (38)

Fraction 12 of the ethylacetate extract afforded the known compounds **37** and **38** (scheme 6). Their structures were confirmed by spectroscopic analysis such as HR-ESI-MS, 1D and 2D NMR (see in tab. 32) and also comparison with reported values [Takano *et al*., 1995; Matsuda *et al*., 2001c].



37



38

Table 32. ^{1}H and ^{13}C NMR data of compounds **37-38** (400 MHz, C_5D_5N).

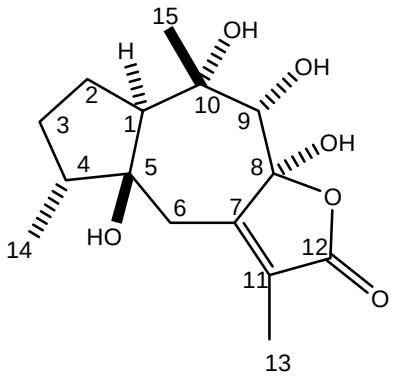
H-Atom	δ_H [ppm]		C-Atom	δ_C [ppm]	
	37	**38**		**37**	**38**
1		3.38 (*ddd*, 3.7/7.6/7.6)	1	75.2	53.1
2 (A)	3.10 (*ddd*, 2.0/9.0/13.1)	1.98 (*m*)	2	35.7	25.3
2 (B)	2.06 (*ddd*, 8.0/11.5/13.1)	1.79 (*m*)			
3 (A)	2.41 (*ddd*, 9.0/11.5/11.5)	2.08 (*m*)	3	41.6	38.2
3 (B)	2.15 (*ddd*, 2.0/8.0/11.5)	1.97 (*m*)			
			4	79.5	80.7
5	3.35 (*dd*, 3.0/12.8)	2.64 (*ddd*, 3.7/3.7/12.8)	5	50.3	52.4
6 (A)	3.21 (*ddd*, 1.5/12.8/17.4)	2.82 (*dd*, 3.7/12.8)	6	22.0	24.6
6 (B)	3.08 (*ddd*, 1.5/3.0/17.4)	2.43 (*dd*, 12.8/12.8)			
9	6.09 (*s*)	2.86 (Abq, 15.5) 2.80 (Abq, 15.5)	7	151.3	161.5
13	1.71 (*br s*)	1.81 (*s*)	8	148.6	106.9
14	1.75 (*br s*)	1.44 (*s*)	9	118.9	44.0
15	1.90 (*s*)	1.58 (*s*)	10	82.7	72.1
			11	125.8	122.7
			12	170.3	173.7
			13	8.5	8.0
			14	23.7	25.6
			15	26.1	32.5

4.2.2.5. 4a,8,9,9a-Tetrahydroxy-3,5,8-trimethyl-4a,5,6,7,7a,8,9,9a -octahydroazuleno[6,5-*b*]furan-2(4*H*)-one (39)

Fraction 8 of the ethylacetate extract was purified by RP-18 chromatography (MeOH : H_2O; 5:5) and HPLC (system 8a) to give the unknown compound **39.**

Compound **39** was obtained as colourless oil. The ESI mass spectrum of **39** did not show the molecular ion. The highest peak in **39** appeared at *m/z* 303.12047 as a dehydrated ion $[M-H_2O+Na]^+$. HR-MS of 303.12047 correlated with $C_{15}H_{20}O_5$, which suggested the molecular formula $C_{15}H_{22}O_6$. The ^{1}H NMR (C_5D_5N) spectrum (tab. 33) indicated the presence of three tertiary methyls [δ 1.874 (*d*, 1.7), 0.712 (*d*, 7.3), 1.471 ppm (*s*)], three sets of methylene protons, two methine protons [δ 3.740 (*dd*, 5.0/3.8), 2.045 (*qd*, 7.3/6.8)] and a methine bearing an oxygen function [δ 3.990 (*s*)]. The ^{13}C NMR spectrum showed signals of an α,β-

unsaturated γ-lactone moiety at δ 158.8, 126.9, and 172.4 ppm. In the HMBC experiment, long-range correlations were observed between the following protons and carbons: 1-H and 3-C; 6-H and 4-C, 5-C, 8-C, 11-C and 7-C; 9-H and 15-C, 1-C, 10-C, 8-C; 13-H_3 and 7-C, 11-C,12-C; 14-H_3 and 4-C, 5-C; 15-H_3 and 1-C, 10-C. Furthermore, the relative configuration of **39** was elucidated by ROESY experiments, in which NOE correlations were observed between the signals of the following proton pairs: 1-H and 6B-H, 2-H; 6A-H and 6B-H, 4-H, 13-H_3, 14-H_3; 9-H and 15-H_3 (therefore 9-H and 15-H_3 are *cis*-β configurated); 14-H_3 and 3B-H, 2-H, 4-H, 6B-H, 6A-H. The configuration of 1-H is *trans*-α because there is no NOE correlation between 1-H and 15-H_3, 9-H. There is no NOE correlation between 1-H and 4-H, therefore 14-H_3 has α-configuration. The NOE correlation between 14-H_3 and 6B-H, 6A-H suggested that 5-OH has β-configuration. Therefore the relative configuration of **39** was assigned, except for the 8-position. Modelling, however, reveals that only an α-orientation of the 8-hydroxy is reasonable.



39

4.2.2.6. Alismoxide (40), 7-(1-hydroxy-1-methylethyl)-1,4-dimethyl-1,2,3,3a,4,5,8,8a-octahydroazulene-1,4-diol (41), gajutsulactone B (42)

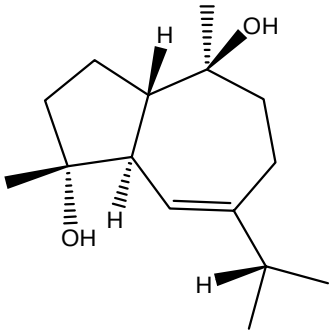
Fraction 7 and fraction 12 of the ethyl acetate extract were chromatographed according to scheme 6 to obtain the known compound **40** and the unknown compound **41**, respectively. The known gajutsulactone (**42**) was purified from the *n*-hexane soluble portion as shown in scheme 5.

Compound **40** and **42** were identified by comparison with literature spectral data [Yoshikawa *et al*., 1992; Matsuda *et al*., 2001c]. The ^{1}H and ^{13}C NMR are shown in tab. 34. Compound **41** was obtained as a colourless oil and the molecular formula was determined as $C_{15}H_{26}O_3$ by high resolution mass spectrometry.

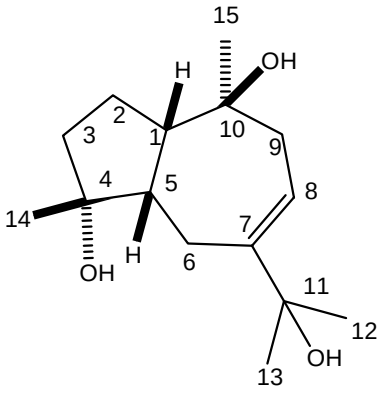
Table 33. NMR data of the new sesquiterpenoid **39** (500 MHz, C_5D_5N).

H-Atom	δ_H [ppm]	$^nJ_{HH}$ coupling δ_H [ppm]		C-Atom	δ_C [ppm]	$^nJ_{CH}$ coupling δ_H [ppm]
		COSY	ROESY			HMBC
1	3.740 (*dd*, 5.0/3.8)	1.76 (2)	1.76 (2) 2.87 (6B)	1	43.3	1.76 (2), 1.47 (15), 2.04 (4)
2	1.767 (*m*)		0.71 (14) 1.43 (3B)	2	25.0	2.35 (3A), 2.04 (4)
3	2.356 (*dddd*, 11.4/11.4/ 10.7/6.8) 1.430 (*m*)	1.76 (2) 2.35 (3A)	2.04 (4), 0.71 (14) 1.76 (2) 2.35 (3A)	3	33.2	1.76 (2), 3.74 (1), 0.71 (14)
4	2.045 (*qd*, 7.3/6.8)	0.71 (14)	0.71 (14)	4	42.9	3.99 (9), 2.35 (3A), 1.43 (3B), 0.71 (14)
				5	92.2	3.28 (6A), 2.87 (6B), 2.04 (4), 1.76 (2), 0.71 (14)
6	3.280 (*d*, 15.6) 2.870 (*dq*, 15.6/1.7)	2.87 (6B) 1.87 (13)	2.04 (4), 1.87 (13) 0.71 (14)	6	32.2	0.71 (14)
				7	158.8	3.28 (6A), 2.87 (6B), 1.87 (13)
8				8	108.6	3.99 (9), 3.28 (6A)
9	3.990 (*s*)		1.47 (15)	9	81.1	1.47 (15)
				10	82.0	3.99 (9), 1.76 (2), 1.47 (15)
				11	126.9	3.28 (6A), 2.87 (6B), 1.87 (13)
				12	172.4	1.87 (13)
13	1.874 (*d*, 1.7)			13	8.7	
14	0.712 (*d*, 7.3)			14	14.2	2.04 (4), 2.35 (3A)
15	1.471 (*s*)			15	19.7	3.99 (9)

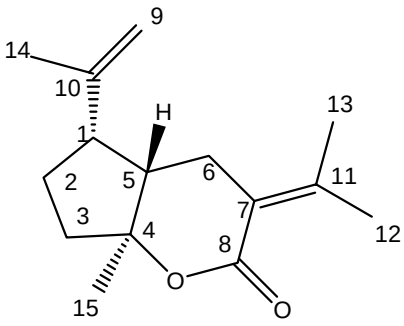
The ^{1}H NMR spectrum showed four methyl signals [δ 1.361 ppm (*s*, H_3-15), 1.568 ppm (*s*, H_3-12, 13), 1.624 ppm (*s*, H_3-14)], attached to oxygenated carbon. The ^{13}C NMR spectrum (in C_5D_5N) showed the signals due to a trisubstituted double bond (δ 150.9 and 118.8 ppm) and three carbinol carbons (δ 80.9, 70.6, 72.7 ppm), together with those due to two methine (δ 54.2 and 49.4 ppm), four methylene (δ 25.5, 37.5, 26.2 and 35.4 ppm) and four methyl (δ 29.2, 29.4, 26.3 and 31.6 ppm) carbons. In the HMBC experiment, long-range correlations were observed between the following protons and carbons: 1-H and 2-C, 9-C, 5-C, 10-C; 5-H and 3-C, 4-C, 14-C and 7-C; 8-H and 9-C, 11-C; 9-H and, 1-C, 7-C, 8-C, 10-C, 11-C, 15-C; 13-H_3 and 7-C, 11-C,12-C; 14-H_3 and 3-C, 4-C, 5-C; 15-H_3 and 1-C, 5-C, 9-C, 10-C. In addition, the relative configuration was clarified by ROESY, in which correlations were observed between the following protons (1-H and 5-H, 2A-H, 15-H_3; 8-H and 9B-H, 12-H_3; 9A-H and 9B-H, 6B-H, 2B-H, 15-H_3; 6A-H and 5-H, 6B-H, 14-H_3, 13-H_3; 15-H_3 and 9A-H, 9B-H, 2A-H; 14-H_3 and 6A-H, 5-H, 3A-H, 3B-H).



40



41



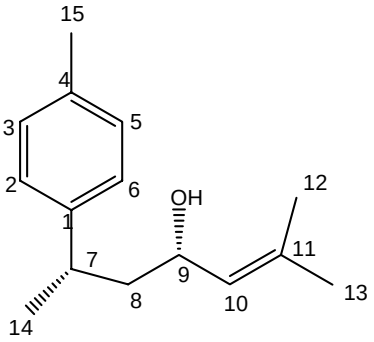
42

Table 34. NMR data of new sesquiterpenoid **41** (500 MHz, C_5D_5N).

H-Atom	δ_H [ppm]	$^nJ_{HH}$ coupling δ_H [ppm]		C-Atom	δ_C [ppm]	$^nJ_{CH}$ coupling δ_H [ppm]
		COSY	ROESY			HMBC
1	3.483 (*m*)	2.41 (5)	2.41 (5)	1	54.2	2.78 (9A), 2.26 (9B) 2.52 (6A), 2.15 (6B) 1.77 (2B), 1.36 (15)
2	1.957 (*m*) 1.775 (*m*)	3.48 (1) 3.48 (1)	3.48 (1) 2.78 (9A)	2	25.5	3.48 (1), 2.41 (5) 2.02 (3A), 1.84 (3B) 1.36 (15)
3	2.020 (*m*) 1.845 (*m*)	1.84 (3B)	1.62 (14) 1.62 (14)	3	37.5	2.41 (5), 1.95 (2A) 1.77 (2B), 1.62 (14)
4				4	80.9	2.41 (5), 2.52 (6A) 2.15 (6B), 1.84 (3B) 1.62 (14)
5	2.414 (*dd*, 12.8/4.9)	3.48 (1)	1.62 (14) 1.56 (13)	5	49.4	3.48 (1), 2.52 (6A) 2.15 (6B), 1.84 (3B) 1.62 (14)
6	2.523 (*d*, 13.9) 2.152 (*dd*, 13.9/12.8)	2.15 (6B) 2.41 (5)	1.62 (14) 2.41 (5) 2.41 (5)	6	26.2	3.48 (1), 2.41 (5) 1.62 (14), 6.15 (8)
7				7	150.9	2.52 (6A), 2.15 (6B) 2.78 (9A), 2.26 (9B) 2.41 (5), 1.56 (13) 1.56 (12)
8	6.157 (*br dd*, 8.4/5.2)	2.78 (9A) 2.26 (9B)	2.26 (9B) 1.56 (12)	8	118.8	2.52 (6A), 2.15 (6B) 2.78 (9A), 2.26 (9B) 1.56 (13), 1.56 (12) 1.36 (15)
9	2.780 (*dd*, 14.2/5.2) 2.268 (*dd*, 14.2/8.4)	2.26 (9B) 2.78 (9A)	1.77 (2B) 2.15 (6B) 2.78 (9A)	9	35.4	3.48 (1), 1.36 (15)
10				10	70.6	3.48 (1), 2.78 (9A) 2.26 (9B), 1.36 (15) 1.77 (2B)
11				11	72.7	6.15 (8), 2.78 (9A) 2.52 (6A), 2.15 (6B) 1.56 (13), 1.56 (12)
12	1.568 (*s*)			12	29.2	1.56 (13)
13	1.568 (*s*)		2.52 (6A)	13	29.4	1.56 (12)
14	1.624 (*s*)			14	26.3	
15	1.361 (*s*)		3.48 (1) 2.78 (9A)	15	31.6	2.26 (9B)

4.2.3. The bisaborane type sesquiterpene bisacumol (43)

The known compound bisacumol (**43**) was isolated from the *n*-hexane extract according to scheme 5 and identified by comparison of spectral data (tab. 35) with literature data [Li *et al*., 2003].



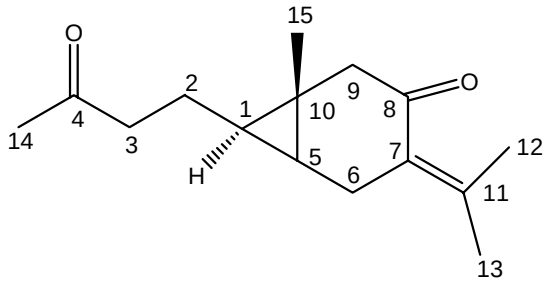
43

Table 35. ^{1}H NMR and ^{13}C NMR data of **40, 42-43** (300 MHz, $CDCl_3$).

H-Atom	δ_H [ppm]		C-Atom	δ_C[ppm]		
	40	**42**		**40**	**42**	**43**
1	1.86 (*m*)	2.88 (*ddd*, 6.4/6.4/9.8)	1	50.9	42.4	144.2
2	1.76 (*m*) 1.62 (*m*)	2.06 (*m*) 1.86 (*m*)	2	21.7	26.2	127.2
3	1.66 (*m*)	1.90 (*m*)	3	40.7	38.0	129.3
4			4	80.5	85.3	135.7
5	2.18 (*m*)	2.30 (*m*)	5	50.5	45.7	129.3
6	5.505 (*br s*)	2.50 (*d*) 2.24 (*d*)	6	121.5	25.7	127.2
7			7	150.0	120.4	36.1
8	2.18 (*m*) 1.92 (*m*)		8	25.3	167.5	46.1
9	1.80 (*m*) 1.46 (*m*)	5.01 (*br s*) 4.84 (*br s*)	9	42.8	111.9	67.1
10			10	75.6	145.2	128.6
11	2.24 (*m*)		11	37.5	151.8	135.0
12	0.989 (*d*, 3.9)	2.183 (*s*)	12	21.7	23.3	18.4
13	0.972 (*d*, 3.5)	1.856 (*s*)	13	21.6	23.5	26.0
14	1.216 (*s*)	1.783 (*s*)	14	22.8	25.2	23.3
15	1.273 (*s*)	1.217 (*s*)	15	21.4	19.9	21.3

4.2.4. The carabrane type sesquiterpene curcumenone (44)

The known compound **44** was purified from fraction 7 of the ethyl acetate extract (scheme 6). The structure of **44** was in agreement with (+)-curcumenone isolated from *Curcuma zedoaria* [Shiobara *et al*., 1985]. The ^{1}H and ^{13}C NMR spectra data were shown in tab. 36.



44

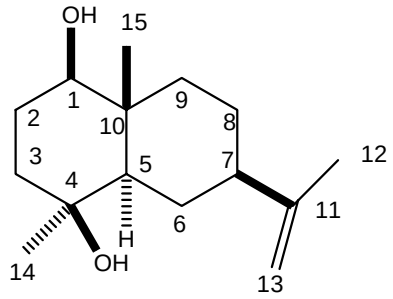
Table 36. ^{1}H NMR and ^{13}C NMR data of **44** (300 MHz, $CDCl_3$).

H-Atom	δ_H [ppm]	Multiplicity	J_{HH} [Hz]	C-Atom	δ_C[ppm]
1	0.45	*dt*	7.3/4.4	1	24.3
2	1.60	*q*	7.3	2	23.6
3	2.47	*t*	7.3	3	44.2
4				4	209.1
5	0.67	*q*	4.4	5	24.3
6	2.81	*m*		6	28.3
7				7	128.3
8				8	202.0
9	2.55	*d*	15.6	9	49.2
	2.52	*d*	15.6		
10				10	20.4
11				11	147.7
12	2.09	*br s*		12	23.7
13	1.79	*br s*		13	23.7
14	2.13	*s*		14	30.3
15	1.12	*s*		15	19.3

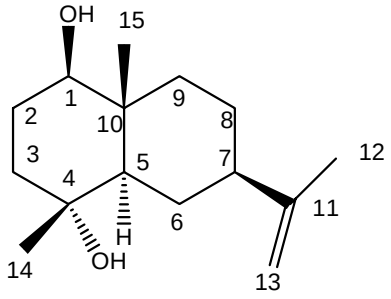
4.2.5. The eudesmane type sesquiterpene 7-isopropenyl-1,4a-dimethyldeca hydronaphthalene-1,4-diol (45)

Fraction 7 of the ethylacetate extract were rechromatographed according to scheme 5 to give the new compound **45**.

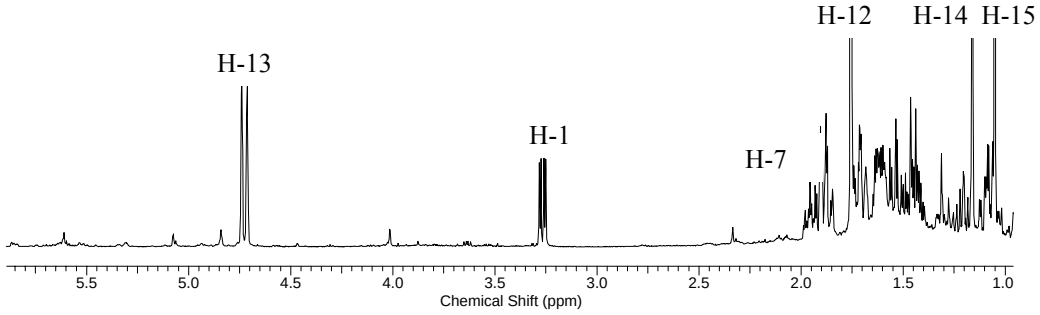
Compound **45** exhibited the molecular formula $C_{15}H_{26}O_2$ and was optically active ($[\alpha]_D$ = -42.3 °(c = 1.10, MeOH)). The IR spectrum showed absorption bands at 3389, 1644, and 890 cm^{-1} ascribable to hydroxyl and olefinic methylene functions. The proton spectra of **45** showed three methyls signals at δ 1.160, 1.051 *s*, 1.755 *s* ppm (H_3-15, H_3-14, H_3-13), a methine bearing the oxygen function at δ 3.266 ppm (H-1, *dd*, *J* = 12.7, 4.2 Hz), two methines at δ 1.071 ppm (H-5, *dd*, *J* = 12.4, 2.6 Hz), δ 1.955 ppm (H-7, *dddd*, *J* = 12.4, 12.4, 4.0, 4.0 Hz), and an olefinic methylene at δ 4.739 ppm (H-12 *Z*), 4.713 (H-12 *E*) together with five methylenes (H_2-2, H_2-3, H_2-6, H_2-8, H_2-9). The ^{13}C ($CDCl_3$) spectrum contains 15 carbon signals showed in tab. 37. In the HMBC experiment, long range correlations were observed between the following proton and carbon pairs (H-1 and C-2, C-10, C-14; H-5 and C-15, C-14, C-6, C-10; H_2-12 and C-7, C-13; H_3-13 and C-8, C-7, C-11, C-12; H_3-14 and C-10, C-9, C-5; H_3-15 and C-2, C-3, C-5). The relative configuration of **45** was determined by a NOE experiment as shown in fig. 30. In the NOE experiment, the correlations between H-1 and H-5; H-5 and H-15, H-7; H-15 *eq* and H-5 *ax*, 6 α *eq*, 3 α *ax* were observed. There is no correlation between H_3-14 and H_3-15. According to the above data, compound **45** is an epimer (at C-4) of cyperusol C.



45



Cyperusol C



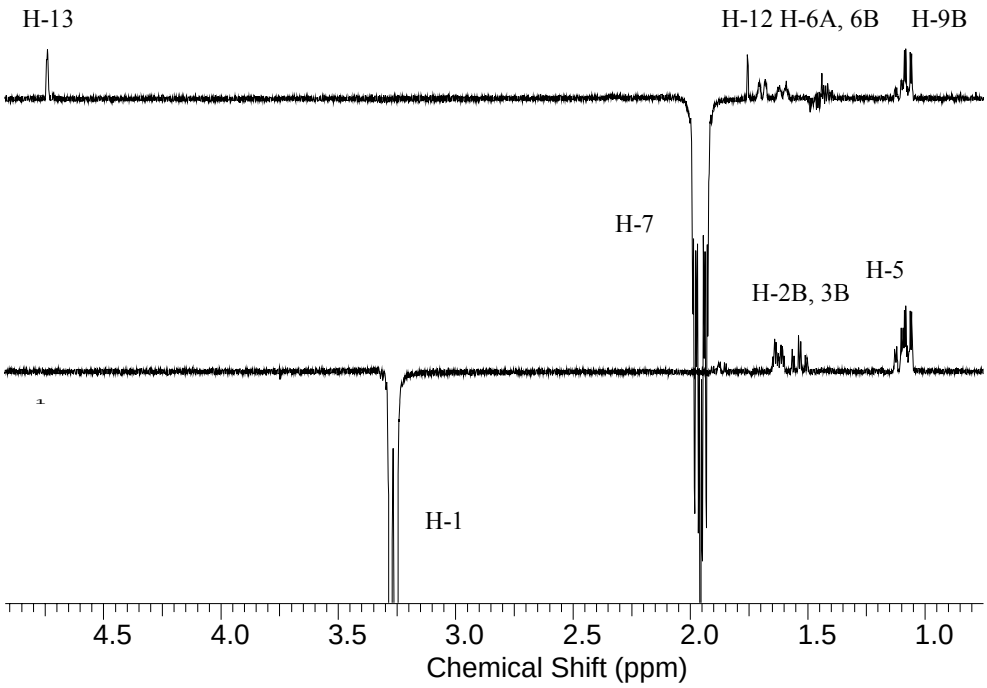


Figure 30. ^{1}H and NOE NMR spectra of the new sesquiterpenoid **45** in $CDCl_3$ (500 MHz).

4.2.6. The diarylheptanoids curcumin (46), demethoxycurcumin (47), bisdemethoxycurcumin (48), (3*S*, 5*S*)-3,5-diacetoxy-1,7-bis(3,4-dihydroxyphenyl)heptane (49)

Fraction 2 and 3 of the ethylacetate extract were chromatographed as shown in scheme 6 to give 3 known diarylheptanoids (**46**, **47**, **48**). The known compound **49** was isolated from fraction 12 (see in scheme 1 and 6) [Kikuzaki *et al*., 1991]**.**

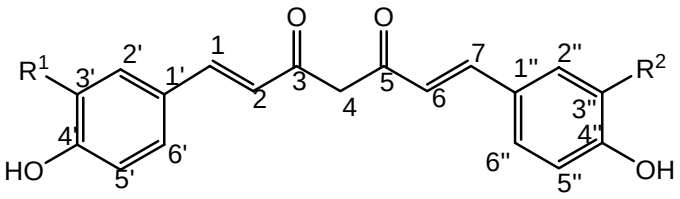
The three curcuminoids **46-48** are found as common pigments in *Curcuma* species. Their structure elucidation was performed by IR, UV, 1D NMR and MS and also confirmed by the comparison with reported values [Kiuchi *et al*., 1993].

Compound **49** gave a $[M+Na]^+$ ion peak at *m/z* 455.16763 in the HR-MS mass spectrum ($C_{23}H_{28}O_8Na$). The IR spectrum showed a strong and broad hydroxyl absorption band at 3584 cm^{-1} and an ester band at 1740 cm^{-1}. In the ^{1}H NMR spectrum (tab. 38), the signal at δ 2.0 ppm (6H, *s*) indicated the presence of two acetyl groups, which was supported by mass fragments of *m/z* 372 $[M–60\ (MeCO_2H)]^+$ and *m/z* 312 $[372–60]^+$. The presence of two 3,4-dihydroxyphenyl groups was suggested by the ^{1}H and ^{13}C NMR spectrum (tab. 39) and the stable fragment ion at *m/z* 123 as base peak in the mass spectrum. The observation of only 11 signals in the ^{13}C NMR spectrum suggested that **49** is symmetric, whereby the uneven C-number indicates a central C-atom in the symmetry element. However, the specific rotation

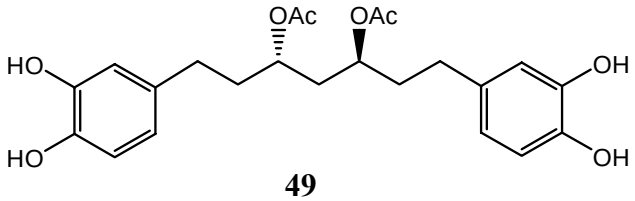
was + 3.0 °, which suggested that symmetry was not of the *meso* type. Overall, structure **49** fits all observations.

Table 37. NMR data of new sesquiterpenoid **45** (500 MHz, $CDCl_3$).

H-Atom	**δ_H [ppm]**	**$^nJ_{HH}$ coupling δ_H [ppm]**		**C-Atom**	**δ_C [ppm]**	**$^nJ_{CH}$ coupling δ_H [ppm]**
		COSY	**ROESY**			**HMBC**
1	3.266 (*dd*, 12.7/4.2)	1.87 (2A) 1.62 (2B)	1.87 (2A) 1.62 (2B)	1	79.7	1.87 (2A), 1.62 (2B), 1.72 (3A), 1.05 (15)
2	1.874 (*m*) 1.620 (*m*)	1.72 (3A) 1.53 (3B)	1.72 (3A) 1.53 (3B)	2	26.8	3.26 (1), 1.53 (3B), 1.16 (14)
3	1.720 (*m*) 1.533 (*ddd*, 14.1/14.1/4.5)		1.07 (5) 3.26 (1)	3	39.4	1.87 (2A), 1.62 (2B), 1.16 (14)
4				4	71.4	1.72 (3A), 1.53 (3B), 1.16 (14)
5	1.071 (*dd*, 12.4/2.6)		3.26 (1)	5	50.4	1.72 (3A), 1.68 (6A), 1.45 (6B) 1.05 (15), 1.16 (14)
6	1.682 (*m*) 1.450 (*m*)		1.93 (7) 1.07 (5)	6	25.6	1.93 (7), 1.45 (8B), 1.16 (14) 1.07 (5),
7	1.938 (*m*)		1.75 (12) 1.07 (5)	7	46.1	1.75 (12), 1.45 (6B), 1.45 (8B) 1.10 (9B)
8	1.620 (*m*) 1.450 (*m*)	1.87 (9A) 1.10 (9B)	1.87 (9A) 1.10 (9B)	8	26.4	1.93 (7), 1.87 (9A), 1.75 (12) 1.10 (9B)
9	1.874 (*m*) 1.108 (*dd*, 13.2/3.7)		1.05 (15) 3.26 (1)	9	39.3	1.93 (7), 1.45 (8B), 1.05 (15)
10				10	38.9	3.26 (1), 1.05 (15)
11		1.75 (12)	1.75 (12) 1.93 (7) 1.45 (6B)	11	150.5	4.73 (13 Z), 4.71 (13 E), 1.75 (12)
12	1.755 (*s*)			12	20.7	4.73 (13 Z), 4.71 (13 E), 1.62 (8 A)
13	4.739 (Z) 4.713 (E)		1.07 (5)	13	108.6	1.75 (12)
14	1.160 (*s*)			14	30.0	1.53 (3B)
15	1.051 (*s*)			15	12.6	3.26 (1), 1.07 (5)



46 $R^1 = R^2 = OMe$
47 $R^1 = OMe$, $R^2 = H$
48 $R^1 = H$, $R^2 = H$



49

Table 38. ^{1}H NMR data of **46, 47** and **49** (400 MHz, a = C_5D_5N, b = $CDCl_3$, c = $CDCl_3$: C_5D_5N 5:1).

H-Atom	δ_H [ppm] 46[a]	47[b]	49[c]
1	8.02 (*d*, 16.0)	7.59 (*d*, 15.6)	2.45 (*ddd*, 7/9/14)
			2.51 (*ddd*, 7/9/14)
2	6.95 (*d*, 16.0)	6.49 (*d*, 15.8)	1.83 (*tdd*, 7/9/14)
			1.74 (*dddd*, 5/7/9/14)
3			4.94 (*q*, 6)
4	6.14 (*s*)	5.79 (*s*)	1.79 (*t*, 7)
5			4.94 (*q*, 6)
6	6.95 (*d*, 16.0)	6.49 (*d*, 15.8)	1.83 (*tdd*, 7/9/14)
			1.74 (*dddd*, 5/7/9/14)
7	8.02 (*d*, 16.0)	7.61 (*d*, 15.6)	2.45 (*ddd*, 7/9/14)
			2.51 (*ddd*, 7/9/14)
2′	7.38 (s)	7.12 (*d*, 1.5)	6.68 (*d*, 2)
3′ (OMe)	3.78 (*s*)	3.95 (*s*)	
5′	7.24 (*d*, 8.0)	6.93 (*d*, 8.0)	6.77 (*d*, 8)
6′	7.32 (*dd*, 8.2)	7.12 (*dd*, 8.0/1.5)	6.54 (*dd*, 2/8)
2″	7.38 (s)	7.47 (*d*, 8.6)	6.68 (*d*, 2)
3″		6.86 (*d*, 8.6)	
3″(OMe)	3.78 (*s*)		
5″	7.24 (*d*, 8.0)	6.86 (*d*, 8.6)	6.77 (*d*, 8)
6″	7.32 (*dd*, 8.2)	7.47 (*d*, 8.6)	6.54 (*dd*, 2/8)

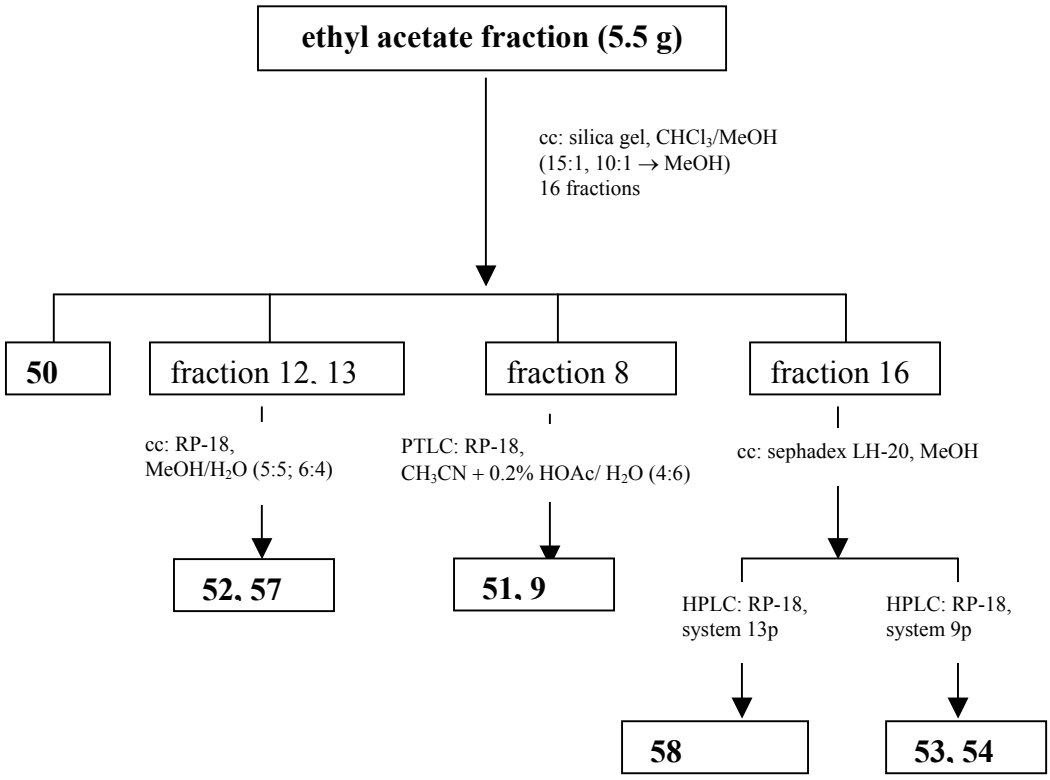
Table 39. ^{13}C NMR data of **46** and **49** (400 MHz, a = C_5D_5N, c = $CDCl_3$: C_5D_5N 5:1).

C-Atom	δ_C [ppm] **46**[a]	C-Atom	δ_C [ppm] **49**[c]
1,7	141.4	1	30.9
2,6	121.6	2	36.7
3,5	184.2	3	69.9
4	50.6	4	38.4
1′, 1″	127.2	5	69.9
2′, 2″	101.7	6	36.7
3′, 3″	148.9	7	30.9
4′, 4″	151.1	1′	132.9
5′, 5″	111.6	2′	115.6
6′, 6″	116.9	3′	145.9
2OMe	55.8	4′	144.1
		5′	115.8
		6′	119.3
		1″	132.9
		2″	115.6
		3″	145.9
		4″	144.1
		5″	115.8
		6″	119.3
		3,5 OAc	170.5

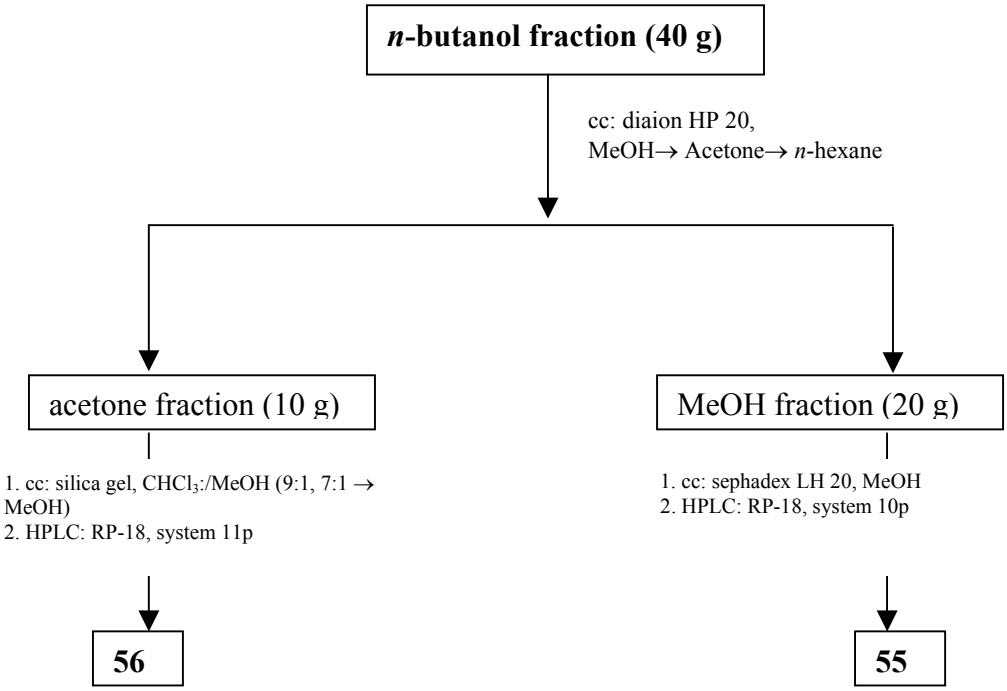
5. Investigation of bioactive constituents from *Vitis repens* rhizomes

5.1. Extraction and isolation of phytoconstituents

Scheme 8. Isolation of phytoconstituents from the ethyl acetate fraction



Scheme 9. Isolation of phytoconstituents from the *n*-butanol fraction

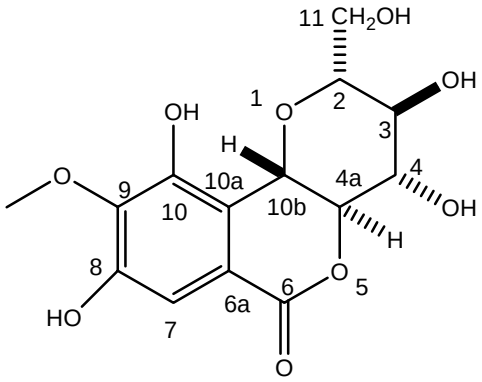


5.2. Characterization of compounds isolated from the rhizome of *V. repens*

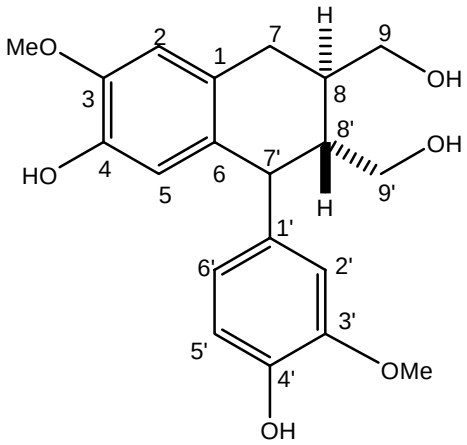
5.2.1. Bergenin (50), isolariciresinol (51), 1-[(3-methylbutyryl)phloroglucinol]-*β-D*-glucopyranoside (52)

According to scheme 1 and 8 dried powdered rhizomes of *Vitis repens* were extracted and afforded the known compounds bergenin (**50**), isolariciresinol (**51**) and 1-[(3-methylbutyryl) phloroglucinol]-*β-D*-glucopyranoside (**52**). The structures of compounds **50-52** were elucidated by comparison of their spectroscopic data (HR-ESI-MS, ^{13}C and ^{1}H NMR) with reported data [Yoshida *et al*., 1982; Jiang *et al*., 2001]. The structures of **51** and **52** were also confirmed by 2D NMR (tab. 41, 42).

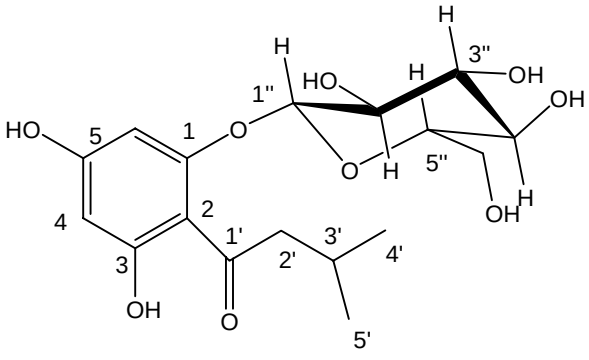
Compound **52** (*m/z* 371.13567 $[M–H]^-$ calc. for $C_{17}H_{23}O_9$) was obtained as white amorphous powder, mp. 114-116 °C. The IR spectrum revealed absorption bands for hydroxy-group (3467 cm^{-1}), aromatic ring (1604, 1456 cm^{-1}) and conjugated carbonyls (1628 cm^{-1}). ^{13}C NMR spectrum analysis showed the presence of one hexosyl moiety attached at position 1. The position of the sugar on the aglycone was confirmed by a HMBC experiment, which showed a long-range correlation between the anomeric proton at δ 5.72 ppm (H-1′′) and the carbon-signal at 161.7 ppm (C-1).



50



51



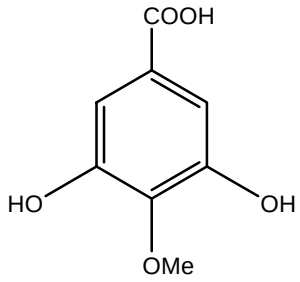
52

Table 40. ^{1}H and ^{13}C NMR data of compound **50** (400 MHz, C_5D_5N).

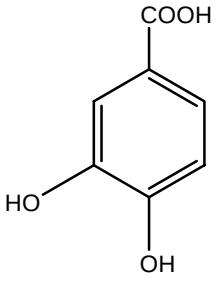
No of H	δ_H [ppm]	multiplicity	J_{HH} [ppm]	C-Atom	δ_C [ppm]
3H	3.89	*s*		2	83.3
3H	4.15	*m*		3	71.9
1H	4.40	*t*	8.6	4	75.4
1H	4.55	*t*	9.8	4a	81.2
1H	4.61	*d*	10.9	6	164.4
1H	5.18	*d*	10.1	6a	119.4
1H	7.65	*s*		7	111.0
				8	152.6
				9	141.8
				10	149.8
				10a	116.5
				10b	73.8
				11	62.5
				OMe	60.3

5.2.2. 4-*O*-Methyl gallate (53), protocatechuic (54), gallic acid (55)

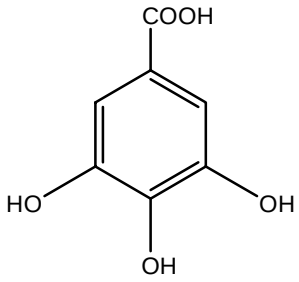
According to scheme 8 the ethyl acetate extract was separated on silica gel. Fraction 16 was purified on sephadex LH 20 (MeOH) and prep. HPLC (system 9p) to give the known compounds **53** and **54**. The *n*-butanol extract was separated according to scheme 9 and afforded the known compound **55**. The structures of **53-55** were elucidated by their spectroscopic data.



53



54



55

Table 41. NMR data of compound **51** (500 MHz, C_5D_5N).

H-Atom	δ_H [ppm]	$^nJ_{HH}$ coupling δ_H [ppm]		δ_C [ppm]	$^nJ_{CH}$ coupling δ_H [ppm]
		COSY	ROESY		HMBC
1				128.0	6.97 (5), 6.89 (2), 4.37 (7′), 3.25, 3.14 (7), 2.59 (8)
2	6.897 (s)	3.80 (3-OMe)	3.80 (3-OMe)	112.5	6.97 (5), 3.25, 3.14 (7)
3				147.0	3.80 (3-OMe)
3-OMe	3.804 (s)			55.9	
4				146.2	6.97 (5), 6.89 (2)
5	6.977 (*br s*)	4.37 (7′)		117.9	6.89 (2)
				134.3	6.97 (5), 6.89 (2), 6.97 (7′), 3.25, 3.14 (7)
7	3.251 (*dd*, 11.0/15.4) 3.140 (*dd*, 4.6/15.6)	6.89 (2) 6.89 (2)	6.89 (2) 6.89 (2)	33.6	6.89 (2), 2.59 (8), 4.23 (9)
8	2.595 (*m*)	3.25 (7A) 3.14 (7B)	3.14 (7B)	40.4	6.97 (7′), 4.23 (9), 3.25, 3.14 (7)
9	4.233 (*t*, 5.1)	2.59 (8)	2.59 (8)	65.6	3.25, 3.14 (7), 2.59 (8)
1′				138.0	7.19 (5′), 7.07 (2′), 4.37 (7′)
2′	7.073 (*d*, 1.4)	3.54 (3′-OMe)	3.54 (3′-OMe)	113.5	7.19 (5′), 6.97 (6′), 4.37 (7′)
3′				148.6	7.19 (5′), 7.07 (2′)
3′-OMe	3.548 (*s*)			55.6	
4′				146.5	7.07 (2′), 7.19 (5′), 6.97 (6′), 4.37 (7′)
5′	7.199 (*d*, 7.8)	6.97 (6′)	6.97 (6′)	116.3	7.07 (2′), 6.97 (6′)
6′	6.975 (*dd*, 1.7/7.9)	7.07 (2′)	4.37 (7′)	123.0	7.07 (2′)
7′	4.373 (*d*, 10.6)	3.25 (7A) 2.36 (8′)	2.59 (8)	47.9	7.07 (2′), 6.97 (6′), 4.23 (9), 3.25, 3.14 (7)
8′	2.366 (*m*)	4.25 (9′A) 3.942 (9′B) 2.59 (8)	6.97 (6′) 4.25 (9′A) 2.59 (8) 3.25 (7A)	48.1	7.07 (2′), 6.97 (6′), 4.37 (7′), 2.59 (8), 4.25, 3.94 (9′)
9′	4.259 (*dd*, 2.5/10.8) 3.942 (*dd*, 4.1/11.0)			61.8	4.37 (7′), 2.59 (8)

Table 42. NMR data of compound **52** (500 MHz, C_5D_5N).

Atom	δ_H [ppm]	$^nJ_{HH}$ coupling δ_H [ppm]		δ_C [ppm]	$^nJ_{CH}$ coupling δ_H [ppm]
		COSY	ROESY		HMBC
1				161.7	6.89 (6), 6.60 (4), 5.72 (1″)
2				105.8	6.89 (6), 6.60 (4)
3				167.5	6.60 (4)
4	6.603 (*d*, 1.5)	6.89 (6)		97.9	6.89 (6)
5				166.3	6.89 (6), 6.60 (4)
6	6.890 (*d*, 1.9)		5.72 (1″)	95.0	6.60 (4)
1′				205.6	3.54 (2′A), 3.23 (2′B), 6.89 (6), 6.60 (4), 2.44 (3′)
2′	3.543 (*dd*, 5.8/15.6) 3.238 (*dd*, 7.8/15.6)	2.44 (3′)	3.23 (2′B)	52.9	2.44 (3′), 0.91 (4′, 5′)
3′	2.445 (*m*)			25.0	3.54 (2′A), 3.23 (2′B), 0.91 (4′), 0.91 (5′)
4′	0.916 (*d*, 6.6)	2.44 (3′)	2.44 (3′)	22.2	3.54 (2′A), 3.23 (2′B), 0.91 (5′)
5′	0.919 (*d*, 6.6)	2.44 (3′)	2.44 (3′)	22.7	3.54 (2′A), 3.23 (2′B), 0.91 (4′)
1″	5.729 (*d*, 7.4)	4.35 (2″) 4.35 (5″)	4.35 (2″) 3.96 (3″)	101.6	4.35 (2″), 4.35 (5″)
2″	4.357 (*m*)	3.96 (3″)	3.96 (3″)	74.4	4.35 (4″)
3″	3.969 (*dd*, 3.5/5.0)	4.35 (4″)	4.35 (4″) 4.35 (5″)	78.6	4.35 (2″), 4.35 (5″), 4.35 (4″)
4″	4.357 (*m*)			70.4	4.35 (5″), 4.35 (6″)
5″	4.357 (*m*)			78.6	4.35 (6″)
6″	4.357 (*m*)			61.5	4.35 (5″)

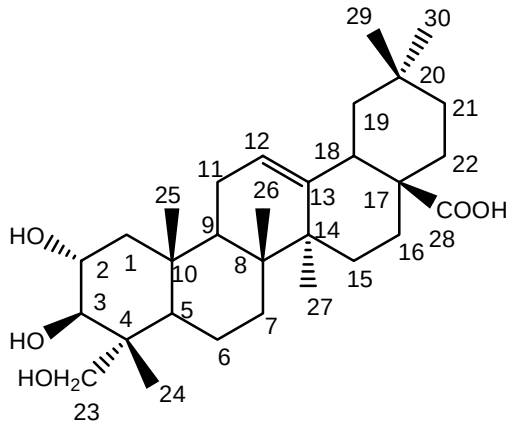
5.2.3. 2α,3β,23-Trihydroxy-olean-12-en-28-oic-acid (9), 3-*O*-galloyl bergenin (56), pallidol (57)

Fraction 8 of the ethylacetate extract was purified on a RP-18 PTLC plate according to scheme 8 to give the known compound **9.** The ^{1}H NMR and ^{13}C NMR spectra were identical to those of 2α,3β,23-trihydroxy-olean-12-en-28-oic-acid obtained from *S. tomentosum*.

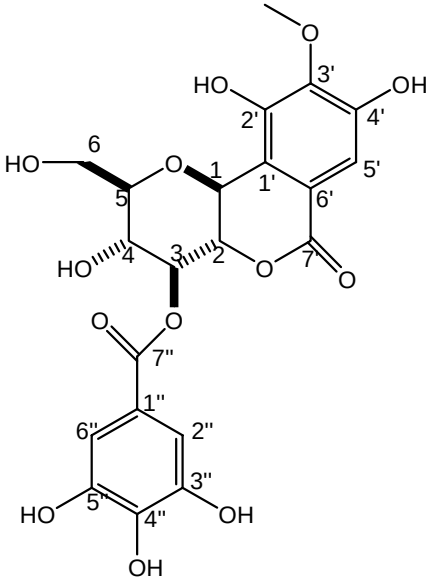
Compound **56** was isolated from the *n*-butanol fraction according to scheme 9. Compound **56** was identified as monogalloyl ester of bergenin (**50**) by ^{1}H NMR, ^{13}C NMR and 2D NMR

(tab. 43). The ^{1}H NMR and ^{13}C NMR spectra were identical to those reported for 3-*O*-galloylbergenin [Yoshida *et al*., 1982].

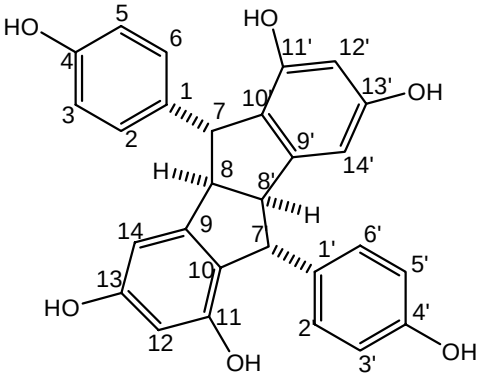
Fraction 12 of the ethylacetate extract was also rechromatographed on RP-18 to give the known compound pallidol **57**. It showed an $[M–H]^-$ ion peak at *m/z* 453.13550 (calc. for $C_{28}H_{21}O_6$ 453.1343) in its HR-ESI-MS mass spectrum. Its IR spectrum showed strong hydroxyl absorption. The ^{1}H NMR spectrum of pallidol (**57**) in C_5D_5N showed the presence of 12 protons which must belong to two sets. The structure was fully assigned by 2D NMR experiments (tab. 44). In the ^{1}H-^{1}H long range COSY spectrum, the benzylic protons at δ 5.352 ppm (H-7, 7′) correlated with the methine hydrogens at δ 4.424 ppm (H-8, 8′) and the *ortho* coupled aromatic hydrogens at δ 7.466 ppm (H-2, 2′, 6, 6′). The broad *meta*-coupled aromatic hydrogen at δ 7.305 ppm (H-14, 14′) in a 1,2,3,5-tetrasubstituted benzene ring was coupled with the aliphatic methine hydrogen at δ 4.424 ppm (H-8, 8′). NOE interactions were observed between H-7 (7′) and H-8 (8′), H-14 (14′), H-2 (6); H-8 (8′) and H-2 (6), H-14 (14′); H-2 (6) and H-3 (5) [Khan *et al*., 1986].



9



56



57

Table 43. NMR data of **56** (500 MHz, C_5D_5N).

Atom	δ_H [ppm]	$^nJ_{HH}$ coupling δ_H [ppm]		δ_C [ppm]	$^nJ_{CH}$ coupling δ_H [ppm]
		COSY	ROESY		HMBC
1	5.39 (*d*, 10.6)	4.81 (2)	6.36 (3)	73.8	4.81 (2)
2	4.81 (*t*, 10.2)	6.36 (3)	4.42 (4)	78.5	5.39 (1), 6.36 (3)
3	6.36 (*t*, 9.0)	4.42 (4)		76.1	5.39 (1), 4.81 (2), 4.42 (4)
4	4.42 (*m*)	4.36 (5)		69.9	6.36 (3)
5	4.36 (*m*)	4.32 (6B)	5.39 (1)	83.5	5.39 (1), 4.42 (4), 4.32 (6B)
6	4.68 (*d*, 10.6), 6A 4.32 (*m*), 6B	4.32 (6B)		62.2	4.42 (4)
1′				116.1	7.74 (5′), 5.39 (1), 4.81 (2)
2′				149.2	7.74 (5′), 5.39 (1)
3′				141.9	7.74 (5′), 5.39 (1), 3.98 (OMe)
4′				152.8	7.74 (5′)
5′	7.74 (*s*)			111.1	
6′				119.4	7.74 (5′), 5.39 (1)
7′				163.5	7.74 (5′)
3′-OMe	3.98 (*s*)			60.3	
1″				120.8	7.90 (2″)
2″, 6″	7.90 (*s*)			110.6	7.90 (6″), 7.90 (2″)
3″, 5″				147.5	7.90 (2″), 7.90 (6″)
4″				141.1	7.90 (2″), 7.90 (6″)
7″				166.7	6.36 (3), 7.90 (6″)

Table 44. NMR data of compound **57** (500 MHz, C_5D_5N).

Atom	δ_H [ppm]	$^nJ_{HH}$ coupling δ_H [ppm]		δ_C [ppm]	$^nJ_{CH}$ coupling δ_H [ppm]
		COSY	ROESY		HMBC
1, 1′				137.8	7.17 (3, 3′, 5, 5′), 5.35 (7, 7′), 4.42 (8, 8′)
2, 6 2′, 6′	7.466 (*d*, 8.3)	7.17 (3, 5)	7.17 (3, 5), 5.35 (7, 7′), 4.42 (8, 8′)	129.1	5.35 (7, 7′)
3, 5 3′, 5′	7.178 (*d*, 8.3)			116.2	7.46 (2, 2′, 6, 6′)
4, 4′				157.1	7.46 (2, 2′,6, 6′), 7.17 (3, 3′ 5, 5′)
7, 7′	5.352 (*s*)	4.42 (8, 8′)	4.42 (8, 8′)	54.5	7.46 (2, 2′, 6, 6′), 4.42 (8, 8′)
8, 8′	4.424 (*s*)			60.6	5.35 (7, 7′), 7.30 (14, 14′)
9, 9′				150.6	5.35 (7, 7′), 4.42 (8, 8′)
10, 10′				123.1	6.85 (12, 12′), 7.30 (14, 14′), 5.35 (7, 7′), 4.42 (8, 8′)
11, 11′				156.4	6.85 (12, 12′), 5.35 (7, 7′)
12, 12′	6.855 (*d*, 1.9)	7.30 (14, 14′)		102.9	7.30 (14, 14′)
13, 13′				160.3	4.42 (8, 8′), 6.85 (12, 12′)
14, 14′	7.305 (*d*, 1.7)		5.35 (7, 7′), 4.42 (8, 8′)	103.3	

6. Bioactivities of isolated compounds

6.1. Antiproliferative activity of cardenolides

Six cardenolides isolated from *Streptocaulon tomentosum* were tested for their antiproliferative activity *in vitro* against MCF-7 (human breast cancer cell line) and L 929 (mouse fibroblast cell line) by acid phosphatase method [Yang *et al*., 1996]. The antiproliferative activity of compounds **4, 10, 11, 12, 13, 15** and **17** are summarized in tab. 45. Cardenolides **10, 11, 12, 13, 15** and **17** show significant antiproliferative activity against MCF 7 cells (IC_{50} < 1 μM - 15,3 μM after 2 days; IC_{50} < 1 μM - 4,31 μM after 5 days incubation). However, cardenolides **11** and **12** possess considerable activity against L 929 (IC_{50} 24.2 and 32.1 μM after 5 days), while other cardenolides show no activity (IC_{50} > 100 μM). Lupeol acetate (**4**) shows also weak antiproliferative activity against L 929 (IC_{50} 79,4 μM after 5 days incubation), whereas no activity against MCF 7 (IC_{50} > 100 μM) could be detected. The antiproliferative activities of monoglycosidic cardenolides **11, 12** attached to digitoxose are stronger, while those of **13** attached to a disaccharide is weaker than the activity of the aglycone **10**. In addition, the antiproliferative activities of **11** and **12** are also stronger than that of **15**. Therefore, the configuration of the γ-lactone ring is also significant. The 17α-configuration of the lactone ring correlates with a weaker effect than the 17β-configuration. Similarly, the induction of apoptosis by compounds **11** and **12** in tumor and U 937 cell lines is stronger in comparison to the other compounds.

6.2. Cellular viability and cell cycle analysis of cardenolides

Four cardenolides (**11, 12, 15,** and **17**) were examined for cellular viability in the tumor cell line and U 937 (human leukemic cell line) at concentrations 100 μM, 10 μM, and 1 μM. All these four cardenolides show toxicity induction of apoptosis at high concentration (> 10 μM) (tab. 46) in both cell lines. Compound **11** is the most detrimental at higher concentration in both of cell lines whereas compounds **15** and **17** show less activity. The most interesting observation is the higher activity of compound **11** against tumor cells vs U 937-cells at low concentration (1 μM). The same cardenolides (**11, 12, 15,** and **17**) were also analysed for the percentage of cells in G0, S, G2, G1 phases of the cell life cycle using flow cytometry. 2 cell lines were used, these are human U 937 myeloid leukemia cell line and tur cell line. Compounds **11** and **12** cause a block at the G_2/M-phase at 100 μM and 10 μM in both of cell lines whereas compounds **15** and **17** block at the G_2/M-phase at 100 μM (see in tab. 47, 48).

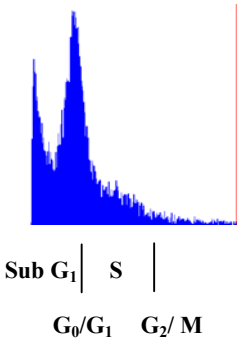
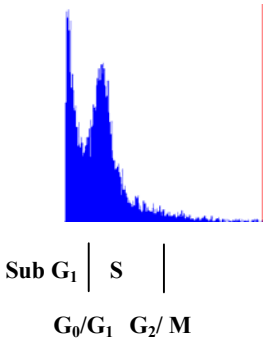
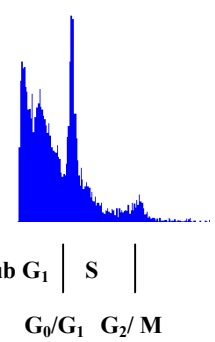
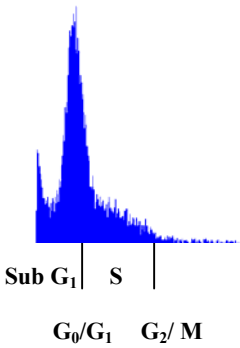
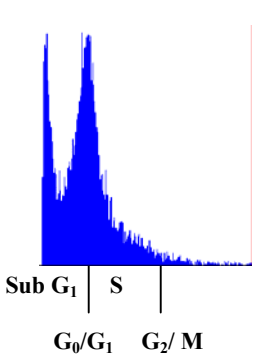
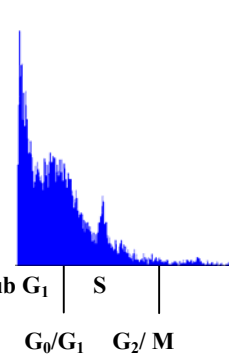
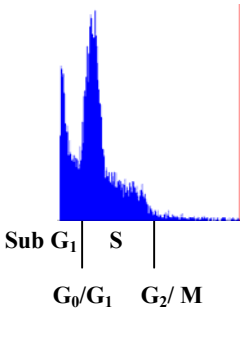
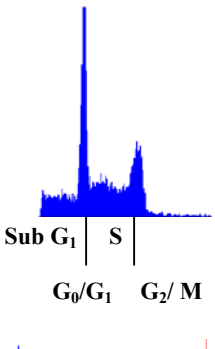
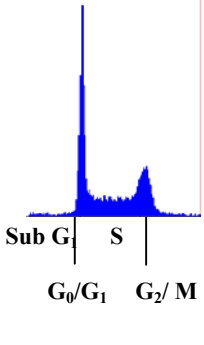
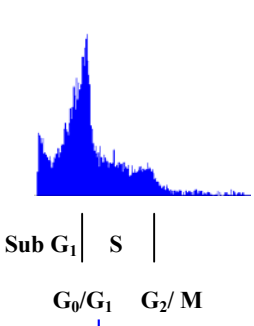
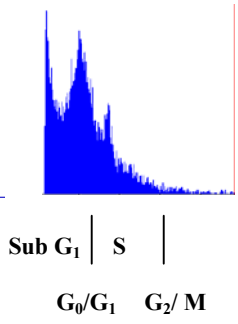
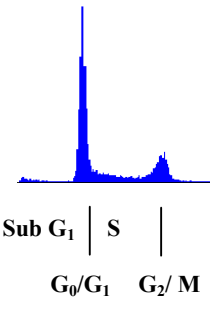
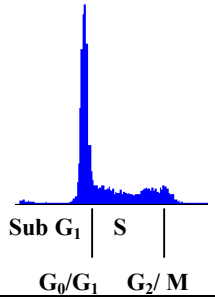
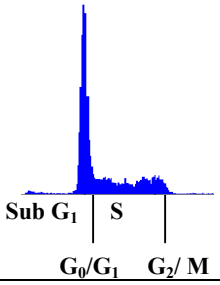
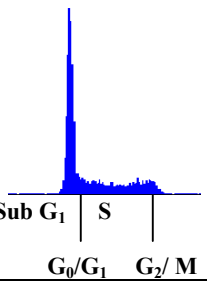
Table 45. Antiproliferative activities of constituents isolated from *S. tomentosum* in MCF-7 (human breast cancer cell line) and L 929 (mouse fibroblast cell line).

No	Compound	IC_{50} (μM) (2 days incubation)		IC_{50} (μM) (5 days incubation)	
		MCF-7	**L 929**	**MCF-7**	**L 929**
4	lupeol acetate	> 100	> 100	> 100	79.4
10	17*α*-H-periplogenin	5.29	> 100	2.57	> 100
11	17*α*-H-periplogenin-3-*O*-*β*-*D*-digitoxoside	< 1	51.5	< 1	24.2
12	17*α*-H-periplogenin-3-*O*-*β*-*D*-cymaroside	< 1	64.0	< 1	32.1
13	17α-H-periplogenin-*β*-glucopyranosyl-(1→4)-2-*O*-acetyl-*β*-digitalopyranoside	15.3	> 100	4.31	> 100
15	17*β*-H-periplogenin-*3*-*O*-*β*-*D*-digitoxoside	7.19	> 100	3.73	> 100
17	17*α*-H-digitoxigenin	4.16	> 100	< 1	> 100
control	camptothecin	0,0804	0,179	0,0122	0,0285
control	doxorubicin	0,207	0,359	0,0049	0,0168

Table 46. Cell viability of constituents isolated from *S. tomentosum* in U 937 (human leukemic cell line) and tumor cell in %.

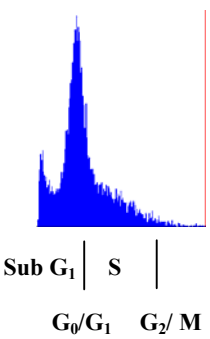
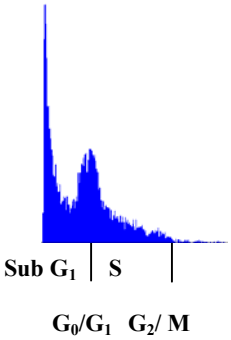
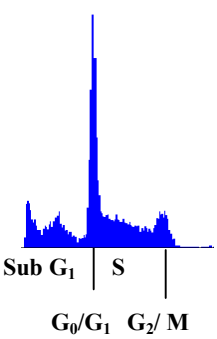
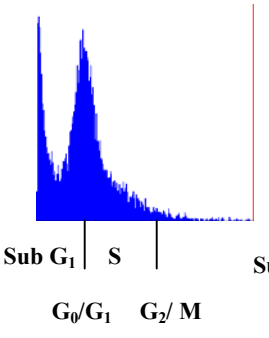
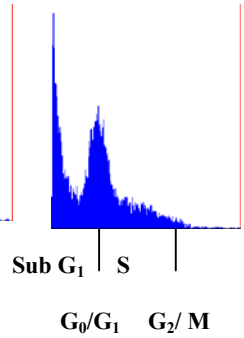
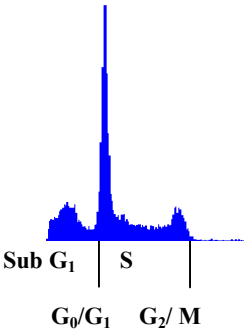
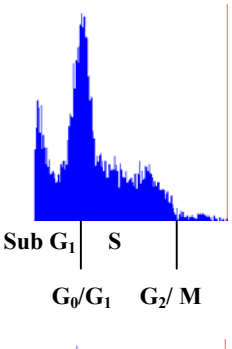
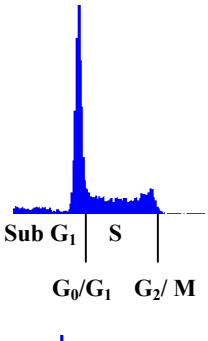
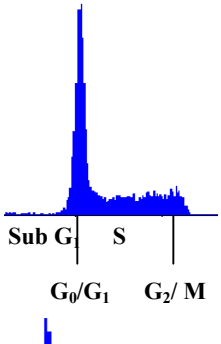
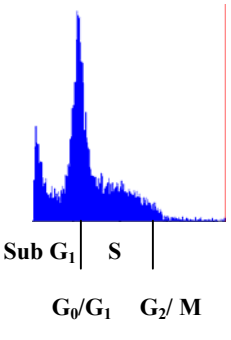
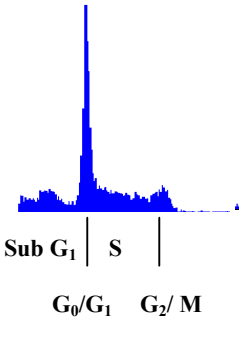
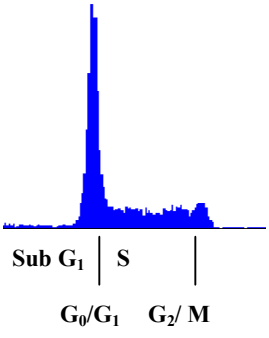
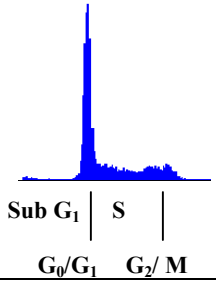
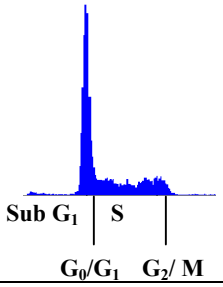
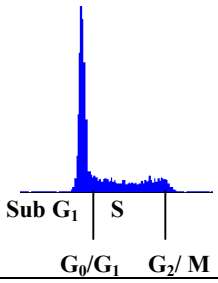
compound	U 937			Tumor		
	100 μM	**10 μM**	**1 μM**	**100 μM**	**10 μM**	**1 μM**
11	14,60%	26,10%	82,80%	21,50%	11,10%	69,50%
12	26,90%	28,90%	68,10%	42,30%	11,50%	27,60%
15	28,60%	93,90%	97,10%	28,00%	70,20%	91,2%
17	26,70%	77,50%	97,30%	28,90%	32,10%	89,8%
control (1% DMSO)	98,20%	98,40%	98,7%	94,30%	94,30%	96,1%

Table 47. Cell cycle analysis on human tumor myeloid leukemic cell line.

Compound	100 µM	10 µM	1 µM
17α H-periplogenin 3-*O*-β-*D*-digitoxoside ($C_{29}H_{44}O_8$) **11**			
17α H-periplogenin 3-*O*-β-*D*-cymaroside ($C_{30}H_{46}O_8$) **12**			
17β H-periplogenin *3*-*O*-β-*D*-digitoxoside ($C_{29}H_{44}O_8$) **15**			
17α H-digitoxigenin ($C_{23}H_{34}O_4$) **17**			
control DMSO			

X-axis: DNA content; Y-axis; Cell number

Table 48. Cell cycle analysis on U 937 cell line.

Compound	100 µM	10 µM	1 µM
17α H-periplogenin 3-*O*-β-*D*-digitoxoside ($C_{29}H_{44}O_8$) **11**			
17α H-periplogenin 3-*O*-β-*D*-cymaroside ($C_{30}H_{46}O_8$) **12**			
17β H-periplogenin *3*-*O*-β-*D*-digitoxoside ($C_{29}H_{44}O_8$) **15**			
17α H-digitoxigenin ($C_{23}H_{34}O_4$) **17**			
control DMSO			

X-axis: DNA content; Y-axis; Cell number

6.3. Screening of cellular viability of sesquiterpenes from *Curcuma comosa* and polyphenols from *Vitis repens*

Some sesquiterpenes (**22, 24, 26, 34, 35, 37, 38**) isolated from *C. comosa* and polyphenols (**51** and **52**) isolated from *V. repens* were examined for cellular viability in the tumor cell and U 937 (human leukemic cell line) at concentrations 100 μM, 10 μM, and 1 μM. Only compound **24** [(1*S*, 10*S*), (4*S*, 5*S*)-Germacrone-1(10), 4(5)-diepoxide] shows some limited growth inhibition (viability 71.60%) at 100 μM.

6.4. Antifungal activity

Dried powdered root of *Streptocaulon tomentosum*, rhizome of *Curcuma comosa*, rhizome of *Vitis repens*, *Aristolochia tagala* Cham. and *Spermacoce hispida* L. were extracted with 80% EtOH and evaporated until the water layer remained. Then water layer was successively partitioned between organic solvents (*n*-hexane, ethyl acetate, *n*-butanol) and water. Four extracts of each plant, three curcuminoids (**46-48**) from the ethylacetate extract of *C. comosa* and cardenolides (**10-18**) from *S. tomentosum* were tested their antifungal properties against *Cladosporium cucumerinum* according to Gottstein *et. al*. (1982). The result is shown in fig. 31 and tab. 49. Three curcuminoids (**46-48**) show antifungal activity at 20 μg (each inhibition zone 154 mm^2) while cardenolides (**10-18**) show no activity. Overall, the potential of these extracts as antifungals is quite limited.

Table 49. Inhibition zones in mm^2 (non-growth of *Cladosporium cucumerinum* on silica plates) to indicate antifungal activity of five medicinal plants.

	C. comosa		***A. tagala***		***V. repens***		***S. hispida***		***S. tomentosum***	
	250 μg	500 μg	250 μg	500 μg	250 μg	500 μg	250μg	500 μg	200 μg	400 μg
n-Hexane	154	227	64	113	64	95	64	113	-	50
Ethylacetate	113	154	50	78	-	-	-	-	64	227
n-Butanol	64	95	-	-	-	-	-	-	-	-
Water	-	-	-	-	-	78	-	-	-	-

(values ≤ 78 mm^2 show inactive compounds)

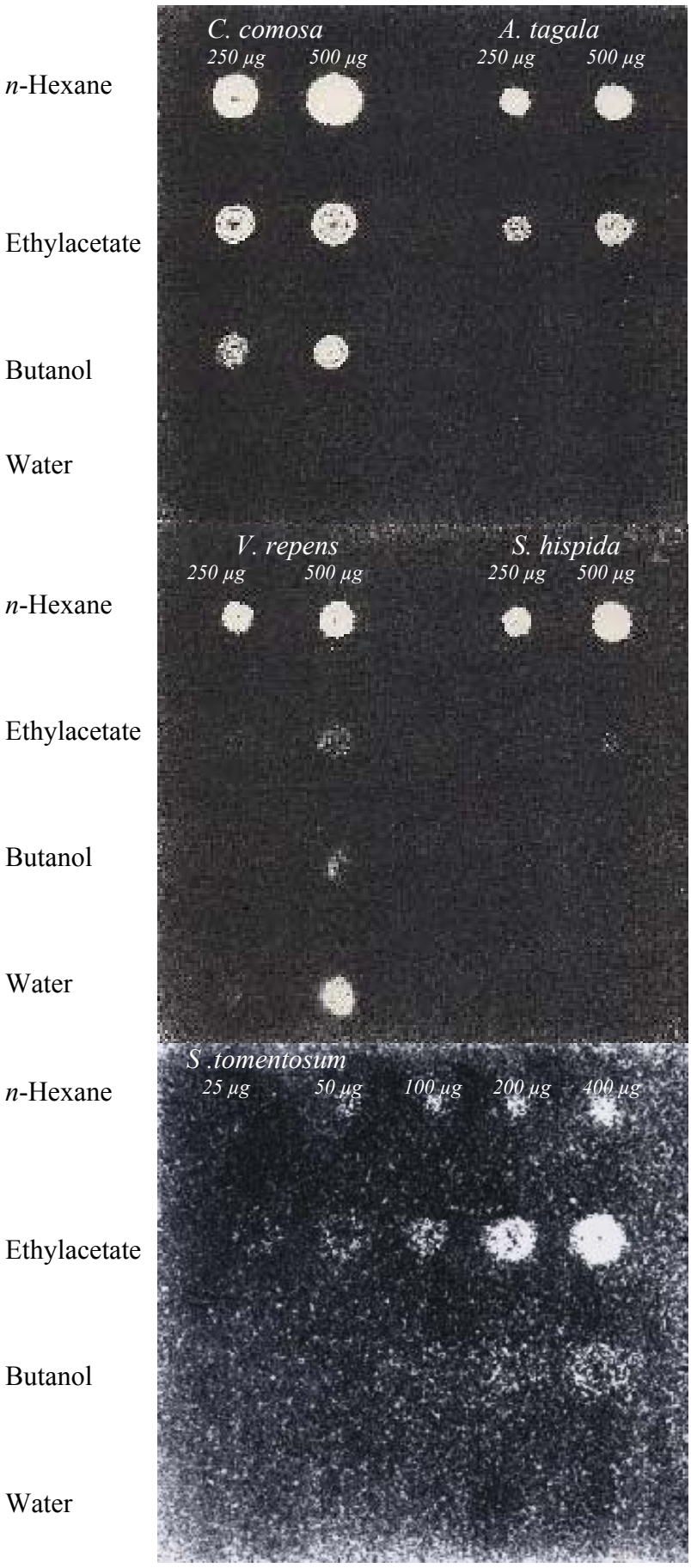


Figure 31. Antifungal activity zone of four extracts of Myanmar medicinal plants.

IV. EXPERIMENTAL SECTION

7. Instruments and materials

NMR spectra

1D NMR spectra (^{1}H, ^{13}C) were recorded from a Varian Unity 400 at 400 MHz for ^{1}H, and at 100 MHz for ^{13}C NMR. 2D NMR spectra (HSQC, HMBC, COSY, ROESY) were recorded from a Varian Inova 500 at 500 MHz for ^{1}H. Chemical shifts in ppm were referenced to the internal TMS (δ = 0 ppm) for ^{1}H and C_5D_5N (δ = 149.81, 135.48, 123.50 ppm), $CDCl_3$ (δ = 77.0 ppm), CD_3OD (δ = 49.00 ppm) and CD_3COCD_3 (δ = 29.80, 205.89 ppm) for ^{13}C, respectively.

ESI mass spectra

ESI mass spectra were measured from a API-150EX mass spectrometer (Applied Biosystems) with a turbo ions pray source.

HR-ESI-MS spectra

The high resolution positive ion ESI mass spectra were obtained from a Bruker Apex III 70 e Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with a 7.0 Tesla superconducting magnet, an RF-only hexapole ion guide and an external electrospray ion source. The sample solutions were introduced continuously via a syringe pump with a flow rate of 120 µl/h.

GC-MS spectra

The GC-MS measurements were performed with a GC-MS system (Voyager, ThermoQuest): 70 eV EI, source temp. 200 °C, column DB5MS (30 m x 0.25 mm, 0.25 µm film thickness), injection temperature 250 °C, interface temperature 300 °C, carrier gas He, flow rate 1.0 ml/min, constant flow mode, splitless injection, column temperature programm: 60 °C for 1 min, then raised to 300 °C at a rate of 10 °C/min, then isothermal at 300 °C for 20 min.

IR spectra

IR spectra were measured on an Bruker IFS 28 infrared spectrophotometer as KBr pellets. The wavelenth is indicated in cm^{-1}.

CD and UV spectra

CD spectra were obtained in methanol on a JASCO J-710 and UV spectra on a JASCO V 560.

Melting points

Melting points were obtained on a VMTG apparatus (Leica, Germany) and are uncorrected.

Specific rotation

The specific rotation was measured with a JASCO DIP-1000 polarimeter.

TLC

Analytical TLC was performed on the precoated aluminium TLC plates with silica gel 60 F_{254} (Merck, 0.25 mm) (normal-phase) and RP-18 F_{254} (Merck, 0.25 mm). Prep. TLC was carried out on the precoated glass plates with silica gel 60 F_{254} (Merck, 0.25 mm, 1 mm, 2 mm) (normal-phase) and RP-18 F_{254} (Merck, 0.25 mm). Spots were detected by UV (254, 360 nm) or by vanillin-H_2SO_4 (1.2 g of vanillin dissolved in 212.48 ml MeOH + 25 ml acetic acid + 11 ml H_2SO_4 dropwise) and 1% $Ce(SO_4)_2$-10% aqueous H_2SO_4 followed by heating.

silica gel 60 F_{254}

Solvent system T_1: *n*-hexane : $CHCl_3$ (5.4:6.6 v/v)

Solvent system T_2: $CHCl_3$: MeOH (12:1 v/v)

Solvent system T_3: $CHCl_3$: MeOH (10:1 v/v)

Solvent system T_4: $CHCl_3$: MeOH (8:2 v/v)

Solvent system T_5: *n*-hexane : acetone (4:1 v/v)

Solvent system T_6: *n*-hexane : acetone (13:7 v/v)

Solvent system T_7: *n*-hexane : acetone (1:1 v/v)

Solvent system T_8: $CHCl_3$: MeOH (9:1 v/v)

Solvent system T_9: $CHCl_3$: MeOH (6:4 v/v)

Column chromatography

Column chromatography was carried out on Kieselgel 60 (70-230 mesh, 230-400 mesh) (Merck), Lichroprep RP-18 (40-63 μm) (Merck), diaion HP 20 (250-850 μm) and sephadex LH 20 (25-100 μm) (Merck).

Column size: (id 3 cm × 90 cm), (id 1.5 cm × 40 cm), (id 1 cm × 60 cm), (id 2 cm × 60 cm)

HPLC

1. Knauer , UV detector,

Analytical HPLC: LiChrospher100 RP-18, 5 μm, 125 × 4 mm + VS (Nr. 51)

Solvent system 1a: A : B (25:75 v/v); flow rate 1 ml/min, 210 nm

Solvent A: Acetonitrile

Solvent B: Water

Solvent system 2a: A : B (30:70 v/v); flow rate 1 ml/min, 210 nm

Solvent A: Acetonitrile

Solvent B: Water

Solvent system 3a: A : B (45:55 v/v); flow rate 1 ml/min, 210 nm

Solvent A: Acetonitrile

Solvent B: Water

Solvent system 4a: A : B (30:70 v/v); flow rate 1 ml/min, 210 nm

Solvent A: Acetonitrile

Solvent B: Water

Solvent system 5a: A : B (25:75 v/v); flow rate 1 ml/min, 210 nm

Solvent A: Acetonitrile

Solvent B: Water

Solvent system 6a: A : B (15:85 v/v); flow rate 1 ml/min, 210 nm

Solvent A: Acetonitrile

Solvent B: Water

Solvent system 7a: A : B (5:95 v/v); flow rate 1 ml/min, 240 nm

Solvent A: Acetonitrile + 0.2% TFA

Solvent B: Water + 0.2% TFA

Preparative HPLC: YMC ODS-A 5 μm 120Å 150 × 20 mm

Solvent system 1p: A : B (20:80 v/v); flow rate 9.2 ml/min, 210 nm

Solvent A: Acetonitrile

Solvent B: Water

Solvent system 2p: A : B (30:70 v/v); flow rate 10 ml/min, 210 nm

Solvent A: Acetonitrile

Solvent B: Water

Solvent system 3p: A : B (45:55 v/v); flow rate 9.2 ml/min, 210 nm

Solvent A: Acetonitrile

Solvent B: Water

Solvent system 4p: A : B (30:70 v/v); flow rate 20.0 ml/min, 210 nm

Solvent A: Acetonitrile

Solvent B: Water

Solvent system 5p: A : B (25:75 v/v); flow rate 9.2 ml/min, 210 nm

Solvent A: Acetonitrile

Solvent B: Water

Solvent system 6p: A : B (15:85 v/v); flow rate 9.2 ml/min, 210 nm

Solvent A: Acetonitrile

Solvent B: Water

Solvent system 7p: A : B (5:95 v/v); flow rate 10 ml/min, 240 nm

Solvent A: Acetonitrile

Solvent B: Water

2. Merck Hitachi D-7000 system, L-7450A diode array detector, pump L-7100

Analytical HPLC: Lichrospher100, RP-18 (5 μm), 3 × 125 mm

Solvent system 8a: A : B (10:90 – 45 min→15:85 v/v); flow rate 0.6 ml/min, 240 nm

Solvent A: Acetonitrile

Solvent B: Water

Solvent system 9a: A : B (0:100– 30 min→ gradient); flow rate 0.6 ml/min, 250 nm

Solvent A: MeOH

Solvent B: 5% MeOH + 0.1% Formic acid

Solvent system 10a: A : B (0:100– 30 min→ gradient); flow rate 0.6 ml/min, 250 nm

Solvent A: MeOH

Solvent B: 5% MeOH + 0.1% Formic acid

Solvent system 11a: B (100%– 30 min); flow rate 0.6 ml/min, 250 nm

Solvent A: MeOH

Solvent B: 5% MeOH + 0.1% Formic acid

Solvent system 12a: A : B (20:80– 30 min); flow rate 0.6 ml/min, 250 nm

Solvent A: Acetonitrile

Solvent B: 1% Acetonitrile

Prep HPLC: Lichrospher100, RP-18 (10 μm), 10 × 250 mm

Solvent system 9p: B (100%– 60 min); A : B (0:100 gradient – 60-120 min);

flow rate 5 ml/min, 250 nm

Solvent A: MeOH

Solvent B: 5% MeOH + 0.1% Formic acid

Solvent system 10p: A : B (0:100 gradient – 120 min); flow rate 5 ml/min, 250 nm

Solvent A: MeOH

Solvent B: 5% MeOH + 0.1% Formic acid

Solvent system 11p: B (100% – 90 min); flow rate 5 ml/min, 250 nm

Solvent A: MeOH

Solvent B: 5% MeOH + 0.1% Formic acid

8. Investigation of bioactive constituents from *Streptocaulon tomentosum* Root

8.1. Plant material

Streptocaulon tomentosum Wight & Arn. (Asclepiadaceae) roots were collected in May 2002 at Mawlamyine, District Mawlamyine, Myanmar (leg./det. Dr Daw Hla Ngwe). The species was identified by Prof. Dr Aung Aung Min, Department of Botany, University of Yangon. A voucher specimen of the clamberer (No.Y.H.V. 1004) is deposited at the University of Yangon, Department of Botany.

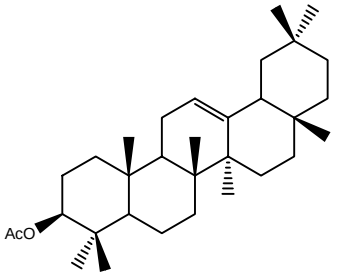
8.2. Extraction and isolation

Dried powdered root of *Streptocaulon tomentosum* (Asclepiadaceae) (1 kg) was extracted with 80% EtOH (1 L × 3) for one week. The solvent was evaporated to the remaining water layer. Then the water layer was successively partitioned between organic solvents (*n*-hexane, ethyl acetate, *n*-butanol 300 mL × 3) and water (see in scheme 1). The *n*-hexane fraction (48 g) was chromatographed over silicagel 60 (70-230 mesh, Merck), using a stepwise gradient of *n*-hexane : ethylacetate (9.5:0.5, 9:1,.....2:1, increasing polarity) to give four fractions. Fraction 1 (8 g) and fraction 2 (4 g) were rechromatographed on silicagel 60 column (230-400 mesh, column size id 2 cm × 60 cm) with the solvent system *n*-hexane : chloroform (3:1) and (9:1-4:1) to give *β*-amyrin acetate (**1**), α-amyrin acetate (**2**), cycloartenol (**3**)**,** and lupeol acetate (**4**) (see in scheme 2). The ethyl acetate fraction (12 g) was separated on a silicagel column (70-230 mesh, column size id 3 cm × 60 cm) and eluted with *n*-hexane : ethyl acetate : methanol (increasing polarity 9:1:0, 7:3:0.5,.....to pure.MeOH) to give 23 fractions (each about 300 mg). Fraction 10 (200 mg), 12 (280 mg) and 14 (300 mg) were rechromatographed on silicagel 60 (230-400 mesh, column size 1.5 cm × 40 cm) using $CHCl_3$: MeOH (9.5:0.5; 4.7:0.3) to give compound **17** (17*α*-H-digitoxigenin), **10** (17*α*-H-periplogenin), and **14** (17*β*-H-periplogenin) respectively. From fraction 18 (400 mg) after rechromatography on sephadex LH 20 in MeOH and purificative by preparative TLC silicagel 60 F_{254}, compound **13** (17*α*-H-periplogenin-*β*-glucosyl (1→4)-2-*O*-acetyl-*β*-digitalose) was obtained. Compounds **5-9, 11, 12, 15, 18-20** were also isolated after repeated column chromatography on silica gel, sephadex LH 20 and RP-18 (see in scheme 3). From the *n*-butanol fraction, compound **16** and **19** were obtained using silicagel and RP-18 (MeOH : H_2O, 8:2; see scheme 4). The fractions collected from column chromatography were checked on TLC plates and detected by spray reagents.

8.3. Characterization of isolated compounds from the root of *S. tomentosum*

8.3.1. Triterpenoids

Compound 1: β-amyrin acetate (mmk 001-a) [lit. 66]



colourless needles

Yield: 100 mg, 0.01%

mp.: 242-243 °C

$[\alpha]_D$: + 80.1 ° (c = 1.10, $CHCl_3$)

TLC: R_f = 0.47 (system T_1, violet colour with vanillin/H_2SO_4, inactive under UV)

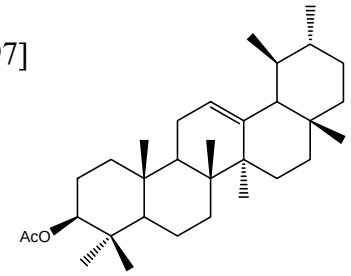
IR: (KBr), ν_{max}= 1722, 1635, 1240, 812 cm^{-1}

^1H NMR: (300 MHz, $CDCl_3$): δ 0.84 (3H, *s*, H-28), 0.88 (12H, *s*, H-23, 24, 29, 30), 0.98 (6H, *s*, H-25, 26), 1,14 (3H, *s*, H-27), 2.07 (3H, *s*, OAc), 4.54 (1H, *dd*, *J* 11.6 Hz, H-3α), 5.21 (1H, *t*, *J* 3.5 Hz, H-12)

GC-MS: RT = 18.03 min, 468 $[M]^+$, 453 (33), 408 $[M\text{-}HOAc]^+$ (5), 393 (40), 281 (70), 218 (100), 203 (68), 69 (90)

EI-MS: (70 ev) *m/z* (rel. int): 468 $[M]^+$, 453 (2), 408 $[M\text{-}HOAc]^+$ (5), 218 (100), 203 (20)

Compound 2: α- amyrin acetate (mmk 001-b) [lit. 6, 97]



colourless needles

Yield: 1.5 g, 0.15%

mp.: 243 °C

$[\alpha]_D$: + 76 ° (c = 1.0, $CHCl_3$)

TLC: R_f = 0.47 (system T_1, violet colour with vanillin/H_2SO_4, inactive under UV)

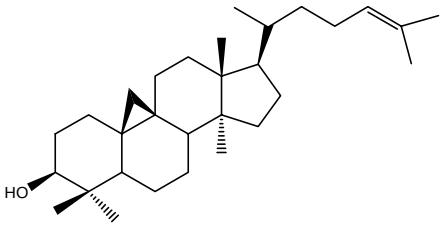
IR: (KBr), ν_{max}= 1730, 1380, 1370, 1250, 1030, 1000, 985, 960 cm^{-1}

^1H NMR: (300 MHz, $CDCl_3$): δ 0.79 (3H, *s*, H-28), 0.88 (12H, *s*, H-23, 24, 29, 30), 0.98 (3H, *s*, H-26), 1.01 (3H, *s*, H-25), 1,07 (3H, *s*, H-27), 2.05 (3H, *s*, OAc), 4.50 (1H, *dd*, *J* 9.7 Hz, H-3α), 5.12 (1H, *t*, *J* 3.6 Hz, H-12)

GC-MS: RT = 18.85 min, 468 $[M]^+$, 453 (45), 408 $[M\text{- }HOAc\text{ }]^+$ (38), 393 (40), 281 (100), 218 (80), 203 (30), 69 (98)

EI-MS: (70 ev) *m/z* (rel. int): 468 $[M]^+$, 453 (10), 408 $[M\text{- }HOAc\text{ }]^+$ (20), 218 (100), 203 (10)

Compound 3: cycloartenol (mmk 002) [lit. 11]



colourless needles

Yield: 100 mg, 0.01%

mp.: 108-109 °C

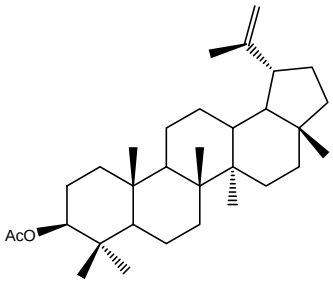
$[\alpha]_D$: + 50 ° (*c* = 1, $CHCl_3$)

IR: (KBr), ν_{max}= 3340, 3060, 2960, 2900, 1495, 1470, 1410, 1235, 1130, 1045, 910 cm^{-1}

^1H NMR: (300 MHz, $CDCl_3$): δ 0.55 (2H, *d*, *J* 4.3 Hz, H-19), 0.81 (3H, *s*, H-30), 0.88 (3H, *d*, *J* 5.7 Hz, H-21), 0.89 (3H, *s*, H-28), 0.96 (6H, *s*, H-18, 29), 1.60 (3H, *s*, H-26), 1.68 (3H, *s*, H-27), 3.23 (1H, *m*, H-3), 5.10 (1H, *t*, *J* 7.0 Hz, H-24)

GC-MS: RT = 16.82 min, 426 $[M]^+$, 393 (27), 281 (35), 218 (25), 203 (10), 69 (100)

Compound 4: lupeol acetate (mmk 003) [lit. 66]



colourless needles

Yield: 40 mg, 0.004%

mp.: 218-220 °C

$[\alpha]_D$: + 35.5 ° (c = 0.35, MeOH-$CHCl_3$)

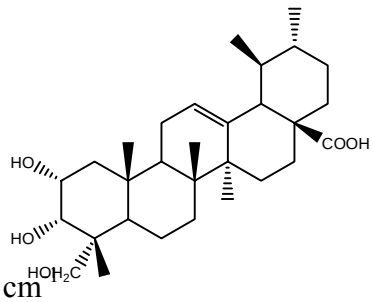
TLC: R_f = 0.41 (system T_1, violet colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max}= 1727, 1239, 3060, 1637, 865 cm^{-1}

^1H NMR: (300 MHz, $CDCl_3$): δ 0.79 (3H, *s*, H-28), 0.85 (9H, *s*, H-23, 24, 25), 0.94 (3H, *s*, H-26), 1.03 (3H, *s*, H-26), 1,70 (3H, *s*, H-30), 2.04 (3H, *s*, OAc), 4.47 (1H, *dd*, *J* 10.6 Hz, H-3α), 4.56, 4.62 (2H, *dd*, *J* 2.2 Hz, H-29), 5.12 (1H, *t*, *J* 3.6 Hz, H-12)

EI-MS: (70 ev) *m/z* (rel. int): 468 $[M]^+$, 453 (22), 408 $[M\text{-}HOAc]^+$, 249 (20), 218 (60), 204 (50), 189 (100)

Compound 5: 2α,3α,23-trihydroxy-urs-12-en-28-oic-acid (mmk 029) [lit. 82]



white powder

Yield: 20 mg, 0.0020%

TLC: R_f = 0.38 (system T_2, blue colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max}= 3452 (OH), 2960, 1745 (COO), 1030 (OH), 1640 (C=C), 827 (C=C) cm^{-1}

^1H NMR: (500 MHz, C_5D_5N): δ 0.876 (3H, *s*, H-24), 0.925 (3H, *d*, *J* 6.2 Hz, H-30), 0.965 (3H, *d*, *J* 6.4 Hz, H-29), 1.009 (3H, *s,* H-25), 1.079 (3H, *s*, H-26), 1.148 (3H, *s*, H-27), 0.96-1.02 (1H, *m*, H-20β), 1.19-2.34 (19H, *m*, H-15, 6, 7, 21,

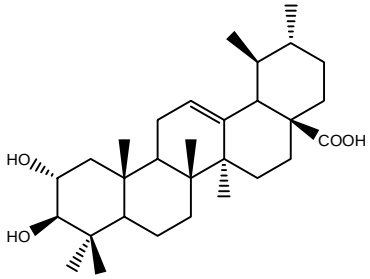
19α, 1, 9α, 5, 22, 11, 16), 2.626 (1H, *br d*, *J* 11.3 Hz, H-18), 3.776 (1H, *d*, *J* 10.8 Hz, H-23B), 3.943 (1H, *d*, *J* 10.8 Hz, H-23A), 4.168 (1H, *d*, *J* 2.3 Hz, H-3), 4.289 (1H, *m*, H-2), 5.480 (1H, *br s*, H-12).

EI-MS: (70 ev) *m/z* (rel. int): 488 $[M]^+$ (10), 248 (100), 203 (80), 191 (20), 173 (40)

HR-ESI-MS: $C_{30}H_{48}O_5Na$ calc. 511.33939 found. 511.33798

Compound 6: 2α,3β-dihydroxy-urs-12-en-28-oic-acid (mmk 038) [lit. 50]

white powder



Yield: 2 mg, 0.0002%

TLC: R_f = 0.34 (system T_2, blue colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max}= 3452 (OH), 2960, 1745 (COO), 1030 (OH), 1640 (C=C), 827 (C=C) cm^{-1}

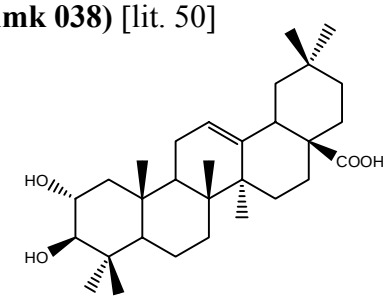
^{1}H NMR: (500 MHz, C_5D_5N): δ 0.960 (3H, *d*, *J* 5.9 Hz, H-30), 0.991 (3H, *s*, H-25), 0.995 (3H, *d*, *J* 4.9 Hz, H-29), 1.0-1.16 (2H, *m,* H-5, 19 or 20), 1.060 (3H, H-26), 1.092 (3H, *s*, H-24), 1.19-1.21 (1H, *m*, H-15), 1.220 (3H, *s*, H-27), 1.291 (3H, *s*, H-23), 1.26-2.36 (16H, *m*, H-21, 19 or 20, 1, 6, 9, 7, 22, 11, 16), 2.641 (1H, *br d*, *J* 11.4 Hz, H-18), 3.420 (1H, *d*, *J* 9.4 Hz, H-3), 4.115 (1H, *ddd*, *J* 11.0/9.4/4.4 Hz, H-2), 5.476 (1H, *m*, H-12).

EI-MS: (70 ev) *m/z* (rel. int): 248 (100), 203 (50), 189 (10), 133 (30)

HR-ESI-MS: $C_{30}H_{48}O_4Na$ $[M+Na]^+$ calc. 495.34448 found. 495.34361

Compound 7: 2α,3β-dihydroxy-olean-12-en-28-oic-acid (mmk 038) [lit. 50]

white powder



Yield: 1.0 mg, 0.0001%

TLC: R_f = 0.34 (system T_2, blue colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max}= 3452 (OH), 2960, 1745 (COO), 1030 (OH), 1640 (C=C), 827 (C=C) cm^{-1}

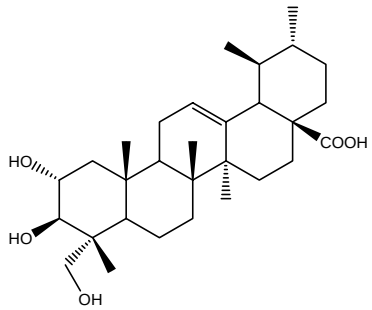
^{1}H NMR: (500 MHz, C_5D_5N): δ0.954 (3H, *s*, H-29), 0.991 (3H, *s*, H-25), 1.014 (3H, *s*, H-30), 1.0-1.16 (1H, *m,* H-5), 1.032 (3H, H-26), 1.092 (3H, *s*, H-24), 1.19-1.21 (1H, *m*, H-15), 1.275 (3H, *s*, H-27), 1.291 (3H, *s*, H-23), 1.26-2.36 (15H, *m*, H-21, 1, 6, 9, 7, 22, 11, 16), 3.314 (1H, *dd*, *J* 13.9/4.0 Hz, H-18), 3.413 (1H, *d*, *J* 9.4 Hz, H-3), 4.115 (1H, *ddd*, *J* 11.0/9.4/4.4 Hz, H-2), 5.476 (1H, *m*, H-12).

EI-MS: (70 ev) *m/z* (rel. int): 248 (100), 203 (50), 189 (10), 133 (30)

HR-ESI-MS: $C_{30}H_{48}O_4Na$ $[M+Na]^+$ calc. 495.34448 found. 495.34361

Compound 8: 2α,3β,23-trihydroxy-urs-12-en-28-oic-acid (mmk 015) [lit. 50]

white needles



Yield: 20 mg, 0.002%

TLC: R_f = 0.24 (system T_2, blue colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max}= 3500 (OH), 1720 (COO), 1030 (OH), 1600 (C=C), 827 (C=C) cm^{-1}

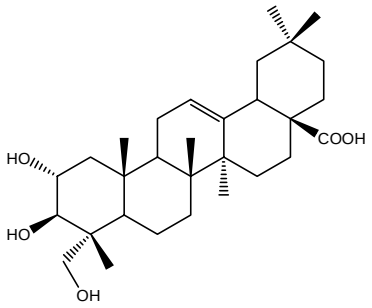
^{1}H NMR: (500 MHz, CD_3OD): δ 0.692 (3H, *s*, H-24), 0.846 (3H, *s*, H-26), 0.8865 (3H, *d*, *J* 6.4 Hz, H-29), 0.86-1.01 (2H, *m,* H-19 or 20, 1B), 0.967 (3H, H-30), 1.042 (3H, *s*, H-25), 1.05-1.11 (1H, *m*, H-15), 1.132 (3H, *s*, H-27), 1.11-2.04 (16H, *m*, H-5, 21, 19 or 20, 6, 9, 22, 11, 16, 1A), 2.202 (1H, *d*, *J* 11.2 Hz, H-18), 3.261 (1H, *d*, *J* 11.0 Hz, H-23B), 3.350 (1H, *dd*, *J* 9.7/2.4 Hz, H-3), 3.498 (1H, *d*, *J* 11.2 Hz, H-23A), 3.687 (1H, *m*, H-2), 5.242 (1H, *m*, H-12).

EI-MS: (70 ev) *m/z* (rel. int): 248 (100), 203 (50), 133 (30)

HR-ESI-MS: $C_{30}H_{48}O_5Na$ calc. 511.33939 found. 511.33798

Compound 9: 2α,3β,23-trihydroxy-olean-12-en-28-oic-acid (mmk 015)

white needles



Yield: 20 mg, 0.002%

TLC: R_f = 0.24 (system T_2, blue colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max}= 3500 (OH), 1720 (COO), 1030 (OH), 1600 (C=C), 827 (C=C) cm^{-1}

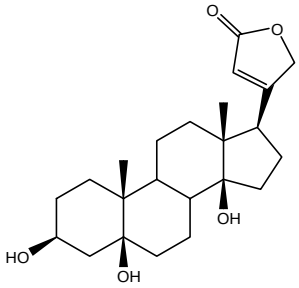
^{1}H NMR: (500 MHz, CD_3OD): δ 0.690 (3H, *s*, H-24), 0.813 (3H, *s*, H-26), 0.86-0.91 (1H, *m,* 1B) 0.907 (3H, *s*, H-29), 0.941 (3H, *s*, H-30), 1.028 (3H, *s*, H-25), 1.05-1.11 (1H, *m*, H-15), 1.175 (3H, *s*, H-27), 1.12-2.04 (16H, *m*, H-5, 21, 19, 6, 9, 22, 11, 16, 1A), 2.849 (1H, *dd*, *J* 13.6/3.9 Hz, H-18), 3.261 (1H, *d*, *J* 11.0 Hz, H-23B), 3.350 (1H, *dd*, *J* 9.7/2.4 Hz, H-3), 3.498 (1H, *d*, *J* 11.2 Hz, H-23A), 3.687 (1H, *m*, H-2), 5.242 (1H, *m*, H-12).

EI-MS: (70 ev) *m/z* (rel. int): 248 (100), 203 (50), 133 (30)

HR-ESI-MS: $C_{30}H_{48}O_5Na$ calc. 511.33939 found. 511.33798

8.3.2. Cardenolides

Compound 10: 17α-H-periplogenin (mmk 022) [lit. 46]



white amorphous powder

Yield: 20 mg, 0.0020%

mp.: 138-141 °C

$[\alpha]_D$: + 16.23 ° (c = 0.086, MeOH)

TLC: R_f = 0.32 (system T_3, dark blue colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max}= 3320, 1775, 1740, 1620 cm^{-1}

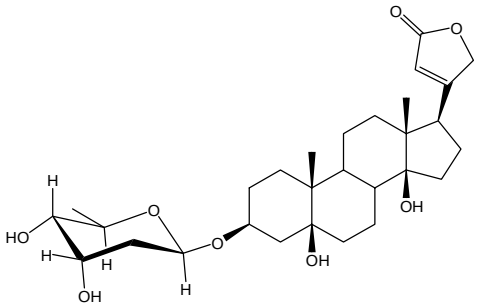
UV: (MeOH), λ_{max} = 218 nm (log ε = 4.3)

^{1}H NMR: (300 MHz, C_5D_5N): δ 0.88 (3H, *s*, H-18), 0.94 (3H, *s*, H-19), 1.21-2.45 (19H, *m*, H-2, 4, 6, 7, 8, 9, 11, 12, 15, 16), 2.84 (1H, *dd*, *J* 9/3 Hz, H-17), 4.46 (1H, *br s*, H-3), 5.08, 5.36 (2H, *dd*, *J* 18.1/1.4 Hz, H-21A, B), 6.17 (1H, *br s*, H-22).

EI-MS: (70 ev) *m/z* (rel. int): 390 $[M]^+$ (1), 372 $[M\text{-}H_2O]^+$ (20), 354 $[M\text{-}2H_2O]^+$ (25), 318 $[C_{19}H_{26}O_4]^+$ (100), 300 $[C_{19}H_{24}O_3]^+$ (8), 262 $[C_{17}H_{26}O_2]^+$ (5), 219 $[C_{15}H_{23}O]^+$ (40), 201 $[C_{15}H_{21}]^+$ (60), 145 $[C_{11}H_{13}]^+$ (30)

HR-ESI-MS: $C_{23}H_{34}O_5Na$ calc. 413.22984 found. 413.23098

Compound 11: 17α-H-periplogenin-β-*D*-digitoxose (mmk 005) [lit. 100]



light brown amorphous solid

Yield: 20 mg, 0.002%

mp.: 130-135 °C

$[\alpha]_D$: + 13.32 ° (c = 0.15, MeOH)

TLC: R_f = 0.23 (system T_3, blue colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max}= 3450, 1740, 1620 cm^{-1}

UV: (MeOH), λ_{max} = 218 nm (log ε = 4.1)

CD: [MeOH, [mdeg] (nm)]: + 3.11 (240.0)

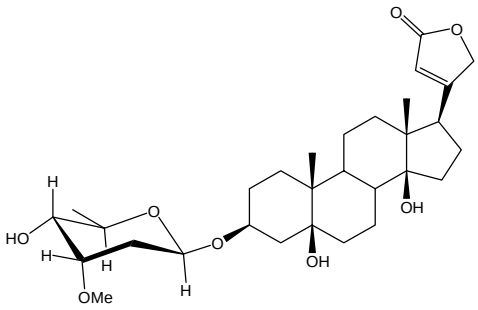
^{1}H NMR: (500 MHz, C_5D_5N): δ 1.036 (3H, *s*, H-18), 1.087 (3H, *s*, H-19), 1.267-1.550 (7H, *m*, H-7B, 11, 12, 1B, 6B), 1.597 (3H, *d*, *J* 6.1 Hz, H_3-6′), 1.642 (1H, *m*, H-9), 1.725-1.755 (2H, *m*, H-2B, 4B), 1.831-1.931 (3H, *m*, H-8, 15B, 6A,), 1.96 (1H, *m*, H-2′B), 1.951-2.312 (7H, *m*, H-16B, 2A, 1A, 16B, 15A, 4A, 7A), 2.391 (1H, *br d*, *J* 13.2 Hz, H-2′A), 2.817 (1H, *d*, *J* 8.7 Hz, H-17), 3.623 (1H, *dd, J* 2.4/9.3 Hz, H-4′), 4.305 (1H, *m*, H-5′), 4.397 (1H, *br s*, H-3), 4.426 (1H,

d, *J* 2.7 Hz, H-3′), 5.063, 5.344 (2H, *dd*, *J* 18.1/1.4 Hz, H-21 A,B), 5.465 (1H, *dd*, *J* 9.7/1.4 Hz, H-1′), 6.158 (1H, *br s*, H-22).

EI-MS: (70 ev) *m/z* (rel. int): 391 (12), 372 (18), 355 (50), 318 (80), 113 (100)

HR-ESI-MS: $C_{29}H_{44}O_8Na$ calc. 543.292839 found. 543.29240

Compound 12: 17α-H-periplogenin-β-*D*-cymaroside (mmk 010) [lit. 100]



light brown needles

Yield: 20 mg, 0.002%

mp.: 182-192 °C (needle), 135 °C (amorphous)

$[\alpha]_D$: + 15.18 ° (c = 0.17, MeOH)

TLC: R_f = 0.43 (system T_3, blue colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max}= 3450, 1740, 1620 cm^{-1}

UV: (EtOH), λ_{max} = 215 nm (log ε = 4.1)

CD: [MeOH, [mdeg] (nm)] : + 4.74 (238.6), – 1.85 (202.2)

^{1}H NMR: (500 MHz, C_5D_5N): δ 1.038 (3H, *s*, H-18), 1.093 (3H, *s*, H-19), 1.26-1.48 (6H, *m*, H-7B, 11, 12, 1B), 1.540 (3H, *d*, *J* 6.1 Hz, H_3-6′), 1.546 (1H, *m*, H-6B), 1.60-1.68 (1H, *m*, H-9), 1.70-1.78 (3H, *m*, H-2B, 4B, 2′B), 1.81-1.93 (3H, *m*, H-8, 15B, 6A,), 1.95-2.31 (7H, *m,* H-16B, 2A, 1A, 16B, 15A, 4A, 7A), 2.286 (1H, *m*, H-2′A), 2.818 (1H, *d*, *J* 8.0 Hz, H-17), 3.417 (3H, *s*, H-3′OMe), 3.536 (1H, *dd, J* 2.4/9.3 Hz, H-4′), 3.554 (1H, *d*, *J* 2.9 Hz, H-3′), 4.233 (1H, *m*, H-5′), 4.373 (1H, *br s*, H-3), 5.066, 5.347 (2H, *dd*, *J* 18.1/1.4 Hz, H-21A, B), 5.177 (1H, *dd*, *J* 9.7/1.8 Hz, H-1′), 6.159 (1H, *br s*, H-22).

EI-MS: (70 ev) *m/z* (rel. int): 390 $[M]^+$, 372 $[M-H_2O]^+$ (20), 355 (100), 318 (48), 145 (32), 113 (57)

HR-ESI-MS: $C_{30}H_{46}O_8Na$ calc. 557.3084895 found. 557.308882

X-ray crystal data: $C_{31}H_{50}O_9$; M.W. = 566.71; monoclinic; space group I 2; lattice constants: a = 12.644(4), b = 7.6341(17), c = 32.153(10), Å, α = 90 °, β = 94.04(4) °, γ = 90 °, *U* = 3095.8(16) $Å^3$, *Z* = 4, D_{calc}= 1.216 Mg/m^3; Final R indices [I>2sigma(I)]: R1 = 0.0554, wR2 = 0.0811

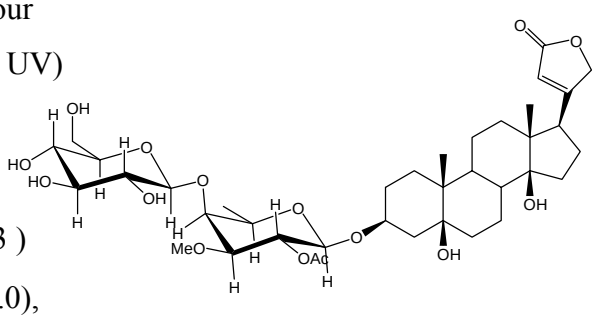
Compound 13: 17α-H-periplogenin-β-glucosyl-(1-4)-2-*O*-acetyl-digitalose (mmk 013)

colourless powder

Yield: 4 mg, 0.0004%

mp.: 191-193 °C

TLC: R_f = 0.44 (system T_4, dark blue colour with vanillin/H_2SO_4, inactive under UV)



IR: (KBr), ν_{max}= 3420, 1780, 1740, 1635, 1123 cm^{-1}

UV: (MeOH), λ_{max} = 218 nm (log ε = 4.3)

CD: [MeOH, [mdeg] (nm)]: + 1.97 (238.0), – 3.69 (200.8)

^{1}H NMR: (500 MHz, CD_3OD): δ 0.875 (3H, *s*, H-18), 0.917 (3H, *s*, H-19), 1.304 (3H, *d*, *J* 6.4 Hz, H-6′), 2.834 (1H, *m*, H-17), 2.088 (3H, *s*, 2′-OCOC<u>H</u>$_3$), 3.431 (3H, *s*, 3′-OC<u>H</u>$_3$), 3.474 (1H, *dd*, *J* 10.1/3.0 Hz, H-3′), 3.640 (1H, *dd*, *J* 11.6/6.1 Hz, H-6′′b), 3.880 (1H, *dd*, *J* 11.6/1.4 Hz, H-6′′a), 4.124 (1H, *br s*, H-3), 4.234 (1H, *br d*, *J* 3.0 Hz, H-4′), 4.530 (1H, *d*, *J* 8.0 Hz, H-1′), 4.542 (1H, *d*, *J* 7.7 Hz, H-1′′), 4.910 (1H, *dd*, *J* 18.8, 1.7 Hz, H-21b), 5.024 (1H, *dd*, *J* 18.8/1.7 Hz, H-21a), 5.095 (1H, *dd*, *J* 10.1/8.0 Hz, H-2′), 5.894 (1H, *br s*, H-22).

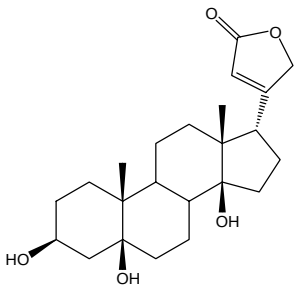
(500 MHz, C_5D_5N): δ 1.001 (3H, *s*, H-18), 1.029 (3H, *s*, H-19), 1.557 (3H, *d*, *J* 6.4 Hz, H-6′), 2.818 (1H, *m*, H-17), 2.225 (3H, *s*, 2′-OCOC<u>H</u>$_3$), 3.457 (3H, *s*, 3′-OC<u>H</u>$_3$), 3.608 (1H, *dd*, *J* 10.2/3.0 Hz, H-3′), 3.738 (1H, *m*, H-5′), 4.190 (1H, *dd*, *J* 9.4/8.8 Hz, H-4′′), 4.248 (1H, *dd*, *J* 8.8/8.8 Hz, H-3′′), 4.448 (1H, *br d*, *J* 3.0 Hz, H-4′), 4.605 (1H, *br d*, *J* 11.5 Hz, H-6′′a), 4.841 (1H, *d*, *J* 8.0 Hz, H-1′), 5.149 (1H, *d*, *J* 7.7 Hz, H-1′′), 5.342 (1H, *dd*, *J* 18.4/1.7 Hz, H-21a), 5.828 (1H, *dd*, *J* 10.2/8.0 Hz, H-2′), 6.161 (1H, *br s*, H-22).

EI-MS: (70 ev) *m/z* (rel. int): 318 (2), 253 (2), 147 (20), 57 (100)

HR-ESI-MS: $C_{38}H_{58}O_{15}Na$ ($[M+Na]^+$) calc. 777.36679 found. 777.36606

Compound 14: 17*β*-H-periplogenin (mmk 012) [lit. 20]

white powder



Yield: 1.5 mg, 0.00015%

mp.: 266-267 °C

$[\alpha]_D$: + 16.4 ° (c = 0.15, MeOH)

TLC: R_f = 0.32 (system T_4, dark blue colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max}= 3420, 1780, 1755, 1635 cm^{-1}

UV: (EtOH), λ_{max} = 215 nm (log ε = 4.5)

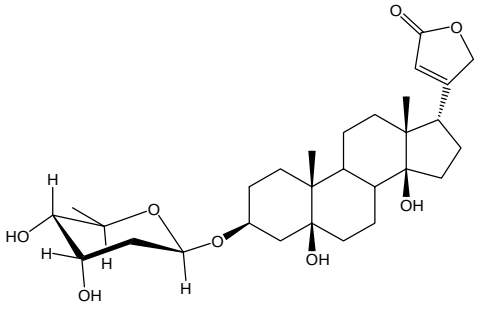
^{1}H NMR: (500 MHz, C_5D_5N): δ 1.11-1.20 (2H, *m*, H-12), 1.186 (3H, *s*, H-19), 1.227 (3H, *s*, H-18), 1.22-1.52 (4H, *m*, H-7B, 11, 1B), 1.62-1.72 (2H, *m*, H-6B, 9), 1.80-2.20 (7H, *m*, H-4B, 8, 15B, 2, 1B, 6A), 2.08-2.38 (5H, *m*, H-16A, 15A, 1A, 7A, 4A), 3.463 (1H, *t*, *J* 9.5 Hz, H-17), 4.483 (1H, *br d*, H-3), 4.849, 4.991 (2H, *dd*, *J* 17.5/1.4 Hz, H-21A, B), 6.162 (1H, *br s*, H-22)

EI-MS: (70 ev) *m/z* (rel. int): 390 $[M]^+$, 372 $[M\text{-}H_2O]^+$, 354 $[M\text{-}2\times H_2O]^+$, 336 $[M\text{-}3\times H_2O]^+$, 318 $[C_{19}H_{26}O_4]^+$ (100), 300 $[C_{19}H_{24}O_3]^+$, 231, 219 $[C_{15}H_{23}O]^+$, 201 $[C_{15}H_{21}]^+$, 145 $[C_{11}H_{13}]^+$

HR-ESI-MS: $C_{23}H_{34}O_5Na$ calc. 413.22984 found. 413.22997

Compound 15: 17*β*-H-periplogenin-*β*-*D*-digitoxoside (mmk 044)

white powder



Yield: 5 mg, 0.0005%

mp.: 165-168 °C

$[\alpha]_D$: + 26.7 °(c = 0.132, MeOH)

TLC: R_f = 0.25 (system T_3, dark blue colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max}= 3420, 1780, 1740, 1635 cm^{-1}

UV: (MeOH), λ_{max} = 218 nm (log ε = 4.5)

CD: [MeOH, [mdeg] (nm)]: + 5.94 (238.4), – 3.39 (216.2)

^{1}H NMR: (500 MHz, C_5D_5N): δ 1.08-1.18 (2H, *m*, H-12), 1.127 (3H, *s*, H-19), 1.198 (3H, *s*, H-18), 1.30-1.58 (5H, *m*, H-7B, 11, 1B, 4B), 1.60-2.34 (12H, *m*, H-9, 4A, 6, 15, 2, 16, 1A, 7A), 1.602 (3H, *d*, *J* 6.2 Hz, H_3-6′), 1.988 (1H, *ddd*, *J* 13.2/9.6/2.6 Hz, H-2′*ax*), 2.405 (1H, *ddd*, *J* 13.2/3.6/1.9 Hz, H-2′*eq*), 3.438 (1H, *br dd*, *J* 9.6/9.6 Hz, H-17), 3.633 (1H, *m*, H-4′), 4.319 (1H, *dq* , *J* 9.4/6.2 Hz, H-5′), 4.426 (1H, *brs*, H-3), 4.439 (1H, H-3′), 4.829 (1H, *br d*, *J* 17.6 Hz, H-21B), 4.988 (1H, *dd*, *J* 17.6/1.8 Hz, H-21A), 5.489 (1H, *dd*, *J* 9.6/1.9 Hz, H-1′), 6.133 (1H, *br s*, H-22)

EI-MS: (70 ev) *m/z* (rel. int): 391 (2), 373(5), 355 (12), 318(40), 57 (100)

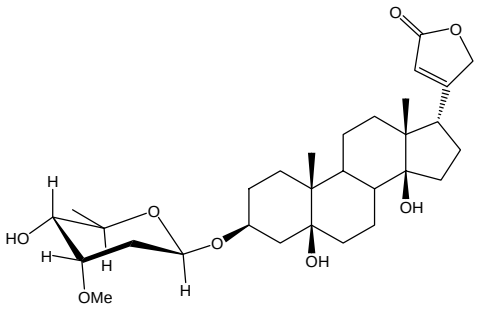
HR-ESI-MS: $C_{29}H_{44}O_8Na$ $[M+Na]^+$ calc. 543.29283 found. 543.29240

Compound 16: 17*β*-H-periplogenin-*β*-*D*-cymaroside (mmk 052)

white powder

Yield: 2 mg, 0.0002%

$[\alpha]_D$: + 18 ° (c = 0.065, MeOH)



TLC: R_f = 0.40 (system T_3, dark blue colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max}= 3450, 1740, 1620 cm^{-1}

UV: (MeOH), λ_{max} = 218 nm (log ε = 4.5)

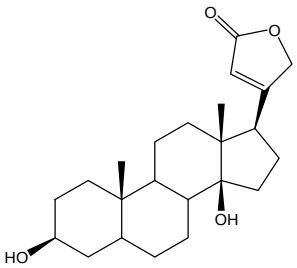
^{1}H NMR: (500 MHz, C_5D_5N): *δ* 1.08-1.18 (2H, *m*, H-12), 1.133 (3H, *s*, H-19), 1.201 (3H, *s*, H-18), 1.22-1.54 (4H, *m*, H-7B, 11, 1B), 1.54-2.34 (14H, *m*, H-9, 4, 6, 8, 15, 2, 16, 1A, 7A), 1.544 (3H, *d*, *J* 6.2 Hz, H_3-6′), 1.92-2.00 (1H, *m*, H-2′*ax*), 2.26-2.34 (1H, *m*, H-2′*eq*), 3.417 (3H, *s*, H-3′OMe), 3.447 (1H, *br dd*, *J* 9.6/9.6 Hz, H-17), 3.562 (1H, *m*, H-4′), 3.734 (1H, *d*, *J* 2.9 Hz , H-3′), 4.135 (1H, *dq*, *J* 9.4/6.2 Hz, H-5′), 4.424 (1H, *br s*, H-3), 4.829 (1H, *br d*, *J* 17.6 Hz, H-21B), 4.988 (1H, *dd*, *J* 17.6/1.8 Hz, H-21A), 5.186 (1H, *dd*, *J* 9.6/1.9 Hz, H-1′), 6.131 (1H, *br s*, H-22)

EI-MS: (70 ev) *m/z* (rel. int): 390 (3), 373 (10), 355 (55), 318 (50), 275 (7), 201 (10), 145 (50), 113 (100), 69 (48)

HR-ESI-MS: $C_{30}H_{46}O_8Na$ calc. 557.3084895 found. 557.30823

Compound 17: 17*α*-H-digitoxigenin (mmk 017) [lit. 100]

colourless amorphous powder



Yield: 17 mg, 0.0017%

$[\alpha]_D$: + 17.17 °(c = 0.08, MeOH)

TLC: R_f = 0.45 (system T_3, blue colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max}= 3420, 1780, 1755, 1635 cm^{-1}

UV: (MeOH), λ_{max} = 216 nm(log ε = 4.5)

^{1}H NMR: (300MHz, C_5D_5N): *δ* 0.99 (3H, *s*, H-19), 1.05 (3H, *s*, H-18), 1.16-2.2 (19H, *m*, H-2, 4, 5, 6, 7, 8, 9, 11, 12, 15, 16), 2.84 (1H, *m*, H-17), 4.42 (1H, *br s*, H-3), 5.06, 5.36 (2H, *dd*, *J* 18.1/1.4 Hz, H-21A, B), 6.15 (1H, *br s*, H-22)

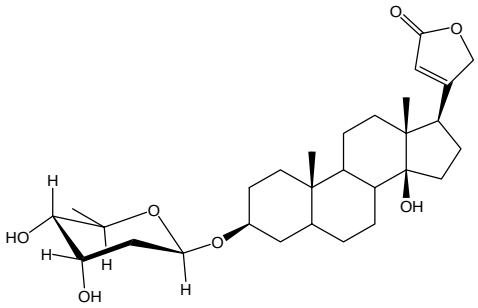
EI-MS: (70 ev) *m/z* (rel. int): 374 $[M]^+$ (7), 356 $[M\text{-}H_2O]^+$ (22), 338 $[M\text{-}2\times H_2O]^+$ (5), 246 $[C_{17}H_{26}O]^+$ (18), 203 $[C_{15}H_{23}]^+$ (100), 162 $[C_{12}H_{18}]^+$ (18), 147 $[C_{11}H_{15}]^+$

HR-ESI-MS: $C_{23}H_{34}O_4Na$ $[M+Na]^+$ calc. 397.23493 found. 397.23581

Compound 18: 17*α*-H-digitoxigenin-*β*-digitoxoside (mmk 015B) [lit. 23]

white powder

Yield: 1.5 mg, 0.00015%



mp.: 164-168 °C

TLC: R_f = 0.37 (system T_4, blue colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max}= 3450, 1740, 1620 cm^{-1}

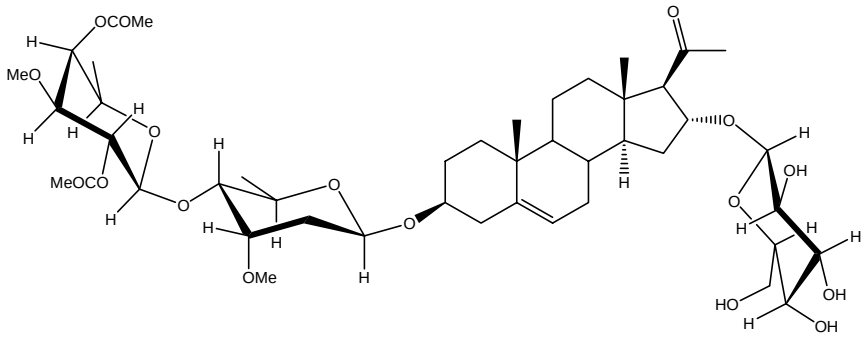
UV: (MeOH), λ_{max} = 218 nm(log ε = 4.2)

^{1}H NMR: (500 MHz, C_5D_5N): δ 0.899 (3H, *s*, H-19), 1.021 (3H, *s*, H-18), 1.16-1.62 (7H, *m*, H-2B, 11, 12, 1B, 4B), 1.634 (3H, *d*, *J* 6.3 Hz, H_3-6′), 1.64-2.22 (14H, *m*, H-2A, 1A, 9, 8, 5, 15, 16, 4A, 7, 2′B), 2.472 (1H, *m,* H-2′A), 2.805 (1H, *m*, H-17), 3.669 (1H, *dd, J* 2.4/9.3 Hz, H-4′), 4.368 (1H, *m*, H-5′), 4.335 (1H, *br s*, H-3), 4.498 (1H, *d*, *J* 2.9 Hz, H-3′), 5.063, 5.344 (2H, *dd*, *J* 18.1/1.4 Hz, H-21A, B), 5.494 (1H, *dd*, *J* 9.7/1.7 Hz, H-1′), 6.156 (1H, *br s*, H-22)

HR-ESI-MS: $C_{29}H_{44}O_7Na$ $[M+Na]^+$ calc. 527.29792 found. 527.29775

8.3.3. Pregnane glycosides

Compound 19: Δ^5-pregnene-3*β*,16*α*-diol-3-*O*-[2,4-*O*-diacetyl-*β*-digitalopyranosyl-(1-4)-*β*-*D*-cymaropyranoside]-16-*O*-[*β*-glucopyranoside] (mmk 056)



white amorphous solid

Yield: 6 mg., 0.0006%

mp.: 146-148 °C

$[\alpha]_D$: – 6.03 ° (c = 0.104, MeOH).

TLC: R_f = 0.24 (system T_3, pale green colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max}= 3417, 1735, 1715, 1680, 1100-1000, 840 cm^{-1}

CD: [MeOH, [mdeg] (nm)]: + 8.31 (286.0)

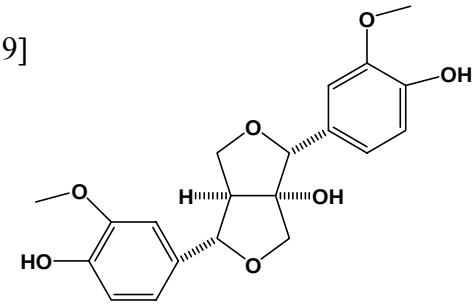
^{1}H NMR: (500 MHz, C_5D_5N): δ 0.626 (3H, *s*, H-18), 0.918 (3H, *s*, H-19), 1.329, 1.480 (3H, *d*, *J* 6.2 Hz, H_3-6′), 1.886 (1H, *m*, H-2′A), 1.934 (3H, *s*, H-4′′CO<u>Me</u>), 2.160 (3H, *s*, H-2′′CO<u>Me</u>), 2.322 (3H, *s*, H-21), 2.365(1H, *m*, H-2′B), 3.025 (1H, *br d*, *J* 6.1Hz, H-17), 3.433 (3H, *s*, H-3′′O<u>Me</u>), 3.526 (3H, *s*, H-3′O<u>Me</u>), 3.554 (1H, *dd*, *J* 9.2/2.6 Hz, H-4′), 3.707 (1H, *dd*, *J* 10.2/3.4 Hz, H-3′′), 3.798 (1H, *m*, H-3), 3.864 (1H, *m*, H-5′′′), 3.966 (1H, *m*, H-5′′), 4.041 (1H, *d*, *J* 3.4 Hz, H-3′), 4.053 (1H, *t*, *J* 8.1 Hz, H-2′′′), 4.233 (1H, *m*, H-5′), 4.274 (1H, *t*, *J* 9.0 Hz, H-3′′′), 4.310 (1H, *t*, *J* 9.1 Hz, H-4′′′), 4.395 (1H, *dd*, *J* 11.7/4.6 Hz, H-6′′′B), 4.487 (1H, *dd*, *J* 9.5/2.2 Hz, H-6′′′A), 4.806 (1H, *d*, *J* 7.8 Hz, H-1′′), 4.924 (1H, *d*, *J* 7.8 Hz, H-1′′′), 5.200 (1H, H-16), 5.271 (1H, *dd*, *J* 9.5/1.7 Hz, H-1′), 5.337 (1H, *d*, *J* 4.9 Hz, H-6), 5.586 (1H, *dd*, *J* 2.4/2.4 Hz, H-4′′), 5.612 (1H, *dd*, *J* 8.0/10.0 Hz, H-2′′)

EI-MS: (70 ev) *m/z* (rel. int): 314, 296, 281, 253, 245 (100), 213, 153, 145

HR-ESI-MS: $C_{45}H_{70}O_{17}Na$ $[M+Na]^+$ calc. 905.4505219 found. 905.4507690

8.3.4. Lignane

Compound 20: 8-hydroxy pinoresinol (mmk 042) [lit. 9]



colourless amorphous powder

Yield: 2 mg, 0.0002%

TLC: R_f = 0.51 (system T_3, blue colour with vanillin/H_2SO_4, inactive under UV)

UV: (MeOH), λ_{max} = 278, 230 nm

^{1}H NMR: (500 MHz, $CDCl_3$: CD_3OD): δ 3.115 (1H, *m*, H-8′), 3.820 (1H, *dd*, *J* 6/9 Hz, H-9′B), 3.903 (3H, *s*, H-3′OMe), 3.912 (3H, *s*, H-3 OMe), 3.907 (1H, *d*, *J* 4.7 Hz, H-9B), 4.046 (1H, *d*, *J* 9.2 Hz, H-9A), 4.511 (1H, *t*, *J* 8.7 Hz, H-9′A), 4.764 (1H, *br s*, H-7), 4.857 (1H, *d*, *J* 5.1 Hz, H-7′), 6.871 (4H, *m*, H-5, 5′, 6, 6′), 7.012 (2H, *s*, H-2, 2′)

EI-MS: (70 ev) *m/z* (rel. int): 374 (60), 237 (10), 222 (30), 207 (50), 165 (50), 137 (100), 131 (57)

HR-ESI-MS: $C_{20}H_{22}O_7Na$ $[M+Na]^+$ calc. 397.12577 found. 397.12653

9. Investigation of bioactive constituents from *Curcuma comosa* Rhizome

9.1. Plant material

The rhizome of *Curcuma comosa* Roxb. (Zingiberaceae) was collected in June 2002 at Yangon, Myanmar. The species was identified at the Department of Botany, University of Yangon.

9.2. Extraction and isolation

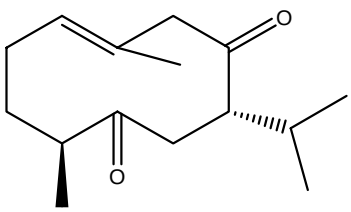
Dried powdered root of *C. comosa* (800 g) was extracted with 80% EtOH (1 L × 3) for one week and the organic solvent evaporated until only the water layer remained. The residue was successively partitioned between organic solvents (*n*-hexane, ethyl acetate, *n*-butanol 300 mL × 3 each) and water (see in scheme 1). The *n*-hexane fraction (11 g) was subjected to silica gel column chromatography by using vacuum and eluted with *n*-hexane, *n*-hexane : acetone (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 50:50). From the 10% acetone fraction, compound **22, 27, 28, 42,** and **43** were isolated using silica gel and RP-18 column chromatography (see scheme 5).

The ethyl acetate soluble fraction (20 g) was subjected to ordinary-phase silica gel column chromatography ($CHCl_3$, increasing polarity to $CHCl_3$: MeOH→ 9:1) to give 13 fractions. Fraction 2 (220 mg) was subjected to silicagel column chromatography (230-400 mesh, *n*-hexane : ethylacetate 6:1) to give known compounds **21, 23, 24, 44,** and **46**. Fraction 3 (1 g) was chromatographed on a silica gel column (230-400 mesh) using *n*-hexane-ethyl acetate solvent gradient (6:1, 5:1, 4:1 to ethyl acetate) to give compounds **25, 47, 48**. The unknown compound **25** was purified by preparative HPLC using an ODS column (solvent system MeCN : H_2O→ 20:80). Fraction 7 (1.5 g) was chromatographed on a silica gel column (230-400 mesh) using $CHCl_3$: MeOH to give 2 fractions. These fraction 7-1 and 7-2 were rechromatographed on silica gel (230-400 mesh) using *n*-hexane : ethyl acetate : methanol (1.95:1.25:0.1) and then on RP-18 column using MeOH : H_2O (6.5:3.5) and (8:2) to give compounds **26, 29, 40,** and **45**. The relative configuration of compound **26** was identified by X-ray crystallography. The isolation of the remaining compounds **31-33, 37-39, 41, 49** is shown in scheme 6. The *n*-butanol fraction of *C. comosa* (10 g) was fractionated on diaion HP 20 (250-850 μm) using successively water, methanol, acetone, and *n*-hexane as solvents (each 500 mL). The methanol fraction (1 g) was subjected to silica gel column chromatography (230-400 mesh) using $CHCl_3$: MeOH (20:1, 9:1) and was submitted to a RP-18 column chromatography (MeOH : H_2O, 5:5) to give compounds **34-36** (see in scheme 7).

9.3. Characterization of isolated compounds from the rhizome of *C. comosa*

9.3.1. Germacrane type sesquiterpenes

Compound 21: curdione (mmk 059) [lit. 55]



white powder

Yield: 300 mg, 0.04%

mp.: 47-49 °C

$[\alpha]_D$: + 216.10 ° (c = 1.6, MeOH)

TLC: R_f = 0.57 (system T_5, violet colour with vanillin/H_2SO_4, active under UV_{254})

IR: (KBr), ν_{max} = 1690, 1460, 1420, 1170, 1060 cm^{-1}

CD: [MeOH, [mdeg] (nm)]: +26655 (309)

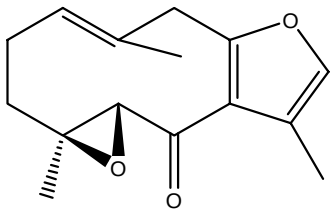
^{1}H NMR: (500 MHz, $CDCl_3$): δ 0.885 (3H, *d*, *J* 6.6 Hz, H-13), 0.951 (3H, *d*, *J* 6.7 Hz, H-12), 0.984 (3H, *d*, *J* 7.0 Hz, H-14), 1.58 (1H, *m*, H-3β), 1.657 (3H, *s*, H-15), 1.88 (1H, *m*, H-11), 2.11 (2H, *m*, H-2), 2.12 (1H, *m*, 3α), 2.34 (1H, *m*, H-4), 2.402 (1H, *dd*, *J* 16.6/2.2 Hz, H-6β), 2.71 (1H, *m*, H-6α), 2.851 (1H, *ddd*, *J* 8.8/8.8/2.2 Hz, H-7), 2.940 (1H, *d*, *J* 10.7 Hz, H-9β), 3.069 (1H, *d*, *J* 10.7 Hz, H-9α), 5.163 (1H, *m*, H-1)

EI-MS: (70 ev) *m/z* (rel. int): 236 [M^+]

GC-MS RT = 15.42 min, 236 [M^+], 180 (83), 167 (75), 109 (85), 69 (100)

HR-ESI-MS: $C_{15}H_{24}O_2$ calc. 236.1770 found. 236.1778

Compound 22: zederone (mmk 059b) [lit. 84]



white needles

Yield: 80 mg, 0.01%

mp.: 153-154 °C

$[\alpha]_D$: + 35.3 °(c = 0.085, MeOH).

TLC: R_f = 0.57 (system T_5, violet colour with vanillin/H_2SO_4, active under UV_{254})

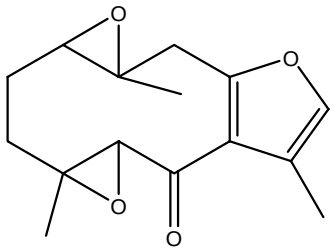
^{1}H NMR: (500 MHz, $CDCl_3$): δ 1.293 (1H, *ddd*, *J* 13.8/13.0/4.2 Hz, H-3β), 1.345 (3H, *d*, *J* 0.6 Hz, H-14), 1.605 (3H, *br s*, H-15), 2.116 (3H, *d*, *J* 1.3 Hz, H_3-13), 2.236 (1H, *m*, H-2β), 2.302 (1H, *ddd*, *J* 13.0/3.6/3.6 Hz, H-3α), 2.524 (1H, *dddd*, *J* 13.8/13.0/13.0/3.6 Hz, H-2α), 3.690 (1H, *br d*, *J* 16.4 Hz, H-9β), 3.757 (1H, *br d*, *J* 16.4 Hz, H-9α), 3.816 (1H, *s*, H-5), 5.485 (1H, *br d*, *J* 12.0 Hz, H-1), 7.090 (1H, *m,* H-12).

EI-MS: (70 ev) *m/z* (rel. int): 246 [M^+] (80), 175 (100), 231, 217, 203, 188, 137, 95, 81, 69

GC-MS: RT = 18.34 min, 246 $[M]^+$ (33), 188 (35), 176 (35), 175 (100), 161 (55), 119 (90), 91 (55), 43 (55)

HR-ESI-MS: $C_{15}H_{18}O_3Na$ $[M+Na]^+$ calc. 269.11481 found. 269.11435

Compound 23: 1a,5,7a-trimethyl-1a,6a,7a,8,9,9a-hexahydrobisoxireno [4,5:8,9]cyclodeca [1,2-*b*]furan-6(2*H*)-one (mmk 061e)



colourless amorphous

Yield: 1.5 mg, 0.00018%

mp.: 147-148 °C

$[\alpha]_D$: + 92.49 ° (c = 0.17, MeOH)

TLC: R_f = 0.81 (system T_7, reddish brown colour with vanillin/H_2SO_4, inactive under UV)

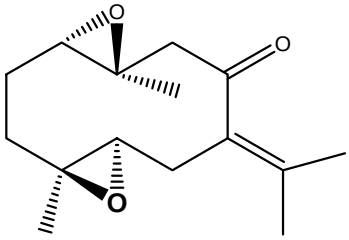
^{1}H NMR: (300 MHz, $CDCl_3$): δ 1.16 (3H, *s*), 1.34 (3H, *s*), 1.51-1.60 (3H, *m*), 2.187 (3H, *d*, *J* 1.3 Hz, H_3-13), 2.22 (1H, *m*), 2.41 (1H, *m*, H-3α), 2.83 (1H, *br d*, *J* 17.2 Hz, H-9*β*), 2.94 (1H, *dd*, *J* 10.5 Hz, H-1), 3.69 (1H, *br d*, *J* 16.7 Hz H-9*α*), 3.78 (1H, *s* , H-5), 7.108 (1H, *m,* H-12).

GC-MS: RT = 19.15 min, 262 $[M]^+$, 233 (18), 175 (35), 149 (40), 135 (40), 122 (87), 94 (65), 43 (100)

HR-ESI-MS: $C_{15}H_{18}O_4Na$ $[M+Na]^+$ calc. 285.10973 found. 285.10940

Compound 24: (1*S*, 10*S*), (4*S*, 5*S*)-germacrone-1(10), 4(5)-diepoxide (mmk 061c)

[lit. 24]



white powder

Yield: 5 mg, 0.00062%

mp.: 84-86 °C

$[\alpha]_D$: + 71.17 ° (c = 0.14, MeOH)

TLC: R_f = 0.31 (system T_5, grey colour with vanillin/H_2SO_4, active under UV_{254})

IR: (KBr), ν_{max} = 1678, 1645 cm^{-1}

UV: (MeOH), λ_{max} = 256 nm (log ε = 4.22), 315 (log ε = 2.30)

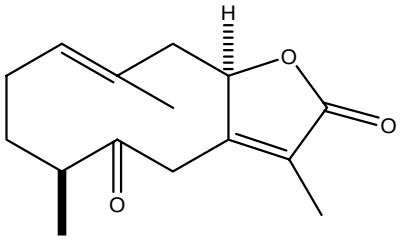
^{1}H NMR: (500 MHz, $CDCl_3$): δ 1.143 (3H, *s*, H_3-14), 1.26-1.32 (1H, *m*, H-3B), 1.444 (3H, *s*, H_3-15), 1.45-1.50 (1H, *m*, H-2B), 1.794 (3H, *s*, H_3-12), 1.862 (3H, *s*, H_3-13), 2.02-2.08 (1H, *m*, H-2A), 2.19-2.24 (1H, *m*, H-3A), 2.260 (1H, *dd*, *J* 14.2/10.8 Hz, H-6B), 2.644 (1H, *d*, *J* 10.8 Hz, H-9B), 2.652 (1H, *dd*, *J* 10.9/2.2

Hz H-5), 2.855 (1H, *dd*, *J* 14.2/2.2 Hz, H-6A), 2.918 (1H, *d, J* 10.8 Hz, H-1), 3.007 (1H, *J* 10.8 Hz, H-9A)

EI-MS: (70 ev) *m/z* (rel. int): 124.9 (100), 122 (80)

HR-ESI-MS: $C_{15}H_{22}O_3Na$ $[M+Na]^+$ calc. 273.14611 found. 273.14575

Compound 25: 3,6,10-trimethyl-7,8,11,11a-tetrahydrocyclodeca[*b*]furan-2,5(4*H*,6*H*)-dione (mmk 062bg)



colourless amorphous

Yield: 10 mg, 0.0012%

$[\alpha]_D$: + 35.2 °(c = 0.15, MeOH).

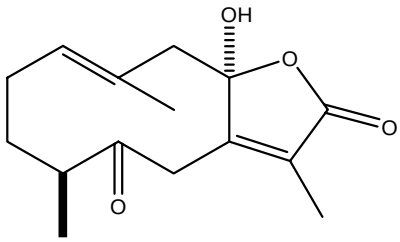
TLC: R_f = 0.20 (system T_5, reddish brown colour with vanillin/H_2SO_4, active under UV_{254})

HPLC: system 1p: R_t = 190,4 min

^{1}H NMR: (500 MHz, $CDCl_3$): δ 1.090 (3H, *d, J* 6.7 Hz, H_3-14), 1.753 (1H, *m*, H-3B), 1.822 (3H, *s*, H_3-15), 1.849 (3H, *s*, H_3-13), 2.048 (3H, *m*, H-2B, 3A, 9B), 2.188 (1H, *m*, H-2A), 2.425 (1H, *m*, H-4), 2.924 (1H, *brs*, H-9A), 3.336 (1H, *d*, *J* 15.9 Hz, H-6), 4.939 (1H, *br s*, H-8), 4.939 (1H, *br s*, H-1).

HR-ESI-MS: $C_{15}H_{20}O_3Na$ $[M+Na]^+$ calc. 271.13046 found. 271.13006

Compound 26: 11a-hydroxy-3,6,10-trimethyl-7,8,11,11a-etrahydrocyclodeca[*b*] furan-2,5(4*H*,6*H*)-dione - methane (mmk 074)



colourless platesheet

Yield: 20 mg, 0.025%

mp.: 147-150 °C

$[\alpha]_D$: + 79.7 °(c = 0.13, MeOH)

TLC: R_f = 0.14 (system T_5, reddish brown colour with vanillin/H_2SO_4, active under UV_{254})

HPLC: system 2p: R_t = 27.8min

CD: [MeOH, [mdeg] (nm)]: + 25.727 (284.2), – 76.970 (250), + 131.763 (224.2)

^{1}H NMR: (500 MHz, $CDCl_3$): δ 1.064 (3H, *d, J* 6.8 Hz, H_3-14), 1.70 (1H, *br d*, *J* 13.7 Hz, H-3B), 1.855 (3H, *s*, H_3-13), 1.933 (3H, *s*, H_3-15), 1.99-2.07 (2H, *m*, H-3A, 2B), 2.23 (1H, *m*, H-2A), 2.309 (1H, *d*, *J* 13.5 Hz, H-9B), 2.458 (1H, *m*, H-4), 2.933 (1H, *d*, *J* 13.4 Hz, H-9A), 3.303 (1H, *d*, *J* 15.7 Hz, H-6B), 3.579 (1H, *d*, *J* 15.4 Hz, H-6A), 4.878 (1H, *d*, *J* 10.7 Hz, H-1).

GC-MS: RT = 20.36 min, 264 $[M]^+$ (10), 246 $[M-H_20]^+$ (12), 121 (53), 82 (100)

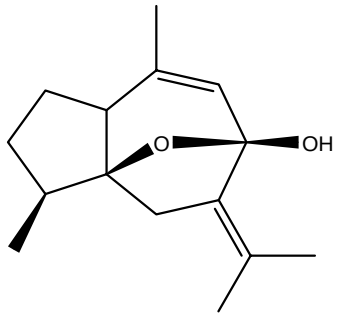
EI-MS: (70 ev) *m/z* (rel. int): 264 $[M]^+$, 246 $[M\text{-}H_20]^+$ (10), 182 (20), 126 (20), 82 (100), 69 (30)

HR-ESI-MS: $C_{15}H_{20}O_4Na$ $[M+Na]^+$ calc. 287.1253802 found. 287.12547

X-ray crystal data: $C_{30}H_{40}O_8$; M.W. = 528.62; orthorhombic; space group P212121; lattice constants: a = 9.4101(16), b = 10.1362(17), c = 29.556(8), Å, α = 90 °, β = 90 °, γ = 90 °, U = 2819.1(10) Å^3, Z = 4, D_{calc}= 1.245 Mg/m^3; Final R indices [I>2sigma(I)]: R1 = 0.0342, wR2 = 0.0717

9.3.2. Guaiane type sesquiterpenes

Compound 27: curcumenol (mmk 062) [lit. 18, 21]



colourless needles

Yield: 200 mg, 0.025%

mp.: 115-117 °C

$[\alpha]_D$: + 166.04 ° (c = 0.13, MeOH)

TLC: R_f = 0.39 (system T_5, reddish brown colour with vanillin/H_2SO_4, inactive under UV)

HPLC: system 3p: R_t = 24.7 min

IR: (KBr), ν_{max} = 3362, 3050,2917, 1670, 1655 cm^{-1}

CD: [MeOH, Δε (nm)]: – 800 (348), + 9000 (260), + 180000 (212)

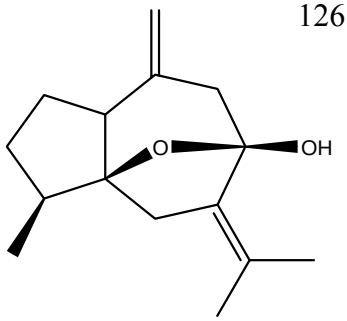
^{1}H NMR: (500 MHz, $CDCl_3$): δ 1.022 (3H, *d*, *J* 6.0 Hz, H_3-14), 1.54-2.00 (6H, *m*, H-1, 4, 2, 3), 1.593 (3H, *s*, H_3-13), 1.660 (3H, *s*, H_3-15), 1.813 (3H, *s*, H_3-12), 2.111 (1H, *br d*, *J* 15.4 Hz, H-6B), 2.657 (1H, *br d*, *J* 15.4 Hz, H-6A), 5.756 (1H, *br s*, H-9)

GC-MS: RT = 15.61 min, 234 $[M]^+$, 191 (40), 189 (68), 147 (66), 133 (80), 105 (100)

EI-MS: (70 ev) *m/z* (rel. int): 234 $[M]^+$

HR-ESI-MS: $C_{15}H_{22}O_2Na$ $[M+Na]^+$ calc. 257.15120 found. 257.15082

X-ray crystal data: $C_{15}H_{22}O_2$; M.W. = 234.33; monoclinic; space group P21; lattice constants: a = 9.405(2), b = 12.649(3), c = 11.865(3), Å, α = 90 °, β = 96.66(3) °, γ = 90 °, U = 1402.0(6) Å^3, Z = 4, D_{calc}= 1.110 Mg/m^3; Final R indices [I>2sigma(I)]: R1 = 0.0374, wR2 = 0.0867



Compound 28: isocurcumenol (**mmk 112)** [lit. 21]

colourless oil

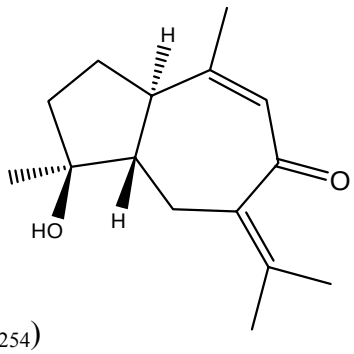
Yield: 2 mg, 0.0002%

IR: (KBr), ν_{max} = 3400, 2920, 1660, 1310, 1100, 980, 880 cm^{-1}

^1H NMR: (400 MHz, $CDCl_3$): δ 1.01 (3H, *d*, *J* 6.4 Hz, H_3-14), 1.5-1.6 (2H, *m*), 1.62 (3H, *s*), 1.65-1.8 (2H, *m*), 1.80 (3H, *s*), 1.9-2.0 (3H, *m*), 2.22 (1H, *t*, *J* 14 Hz), 2.5-2.6 (2H, *m*), 2.67 (1H, *d*, *J* 14 Hz), 2.83 (1H, *br s*), 4.73 (1H, *t*, *J* 2.1 Hz), 4.78 (1H, *t*, *J* 2.1 Hz).

EI-MS: (70 ev) *m/z* (rel. int): 234 $[M]^+$ (12), 219 (10), 216 (14), 201 (11), 191 (84), 173 (22), 147 (37), 133 (28), 121 (100), 105 (87)

HR-ESI-MS: $C_{15}H_{22}O_2Na$ $[M+Na]^+$ calc. 257.15122 found. 257.15084



Compound 29: procurcumenol (mmk 075) [lit. 40]

colourless oil

Yield: 3.5 mg, 0.00043%

$[\alpha]_D$: + 65.04 ° (c = 0.39, MeOH)

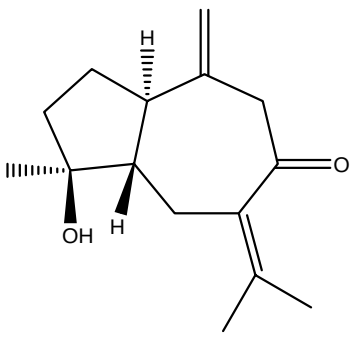
TLC: R_f = 0.48 (system T_6, reddish brown colour with vanillin/H_2SO_4, active under UV_{254})

IR: (KBr), ν_{max} = 3430, 1650, 1440, 1377 cm^{-1}

^1H NMR: (400 MHz, $CDCl_3$): δ 1.21 (3H, *s*), 1.72 (3H, *s*), 1.74 (3H, *s*), 1.86 (3H, *s*), 1.87-2.00 (*m*), 5.85 (1H, *m*, H-9)

GC-MS: RT = 18.13 min, 234 $[M]^+$(12), 216 $[M-H_2O]^+$(65), 123 (80), 43 (100)

HR-ESI-MS: $C_{15}H_{22}O_2Na$ $[M+Na]^+$ calc. 257.15120 found. 257.15120



Compound 30: isoprocurcumenol (mmk 124) [lit. 55]

colourless needles

mp.: 99-100 °C

Yield: 3 mg, 0.00037%

$[\alpha]_D$: – 70.02 ° (c = 0.24, MeOH)

TLC: R_f = 0.74 (system T_7, reddish brown colour with vanillin/H_2SO_4, active under UV_{254})

HPLC: system 4p: R_t = 14.87 min

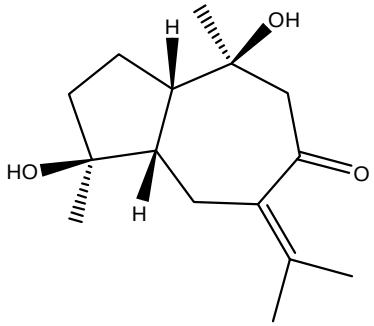
IR: (KBr), ν_{max} = 3450, 1674, 1610 cm^{-1}

CD: [MeOH, Δε (nm)]: – 4043 (321), + 4942 (248)

^{1}H NMR: (400 MHz, $CDCl_3$): δ 1.24 (3H, *s*, H_3-14), 1.82 (3H, *s*, H_3-13), 1.92 (3H, *s*, H_3-12), 1.40-2.0 (*m*), 2.82 (1H, *d*, *J* 14.5 Hz), 4.90 (2H, *d*, *J* 6.6 Hz, H_2-15)

HR-ESI-MS: $C_{15}H_{22}O_2Na$ $[M+Na]^+$ calc. 257.15120 found. 257.15120

Compound 31: isozedoarondiol (mmk 064) [lit. 56]



colorless needles

Yield: 10 mg, 0.0012%

mp.: 150-156 °C ($CHCl_3$)

$[\alpha]_D$: – 83.8 ° (c = 0.24, MeOH)

TLC: R_f = 0.29 (system T_6, reddish brown colour with vanillin/H_2SO_4, active under UV_{254})

IR: (KBr), ν_{max} = 3500, 3330, 1662, 1598, 1378, 1304, 1170 cm^{-1}

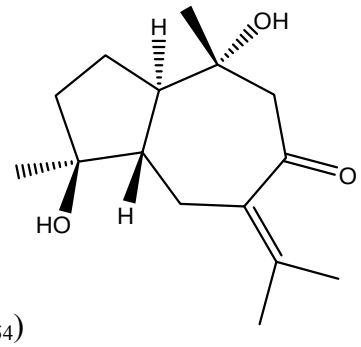
UV: (MeOH), λ_{max} = 252 nm (log ε = 3.94)

CD: [MeOH, Δε (nm)]: – 6323 (313)

^{1}H NMR: (500 MHz, $CDCl_3$): δ 1.22 (3H, *s*, H_3-14 or 15), 1.42 (3H, *s*, H_3-14 or 15), 1.87 (3H, *s*, H_3-12 or 13), 2.01 (3H, *s*, H_3-12 or 13), 1.48-1.84 (6H, *m*, H-2, 3, 1, 5), 2.41 (1H, *d*, *J* 16.0 Hz, H-9B), 2.51 (1H, *d*, *J* 14.0 Hz, H-6B), 2.83 (1H, *m*, H-6A), 3.23 (1H, *d*, *J* 16.0 Hz, H-9A)

HR-ESI-MS: $C_{15}H_{24}O_3Na$ $[M+Na]^+$ calc. 275.16176 found. 275.14028

Compound 32: zedoarondiol (mmk 065) [lit. 56]



colourless needles

Yield: 110 mg, 0.013%

mp.: 134 °C

$[\alpha]_D$: – 26.47 ° (c = 0.21, MeOH)

TLC: R_f = 0.31 (system T_6, reddish brown colour with vanillin/H_2SO_4, active under UV_{254})

IR: (KBr), ν_{max} = 3420, 2970, 1662, 1604 cm^{-1}

UV: λ_{max}(MeOH) nm (log ε): 258 (3.86)

CD: [MeOH, Δε (nm)]: $[\theta]_{313}$ –6468 (313)

^{1}H NMR: (500 MHz, $CDCl_3$): δ 1.175 (3H, *s*, H_3-15), 1.200 (3H, *s*, H_3-14), 1.34 (1H, *t*, *J* 11.0 Hz, H-5), 1.64-1.80 (4H, *m*, H-2, 3), 1.831 (3H, *s*, H_3-12), 1.921 (3H, *s*,

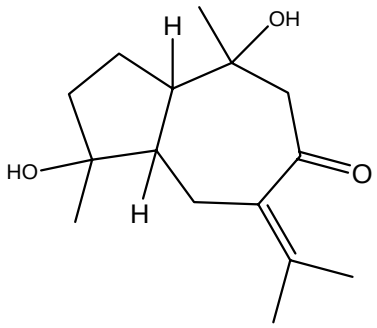
H_3-13), 1.93-2.02 (2H, *m*, H-6B, 1), 2.587 (1H, *d*, *J* 12.6 Hz, H-9B), 2.819 (1H, *d*, *J* 15.1 Hz, H-6A), 2.958 (1H, *d*, *J* 12.6 Hz, H-9A)

EI-MS: (70 ev) *m/z* (rel. int): 252 $[M]^+$ (5), 234 $[M-H_2O]^+$ (43), 216 $[M-2H_2O]^+$ (50), 201 (25), 191 (45), 173 (62), 145 (70), 131 (50), 119 (42), 104 (100)

GC-MS: RT = 16.18 min, 234 $[M-H_2O]^+$, 191 (40), 173 (15), 149 (25), 81 (55), 43 (100)

HR-ESI-MS: $C_{15}H_{24}O_3Na$ $[M+Na]^+$ calc. 275.16176 found. 275.14028

Compound 33: 1,4-dihydroxy-1,4-dimethyl-7-(1-methylethylidene)octahydroazulen-6(1*H*)-one -methane (mmk 090)



colourless amorphous

Yield: 2 mg, 0.00025%

HPLC: system 5p: R_t = 16.59 min

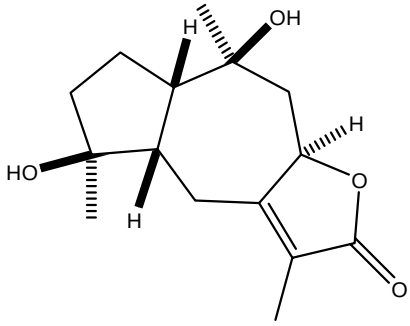
IR: (KBr), ν_{max} = 3420, 2970, 1662, 1604 cm^{-1}

UV: λ_{max}(EtOH) nm (log ε): 255 (3.5)

^{1}H NMR: (400 MHz, $CDCl_3$): δ 1.16 (3H, *s*, H_3-15), 1.26 (3H, *s*, H_3-14), 1.50-1.80 (*m*), 1.81 (3H, *s*, H_3-12 or 13), 1.89 (3H, *d*, H_3-12 or 13), 1.93-2.02 (*m*), 2.51 (1H, *d*, *J* 11.7 Hz, H-9B), 2.83 (1H, *d*, *J* 15.6 Hz, H-6A), 2.92 (1H, *d*, *J* 11.7 Hz, H-9A)

HR-ESI-MS: $C_{15}H_{24}O_3Na$ $[M+Na]^+$ calc. 275.16176 found. 275.14028

Compound 34: zedoalactone A (mmk 068) [lit. 96]



colourless oil

Yield: 37 mg, 0.0046%

$[\alpha]_D$: – 13,26 °(c = 0.1, MeOH)

TLC: R_f = 0.1 (system T_6, reddish brown colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max} = 3390, 2970, 1730, 1680 cm^{-1}

UV: (MeOH), λ_{max} = 223 nm (log ε = 4.00)

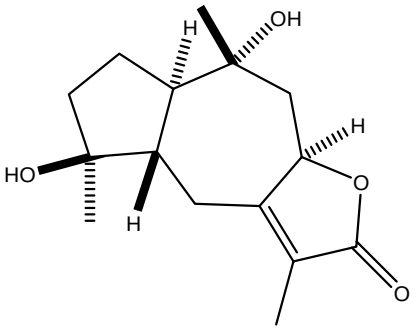
CD: (mdeg): – 7.899 (246.8 nm), + 0.8543 (224.6 nm), – 17.655 (203.6)

^{1}H NMR: (500 MHz, $CDCl_3$): δ 1.237 (3H, *s*, H_3-15), 1.392 (3H, *s*, H_3-14), 1.49 (1H, *m*, H-2B), 1.78 (1H, *m*, H-3B), 1.831 (3H, *d*, *J* 2.0 Hz, H_3-13), 1.85 (2H, *m*, H-2A, 6B), 2.004 (1H, *ddd*, *J* 13.3/ 6.6/3.7 Hz, H-5), 2.093 (1H, *ddd*, *J* 16.0/2.6/0.7 Hz, H-9B), 2.23 (1H, *m*, H-3A), 2.331 (1H, *dd*, *J* 16.0/6.9 Hz, H-9A), 2.71 (2H, *m*, H-1, 6A), 4.920 (1H, *ddq*, *J* 6.9/2.6/2.0 Hz, H-8)

EI-MS: (70 ev) *m/z* (rel. int): 248 $[M\text{-}H_2O]^+$ (17), 230 $[M\text{-}2H_2O]^+$ (33), 226 $[M\text{-}3H_2O]^+$ (100), 215 $[M\text{-}2H_2O\text{–}CH_3]^+$ (23), 201 (37), 187 (20)

HR-ESI-MS: $C_{15}H_{22}O_4Na$ $[M+Na]^+$ calc. 289.141030 found. 289.14139

Compound 35: 5,8-dihydroxy-3,5,8-trimethyl-4a,5,6,7,7a,8,9,9a-octahydroazuleno [6,5- *b*]furan-2(4*H*)-one (mmk 070)



colourless oil

Yield: 13 mg, 0.0016%

$[\alpha]_D$: + 18.60 °(c = 0.164, MeOH)

TLC: R_f = 0.17 (system T_6, reddish brown colour with vanillin/H_2SO_4, inactive under UV)

IR: (KBr), ν_{max} = 3390, 2970, 1730, 1680 cm^{-1}

UV: (MeOH), λ_{max} = 223 nm (log ε = 4.00)

CD: [MeOH, [θ] (nm)]: – 3.623 (228.2)

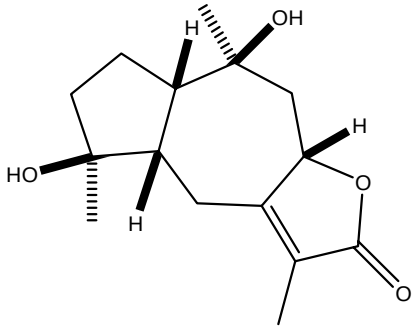
^1H NMR: (500 MHz, $CDCl_3$): δ 1.245 (3H, *s,* H_3-15), 1.281 (3H, *s*, H_3-14), 1.575 (1H, *ddd*, *J* 13.0/9.0/2.8 Hz, H-5), 1.72-1.80 (4H, *m*, H_2-2,3), 1.755 (1H, *dd*, *J* 14.7/11.3 Hz, H-9β), 1.813 (3H, *dd*, H_3-13), 1.971 (1H, *m*, H-1), 2.058 (1H, *dd*, *J* 14.7/13.3 Hz, H-6β), 2.307 (1H, *dd*, *J* 14.7/2.7 Hz, H-9α), 2.997 (1H, *dd*, *J* 15.1/2.8 Hz, H-6α), 5.133 (1H, *d*, *J* 11.2 Hz, H-8).

EI-MS: (70 ev) *m/z* (rel. int): 248 $[M\text{-}H_20]^+$ (100), 230 $[M\text{-}2 \times H_20]^+$, 215 $[M\text{-}2 \times H_20\text{-}CH_3]^+$, 205 (48), 190 (70), 175 (20), 107 (20)

GC-MS: RT = 21.93 min, 248 $[M\text{-}H_2O]^+$ (40), 230 $[M\text{-}2 \times H_20]^+$ (25), 215 $[M\text{-}2 \times H_20\text{-}CH_3]^+$ (10), 205 (25), 190 (45), 175 (10), 107 (15)

HR-ESI-MS**:** $C_{15}H_{22}O_4Na$ $[M+Na]^+$ calc. 289.141030 found. 289.14139

Compound 36: 5,8-dihydroxy-3,5,8-trimethyl-4a,5,6,7,7a,8,9,9a-octahydroazuleno [6,5-*b*]furan-2(4*H*)-one (mmk 069)



colourless oil

Yield: 13 mg, 0.0016%

$[\alpha]_D$: + 46.8 ° (c = 0.15, MeOH)

TLC: R_f = 0.17 (system T_6, reddish brown colour with vanillin/H_2SO_4, inactive under UV)

HPLC: system 6p: R_t = 39.38 min

IR: (KBr), ν_{max} = 3390, 2970, 1730, 1680 cm^{-1}

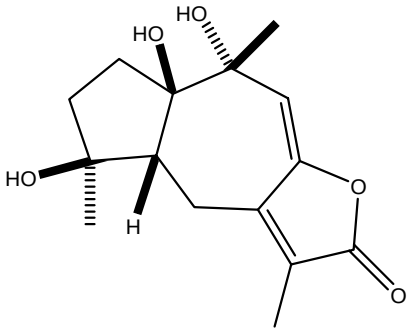
UV: (MeOH), λ_{max} = 223 nm (log ε = 4.00)

^{1}H NMR: (500 MHz, $CDCl_3$): δ 1.317 (3H, *s*, H_3-15), 1.396 (3H, *s*, H_3-14), 1.678 (1H, *dd*, *J* 13.7/11.7 Hz, H-9B), 1.64-1.78 (2H, *m*, H_2-3), 1.790 (3H, *d*, *J* 1.8 Hz, H_3-13), 2.23 (2H, *m*, H-5, 6B), 2.281 (1H, *ddd*, *J* 13.7/3.4/1.7 Hz, H-9A), 2.719 (1H, *m*, H-6A), 2.856 (1H, *dddd*, *J* 12.3/7.9/5.1/1.4 Hz, H-1), 5.279 (1H, *dqd*, *J* 11.7/1.8/1.7 Hz, H-8)

EI-MS: (70 ev) *m/z* (rel. int): 266 $[M]^+$, 248 $[M\text{-}H_20]^+$ (100), 230 $[M\text{-}2 \times H_20]^+$ (80), 205 (40), 190 (95), 175 (30), 107 (20)

HR-ESI-MS**:** $C_{15}H_{22}O_4Na$ $[M+Na]^+$ calc. 289.141030 found. 289.14139

Compound 37: zedoalactone B (mmk 092) [lit. 96]



colourless oil

Yield: 23 mg, 0.0028%

$[\alpha]_D$: + 180.2 ° (c = 0.4, MeOH)

TLC: R_f = 0.17 (system T_6, blue colour with vanillin/H_2SO_4, active under UV_{254})

HPLC: system 7p: R_t = 32.7 min

IR: (KBr), ν_{max} = 3400, 2970, 2940, 2880, 1740, 1660, 1630 cm^{-1}

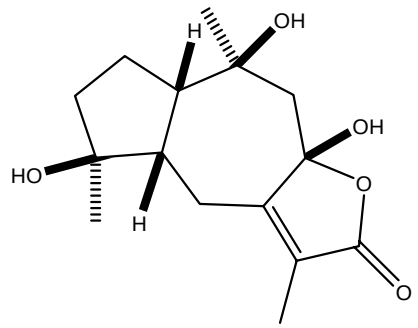
UV: (MeOH), λ_{max}= 273 nm (log ε = 4.33)

^{1}H NMR: (400 MHz, C_5D_5N): δ 1.71 (3H, *br s*, H_3-13), 1.75 (3H, *br s*, H_3-14), 1.90 (3H, *s*, H_3-15), 2.06 (1H, *ddd*, *J* 8.0/11.5/13.1 Hz, H-2α), 2.15 (1H, *ddd*, *J* 2.0/8.0/11.5 Hz, H-3α), 2.41 (1H, *ddd*, *J* 9.0/11.5/11.5 Hz, H-3β), 3.08 (1H, *ddd*, *J* 1.5/3.0/17.4 Hz, H-6β), 3.10 (1H, *ddd*, *J* 2.0/9.0/13.1 Hz, H-2β), 3.21 (1H, *ddd*, *J* 1.5/12.8/17.4 Hz, H-6α), 3.35 (1H, *dd*, *J* 3.0/12.8 Hz, H-5), 6.09 (1H, *s*, H-9α)

EI-MS: (70 ev) *m/z* (rel. int): 262 $[M\text{-}H_2O]^+$ (10), 244 $[M\text{-}2H_2O]^+$ (30), 226 $[M\text{-}3H_2O]^+$ (100), 211 $[M\text{-}3H_2O\text{–}CH_3]^+$ (50)

HR-ESI-MS: $C_{15}H_{18}O_4$ $[M\text{-}H_2O]^+$ calc. 262.1205 found. 262.1195

Compound 38: zedoarolide B (mmk 094-3) [lit. 63]



colourless oil

Yield: 5 mg, 0.00062%

$[\alpha]_D$: – 20.6 ° (c = 1.80, MeOH)

TLC: R_f = 0.31 (system T_7, yellow colour with vanillin/H_2SO_4, active under UV_{254})

HPLC: system 7p: R_t = 50.4 min

IR: (KBr), ν_{max} = 3475, 2940, 1719, 1686, 1000 cm^{-1}

UV: (MeOH), λ_{max} = 223 nm (log ε = 3.82)

CD: [MeOH, Δε (nm)]: + 1.76 (226), – 3.64 (247)

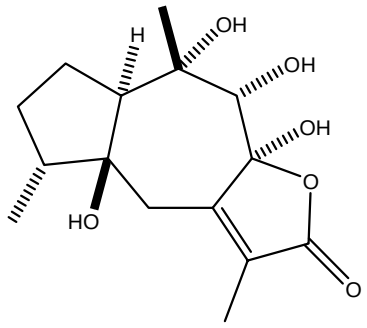
^{1}H NMR: (400 MHz, C_5D_5N): δ 1.44 (3H, *s*, H_3-14), 1.58 (3H, *s*, H_3-15), 1.79 (1H, *m*, H-2α), 1.81 (3H, *s*, H_3-13), 1.97 (1H, *m*, H-3α), 1.98 (1H, *m*, H-2β), 2.08 (1H, *m*, H-3β), 2.43 (1H, *dd*, *J* 12.8/12.8 Hz, H-6β), 2.64 (1H, *ddd*, *J* 3.7/3.7/12.8 Hz, H-5), 2.80, 2.86 (2H, *ABq*, *J* 15.5 Hz, H-9β, 9α), 2.82 (1H, *dd*, *J* 3.7/12.8 Hz, H-6α), 3.38 (1H, *ddd*, *J* 3.7/7.6/7.6 Hz, H-1)

EI-MS: (70 ev) *m/z* (rel. int): 264 $[M-H_2O]^+$ (70), 246 $[M-2H_2O]^+$ (100), 228 $[M-3H_2O]^+$ (20)

HR-ESI-MS**:** $C_{15}H_{23}O_5$ $[M+H]^+$ calc. 283.1546 found. 283.1530

Compound 39: 4a,8,9,9a-tetrahydroxy-3,5,8-trimethyl-4a,5,6,7,7a,8,9,9a-octahydroazuleno[6,5-*b*]furan-2(4*H*)-one (mmk 108)

colourless oil



Yield: 2 mg, 0.00025%

TLC: R_f = 0.69 (system T_7, reddish brown colour with vanillin/H_2SO_4, active under UV_{254})

HPLC: system 8a: R_t = 25.5 min

CD: [MeOH, Δε (nm)]: + 14.601 (242.6), – 21.636 (216.2), + 3.099 (195.2)

^{1}H NMR: (500 MHz, C_5D_5N): δ 0.712 (3H, *d*, *J* 7.3 Hz, H_3-14), 1.430 (1H, *m*, H-3B), 1.471 (3H, *s*, H_3-15), 1.767 (1H, *m*, H_2-2), 1.874 (3H, *d*, *J* 1.7 Hz, H_3-13), 2.045 (1H, *qd*, *J* 7.3/6.8 Hz, H-4), 2.356 (1H, *dddd*, *J* 11.4/11.4/10.7/6.8 Hz, H-3A), 2.870 (1H, *dq*, *J* 15.6/1.7 Hz, H-6B), 3.280 (1H, *d*, *J* 15.6 Hz, H-6A), 3.740 (1H, *dd*, *J* 5.0/3.8 Hz, H-1), 3.990 (1H, *s*, H-9)

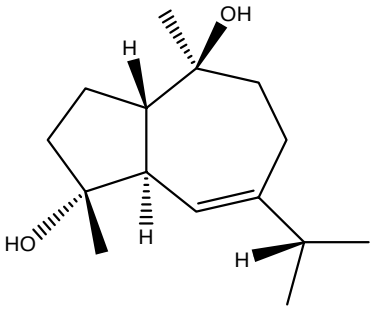
EI-MS: (70 ev) *m/z* (rel. int): 280 $[M-H_2O]^+$ (2), 262 $[M-2H_2O]^+$ (20), 244 $[M-3H_2O]^+$ (15), 219 (90), 201 (100)

HR-ESI-MS**:** $C_{15}H_{20}O_5Na$ $[M–H_2O+ Na]^+$ calc. 303.12 found. 303.12047

Compound 40: alismoxide (mmk 072) [lit. 107]

colourless prisms

Yield: 4 mg, 0.0005%



mp.: 142-144 °C

$[\alpha]_D$: – 2.8 ° (c = 0.18, MeOH)

TLC: R_f = 0.46 (system T_6, reddish brown colour with vanillin/H_2SO_4, active under UV_{254})

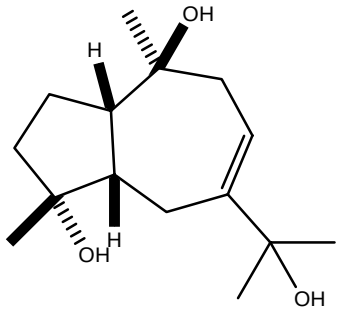
IR: (nujol), ν_{max} = 3280, 1655 cm^{-1}

^1H NMR: (500 MHz, $CDCl_3$): δ 0.972 (3H, *d*, *J* 3.5 Hz, H_3-13), 0.989 (3H, *d, J* 3.9 Hz, H_3-12), 1.216 (3H, *s*, H_3-14), 1.273 (3H, *s,* H_3-15), 1.42-1.48 (1H, *m*, H-9B), 1.58-1.70 (3H, *m*, H_2-3, 2B), 1.74-1.96 (4H, *m*, H-2A, 9A, 1, 8B), 2.14-2.26 (3H, *m*, H-8B, 5, 11), 5.505 (1H, *br s*, H-6).

GC-MS: 220 $[M-H_2O]^+$ (12), 205 (12), 162 (77), 159 (33), 147 (45), 134 (40), 119 (65), 107 (41), 93 (48), 43 (100)

EI-MS: (70 ev) *m/z* (rel. int): 220 $[M-H_2O]^+$

Compound 41: 7-(1-hydroxy-1-methylethyl)-1,4-dimethyl-1,2,3,3a,4,5,8,8a-octahydroazulene-1,4-diol (mmk 095)



colourless oil

Yield: 5 mg, 0.0006%

TLC: R_f = 0.21 (system T_5, blue colour with vanillin/H_2SO_4, active under UV_{254})

CD: [MeOH, Δε (nm)]: 6.602 (202.4)

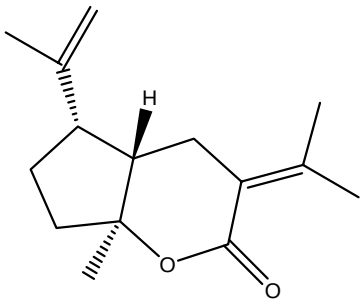
^1H NMR: (500 MHz, C_5D_5N): δ 1.361 (3H, *s,* H_3-15), 1.568 (6H, *s,* H_3-12, 13), 1.624 (3H, *s*, H_3-14), 1.775 (1H, *m,* H-2 *ax*), 1.845 (1H, *m*, H-3 *ax*), 1.957 (1H, *m*, H-2 *eq*), 2.020 (1H, *m*, H-3 *eq*), 2.152 (1H, *dd*, *J* 13.9/12.8 Hz, H-6 *ax*), 2.268 (1H, *dd*, *J* 14.2/8.4 Hz, H-9 B), 2.414 (1H, *dd*, *J* 12.8/4.9 Hz, H-5), 2.523 (1H, *d*, *J* 13.9 Hz, H-6 *eq*), 2.780 (1H, *dd*, *J* 14.2/5.2 Hz, H-9A), 3.483 (1H, *m*, H-1), 6.157 (1H, *dd*, *J* 8.4/5.2 Hz, H-8)

GC-MS: RT = 17.71 min, 236 (3), 218 (11), 203 (10), 175 (72), 133 (66), 81 (40), 43 (100)

EI-MS: (70ev) *m/z* (rel. int): 236 $[M–H_2O]^+$ (5), 218 $[M–2H_2O]^+$ (20), 203 (15), 175 (100), 160 (30), 133 (60), 81 (30)

HR-ESI-MS: $C_{15}H_{26}O_3Na$ $[M+Na]^+$ calc. 277.17741 found. 277.17702

Compound 42: gajutsulactone B (mmk 118) [lit. 63]



colourless oil

Yield: 5 mg, 0.0006%

$[\alpha]_D$: - 35.0 ° (c= 0.10, $CHCl_3$)

IR: (film), ν_{max} = 2973, 1713, 1646, 1620, 1067 cm^{-1}

UV: (MeOH), λ_{max} = 232 nm (log ε = 4.37)

CD: [MeOH, Δε (nm)]: –3.03 (239)

^{1}H NMR: (500 MHz, $CDCl_3$): δ 1.217 (3H, *s*, H_3-15), 1.783 (3H, *s*, H_3-14), 1.856 (3H, *s*, H_3-13), 1.87-2.01 (4H, *m,* H_2-2,3), 2.183 (3H, *s,* H_3-12), 2.24 (1H, *d* , H-6B), 2.30 (1H, *m*, H-5), 2.50 (1H, *d*, H-6A), 2.88 (1H, *ddd*, *J* 6.4/6.4/9.8 Hz, H-1), 4.84, 5.01 (1H each, both *br s*, H_2-9).

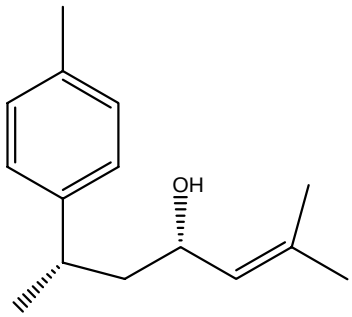
GC-MS: RT = 17.50 min, 234 $[M]^+$ (3), 219 $[M–CH_3]^+$ (15), 191 (65), 107 (100)

EI-MS: (70ev) *m/z* (rel. int): 234 $[M]^+$ (5), 107 (100)

HR-ESI-MS: $C_{15}H_{22}O_2Na$ $[M+Na]^+$ calc. 257.15120 found. 257.15119

9.3.3. Bisaborane type

Compound 43: bisacumol (mmk 119) [lit. 59]



colourless oil

Yield: 6 mg, 0.0007%

$[\alpha]_D$: + 14.5 ° (c = 0.50, EtOH)

IR: (CCl_4), ν_{max} = 3630 cm^{-1}

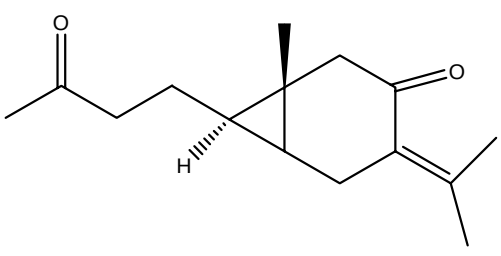
^{1}H NMR: (400 MHz, $CDCl_3$): δ 1.23 (3H, *d, J* 7.1 Hz, H_3-14), 1.53 (3H, *d*, *J* 1.5 Hz, H_3-13), 1.67 (3H, *d*, *J* 1.2 Hz, H_3-12), 1.78-1.86 (2H, *m*), 2.32 (3H, *s*, H_3-15), 2.86 (1H, *m*), 4.17 (1H, *m*), 5.16 (1H, *d*, *J* 9.2 Hz, H-10)

GC-MS: RT = 14.28 min, 218 $[M]^+$ (25), 200 $[M–H_2O]^+$ (20), 157 (20), 119 (100), 85 (98)

EI-MS: (70 ev) *m/z* (rel. int): 218, 203, 200, 185, 157, 119, 85

9.3.4. Carabrane type

Compound 44: curcumenone (mmk 061b) [lit. 56]



colourless needles

Yield: 50 mg, 0.0062%

mp.: 28 °C

$[\alpha]_D$: – 7.83 ° (c = 0.14, MeOH)

TLC: R_f = 0.37 (system T_5, reddish brown colour with vanillin/H_2SO_4, active under UV_{254})

IR: (KBr), ν_{max} = 1718, 1675, 1600, 1360, 1170 cm^{-1}

UV: (MeOH), λ_{max} = 234 nm (log ε = 3.92)

CD: [MeOH, Δε (nm)]: $[\theta]_{314}$ +1884

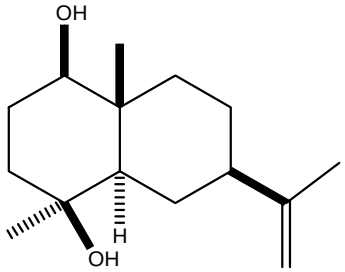
^{1}H NMR: (300 MHz, $CDCl_3$): δ 0.45 (1H, *dt*, *J* 7.3/4.4 Hz, H-1), 0.67 (1H, *q*, *J* 4.4 Hz, H-5H), 1.12 (3H, *s*, H-15), 1.60 (2H, *q*, *J* 7.3 Hz, H-2), 1.79 (3H, *br s*, H-13), 2.09 (3H, *br s*, H-12), 2.13 (3H, *s*, H-14), 2.47 (2H, *t*, *J* 7.3 Hz, H-3), 2.52 (1H, *d*, *J* 15.6 Hz, H-9B), 2.55 (1H, *d*, *J* 15.6 Hz, H-9A), 2.81 (2H, *m*, H-6)

EI-MS: (70 ev) *m/z* (rel. int): 234 (29), 219 (13), 191 (17), 176 (60), 167 (32), 163 (31),161 (32), 149 (40), 121 (33), 107 (30), 68 (100), 67 (48), 43 (65)

HR-ESI-MS: $C_{15}H_{22}O_2$ Na $[M+Na]^+$ calc. 257.15120 found. 257.15120

9.3.5. Eudesmane type sesquiterpene

Compound 45: 7-isopropenyl-1,4a-dimethyldecahydronaphthalene-1,4-diol (mmk 076)



colourless oil

Yield: 6 mg, 0.00075%

$[\alpha]_D$: – 4.8 ° (c = 0.097, MeOH)

TLC: R_f = 0.69 (system T_7, blue colour with vanillin/H_2SO_4, active under UV_{254})

IR: (film), ν_{max} = 3389, 1644, 1385, 1169, 1075, 890 cm^{-1}

^{1}H NMR: (500 MHz, $CDCl_3$): δ 1.051 (3H, *s,* H_3-15), 1.071 (1H, *dd*, *J* 12.4/2.6 Hz H-5), 1.108 (1H, *dd*, *J* 13.2/3.7 Hz, H-9α), 1.160 (3H, *s*, H-14), 1.450 (1H, *m*, H-8β), 1.450 (1H, *m*, H-6β), 1.533 (1H, *ddd*, *J* 14.1/14.1/4.5 Hz, H-3α), 1.620 (1H, *m*, H-8α), 1.620 (1H, *m*, H-2β), 1.682 (1H, *m*, H-6α), 1.720 (1H, *m*, H-3β), 1.755 (3H, *s*, H_3-12), 1.874 (1H, *m*, H-2α), 1.874 (1H, *m*, H-9β), 1.938 (1H, *m*, H-7), 3.266 (1H, *dd*, *J* 12.7/4.2 Hz, H-1), 4.739 (1H, H-13 *Z*), 4.713 (1H, H-13 *E*).

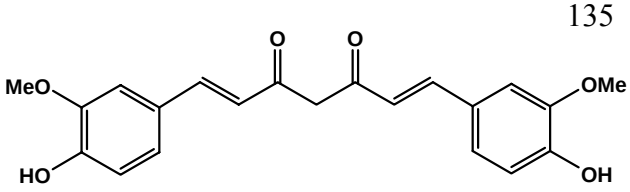
GC-MS: RT = 16.91min, 238 $[M]^+$ (5), 220 $[M–H_2O]^+$ (27), 162 (30), 121 (32), 43 (100)

HR-ESI-MS: $C_{15}H_{25}O_2$ $[M–H]^–$ calc. 237.18490 found. 237.18483

9.3.6. Diarylheptanoids

Compound 46: curcumin (mmk 063) [lit. 49]

yellow needles



Yield: 7 mg, 0.00087%

mp.: 186-188 °C

IR: (KBr), ν_{max} = 3447, 2925, 2850, 1627, 1602, 1510, 1458, 1429, 1283, 1155, 963 cm^{-1}

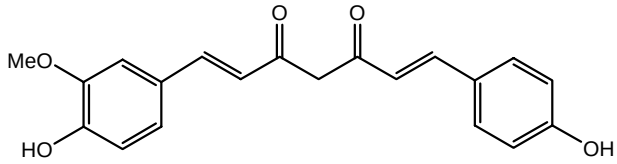
UV: (MeOH), λ_{max} = 260, 425 nm (log ε = 3.5, 5.5)

^{1}H NMR: (400 MHz, DMSO-d_6): δ 3.92 (6H, *s*, H_3-3′, 3′′OMe), 5.86 (1H, *s*, H-4), 6.50 (2H, *d*, *J* 15.6 Hz, H-2, 6), 6.90 (2H, *d*, *J* 8.0 Hz, H-5′, 5′′), 7.07 (2H, *dd*, *J* 8.0/1.5 Hz, H-6′, 6′′), 7.09 (2H, *d*, *J* 1.5 Hz, H-2′, 2′′), 7.55 (2H, *d*, *J* 15.6 Hz, H-1, 7). (400 MHz, C_5D_5N): δ 3.78 (6H, *s*, H_3-3′, 3′′OMe), 6.14 (1H, *s*, H-4), 6.95 (2H, *d*, *J* 16.0 Hz, H-2, 6), 7.24 (2H, *d*, *J* 8.0 Hz, H-5′, 5′′), 7.32 (2H, *dd*, *J* 8.2 Hz, H-6′, 6′′), 7.38 (2H, *s* , H-2′, 2′′), 8.02 (2H, *d*, *J* 16.0 Hz, H-1, 7).

EI-MS: (70 ev) *m/z* (rel. int): 368 $[M]^+$ (50), 350 (70), 335 (10), 272 (20), 190 (90), 77 (100)

Compound 47: demethoxycurcumin (mmk 063-b) [lit. 49]

orange crystaline powder



Yield: 2 mg, 0.00025%

mp.: 177-179 °C

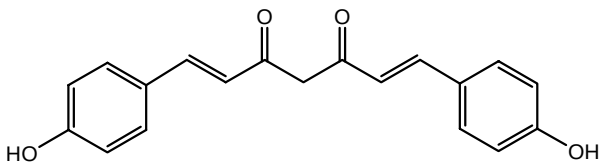
IR: (KBr), ν_{max} = 3447, 2925, 2850, 1627, 1602, 1510, 1458, 1429, 1283, 1155, 963 cm^{-1}

UV: (MeOH), λ_{max} = 250, 419 nm (log ε = 3.5, 5.1)

^{1}H NMR: (300 MHz, $CDCl_3$): δ 3.95 (3H, *s*, H_3-3′OMe), 5.79 (1H, *s*, H-4), 6.49 (2H, *d*, *J* 15.8 Hz, H-2,6), 6.86 (2H, *d*, *J* 8.6Hz, H-3′′, 5′′), 6.93 (1H, *d*, *J* 8.0 Hz, H-5′), 7.12 (1H, *d* , *J* 1.5 Hz, H-2′), 7.12 (1H, *dd* , *J* 8.0/1.5 Hz, H-6′), 7.47 (2H, *d*, *J* 8.6 Hz, H-2′′, 6′′), 7.59 (1H, *d*, *J* 15.6 Hz, H-1), 7.61 (1H, *d*, *J* 15.6 Hz, H-7)

Compound 48: bisdemethoxycurcumin (**mmk 063-c)** [lit. 49]

orange crystaline powder



Yield: 2 mg, 0.00025%

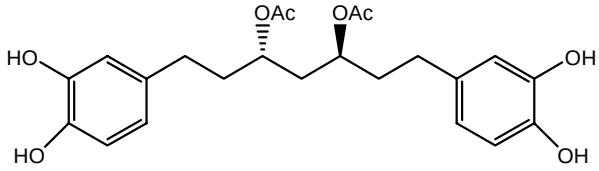
mp.: 177-179 °C

UV: (MeOH), λ_{max} = 245, 415 nm (log ε = 4.3, 5.5)

^{1}H NMR: (300 MHz, $CDCl_3$): δ 5.98 (1H, *s*, H-4), 6.66 (2H, *d*, *J* 16.0 Hz), 6.91 (4H, *d*, *J* 8.0 Hz), 7.57 (4H, *d*, *J* 8.0 Hz), 7.61 (2H, *d* , *J* 16.0 Hz).

Compound 49: (3*S*, 5*S*)-3,5-diacetoxy-1,7-bis(3,4-dihydroxyphenyl)heptane (mmk 093)

[lit. 48]



yellow oil

Yield: 6 mg, 0.00075%

$[\alpha]_D$: + 3 ° (c = 1.3, EtOH)

TLC: R_f = 0.1 (system T_7, reddish brown colour with vanillin/H_2SO_4, active under UV_{254})

IR: (film), ν_{max} = 3584, 1740, 1608, 1520 cm^{-1}

UV: (EtOH), λ_{max} = 283 nm (log ε = 3.70)

^{1}H NMR: (300 MHz, $CDCl_3$): δ 1.74 (2H, *dddd, J* 5/7/9/14 Hz, 2A, 6A), 1.79 (2H, *t*, *J* 7 Hz, H_2-4), 1.83 (2H, *tdd*, *J* 7/9/14 Hz, H-2B, 6B), 2.00 (6H, *s*, H_3-3, 5 OAc), 2.45 (2H, *ddd, J* 7/9/14 Hz, H-1A, 7A), 2.51 (2H, *ddd, J* 7/9/14 Hz, H-1B, 7B), 4.94 (2H, *q*, *J* 6 Hz, H-3, 5), 6.54 (2H, *dd*, *J* 2/8 Hz, H-6′, 6″), 6.68 (2H, *d*, *J* 2 Hz, H-2′, 2″), 6.77 (2H, *d*, *J* 8 Hz, H-5′, 5″)

EI-MS: (70 ev) *m/z* (rel. int): 432 $[M]^+$ (13), 372 $[M–HOAc]^+$ (13), 312 $[372–HOAc]^+$ (10), 189 (15), 176 (17), 149 (36), 136 (14), 123 (100)

HR-ESI-MS: $C_{23}H_{28}O_8Na$ $[M+Na]^+$ calc. 455.16763 found. 455.16777

10. Investigation of bioactive constituents from *Vitis repens* rhizome

10.1. Plant material

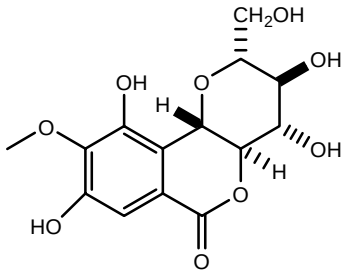
The rhizome of *Vitis repens* Wight & Arm. (Vitaceae) was collected in June 2002 at Taung Gyi, Shan Division, Myanmar. The species was identified at the Department of Botany, University of Yangon.

10.2. Extraction and isolation

Dried powdered rhizomes of *Vitis repens* (800 g) were extracted with 80% EtOH (1 L × 3) for one week and the organic solvent evaporated until only the water layer remained. The residue was successively partitioned between organic solvents (*n*-hexane, ethyl acetate, *n*-butanol 300 mL each × 3) and water. The ethyl acetate fraction and *n*-butanol fraction was fractioned further on silica gel, RP-18, diaion HP 20 (250-850 μm) and purified by HPLC to give compounds **50-58** (see schemes 8, 9).

10.3. Characterization of isolated compounds from the rhizome of *V. repens*

Compound 50: bergenin (mmk 077) [lit. 106]



colourless prisms

Yield: 200 mg, 0.020%

mp.: 137-139 °C

$[\alpha]_D$: – 37.25 ° (c = 0.21, MeOH)

TLC: R_f = 0.23 (system T_8, green with vanillin/H_2SO_4, active under UV_{254})

IR: (KBr), ν_{max} = 3400 (OH), 1695 (>C=O), 1600, 1520, 1455, 1360, 1340, 1325, 1225, 1130, 1120, 1080, 1060, 1038, 980, 955, 900, 855, 760, 720 cm^{-1}

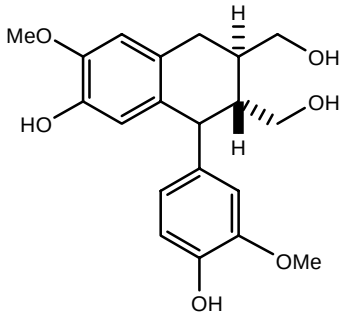
UV: (MeOH), λ_{max} = 219 nm (log ε = 4.34), 276 (3.84), 314 (3.46)

^1H NMR: (400 MHz, C_5D_5N): δ 3.89 (3H, *s*, 9 OMe), 4.15 (3H, *m*), 4.40 (1H, *t*, *J* 8.6 Hz), 4.55 (1H, *t*, *J* 9.8 Hz), 4.61 (1H, *d*, *J* 10.9 Hz), 5.18 (1H, *d*, *J* 10.1 Hz), 7.65 (1H, *s*)

EI-MS: (70 ev) *m/z* (rel. int): 328 $[M]^+$ (28), 237 (5), 222 (20), 208 $[C_{10}H_8O_5]^+$ (100), 195 (16), 180 (23), 165 (12), 152 (20)

HR-ESI-MS: $C_{14}H_{16}O_9Na$ $[M+Na]^+$ calc. 351.06865 found. 351.06859

Compound 51: isolariciresinol (mmk 080) [lit. 41]



pale yellow amorphous powder

Yield: 9 mg, 0.0009%

$[\alpha]_D$: + 26.01 ° (c = 0.15, MeOH)

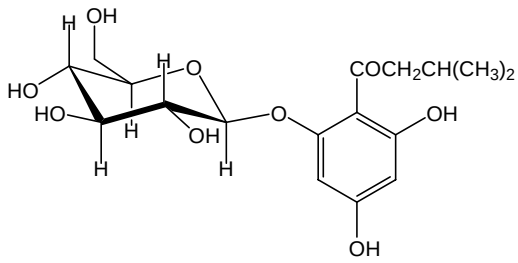
TLC: R_f = 0.59 (system T_8, red colour with vanillin/H_2SO_4, active under UV_{254})

^{1}H NMR: (500 MHz, C_5D_5N): δ 2.366 (1H, *m*, H-8′), 2.595 (1H, *m*, H-8), 3.140 (1H, *dd*, *J* 4.6/15.6 Hz, H-7B), 3.251 (1H, *dd*, *J* 11.0/15.4 Hz, H-7A), 3.548 (3H, *s*, H-3′OMe), 3.804 (3H, *s*, H-3OMe), 3.942 (1H, *dd, J* 4.1/11.0 Hz, H-9′B), 4.233 (1H, *t*, *J* 5.1 Hz, H-9), 4.259 (1H, *dd*, *J* 2.5, 10.8 Hz, H-9′A), 4.373 (1H, *d*, *J* 10.6 Hz, H-7′), 6.897 (1H, *s*, H-2), 6.975 (1H, *dd*, *J* 1.7/7.9 Hz, H-6′), 6.977 (1H, *br s*, H-5), 7.073 (1H, *d*, *J* 1.4 Hz, H-2′), 7.199 (1H, *d*, *J* 7.8 Hz, H-5′)

EI-MS: (70 ev) *m/z* (rel. int): 360 $[M]^+$, 342 $[M–H_2O]^+$, 311 (100), 284 (30), 241 (20)

HR-ESI-MS: $C_{20}H_{23}O_6$ $[M–H]^-$ calc . 359.150012 found. 359.15019

Compound 52: 1-[(3-methylbutyryl) phloroglucinol]- β-D-glucopyranoside (mmk 084)



pale yellow amorphous powder

Yield: 9 mg, 0.0009%

mp.: 114-116 °C

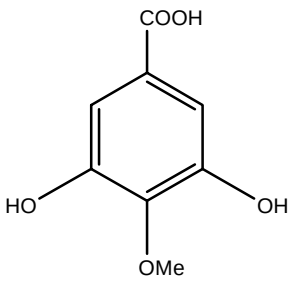
TLC: R_f = 0.18 (system T_8, red colour with vanillin/H_2SO_4, active under UV_{254})

IR: (KBr), ν_{max}= 3467, 1628, 1604, 1456 cm^{-1}

^{1}H NMR: (500 MHz, C_5D_5N): δ 0.916 (3H, *d*, *J* 6.6 Hz, H-4′), 0.919 (3H, *d*, *J* 6.6 Hz, H-5′), 2.445 (1H, *m*, H-3′), 3.238 (1H, *dd*, *J* 7.8/15.6 Hz, H-2′B), 3.543 (1H, *dd*, *J* 5.8/15.6 Hz, H-2′A), 3.969 (1H, *dd, J* 3.5/5.0 Hz, H-3′′), 4.357 (5H, *m*, H-2′′, 4′′, 5′′, 6′′), 5.729 (1H, *d*, *J* 7.4 Hz, H-1′′), 6.603 (1H, *d*, *J* 1.5 Hz, H-4), 6.890 (1H, *d*, *J* 1.9 Hz, H-6)

HR-ESI-MS: $C_{17}H_{23}O_9$ $[M–H]^-$ calc. 371.13475 found. 371.13567

Compound 53: 4-*O*-methyl gallate (mmk 097)



pale brown amorphous powder

Yield: 2 mg, 0.0002%

TLC: R_f = 0.72 (system T_9, blue colour with

1% $Ce(SO_4)_2$ /H_2SO_4, active under UV_{254})

HPLC: system 9p: R_t = 63.5 min

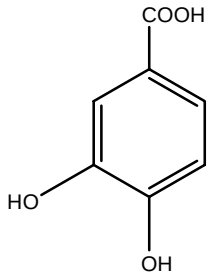
^{1}H NMR: (400 MHz, CD_3OD): δ 3.84 (3H, s, OMe), 7.03 (2H, s)

^{13}C NMR: (400 MHz, CD_3OD): δ 59.5 (OMe), 109.2, 139.7, 150.4, 169.0

EI-MS: (70 ev) *m/z* (rel. int): 184 $[M]^+$ (100), 169 (95), 141 (50), 113 (40)

HR-ESI-MS: $C_8H_8O_5Na[M+Na]^+$ calc . 207.02639 found. 207.02640

Compound 54: protocatechuic (mmk 098)



pale brown amorphous powder

Yield: 3 mg, 0.0003%

TLC: R_f = 0.62 (system T_9, blue colour with 1% $Ce(SO_4)_2$ /H_2SO_4, active under UV_{254})

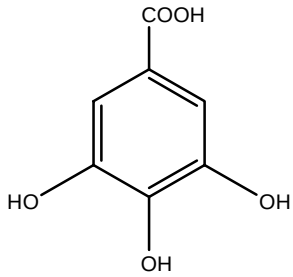
HPLC: system 9P: R_t = 27.7 min

^{1}H NMR: (400 MHz, C_5D_5N): δ 7.16 (1H, *s*), 7.27 (1H, *d*, *J* 8.2 Hz), 8.05 (1H, *d*, *J* 8.2 Hz)

EI-MS: (70 ev) *m/z* (rel. int): 154 $[M]^+$ (100), 137 (95), 110 (60)

HR-ESI-MS: $C_7H_5O_4$ $[M-H]^-$ calc . 153.01933 found. 153.01936

Compound 55: gallic acid (mmk 107)



pale brown amorphous powder

Yield: 6 mg, 0.0006%

TLC: R_f = 0.55 (system T_9, blue colour with 1% $Ce(SO_4)_2$ /H_2SO_4, active under UV_{254})

HPLC: system 10p: R_t = 12 min

^{1}H NMR: (400 MHz, C_5D_5N): δ 8.12 (2H, *s*)

^{13}C NMR: (400 MHz, C_5D_5N): δ 110.5, 122.9, 140.5, 147.6, 169.6

EI-MS: (70 ev) *m/z* (rel. int): 170 $[M]^+$ (70), 153 (100), 135 (20), 126 (45)

HR-ESI-MS: $C_7H_5O_5[M-H]^-$ calc. 169.01424 found. 169.01399

Compound 56: 3-*O*-galloyl bergenin (mmk 103) [lit. 106]

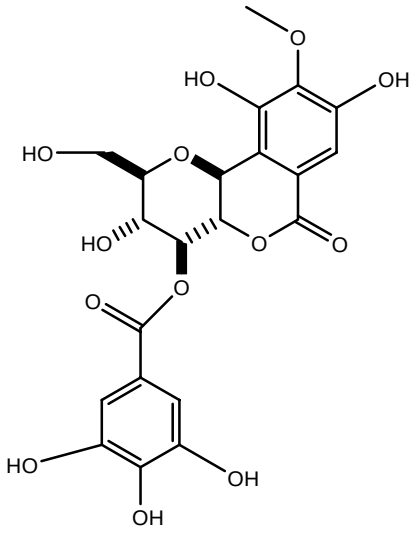
pale brown amorphous powder

Yield: 2 mg, 0.0002%

$[\alpha]_D$: − 53 ° (c = 1.1, MeOH)

TLC: R_f = 0.72 (system T_9, blue colour with

1% $Ce(SO_4)_2$ /H_2SO_4, active under UV_{254})



HPLC: system 11p: R_t = 40.1 min

IR: (KBr), ν_{max}= 3300, 1710, 1610, 1530, 1510, 1210 cm^{-1}

UV: (EtOH), λ_{max} = 277 nm (log ε = 4.17)

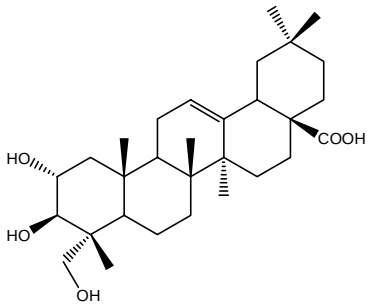
^{1}H NMR: (400 MHz, C_5D_5N): δ 3.98 (3H, *s*, OMe), 4.37 (3H, *m*), 4.68 (1H, *d*, *J* 10.6 Hz), 4.81 (1H, *t*, *J* 10.2 Hz), 5.39 (1H, *d*, *J* 10.6 Hz), 6.36 (1H, *t*, *J* 9.0 Hz), 7.74 (1H, *s*), 7.90 (2H, *s*)

EI-MS: (70 ev) *m/z* (rel. int): 328 (43), 237 (10), 208 (100), 170 (12)

HR-ESI-MS**:** $C_{21}H_{20}O_{13}$ Na$[M+Na]^+$ calc. 503.079611 found. 503.08002

Compound 9: 2α,3β,23-trihydroxy-olean-12-en-28-oic-acid (mmk 081)

white needles



Yield: 1.5 mg, 0.00015%

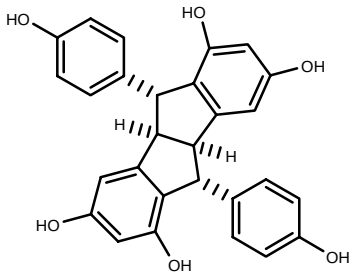
IR: (KBr), ν_{max}= 3500 (OH), 1720 (COO), 1030 (OH), 1600 (C=C), 827 (C=C) cm^{-1}

^{1}H NMR: (500 MHz, C_5D_5N): δ 0.907 (3H, *s*, H-29), 0.981 (3H, *s*, H-30), 1.036 (3H, *s,* H-26), 1.058 (6H, *s*, H-24, 25), 1.11-1.14 (1H, *m*, H-15), 1.16 (3H, *s*, H-27), 1.14-2.5 (18H, *m*, H-16, 7, 1, 21, 19, 6, 5, 9, 22, 11), 3.2-3.3 (1H, *m*, H-18), 3.723 (1H, *d*, *J* 10.4 Hz, H-23B), 4.194-4.223 (2H, H-3, 23A), 4.22-4.28 (1H, *m*, H-2), 5.453 (1H, *br s*, H-12).

EI-MS: (70 ev) *m/z* (rel. int): 248 (100), 203 (50), 133 (30)

HR-ESI-MS: $C_{30}H_{48}O_5$Na $[M+Na]^+$ calc. 511.33939 found. 511.33989

Compound 57: pallidol (mmk 086) [lit. 47]



brown solid

Yield: 2 mg , 0.0002%

$[\alpha]_D$: – 25.4 ° (c = 0.10, MeOH)

TLC: R_f = 0.12 (system T_8, orange colour with vanillin/H_2SO_4, active under UV_{254})

HPLC: system 12a: R_t = 10.1 min

IR: (KBr), ν_{max}= 3300, 1600 cm^{-1}

UV: (MeOH), λ_{max} = 226, 285, 321 nm

^{1}H NMR: (500 MHz, C_5D_5N): δ 4.424 (2H, *s*, H-8, 8′), 5.352 (2H, *s*, H-7, 7′), 6.855 (2H, *d, J* 1.9 Hz, H-12, 12′), 7.178 (4H, *d*, *J* 8.3 Hz, H-3, 3′, 5, 5′), 7.305 (2H, *d*, *J* 1.7 Hz, H-14, 14′), 7.466 (4H, *d*, *J* 8.3 Hz, H-2, 2′, 6, 6′)

HR-ESI-MS: $C_{28}H_{21}O_6$ $[M-H]^-$ calc. 453.13436 found. 453.13550

11. Bioactivities

11.1. Antifungal test

Fungal culture *Cladosporium cucumerinum*

Yeast nutrient solution

Mannitole	50 g
Saccharose	50 g
Succinic acid	5.4 g
Yeast-Extract	3.0 g
KH_2PO_4	0.1 g
$MgSO_4 \times 7\ H_2O$	0.3 g
$FeSO_4 \times 7\ H_2O$	0.01 g
$ZnSO_4 \times 7\ H_2O$	0.0044 g
H_2O	1000 ml

pH 5.4

The antifungal testing was carried out by spraying hand-made TLC plates (glass plate, 20 × 20 cm, kiesel gel 60 HF_{254}, thick 0,5 mm were dried at 120 °C for 30 min in the oven) with conidia of *Cladosporium cucumerinum* [Gottstein *et al*., 1982]. The crude extracts and pure substances were loaded on the TLC plates by using a microliter syringe. Test amount were 250 µg, 500 µg for crude extracts and 20 µg for pure substances. Loaded samples give 1 cm diameter (area 78 mm^2). Crude extracts were also chromatographed on TLC plates with *n*-hexane:ethyl acetate and chloroform:methanol. The dried plates were sprayed with 10 ml spore suspension of *Cladosporium cucumerinum* (spore density ca. 2.5 × 10^6 spore/ml) and dried at room temperature for a few minutes. Finally, these plates were placed in a TLC chamber containing moistured filter paper and the fungus was cultured in a incubator at 25 °C for 2 days.

11.2. Antiproliferative activity

Acid phosphatase assay: Cells were grown in 96-well plates at densities upto 100,000 cells per well. The culture medium was removed from these cells with a multichannel pipettor (Wheaton), and each well was washed once with 200 µl phosphate-buffered saline (PBS, pH 7.2). For nonadherent cells, to remove solution from plates, the 96-well plates were centrifuges at 2500 rpm for 10 min (Beckman GS-15R centrifuge). To each well, 100 µl of buffer containing 0.1 M sodium acetate (pH 5.0), 0.1% Triton X-100, and 5 mM *p*-nitrophenyl phosphate was added. The plates were placed in a 37 °C incubator for 2 h. The reaction was stopped with the addition of 10 µl of 1 N NaOH, and color development was assayed at 405 nm using a microplate reader (THERMOmax plate reader, Molecular Devices, Inc.). The nonenzymatic hydrolysis of the pNPP substrate was determined for each assay by including wells that did not contain cells. This background value was typically 0.07-0.2 absorbance units [Yang *et al*., 1996].

11.3. Cellular viability

Cell culture and stimulation

Human U 937 myeloid leukemia cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 µg/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine (Gibco, Grand Island, N.V., USA). Human TUR myeloid leukemic cells (ATCC #2367) were grown in a similar medium supplemented with 400 µg/ml G418 (Sigma Chemical Co., St. Louis, MO/USA). The maintenance of the TUR cells in the presence of G418 was terminated one week before the appropriate experiments. U 937 and TUR cells were treated with the appropriate substances at a density of 2 x 10^5cells/ml for up to 72h, respectively. The cell number and viability of each culture was assessed by a Vi-Cell cell viability analyzer (Beckman Coulter) using an assay kit and the quantification software Vi-Cell version 1.01 according to the manufacturers protocol (Beckman Coulter).

Cell cycle analysis

Following an appropriate incubation the cells were fixed in 70% (v/v) ice-cold ethanol at 4 °C for 24 h. The fixed cells were stained with CyStain DNA 2 step kit (Partec GmbH, Münster, Germany) and filtered through a 50 µm filter. The samples were then analyzed in a Galaxy flow cytometer (Dako-Cytomation GmbH, Hamburg, Germany) using FloMax analysis software (Partec) and the MultiCycle cell cycle software (Phoenix Flow Systems Inc., San Diego, CA.

V. APPENDIX

12. X-ray datas of compounds 12, 26, 27

12.1. X-ray datas of compound 12

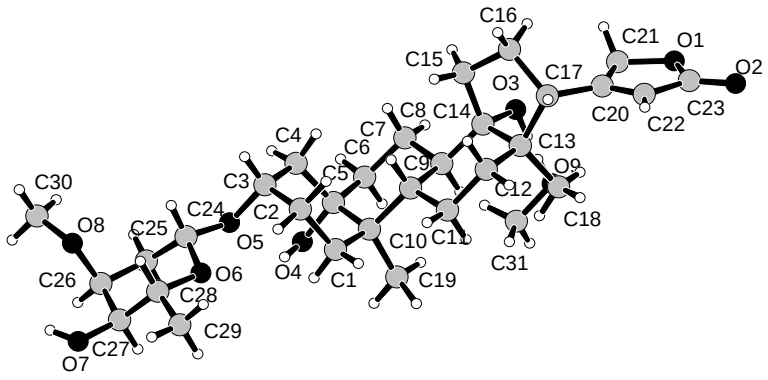


Table 50. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for compound **12**. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
C(1)	2950(4)	4639(7)	270(1)	32(1)
C(2)	3557(4)	2937(8)	233(2)	41(2)
C(3)	2882(4)	1389(8)	349(1)	35(1)
C(4)	2457(4)	1638(7)	776(1)	30(1)
C(5)	1854(3)	3377(7)	832(1)	28(1)
C(6)	1515(4)	3543(7)	1275(1)	35(1)
C(7)	2426(3)	3845(7)	1603(1)	32(1)
C(8)	3089(3)	5460(6)	1491(1)	25(1)
C(9)	3466(3)	5267(7)	1049(1)	28(1)
C(10)	2538(4)	4987(7)	704(1)	28(1)
C(11)	4176(4)	6802(7)	951(1)	38(1)
C(12)	5088(4)	7023(7)	1286(1)	35(1)
C(13)	4727(3)	7310(6)	1733(1)	24(1)
C(14)	3978(3)	5765(6)	1830(1)	26(1)
C(15)	4735(3)	4246(7)	1931(1)	33(1)
C(16)	5718(4)	5046(7)	2165(2)	40(1)
C(17)	5731(3)	7042(7)	2042(1)	29(1)
C(18)	4231(4)	9115(7)	1758(2)	39(2)
C(19)	1846(5)	6646(8)	662(2)	41(2)
C(20)	5817(4)	8237(7)	2414(1)	32(1)
C(21)	5244(4)	8005(8)	2803(2)	56(2)
C(22)	6452(4)	9592(8)	2463(2)	41(2)

C(23)	6298(5)	10410(9)	2859(2)	56(2)
C(24)	2101(4)	420(6)	-323(1)	28(1)
C(25)	1013(4)	-219(7)	-505(1)	33(1)
C(26)	1077(4)	-865(8)	-946(1)	38(1)
C(27)	1583(4)	495(8)	-1212(1)	44(2)
C(28)	2655(4)	1001(8)	-1005(1)	41(1)
C(29)	3224(5)	2384(8)	-1243(2)	71(2)
C(30)	1217(5)	-3967(9)	-852(2)	68(2)
C(31)	1070(5)	8327(11)	1926(2)	94(3)
O(1)	5579(3)	9472(6)	3068(1)	57(1)
O(2)	6709(3)	11711(7)	3020(1)	85(2)
O(3)	3512(2)	6207(4)	2226(1)	31(1)
O(4)	887(2)	3306(4)	569(1)	35(1)
O(5)	1955(2)	1193(4)	61(1)	31(1)
O(6)	2523(2)	1673(5)	-593(1)	35(1)
O(7)	1696(3)	-65(5)	-1631(1)	56(1)
O(8)	1733(3)	-2387(5)	-956(1)	42(1)
O(9)	1812(3)	8511(6)	2255(1)	67(1)

12.2. X-ray datas of compound 26

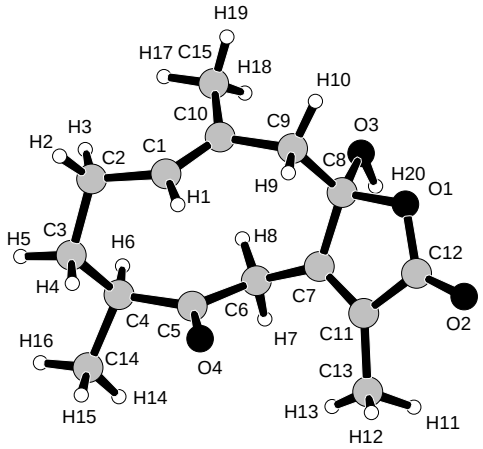


Table 51. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) forcompound **26**. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
C(1)	11414(3)	3359(3)	1989(1)	41(1)
C(2)	9974(3)	3972(4)	1929(1)	56(1)

C(3)	9433(3)	3772(3)	1449(1)	54(1)
C(4)	10385(3)	4337(3)	1079(1)	44(1)
C(5)	11771(2)	3577(3)	1044(1)	35(1)
C(6)	13169(3)	4334(2)	1078(1)	36(1)
C(7)	14377(2)	3500(2)	1235(1)	32(1)
C(8)	14952(2)	3588(2)	1710(1)	36(1)
C(9)	13981(3)	3162(3)	2097(1)	39(1)
C(10)	12611(3)	3930(2)	2130(1)	38(1)
C(11)	15122(2)	2624(2)	995(1)	37(1)
C(12)	16188(3)	2042(3)	1303(1)	43(1)
C(13)	15047(4)	2234(4)	509(1)	51(1)
C(14)	9648(4)	4259(4)	615(1)	63(1)
C(15)	12689(4)	5297(3)	2326(1)	54(1)
C(16)	12162(3)	8115(3)	640(1)	45(1)
C(17)	13572(3)	8678(4)	786(1)	58(1)
C(18)	13756(3)	8625(3)	1299(1)	48(1)
C(19)	12603(2)	9359(3)	1565(1)	39(1)
C(20)	11189(2)	8644(2)	1522(1)	35(1)
C(21)	9892(3)	9414(2)	1358(1)	35(1)
C(22)	8796(2)	8547(2)	1141(1)	32(1)
C(23)	8530(2)	8559(2)	635(1)	36(1)
C(24)	9735(3)	8034(3)	335(1)	39(1)
C(25)	11151(3)	8715(2)	402(1)	37(1)
C(26)	7935(2)	7676(2)	1341(1)	33(1)
C(27)	7066(2)	7065(2)	984(1)	36(1)
C(28)	7747(3)	7342(3)	1826(1)	45(1)
C(29)	12969(3)	9442(3)	2067(1)	53(1)
C(30)	11291(4)	10043(3)	185(1)	52(1)
O(1)	16119(2)	2612(2)	1709(1)	44(1)
O(2)	17026(2)	1154(2)	1223(1)	59(1)
O(3)	15509(2)	4827(2)	1809(1)	44(1)
O(4)	11770(2)	2398(2)	966(1)	44(1)
O(5)	7353(2)	7612(2)	582(1)	42(1)
O(6)	6197(2)	6176(2)	1021(1)	46(1)
O(7)	8094(2)	9783(2)	476(1)	47(1)
O(8)	11086(2)	7491(2)	1638(1)	47(1)

12.3. X-ray datas of compound 27

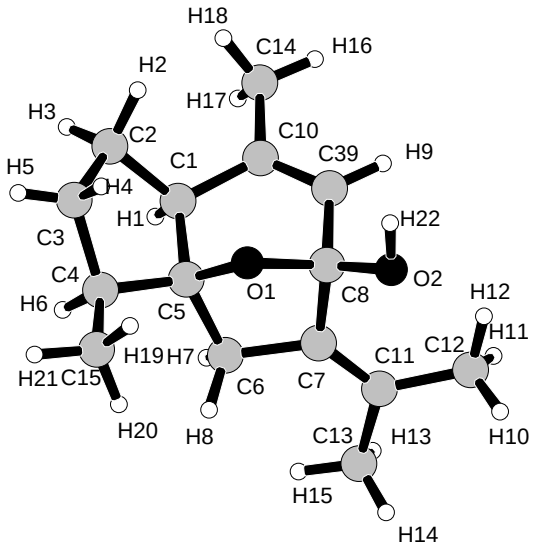
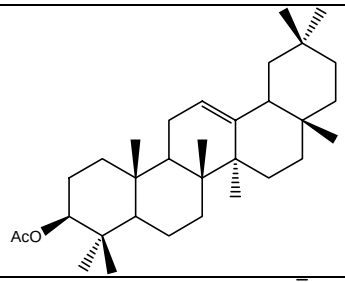
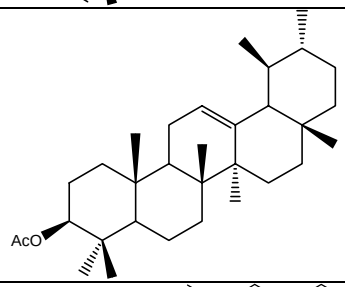
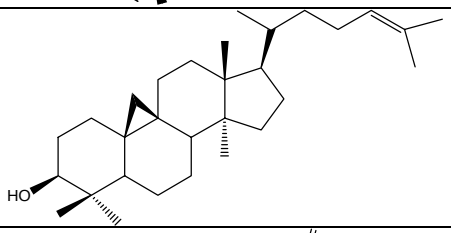
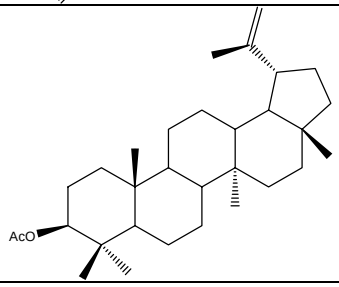


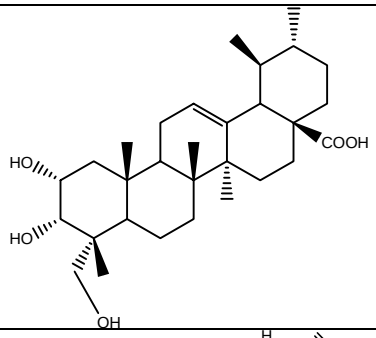
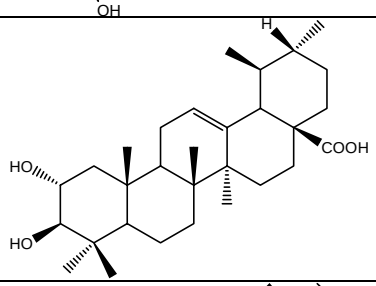
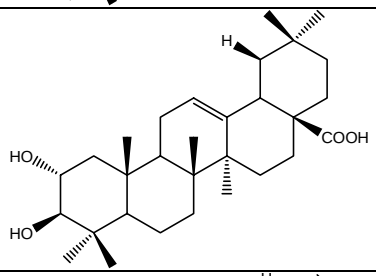
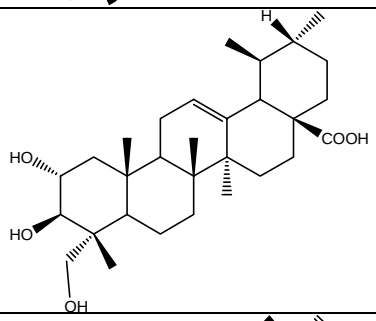
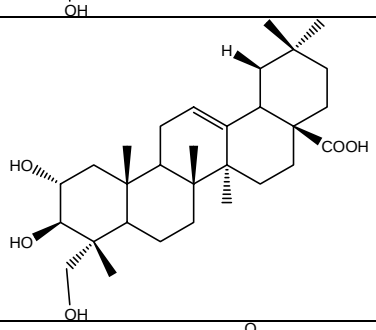
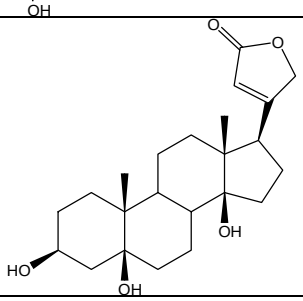
Table 52. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for compound **27**. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

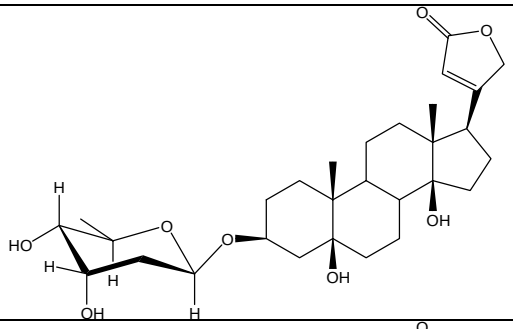
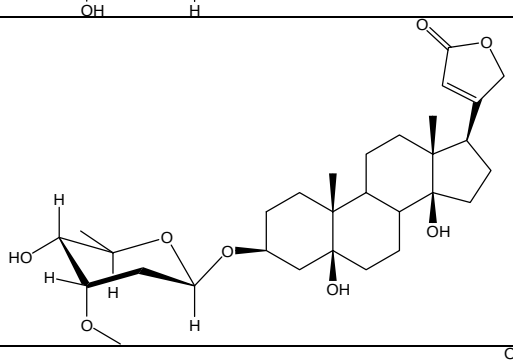
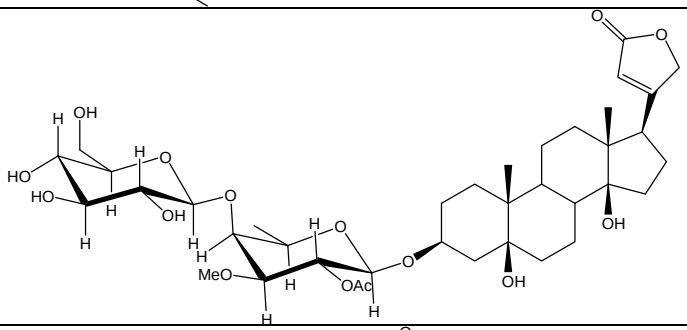
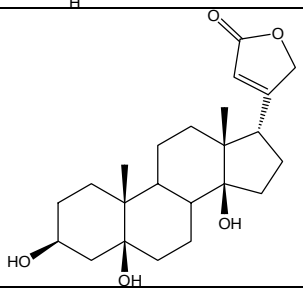
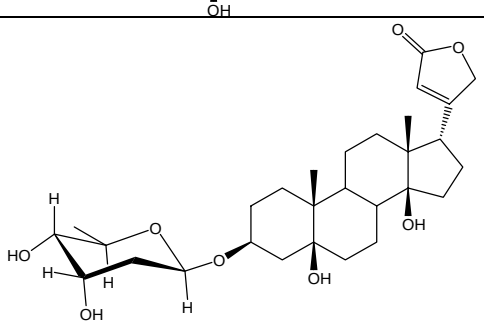
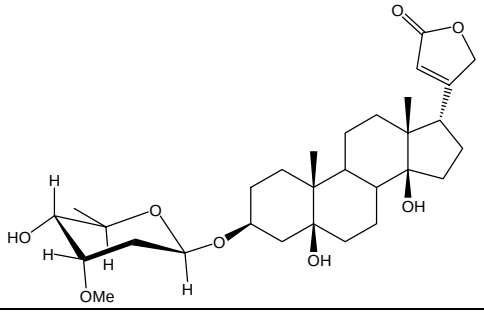
	x	y	z	U(eq)
C(1)	9514(2)	2164(2)	3622(2)	36(1)
C(2)	10696(3)	2458(2)	2844(2)	49(1)
C(3)	10940(3)	1466(2)	2162(2)	47(1)
C(4)	10279(3)	546(2)	2775(2)	41(1)
C(5)	8998(2)	1057(2)	3228(1)	31(1)
C(6)	8232(2)	433(2)	4098(2)	35(1)
C(7)	6694(2)	793(1)	3884(1)	30(1)
C(8)	6652(2)	1539(1)	2870(1)	26(1)
C(10)	8313(2)	2951(2)	3616(2)	35(1)
C(11)	5636(2)	547(2)	4498(2)	37(1)
C(12)	4164(3)	989(3)	4298(2)	55(1)
C(13)	5891(3)	-194(2)	5502(2)	55(1)
C(14)	8698(4)	4045(2)	4039(2)	55(1)
C(15)	9975(3)	-435(2)	2066(2)	58(1)
C(16)	6069(2)	4636(1)	282(2)	34(1)
C(17)	5350(3)	4937(2)	1360(2)	50(1)
C(18)	3958(3)	4324(2)	1299(2)	51(1)
C(19)	3666(3)	3924(2)	71(2)	40(1)
C(20)	5146(2)	3718(1)	-270(1)	29(1)
C(21)	5298(2)	3538(2)	-1531(2)	33(1)
C(22)	6568(2)	2799(1)	-1513(1)	30(1)

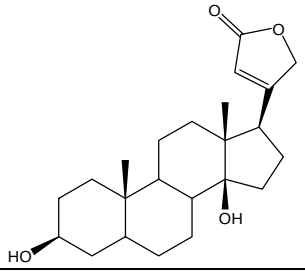
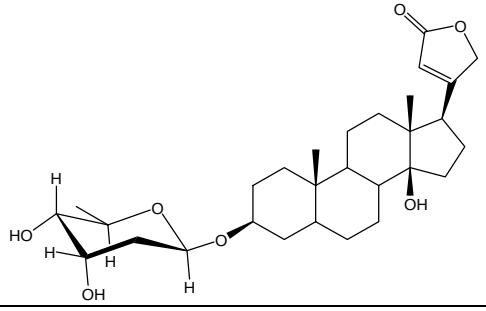
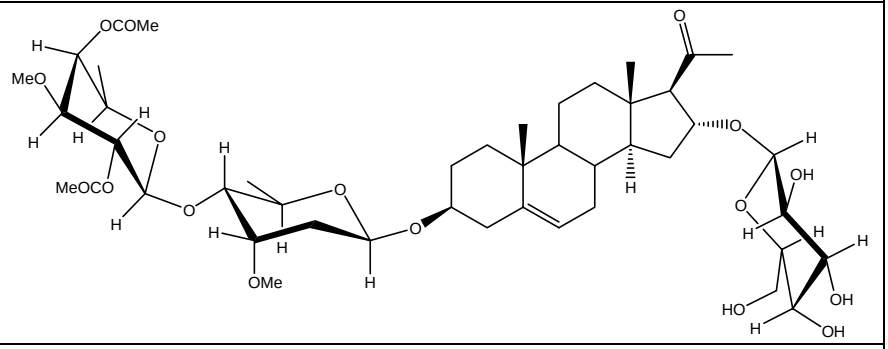
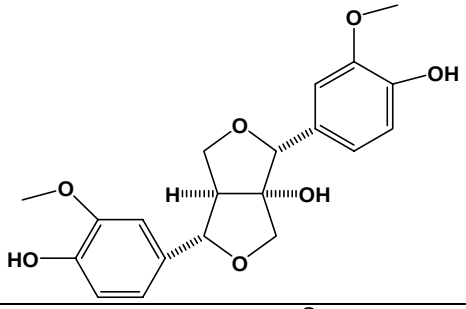
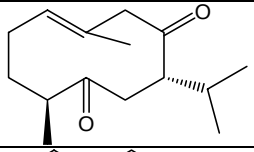
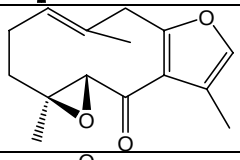
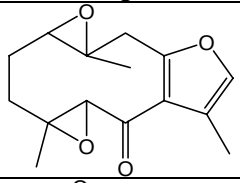
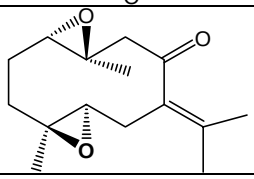
C(23)	7012(2)	2562(1)	-263(1)	27(1)
C(24)	8062(2)	3385(2)	230(1)	31(1)
C(25)	7630(2)	4347(2)	486(1)	34(1)
C(26)	7199(2)	2473(2)	-2409(1)	36(1)
C(27)	8501(3)	1785(2)	-2346(2)	48(1)
C(28)	6629(4)	2834(2)	-3583(2)	57(1)
C(29)	8656(3)	5190(2)	953(2)	52(1)
C(30)	2637(3)	3000(2)	-83(3)	56(1)
C(39)	6985(2)	2654(2)	3271(1)	31(1)
O(1)	7864(1)	1181(1)	2308(1)	27(1)
O(2)	5421(1)	1445(1)	2121(1)	31(1)
O(3)	5696(1)	2733(1)	242(1)	27(1)
O(4)	7461(2)	1532(1)	-49(1)	33(1)

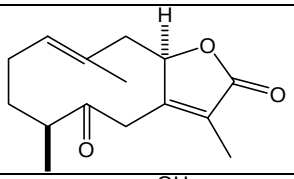
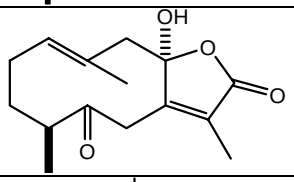
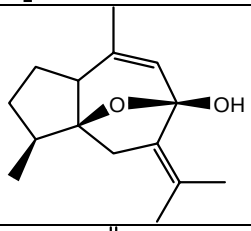
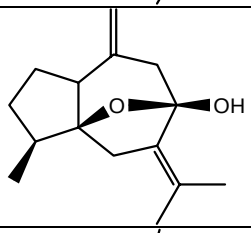
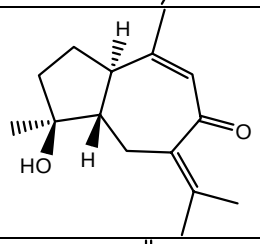
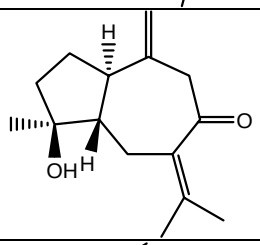
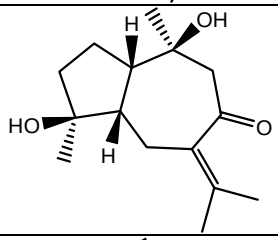
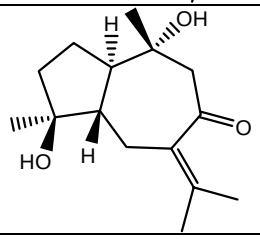
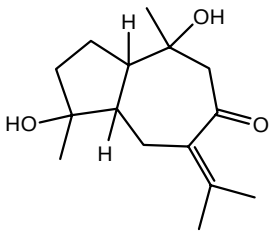
13. List of compounds

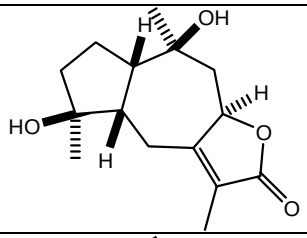
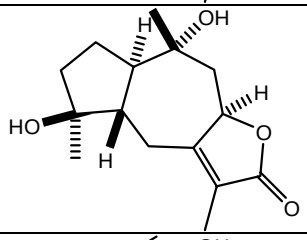
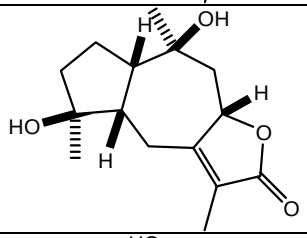
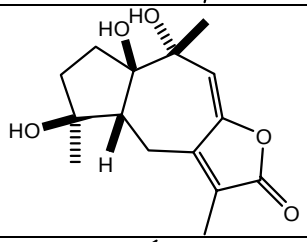
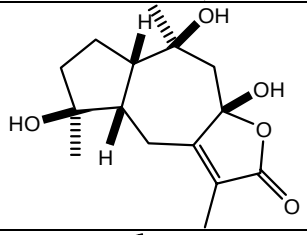
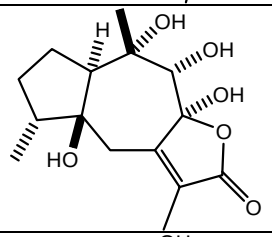
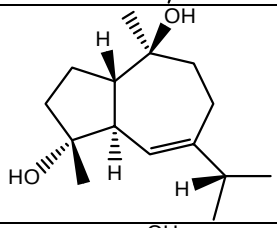
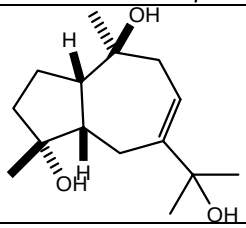
number	structure
1	
2	
3	
4	

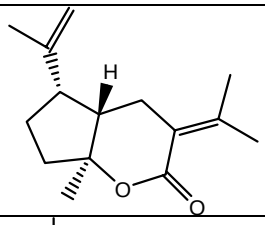
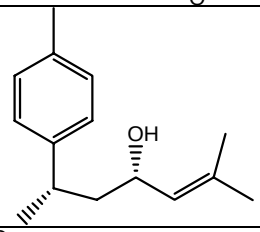
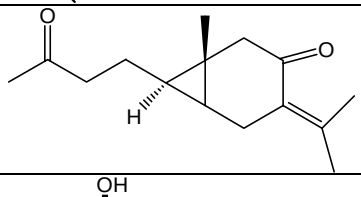
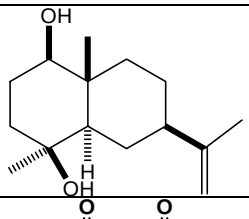
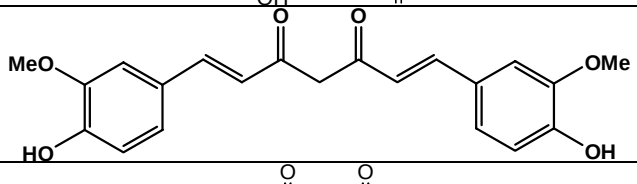
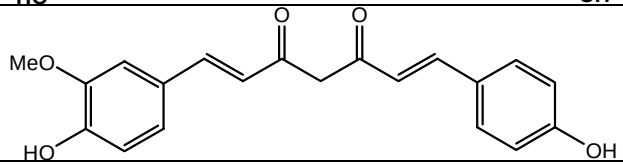
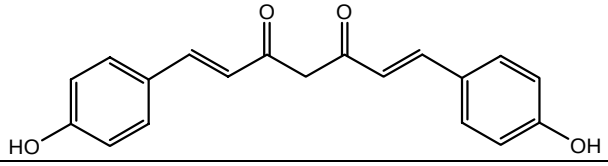
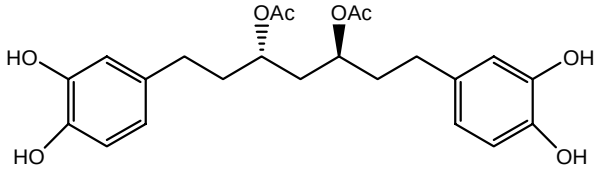
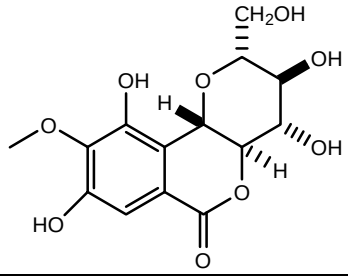
5	
6	
7	
8	
9	
10	

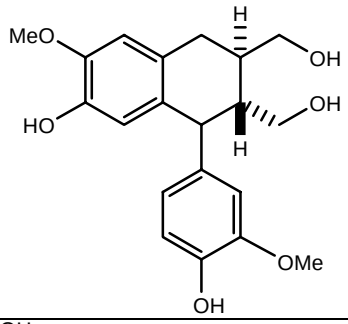
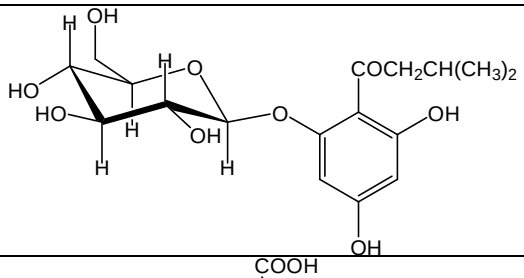
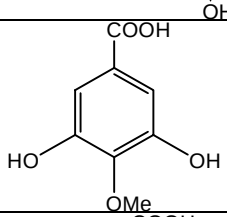
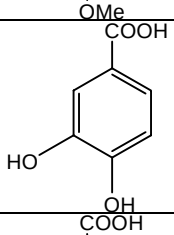
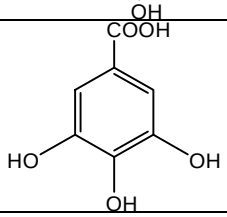
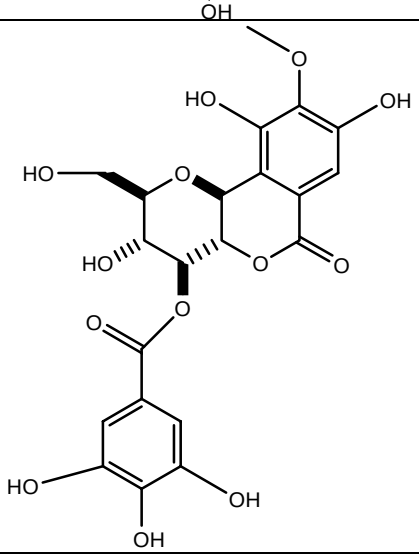
11	
12	
13	
14	
15	
16	

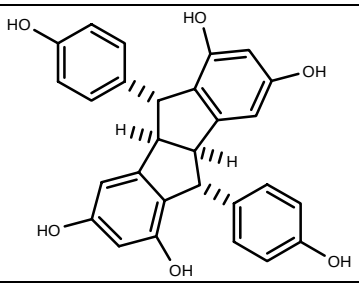
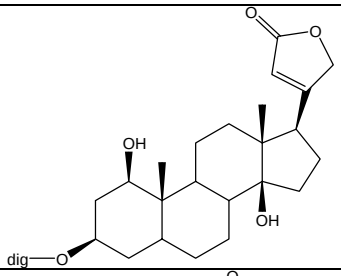
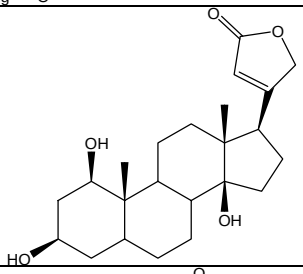
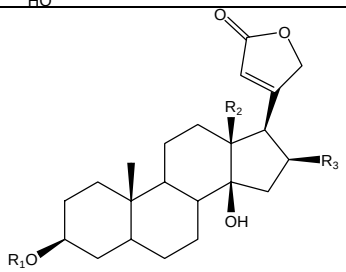
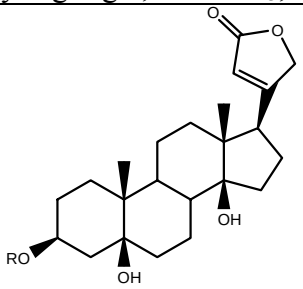
17	
18	
19	
20	
21	
22	
23	
24	

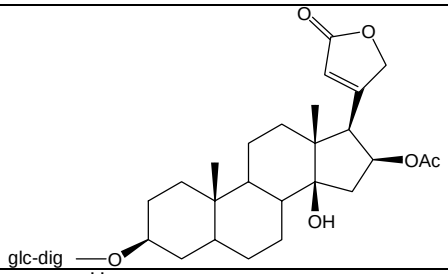
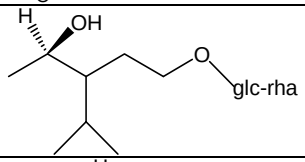
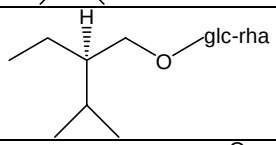
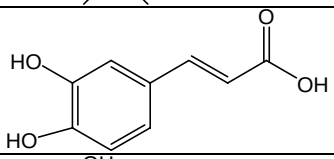
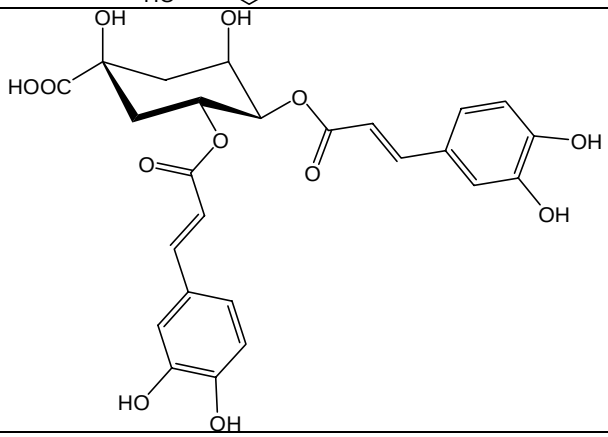
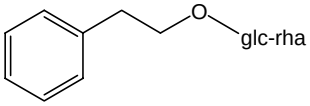
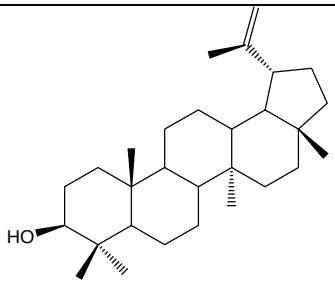
25	
26	
27	
28	
29	
30	
31	
32	
33	

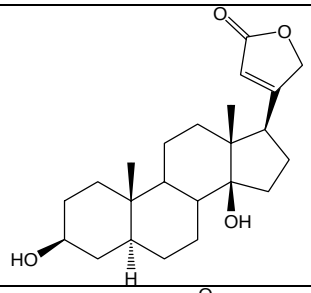
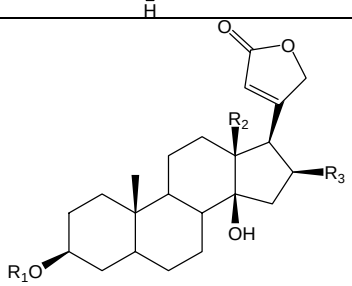
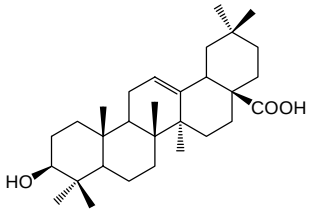
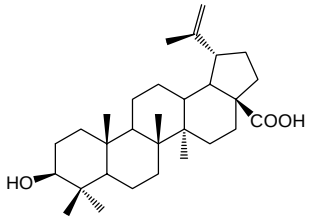
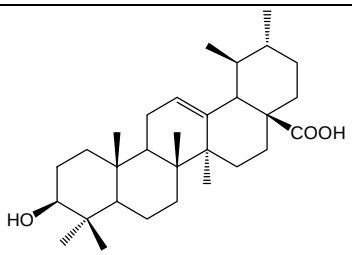
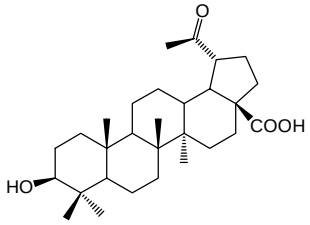
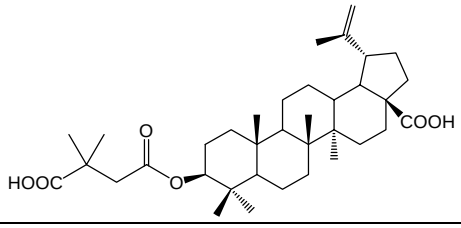
34	
35	
36	
37	
38	
39	
40	
41	

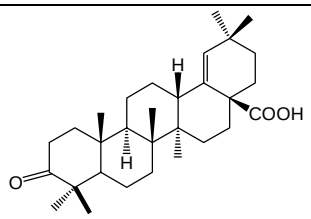
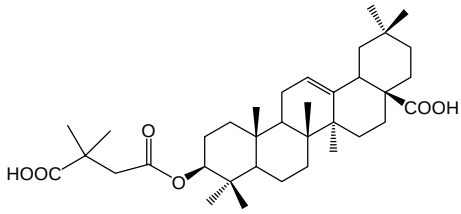
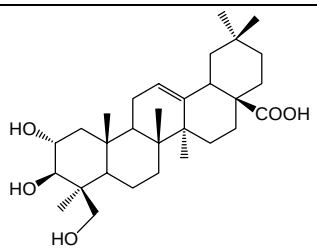
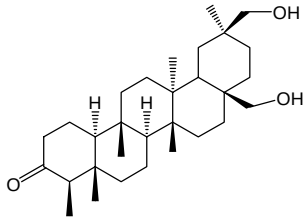
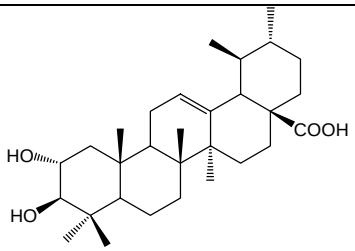
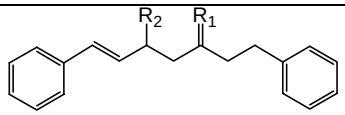
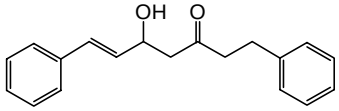
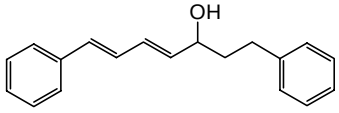
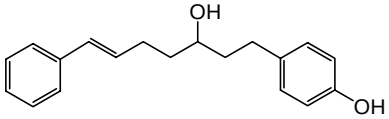
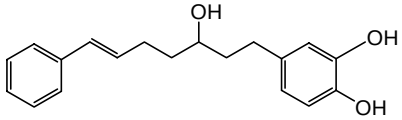
42	
43	
44	
45	
46	
47	
48	
49	
50	

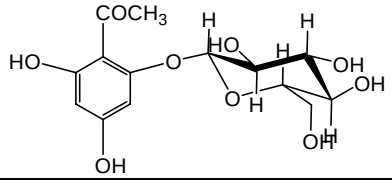
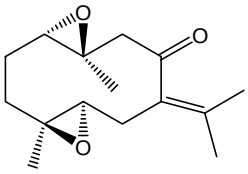
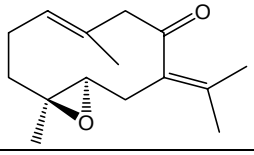
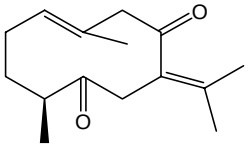
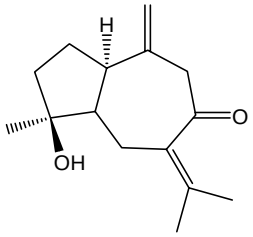
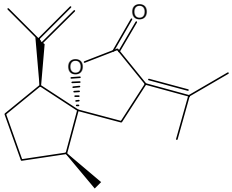
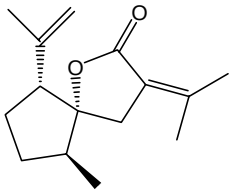
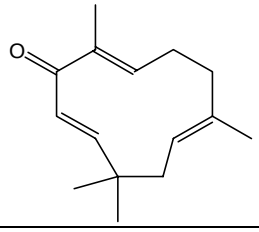
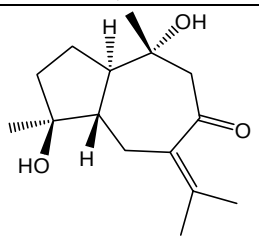
51	
52	
53	
54	
55	
56	

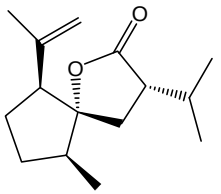
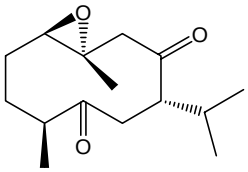
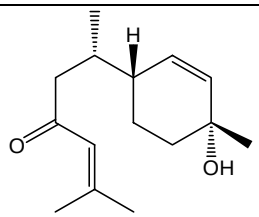
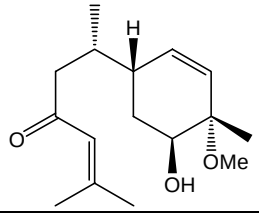
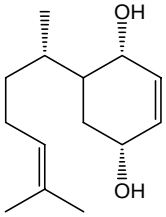
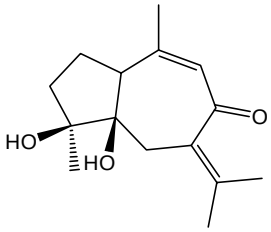
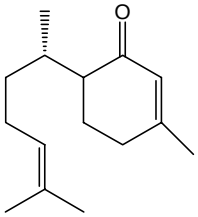
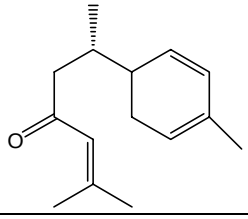
57	
58	not determined
59	
60	
61	 R_1 = glc-glc, R_2 = CH_3, R_3 = H
62	R_1 = (3-*O*-Ac-dig)-glc-glc, R_2 = CH_3, R_3 = H
63	R_1 = cym-dtl-glc-glc, R_2 = CH_3, R_3 = H
64	R_1 = dig-glc-glc, R_2 = CH_3, R_3 = H
65	R_1 = glc-glc, R_2 = CH_3, R_3 = H
66	R_1 = cym-glc-glc, R_2 = CH_3, R_3 = H
67	 R = dtl-glc
68	R = glc
69	R = dig-glc

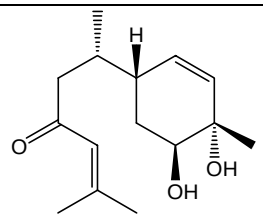
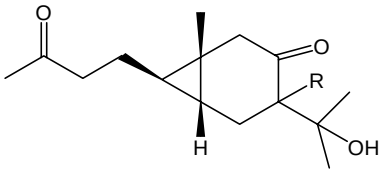
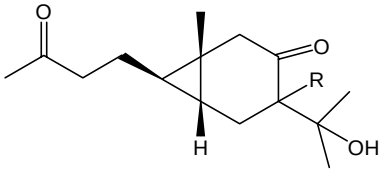
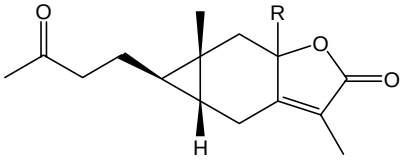
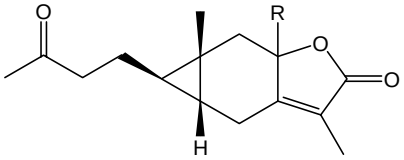
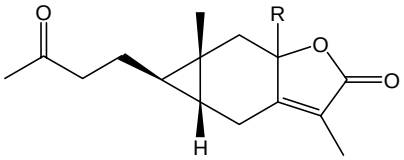
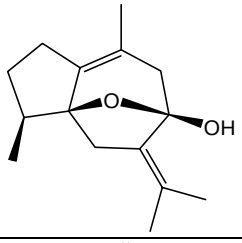
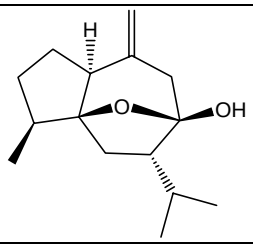
70	
71	
72	
73	
74	
75	
76	

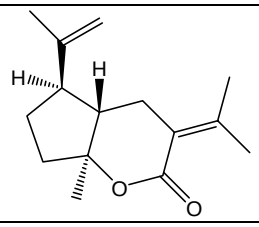
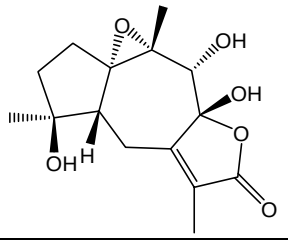
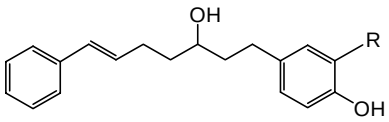
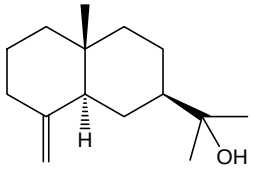
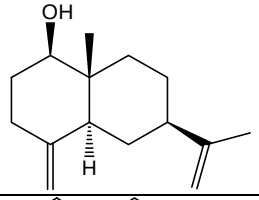
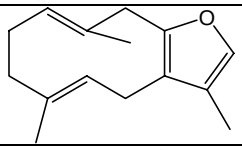
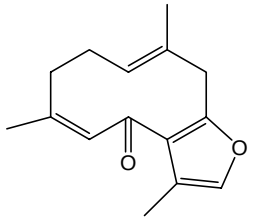
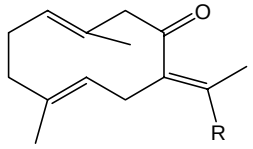
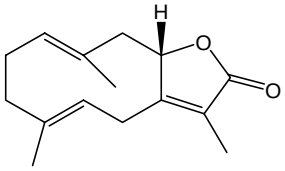
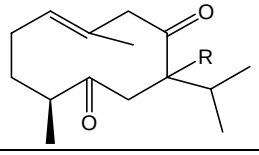
77	
78	 R_1 = dig-dig-dig, R_2 = CH_3, R_3 = H
79	R_1 = dig-dig-dig, R_2 = CH_3, R_3 = H
80	R_1 = dig-dig-dig, R_2 = CH_3, R_3 = OH
81	
82	
83	
84	
85	

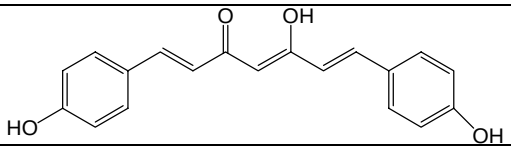
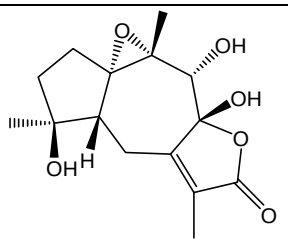
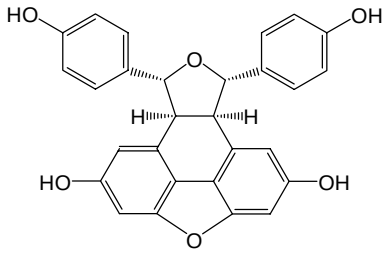
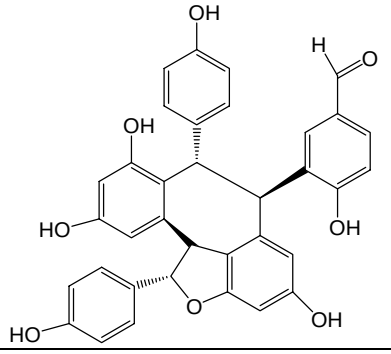
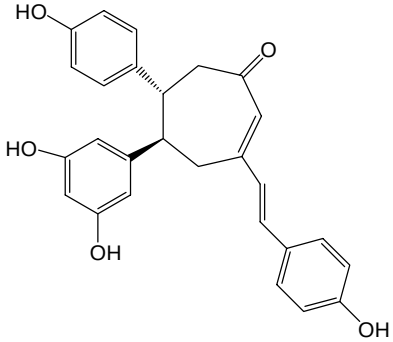
86	
87	
88	
89	
90	
91	 R_1 = H, $OCOCH_3$; R_2 = H
92	R_1 = O; R_2 = H
93	R_1 = H, OH; R_2 = H
94	R_1 = H, OH; R_2 = H; $\Delta^{4,5}$
95	
96	
97	
98	

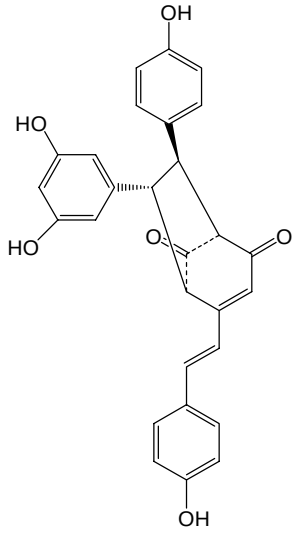
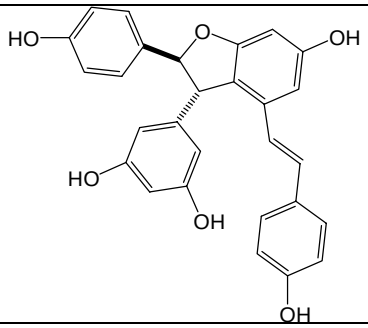
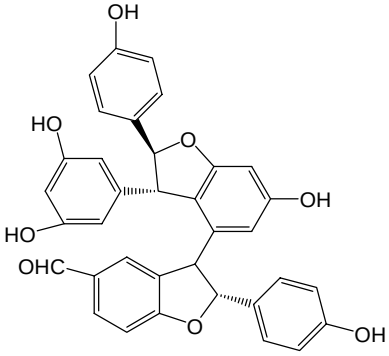
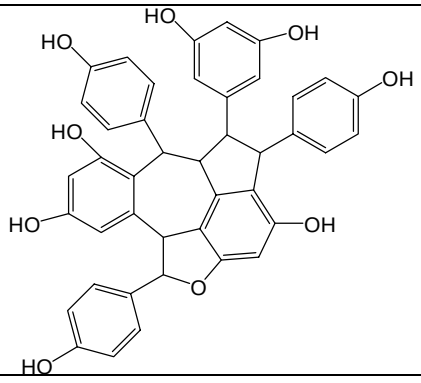
99	
100	 R = H
101	
102	
103	
104	
105	
106	
107	

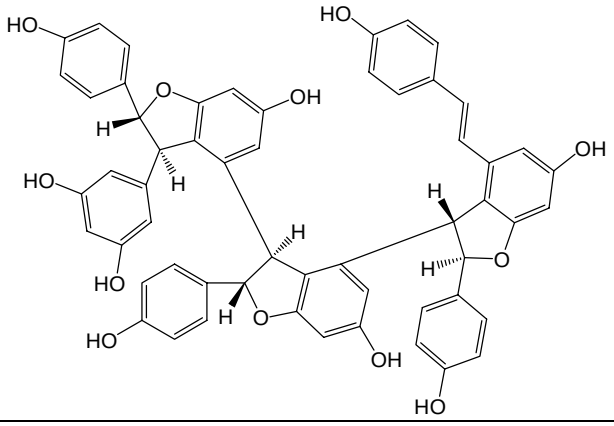
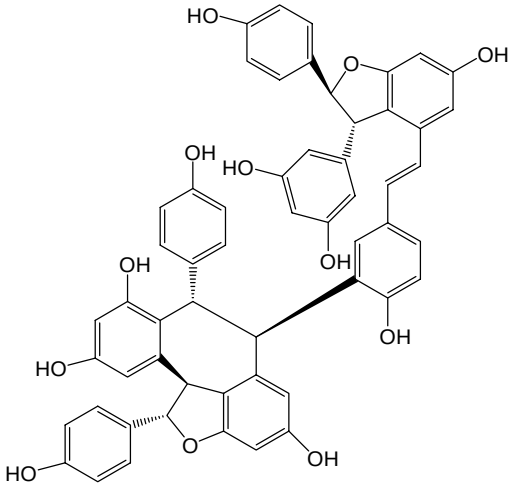
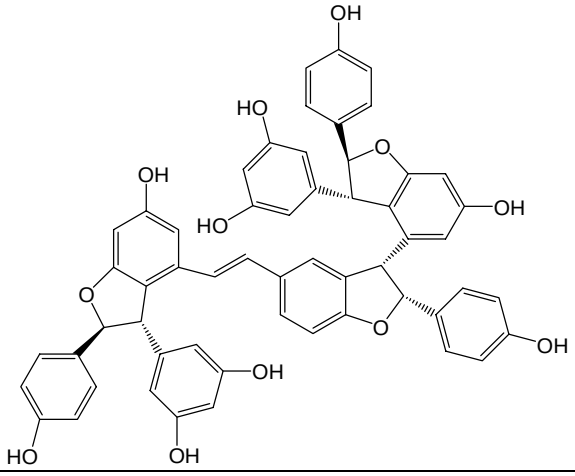
108	
109	
110	
111	
112	
113	
114	
115	

116	
117	 R = β-H
118	 R = α-H
119	 R = β-H
120	 R = α-H
121	 R = α-OH
122	
123	

124	
125	
126	 $R_1 = O; R_2 = OH$
127	
128	
129	
130	
131	 $R = CH_2OH$
132	
133	

	R = α-H
134	
135	
136	
137	
138	

139	
140	
141	
142	

143	
144	
145	

14. Abbreviations

calc.	Calculated
CD	Circular Dichroism
$Ce(SO_4)_2$	
COSY	Correlation Spectroscopy
cym.	β-cymarose
dig.	β-digitoxose
dtl.	β-digitalose
TLC	Thin layer chromatography
EE	Ethylacetate
EI	Electron ionization
ESI	Electron spray ionization
EtOH	Ethylalcohol
GC	Gas chromatography
glc.	β-glucose
HCl	Hydrochloric acid
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High Performance Liquid Chromatography
HR	High Resolution
H_2SO_4	sulphuric acid
HSQC	Heteronuclear Single Quantum Correlation
IR	Infraredspectroscopy
MeOH	Methanol
min	minute
mp	melting point
MS	Mass Spectrometry
m/z	mass/charge
NMR	Nuclear Magnetic Resonance (Spectroscopy)
NOE	Nuclear-Overhauser-Enhancement
Prep.	preparative
rha.	α-rhamnose
R_f	Retention Factor
R_t	Retention Time
ROESY	Rotating Frame Overhauser Enhancement Spectroscopy

RP	Reversed Phase
Tab.	Table
TFA	Trifluoroaceticacid
UV/VIS	Ultraviolet/visible

VI. REFERENCES

1. Abrantes-Metz, R.; Adams, C.; Metz, A. **2003.** *Pharmaceutical Development Phases: a Duration Analysis*. *The Federal Trade Commission*.
2. Aggarwal, B. B.; Kumar, A.; Bharti. A. C. **2003**. *Anticancer Res. 23*, 363-398.
3. Akera, T.; Brody, T. M. **1978**. *Pharmacol. Rev*. *29*, 187-220.
4. Arvigo, R.; Balick, M. **1993**. *Rainforest Remedies*, Lotus Press, Twin Lakes.
5. Budzikiewicz, H.; Wilson, J. M.; Djerassi, C. **1963**. *J. Am. Chem. Soc. 85*, 3688.
6. Chen, Z. S.; Lee, G. H.; Kuo, Y. H. **1993**. *Phytochemistry 34*, 783-786.
7. Chien, M. M.; Zahradka, K. E.; Newell, M. K.; Freed, J. H. **1999**. *J. Biol. Chem*. *274*, 7059-7066.
8. Connolly, J. D.; Hill, R. A. **2002**. *Nat. Prod. Rep*. *19*, 494-513.
9. Cowan, S.; Stewart, M.; Abbiw, D. K.; Latif, Z.; Sarker, S. D.; Nash, R. J. **2001**. *Fitoterapia 72*, 80-82.
10. Danieli, N.; Mazur, Y.; Sondheimer, F. **1966**. *Tetrahedron 22*, 3189-3193.
11. De Pascual Teresa, J.; Urones, J. G.; Marcos, I. S.; Basabe, P.; Sexmero Cuadrado, M. J.; Fernandez Moro, R. **1987**. *Phytochemistry 26*, 1767-1776.
12. Dey, P. M.; Harborne, J. B. **1991**. *Methods in Plant Biochemistry 7*, 341-342.
13. Dev, S. **1999.** *Environ. Health Perspect 107***,** 783.
14. Dong, M.; Feng, X. Z.; Wang, B. X.; Wu, L. J.; Ikejima, T. **2001**. *Tetrahedron 57*, 501-506.
15. Dou, D. Q.; Ren, J.; Cooper, M.; He, Y. H.; Pei, Y. P.; Takaya, Y.; Niwa, M.; Chen, Y. J.; Yao, X. S.; Zhou, R. P. **2003**. *J. Chin. Pharm. Sci*. *12*, 57-59.
16. Fallarino, M. **1994**. *Herbalgram 31*, 38.
17. Farnsworth, N. R.; Akerele, O.; Bingel, A. S.; Soejarto, D. D.; Guo, Z. **1985**. *Bull. WHO*. *63*, 965
18. Firman, K.; Kinoshita, T.; Itai, A.; Sankawa, U. **1988**. *Phytochemistry 27*, 3887-3891.
19. Fujioka, T.; Kashiwada, Y.; Kilkuskie, R. E.; Cosentino, L. M.; Ballas, L. M.; Jiang, J. B.; Janzen, W. P.; Chen, I. S.; Lee, K. H. **1994**. *J. Nat. Prod*. *57*, 243-247.
20. Furuya, T.; Kawaguchi, K.; Hirotani, M. **1988**. *Phytochemistry* 27, 2129-2133.
21. Giang, P. M.; Son, P. T. **2002**. *Journal of Chemistry 40*, 108-112.
22. Gottstein, D.; Gross, D.; Lehmann, H. **1982**. *Arch. Phytopathol*. *Pfl*. *20*, 111-116.
23. Habermehl, G. G.; Hammann, P. E.; Wrag, V. **1985**. *Magn. Reson. Chem*. *23*, 959-963.
24. Harimaya, K.; Gao, J. F.; Ohkura, T.; Kawamata, T.; Iitaka, Y.; Guo, Y. T.; Inayama, S. **1991**. *Chem. Pharm. Bull. 39,* 843-853.

25. Haux, J. **1999**. *Medical Hypotheses*. *53*, 543-548.
26. Hikino, H.; Konno, C.; Takemoto, T. **1971**. *Chem. Pharm. Bull*. *19*, 93-96.
27. Hikino, H.; Sakurai, Y.; Numabe, S.; Takemoto, T. **1968**. *Chem. Pharm. Bull*. *16*, 39-42.
28. Hikino, H.; Takahashi, H.; Sakurai, Y.; Takemoto, T.; Bhacca, N. S. **1966**. *Chem. Pharm. Bull. 14*, 550-551.
29. Hisham, A.; Kumar, G. J.; Fujimoto, Y.; Hara, N. **1995**. *Phytochemistry 40*,1227.
30. http://fajerpc.magnet.fsu.edu/Education/2010/Lectures/12_Memberane_Transport_files/image036.jpg, 1.11.05.
31. http://www.aaas.org/international/africa/gbdi/mod1b.html, 27.10.05.
32. http://www.iupac.org/symposia/proceedings/phuket97/sririrugsa.pdf, 24.09.05.
33. http://www.tuninst.net/MyanMedPlants/DMB-USG/hypoten/hypo.htm#Curcuma-Comosa, 25.10.05.
34. http://www.wellcome.ac.uk/en/genome/tacklingdisease/hg09b005.html, 27.10.05.
35. Huang, Y. L.; Tsai, W. J.; Shen, C. C.; Chen, C. C. **2005**. *J. Nat. Prod*. *68*, 217-220.
36. Hughes, F. M.; Evans-Storms, R. B.; Cidlowski, J. A. **1998**. *Cell Death Differ 5*, 1017-1027.
37. Inayama, S.; Gao, J. F.; Harimaya, K.; Hikichi, M.; Iitaka, Y.; Guo, Y. T.; Kawamata, T. **1985**. *Chem. Pharm. Bull*. *33*, 2179-2182.
38. Ito, J.; Gobaru, K.; Shimamura, T.; Niwa, M. **1998**. *Tetrahedron 54*, 6651-6660.
39. Ito, J.; Niwa, M. **1996**. *Tetrahedron 52*, 9991-9998.
40. Jang, M. K.; Sohn, D. H.; Ryu, J. H. **2001**. *Planta Med*. *67*, 550-552.
41. Jiang, Z. H.; Tanaka, T.; Sakamoto, M.; Jiang, T.; Kouno, I. **2001**. *Chem. Pharm. Bull*. *49*, 1036-1038.
42. Jozova, B.; Novotny, L. **2000**. *Chem. Abstr*. *133*, 187458.
43. Jozova, B.; Novotny, L. **2000**. *Farm. Obz*. *69*, 115.
44. Jurgens, T. M.; Frazier, E. G.; Schaeffer, J. M.; Jones, T. E.; Zink, D. L.; Borris, R. P. **1994**. *J. Nat. Prod. 57*, 230-235.
45. Kashiwada, Y.; Hashimoto, F.; Cosentino, L. M.; Chen, C. H.; Garrett, P. E.; Lee, K. H. **1996**. *J. Med. Chem*. *39*, 1016-1017.
46. Kawaguchi K.; Hirotani M.; Furuya. T. **1988**. *Phytochemistry 27*, 3475-3479.
47. Khan, M. A.; Nabi, S. G. Prakash, S.; Zaman, A. **1986**. *Phytochemistry 25*, 1945-1948.
48. Kikuzaki, H.; Kobayashi, M.; Nakatani, N. **1991**. *Phytochemistry 30*, 3647-3651.
49. Kiuchi, F.; Goto, Y.; Sugimoto, N.; Akao, N.; Kondon, K.; Tsuda, Y. **1993**. *Chem. Pharm. Bull*. *41*, 1640-1643.

50. Kojima, H.; Ogura, H. **1986**. *Phytochemistry 25,* 729-733.
51. Konoshima, T.; Takasaki, M. **2000**. *Stud. Nat. Prod. Chem*. *24*, 607.
52. Kouno, I.; Kawano, N. **1985**. *Phytochemistry 24,* 1845-1847.
53. Kress, W. J.; Defilipps, R. A.; Faer, E.; Kyi, Y. Y. **1964**. *A checklist of the trees, shrubs, herbs and climbers of Myanmar*. *45,* 1-590. http://persoon.si.edu/myanmar/, 1. 11. 05.
54. Kress, W. J.; Prince, L. M.; Williams, K. J. **2002**. *Am. J. Bot. 89,* 1682-1696.
55. Kuroyanagi, M.; Ueno, A.; Koyama, K.; Natori, S. **1990**. *Chem. Pharm. Bull*. *38*, 55-58.
56. Kuroyanagi, M.; Ueno, A.; Ujiie, K.; Sato, S. **1987**. *Chem. Pharm. Bull*. *35*, 53-59.
57. Lázaro, M. L; Pastor, N.; Azrak, S. S.; Ayuso, M. J.; Austin, C. A.; Cortés, F. **2005**. *J. Nat. Prod. 68*, 1642-1645.
58. Lee, K. H. **2004**. *J. Nat. Prod*. *67*, 273-283.
59. Li, A.; Yue, G.; Li, Y.; Pan, X.; Yang, T. K. **2003**. *Tetrahedron Asymmetry 14*, 75-78.
60. Ma, C.; Nakamura, N.; Miyashiro, H.; Hattori, M.; Shimotohno, K. **1998**. *Phytother. Res. 12*, 138-142.
61. Matsuda, H.; Morikawa, T.; Ninomiya, K.; Yoshikawa, M. **2001**. *Bioorg. Med. Chem*. *9*, 909-916.
62. Matsuda, H.; Morikawa, T.; Ninomiya, K.; Yoshikawa, M. **2001**. *Tetrahedron 57*, 8443-8453.
63. Matsuda, H.; Morikawa, T.; Toguchida, I.; Ninomiya, K.; Yoshikawa, M. **2001**. *Chem. Pharm. Bull*. *49*, 1558-1566.
64. Matsuda, H.; Morikawa, T.; Toguchida, I.; Ninomiya, K.; Yoshikawa, M. **2001**. *Heterocycles 55*, 841-846.
65. Matsuda, H.; Ninomiya, K.; Morikawa, T.; Yoshikawa, M. **1998**. *Bioorg. Med. Chem. Lett. 8*, 339-344.
66. Matsunaga, S.; Tanaka, R.; Akagi, M. **1988**. *Phytochemistry 27*, 535-537.
67. Mengoni, F.; Lichtner, M.; Battinelli, L.; Marzi, M.; Mastroianni, C. M.; Vullo, V.; Mazzanti, G. **2002**. *Planta Med*. *68*, 111-114.
68. Nnadozi, K.; Sodipo; Amoo, V.; Cragg, G.; Keller, M.; Artuso, A. **2000**. *GBDI/IITA Biodiversity, Biotechnology, and Law Training Course*. West Africa, 5-7.
69. Newman, D. J.; Cragg, G. M.; Snader, K. M. **2000**. *Nat. Prod. Rep*. *17*, 215-234.
70. Ohshiro, M.; Kuroyanagi, M.; Ueno, A. **1990**. *Phytochemistry 29*, 2201-2205.
71. Ohtsu, H.; Xiao, Z.; Ishida, J.; Nagai, M.; Wang, H. K.; Itokawa, H.; Su, C. Y.; Shih, C.; Lee, Y.; Tsai, M. Y.; Chang, C.; Lee, K. H. **2002**. *J. Med. Chem. 45*, 5037-5042.

72. Petersen, O. G. **1889**. *Musaceae, Zingiberaceae, Cannaceae, Marantaceae. In A. Engler and K. Prantl [eds.], Die Natürlichen Pflanzenfamilien, 1st ed., 2,* 1–43. Verlag von Wilhelm Engelmann, Leipzig, Germany.

73. Pettit, G. R.; Pierson, F. H.; Herald, C. L. **1994**. *Anticancer drugs from animals, plants, and microorganisms,* John Wiley & Sons, INC. New York Chichester, 62-63.

74. Ping, T; Michael, G.; Gilbert, W.; Douglas, S. http://flora.huh.harvard.edu/ china/mss/ volume16/Asclepiadaceae.published.pdf, 27.10.05.

75. Press, J. B.; Reynolds, R. C.; May, R. D.; Marciani, D. J. **2000**. *Stud. Nat. Prod. Chem. 24,* 131.

76. Rajakrishnan, V.; Menon, V. P.; Rajashekaran, K. N. **1998**. *Phytotherapy Research, 12,* 55-56.

77. Rasmussen, H. B.; Christense, S. B.; Kvist, L. P.; Karazmi, A. **2000**. *Planta Med. 66,* 396-398.

78. Repke, K. **1963**. Effects of digitalis on membrane adenosine triphosphatase of cardiac muscle, In: W. Willbrandt, ed. New Aspects of Cardiac Glycosides. Pergamon Press, New York, *3*, 47-73.

79. Repke, K. R. H.; Benga, Gh.; Tager, J. M. (eds). **1988**. *Biomembranes, Basic and Medical Research. Berlin, Springer Verlag*. 161-173.

80. Rios, J. L.; Recio, M. C.; Manez, S.; Giner, R. M. **2000**. *Stud. Nat. Prod. Chem. 24*, 93-95.

81. Roth, G. N.; Chandra, A.; Nair, M. G. **1998**. *J. Nat. Prod. 61*, 542-545.

82. Sashida, Y.; Ogawa, K.; Mori, N.; Yamanouchi, T. **1992**. *Phytochemistry 31*, 2801-2804.

83. Sen, C. K.; Sashwati, R.; Packer, L. **1999**. *Cell Death Differ 6,* 481-491.

84. Shibuya, H.; Hamamoto, Y.; Cai, Y.; Kitagawa, I. **1987**. *Chem. Pharm. Bull. 35*, 924-927.

85. Shiobara, Y.; Asakawa, Y.; Kodama, M.; Yasuda, K.; Takemoto, T. **1985**. *Phytochemistry 24*, 2629-2633.

86. Shiratori, O. **1967**. *Gann 58,* 521-528.

87. Simon, A.; Allais, D. P.; Duroux, J. L.; Basly, J. P.; Durand-Fontanier, S.; Delage, C. **1998**. *Cancer Lett. 129*, 111-116.

88. Skou, J. C. **1965**. *Physiol. Rev. 45*, 596-617.

89. Soudamini, K. K.; Unnikrishnan, M. C.; Soni, K. B.; Kuttan, R. **1992**. *J. Physiol. Pharmacol. 365,* 239-243.

90. Stenkvist, B. **1999**. *Oncology Reports 6*, 493-496.
91. Stenkvist, B.; Bengtsson, E.; Dahqvist, B.; Eriksson, O.; Jarkrans, T.; Nordin, B. N. **1982**. *Engl. J. Med. 306*, 484.
92. Stenkvist, B.; Bengtsson, E.; Eklund, G.; Eriksson, O.; Holmquist, J.; Nordin, B.; Westman-Naeser, S. **1980**. *Anal. Quant. Cytol. 2*, 49-54.
93. Stenkvist, B.; Bengtsson, E.; Eriksson, O.; Holmquist, J.; Nordin, B.; Westman-Naeser, S. **1979**. *Lancet 1*, 563.
94. Suksamrarn, A.; Eiamong, S.; Piyachaturawat, P.; Byrne, L. T. **1997**. *Phytochemistry 45*, 103-105.
95. Sun, I. C.; Kashiwada, Y.; Morris-Natschke, S. L.; Lee, K. H. **2003**. *Curr. Top. Med. Chem. 3*, 155-169.
96. Takano, I.; Yasuda, I.; Takeya, K.; Itokawa, H. **1995**. *Phytochemistry 40*, 1197-1200.
97. Tam, P. T.; Hung, T. **2002**. *Tap Chi Duoc Hoc. 1*, 13-15.
98. Tan, X. G.; Zhang, X. R.; Wang, M. K.; Peng, S.; Ding, L. S. **2002**. *Chinese Chemical Letters 13*, 547-548.
99. Trujillo, J. M.; Hernandez, O.; Navarro, E. **1990**. *J. Nat. Prod. 53*, 167-70.
100. Ueda, J. Y.; Tezuka, Y.; Banskota, A. H.; Tran, Q. L.; Tran, Q. K.; Saiki, I.; Kadota, S. **2003**. *J. Nat. Prod. 66*, 1427-1433.
101. Ueda, J. Y.; Tezuka, Y.; Banskota, A. H.; Tran, Q. L.; Tran, Q. K.; Saiki, I.; Kadota, S. **2003**. *Biol. Pharm. Bull. 26*, 1431-1435.
102. Yaguchi, Y.; Sakurai, N.; Nagai, M.; Inoue, T. **1988**. *Chem. Pharm. Bull. 36*, 1419-1424.
103. Yang, T. T.; Sinai, P.; Kain, S. R. **1996**. *Analytical biochemistry 241*, 103-108.
104. Yasmuawa, K.; Akihisa, T. **2000**. *Chem. Abstr. 133*, 83607.
105. Yasmuawa, K.; Akihisa, T. **2000**. *Nihon Yukagakkaishi 49*, 571.
106. Yoshida, T.; Seno, K.; Takama, Y.; Okuda, T. **1982**. *Phytochemistry 21*, 1180-1182.
107. Yoshikawa, M.; Hatakeyama, S.; Tanaka, N.; Fukuda, Y.; Murakami, N.; Yamahara, J. **1992**. *Chem. Pharm. Bull. 40*, 2582-2584.
108. Yoshikawa, M.; Murakami, T.; Morikawa, T.; Matsuda, H. **1998**. *Chem. Pharm. Bull. 46*, 1186-1188.
109. Zhu, Y. M.; Shen, J. K.; Wang, H. K.; Cosentino, L. M.; Lee, K. H. **2001**. *Bioorg. Med. Chem. Lett. 11*, 3115-3118.

CURRICULUM VITAE

1. Name : Myint Myint Khine
2. Sex : Female
3. Date of Birth : 24th September 1964
4. Place of Birth : Yangon, Myanmar
5. Nationality : Myanmar
6. Education

Year	**Type of Institutions**	**Certificates Diploma and Degrees**	**Grade***
2002-2006	Leibniz Institute of Plant Biochemistry, Halle/Saale Germany	Ph.D Candidate	
1999 - 2002	University of Yangon, Myanmar	Ph.D Candidate	Thesis passed with credit
1996 - 1998	University of Foreign Language, Yangon, Myanmar	German Diploma	
1987 - 1993	University of Yangon, Myanmar	Master of Science	4
1985 - 1986	University of Yangon, Myanmar	B.Sc (Hons.) in Chemistry	4.5
1984 - 1985	University of Yangon, Myanmar	Bachelor of Science Degree	5
1980 - 1981	High School No.(4), Tarmway Yangon, Myanmar (Government of Matriculation)	Passed with Distinction	

7. Professional Experience : (1999 - up to now) Assistant Lecturer
(1991- 1999) Demonstrator
Chemistry Department
[Yangon University]

Grade 5 is the highest
Grade 1 is the lowest